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# Microbial composition of Panchagavya

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**ABSTRACT**

Panchagavya (PG) prepared with five cow products was investigated for documenting the microbes at 7, 15, 30 and 50 days after its preparation. The isolated microbes consisted of aerobic heterotrophic bacteria, lactic acid bacteria, yeast like fungi and anaerobic bacteria. The highest microbial load were recorded in the 7 days old PG. Though a gradual reduction in the microbial load was observed up to 50 days, the microbial population reduced significantly after 30 days, suggesting that the use of PG should be restricted upto 30 days of its preparation to derive maximum benefits.

**Key words**: Microbes, Panchagavya

Panchagavya (PG) is an organic formulation prepared with five products of cow *viz*., milk, ghee, curd, dung and urine (Natarajan, 2002) and its positive effect on growth and productivity of crops has been reviewed and documented earlier by Somasundaram and Amanullah (2007). Due to presence of macro (N. P, K and Ca) and micro (Zn, Fe, Cu, Mn) nutrients (Papen *et al*., 2002), biofertilizers such as azospirillum, azotobactor, phosphobacteria and Pseudomonas (Yadav and Lourduraj, 2005), growth promoting enzymes along with essential plant nutrients (Vasumathi, 2001; Perumal *et al*., 2006; Sanjutha, *et al*.,2008) and beneficial microorganisms ( Swaminathan, *et al*., 2007; Sreenivas, *et al*., 2011), panchagavya is now gaining attention as an efficient organic growth promoter (Naik, *et al*.,2009). In view of the above, the attempts were made to document the microbial composition of PG at 7, 15, 30 and 50 days after its preparation, so as to exploit the maximum load of microbes for its efficient use in agricultural crops.

## MATERIALS AND METHODS

The investigation on the microbial composition of panchagavya was carried out during 2010 in the department of Entomology, College of Agriculture, OUAT, Bhubaneswar. About two liters of PG was freshly prepared in a sequence of (a) 300ml of cow urine and coconut water (100ml) were mixed in a container on day one; (b) after 3 days cow ghee (100gms) was added to cow dung (500 gms) and thoroughly mixed in a separate earthen pot; (c) on 6$^{th}$ day cow urine was added to the cow dung and ghee; (d) on the 8$^{th}$ day all the remaining ingredients like curd (300ml), cow milk (200ml), jaggery (50 gms), ripe banana (2 nos.), fresh coconut water (300ml) and toddy water (200ml) were added to the cow dung and mixed thoroughly. The toddy water was prepared by fermenting fresh coconut water for 3-4 days. Panchagavya so prepared in an earthen pot was stirred twice every day (i.e morning and evening) to aerate the contents and to prevent foul smell. The earthen pot with PG was kept in a shade and covered with mosquito net to prevent dipterous flies from laying eggs. On 15$^{th}$ day the PG was filtered through double-layered muslin to remove fiber and unwanted debris. Sample of PG was collected on 7, 15, 30 and 50 days after its preparation and was subjected to different microbial techniques to ascertain various microbial population. The standard microbiological methods of serial dilution and spread plate technique was used for isolation of total aerobiac heterotrophic bacteria, anaerobic bacteria, fungi, lactic acid bacteria in different agar media plates such as nutrient agar (NA), lactic acid differential agar (LDA), potato dextrose agar (PDA) and anaerobic agar procured from Hi Media (Mumbai). The plates were incubated at 28 ± 2 $^0$C up to one week and then colony counts were recorded. The anaerobic agar plates were incubated in an anaerobic jar with a gas pack and indicator system. The cfu /ml of PG were calculated by taking a mean plate count of three replicates. Further, pure culture of the isolates was prepared and the Gram's reaction was conducted to observe the cell morphology, Gram variability and size of the cells.

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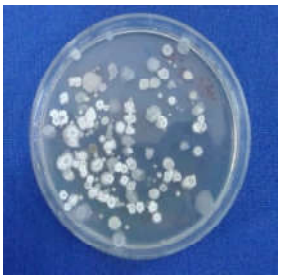
## RESULTS AND DISCUSSION

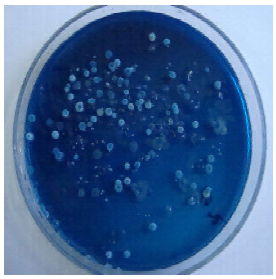
The microbes isolated from Panchagavya using different media showed the presence of aerobic heterotrophic bacteria, lactic acid bacteria, and yeast like fungi (Fig.1a – c) and anaerobic bacteria (Fig.1d). The Gram reaction of the different colonies isolated during the study showed that all the bacterial isolates belongs to Gram +ve rods of varying shapes and sizes.(Fig 2a &2b).The Potato Dextrose Agar showed the growth of non-filamentous smooth fungal colonies. Among the microbes the yeast like fungi ($1.3\times10^6$ CFU/ml) was observed to be predominant at 7 days after preparing PG and this was followed by the anaerobic bacteria ($1.0\times10^6$ CFU/ml) (Table 1).

**Table 1:** Microbes isolated from panchagavya in different media

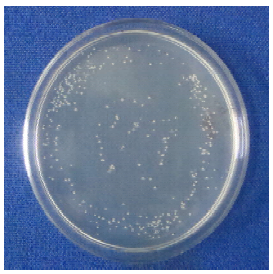
| Media used | Microbes observed | Microbial load (cfu/ml) days after preparing 'Panchagavya' | | | |
|---|---|---|---|---|---|
| | | 7 | 15 | 30 | 50 |
| Nutrient agar | Aerobic heterotrophic bacteria | $7.1x10^5$ | $1.5x10^4$ | $1.1x10^4$ | $8.0x10^3$ |
| Lactic bacteria differential agar | Lactic acid bacteria | $9.5x10^5$ | $2.0x10^4$ | $1.2x10^4$ | $8.6x10^3$ |
| Potato dextrose agar | Fungi (yeast like cell) | $1.3x10^6$ | $3.5x10^3$ | $6.5x10^3$ | $9.4x10^3$ |
| Anaerobic agar | Anaerobic bacteria | $1.0x10^6$ | $1.9x10^4$ | $1.3x10^4$ | $1.3x10^4$ |



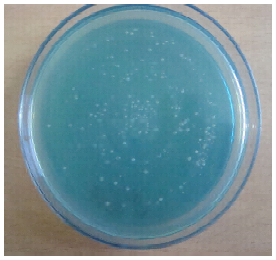
(a) Nutrient Agar (NA) Media



(b) Lactic acid bacteria Differential (LDA)Media

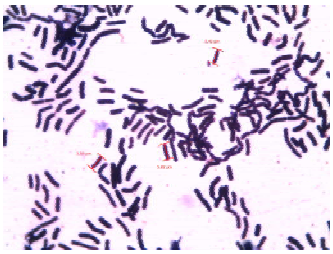


(c) Potato Dextrose Agar (PDA) Media (non filamentous fungi)

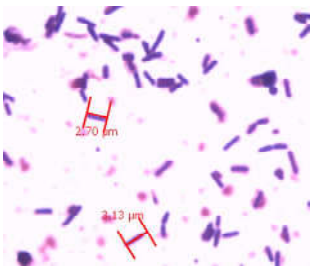


(d) Anaerobic agar (Relatively large & minute bacteria)

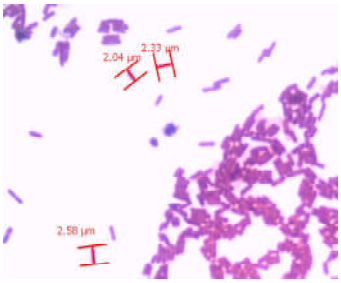
**Fig. 1:** Growth patterns of aerobic and anaerobic microbes in different media



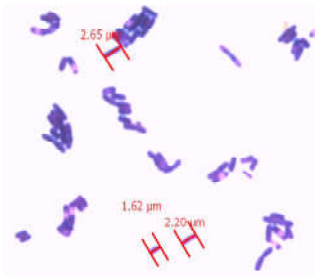
Gram -+ ve rods (3.00 ± 0.54 µm)



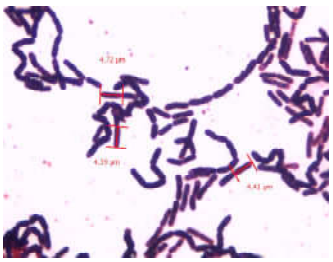
Gram +ve rods (2.48 ± 0.31 µm)



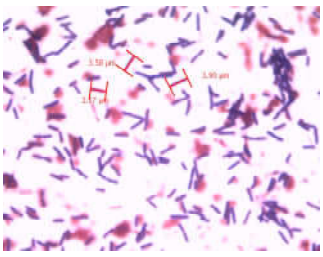
Gram +ve rods (2.32 ± 0.27 µm)



Gram +ve (2.26 ± 0.49 µm) rod shaped with spores

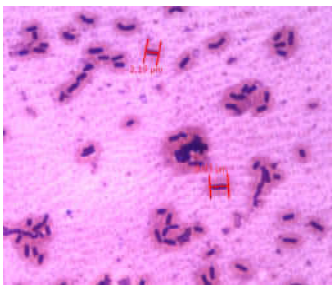


Gram +ve (4.37±0.30 µm) rods in chains

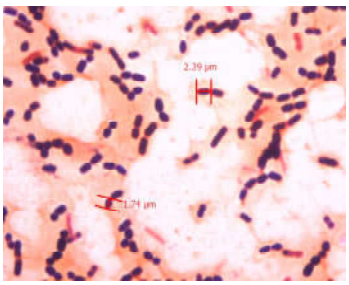


Gram +ve (3.38 ± 0.45 µm) rod

**Fig. 2a:** Microscopic view of different aerobic bacteria and their dimensions



Gram +ve (2.54 ± 0.44 µm) rod; single rods; not in chains



Gram +ve (2.25 ± 0.46 µm) rod; mostly arranged in chains

**Fig. 2b:** Microscopic view of different anaerobic bacteria and their dimensions

Similarly, a significant load of lactic acid bacteria and aerobic bacteria were recorded in 15 ($2.0\times10^4$ CFU/ml) and 30 days ($1.3\times10^4$ CFU/ml) old PG. At 50 days the predominance of anaerobic bacteria ($1.3\times10^4$ CFU/ml) was observed which was followed by the yeast like fungi ($9.4\times10^3$ CFU/ml). High counts of anaerobic bacteria and yeast like fungi at 7, 30, and 50 days old PG might be due to anaerobic condition. Dhama *et al*. (2005) reported the *Lactobacillus* counts as 8.71 log cfu/g at 30 days fermentation and according to them this might be due to the presence of curd and milk in panchagavya.

However, the beneficial microbe i.e. lactic acid bacteria were noticed in appreciable densities in 30 days old PG. Mathivanan *et al*. (2006) also opined that PG at 30 days of age recorded better proposition of chemical and microbial composition favourable for utilization as a growth promoter. Moreover, they also indicated that PG did not have direct antibacterial activity. According to Xu (2001), the effective micro organisms (EMO) in panchagavya were the mixed culture of naturally occurring, beneficial microbes, mostly lactic acid bacteria (*Lactobacillus*), yeast (*Saccharomyces*), actinomyces (*Streptomyces*), photosynthetic bacteria (*Rhodopsuedomonas*) and certain fungi (*Aspergillus*). In view of the fact that panchagavya contains naturally occurring beneficial microorganisms (Swaminathan, 2005), some of which are nitrogen fixers and P-solubilizers (Sreenivas, *et al*., 2011), it can be considered as an ideal organic growth promoter. However, it is advised to be used within 30 days of its preparation to achieve success.

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# Promotion of rice seedling growth characteristics by development and use of bioformulation of *Pseudomonas fluorescens* RRb-11

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**ABSTRACT**

The experiments carried out to study the effect of various carrier based bioformulation on germination, plant height, dry matter and yield revealed that the seed bacterisation increased seed germination by 4.1 to 11.7 percent and by 3.5 to 11.2 percent over the control in pot experiment and by 3.0 to 12.1 percent and 2.0 to 11.8 percent over the control in the microplot experiment during 2006 and 2007, respectively. Seed bacterisation with talc based bioformulation increased plant height significantly. Similarly, the carrier based bioformulation increased dry matter and yield in a significant manner. Though the wheat and soybean based bioformulation was found ineffective in enhancing growth parameters but talc and kaolinite based bioformulation were equally at par in enhancing plant growth characteristics. Seed treatment and spray with RRb-11 bioformulation and chemicals were found at par in reducing disease intensity and increase in the yield.

**Key words:** *Pseudomonas fluorescens*, PGPR, Plant Growth Promotion, Plant height, Germination, drymatter

Plant growth-promoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth to describe soil bacteria that colonize the roots of plants following inoculation onto seed and enhance plant growth. (Kloepper *et al.,* 1980). The prospects of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria to increase plant growth has shown considerable promise in laboratory and green house studies, but responses have been variable in the field (Bowen and Rovira, 1999). PGPR enhance plant growth by direct and indirect means, but the specific mechanisms involved have not been well-characterized. Direct mechanisms of plant growth promotion by PGPR can be demonstrated in the absence of plant pathogens or other rhizosphere microorganisms, while indirect mechanisms involve the ability of PGPR to reduce the deleterious effects of plant pathogens on crop yield. PGPR have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Glick, 1995). Direct enhancement of mineral uptake due to increase in specific ion fluxes at the root surface in the presence of PGPR has also been reported (Bertrand *et al*., 2000).

The rhizosphere microfloras are predominantly composed of gram negative pseudomonads .The fluorescent *Pseudomonads* constitute a large proportion of rhizosphere microflora presumably due to their nutritional versatility, being able to utilize large number of organic substances (Stolp and Gadkari, 1981). Suppression of plant disease by fluorescent *Pseudomonads* species involves the production of sederophores, antibiotics and aggressive root colonization. These are often grouped under PGPR because of their ability to promote the plant growth and suppress plant diseases. Previously, Sakthivel et al., 1986 reported 27% increase in plant height over the control. A PGPR *Pseudomonas fluorescens* B16, isolated from the roots of graminaceous plants has been shown to colonize the roots of various plants, and to increase the height, flower number, fruit number and total fruit weight of tomato plants (Minorsky, 2008). Apart from plant height (PH) and drymatter, other yield components such as germination%, panicle length (PL) and the number of spikelets per panicle (SPP) directly contribute to grain yield and exhibit higher heritability than yield itself (Liu *et al*. 2010). Therefore, it is more feasible to focus on yield components rather than yield as a whole. In this study the plant growth promoting capacity of RRb-11 isolate of *Pseudomonas fluorescens* is reported.

## MATERIAL AND METHODS

Pusa Basmati-1, a susceptible variety of rice to bacterial leaf blight pathogen, *Xanthomonas oryzae pv. oryzae* (Xoo), was selected for the experiment in glass house and microplot. The highly virulent culture of Xoo (Kaul Isolate) isolated

from Haryana was used for the experiment. Inoculation of pathogen on leaf was done by leaf clip method (Kauffman *et al.*, 1973). Leaf tips of 6 week old rice plant were clipped off by sterilized scissor and the cut end of the leaves were submerged in the Xoo suspension. Rhizosphere bacteria (RRb-11) strain was obtained from Bacteriology unit of Division of Plant Pathology, IARI, New Delhi, which was screened previously *in vitro* experiment for its plant growth promoting traits i.e. production of indoleacetic acid (IAA), ammonia (NH3), hydrogen cyanide (HCN), siderophore and 2, 4- diacetylphloroglucinol (DAPG).RRb-11 culture was routinely maintained on NA medium and was subcultured once in a month.

**Preparation of bioformulation**

The two mineral carriers of talc and kaolinite and three powdered organic compounds of wheat bran, barley bran and soybean bran were chosen as carriers. These were steam sterilized at 140 kPa for 30 min and dried aseptically in plastic trays for 12 h at 50°C before use. One loopful of *P. fluorescens* (RRb-11) isolate was inoculated in nutrient agar (NA) broth and incubated on a shaker incubator at 150 rpm for 48 h at 27°C. After 48 h of incubation, the broth containing 1 X $10^8$ cfu per ml was used for preparation of talc, kaolinite, wheat bran, barley bran and soybean bran based formulations. The formulation was developed as described by Amer and Utkhede, 2000 with some modification. The CMC, carrier and bacterial suspension in broth ($10^8$ c.f.u. $ml^{-1}$ ) were used in the ratio of 1:50:4 The bioformulation was prepared as: talc powder (5.0g carboxy methyl cellulose (CMC) + 250 g talc powder (autoclaved at $121^0$C at 15 p.s.i. for 30 min) + 20 ml of bacterial suspension in broth ; kaolinite powder (5.0g CMC + 250 g autoclaved kaolinite powder + 20 ml of bacterial suspension in broth); wheat bran (5.0g CMC + 250 g autoclaved wheat bran + 20 ml of bacterial suspension in broth) ; barley bran (5.0g CMC + 250 g autoclaved barley + 20 ml of bacterial suspension in broth) ; soybean bran (5.0g CMC + 250 g autoclaved soybean bran + 20 ml of bacterial suspension in broth) and 20 ml of bacterial broth suspension alone as a control. The materials were shade dried and stored in sealed plastic bags at room temperature. Three independent samples were analysed with three replications for each analysis. The experiment was set up in completely randomised design (CRD) for glass house and phytotron study and in randomised block design (RBD) for miniplot (1 X 1 $m^2$) study.

***In vitro* evaluation of bioformulations**

**Seed bacterisation**: Seeds of Pusa Basmati-1 were surface sterilized with 1% sodium hypochlorite for 1-2 min., washed and rinsed in sterilized distilled water (SDW) four times and dried overnight under shade. The rhizobacterial culture was separately grown in nutrient broth for 48 hr at 28°C in shaker incubator. The broth obtained was dissolved in sterilized distilled water to obtain the population density of $10^8$ cfu $ml^{-1}$ (adjusted with spectrophotometer).The suspension was mixed with 2 % CMC and different carriers. The seeds were allowed to dry overnight in aseptic condition after coating with carrier mixed bacterial culture and CMC. Care was taken to avoid clumping of seeds. Seeds coated with slurry of CMC (without bacteria) served as control.

**Glass house study**: The formulations were assessed for their efficacy on the plant growth parameters in glass house conditions. The soil collected from rice research fields and sterilized at 85°C for 3 h in steam oven was filled in plastic pots (20 cm). Eight rice seeds treated with different bioformulations were sown in each pot. The number of emerged and healthy seedlings was cut inoculated with Xoo suspension by Kauffman's method as discussed earlier. The pot culture study was undertaken by using completely randomised block design (CRD). An untreated control was also placed for comparison.

**Microplot study**: The seeds treated with different bioformulations were grown in the nursery. The 21 days old seedlings were then transplanted in microplot (1 X 1 $m^2$). The study was framed with three replications for different treatments in randomised block besign (RBD).

**Field study:** A separate field experiment was conducted at Agriculture Research Station, Banswara, a hot spot for bacterial leaf blight of rice, in 2009-10 and 2010-11 to test the efficacy of best performed bioformulation against bacterial leaf blight zone. The experiment was carried out with GP Dhan, as susceptible cultivar, under natural infection. Plot size of the experiment was 2 X 1 $m^2$ with three replications and the experiment was laid out in randomized block design (RBD). The treatments included were hot water treatment (at 52°C for 15 min.), seed treatment and sprays with copper oxychloride (2.5g/kg seeds), streptomycin (100 ppm), 2-bromo-2 nitro propane-1,3- diol (Bactinash-200) (500 ppm) and talc based bioformulation of *P. fluorescens* (5g/kg seeds). The control treatment was maintained without any spray. The disease intensity percent was recorded by following formula:

$$(\%)\ \text{Disease Intensity} = \frac{\text{Leaf length} - \text{Lesion Length}}{\text{Leaf length}} \times 100$$

N, $P_2O5$, and $K_2O$ fertilizers were applied at the rate of 70: 35: 35 kg $ha^{-1}$. The prophylactic sprayings were given at 40 days after sowing (DAS) and second curative spray at the

appearance of the disease ( around 60-65 DAS). The disease intensity was recorded at 20, 40 and 60 days after transplanting (DAT).

**Effect of PGPR on seed germination and other plant growth parameters conditions**

A PGPR strain RRb 11 when mixed with different carriers; bioformulation developed were treated to rice seeds singly or in combination at the rate of $10^8$ CFU/ml. Seeds were gently shaken on an orbital shaker in rhizobacterial suspension prepared in sterile distilled water for 24 h. Seeds soaked in sterile distilled water served as control.

Germination tests were carried out by the paper towel method (ISTA 2003). The germination paper was soaked in distilled water, the PGPR treated and untreated seeds were seeded onto paper towels at the rate of 100 seeds/paper towel, rolled and wrapped with polythene to prevent drying, then incubated at 28°C for seven days. After seven days of incubation, the towels were unrolled and the number of seed germinated were counted for percentage germination from 8 to 12 days after sowing (DAS) . Morphological parameters such as plant height from field surface to the top of the highest panicle of each plant was measured at 50 days after transplanting (DAT).Ten plants in the middle of the two rows of each plot were selected for trait measurements. Plant samples (above ground portions) were collected at 50 days after planting (DAT) to estimate total dry matter content. Grain yield was recorded at harvest.

**Statistical analysis**

The disease intensity data was arcsine transformed before analysis of variance (ANOVA). The package used for analysis was Web Agri Stat Package 2.0 (WASP 2.0) developed by ICAR Research Complex for Goa, Panjim.

**RESULT AND DISCUSSION**

**Effect of seed bacterisation with bioformulation on seed germination and plant growth parameter in glass house experiment**

Seed bacterisation with different bioformulation of *P.fluorescens* influenced seed germination very effectively. It was found that the seed bacterisation increased seed germination by 4.1 to 11.7% and 3.5 to 11.2 per cent over the control in 2006, 2007, respectively. The maximum seed germination was recorded when seeds were treated with talc based bioformulation during both the years. Seed treatment with different carriers based bioformulation improved plant growth parameters viz. plant height (cm), dry matter (g/pot) and yield (g/pot). Talc based bioformulation significantly increased the plant height to 104.1cm and 101.3cm as compared to plant height in control (79.0cm and 74.3cm) in 2006 and 2007 experiment, respectively. Kaolinite and talc+ kaolinite based formulations also showed good results during both the years. (Table 1). Inoculation of wheat plants with *P. fluorescens* in potted soil naturally infested with *Gaeumannomyces graminis* var. *tritici*, showed 29 per cent higher grain yield over untreated control (Mroz *et al.*, 1994).

Similarly, these carrier based bioformulation treatment significantly increased the dry matter content to 328.1 g/pot and 317.3g/pot in talc based bioformulation treatment in 2006 and 2007, respectively. Kaolinite (321.0g/pot), barley (315.1g/pot) and talc + kaolinite (318.4 g/pot) based combination in 2006 experiment while kaolinite (304 g/pot) in 2007 were also equally effective as compared to control. Seeds of PB-1 treated with RRb-11 based bioformulation also showed significant increase in yield. In 2006 experiment, seed treatment with talc based bioformulation significantly increased the yield to 72.1 g/pot as compared to 66.1 g/pot in 2007. Treatment with kaolinite (69.4 g/pot) and talc + kaolinite combination (67.4 g/pot) in 2006 experiment showed better results as compare with control (51.1 g/pot).

Pal *et al.* (2003) reported that four PGPR strains belonging to fluorescent *Pseudomonas* group increased root length, shoot length and pod yield of groundnut which were attributed to production of siderophores and IAA like substances. Two strains of fluorescent *Pseudomonas* isolated from potato epidermis and celery roots significantly increased growth of potato plants upto 50 per cent higher than control in greenhouse assays (Kleopper *et al.*, 1980). Vrany and Fiker (1984) reported that inoculation of tuber pieces with the isolates of *P. fluorescens* caused better growth of potato, tomato, cucumber and lettuce. Potato plants bacterized with plant growth promoting *Pseudomonas* strain showed increased root and shoot fresh weight and observed simultaneous suppression of deleterious pathogenic microflora (Vanpeer and Schippers, 1989).

**Effect of seed bacterisation with bioformulations on seed germination and plant growth parameter in microplot experiment**

Similar to the seed bacterisation in glass house, the seed bacterisation with different bioformulations in microplot was also found effective in plant growth promotion. The treatment with different bioformulation increased seed germination by 3.0 to 12.1% over control in 2006 and by 2.0 to 11.8% over control in 2007. Maximum seed germination was recorded when seeds were treated with talc based bioformulation during both the years. The talc

**Table 1:** Effect of seed treatment with bioformulation of *P.fluorescens* on plant growth parameters during 2006 and 2007

| Treatments | Plant Growth Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Seed germination (%) | | Plant height (cm) | | Dry matter (g/pot) | | Yield (g/pot) | |
| | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| Kaolinite powder | 90.3 | 91.2 | 100.3 | 96.4 | 321.0 | 304.1 | 69.3 | 61.5 |
| Talc powder | 94.1 | 93.2 | 104.1 | 101.3 | 328.1 | 317.3 | 72.1 | 66.1 |
| Soybean bran | 88.2 | 86.5 | 86.1 | 84.2 | 273.0 | 269.5 | 61.0 | 58.0 |
| Barley bran | 90.2 | 91.2 | 97.4 | 93.1 | 315.1 | 296.2 | 65.7 | 60.3 |
| Wheat bran | 86.5 | 85.5 | 88.1 | 84.0 | 275.1 | 270.5 | 62.1 | 60.5 |
| Soybean bran + Barley bran | 90.0 | 91.7 | 90.3 | 87.3 | 297.5 | 290.3 | 63.0 | 65.5 |
| Soybean + wheat bran | 85.3 | 84.5 | 83.0 | 83.0 | 271.5 | 268.1 | 59.5 | 57.0 |
| Barley bran + Talc powder | 91.5 | 92.0 | 93.1 | 90.5 | 309.3 | 290.0 | 63.1 | 59.3 |
| Soybean bran + Kaolinite powder | 89.5 | 90.0 | 86.3 | 85.1 | 273.0 | 275.5 | 61.5 | 63.2 |
| Barley bran + Wheat Bran | 88.0 | 87.0 | 83.2 | 82.3 | 265.0 | 268.5 | 59.5 | 58.5 |
| Soybean bran + Talc powder | 88.5 | 88.3 | 85.4 | 85.3 | 270.1 | 271.3 | 60.5 | 62.1 |
| Barley bran + Kaolinite powder | 91.0 | 92.5 | 91.3 | 89.1 | 298.4 | 280.5 | 63.3 | 58.5 |
| Wheat bran + Talc powder | 87.1 | 89.3 | 89.1 | 83.3 | 292.0 | 288.5 | 63.0 | 60.1 |
| Wheat bran + Kaolinite powder | 89.5 | 89.0 | 88.0 | 82.1 | 277.1 | 278.2 | 62.1 | 59.3 |
| Talc + Kaolinite powder | 92.5 | 92.0 | 98.2 | 93.5 | 318.4 | 298.1 | 67.4 | 61.3 |
| Control | 82.4 | 81.0 | 79.0 | 74.3 | 213.0 | 224.3 | 51.1 | 49.3 |
| CD (P=0.05) | | | 3.21 | 3.01 | 17.58 | 21.3 | 3.52 | 3.41 |

based bioformulation treatment increased plant height to 98.4 cm and 99.3 cm as compared to control (78.1 cm and 78.4 cm), respectively in both the years. Similarly, the treatment with kaolinite and talc + kaolinite based bioformulation was found equally effective in both the years. The treatment with talc based bioformulation significantly increased the dry matter content to 77.1 g/pot and 76.7 g/pot in 2006 and 2007, respectively. Plant growth promoting strains of *P. fluorescens* ANP15 and *P. aeruginosa* 7 NSK-2 were reported to protect maize seeds from cold stock damage and significant increased in germination of maize seeds and enhanced dry matter content of inoculated plants (Hofte *et al*., 1991). In 2006, the seed treatment with talc based bioformulation significantly increased the yield to 366.1 g/$m^2$ as compared with 365.1 g/$m^2$ in 2007. (Table 2).

The results showed that most bioformulations promoted growth characteristics of the paddy seedlings as compared to the control. This may probably be because of bioformulations playing effective roles in increasing and establishment of durability of antagonistic microorganisms in soil and possibly producing antibiotics, siderophores, hydrolytic enzymes, phytohormones and/or other volatile extra-cellular metabolites.

**Effect of seed treatment of different carrier based bioformulation on bacterial leaf blight of rice**

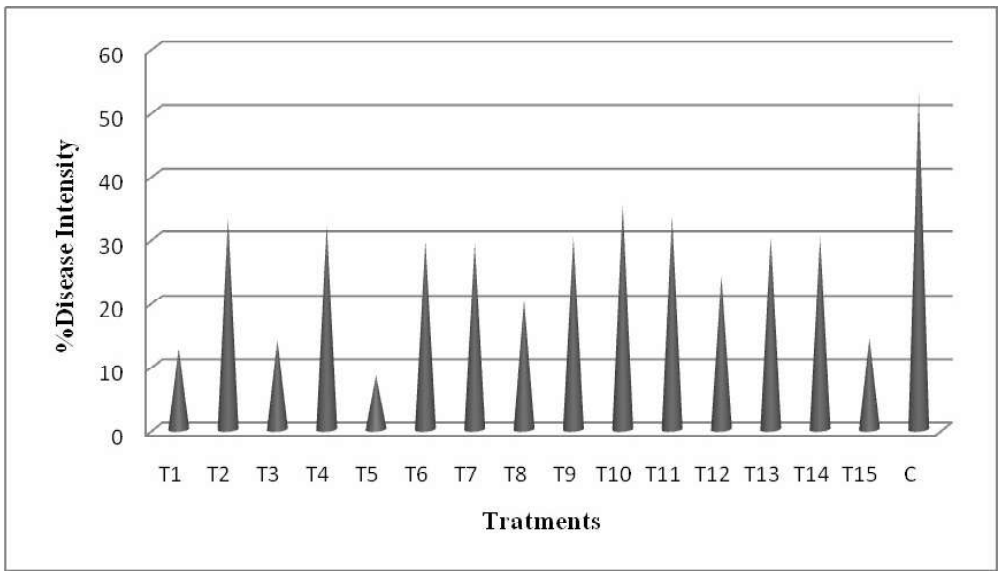
When seeds were treated with various carrier based bioformulation of *P. fluorescens* RRb-11 in glass house the talc based bioformulation treatment showed minimum disease intensity of 8.47 percent and reduced disease intensity to the extent of 83.87 percent against the control. The seed treated with kaolinite based bioformulation exhibited disease intensity of 12.66 percent which reduced disease intensity by 75.9 percent as compared to control. The percent disease intensity in untreated control was 52.6 percent. Talc based formulation of *P. fluorescens* Pf1 was coated on to seeds at the rate of 4g/Kg ($10^7$cfu/g) of chickpea seeds (cv.Shoba) for the management of chickpea wilt. Sowing of treated chickpea seeds resulted in establishment of rhizobacteria on chickpea rhizosphere (Vidhyasekaran and Muthamilan, 1995). Treatment of pigeonpea seeds with talc based formulation of *P. fluorescens* (Pf1) effectively controlled fusarial wilt of pigeonpea under greenhouse and field conditions (Vidhyasekaran *et al*., 1997). Soaking of rice seeds in water containing 10g of talc based formulation of *P. fluorescens,* consisting mixture of PF1 and PF2 ($10^8$cfu/g), for 24h controlled rice sheath blight under field condition (Nandakumar *et al*., 2001).

**Table 2:** Effect of seed treatment with bioformulation of *P.fluorescens* on plant growth parameters during 2006 and 2007

| Treatments | Plant Growth Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Seed Germination (%) | | Plant height (cm) | | Dry matter (g/plant) | | Yield (g/m²) | |
| | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| Kaolinite powder | 89.4 | 88.7 | 97.0 | 93.8 | 71.5 | 71.0 | 353.0 | 342.4 |
| Talc powder | 92.5 | 91.3 | 98.4 | 96.4 | 77.1 | 74.5 | 366.1 | 365.1 |
| Soybean bran | 86.4 | 85.5 | 83.0 | 85.6 | 56.0 | 55.6 | 314.0 | 308.5 |
| Barley bran | 90.0 | 87.5 | 95.1 | 88.1 | 70.3 | 69.0 | 347.1 | 340.5 |
| Wheat bran | 87.5 | 84.3 | 81.1 | 78.5 | 61.2 | 58.5 | 321.4 | 307.2 |
| Soybean bran + Barley bran | 88.5 | 88.0 | 83.1 | 81.5 | 58.5 | 55.0 | 315.4 | 311.5 |
| Soybean + wheat bran | 83.4 | 81.5 | 80.1 | 83.5 | 51.9 | 52.4 | 303.1 | 298.5 |
| Barley bran + Talc powder | 89.5 | 87.1 | 89.3 | 87.4 | 68.4 | 69.4 | 337.3 | 332.4 |
| Soybean bran + Kaolinite powder | 86.2 | 89.4 | 83.3 | 82.5 | 56.4 | 55.1 | 313.2 | 310.0 |
| Barley bran + Wheat Bran | 85.2 | 86.4 | 84.2 | 85.0 | 53.3 | 55.4 | 307.0 | 305.5 |
| Soybean bran + Talc powder | 87.4 | 86.0 | 83.4 | 81.4 | 55.3 | 51.2 | 311.1 | 313.2 |
| Barley bran + Kaolinite powder | 88.0 | 88.5 | 89.0 | 86.1 | 65.7 | 67.5 | 334.0 | 334.5 |
| Wheat bran + Talc powder | 85.0 | 88.1 | 84.1 | 85.3 | 60.0 | 62.4 | 325.4 | 314.1 |
| Wheat bran + Kaolinite powder | 88.2 | 86.4 | 85.4 | 87.5 | 61.8 | 64.5 | 329.4 | 325.1 |
| Talc + Kaolinite powder | 91.5 | 90.5 | 97.2 | 95.3 | 73.2 | 71.3 | 357.1 | 349.5 |
| Control | 80.4 | 79.5 | 78.1 | 78.4 | 42.0 | 50.1 | 272.1 | 285.4 |
| CD (P=0.05) | | | 2.01 | 3.52 | 7.42 | 4.31 | 27.45 | 21.45 |

**Table 3:** The comparative effect of chemical treatment and talc based bioformulation of *Pseudomonas fluorescens* RRb-11 against bacterial leaf blight of rice.

| Treatments | 2009-10 | | 2010-11 | | Cumulative mean %DI | | Yield (qt/ha) | |
|---|---|---|---|---|---|---|---|---|
| | %DI | % ROC | % DI | % ROC | % DI | % ROC | Mean | % IOC |
| Seed treatment with COC+ Streptomycin | 21.2 (27.4)$^{b}$ | 27.8 | 19.3 (26.0)$^{ab}$ | 25.5 | 20.2 | 26.8 | 28.43 | 13.4 |
| Spray with Bactinash-200 | 22.8 (28.5)$^{ab}$ | 22.4 | 19.8 (26.3)$^{ab}$ | 23.5 | 21.3 | 22.8 | 28.15 | 12.4 |
| T1 +2 Spray of COC + Streptomycin | 10.2 (18.2)$^{d}$ | 65.3 | 8.5 (16.9)$^{d}$ | 67.2 | 9.3 | 66.3 | 34.22 | 36.6 |
| Hot water treatment +2 Spray of COC + Streptomycin | 14.7 (22.5)$^{cd}$ | 50.0 | 11.4 (19.6)$^{cd}$ | 66.0 | 13.1 | 52.5 | 31.27 | 24.8 |
| ST with *Pseudomonas fluorescens* bioformulation | 18.1 (25.4)$^{bc}$ | 38.4 | 15.1 (22.7)$^{bc}$ | 41.7 | 16.6 | 39.8 | 30.88 | 23.3 |
| ST + Spray with *P.fluorescens* bioformulation | 12.2 (20.4)$^{d}$ | 58.5 | 12.2 (20.4)$^{cd}$ | 52.9 | 12.2 | 55.8 | 32.46 | 29.6 |
| Hot water treatment + Spray of Bactinash-200 | 14.1 (22.0)$^{cd}$ | 52.0 | 11.9 (20.1)$^{cd}$ | 54.0 | 13.0 | 52.9 | 31.08 | 24.1 |
| Control | 29.4 (32.8)$^{a}$ | | 25.9 (30.6)$^{a}$ | | 27.6 | | 25.05 | |
| CV | 11.2 | | 11.7 | | | | | |
| CD at 5% | 4.8 | | 4.7 | | | | | |




T1-Kaolinite powder;T2-wheat bran;T3-Barley bran;T4-Soybean bran;T5-Talc powder; T6- Soybean bran + Barley bran;T7- Soybean + wheat bran;T8- Barley bran + Talc powder;T9- Soybean bran + Kaolinite powder; T10- Barley bran + Wheat Bran;T11- Soybean bran + Talc powder;T12- Barley bran + Kaolinite powder;T13- Wheat bran + Talc powder;T14- Wheat bran + Kaolinite powder;T15- Talc + Kaolinite powder; C-Control

**Fig 1:** Effect of seed treatment with different carrier based bioformulation of *P. fluorescens* against bacterial leaf blight of rice.

**The comparative effect of chemical treatment and talc based bioformulation of *P. fluorescens* against bacterial leaf blight of rice.**

The results encouraged us to test the talc based bioformulation against bacterial leaf blight of rice. Thus, an experiment was framed to test the comparative effect of chemical treatment and talc based bioformulation of *P. fluorescens* against bacterial leaf blight (BLB) of rice. The treatments comprising chemicals and biological control agent, a powder formulation of *P. fluorescens* RRb-11, was tested against BLB. There was a significant reduction in disease intensity in most of the treatments except those where only prophylactic spray was done i. e. seed treatment with copper oxychloride (COC) and streptomycin and one spray with 2- Bromo-2 Nitro Propane-1,3- Diol (Bactinash-200). Maximum reduction of 65.3 percent and 67.2 percent in disease intensity over control was recorded when seed treatment and two sprays with copper oxychloride and streptomycin was done in both the years, respectively. Significant reduction in disease intensity was also recorded when seed treatment and spray with talc based bioformulation of *P.fluorescens* RRb-11 was done. This treatment reduced disease intensity by 58.5 percent and 52.9 percent in 2009 and 2010, respectively. In 2009-10, the seed treatments and spray with COC+ streptomycin and *P. fluorescens* showed at par results. Lowest cumulative mean percent disease intensity of 9.3 percent was recorded when seeds were treated and plants were sprayed with copperoxychloride and streptomycin. Interestingly the seed treatment and spray with talc based bioformulation also showed significantly low cumulative mean percent disease intensity of 12.2 percent (Table 3). The mean maximum yield of 34.22 qt/ha was recorded when seeds were treated and plants were sprayed with copperoxychloride and streptomycin which is very closely followed by seed treatment and spray with talc based bioformulation of *P.fluorescens.*

The study revealed that seed treatment with bioformulation of *P. fluorescens* RRb-11 recorded overall increase in plant growth promoting parameters. The treatment with talc based bioformulation not only reduced the disease intensity but also enhanced germination, increased height, dry matter and ultimately the yield. The field trial also revealed at par efficacy against bacterial leaf blight of rice. Therefore, this bioformulation can be exploited commercially.

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# Utilization of bagasse hydrolyzate for lactic acid production by fed batch fermentation

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**ABSTRACT**

Comparative study of lactic acid production, conducted through the fed batch fermentation and batch production method utilizing sugars from acid hydrolyzed sugarcane bagasse partially substituting glucose in production media and pure strains of *Lactobacilli* (1) *L. delbreuckii* (NCIM2025) (2) *L. pentosus* (NCIM 2912) (3)*Lactobacillus sp.*(NCIM 2734 (4) *Lactobacillus sp.* (NCIM2084) and coculture of first two strains revealed that the strain-3 provided the highest lactic acid production of 89.92 g/l and 109.45 g/l, closely followed by that of coculture, 88.79 g/l and 108.94 g/l at 120 g/l total sugar input, while at same sugar input the productivity values of coculture 1.8497 and 1.8156 g/l/h in cases of batch and fed batch production were overall highest in comparison to all the pure strains. The experiments highlighted the potential, advantages of the coculture over pure culture (based on productivity) and fed batch production over the batch one, in terms of maximum lactic acid production, and operating without inhibition at high sugar concentrations.

**Keywords**: Batch, Bagasse, Coculture, Fed batch, Fermentation, hydrolyzate, *Lactobacillus.*

Lactic acid (hydroxy carboxylic acid) and its predominant producer bacteria, *Lactobacillus sp* , are in high demand for their roles as acidulant, preservative, flavoring agent, probiotics and bacteriocins in food and dairy industries and act as moisturizing agents in cosmetics (Adsul et al. 2007; Narayanan et al. 2004; Adnan et al. 2007 ). Lactic acid (hydroxy carboxylic acid) is a high utility organic acid that finds various applications in food, dairy, chemical, textile, pharmaceutical industries and in biomedical sector ( Ghosh and Ghosh, 2011a).The biopolymer industry has high requirement of lactic acid as feedstock , which is evidenced by consumption of 20-30 per cent of global production of lactic acid in 2005 (Zhang et. al., 2007). The fermentative production (about 90%) of lactic acid obtained through microbes is preferred over chemical synthesis methods, as it provides steriospecific L(+) and D(-) forms of high purity lactic acid, while later provides a racemic mixture DL lactic acid, which requires high cost in separation (Altaf et al., 2006; Adsul et. al., 2007). Sugarcane is abundantly cultivated in India engaging about 4.36 million hectares of total cropped area and production over 250 million tons per year **(**Trivedi, 2008). The sugar mills and juice extraction units produce large amount of sugarcane bagasse as agroindustrial waste, which under the dried conditions may cause fire hazards and lung problems such as bagassosis due to its inhalation at the work place **(**Phoolchand, 1991**)**. In India the processing of 100 tons of sugarcane provides approximately 30-34 tons of bagasse out of which, 22-24 tons are used in processing while 8-10 tons are still left as waste (Shrivastava and Solomon, 2008). Over 60,000,000 tons of sugarcane bagasse is produced by Brazil while Asia shares about 44 percent of sugarcane bagasse (Kim and Dale, 2004; Pessoa et al., 1997). In the light of the above mentioned facts about the abundance, availability and risks involved with dried bagasse, the hydrolysis of bagasse is a better option for its agroindustrial waste management through cost effective chemical production, because its hydrolyzate consists of variety of fermentable sugars that can be utilized for production of chemicals and energy (Han et al., 1983).

The objectives of the present study were (1) to compare the lactic acid production through the batch and fed batch fermentations (2) to determine the effect of different doses of sugarcane bagasse hydrolyzate (used after lignin removal, acid hydrolysis and neutralization) and glucose on lactic acid production by the *Lactobacillus* strains under study (3) to evaluate advantages of application of coculture over the pure strains of lactobacilli, in terms of lactic acid production and productivity.

## MATERIALS AND METHODS

The chemicals used in the experiments were of Merck and High media make. Pure cultures of *Lactobacilli* (1) *L. delbrueckii* (NCIM2025) (2) *L. pentosus* (NCIM 2912) (3) *Lactobacillus sp.* (NCIM 2734) (4) *Lactobacillus sp.* (NCIM2084) were acquired from National Chemical Laboratory(NCL) Pune. The inoculum for the *Lactobacilli* strains were prepared

in MRS (de Mann Rogosa Sharpe) media at $35^0$ C, 180 rpm for fourteen hours .Composition of one liter MRS medium is : 10g proteose peptone, 5g yeast extract, 10g beef extract, 20g dextrose, 1g tween 80, 2g ammonium citrate, 5g sodium acetate, 0.1g $MgSO_4.7H_2O$, 0.05 g $MnSO_4$, 2g $K_2HPO_4$ in distilled water as solvent. For preparation of the coculture the volumes of the liquid cultures corresponding to 1.55 g/L cell dry weight in the strains(1) and strain(2) were mixed.

The hydrolysis of bagasse was carried out with 1 percent sulphuric acid, taking 1/10 w/v ratio under autoclaving conditions,$121^0$ C,15 lbs for 30 minutes. This was later cooled, detoxified with calcium carbonate and neutralized for further use in production media.

One liter of production media consists of : Various initial doses of total sugar (60, 80 ,100 and 120)g/L were applied in production media and other components were, 15g yeast extract, 1g sodium acetate, 0.03g $MnSO_4.H_2O$, 0.10g $MgSO_4.7H_2O$, 0.25g $KH_2PO_4$, 0.25g $K_2HPO_4$ and 0.03g $FeSO_4$. The portion of pure glucose in 60, 80,100 and 120 g total sugar dose in one liter were 45, 60, 70 and 80 g and the rest was contributed by the sugars from bagasse hydrolyzate.

For investigation of comparative advantages of fed batch fermentation utilizing bagasse hydrolyzate sugars at high dose 120 g/l, a set of batch experiments were performed with pure glucose at 120 g/l.

Earlenmayer flasks of 250 ml containing 100ml production media, were inoculated with 1.55 g/l cell dry weight keeping the initial pH 6.5.These were kept at $35^o$ C,180 rpm for 108 hours incubation conditions with an addition of 2 percent sodium hydroxide neutralizer every 12 hours to maintain the pH. In the fed batch experiments, 10 ml feeding of acid hydrolyzed, detoxified and neutralized bagasse hydrolyzate solutions was performed every 12 hours. Sodium hydroxide (2%) was preferred over calcium carbonate due the inhibitory effects of calcium carbonate on the bacterial strains under study (Ghosh and Ghosh, 2008; 2009;2011b).

The cell dry weight of bacterial cells was determined by centrifugation of preculture MRS broth obtained after 14 hours, at 8000 rpm for 10 minutes, to separate the cells from supernatant followed by washing with 0.85% NaCl solution and drying in preweighed microporous papers at $70^0$C till constant weights were attained.

The pentosan and lignin in the sugarcane bagasse were determined spectrophotometrically( Ghosh and Ghosh, 2011b).The glucose in the hydrolyzate was estimated by DNS method.

**Determination of pentoses in sugarcane bagasse hydrolyzate**

This is a colorimetric method developed by M.V. Tracey, is helpful in the quantitative determination of pentose sugars even in the presence of large excess of hexoses. The reagent was prepared by mixing 100 ml glacial acetic acid, 10 ml 5 per cent aqueous acetic acid,24 ml water 16ml colorless aniline were mixed and stored in dark glass bottles. Six ml of the reagent were mixed in a test tube with 2ml sugar sample to be determined and these test tubes were kept in dark at room temperature for twenty hours, xylose was used as standard. The color developed was measured by spectrophotometrically at 480nm (Tracey, 1950).This method was applied for determining of the pentose from the sugarcane acid treated bagasse extract. But prior to the autoclaving or boiling treatment of bagasse with 1per cent sulphuric acid the bagasse was subjected to lignin removal by ammonium hydroxide (Laopaiboon et al., 2010).Then delignified bagasse in dried and ground form was used. Subsequent to boiling or autoclaving treatments the extract was detoxified with calcium hydroxide (Pessoa et al., 1997).

**Estimation of lactic acid by Kimberley Taylor method**

The lactic acid present in the fermentation broths were quantitatively assayed by Kimberly Taylor method, which utilizes hot concentrated sulphuric acid effects , that include oxidation of lactic acid to acetaldehyde, which subsequently forms a chromogenic complex with p- phenyl phenol in presence of copper. The fermented broths were centrifuged at 8000g and the supernatants were used for lactic acid estimation. 0.5 ml of supernatant was added with 3ml of 96 percent sulphuric acid, followed by heating for ten minutes in boiling water bath for ten minutes, then cooling it to room temperature for about 30 minutes. The cool solution was added with 50 micro liter 4 per cent copper sulphate and 100 micro liter p- phenyl phenol (prepared by dissolving 1.5 percent of the reagent in 95 percent ethyl alcohol) which provided a chromogenic complex. The absorbance for lactic acid is measured in a UV-VIS double beam spectrophotometer at 570nm (Taylor, 1996 and Mirdamadi et al., 2002).

The figures for trend line graphs and the trend line equations for different doses of sugar input and lactic acid output have been provided with the help of MS-Excel.

## RESULTS AND DISCUSSIONS

The sugarcane bagasse used for acid hydrolysis was found to contain 25.68 percent pentosans and 20.06 percent lignin by weight. The acid hydrolyzate of sugarcane predominantly consisted of the pentoses such as xylose 11.62 g/l and arabinose 1.24 g/l besides glucose, 2.85 g/l. The

**Table 1**: Lactic acid formation by the different strains of *Lactobacilli* through batch production method.

| Bacterial strains | Total Sugar 60 g/l | | Total Sugar 80 g/l | | Total Sugar 100 g/l | | Total Sugar 120 g/l | | Pure Glucose 120 g/l | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LA | P | LA | P | LA | P | LA | P | LA | P |
| *L. delbrueckii* NCIM2025 (Strain-1) | 37.08±0.91 | 0.7725 | 49.87±1.25 | 1.0389 | 60.85±1.21 | 1.2677 | 71.34±1.22 | 1.1890 | 71.01±1.23 | 1.1835 |
| *L. pentosus* NCIM2912 (Strain-2) | 48.34±1.26 | 1.0070 | 64.66±1.36 | 1.3470 | 72.01±1.23 | 1.5002 | 82.06±1.39 | 1.1397 | 59.07±1.27 | 0.9848 |
| Coculture of strains 1 , 2 | 49.76±1.30 | 1.0366 | 67.93±1.41 | 1.4152 | 78.66±1.35 | 1.6387 | 88.79±1.43 | 1.8497 | 76.85±1.24 | 1.6012 |
| *Lactobacillus sp.* NCIM2734 (Strain-3) | 47.64±1.24 | 0.9925 | 69.54±1.28 | 1.1590 | 80.02±1.41 | 1.3336 | 89.92±1.45 | 1.2486 | 58.98±1.21 | 1.2287 |
| *Lactobacillus sp.* NCIM2084 (Strain-4) | 36.51±0.98 | 0.6085 | 48.37±1.25 | 0.6718 | 57.35±1.27 | 0.7965 | 66.89±1.39 | 0.9290 | 60.07±1.28 | 1.0011 |

**Table 2**: Lactic acid formation by the different strains of *Lactobacilli* in batch formation.

| Bacterial strains | Total Sugar 60 g/l | | Total Sugar 80 g/l | | Total Sugar 100 g/l | | Total Sugar 120 g/l | |
|---|---|---|---|---|---|---|---|---|
| | LA | P | LA | P | LA | P | LA | P |
| *L. delbrueckii* NCIM2025 (Strain-1) | 43.26±1.18 | 0.7210 | 57.06±1.28 | 0.9510 | 69.90±1.34 | 1.1650 | 78.80±1.43 | 1.3133 |
| L. pentosus NCIM2912 (Strain-2) | 53.77±1.24 | 0.8961 | 67.89±1.32 | 1.1315 | 85.76±1.56 | 1.4293 | 96.42±1.80 | 1.1478 |
| Coculture of strains 1 and 2 | 54.89±1.26 | 0.9148 | 71.68±1.29 | 1.1946 | 90.23±1.63 | 1.5038 | 108.94±1.87 | 1.8156 |
| *Lactobacillus sp.* NCIM2734 (Strain-3) | 53.01±1.20 | 0.8835 | 72.90±1.38 | 1.2150 | 91.08±1.72 | 1.0842 | 109.45±1.94 | 1.3031 |
| *Lactobacillus sp.* NCIM2084 (Strain-4) | 44.97±1.12 | 0.7495 | 53.89±1.22 | 0.7484 | 64.39±1.30 | 0.8943 | 72.44±1.35 | 1.0061 |

lactic acid production data in the tables 1 and 2 have been expressed as mean ± standard deviation values obtained from different trials and the productivities given are around the mean values. The results in Table 1, for batch fermentation indicate that for all the doses of sugar except at 60 g/l, the strain-3 showed higher lactic acid production very closely followed by the coculture, but still the coculture which has showed higher productivity values for every dose of sugar with the overall highest being 1.8497 g/l/h can be considered best option for lactic acid production. In Table 1 the strain-3 and strain-2 which utilize both hexoses and pentoses, show higher lactic acid production than the strain-1, because the strain -1 (*L. delbrueckii*) is known only to utilize hexose sugars but the bagasse hydrolyzate used in the production medium as carbon source, predominantly adds to pentose sugars.

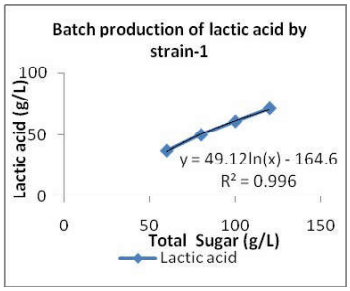
LA- Maximum Lactic acid concentration (g/l); P- Corresponding productivity (g/l/h); The fermentation conditions applied: 1.55 g/l inoculums (cell dry weight), 2% NaOH neutralizer (added every 12 hours to maintain pH) at $35^0$C, initial pH 6.5,180 rpm shaking for 108 hours.

With the increasing doses of total sugar input, comprising of glucose and bagasse hydrolyzate from 60 to 120 g/l, all the *Lactobacillus* strains and the coculture showed an increase in lactic acid production while the 120 g/l pure glucose input led to the decline in lactic acid production of all the strains. The lactic acid production (Table1) by strain-1 at 120 g/l hydrolyzate containing total sugar is approximately similar to that with 120 g/l pure glucose input, still it can be concluded that the strain-1, performs better in 120 g/l hydrolyzate containing media because that only contained 80 g/l of initial pure glucose (since strain-1 cannot utilize pentose sugars) decline with the 120 g/l dose of pure glucose. The level of glucose applied in the medium for fermentative production of lactic acid affects several components of glycolysis pathway and uptake rate, hence the resultant lactic acid production may be consequently at

higher level or lower (substrate inhibition) level (Papagianni et al., 2007). The study revealed that, at optimum level of glucose, key glycolytic enzymes PFK (phospho-fructo kinase), PYK (pyruvate kinase) and the LDH (lactate dehydrogenase), showed highest specific activities (also highest glycolytic flux) but very high level of glucose application led to the reduction in the glycolytic flux and decline in PFK activity. The reduction in PFK enzyme activity, simultaneously showed a decrease in glycolytic and lactate fluxes (Papagianni et al., 2007). It has been reported that very high doses of glucose can lead to, accumulation of un-phosphorylated intracellular glucose through un-facilitated diffusion (due to saturation of carriers) and lower amounts of phosphorylated sugars, which suggest inhibition of PFK enzyme. The phosphorylated sugars such as fructose bis phosphate FBP, activate the PYK and LDH and direct the flux towards lactate production, while its lower levels inactivate the LDH (Papagianni et al., 2007). The LDH enzyme catalyzes the conversion of pyruvic acid to lactic acid hence its inactivation may lower the lactic acid production significantly.

LA- Maximum Lactic acid concentration (g/l); P- corresponding productivity (g/l/h); The fermentation conditions: utilizing 1.55 g/l inoculum, 2% NaOH neutralizer (added every 12 hours to maintain pH) at 35$^0$C, initial pH 6.5,180 rpm shaking for 108 hours incubation. Aliquots of 10 ml solution of acid hydrolyzed, detoxified and neutralized sugarcane bagasse, were fed after each 12 hours in the corresponding set of flasks.

The data for fed batch fermentation (Table 2) indicate the attainment of highest individual lactic acid production values and productivities for all the strains at 120 g/l sugar dose. The highest overall lactic acid production was attained by the strain-3 closely followed by the coculture while the strain-4 showed minimum lactic acid production with all the doses of total sugar. The strain-2 and strain-3 had higher lactic acid production than strain-1, similar to the trend found in Table 1. From the Table1 and 2 it is evident that the fed batch fermentation provides higher lactic acid concentrations for all the sugar doses while the batch production achieves maximum productivities, except for strain-4, till 100 g/l total sugar dose while at 120 g/l sugar dose fed batch fermentation has both higher lactic acid production and productivities than the batch one. The fed batch lactic acid production (utilizing hydrolyzate substituted carbon source) and corresponding productivities in Table-2, are much higher than these values obtained for the 120 g/l pure glucose input (Table-1). Hence fed batch fermentation utilizing higher doses of bagasse hydrolyzate with the coculture may prove beneficial for lactic acid production industries.

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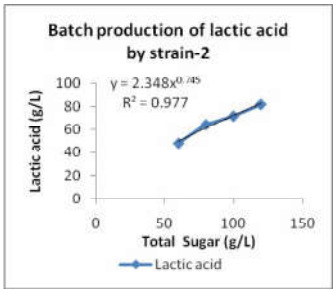


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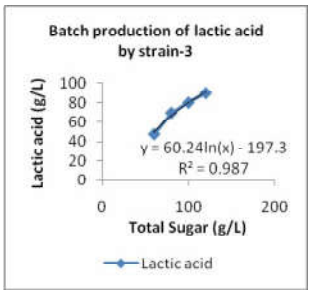


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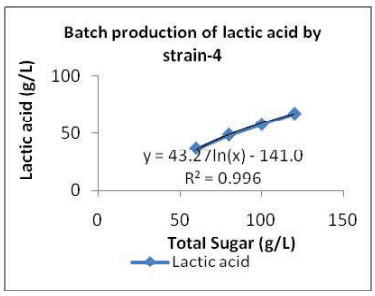
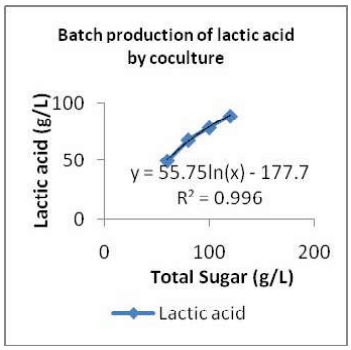


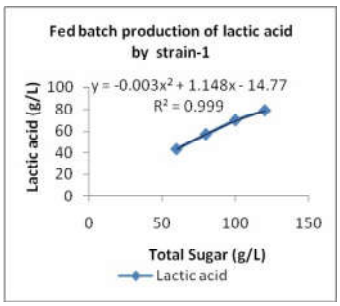

1 (d)



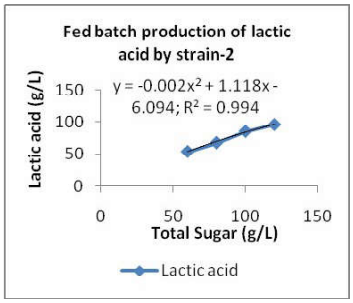

1 (e)

**Fig. 1.** Mathematical relations between total sugar input and lactic acid output with batch fermentation utilizing acid hydrolyzate of sugarcane bagasse in production media at 35$^0$C, initial pH 6.5,1.55g/l inoculum (cell dry weight) 2%NaOH neutralizer, 180 rpm for 108 hours incubation.

The Fig.1(a), 1(b), 1(c), 1(d) and 1(e) show the mathematical relations between the total sugar input (utilizing a combination of different sugars from acid hydrolyzate of sugarcane bagasse and glucose) and lactic acid output with the respective trend line, resulting from the batch fermentation for the strains 1,2,3,4 and coculture. Apart from the strain-2 (power function) all the other strains and coculture show logarithmic function for the lactic acid production. Higher value of the coefficient of correlation $R^2$ has been taken into account for selection of these mathematical relations between the total sugar input and lactic acid output in case of each of the microbial strains.




2(a)

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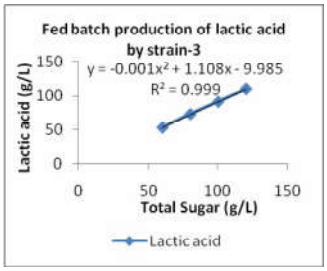


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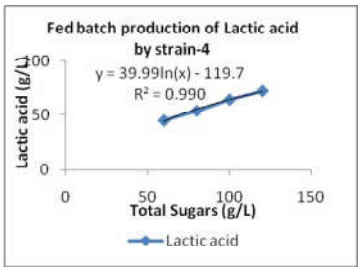


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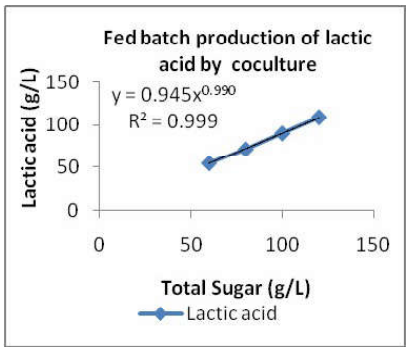


2(e)

**Fig. 2.** Mathematical relations between total sugar input and lactic acid output with fed batch fermentation utilizing acid hydrolyzate of sugarcane bagasse in production media at 35$^0$C, initial pH 6.5,1.55g/l inoculum (cell dry weight) 2%NaOH neutralizer, 180 rpm for 108 hours incubation. Aliquots of 10 ml detoxified, neutralized sugarcane bagasse hydrolyzate were fed every 12 hours.

The Fig.2. (a), 2(b), 2(c), 2(d) and 2(e) show the mathematical relations between the total sugar input (utilizing a combination of different sugars from acid hydrolyzate of sugarcane bagasse and glucose) and lactic acid output with the respective trend line, resulting from the fed batch fermentation for the strains 1,2,3,4 and coculture. Higher value of the coefficient of correlation $R^2$ has been basis of selection of these mathematical relations. In Fig. 2., the lactic acid production in follows a quadratic function for the strains-1,2 and 3, while the strain-4 and coculture show a logarithmic function and power function with the total sugar input.

The strains-1,2,3,4 and coculture had lowest pH values of 4.16,3.99,3.90,4.21 and 3.96 respectively, at 120 g/l total dose in batch production while lowest pH values of 4.08,4.40, 3.78, 4.17 and 3.82 respectively were evidenced at 120 g/l total level in fed batch production. The above mentioned pH values, suggest that the strain-2,3 and coculture can withstand very low pH values (highly acidic conditions) and perform efficiently under the given conditions to provide high values of lactic acid production, with high dose of sugar input applied for batch and fed batch fermentations.

The investigations showed that the lactic acid production was highest in case of the fed batch fermentation while the productivity was higher in batch fermentation up to 100 g/l starch input. Higher lactic acid production as well as productivity, were provided by fed batch fermentation for all the pure strains and coculture of lactobacilli at 120 g/l sugar dose containing bagasse hydrolyzate. The batch fermentation with 120 g/l dose of pure glucose provided lower lactic acid production as compared to that with 120 g/l total sugar input with bagasse hydrolyzate. Hence, it can be inferred that fed batch fermentation is better than the batch one at higher sugar inputs that are inhibitory to the batch production. Considering the cheaper cost, abundance, wide distribution and easy availability of sugarcane bagasse, the detoxified neutralized, acid hydrolyzate of bagasse obtained from it has the potential to provide significant amount of low cost carbon source for microbial production of lactic acid. The results of these batch and fed batch experiments suggested that the strain-2 and 3 had higher lactic acid production even more than strain-1 of total sugar input containing hydrolyzate due to their pentose sugar utilizing capability which lacks in strain-1. The studies with batch and the fed batch fermentations showed that the coculture had significantly higher lactic acid production than its constituent strains and the other pure strains except strain-3, at all levels of total sugar input, hence the coculture may be found more suitable for the lactic acid fermentation industries utilizing high doses of bagasse hydrolyzate as carbon source. The investigation further indicated that production and the productivities of all the *Lactobacillus* strains under study are at their peak while undergoing fed batch fermentation at high sugar input from mixture of sugarcane bagasse hydrolyzate and pure glucose, but if bagasse hydrolyzate is used as sole carbon source, the strain-1 (*L. delbrueckii*) which is well known strict hexose utilizer, can provide very low amounts of lactic acid, because the acid hydrolyzate of bagasse predominantly consists of pentose sugars.

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# Efficacy of neem seed oil for bamboo protection against degrading agencies

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**ABSTRACT**

The experiment carried out to test the effectivity of different neem seed oil concentrations in bamboo protection, done through Boucheriee process, revealed that the treated *Dendrocalamus strictus* and *Bambusa nutans* performed excellently till one year. None of the bamboo species was protected by neem seed oil treatment after 24 months. Results revealed that the specimens above ground were in sound condition, so neem seed oil can be recommended for interior use.

**Key words:** *Bambusa nutans,* CCA, CCB, *Dendrocalamus strictus* and neem seed oil

Bamboo, belonging to the sub family *Bambusoideae* and family *Poacae,* is the most versatile forest produce world wide. There are more than 1,250 species under genera of bamboo, which are unevenly distributed in the various parts of the humid tropical, subtropical and temperate regions of the earth. This natural resource plays an important role in the livelihood of rural people and in the cottage industry. It is an economic substitute of wood. Though more than half of the bamboo produced in India is turned into paper, it is also being commonly used as constructional material, poles, posts, mats, sea folding, ladders, bridges, fences etc. and to make a wide variety of products such as furniture, doors , windows, fans, toys and several handicrafts articles.

Bamboo used for construction purpose, handicrafts, decorative items and furniture prepared from bamboo falls an easy prey to deteriorating agencies like fungi, insects and termites. These items treated with conventional preservatives like boron, CCA etc., used for both exterior and interior purpose cause a serious threat to the environment and to the health of living beings due to their toxicity. Therefore, a strong need has been felt towards the development of eco-friendly preservatives from natural products to enhance the life of bamboo.

Neem, widely recognized for its medicinal, insecticidal and anti-microbial properties, is considered to be a store house of various biological active compounds such as, azadirachtin, salanin, nimbin, quercetin, and is now drawing attention throughout the world. Extensive research work is being carried out to evaluate the entomological and pathological properties of neem seed oil, however, very little work is reported on wood/ bamboo destroying agencies.

## MATERIALS AND METHODS

Extraction of neem seed oil was done with petroleum ether following extraction methods described by Pant, 2010**.** Fresh neem fruits were collected from Saharanpur (Latitude 29$^{o}$. 58′N and longitude 77$^{o}$. 23′E), Uttar Pradesh, India.

The fruits were de-pulped and the seeds obtained were washed with water and then shade dried. The seeds were decorticated and the kernels obtained were pulverized to 40-60 mesh. Pulverized powder (300g) was introduced into a Soxhlet apparatus and extracted with petroleum ether (60-80$^{o}$C). The oil obtained was dried over anhydrous sodium sulphate and was then separated from the solvent resulting in pale yellow oil (yield 13%) (Pant and Tripathi, 2011).

### Treatment of bamboo with neem seed oil

Neem seed oil was diluted to concentration levels of 5, 10, 15 and 20% in kerosene oil. Different bamboo species i.e. *Dendrocalamus strictus* and *Bambusa nutans* were procured from Forest Research Institute, Dehradun campus. *Bambusa nutans* and *D. strictus* of about 10 feet in length were fixed firmly to the boucherie apparatus with the help of hose pipe and clamps. Treating solution was introduced in the preservative tank of the boucherie apparatus. An air pressure of 10-15 mm of Hg was applied to the boucherie apparatus with the help of bicycle pump. The valve was opened and the preservative was allowed to flow. The sap was displaced

by the preservative and collected at the other end. Simultaneously with the displacement of sap, preservative solution flowed throughout the length of bamboo. The treatment process took about 5-6 h and retention of the preservative in bamboo was calculated. Treated bamboos were left over for 15 days for fixation of preservative (Kumar and Dev, 1993).

**Preparation of samples and installation in test yard**

Samples (one foot) were prepared from treated and untreated green bamboo. These were installed in test-yard at a distance of one feet, half buried in soil and half above ground and the observations were made after regular intervals for the fungal and termite attack and scores were assigned according to (Purushottam *et al.*, 1967) (Table -1). Six replicates were taken for each concentration, control with solvent and control.

**Table 1:** Extent of decay caused by fungus and termites and their scores

| Symbol | Score | Detailed description of attack |
|---|---|---|
| N | 0 | No attack (Normal) |
| Vsw | 0.5 | Very slight termite attack |
| Vsf | 0.5 | Very slight fungal attack |
| Vswf | 0.75 | Very slight termite and fungal attack |
| Sw | 1 | Slight termite attack |
| Sf | 1 | Slight fungal attack |
| Sf+Vsw | 1.25 | Slight fungal and very slight termite attack |
| Sw+Vsf | 1.25 | Slight termite and very slight fungal attack |
| Swf | 1.5 | Slight termite and fungal attack |
| Mw | 2 | Moderate termite attack |
| Mf | 2 | Moderate fungal attack |
| Mf+ Vsw | 2.25 | Moderate fungal and very slight termite attack |
| Mw+Vsf | 2.25 | Moderate termite and very slight fungal attack |
| Mf+ Sw | 2.5 | Moderate fungal and slight termite attack |
| Mw+Sf | 2.5 | Moderate termite and slight fungal attack |
| Mwf | 3 | Moderate termite and fungal attack |
| Bw | 3 | Heavy termite attack |
| Bf | 3 | Heavy fungal attack |
| Bf+VSw | 3.25 | Heavy fungal and very slight termite attack |
| Bw+Vsf | 3.25 | Heavy termite and very slight fungal attack |
| Bf+Sw | 3.5 | Heavy fungal and slight termite attack |
| Bw+Sf | 3.5 | Heavy termite and slight fungal attack |
| Bf+Mw | 4 | Heavy fungal and moderate termite attack |
| Bw+Mf | 4 | Heavy termite and moderate fungal attack |
| Bwf | 4.5 | Heavy termite and fungal attack |
| Dw | 5 | Destroyed by termite attack |
| Df | 5 | Destroyed by fungal attack |
| Dwf | 5 | Destroyed by termite and fungal attack |

**RESULTS**

*Bambusa nutans* treated with 5, 10, 15 and 20 percent of neem seed oil showed retention of 4.38, 9.45, 14.01 and 21.81 kg/m$^3$, respectively. Table-2 shows that samples of *B. nutans* treated with 5, 10, 15 and 20 percent of neem seed oil were in normal (N) condition till six months as compared to control (untreated) which were slightly attacked by termites (Sw) after three months. Control specimens were moderately attacked by termites and very slightly attacked by fungi (Mw-Vsf) after six months of installation. Inspection made after nine months of installation indicated that samples treated with 5 percent neem seed oil showed very slight infestation of termites (Vsw) as compared to those treated with 10 and 15 percent which were in normal condition (N). Controls were moderately attacked by termites and showed very slight fungal attack (Mw-Vsf). After 12 months of installation, samples treated with 15 and 20 percent concentrations of neem seed oil were in normal condition as compared to specimens treated with 5 and 10 percent. Control were completely destroyed by the termites and fungi (Fig. 1A). Inspection made after 24 months of installation revealed that bamboo samples treated with 5% were completely destroyed by termites (Dw), whereas samples treated with 10 percent were badly destroyed by termites and also showing very slight fungal attack (Bw-Vsf). In case of 15 and 20 percent treatment, bamboo specimens showed moderate termite and very slight fungal attack (Mw-Vsf) as compared to control samples which were completely destroyed by termites and fungi. Control and control with solvent specimens showed similar results (Table-2, Fig. 1A).

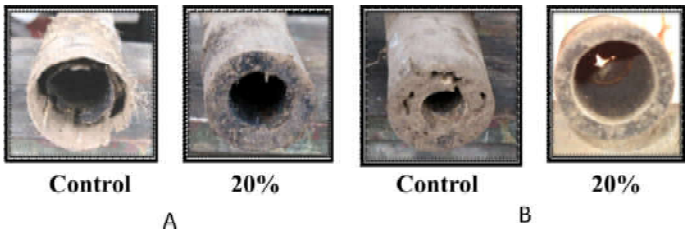


Fig. 1 : Conditions of untreated and treated samples of *Bambusa nutans* (A) and *Dendrocalamus strictus* (B) removed after 12 months

In *D. strictus,* retention of about 5.76, 9.43, 10.01 and 23.11 kg/m$^3$ was observed in specimens treated with 5, 10, 15 and 20 percent concentrations of neem seed oil. Samples treated with 5, 10, 15 and 20 percent of neem seed oil were in sound and normal condition upto nine months as compared to control specimens showing very slight termite attack (Vsw). Observations made after 12 months of installation indicated moderate termite infestation (Msw) in control specimens while specimens treated with 20 and 15 percent were in normal condition (N) (Fig. 1B). Specimens treated with 10

**Table 2:** Condition of treated and untreated samples of bamboo in field

| Duration of observation | Conc. (%) | Species | |
|---|---|---|---|
| | | *B. nutans* | *D. strictus* |
| **3 months** | Control | Sw (1) | N (0) |
| | 5 | N (0) | N (0) |
| | 10 | N(0) | N (0) |
| | 15 | N(0) | N (0) |
| | 20 | N(0) | N (0) |
| **6 months** | Control | Mw-Vsf (2.25) | Vsw (0.5) |
| | 5 | N (0) | N (0) |
| | 10 | N(0) | N (0) |
| | 15 | N(0) | N (0) |
| | 20 | N(0) | N (0) |
| **9 months** | Control | Mw-Vsf (2.25) | Vsw (0.5) |
| | 5 | Mw (2) | Vsw (0.5) |
| | 10 | Vsw (0.5) | N (0) |
| | 15 | N(0) | N (0) |
| | 20 | N(0) | N (0) |
| **12 months** | Control | Bw-Mf (4) | Mw (2) |
| | 5 | Mw (2) | Sw (1) |
| | 10 | Sw (1) | Vsw (0.5) |
| | 15 | N(0) | N (0) |
| | 20 | N(0) | N (0) |
| **24 months** | Control | Dwf (5) | Bw (3) |
| | 5 | Dw (5) | Mw-Vsf (2.25) |
| | 10 | BwVsf (3.25) | Mw (2) |
| | 15 | Mw-Vsf (2.25) | Swf (1.5) |
| | 20 | Mw-Vsf (2.25) | Sw (1) |

Values in parenthesis are scores based on visual observations.

and 5 percent of neem seed oil showed very slight to slight infestation by termites after 12 months. After 24 months it was observed that 5 percent neem seed oil treated samples were moderately destroyed by termites and very slightly attacked by fungi (Mw-Vsf), while moderate termite attack (Mw) was observed in specimens treated with 10 percent. Specimens treated with 15 percent concentration showed slight termite and fungal attack (Swf). At 20 percent slight termite attack (Sw) was observed as compared to control which were badly destroyed by termites (Bw). Control and control with solvent specimens showed similar results i.e., the treatment of kerosene oil alone was not found effective. In both the species, the treated specimens above the ground were observed in sound condition after 24 months (Table-2, Fig. 1B).

Quercetin isolated from water soluble fraction of the crude neem leaves extract possesses anti-fungal property (Tewari, 1992). Aqueous neem leaves extract inhibits

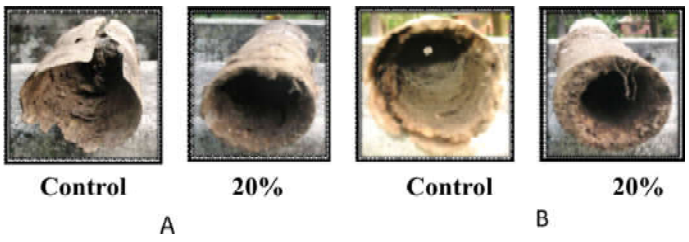


Fig. 2: Conditions of untreated and treated samples of *Bambusa nutans* (A) and *Dendrocalamus strictus* (B) removed after 24 months

aflatoxin production in *Aspergillus flavus* and *A. parasiticus* and reported fungi toxicity against *Rhizoctonia solani* (Bhatnagar and McCormick, 1988; Kurucheve *et al*., 1997). Extracts of neem leaves, neem oil and seed kernels was found effective against certain human fungi including *Trichophyton*, *Epidermophyton*, *Microsporum*, *Trichosporon*, *Geotricum* and *Candida* (Khan and Wassilew, 1987). Neem oil showed anti-fungal activity against *Tricophyton mentagrophytes* (Tewari, 1992). Kumar *et al*., (1990) observed that 1 percent kerosene in combination with 4 percent neem extract was effective in the integrated pest management of *Coccus viridis* on coffee. Sharma *et al*., (1998) found that neem seed kernel fraction impregnated into specimens of mango and tested against *Microcerotermes beesoni*, imparted four times protection to the test blocks, as compared to control. CSIRO research has suggested that Australian neem extract, which when vacuum coated into wooden blocks, was capable of deterring termite feeding. Small amounts of ethanolic extracts of neem leaves resulted in the complete mortality of *Microcerotermes turneri*, a wood-destroying termite (Friend, 1995). Venmalar and Nagveni (2005) reported the efficacy of neem oil and copper complex of neem oil against wood destroying termites. Neem seed oil was also found effective against wood destroying termites in laboratory and termite mound (Dhyani, 2008). Pant and Tripathi, 2011 reported the fumigant activity of neem seed oil against wood degrading agencies.

Eco-friendly formulation prepared from neem will help in phasing out conventional preservatives which are raising an alarm world-wide. The product will be used by the cottage industry as well as by the handicraft industry to enhance the durability of their handicrafts items against degrading agencies.

The study revealed that *Dendrocalamus strictus* is comparatively more naturally durable than *Bambusa nutans* but none of the species could be completely protected by neem seed oil after two years under exterior ground contact condition. However, neem seed oil treatment may be recommended for interior use.

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# Utilization of cheese whey for lactic acid production by batch and fed batch fermentation

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**ABSTRACT**

The studies on lactic acid production through the batch and fed batch fermentation methods, utilizing combination of glucose and cheese whey as carbon source and pure strains of *Lactobacilli* (1) *L. delbreuckii* (NCIM2025) (2) *L. pentosus* (NCIM 2912) (3) *Lactobacillus sp.* (NCIM 2734 (4) *Lactobacillus sp.* (NCIM2084) and coculture of first two strains, revealed that in batch and fed batch fermentations, the coculture provided the highest lactic acid production of 96.01 g/l and 112.56 g/l, followed by that of strain -1, 89.34 g/l and 104.26 g/l at 120 g/l total sugar input. Higher productivity values of strains were also evidenced at 120 g/l total sugar dose under fed batch fermentation as compared to batch production with pure glucose or whey mixed glucose. The experiment highlights the potential, advantages of the coculture over pure culture and fed batch production over the batch one, in terms of maximum lactic acid production.

**Keywords**: Batch, Coculture, Fed batch, Fermentation, *Lactobacillus.*

Lactic acid (2-hydroxy propanoic acid) has a wide range of applications in dairy, chemical, food, textile, pharmaceutical industries and in biomedical sector (Ghosh and Ghosh, 2011a). Lactic acid and its major producer, *Lactobacillus sp.*, play an important roles as acidulant, preservative, flavor enhancer, probiotics and bacteriocins in food and dairy industries and as moisturizing agents in cosmetics (Adsul et al. 2007; Narayanan et al. 2004; Adnan et al. 2007). In the year 2005, biopolymer industry utilized about 20-30 percent of its global production for the production of biodegradable polymer poly lactic acid (Zhang et. al., 2007). The major portion (about 90%) of the global lactic acid is obtained through fermentative production which provides highly pure steriospecific L(+) and D(-) forms of lactic acid, hence it has advantage over the chemical synthesis methods, that result in a racemic mixture ( DL lactic acid) involving expensive separation methods (Altaf et al., 2006; Adsul et. al., 2007). The economy in fermentative lactic acid production can be further enhanced by partially or totally substituting the pure sugar requirement with the sugars available in the abundant waste materials, such as whey (over $10^8$ tons/year) that has approximately 5 percent lactose, 0.06 percent fat, and 0.8 to 1 percent nitrogenous compounds, BOD of 38,000 to 46,000 ppm and COD 80 g/l (Ghasemi et al., 2009; Bullerman and Berry, 1966). Large amounts of whey are liberated from dairies in different countries. In Denmark it was about 1.8 million tons/year, while in Canada it was $2.72 \times 10^6$ metric tons/year, out of which about 50 percent had to be disposed of as waste causing serious pollution problems (Ghaly et al., 2003**).** Lactose sugar contained in whey can be bio-converted into various fermentation products such as lactic acid, ethanol and single cell proteins, which can not only prove cost effective for lactic acid production but also potentially bring down the cost of activated sludge treatment (Panesar et al. 2007; Ghasemi et al., 2009; Aggarwal et al. 2008).

The objectives of the present study were (1) To compare the lactic acid production through the batch and fed batch fermentations (2) To determine the effect of different doses of whey on lactic acid production by the *Lactobacillus* strains under study (3) To evaluate advantages of application of coculture over the pure strains of lactobacilli, in terms of lactic acid production and productivity.

## MATERIALS AND METHODS

The chemicals used were of Merck (Darmstadt, Germany) and Hi Media (Mumbai, Maharashtra, India) make. Pure cultures of *Lactobacilli* (1) *L. delbrueckii* (NCIM2025) (2) *L. pentosus* (NCIM 2912) (3)*Lactobacillus sp.*(NCIM 2734) (4) *Lactobacillus sp.* (NCIM2084) were acquired from National Chemical Laboratory(NCL) Pune. The inoculum preparation for the *lactobacilli* strains were carried out in MRS (de Mann Rogosa Sharpe) media at $33^0$ C, 180 rpm for fourteen hours. Composition of one liter MRS medium is of : 10g proteose peptone, 5g yeast extract, 10g beef extract, 20g dextrose, 1g tween 80, 2g ammonium citrate, 5g sodium acetate, 0.1g $MgSO_4.7H_2O$, 0.05 g $MnSO_4$, 2g $K_2HPO_4$ in distilled water

as solvent. For preparation of the coculture, the volumes of the liquid cultures corresponding to 1.55 g/l cell dry weight in the strains- (1) and strain (2) were mixed. The cheese whey, to be utilized, was vacuum filtered and neutralized before mixing with the production media.

One liter of production media consisted of : total sugar doses (60, 80 ,100 and 120)g/l applied in production media with 15g yeast extract, 1g sodium acetate, 0.03g $MnSO_4.H_2O$, 0.10g $MgSO_4.7H_2O$, 0.25g $KH_2PO_4$, 0.25g $K_2HPO_4$ and 0.03g $FeSO_4$. The total sugar doses 60, 80, 100 and 120 g consisted of 30, 40, 50 and 70 g pure glucose while the rest amount of the sugar was contributed by lactose component of the cheese whey. For investigation of comparative advantages of fed batch fermentation, utilizing whey mixed glucose as carbon source at high dose 120 g/l, a set of batch experiments were performed with pure glucose at 120 g/l.

Flasks of 250 ml capacity filled upto 100ml production media, were inoculated with 1.55 g/l cell dry weight of the strains under study, keeping the initial pH 6.5.These flasks were kept at 33° C,180 rpm for 108 hours incubation conditions with an addition of 2 percent sodium hydroxide neutralizer every 12 hours to maintain the pH. In the fed batch experiments, 10 ml filtered and neutralized cheese whey was fed every 12 hours in the flasks. Sodium hydroxide (2%) was preferred over calcium carbonate as neutralizer (added every 12 hours periodically to maintain the pH 6.5), due the inhibitory effects of calcium carbonate on the bacterial strains under study (Ghosh and Ghosh, 2008; 2009; 2011b).

The cell dry weight of bacterial cells was determined by centrifugation of preculture MRS broth obtained after 14 hours, at 8000 rpm for 10 minutes ,to separate the cells from supernatant followed by washing with 0.85 percent NaCl solution and drying in preweighed microporous papers at $70^0$C till constant weights were attained.

**Lactose estimation in cheese whey**

Lactose content of the cheese whey was estimated by the colorimetric method of Nickerson et al. (1976). Panesar et al. (2007) described the steps of this method, whereby 5 ml of whey sample was prepared by prior treatment with zinc acetate–phosphotungstic acid reagent for removal of proteins and sodium hydroxide. This was further added with 5 ml of glycine–NaOH buffer and 0.5 ml each of methylamine–HCl and sodium sulfite solution followed by thorough mixing and kept at 65°C in a water bath for 25 min. The sample mixture was cooled immediately in an ice-water bath for 2 min and the absorbance was measured at 540 nm, using a UV- visible double beam spectrophotometer (Systronics, Ahmedabad, India).

**Estimation of lactic acid by Kimberley Taylor method**

The lactic acid present in the extracts were quantitatively assayed by Kimberly Taylor method, which utilizes hot concentrated sulphuric acid effects , that include oxidation of lactic acid to acetaldehyde, which subsequently forms a chromogenic complex with p- phenyl phenol in presence of copper. The extracts were centrifuged at 8000g and the supernatants were used for lactic acid estimation. 0.5 ml of supernatant was added with 3ml of 96 percent sulphuric acid, followed by heating for ten minutes in boiling water bath for ten minutes, then cooling it to room temperature for about 30 minutes. The cool solution was added with 50 micro liter, 4 percent copper sulphate and 100 micro liter, p- phenyl phenol(prepared by dissolving 1.5 percent of the reagent in 95 percent ethyl alcohol) which provided a chromogenic complex. The absorbance for lactic acid is measured in a UV-VIS double beam spectrophotometer at 570nm (Taylor, 1996 and Mirdamadi et al., 2002).

The figures for trend line graphs and the trend line equations for different doses of sugar input and lactic acid output have been provided with the help of MS-Excel.

**RESULTS AND DISCUSSIONS**

The lactic acid production data in the tables 1 and 2 have been expressed as mean ± standard deviation values obtained from different trials and the productivities given are around the mean values. The results in Table 1, for batch fermentation indicate that, for every dose of sugar, the coculture showed higher lactic acid production, very closely followed by the strain-1, while the strain-2 (*L. pentosus*), which is primarily pentose sugar utilizer, showed the least lactic acid production because the carbon source consists of glucose and lactose. A rising trend of lactic acid production is evidenced in Table 1, with enhancement in total sugar doses (containing whey lactose) till 120 g/l because the stimulatory substances from whey help in growth and production of microbial cells, while there is a sharp decline in lactic acid production and productivity at 120 g/l of pure glucose application, due to high sugar dose inhibition. In Table 1 there is a rising trend of productivities with incremental doses of total sugar till 100 g/l after which the productivities decline at 120 g/l, although the lactic acid productions reach the maximum for all the strains. This observation indicated that more time was required to metabolize the sugars and produce lactic acid. The 120 g weight of total sugar component actually contains 50 g lactose and high dose of glucose (70 g), while equal amounts of glucose and lactose constituted the other doses such as 60, 80 and100 g. This extra amount of glucose could have possibly delayed the uptake of lactose in case of diauxic growth of lactobacilli( preferential uptake of sugars) or may have inhibited lac operon mediated uptake

**Table 1**: Lactic acid formation by the different strains of *Lactobacilli* corresponding to various total sugar doses comprising of glucose and whey combination.

| Bacterial strains | Total Sugar 60 g/l | | Total Sugar 80 g/l | | Total Sugar 100 g/l | | Total Sugar 120 g/l | | Pure Glucose 120 g/l | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LA | P | LA | P | LA | P | LA | P | LA | P |
| *L. delbrueckii* NCIM2025 (Strain-1) | 41.06±1.02 | 1.1406 | 58.95±1.27 | 1.2281 | 82.95±1.59 | 2.3041 | 89.34±1.70 | 1.2202 | 71.01±1.37 | 1.1835 |
| L. pentosus NCIM2912 (Strain-2) | 30.97±0.88 | 1.2904 | 49.88±1.16 | 1.3855 | 68.84±1.33 | 1.9122 | 77.95±1.48 | 1.2642 | 59.09±1.28 | 0.9848 |
| Coculture of strains 1, 2 | 45.63±1.06 | 1.2675 | 66.67±1.34 | 1.8519 | 87.89±1.68 | 2.4413 | 96.01± | 1.3625 | 76.86±1.40 | 1.6012 |
| *Lactobacillus sp.* NCIM2734 (Strain-3) | 37.69±0.98 | 0.7852 | 52.93±1.19 | 1.4700 | 63.64±1.25 | 1.3258 | 71.89±1.43 | 1.3066 | 58.98±1.23 | 1.2287 |
| *Lactobacillus sp.* NCIM2084 (Strain-4) | 35.72±0.95 | 0.9952 | 56.95±1.20 | 1.5819 | 72.86±1.44 | 2.0241 | 78.96±1.51 | 1.3272 | 60.07±1.32 | 1.0010 |

LA- Maximum Lactic acid concentration (g/l); P-Corresponding productivity (g/l/h)

**Table 2**: Lactic acid formation by the different strains of *Lactobacilli* corresponding to various doses of glucose and whey combination.

| Bacterial strains | Total Sugar 60 g/l | | Total Sugar 80 g/l | | Total Sugar 100 g/l | | Total Sugar 120 g/l | |
|---|---|---|---|---|---|---|---|---|
| | LA | P | LA | P | LA | P | LA | P |
| *L. delbrueckii* NCIM2025 (Strain-1) | 48.69±1.14 | 1.0144 | 66.95±1.34 | 1.3947 | 88.74±1.54 | 1.8487 | 104.26±1.92 | 2.1721 |
| L. pentosus NCIM2912 (Strain-2) | 38.94±0.85 | 0.8113 | 63.02±1.29 | 1.3129 | 79.38±1.38 | 1.6537 | 96.08±1.87 | 2.0016 |
| Coculture of strains 1 and 2 | 52.76±1.18 | 1.0991 | 74.66±1.38 | 1.5554 | 90.16±1.85 | 1.8783 | 112.56±2.69 | 2.3450 |
| *Lactobacillus sp.* NCIM2734 (Strain-3) | 45.54±1.10 | 0.9487 | 58.09±1.20 | 1.2102 | 74.69±1.36 | 1.5560 | 98.38±1.91 | 2.0495 |
| *Lactobacillus sp.* NCIM2084 (Strain-4) | 43.26±0.95 | 0.9013 | 66.24±1.31 | 1.3800 | 79.76±1.43 | 1.6617 | 90.99±1.75 | 1.8956 |

LA- Maximum Lactic acid concentration (g/l) ; P-Corresponding productivity (g/l/h)

and hydrolysis of lactose by the *Lactobacillus* cells for the conversion to lactic acid.

Besides the first reports in *E. coli,* the lac operon has also been found in various *Lactobacilli* including *L. casei* and *L .delbrueckii* etc., for the uptake and initiation of the lactose metabolism. Lactose permease and â-galactosidase enzyme systems have been identified in some of the *Lactobacilli,* while in the others, additionally the lactose specific phosphoenolpyruvate dependent phosphotransferase system (lac-PTS) has been reported, for uptake of lactose (Freifelder, 1998;Alpert and Siebers, 1997).

The stimulatory substances from whey encourage higher cell numbers that may consume glucose and bring down its level so that uptake of lactose is facilitated. However, the cheese whey also reported to contain minerals such as sodium, potassium, calcium, magnesium and zinc etc. which may be helpful in the transport processes and metabolic processes of the *Lactobacillus* strains (Goyal and Gandhi, 2008**)**. From Table 1 it is seen that 120 g/l of pure glucose carbon source without any whey lactose, results to relatively low production of lactic acid as compared with that obtained in case of 120 g/l whey substituted glucose input.

The Table 2 data for fed batch fermentation indicate highest individual lactic acid production values and productivities for all the pure strains and coculture at 120 g/l total sugar comprising of glucose and whey lactose, while the same dose with pure glucose proved to be inhibitory. Hence, all the strains exhibit a sharp decline in both the production and productivity (Table 1).The highest overall lactic acid production was attained by the coculture, closely followed by the strain-1. The coculture showed higher lactic acid production and productivity than its component strains 1 and 2, with all the doses of total sugar (Table 2). In Table 2, the strain-2 lags behind the strain-4 in terms of lactic acid production till 100 g/l total sugar dose but at a higher dose of 120 g/l, it showed higher acid production than the strain-4, indicating capability of strain-2 to perform better with higher doses of whey mixed glucose.

From the Table 1 and 2, it is evident that the fed batch fermentation provides higher lactic acid concentrations while the batch production achieves maximum productivities till 100 g/l total sugar dose while at 120 g/l total sugar dose fed batch fermentation has both lactic acid production and productivities higher than the batch fermentation with pure or whey mixed glucose. Hence fed batch fermentation can be successfully applied in the fermentation industries which produce lactic acid by utilizing higher doses of whey mixed glucose.

The strains-1,2,3,4 and coculture had lowest pH values of 3.97, 4.15,4.24,4.12 and 3.89, respectively, at 120 g/l total dose in batch production while lowest pH values of 3.81,3.90, 3.87, 3.96 and 3.73, respectively were evidenced at 120 g/l

total level in fed batch production. The above mentioned pH values, suggest that the strain -1 and coculture can withstand very low pH values (highly acidic conditions) and perform efficiently under the given conditions to provide high values of lactic acid production, with high dose of sugar input applied for batch and fed batch fermentations.

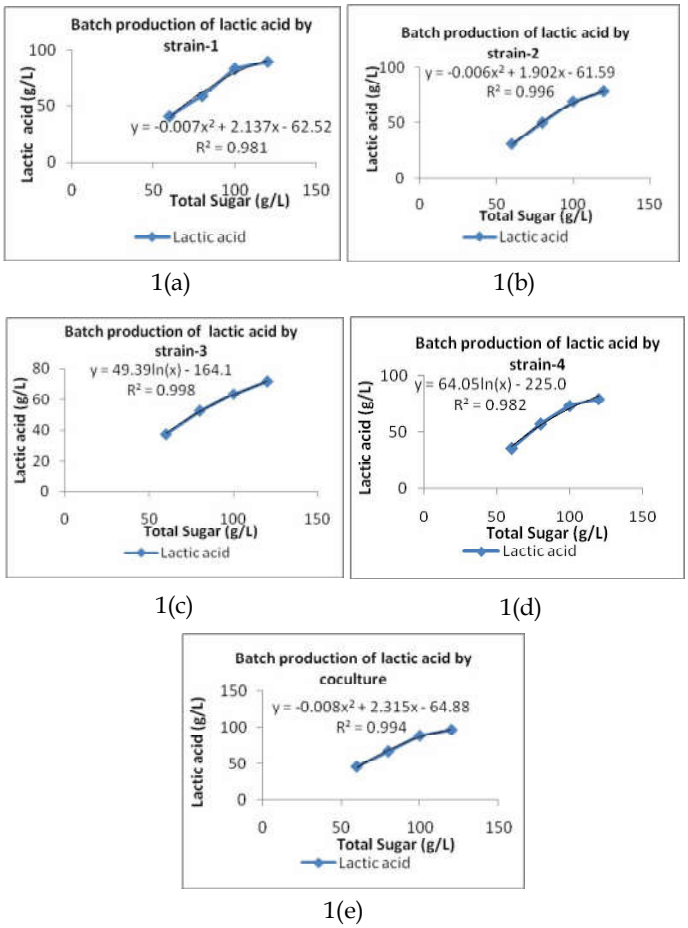


**Fig. 1.** Mathematical relations between total sugar input and lactic acid output with batch fermentation utilizing whey substituted glucose as carbon source in production media at 35$^0$C, initial pH 6.5,1.55 g/l inoculum (cell dry weight) 2%NaOH neutralizer, 180 rpm for 108 hours incubation.

The Fig.1(a), 1(b), 1(c), 1(d) and 1(e) show the mathematical relations between the total sugar input (utilizing lactose component of whey and glucose) and lactic acid output with the respective trend line, resulting from the batch fermentation for the strains 1,2,3,4 and coculture. The lactic acid production in the strains 1, 2 and coculture, follow quadratic function, while the strains 3 and 4 show a logarithmic function for production. Higher value of the coefficient of correlation $R^2$ has been taken into account for selection of these mathematical relations between the total sugar input and lactic acid output in case of each of the microbial strains.

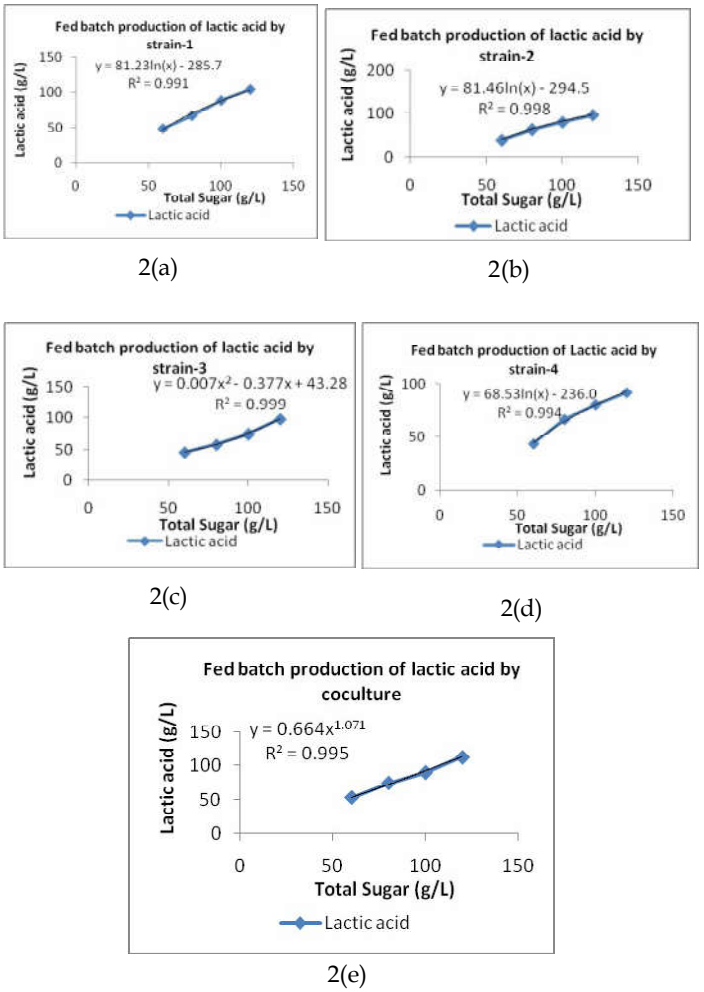


**Fig. 2.** Mathematical relations between total sugar input and lactic acid output with batch fermentation utilizing whey substituted glucose as carbon source in production media at 35$^0$C, initial pH 6.5, 1.55 g/l inoculum (cell dry weight) 2%NaOH neutralizer, 180 rpm for 108 hours incubation. Aliquots of 10 ml filtered whey added every 12 hours in the media.

The Fig.2. (a), 2(b), 2(c), 2(d) and 2(e) show the mathematical relations between the total sugar input (utilizing lactose component of whey and glucose) and lactic acid output with the respective trend line, resulting from the fed batch fermentation for the strains 1,2,3,4 and coculture. The lactic acid production in the strains 1, 2 and 4, follow a logarithmic function, while the strains 3 and coculture show quadratic function and power function, respectively. Higher value of the coefficient of correlation $R^2$ has been taken into consideration for these mathematical relations.

The studies revealed that the lactic acid production was highest in case of the fed batch fermentation while the productivity was higher in batch fermentation in majority of cases up to 100 g/l total sugar input. Fed batch fermentation

provided higher lactic acid production as well as productivity for all the pure strains and coculture of lactobacilli at 120 g/l dose of whey mixed glucose as carbon source, while the batch production gets inhibited. Hence, fed batch fermentation served better than the batch one at higher sugar inputs that are inhibitory to the batch production. Taking into account the abundance, cost free easy availability of lactose sugars and stimulatory substances and its compatibility with the *Lactobacillus* strains, the cheese whey can be considered as an ideal renewable carbon source for the lactic acid fermentation industries. The bio-utilization of whey in fermentation industries has additional benefits in reducing the treatment costs and prevention of water pollution loads. The results of these batch and fed batch experiments suggested that the coculture had significantly high lactic acid production and productivity for every dose of whey substituted glucose as compared to the other pure strains. Hence, the coculture may be found more suitable for the lactic acid fermentation industries.

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# Effect of water stress on essential oil, biochemical's and growth in different varieties of Japanese mint

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**ABSTRACT**

The pot culture experiment conducted with Japanese mint (*Mentha arvensis* L. var. piperascens Mal.) cultivars exposed to water stress and recovery for growth and essential oil metabolism revealed reduction in herbage yield, relative water content, water potential and nitrate reductase activity and increase in proline, menthol and sugar contents. Oil content increased in Code –A, Himalaya, decreased in Gomti & Shivalik while it remained unchanged in Hy-77. Code –A and Himalaya showed better adaptability than other varieties under drought.

**Key words**: *M.arvensis*, essential oil, Proline, nitrate reductase, sugar content

Japanese mint, also called Menthol mint *(Mentha arvensis)*, of family Lamiaceae, is one of the most popular essential oil crop widely cultivated in subtropical region of the world for volatile oil production. The oil and its constituents (menthol, menthone & menthy acetate) are used in pharmaceutical, flavor and perfumery industries (Farooqi *et al.* 1999, Mathur & Farooqi 2005). Presently in India, it is cultivated in different agro climatic zones, covering an area of 1, 50,000 ha with estimated production of 15,000 tones of essential oil that accounts for about 80 percent of total menthol mint production of the world. The crop being drought sensitive incur high herbage yield loss under water stress conditions, particularly in the north Indian plains during summer (May- June), where harvesting time coincides with hot- humid climate. Since last two decades Uttar Pradesh and other parts of India has experienced freak weather conditions. In a large part of the agricultural areas in the world, water is an important factor for growth and productivity. Genotypic variability reported in various plants in relation to drought response can be exploited for development of drought tolerant varieties (Premchand *et al.* 1987). Significant details of the physiological and metabolic response of food crops of dry environment are available (Zeevart and Creelmann, 1988), however, informations on the behavior of medicinal and aromatic plants under limited moisture availability are scant (Singh, Sangwan *et al.* 1994, Sangwan *et al.* 1993, Charles 1990). Therefore, the present study to evaluate the variability within cultivated genotypes of *M. arvensis* under water stress, imposed by limited supply of soil moisture, was taken up in pot culture experiment.

## MATERIALS AND METHODS

The experiment was laid down with Japanese mint varieties viz., Gomti, Shivalik & Hy-77. The plants were grown in earthen pots for two months and kept in glasshouse at 25°C - 47 °C with and irradiance of 80 - 1000 µEm-2S-1 and a day length of 12 hr. After two months of growth, the water stress treatment was started. For each variety, four pots each as control and stress treatment were kept. Water stress in plants was developed by watering on alternate days and maintaining soil moisture contents at 5 - 6 percent level. The plants in other set were maintained at soil moisture level of 28 percent by giving water every day. After 30 days of the treatment the plants in the stress treatment showed decrease in growth and wilting of leaves. Effect of water stress on Water potential (Y), relative water content (RWC), NR activity, chlorophyll content, plant height, leaf area were investigated. Oil content and composition were also analyzed.

In another experiment, the effect of water stress and recovery on growth and oil yield Gomti, Himalaya, Code-A and MAH-3 in varieties were investigated. The pots with 60 days old plants were divided into three sets. In the first set (control), watering was continued as before. In the second and third set, no water was given and the plants were stressed up to reversible leaf wilting stage. The plants in the third set the pots were rewatered and analyzed when fully recovered. Measurement of different parameters was made for examination of stress of recovery effect. The data on growth parameters, oil contents and composition, chlorophyll contents, sugar contents and nitrate reductase activity were recorded.

The water potential was measured by a thermocouple psychrometer and relative water content was determined by the method given by Mathur *et al*. (2001). Leaf area was measured using a Li-cor LI-3100 leaf area meter. Proline was estimated colorimetrically by the method of Bates *et al.* (1973). NR activity was measured by in vivo method according to Hageman and Hucklesky (1971). Total sugar was estimated by anthrone reagent according to Yemm and Wills (1954). The essential oil was estimated by Clevenger type apparatus (Clevenger, 1928). The major oil constituents: Menthol, Menthone etc. were determined by gas liquid chromatography (Perkin Elmer model 3920B). The experiment was conducted in a layout of a 3 x 3 factorial randomized block design. Treatmental at differences were compared using critical difference (CD) at 5% and 1% level of significance for the species x level interaction effect. The cultivar X treatment interaction effect was computed in the final experiment. Simple linear correlation coefficients (r value) were calculated to estimate the interrelation between characters under study (Das and giri1974, Gupta and kapoor1983).

## RESULTS AND DISCUSSION

In first experiment (Table-1, 2) herbage yield, plant height, root stem ratio, leaf area, water potential (Y), relative water content (RWC) decreased significantly in all the cultivars. Reduction in plant height was significantly maximum in Gomti (20.01) and minimum in Hy-77 (09.09%). Stem root ratio was affected by water stress and it was significantly reduced to 34.62, 40.00 and 32.00 percent, respectively in Gomti, Shivalik and Hy-77. Herb yield was significantly inhibited due to stress. The maximum reduction was in Shivalik (72.86%). Branching and leaf area was significantly affected in stressed plants. Relative water content (RWC) and water potential (y) decreased significantly in all the varieties under stress. Decrease in water potential was maximum in Gomti (200%) over control and minimum in Hy-77 (33.33%). Relative water content was 80-81 percent under unstressed plants. It decreased to 44–48 percent in stressed plants, maximum in Gomti (45.27%) and minimum in Hy-77 (27.64%). No significant change in Proline content under stress condition was observed; however it was maximum accumulated in Hy-77 (58.33%). Oil content decreased significantly under stress, whereas it remained the same in Hy-77 (0.87 g / 100 g fresh wt). Menthol content in Hy-77 increased whereas in Shivalik and Gomti it remained unaffected. Chlorophyll content under stress increased in each variety although insignificantly. Chlorophyll b significantly increased in Shivalik and Hy-77 under stress and decreased in Gomti. Total chlorophyll increased to 13.33, 20.97, and 5.97 percent in Gomti, Shivalik and Hy-77, respectively. Correlation studies (Table-3) suggested that NR activity was positively correlated to water potential, relative water content, plant height, leaf / stem ratio, herb yield, leaf area, dry wt. and total chlorophyll. Oil contents were positively correlated to menthol. Plant height was positively correlated to stem root ratio, herb yield, no of branches / plant, leaf area and negatively with dry wt and chlorophyll. Water potential was positively significant to soil moisture content, plant height, stem root ratio, no of branches / plant, leaf area & negatively correlated with oil content. Proline, dry wt and total chlorophyll. RWC, is negatively correlated with dry wt and positively with soil moisture content, plant height, stem root ratio, herb yield, no of branches / plant and leaf area. Herb yield is positively correlated to no of branches / plant, leaf area and negatively with dry wt. No of branches / plant is positively correlated with leaf area and negatively with dry wt. Leaf area is negatively correlated to dry wt., was experiment. The effect of water stress on height, leaf area, no of branches / plant under was short-term water stress unaffected significantly (Table 4). Himalaya recovered best (96.0%) in terms of herb yield when rewatered. Height and leaf area was decreased maximum in MAH-3. Number of branches was not changed in Code-A variety under stress condition.

The study on the water relations showed that the water potential (y) and relative water content (RWC) decreased in all the varieties under stress condition. Water potential decreased to maximum of 100 percent in MAH-3 and Himalaya. In Code-A recovery of $y_w$ was 100 percent. Relative water content decreased to maximum in Gomti (39.85%) whereas in Code-A, RWC it was minimum (19.46% over control). Himalaya and Code-A recovered best when rewatered. Oil content increased under stress condition in Himalaya and Code-A by 5.63 and 8.0 percent, respectively, while decreased in Gomti and MAH-3 by 16 and 20 percent over control. Rewatering showed no specific change in oil content. Menthol content under stress condition increased in the range of 3.0 to 15.40 percent over control and 3.0 percent to 23 percent over control in recovery. Menthone content decreased in Himalaya and MAH-3. While nitrate reductase activity decreased in water stress condition. It was maximum in MAH-3 (35.78%) and Gomti (38.72%). In Code-A, NR activity decreased by 21.2 percent and recovered on rewatering. In Himalaya, NR activity decreased from 12.4 to 8.2 mmmole/g fresh wt. and was 10.49 mg/g on rewatering. Chlorophyll content increased in each variety except in MAH-3 under stress condition. In Code-A, total Chlorophyll content increased from 0.849 - 1.09 mg/g. In Gomti it did not increase significantly. In MAH-3 chlorophyll content decreased from 0.397 - 0.381 mg /g fresh wt in water stress as well as after recovery. Sugar accumulated in all the variety under stress condition. It was maximum in Code-A (from

**Table 1:** Effect of water stress on growth parameter in different varieties of *M. arvensis*

| Variety | Treatment | Plant height | Stem: root ratio | Herb yield (g/plant) | No of branches /plant | Leaf area ($cm^2$ ) | Dry wt (g/100g fr. Wt.) |
|---|---|---|---|---|---|---|---|
| **GOMTI** | Control | 38.33 | 2.60 | 30.09 | 10.66 | 13.76 | 24.70 |
| | Stress | 30.66 | 1.70 | 8.88 | 1.3 | 8.81 | 29.88 |
| | | (20.01) | (34.62) | (70.49) | (87.80) | (35.97 | (-20.97) |
| **SHIIVALIK** | Control | 39.00 | 2.5 | 37.88 | 16.66 | 14.05 | 24.34 |
| | Stress | 32.00 | 1.50 | 10.28 | 3.0 | 8.09 | 30.30 |
| | | (17.95) | (40.00) | (72.86) | (81.99) | (42.42) | (-24.49) |
| **Hy-77** | Control | 33.00 | 2.50 | 34.13 | 12.00 | 13.02 | 25.04 |
| | Stress | 30 | 1.70 | 12.76 | 3.30 | 9.66 | 30.5 |
| | | (9.09) | (32.00) | (62.61) | (72.52) | (25.81) | (-21.81) |
| **CD 5%** | | 7.40 | 0.25 | 7.90 | 3.10 | 3.10 | 1.80 |
| **CD 1%** | | 10.61 | 0.36 | 11.33 | 4.4 0 | 4.40 | 2.50 |

[Figure in parthenses show % increase (-) and % decrease against control]

**Table 2:** Effect of water stress on water relation, oil content and composition in different *M . arvensis* varieties

| Variety | Treatment | Water potential (-Mpa)l | RWC (%) | Prolie (µmole/g fr wt) | Oil contet g/100g fr.wt. | Menthoe (%) | Menthl (%) | chl a (mg/g fr.wt) | chl b (mg/g fr.wt) | chl a+b (mg/g fr.wt) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 0.02 | 80.4 | 0.12 | 0.65 | 5.2 | 86.31 | 0.22 | 0.204 | 0.45 |
| GOMTI | stress | 0.06 | 44.4 | 0.14 | 0.53 | 3.1 | 86.13 | 0.32 | 0.136 | 0.51 |
| | | (-200.0) | (44.78) | 16.67) | (18.46) | (40.38) | (0.21) | (-45.45) | (33.33) | (-13.33) |
| | Control | 0.03 | 80.4 | 0.11 | 0.7 | 2.3 | 90.11 | 0.27 | 0.173 | 0.472 |
| SHIVALIK | stress | 0.06 | 53.3 | 0.17 | 0.65 | 2.5 | 90.66 | 0.3 | 0.273 | 0.57 |
| | | (-100.0) | (33.71) | (-54.55) | (7.14) | (-8.70) | (-0.61) | (-11.11) | (-57.80) | (-20.97) |
| | Control | 0.03 | 81.4 | 0.12 | 0.87 | 0.64 | 91.12 | 0.35 | 0.232 | 0.553 |
| HY-77 | stress | 0.04 | 28.9 | 0.19 | 0.87 | 0.67 | 96.86 | 0.37 | 0.357 | 0.586 |
| | | (-33.33) | (64.50) | (- 58.33) | (0.00) | (-4.69) | (-6.30) | (-5.71) | (-53.88) | (-5.97) |
| CD 5% | | 0.006 | 9.5 | 0.12 | 0.01 | 1.5 | 1.9 | 0.33 | 0.2 | 0.26 |
| CD 1% | | 0.009 | 13.5 | 0.18 | 0.08 | 2.2 | 2.8 | 0.48 | 0.29 | 0.37 |

[Figure in parthenses show % increase (-) and % decrease against control]

**Table 3:** Correlation coefficient ®among the various characters of *M. arvensis*

| | Relative Water Content | herb yield | No of branches /plant | Leaf area | Plant height | Total chlorophyll | Proline | oil content | Menthol |
|---|---|---|---|---|---|---|---|---|---|
| W P | 0.70* | 0.80* | 0.76* | 0.83* | 0.84* | -0.87* | -0.99** | -0.25 | -0.17 |
| RWC | | .96* | 0.92* | 0.95* | 0.83 | -0.46 | 0.98** | -0.44 | -0.72 |
| Herb yield | | | 1.00* | 0.97** | 0.85** | -0.56 | -0.87** | -0.32 | -0.411 |
| No of branches /plant | | | | 0.94** | 0.88* | -0.58* | -0.83* | -0.28 | -0.43 |
| Leaf area | | | | | 0.88* | -0.83* | -0.89* | -0.26 | -0.24 |
| Plant height | | | | | | -0.83* | -0.89* | -0.37 | -0.007 |
| Total chlorophyll | | | | | | | 0.84* | 0.52 | 0.48 |
| Proline | | | | | | | | 0.37 | 0.08 |
| Oil content | | | | | | | | | 0.84** |

*=Significant at 5%
* *=Significant at 1%

**Table 4:** Effect of water stress and recovery on growth on different varieties

| Varieties | Treatment | Herb yield (g/plant) | Height (cm) | Leaf area ($cm^2$) | No of branches /plant | Dry wt (g/100gm fr.wt) |
|---|---|---|---|---|---|---|
| **Himalaya** | Control | 49.5 | 39.3 | 29.0 | 12.0 | 25.69 |
| | Stress | 38.03 (23.17) | 38.13 (2.98) | 23.7 (18.28) | 9.3 (22.50) | 37.07 (-44.30) |
| | Recovery | 47.16 (4.73) | 38.14 (2.95) | 27.8 (4.14) | 9.3 (22.50) | 25.4 (1.13) |
| **Gomti** | Control | 40.5 | 49.8 | 25.8 | 13.3 | 23.83 |
| | Stress | 30.5 (24.69) | 46.4 (6.83) | 22.16 (14.11) | 12.0 (9.77) | 33.1 (-38.90) |
| | Recovery | 31.6 (21.98) | 46.2 (7.23) | 25.16 (2.48) | 12.0 (9.77) | 24.7 (-3.65) |
| **Code-A** | Control | 44.3 | 52.7 | 36.16 | 12.0 | 22.0 |
| | Stress | 34.9 (21.22) | 51.1 (3.04) | 31.1 (13.99) | 12.0 (0.00) | 28.3 (-28.64) |
| | Recovery | 37.6 (15.12) | 50.7 (3.80) | 29.6 (18.14) | 12.0 (0.00) | 24.9 (-13.18) |
| **MAH-3** | Control | 29.6 | 31.6 | 30.1 | 15.0 | 24.1 |
| | Stress | 20.5 (30.74) | 28.2 (10.76) | 22.5 (25.25) | 10.0 (33.33) | 36.3 (-50.62) |
| | Recovery | 24.6 (16.89) | 28.0 (11.39) | 23.4 (22.26) | 11.3 (24.67) | 38.98 (-61.74) |
| **CD5%** | | 8.2 | 7.5 | 7.1 | 5.7 | 5.4 |
| **CD1%** | | 11.2 | 10.2 | 9.7 | 7.8 | 7.3 |

[Figure in parthenses showing % increase (-) and % decrease comparison to control]

**Table 5:** Effect of water stress and recovery on oil content and composition in different varieties of *M . arvensis*

| Varieties | Treatment | Water potential | RWC (%) | Oil Content (g/100g fr.wt.) | Menthol (%) | Menthone (%) | NR activity (mμ mole/fr.wt) | Chl a mg/g fr.wt.) | Chl b mg/g fr.wt.) | Chl a+b mg/g fr.wt.) | Sugar content mg/g dry wt |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Himalaya** | Control | 0.02 | 80.2 | 0.71 | 83.00 | 2.40 | 12.4 | 0.31 | 0.19 | 0.41 | 100.1 |
| | Stress | 0.04 | 63.39 | 0.75 | 89 | 1.60 | 9.2 | 0.36 | 0.14 | 0.5 | 146.8 |
| | | (100.00) | (21.07) | (-5.63) | (-7.23) | (33.33) | (25.81) | (6.13) | (6.32) | 21.95) | |
| | Recovery | 0.03 | 77.6 | 0.73(- | 85.5 | 2.60 | 10.49 | 0.35 | 0.14 | 0.55 | 98.93 |
| | | (-50.00) | (3.24) | 2.82) | (-3.01) | (-8.33) | (15.40) | (-12.90) | (26.32) | (-34.15) | (1.17) |
| **Gomti** | Control | 0.03 | 82.79 | 0.66 | 72.10 | 5.30 | 15.83 | 0.23 | 0.23 | 0.38 | 67.9 |
| | Stress | 0.05 | 49.8 | 0.55 | 83.2 | 5.40 | 9.7 | 0.24 | 0.16 | 0.41 | 75.53 |
| | | (-66.67) | (39.85) | (16.67) | (-15.40) | (-1.89) | (38.72) | (-4.35) | (30.43) | (-7.89) | (-11.24) |
| | Recovery | 0.04 | 60.3 | 0.55 | 88.9 | 2.8 | 9.70 | 0.25 | 0.15 | 0.40 | 62.9 |
| | | (-33.33) | (27.17) | (16.67) | (-23.30) | (47.17) | (38.72) | (-8.70) | (34.78) | (-5.26) | (7.36) |
| **Code-A** | Control | 0.02 | 85.3 | 0.75 | 85.1 | 2.6 | 25.10 | 0.44 | 0.35 | 0.84 | 142.13 |
| | Stress | 0.04 | 68.7 | 0.81 | 88.4 | 3.8 | 19.80 | 0.74 | 0.37 | 1.09 | 180.66 |
| | | (-100.00) | (19.46) | (-8.00) | (-3.88) | (46.15) | (21.12) | (-68.18) | (-5.71) | (-29.76) | (-27.11) |
| | Recovery | 0.02 | 77.5 | 0.81 | 86.4 | 2.7 | 22.94 | 0.74(- | 0.36(- | 1.0 | 100.55(29.2 |
| | | (0.00) | (9.14) | (-8.00) | (-1.53) | (-3.85) | (8.61) | 68.18) | 2.86) | (-19.05) | 5) |
| **MAH-3** | Control | 0.02 | 77.89 | 0.54 | 85.4 | 2.60 | 14.16 | 0.22 | 0.19 | 0.39 | 68.43 |
| | Stress | 0.04 | 51.5 | 0.43 | 87.4 | 1.30 | 9.20 | 0.27 | 0.10 | 0.38 | 58.4 |
| | | (-100.00) | (33.88) | (20.37) | (-2.34) | (50.00) | (35.03) | (--22.73) | (47.37) | (2.56) | (14.66) |
| | Recovery | 0.03 | 56.7 | 0.41 | 91.3 | 1.2 | 9.23 | 0.23 | 0.10 | 0.33 | 58.23 |
| | | (-50.00) | (27.21) | (24.07) | (-6.91) | (53.85) | (34.82) | (-4.55) | (47.37) | (15.38) | (14.91) |
| CD5% | | 0.004 | 11.5 | 0.13 | 6.90 | 1.80 | 3.80 | 0.49 | 0.52 | 0.10 | 17.45 |
| CD1% | | 0.009 | 15.6 | 0.17 | 9.40 | 2.40 | 5.10 | 0.67 | 0.71 | 0.13 | 23.72 |

[Figure in parthenses showing % increase (-) and % decrease comparison to control]

**Table 6:** Correlation coefficient (r) among the various characters of *M. arvensis*

| | RWC | leaf area | Herb yield | Total chlorophyll | Menthol | Oil content | Sugar | N R activity |
|---|---|---|---|---|---|---|---|---|
| WP | .87* * | .71* | 0.49 | -0.38 | -0.32 | -0.31 | -0.1 | 0.71* |
| RWC | | 0.79* | 0.77* | -0.29 | -0.48 | -0.65* | -0.08 | 0.56 |
| leaf area | | | 0.46 | -0.07 | -0.07 | -0.62* | -0.4 | 0.45 |
| herbyield | | | | -0.44 | -0.41 | -0.70* | -0.71 | 0.05 |
| Total chlorophyll | | | | | 0.64* | 0.24 | 0.85** | -0.62* |
| Menthol | | | | | | 0.44 | 0.21 | -0.41 |
| Oil content | | | | | | | 0.41 | -61* |
| Sugar | | | | | | | | -0.62* |

* significant at 5%
** significant at 1 %

142.13 - 180.66 mg/g) and decreased 29.5 percent after recovery. Sugar accumulation was least in MAH-3 (14%) and on recovery it remained the same. In Himalayan, sugar accumulated to the extent of 16 percent under water stress condition. Correlation study (Table-6) indicates that oil content was significantly positively related with RWC, Herb yield, height and leaf area. NR was negatively correlated with water potential chlorophyll and positively with no of branches. Menthol was negatively correlated with menthone and positively with Chla while menthone was positively correlated with sugar. Height was significantly related with sugar while, leaf area was positively related with dry wt. Water potential was negatively correlated with RWC, leaf area, no of branches and positively correlated with dry wt. RWC was positively correlated with herb yield & leaf area and negatively with dry wt.

There was a significant decrease in growth in all the varieties due to the water stress. Decrease in herbage yield under water stress is reported in many medicinal and aromatic plants like aromatic grasses and *M .piperita* (Singh Sangwan *et al.* 1989,charles 1990). In the present experiment the cultivars differed in growth response to stress condition, which indicated a genetic variability among them.. Decrease in herbage yield, plant height, number of branches, leaf stem ratio was found maximun in Gomti, shivalik, & MAH-3, while minimum in code –A, Hy-77 and Himalaya. Thus, code –A, Hy-77 & Himalaya were considered tolerant over Gomti, Shivalik and MAH-3. These varieties also showed tolerance under osmotic stress, and stress under field condition (Mathur *et al*. 2001, 2005). Similarly, reduction in herbage yield in sensitive verities under water stress is also reported by Fatima *et al.* (2000).Hy-77 was most responsive in maintaining Y and RWC under water deficit. Code-A and Himalaya showed better ability to recover from wilting in terms of RWC. Thus, these cultivars are more suitable for cultivation under water scarcity than other genotype. High RWC and the maintenance of a high water potential was also reported in drought resistant cultivars of winter wheat (Scanfield *et al.* 1998). There was a significant increase in proline content under water stress, and maximum in Hy-77. Singh –Sangwan (1994), Ansari *et al.* (1998), Mathur *et al.* (1998) also reported accumulation of proline under drought and salanity stress . Proline accumulation under stress is viewed as a primitive metabolic response in cellular milieu (Hanson and Hitz 1982). Decrease in NR activity may be due to decrease in availability of NADH or due to inactivation of enzyme under stress (Sawhney *et al.* 1982).NR activity does not affect much in tolerant cultivars like Code –A, and Himalaya and recovery was also good. Prakash (1982) also found similar result in wheat. Increase in sugar content under water stress in different verities of *M. arvensis* is in accordance with the report on other plants (Iijin 1975, Ford and Wilson 1981, Schubert 1995). Solute accumulation under water stress lead to maintenance of turgor potential .More increase in sugar content under stress in verities (Code-A, Himalaya) as compared to other varieties indicates that these varieties may be relatively more resistant. Many factors including age ,seasonal variation, nutrition and temperature have an influence on accumulation of secondary products (Bardzik *et al*.1971).Stress induced changes in oil accumulation are thought to be a result of the effect of stress on plant growth and differentiation rather than its direct effect on oil synthesis (Charles, 1990). This can be the reason for significant decrease in oil content in Shivalik and Gomti, under stress while decrease was not found in Hy-77. In the second experiment, oil content increased in Himalaya and code –A., while decreased in Gomti & MAH –3 under stress. It is believed that this was due to direct effect of stress on oil biosynthesis. Thus, dehydration may be responsible for the direct effect on enzyme catalyzing relevant secondary biochemical reactions. Therefore, the effect of water stress on oil accumulation is direct as well as indirect. This was demonstrated that metabolic levels may increase, decrease or have no change under water stress (Sangwan *et al.* 2001). Changes in oil content and composition as a result of drought

have been reported also for mint (Charles *et al*.1990) and sweet basil (Simon, 1992). Earlier reports also suggest that level of the oil decreased, increased or remains unchanged in Cymbopogan species (Sangwan *et al*. 1993b, Singh sangwan *et al.* 1994, Mathur *et al.*1998). Similar results were reported in osmotic stress and field experiments (Mathur *et al.* 2001, Mathur *et al.* 2005). Code –A, and Himalaya showed better adaptability under drought than the other studied varieties and can therefore be evaluated for cultivation in drought prone area.

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# Post-harvest soil nutrient status as influenced by rice varieties, sowing time, and nitrogen levels under rainfed upland conditions

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**ABSTRACT**

Field experiments were carried out for two consecutive *kharif* seasons of 1998 and 1999 at Agricultural Research Station, Ragolu of North Coastal Agro climactic Zone of Andhra Pradesh to study the influence of varieties, time of sowing and nitrogen levels on post-harvest soil nutrient status in rainfed upland rice. Post harvest available N status of soil was altered significantly due to rice varieties and variation in time of sowing as well as nitrogen levels. The interaction effects among these factors were also statistically measurable during both the instances of study. While, the post harvest soil available phosphorus and potassium status did not alter significantly. The post harvest soil available nitrogen, phosphorus and potassium status was found to be the highest with the treatments recording the lowest yield.

**Key words:** Post harvest, Nutrient status. Nitrogen, Phosphorus, Potassium

Upland rice constitutes one of the important ecosystems of the country and its improvement would contribute greatly in meeting future rice demands. Mishra (1999) defined upland rice as the rice grown in fields, bunded or unbunded, flat or slopy, rainfed, where field preparation and seeding are done under dry conditions and there is no standing water on the soil surface within 48 hours after cessation of rains and there is varying degree of soil moisture stress at different stages of crop. Rainfed upland rice is cultivated generally under 700-1100 mm rainfall zones and occupies 7.1 m. ha accounting for 17 per cent of the total rice area of the country. However, its contribution to rice production is a mere 10 per cent (Sinha *et al*., 1996). A number of factors like soil moisture stress, delayed sowing, heavy weed infestation, poor native soil fertility status and poor spread of improved upland cultivars have been identified as important constraints in realization of enhanced productivity levels under rainfed upland situations.

## MATERIALS AND METHODS

Field experiments were carried out for two consecutive *kharif* seasons of 1998 and 1999 at Agricultural Research Station, Ragolu (North coastal Agro- climatic Zone of Andhra Pradesh) of Acharya N.G.Ranga Agricultural University. The soils of the experimental site are sandy clay loam in texture, neutral in pH, low in organic carbon and available nitrogen, medium in available phosphorus and potassium. The treatments consisted of three varieties ($V_1$- Pushkala, $V_2$ – RGL-2539 and $V_3$-MTU –1006), three dates of sowing ($D_1$- second fortnight of June, $D_2$- first fortnight of July and $D_3$ – second fortnight of July) and four levels of nitrogen ($N_1$ - control, $N_2$ - 30 kg $ha^{-1}$, $N_3$ - 60 kg $ha^{-1}$ and $N_4$ - 90 kg $ha^{-1}$). The experiment was laid out in split plot design with two factors (three varieties and three dates of sowing) assigned to the main plots and four levels of nitrogen to the sub-plots; each treatment was replicated thrice. The soils were sandy clay loam in texture, low in organic carbon content and available nitrogen, medium in available phosphorous and available potassium (Table 1)

## RESULTS AND DISCUSSION

### Soil Available Nitrogen

Post harvest available N status of soil altered significantly due to rice varieties and variation in time of sowing as well as nitrogen levels. The interaction effects among these factors were also statistically measurable during both the instances of study.

Significantly higher post harvest soil available nitrogen was associated with MTU 1006 ($V_3$), while it was the lowest with Pushkala ($V_1$), during both the years of experimentation.

Time of sowing influenced the post harvest available nitrogen status of the soil significantly. Among the three staggered sowing dates, $D_3$ (July second fortnight sowing) registered the highest quantity of post harvest soil available

**Table 1:** Physico-chemical properties of the experimental field's soil

| Properties | Value | | Method |
|---|---|---|---|
| | 1998 | 1999 | |
| **Physical characteristics** | | | |
| Sand (%) | 74.5 | 73.0 | International pipette method (Piper, 1966) |
| Silt (%) | 2.5 | 3.5 | |
| Clay (%) | 23.0 | 23.5 | |
| Soil texture | Sandy clay loam | | |
| **Chemical characteristics** | | | |
| Soil $P^H$ (1:2.5 soil water) | 7.4 | 7.0 | Glass electrode $P^H$ meter (Jackson, 1973) |
| EC (1:2.5 soil water extract) (ds $m^{-1}$) | 0.1 | 0.15 | Conductivity bridge (Jackson 1973) |
| Organic carbon (%) | 0.40 | 0.43 | Wet digestion method (Walkely and Black, 1934) |
| Available nitrogen (kg $ha^{-1}$) | 178.5 | 169.3 | Alkaline permanganate method (Subbiah and Asija, 1956) |
| Available $P_2O_5$ (kg $ha^{-1}$) | 55.3 | 53.5 | Olsen's method (Olsen *et al*., 1954) |
| Available $K_2O$ (kg $ha^{-1}$) | 240.0 | 236.3 | Flame photometry (Jackson, 1973) |
| Available Zn (ppm) | 0.85 | 0.93 | DTPA soil test (Lindsay and Norvel 1978) |
| Available Fe (ppm) | 19.61 | 17.27 | DTPA soil test (Lindsay and Norvel 1978). |

**Table 2:** Post harvest available nitrogen status (kg $ha^{-1}$) of soil as influenced by rice varieties, time of sowing and nitrogen levels *kharif*, 1998.

| | $V_1$ | $V_2$ | $V_3$ | $D_1$ | $D_2$ | $D_3$ | Mean |
|---|---|---|---|---|---|---|---|
| $N_1$ | 129.07 | 128.84 | 150.23 | 131.05 | 136.88 | 140.20 | 136.05 |
| $N_2$ | 142.08 | 147.16 | 160.14 | 141.48 | 149.09 | 158.81 | 149.79 |
| $N_3$ | 163.61 | 162.58 | 181.65 | 162.30 | 165.27 | 180.27 | 169.28 |
| $N_4$ | 189.89 | 196.09 | 208.70 | 188.60 | 200.00 | 206.07 | 198.23 |
| $D_1$ | 146.70 | 150.99 | 169.90 | - | - | - | 155.86 |
| $D_2$ | 156.79 | 155.71 | 175.94 | - | - | - | 162.81 |
| $D_3$ | 165.00 | 169.31 | 179.71 | - | - | - | 171.34 |
| Mean | 156.16 | 158.67 | 175.18 | - | - | - | - |

| | S Em ± | CD (0.05) |
|---|---|---|
| V | 0.85 | 2.10 |
| D | 0.85 | 2.10 |
| N | 4.97 | 11.57 |
| V X D | 1.47 | 3.64 |
| V at N | 7.50 | 17.46 |
| N at V | 8.61 | 20.02 |
| D at N | 7.50 | 17.46 |
| N at D | 8.61 | 20.02 |

**Table 3:** Post harvest available nitrogen status (kg $ha^{-1}$) of soil as influenced by rice varieties, times of sowing and nitrogen levels – *kharif*, 1999

| | $V_1$ | $V_2$ | $V_3$ | $D_1$ | $D_2$ | $D_3$ | Mean |
|---|---|---|---|---|---|---|---|
| $N_1$ | 125.84 | 120.74 | 124.43 | 109.83 | 119.65 | 131.53 | 120.34 |
| $N_2$ | 126.93 | 133.46 | 140.19 | 122.44 | 129.56 | 148.59 | 133.53 |
| $N_3$ | 144.19 | 152.57 | 157.14 | 141.92 | 147.60 | 164.38 | 151.30 |
| $N_4$ | 166.31 | 175.62 | 181.09 | 163.83 | 171.37 | 187.82 | 174.34 |
| $D_1$ | 126.97 | 135.70 | 140.94 | - | - | - | 134.54 |
| $D_2$ | 135.85 | 142.90 | 147.18 | - | - | - | 141.98 |
| $D_3$ | 151.94 | 158.29 | 164.01 | - | - | - | 158.08 |
| Mean | 140.82 | 145.60 | 150.71 | - | - | - | - |

| | S Em ± | CD (0.05) |
|---|---|---|
| V | 0.69 | 1.71 |
| D | 0.69 | 1.71 |
| N | 4.00 | 9.31 |
| V X D | 1.20 | 2.97 |
| V at N | 6.04 | 14.06 |
| N at V | 6.93 | 16.12 |
| D at N | 6.04 | 14.06 |
| N at D | 6.93 | 16.12 |

N, while the lowest value being associated with $D_1$ (June second fortnight sowing), during both the years of investigation.

Regarding the levels of nitrogen, significant increase in the post harvest soil available N values was noticed with added levels of nitrogen. Maximum value of post harvest soil available N was associated with $N_4$ (90 kg N $ha^{-1}$), while non-supply of nitrogen ($N_1$) has resulted in the lowest soil available N status, during both the years under the course of investigation. The interaction effect between varieties and time of sowing, varieties and N levels as well as time of sowing and N levels was figured.

Regardless of the varieties, the highest value of post harvest soil available nitrogen was registered with $D_3$ (sowing in second fortnight of July) compared to earlier sowings ($D_2$ and $D_1$). The post-harvest soil available N status was the highest with MTU 1006 ($V_3$) sown during second

**Table 4:** Post harvest soil available phosphorus ($P_2O_5$) and available Potassium ($K_2O$) content as influenced by rice varieties, time of sowing and nitrogen levels – *kharif,* 1998 and 1999

| Treatments | Available phosphorus (kg ha$^{-1}$) | | Available potassium (kg ha$^{-1}$) | |
|---|---|---|---|---|
| | 1998 | 1999 | 1998 | 1999 |
| **Varieties** | | | | |
| $V_1$ | 75.79 | 78.41 | 173.38 | 189.05 |
| $V_2$ | 78.90 | 80.07 | 177.93 | 194.68 |
| $V_3$ | 81.62 | 81.13 | 194.74 | 200.85 |
| S Em ± | 0.54 | 0.43 | 1.67 | 1.58 |
| CD (0.05) | NS | NS | NS | NS |
| **Sowing time** | | | | |
| $D_1$ | 75.40 | 77.55 | 169.98 | 183.90 |
| $D_2$ | 79.23 | 79.40 | 181.24 | 190.11 |
| $D_3$ | 81.68 | 82.66 | 194.83 | 210.57 |
| S Em ± | 0.54 | 0.43 | 1.67 | 1.58 |
| CD (0.05) | NS | NS | NS | NS |
| **Nitrogen levels** | | | | |
| $N_1$ | 84.32 | 86.09 | 213.67 | 234.75 |
| $N_2$ | 78.15 | 80.84 | 182.65 | 196.70 |
| $N_3$ | 75.67 | 77.91 | 170.49 | 177.82 |
| $N_4$ | 74.86 | 75.83 | 164.29 | 172.54 |
| S Em ± | 1.11 | 0.93 | 3.67 | 3.26 |
| CD (0.05) | NS | NS | NS | NS |

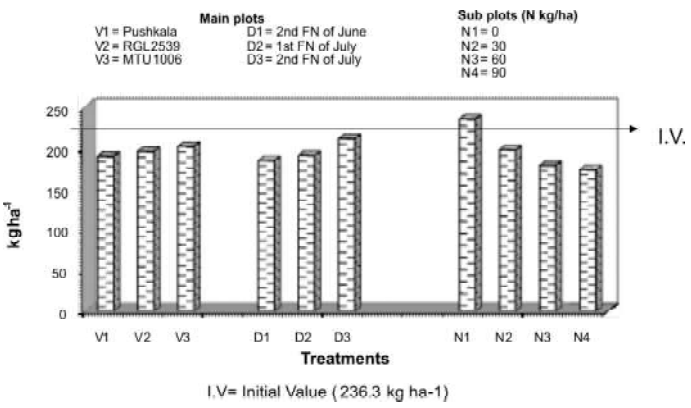


Fig. 1: Post harvest soil available nitrogen status (kg ha$^{-1}$) as influenced by rice varieties, sowing time and nitrogen levels - *kharif*, 1998

fortnight of July ($D_3$), while it was lowest with early sowing ($D_1$) of Pushkala ($V_1$).

The post harvest soil available N tended to increase with increasing levels of N up to 90 kg ha$^{-1}$ ($N_4$). At any given level of N, the variety MTU 1006 ($V_3$) registered the

**Table 5:** Grain yield, straw yield and Harvest index of rice varieties as influenced by time of sowing and nitrogen levels.

| Treatments | Grain yield (kg ha$^{-1}$) | | Straw Yield (kg ha$^{-1}$) | | Harvest index (%) | |
|---|---|---|---|---|---|---|
| | 1998 | 1999 | 1998 | 1999 | 1998 | 1999 |
| **Varieties** | | | | | | |
| V1 | 2330 | 1890 | 3080 | 2370 | 42.8 | 43.7 |
| V2 | 2090 | 1660 | 3140 | 2220 | 39.42 | 42.29 |
| V3 | 1560 | 1530 | 2660 | 2080 | 36.33 | 41.78 |
| S Em ± | 48.58 | 40.48 | 30.0 | 40.0 | 0.18 | 0.23 |
| CD (0.05) | 120.0 | 100.0 | 70.0 | 110.0 | 0.44 | 0.56 |
| **Sowing time** | | | | | | |
| D1 | 1700 | 1760 | 3190 | 2500 | 40.59 | 48.56 |
| D2 | 1570 | 1580 | 2950 | 2350 | 40.1 | 41.75 |
| D3 | 1400 | 1250 | 2740 | 1810 | 37.87 | 42.46 |
| S Em ± | 48.58 | 40.48 | 30.0 | 40.0 | 0.18 | 0.23 |
| CD (0.05) | 120.0 | 100.0 | 70.0 | 110.0 | 0.44 | 0.56 |
| **Nitrogen levels** | | | | | | |
| N1 | 1360 | 940 | 2500 | 1430 | 34.8 | 39.1 |
| N2 | 1970 | 1640 | 3000 | 2270 | 39.27 | 41.76 |
| N3 | 2270 | 2020 | 3140 | 2540 | 41.72 | 44.36 |
| N4 | 2370 | 2170 | 3200 | 2640 | 42.27 | 45.13 |
| S Em ± | 107.29 | 128.75 | 40.0 | 60.0 | 0.25 | 0.36 |
| CD (0.05) | 250.0 | 300.0 | 80.0 | 130.0 | 0.59 | 0.84 |

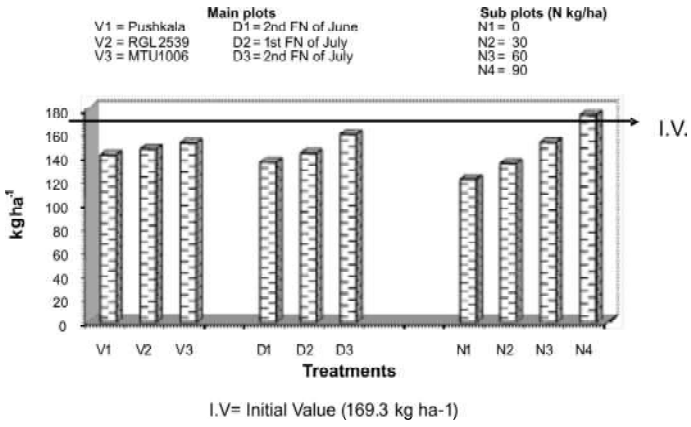


Fig. 2: Post harvest soil available nitrogen status (kg ha$^{-1}$) as influenced by rice varieties, sowing time and nitrogen levels - *kharif*, 1999

highest values of post harvest soil nitrogen compared to other two varieties. The highest post harvest soil available N was associated with 90 kg N ha$^{-1}$ ($N_4$) to MTU 1006 ($V_3$), while it was the lowest with non-supply of N ($N_1$) to Pushkala ($V_1$), which was however, comparable with other varieties at the same N level ($N_1$).

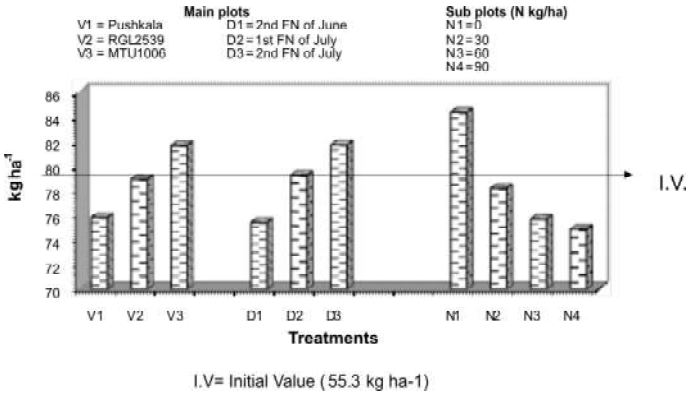


Fig. 3: Post harvest soil available phosphorus status (kg $ha^{-1}$) as influenced by rice varieties, sowing time and nitrogen levels - *kharif*, 1998

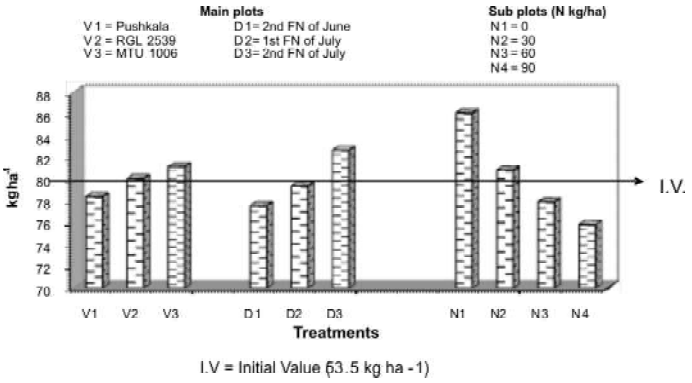


Fig.4: Post harvest soil available phosphorus status (kg $ha^{-1}$) as influenced by rice varieties, sowing time and nitrogen levels - *kharif*, 1999

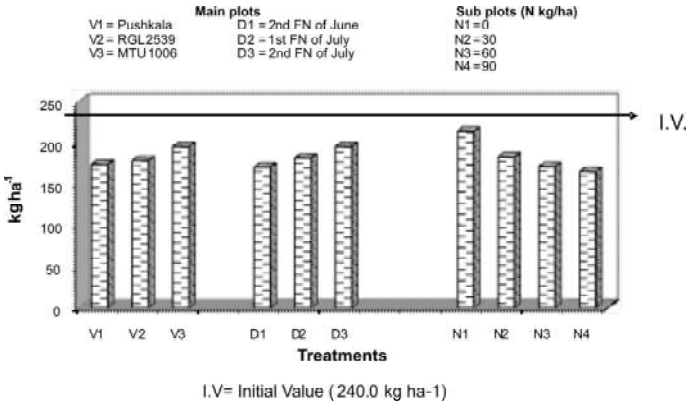


Fig.5: Post harvest soil available potassium status (kg $ha^{-1}$) as influenced by rice varieties, sowing time and nitrogen levels - *kharif*, 1998

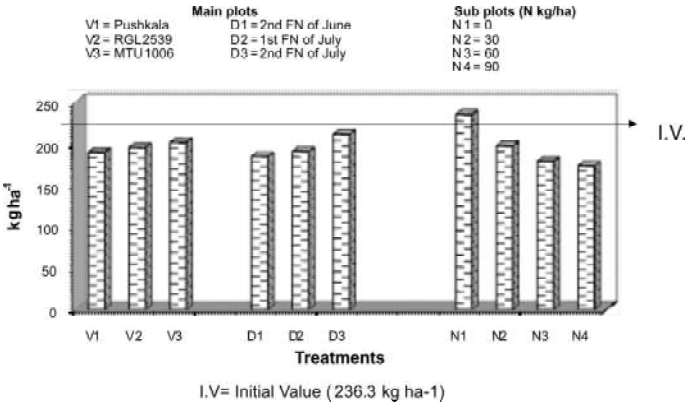


Fig. 6: Post harvest soil available potassium status (kg $ha^{-1}$) as influenced by rice varieties, sowing time and nitrogen levels - *kharif*, 1999

The post harvest N status of soil increased irrespective of time of sowing up to 90 N kg $ha^{-1}$ ($N_4$). Crop sown during second fortnight of July ($D_3$) and received 90 kg $ha^{-1}$ ($N_4$) registered the highest value of post harvest soil available nitrogen, while it was the lowest with non-supply of nitrogen ($N_1$) to Pushkala ($V_1$).

**Soil available phosphorus and potassium**

Post harvest soil available phosphorus and potassium status did not alter significantly due to different rice varieties, varied time of sowing as well as nitrogen levels to a statistically detectable magnitude during both the years of experimental

**Grain and straw yield**

The highest grain yield was recorded with the variety Pushkala ($V_1$), which was significantly superior to other two varieties. The variety MTU 1006 ($V_3$) produced the lowest grain yield. Significantly higher grain yield was registered with early sowing ($D_1$), while it was the lowest with the most delayed sowing ($D_3$). The grain yield was maximum with 90 Kg N $ha^{-1}$ ($N_4$), which was however in parity with 60 Kg $ha^{-1}$ ($N_3$). The lowest grain yield was recorded with control ($N_1$). Similar trend as in the case of grain yield was observed with straw yield and HI. Enhanced yield of rice with added levels of N has been an undisputed fact and universally acceptable proposition as could be visualized from the widely documented research evidence (Rekhi *et al*, 1989 and Kathirasan *et al*, 1997). Usually, the response of a given rice variety would be much lesser under upland conditions than under low land situation at the same location, in the same season. Upland rice often tends to show low nitrogen response, since it would prefer to absorb as high as possible N from soil. Thus, in the present investigation, the situation of non-responsiveness of upland rice beyond 60 kg N $ha^{-1}$ has been encountered. Response of upland rice to the limited supply of fertilizer N and

uneconomic response with higher level of fertilizer N has been widely documented across 111 rice growing countries in the world (Mishra *et al*., 1988; Mahapatra 1989; Stone and Pereira, 1994; Naseem, 1995 and Roy and Mishra, 1999).

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# Use of organic formulations in enchancing mulberry productivity in sericulture

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**ABSTRACT**

The organic based foliar sprays viz., vermiwash, biodigester and panchagavya were sprayed at 1, 3 and 5 per cent on M-5 mulberty on 15 and 30$^{th}$ day of pruning. Vermiwash at 5 per cent significantly stimulated the plant growth, leaf yield and a biochemical constituent of mulberry over vipul and unsprayed leaves. The silkworm growth, cocoon and silk traits increased correspondingly with foliar spray of vermiwash at 5 per cent on mulberry.

**Keywords:** Mulberry, organic foliar spray (vermiwash, biodigester and panchagavya), leaf yield and quality, silkworm, *Bombyx mori*, bioassay.

The silkworm (Bombyx mori L.), a monophagus insect, derives almost all the nutrients for its growth from mulberry leaf (Bose and Majumdar, 1995), the quantity and quality of which influence the physiological, biochemical and commercial traits of mulberry silkworm. Among the various factors influencing the success of cocoon crop, the leaf quality alone contributes for 38.30 per cent (Juyal et al., 2003). Thus, production of quality mulberry leaves plays an important role in silk production (*Singhvi et al.,* 2000). This may be increased by physiological manipulation through the foliar sprays in addition to the recommended cultivation practices. Besides, foliar sprays of major and minor nutrients and growth regulators are also reported to enhance the yield and biochemical constituents of mulberry (Mishra *et al.,* 1993; Tikku and Bindroo, 1996; Singhvi *et al*., 2001 and 2003; Rajegowda and Raju, 2011).

There is an increasing demand for naturally derived agro-chemicals for sustainable production in organic farming system (Suthar, 2010). The organic based foliar sprays formulations viz., vermiwash, biodigester and panchgavya are the liquid extractions during the advanced stage of decomposition and are known to supplement major and minor nutrients, growth stimulants and other beneficial substances to the plants (Sebastian and Christopher, 2007). Observed effects of organic foliar spray formulations include increase in plant growth, yield and quality of several crop plants (Subhashini *et al*., 2001; Natarajan, 2002; Sridhar, 2003; Venkataramana et al., 2009; Sudhakar et al., 2011). The present study was aimed to find out the beneficial effects of organic foliar spray formulations on mulberry and silkworm, *Bombyx mori* L. production.

## MATERIAL AND METHODS

Ten years old mulberry tree of variety M-5 was used for study. The plants were pruned during May, 2010 and leaves were raised by following the standard cultivation practices (Dandin *et al*., 2000). The plants were sprayed with organic based foliar spray formulations *viz*.; vermiwash, biodigester, and panchgavya at 1, 3 and 5 per cent on 15$^{th}$ and 30$^{th}$ days of pruning and compared with recommended vipul spary (250 ml/ha/crop). On 50$^{th}$ day of pruning the plant growth and yield traits were recorded (Table-1). On the same day the biochemical constituents of leaf *viz*., leaf moisture (A.O.A.C., 1970), chlorophyll 'a' and 'b' and total chlorophyll (Shoof and Lium, 1996), total protein (Jackson, 1973) and total sugar (Highkin and Frankel, 1962) were estimated (Table-1). Bioassay study was conducted by using Pure Mysore x CSR-2 cross breed hybrid silkworms. Silkworms of one disease free laying were reared in three replications by adapting standard rearing practices (Dandin et al, 2000). Observations on silkworm growth and development, cocoon and silk traits were recorded (Table-2). Fibroin and sericin content in silk was estimated by following the procedure suggested by Orlandi (1954). Data were statistically analyzed as per Gomez and Gomez (1984).

**Table 1:** Effect of foliar spray of organic formulations on mulberry growth, yield and biochemical constituents

| Treatments | Plant growth and yield | | | | Biochemical constituents | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | Number of leaves/ shoot | Leaf area ($dm^2$) | Leaf yield (kg/plant) | Leaf moisture (%) | Chlorophyll 'a (mg/g) | Chlorophyll 'b' (mg/g) | Total chlorophyll (mg/g) | Total sugar (%) | Protein (%) |
| Panchagavya 1% | 105.90$^{ef}$ | 17.51$^{e}$ | 268.50$^{f}$ | 350.00$^{d}$ | 72.41 (58.37)$^{d}$ | 0.69$^{d}$ | 0.31$^{e}$ | 0.98$^{e}$ | 8.29 (16.73)$^{ef}$ | 20.02 (26.52)$^{c}$ |
| Panchagavya 3% | 109.84$^{de}$ | 17.88e | 271.19$^{f}$ | 482.00$^{abc}$ | 73.52 (59.06)$^{bcd}$ | 0.71$^{d}$ | 0.44$^{de}$ | 1.13$^{e}$ | 8.92 (17.39)$^{cde}$ | 20.32 (26.78)$^{bc}$ |
| Panchagavya 5% | 114.73$^{cd}$ | 18.98d$^{e}$ | 284.45$^{e}$ | 455.00$^{bcd}$ | 75.60 (60.43)$^{bcd}$ | 0.91$^{d}$ | 0.43$^{de}$ | 1.32$^{cde}$ | 8.59 (17.04)$^{def}$ | 20.68 (27.00)$^{bc}$ |
| Biodigester 1% | 119.33$^{bc}$ | 19.61$^{cd}$ | 292.65$^{d}$ | 532.00$^{ab}$ | 74.33 (59.56)$^{bcd}$ | 0.89$^{cd}$ | 0.68$^{b}$ | 1.54$^{cd}$ | 8.72 (17.16)$^{cdef}$ | 21.33 (27.42)$^{bc}$ |
| Biodigester 3% | 120.86$^{bc}$ | 17.78$^{e}$ | 284.69$^{e}$ | 520.00$^{ab}$ | 75.00 (60.07)$^{bcd}$ | 0.93$^{cd}$ | 0.64$^{bc}$ | 1.54$^{cd}$ | 9.43 (17.88)$^{bcd}$ | 22.00 (28.00)$^{b}$ |
| Biodigester 5% | 123.87$^{ab}$ | 20.07$^{bcd}$ | 301.86$^{c}$ | 513.00$^{abc}$ | 76.64 (61.19)abcd | 1.02$^{bc}$ | 0.68$^{b}$ | 1.68$^{bc}$ | 9.10 (17.55)$^{cde}$ | 22.08 (28.06)$^{b}$ |
| Vermiwash 1% | 122.47$^{ab}$ | 21.24$^{abc}$ | 304.06$^{bc}$ | 530.00$^{ab}$ | 75.56 (60.56)$^{abc}$ | 1.04$^{bc}$ | 0.66$^{bc}$ | 1.64$^{bc}$ | 9.55 (17.99)$^{abc}$ | 22.30 (28.19)$^{b}$ |
| Vermiwash 3% | 126.79$^{ab}$ | 21.42$^{ab}$ | 309.53$^{b}$ | 553.00$^{ab}$ | 76.95 (61.49)$^{ab}$ | 1.33$^{a}$ | 0.83$^{a}$ | 2.06$^{a}$ | 10.29 (18.70)$^{ab}$ | 25.14 (30.01)a |
| Vermiswash 5% | 129.16$^{a}$ | 22.19$^{a}$ | 322.98a | 566.00a | 79.75 (63.50)$^{a}$ | 1.37$^{a}$ | 0.89$^{a}$ | 2.16$^{a}$ | 10.38 (18.81)$^{a}$ | 26.93 (31.30)$^{a}$ |
| Vipul (250 ml/ha) | 125.83$^{ab}$ | 21.46$^{ab}$ | 310.61$^{b}$ | 533.00$^{ab}$ | 76.84 (61.32)$^{abc}$ | 1.22$^{ab}$ | 0.84$^{a}$ | 1.97$^{ab}$ | 10.01 (18.46)$^{ab}$ | 26.25 (30.80)$^{a}$ |
| Water | 106.70$^{ef}$ | 14.30$^{f}$ | 259.92$^{g}$ | 480.00$^{abc}$ | 75.08 (60.08)$^{bcd}$ | 0.82$^{cd}$ | 0.61$^{bc}$ | 1.36$^{cde}$ | 8.79 (16.74)$^{ef}$ | 21.13 (27.39)$^{bc}$ |
| Untreated | 100.41$^{f}$ | 14.50$^{f}$ | 257.44$^{g}$ | 403.00$^{cd}$ | 72.85 (58.60)$^{cd}$ | 0.79$^{cd}$ | 0.52$^{cd}$ | 1.20$^{de}$ | 7.91 (16.34)$^{f}$ | 19.68 (26.35)$^{c}$ |
| **S.Em. ±** | **2.30** | **0.53** | **2.40** | **0.376** | **0.488** | **0.047** | **0.025** | **0.070** | **0.161** | **0.251** |

Values in vertical columns with same letters do not differ statistically by Duncan's Multiple Range Test (DMT) at 5 per cent level. Figures in parenthesis are angular transformed transformed values.

**Preparation of organic formulations**

**Vermiwash**: Vermicomposting was carried out in a cement container with a outlet tap at bottom. During advanced stage of vermicomposting, the pit was watered and the washing of earthworms body and feacal matter was collected as Vermiwash from the outlet tap (Giraddi, 2008).

**Biodigester:** The cement container was filled with cow dung and urine, organic waste and locally available plant leaves in equal proportion and thoroughly mixed. The tank was covered with a thin layer of soil, watered and later allowed for decomposition. During advanced stage of decomposition the tank was watered and the liquid drained out was collected as biodigester (Babalad *et al*., 2010).

**Panchagavya :** Seven kilograms of cow dung and 1kg of ghee was thoroughly mixed in a cement tank and kept for two days. After two days, four litres of cow urine + ten litres of water was added and allowed for fermentation for ten days. The mixture was stirred daily twice. After ten days, two litres of cow milk, two litres of curd, three litres of sugarcane juice, two litres of coconut water and twelve riped bananas were added to the tank and allowed for further fermentation for fifteen days. The content was stirred twice daily. Later, the solution was filtered and used as panchagavya (Babalad *et al*., 2010).

**RESULTS AND DISCUSSION**

**Growth and yield**: The foliar spray of organic formulations influenced the plant growth and related traits significantly. The plant height was significantly increased due to spraying of vermiwash 5 per cent (129.16 cm). It was followed by vermiwash 3 per cent (126.79 cm), vipul (125.83 cm), bioigester 5 per cent (123.87 cm) and vermiwash 1 per cent (126.79 cm), vipul (125.83 cm), biodigester 5 per cent (123.87 cm) and vermiwash 1 per cent (122.47 cm) and was on par with vermiwash 5 per cent. The untreated plant recorded 100.41 cm of height. Foliar spray of vermiwash 5 per cent (22.19 leaves/shoot) followed by vipul (21.46), vermiwash 3 per cent (21.42) and 1 per cent (21.24)

**Table 2:** Silkworm, *Bombyx mori* L. growth and development as influenced by foliar spray of organic formulations on mulberry.

| Treatments | Total larval duration (hr) | Mature larval weight (g/10 larvae) | Effective rate of rearing (%) | Cocoon weight (g/10 cocoons) | Cocoon shell weight (g/10 shells) | Cocoon shell ratio (%) | Pupal weight (g/10 pupae) | Cocoon yield (g/10 pupae) | Cocoon filament length (m) | Denier | Fibroin (%) | Sericin (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Panchagavya 1% | 608.00 | 37.50$^{c}$ | 96.74 (76.68)$^{de}$ | 18.40$^{cd}$ | 3.47$^{abc}$ | 19.60 (25.76) | 14.87$^{c}$ | 716.66$^{f}$ | 790.50$^{cd*}$ | 3.04 | 77.36 (61.59)$^{cd}$ | 22.63 (28.40)$^{cde}$ |
| Panchagavya 3% | 624.00 | 37.83$^{c}$ | 97.96 (81.81)$^{cde}$ | 19.19$^{cd}$ | 3.44$^{abc}$ | 17.99 (25.09) | 15.71$^{abc}$ | 756.66$^{c}$ | 812.98$^{c}$ | 2.90 | 74.87 (59.91)$^{e}$ | 25.13 (30.08)$^{b}$ |
| Panchagavya 5% | 612.00 | 37.49$^{c}$ | 97.92 (81.73)$^{cde}$ | 18.05$^{cd}$ | 3.30$^{bc}$ | 18.30 (25.30) | 14.67$^{c}$ | 680.00g | 787.72$^{cd}$ | 2.99 | 75.19 (60.12)$^{de}$ | 24.81 (29.87)$^{bc}$ |
| Biodigester 1% | 608.00 | 35.66$^{d}$ | 97.57 (81.05)$^{cde}$ | 18.87$^{cd}$ | 3.22$^{bc}$ | 17.10 (24.42) | 15.30$^{bc}$ | 726.66e | 734.16$^{de}$ | 2.99 | 76.09 (60.73)$^{de}$ | 23.90 (29.26)$^{bcd}$ |
| Biodigester 3% | 624.00 | 37.96$^{c}$ | 97.69 (81.44)$^{cde}$ | 19.60$^{bc}$ | 3.84$^{ab}$ | 19.55 (26.14) | 15.44$^{bc}$ | 740.00$^{d}$ | 782.93$^{cd}$ | 3.23 | 77.33 (61.57)$^{cd}$ | 24.42 (28.27)$^{de}$ |
| Biodigester 5% | 620.00 | 38.67$^{c}$ | 98.41 (82.84)$^{bcd}$ | 18.80$^{cd}$ | 3.44$^{abc}$ | 18.30 (25.35) | 15.19$^{bc}$ | 736.66$^{d}$ | 776.90$^{cd}$ | 3.22 | 75.66 (60.34)$^{de}$ | 24.49 (29.65)$^{bcd}$ |
| Vermiwash 1% | 616.00 | 35.95$^{d}$ | 97.67 (81.40)$^{cde}$ | 17.80$^{d}$ | 3.07$^{c}$ | 17.31 (24.57) | 14.48c | 733.33de | 786.51$^{cd}$ | 2.87 | 79.11 (62.80)$^{bc}$ | 20.88 (27.19)$^{ef}$ |
| Vermiwash 3% | 608.00 | 40.49$^{b}$ | 99.38 (85.97)$^{ab}$ | 20.71$^{ab}$ | 4.02$^{a}$ | 19.83 (26.39) | 16.54$^{ab}$ | 806.66$^{b}$ | 880.46$^{ab}$ | 3.16 | 81.70 (64.68)$^{a}$ | 18.29 (25.31)$^{g}$ |
| Vermiswash 5% | 604.00 | 41.79$^{a}$ | 99.43 (86.18)$^{a}$ | 21.10$^{a}$ | 4.06$^{a}$ | 19.58 (26.21) | 17.03$^{a}$ | 820.00a | 926.54$^{a}$ | 2.97 | 81.36 (64.42)$^{a}$ | 18.65 (25.58)$^{g}$ |
| Vipul | 616.00 | 38.54c | 98.77 (83.94)$^{abc}$ | 19.18$^{cd}$ | 3.28$^{bc}$ | 17.23 (24.55) | 15.85$^{abc}$ | 736.66$^{d}$ | 819.84$^{bc}$ | 2.77 | 80.47 (63.79)$^{ab}$ | 19.50 (26.20)$^{fg}$ |
| Water | 612.00 | 35.99$^{d}$ | 97.07 (80.16)$^{de}$ | 17.63$^{d}$ | 3.06$^{c}$ | 17.65 (24.82) | 14.50$^{c}$ | 656.66$^{h}$ | 682.27$^{e}$ | 2.93 | 74.07 (59.40)$^{e}$ | 25.92 (30.59)$^{b}$ |
| Untreated | 628.00 | 34.59$^{d}$ | 96.01 (78.60)$^{e}$ | 17.65d | 2.93$^{c}$ | 16.66 (24.11) | 14.51$^{c}$ | 640.00$^{i}$ | 688.26$^{e}$ | 2.93 | 70.53 (57.12)$^{f}$ | 29.47 (32.87)$^{a}$ |
| **S.E.m. ±** | **NS** | **0.43** | **1.02** | **0.47** | **0.196** | **NS** | **0.45** | **2.72** | **21.51** | **NS** | **0.48** | **0.48** |

Values in vertical columns with same letters do not differ statistically by Duncan's Multiple Range Test (DMRT).
Figures in parenthesis are angular transformed values.
NS: Non-singificant

significantly increased the number of leaves per shoot as compared to 14.50 leaves/shoot in untreated plants. The leaf area of 322.98 $dm^2$ was observed in vermiwash 5 per cent sprayed leaves and differed significantly over other treatments and untreated leaves (257.44 $dm^2$). The leaf yield per plant was significantly high in vermiwash 5 per cet (566.00g) as compared to unsprayed plant (403.00g). It was followed by vermiwash 3 per cent (553.0g) and vipul (533.00g), biodigester 1 per cent (532.00g), 3 per cent (520.00g) and 5 per cent (513.0g), panchagavua 3 per cnet (482.00g) and water spray (480.0g) and were on par with vermiwash 5 per cent (Table-1).

**Biochemical constituents of mulberry**: Foliar spray of vermiwash 5 per cent significantly enhanced the leaf moisture upto 79.75 per cent as compared to 72.85 per cent in unsprayed leaves. Vermiwash 3 per cent (76.95%), vipul (76.84%), biodigester 5 per cnet (76.64%) and panchagavya 5 per cent (75.60%) were the next best treatments. The chlorophlly a, b and total chlorophyll in mulberry leaves were significantly enchaced due to foliar spray of vermiwash 5 per cent (1.37, 0.89 and 2.16 mg/g) and 3 per cent (1.33, 0.83 and 2.06 mg/g), vipul spray was on per with vermiwash 5 per cent in chlorophyll 'b' (0.84 mg/g) and total chlorophyll (1.97 mg/g) content. While, unsprayed leaves recorded 0.79, 0.52 and 1.20 mg/g of chlorophyll a, b and total chlorophyll. The total sugar in leaves was significantly more in vermiwash 5 per cent (10.38%) and 3 per cent (10.29%) and vipul spray (10.01%) and it was 7.91 per cent in unsprayed leaves. While, the crude protein content in leaves was significantly more in vermiwash 5 per cent (326.93%) and vipul (26.25%) against 19.68 per cent in unsprayed leaves (Table-1).

**Bioassay studies:** The silkworm growth and development varied significantly due to feeding with organic foliar sprayed leaves. The total larval duration did not vary due to organic foliar spray on leaves with unsprayed leaves

and varied from 604 hr (vermiwash 5%) to 624.0 hr (panchagavya and biodigester at 3 per cent) among the foliar spray treatments and it was 628.0 hr in unsprayed leaves. The mature larval weight was significantly high in vermiwash 5 per cent (41.79 g/10 larvae) compared to vipul spray (38.54 g/10 larvae) and unsprayed treatment (34.59 g/10 larvae). High effective rate of rearing was observed in vermiwash 5 per cent (99.43%) and 3 per cent (99.38%) and vipul spra (98.77%) and were superior over unsprayed treatment (96.01%). The cocoon weight and cocoon shell weight were significantly high in vermiwash 5 per cent (21.10 g/10 cocoon and 4.06 g/10 shells), while, vermiwash 3 per cent (20.71 g/10 cocoons) was on par with vermiwash 5 per cent in cocoon weight. Vermiwash 3 per cent (20.71 g/10 cocoons) was on par with vermiwash 5 per cent in cocoon weight. Vermiwash 3 per cent (4.02 g/10 shells), biodigester 3 per cent (3.84 g/10 shells), panchagavya 1 per cent (3.47 g/10 shells) and 3 per cent (3.44 g/10 shells) and biodigester 5 per cent (3.44g) were on par with vermiwash 5 per cent. In untreated treatment 17.65 g/10 cocoons of cocoon weight and 2.93 g/10 shells of shell weight was recorded. The cocoon to shell ratio didn't vary due to organic foliar and vipul spray with unsprayed treatment. The pupal weight increased significantly due to vermiwash 5 percent (17.03 g/10 pupae) and 3 per cent (16.54 g/10 pupae), vipul (15.85 g/10 pupae) and panchagavya 3 percent (15.71 g/10 pupae) and all were on par. In unsprayed treatment, it was 14.15 g/ 10 puape.

The highest cocoon yield of 820 g/dfl was obtained in vermiwash 5 per cent foliar spray as compared to 736.66g and 640 g in vipul spray and unsprayed treatments, respectively. Longer silk filament of 926.54m and 880.46 m was recorded in vermiwash 5 and 3 per cent against 688.26 m silk under untreated treatment. The filament thickness (denier) was non-significant. Vermriwash 3 per cent (81.70%) and 5 per cent (81.36%) and vipul (80.47%) foliar spray significantly increased the fibroin protein in silk and were on par. The above treatments recorded lesser sericin protein in silk.

The results reveal the superiority of foliar sprays in improving the mulberry growth, yield and biochemical constituents. Among the organic foliar spray vermiwash at 3 and 5 per cent significantly increased growth, yield and biochemical composition of leaves over vipul, a commercially recommended foliar spray on mulberry. Vermiwash known to contain nutrients, enzymes and growth promoting hormones might have balanced nutritional requirement of leaf besides stimulating the vigorous growth as compared to panchagavya and biogester which supplement mainly the nutrients while vipul exhibited only growth promoting effect. High chlorophyll in the leaf is indicative of higher photosynthetic efficiency (Patil, 1988), which enhanced the leaf yield by increasing leaf area (Watson, 1952). Venkataramana *et al*., (2009) and Sudhakar *et al*., (2011) reported the beneficial effect of foliar spray of vermiwash on mulberry. The foliar sprays relatively increased the silkworm growth, cocoon yield and silk traits. The importance of leaf quality in successful cocoon harvest was reported by several workers (Juyal, 2003; Venkataramana *et al*., 2009: Sudhakar *et al*., 2011). The beneficial effects of organic based foliar sprays on several agricultural and horticultural crops in improving the yield and quality of products has been well documented (Natarajan, 2002; Sebastian and Christopher, 2007; Sridhar, 2003; Subashini *et al*., 2001). It is thus concluded that Vermiwash at 5 per cent foliar spray substantially increased the mulberry productivity and improved the performance of silkworm growth, cocoon and silk yield over vipul spray and other organic foliar spray. In commercial silkworm rearing, mulberry garden leaf waste and rearing waste can well be utilized for vermicomposting and the collected vermiwash can be used as foliar spray without adding much to the input cost.

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# Utilization of coarse grains for preparation of *Thalipeeth*, its sensory acceptability and nutrient availability

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**ABSTRACT**

The study carried out with the objectives of assessing the acceptability of *Thalipeeth* developed by incorporating bajra, wheat and soy flour mixed in the ratio of 10:80:10 ($T_1$), 20:70:10 ($T_2$), 30:60:10 ($T_2$) against 100% wheat flour (control) and to finding out the nutritive value as well as analyzed elements by TBS indicated that *Thalipeeth* was liked very much by the panelists. Nutrient analysis indicated that *Thalipeeth* contains 47.34% moisture, 1.95% ash, 7.3% fat, 6.39% protein. 0.68% fiber, 2.145 mg iron, 31.32 mg calcium, 36.24% carbohydrate and 237 kcl energy. Elements detected from LIBS spectra for the developed product were carbon, hydrogen, nitrogen, calcium, iron, sodium and magnesium.

**Keywords:** Coarse grain, *Thalipeeth*, nutrient

Coarse grains, referred to the millet along with maize and sorghum, that constitue the food of the economically weaker sections of the population of India, are nowadays gaining popularity amontst people accustomed to softer cereals (wheat and rice) because of the richness in insoluble dietary fiber containing large proportion of cellulose, which has beneficial effects in the gastrointestinal tract and various degenerative diseases. The soluble fractions, which mainly consists of pectin, arabinoxylan and b-glucan, has the ability to lower blood serum cholesterol, through its tendency to increase viscosity in the intestine. (Mridula and Gupta, 2008).

Millets are hardy plants mostly grown in areas with low rainfall, poor irrigation facilities and low soil fertility where most other cereal grains would fail. These are well suited for "dry farming". With the current rate of increase in population and less than adequate irrigational facilities millets adequately meet the demand for additional food supply in developing countries. These are especially beneficial to vegetarians who depend on plant food for their protein nourishment. It is reported that cardiovascular diseases, duodent ulcers and hyperglycemia occurs rarely in regular millet eaters. Since millets, which are rich in fiber are used less as staple though available in plenty it would be worth while considering supplementation study with selected millet (Menon, 2004).

Among millets, bajra (pearl millet) is the predominant fourth most important staple food crop for rural people of dry land regions of India after rice, wheat and sorghum (Singh *et al.,* 2006). Bajra is comparable and even superior in some of the nutritional characteristics to major cereals with respect to its energy value, protein, fat and minerals (Anu *et al,* 2007). Several studies reported possibility of utilization of bajra flour for making diversified food products for human consumption, although its color deterioraes the appearance and colour of the developed products due to presence of complex carbohydrate and lower glycemic response. Bajra is also getting popularity amongst the health conscious consumers.

It was found that supplementation of cereal based diet with soybean can play an important role in combating the protein energy malnutrition because celeals are deficient in lysine and pulses in sulphur containing amino acids. Incorporatoin of soy flour in a small quantity will improve the protein quality of cereals based products. As per Food and Agriculture Organization report, replacing wheat flour with 20% non-what flour for the manufacture of bakery products would result in an estimated savings in foreign currency of US$320 million annually (Mridual and Gupta, 2008). Government of India has recommended that the fortification of soy flour with wheat flour in 1:10 proportion would be beneficial in improving the nutritional status of our chapattis and other products without affecting the texture of food products, (Pandey *et al.,* 2008).

*Thalipeeth,* a recipe of Maharashtra traditionally prepared from *Bajra* flour, is less acceptable inother states because of its grey color. Thus, soy flour and wheat four is incorporated to make it more acceptable and enrich its nutrients. The present study was, therefore, carried out to find the effect of incorporation of different proportion of *Bajra* and soy flour in wheat flour on sensory characteristics and nutrient composition of *Thalipeeth.* Qualitative assessment of value added products by Laser Induced Breakdown Spectorscopy (LIBS) was also done for elemental analysis.

## MATERIALS AND METHODS

*Bajra* and soybean seeds, refined oil, sugar, jaggery, groundnut, bengal gram flour were procured from the local market of Allahabad in the month of January 2010. Bajra and soybean seeds were cleaned, washed, roasted and sun dried for 2 days and then ground in the atta maker to make the flour.

*Thalipeeth* was prepared by using bajra, wheat and soy flour in ratio of 0:100:0 (control). 10:80:10 (T1), 20:70:10 (T2) and 30:60:10 (T3). The ingredients (g) used in preparation of *Thalipeeth* were wheat flour 50, onion 25, garlic 2 cloves, green chili 1 small size, ajwain ¼ tsp, salt ¼ tsp and oil 1 tsp. The dough was prepared by adding all the ingredients. Small ball in the shape of *Paratha* was shallow fried till golden brown on both sides.

The sensory quality of the developed product in respect of colour and appearance, body and texture, flavour, and taste and overall acceptability was judged by five panalist using 9-point Hedonic scale.

Moisture ash and crude fiber were estimated by AOAC method. protein by Micro Kjeldhal method, fat by Soxhlet method, carbohydrate by difference method, iron by colorimetry method, calcium by Titration method and energy (Kcal/100g) (4 X Protein) % + (9 X fat)% + (4 X CHO)%.

Laser-induced breakdown spectroscopy (LIBS) of *Thalipeeth :* A high energy laser is focused on the sample material (solid, liquid or gas) which causes target material to undergo rapid local heating, vaporization, dissociation, ionization of its atoms and the atomic emission from the expanding plasma lume that is formed during the laser matter interaction provides valuable information about the composition of the target material on the screen conneted on line. *Thalipeeth* was subjected for element analysis by LIBS.

The data were subjected to analysis of variance (ANOVA) using Random Block Design. Significant difference between treatments was determined by using critical difference test at $p<0.05$ (Chandel, 2006).

## RESULTS AND DISCUSSION

### Sensory evaluation

**Colour and Appearance:** Table 1 shows that treatment $T_2$ with incorporation of *bajra* flour at 20% level got highest mean score while treatment $T_3$ with 30% *bajra* flour got the least mean score regarding colour and appearance. This is because as the proportion of *bajra* flour increases, its undesirable grey color becomes more dominant which makes the color and appearance of product less acceptable. Singh *et al* (2006) who reported that lower organoleptic scores for type II and IV cakes may be due to high level of pearl millet may give dark color to product.

**Body and texture:** The Table 1 shows that the average score in treatment $T_3$ at 30% level of incorporation of *bajra* flour decreased because its coarse and rough texture become more dominant. Mridula *et al* (2008) also found that the high fiber and low gluten content in *bajra* flour than wheat flour may be attributed to the incresed rough and coarse texture of the biscuits in proportion to the *bajra* flour levels in flour blends.

**Taste and Flavour:** The average scores of *Thalipeeth* was maximum for $T_2$ enriched with 20% *bajra* flour followed by treatments $T_1$, $T_3$ and $T_0$ enriched with 10%, 30% and 0% *bajra* flour, respectively. The results showed that the incorporation of *bajra* flour at 20% was highly acceptable with improved taste and flavour of *Thalipeeth* than control.

**Overall Acceptability:** The mean scores in Table 1 show that *Thalipeeth* prepared from different levels of *bajra* flour with 10% soy flour were accepted by the panelist with highest acceptability at 20% *bajra* flour level.

**Nutritional evaluation :** Table 2 fig 4.2 and 4.3 reveals that *Thalipeeth* contains 47.34% moisture, 1.95% ash, 7.3% fat, 6.39% protein, 0.68% fiber, 2.14 mg iron, 31.32 mg calcium, 36.34% carbohydrate and 237 kcal energy. Singh *et al* (2006)

**Table 1:** Average Sensory scores of *Thalipeeth* prepared by admixture of *Bajra*, soy and wheat flour.

| Parameters / Treatments | Colour and Appearance | Body and Texture | Taste and Flavour | Overall Acceptability |
|---|---|---|---|---|
| **Control** | 7.16 ± 0.11 | 7.4 ± 0.12 | 7.44 ± 0.12 | 7.2 ± 0.27 |
| **$T_1$** | 7.84 ± 0.13 | 7.52 ± 0.09 | 7.64 ± 0.15 | 7.7 ± 0.28 |
| **$T_2$** | 8.04 ± 0.14 | 8.44 ± 0.13 | 8.44 ± 0.12 | 8.1 ± 0.29 |
| **$T_3$** | 7.92 ± 0.10 | 7.64 ± 0.1 | 7.68 ± 0.15 | 7.8 ± 0.27 |
| **CD ($p<0.05$)** | 0.47 | 0.35 | 0.51 | 0.47 |

**Table 2:** Average nutrient content of *Thalipeeth*

| Nutrients | Moisture (%) | Ash (%) | Fat (g) | Protein (g) | Dietary fiber (g) | Carbohydrate (g) | Iron (mg/100) | Calcium (mg/100) | Energy (Kcals/100g) |
|---|---|---|---|---|---|---|---|---|---|
| Average | 47.34 | 1.95 | 7.3 | 6.39 | 0.68 | 36.34 | 0.145 | 31.32 | 431 |

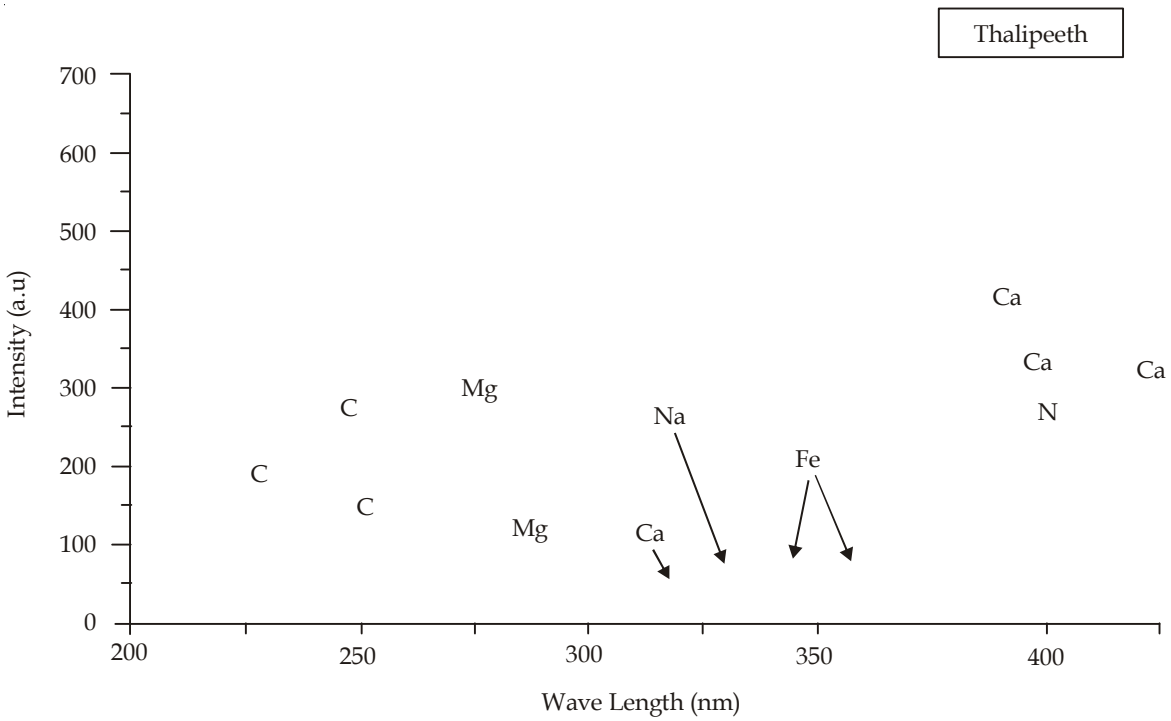


Fig 1: LIBS spectra for Elements of *Thalipeeth* in Treatment $T_2$

reported that moisture, ash, protein, fat, iron, calcium and phosphorus content increased while carbohydrate contnet decreased in soy and *bajra* flour incorporated cake as compared to control.

**Elements analysis of *Thalipeeth* by LIBS:** Fig 1 showed that LIBS spectra of *Thalipeeth* consisted of carbon, hydrogen, nitrogen, calcium, iron, sodium and magnesium.

It is thus concluded that millet like *bajra* flour could be successfull incorporated with wheat and soy flour to enhance the nutritive value of *Thalipeeth.* The level of *bajra* flour was standardized at 20 percent on the basis of evaluation. Among the experimental treatments, $T_2$ (20%) was most acceptable for *Thalipeeth.* The average nutrient content of the developed products showed that an average percentage of *Thalipeeth* contains 47.34 percent moisture. 1.95 percent ash, 7.3 percent fat, 6.39 percent protein, 0.68 percent fibre, 2.145 mg iron, 31.32 mg calcium, 36.34 percent carbohydrate and 237 kcal energy. Similarly, the LIBS spectra of *Thalipeeth* consists of elements as: carbon, hydrogen, nitrogen, calcium, iron, sodium and magnesium.

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# Potential of neem cake in the control of stalk borers, termites and root-knot nematodes in Tanzania

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**ABSTRACT**

In field trials conducted during high stalk borer infestation in Tanzania, application of powdered neem cake at 2g/ plant twice at 4 and 6 weeks after plant emergence increased the grain yield by 30 percent over the untreated control and by 25 percent over thiodan treatment. Application of neem cake at 3g/m$^2$ also reduced lodging due to termite attack in maize crop and registered a 62 percent higher grain yield over the untreated control and 15 percent over furadan treatment. Incorporating neem cake at 40 or 50g/m$^2$ into nematode-infested soil significantly reduced the juvenile (J2) population of the root-knot nematode, *Meloidogyne javanica*, as well as the root galling index in tomato and tobacco seedlings. The broad spectrum of biological activity of neem cake against insect pests as well as parasitic nematodes could confer dual benefits to farmers.

**Key words:** *Azadirachta indica,* neem cake, root-knot nematodes, stalk borers, Tanzania, termites

In Tanzania, stalk borers, such as *Chilo partellus* (Swinhoe) and *Busseola fusca* Fuller, cause severe damage and yield loss in maize (Harris and Nwanze 1992), while gerass termites, *Trinervitermes* spp. during dry spells can cause lodging of the maize crop. *M. javanica*, the most prevalent root-knot nematode, severely reduces tobacco yield in Tanzania and other African countries (Mudulu and Trudgoill 1994). Farmers control these crop pests through chemical pesticides, which often are in short supply, expensive and unaffordable. Moreover, their production and use are hazardous to people and livestock leading to serious implications in environmental degradation. An alternative to synthetic chemicals is organic or botanical pesticides, which are products of plant origin having pest control properties and do not cause similar problems (Arnason *et al.* 1989). Neem, *Azadirachta indica* A. Juss., a botanical cousin of mahogany, is an example of plants whose products have been found to have significant pest control potential for a long time (NRC, 1992). The bioactive principals or the "bitters" in neem have been identified as limonoids, a group of stereochemically homogeneous tetranortriterpenoids. The most important active principal, azadirachtin, has distinct repellent, antifeedant and growth inhibitory effects on insects (Saxena 1989, 2011, Schmutterer 1990).

Over the past three decades, neem has come under close scientific scrutiny as a source of natural pest control materials (Jacobson 1989, Schmutterer 2002a) in a number of crops, including cotton, tobacco, groundnuts and horticultural crops (Jackai 1993). Common neem products tested include seed oil, seed or kernel powder, and seed, leaf or bark extract.

In Shinyanga and Tabora regions of Tanzania, neem has been widely planted and is being promoted in agroforestry and afforestation programmes. In addition to providing fuelwood and timber, neem has gained considerable popularity over the years for its medicinal value for both humans and livestock. Neem is called "Mwarubaini" in Kiswahili, meaning a reliever of 40 disorders, from chronic malaria to diarrhoea. However, due to lack of awareness of its pest control potential, farmers in Tanzania are not yet using neem for pest control. To promote the use of neem by farmers in existing agricultural systems, a series of field trials was conducted to determine the potential of powdered neem cake (NC) for the management of stalk borers in maize, termites in tree establishment and the root-knot nematodes, *Meloidogyne javanica* (Treub) Chitwood on tobacco.

## MATERIALS AND METHODS

### *Study area*

Maize and tobacco trials were conducted respectively at Shinyanga and Tabora areas in western Tanzania. The

Shinyanga and Tabora regions cover 126,884 km$^2$ (13.5% of Tanzania) and support about 3.3 million inhabitants (~11.8% of the country's population). The area is characterised by unimodal plateau (1000 to 1500 m above sea level) with rainfall mostly concentrated between November to April. Shinyanga receives a mean annual rainfall of ~700 mm and Tabora ~808 mm. At Shinyanga, mean monthly maximum temperatures vary between 27.6$^o$C and 30.2$^o$C and minimum temperatures vary between 15$^o$C and 18.3$^o$C. Soils at the experimental sites are clay loams and with vertic properties, pH (water) = 8.0, total N = 0.18%, organic C = 0.2%, and extractable P = 3-5 ppm. At Tabora, mean monthly temperatures range from 26.9$^o$C to 32.3$^o$C and minimum temperatures from 13.4$^o$C to 18.5$^o$C. Soils at the Tabora experimental sites are sandt to sandy loams with pH (water) = 5.0, total N = 0.03%, organic C = 0.4%, and extractable P = 7 ppm. The rainy season is characterised by short dry spells, often detrimental to crop production. Maize is the staple food crop in both regions. Cotton is the main cash crop in Shinyanga and tobacco in Tabora.

**Neem matrials**

Fresh neem seed, collected from ripe fruits, was washed and dried under shade for 3-4 days to 12-14% moisture content and Neem cake (NC) was prepared by cold-pressing whole seeds in a 'Komet' single-screw vegetable oil expeller )IBG Monforts, GmbH & Co., Monchengladbach, Germany). NC was then manually pounded to a fine powder and stored in paper bags. The azadirachtin A content of NC, as determined by high-pressure liquid chromatography at ICIPE's Natural Product Chemistry Laboratory, was found to be 5800 ppm.

**NC against maize stalk borers**

In 1995 and 1996 cropping seasons, 'Cargil 41' maize cultivar was sown in 8 x 9 m plots at a spacing of 0.75 m between rows and 0.60 m between plants within a row. The crop was weeded twice and fertilised with diammonium phosphate at sowing and with calcium ammonium phosphate as a top dress at recommended rates. Powdered NC at 2g/plant was applied once at 4 weeks or twice at 4 and 6 weeks after plant emergence (WE) in comparison with application of thiodan (35%) dust at 4 WE and untreated control plants for stalk borer control. The treatments were arranged in a randomised block design (RBD) and replicated five times. The stalk borer infestation was monitored visually. At harvest, maize grain yield from four 7-m-long central (21 m$^2$) rows was recorded.

**NC against termites in maize**

In 1996 and 1997 cropping seasons, 'Cargil 41' maize was sown in 6 by 9 m plots at a spacing of 0.75 m between rows and 0.60 m between plants within a row. The crop was weeded and fertilised as above. NC application at 1, 2, or 3 g/plant was compared with application of furadan (5G) at 2 g/plant and with no insecticide in the control. The treatments were arranged in a RBD and replicated five times. Termite infestation was monitored in each plot by counting the plants affected and per cent lodging. Grain yield from the middle four 7-m-long (21 m$^2$) rows was recorded.

**Efficacy of NC against root-knot nematodes in tomato and tobacco**

In a trial conducted at the Tumbi Experiment Station, Tabora in 1998, application of NC at various rates was compared with ethylene dibromide (EDB) treatment with or without chemical treatment on nematode infestation. NC was tested at 20, 25, 30, 35, 40, and 50 g/m$^2$ by spreading uniformly and raking into soil in each plot. EDB (92%) was injected into the soil at 62 ml/m$^2$ 14 d before planting tobacco. The treatments, tested in 1 by 2m plots, were arranged in RBD and replicated four times. Before the start of the experiment, nematode susceptible seedlings of 'Money Maker' tomato cultivar, infested with root-knot nematodes, were grown in an experimental plot for eight weeks to ensure build up of uniform nematode inoculum in the soil. Soil samples were taken randomly from 0 to 15cm soil depth from 10 locations within each plot to determine the initial soil nematode population by bioassay technique Luc *et al.* (1990)

At 3 d after applying NC, samples from 0 to 15cm soil depth were collected from each plot for bioassay pot experiment. For each treatment, 10 plastic pots (15 cm diam.) were filled with soil and arranged in a randomized complete block design with 10 replications in a greenhouse. Tobacco was sown in each pot directly by seeds. The seedlings were left to grow for 60 d before sampling for nematode assessment. Nematode infestation was assessed in terms of root gall index (RGI) according to Bridge and Page (1977).

**RESULTS AND DISCUSSION**

*Efficacy of NC application against stalk borers*

The 1995-96 season was extremely favorable to crop growth with good rainfall and low stalk borer infestation, so crop yields were fairly high (Table 1). In view of low pest infestation, there were no significant differences between yield of NC- or thiodan-treated maize and untreated maize plants. Due to the presence of some oil, application of NC caused a slight scorching effect on young maize leaves in whorls, but the leaves that developed subsequently did not

Table 1. Maize grain yield in response to application of neem cake (NC) or thiodan against stalk borers at Shinyanga, Tanzania, 1995-96 and 1996-97 cropping seasons

| Treatment | Grain yield (kg/ha) | |
|---|---|---|
| | 1995-96 | 1996-97 |
| NC applied once | 3220 | 1403 |
| NC applied twice | 3100 | 1537 |
| Thiodan applied once | 3210 | 1233 |
| Untreated (control) | 3560 | 1183 |
| SED | 290 | 145 |

show any such adverse effect. In the second year, however, when there was a noticeable stalk borer infestation, NC applied twice increased grain yield significantly by 30 percent over the untreated control or by 25 percent over the chemical treatment with thiodan. There were no significant differences between yields of maize plants applied once or twice with NC.

**Efficacy of neem cake application against termites**

Lodging of maize crop is an indicator of termite damage. Termite infestation was relatively high in 1995-96, as evident from 63 percent lodging in the untreated control (Table 2). Application of furadan or of NC at 1, 2, or 3g/m$^2$ decreased termite attack and increased crop yields significantly over the untreated control. The beneficial effect of NC was particularly high at 3g/m$^2$, which not only registered 62 percent higher yield over the untreated control but also gave a 15 percent higher yield over the furadan treatment ($P$<0.05). The reduction in lodging and an increase in grain yield due to application of NC at 1 or 2g/m was on par with furadan treatment.

Table 2. Maize grain yield in response to application of powdered neem cake (NC) or furadan 5G against termites, 1995-96 and 1996-97 cropping seasons

| Treatment | Lodging (%) | | Grain yield (kg/ha) | |
|---|---|---|---|---|
| | 1995-96 | 1996-97 | 1995-96 | 1996-97 |
| NC at 1g/plant | 49.8 | 36.4 | 3560 | 1344 |
| NC at 2g/plant | 45.3 | 32.2 | 3700 | 1563 |
| NC at 3g/plant | 35.5 | 27.2 | 4210 | 1651 |
| Furadan 5G at 2g/plant | 43.0 | 41.1 | 3660 | 1215 |
| Untreated (control) | 63.1 | 49.9 | 2600 | 1240 |
| SED | 5.1 | 3.5 | 260 | 151 |

In 1996-97, termite attack was lower than in previous cropping season, as evident from only 50 percent lodging in the untreated control (Table 2). Although application of furadan or NC at 1g/m$^2$ reduced lodging, it did not lead to an increase in grain yield. Only application of NC at 2 or 3g/m$^2$, reduced lodging substantially and increased maize grain yield significantly over the untreated control. A mean yield of 1651 kg/ha was recorded with application of NC at 3g/plant compared with 1240 kg/ha for control and 1215 kg/ha for Furadan treatment. Clearly, NC application was more effective for termite control than a synthetic chemical, such as furadan. As the rate of NC application increased, the number of plants attacked by termites decreased and the maize yield increased. The linear response observed in respect of the reduced termite damage and increased grain yield with neem, suggests the need to determine the optimum rate of NC application.

**Efficacy of neem cake application against root-knot nematodes**

Before treatment, the nematode population in various treatment plots was identically high, indicating that all plots were adequately infested with nematodes. Three days after EDB injection, no nematodes were observed in the soil, while three days after the application of NC at 40 or 50g/m$^2$ into the soil, significantly fewer nematodes were found than in untreated control (Table 3). Also, significant differences in

Table 3. Effect of application of neem cake (NC) at different rates or ethylene dibromide (EDB) on ***M. javanica*** root gall index (RGI) in tomato and tobacco seedlings and juvenile ($J_2$) nematode population in tobacco seedlings. Tabora, Tanzania, 1998

| RGI in seedlings of | | | |
|---|---|---|---|
| Treatment | Tomato[a] | Tobacco | Juveniles[b] |
| NC 20g/m$^2$ | 2.5 | 3.5 | 42bc |
| NC 25g/m$^2$ | 3.0 | 3.3 | 23bc |
| NC 30g/m$^2$ | 2.7 | 3.1 | 27bc |
| NC 35g/m$^2$ | 3.0 | 2.6 | 92c |
| NC 40g/m$^2$ | 1.5 | 1.6 | 27bc |
| NC 50g/m$^2$ | 1.5 | 1.3 | 16b |
| EDB | 0.0 | 0.8 | 0a |
| Untreated (control) | 7.5 | 7.4 | 475d |
| SED | 0.55 | 0.28 | - |
| 'F' probability | <0.01 | <0.01 | <0.01 |

Juvenile means for various treatments followed by the same letter are not significantly different.
[a]Measured by bioassay; RGI before applying treatments was 5.8 $\pm$ 0.62
[b]Juveniles before applying treatments were 577.

root gall index (RGI) were recorded among treatments at 60 days after planting tobacco seedlings. The EDB treatment had the lowest level of juvenile infestation, followed by NC application at 50g/$m^2$, while untreated seedlings had the highest RGI.

Neem seed from which neem came (NC) was prepared for the control of stalk borers, termites and nematodes, is a renewable natural resource with multiple uses. A mature neem tree can produce $\geq$50 kg neem fruits; 50 kg of fruits yield about 30 kg of seed from which one can get 24 kg of NC and 6 kg of valuable neem oil. Neem materials have been found to affect nearly 500 species of noxious insects (Schmutterer and Singh 2002) but also several species of nematodes, including the root-knot nematodes (Saxena 1993). The comparative advantage of the use of neem materials over chemical pesticides is that there are little or negligible side effects on pests' natural enemies and other non-target organisms (Schmutterer 2002b). Compared with costly pesticides, the relatively low production cost of neem materials, some of which can readily be produced using simple tools in rural households, make them ideal for use by resource-limited farmers. Unlike pesticides, which outright kill pests, neem materials have diverse behavioral and physiological effects, which impair the pests' overall fitness (Saxena 1989, 2011, Schmutterer 1990, Mordue *et al*. 1999). Some of these effects on lepidopterans (Koul and Isman 1991) are attributed to the presence of novel chemicals, such as azadirachtin in neem. Likewise, several species of termites, such as *Microtermes* (Ketkar 1976), *Reticulotermes* (Jacobson 1981, Serit *et al*. 1992), *Odontotermes* (Gold *et al*. 1989), and *Coptotermes* (Grace and Yates 1991) are found to be affected by neem materials both in laboratory tests and field trials. Bioactive principals in crude extract of neem seed have also been reported to inhibit the penetration, hatchability and development of nematodes (Mojumder 2002).

The effectiveness of simple neem seed materials, such as NC, in reducing the plant damage caused by some important crop pests such as stalk borers, termites and root-knot nematodes is of practical significance. The broad spectrum of biological activity of neem materials against insect pests as well as parasitic nematodes would confer dual benefits on the users. Increasing awareness of the potential of neem for low-cost and sustainable pest mangement should therefore receive priority in rural development programmes in tropical developing countries.

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# Role of weather factors and total soluble solids on the population buildup of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hompoptera : Pseudococcidae) on grapevine in India

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**ABSTRACT**

Field studies, conducted from December 2004 to March 2005 on Thompson seedless at Bangalore and from December 2006 to March 2007 at Pune, to determine the role of weather factors and total soluble solids (TSS) in the buildup of the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) population in the vineyards revealed that the buildup of the population of *M. hirsutus* was found to increase from 25.30 / plant in December 2004 to 522.90/plant during March 2005 at the time of harvesting in Bangalore. A steady buildup was also observed in the number of mealybug colonies from 0.80 / plant in December 2006 to 7.40 /plant during March 2005 at the time of harvesting at Pune. Further the studies have also shown that the mealybug population buildup coincide with increased with the increase in temperature, decrease in the humidity and advancement in the berry development. Step-wise regression procedure employed to arrive at a multiple regression model showed that 96.1-99.7 % of the mealybug population buildup could be predicted by two factors namely relative humidity and TSS. It is concluded that the weather factors chiefly relative humidity coupled with TSS influenced the buildup of the mealybug population in the vineyards in both the locations.

**Key words**: *Maconellicoccus hirsutus*, grapevine, weather factors, Total Soluble Solids

Mealybugs cause serious losses in grapes in many countries including India. They are found on the growing shoot, leaves, bark, stem, inflorescence and bunches. In India, severe outbreak of mealybugs was reported in vineyards of Andhra Pradesh by Tejkumar *et al.* (1977) and Satyanarayana (1981) and subsequently in Karnataka, Maharashtra and Tamil Nadu (Manjunath, 1985). Grape production is often adversely affected to the extent of damage being as much as 90% in extreme cases (Babu and Azam, 1983). There is fluctuation in the mealybug population and damage in different months and different crop growth stages. It is very difficult to control the mealybugs in the advanced stage of berry development by any method of control. With a view to develop effective mealybug management programme, a study was conducted to determine the role of weather factors and also total soluble solids (TSS) in the berries on the mealybug population build up in vineyards.

## MATERIALS AND METHODS

### Selection of orchard

A field study was conducted on the grape variety Thompson seedless infested with pink hihiscus mealybug at Hosallipalaya in Kolar district of Karnataka from December 2004 to March 2005. Another field study was conducted at NRC for Grapes, Pune on Thompson seedless from December 2006 to March 2007. In both the locations, the study period was restricted from December to March, since berries were available during this time for estimation of TSS.

### Sampling

Observations were recorded at fortnight intervals on 10 randomly selected plants in both the vineyards from December to March in both locations. At Bangalore, the mealybugs were counted on five shoots in each plant. In each shoot, number of mealybugs were recorded on the stem, leaves, flower panicles, and bunches. At Pune, the number of mealybug colonies were counted on the entire plant.

Data on weather parameters viz., maximum and minimum temperatures ($^0$C), relative humidity (%) and rainfall (mm) were collected during the study period. The correlations between the mealybug and TSS and the weather

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factors were worked out to determine their influence on the build up of mealybug population on grapevine.

**Estimation of TSS**

Total soluble solids (TSS) was determined using refracto-meter as suggested by Lodhi and Selvaraj (1974).

**Statistical modelling**

As a first step linear correlation coefficient among mealy bug population in Grapes, observed during the season 2004-05 individually at Bangalore and Pune conditions ( Y )with weather parameters such as minimum temperature ($X_1$), maximum temperature ($X_2$), relative humidity ($X_3$), rainfall ($X_4$) and total soluble solids ($X_5$) were worked out. Then a statistical model relating mealy bug population with all the weather parameters was developed following the procedure of least square technique (Ryan, 1997).

**Measures of model adequacy**

As a measure of goodness-of-fit of the developed models, the values of the Co-efficient of determination ($R^2$) (Agostid'no and Stephens, 1986) and Mean Squared Error (MSE) were calculated as below:

a) Co-efficient of determination ($R^2$):

$$R^2 = 1 - [S(Y_i - \hat{Y})^2 / [S(Yi - \overline{Y})^2]$$

b) Mean Squared Error (MSE):

$$MSE = [S(Y_t - \hat{Y}_t)^2 / n]$$

where $Y_t$ represents the mealy bug population at time t and n is the number of observations.

However, inclusion of an additional independent variable into the selected candidate model will always boost the computed $R^2$ value (Kvalseth, 1985) Hence, to ensure the statistical significance of the computed regression coefficients, they were subjected to t-test statistical analysis (Draper and Smith, 1981). To get further insight into the results obtained, a step-wise regression procedure (Ryan, 1997) was employed, as delineated below, to select the most crucial weather factors, which can influence the variability in mealy bug population.

In step-wise regression, the final regression equation is developed stage by stage. The first independent variable ($X_1$) that enters the regression equation is the one that has the highest simple correlation with the dependent variable (Y). The regression coefficient of Y on $X_1$ is computed and tested for its significance. If it is found to be significant, then based on the highest first order partial correlation coefficient, the next variable to enter is selected. Then the regression equation is fitted with these two variables. Making use of the *F*-test, the two partial regression coefficients are tested to determine if they should be retained in the equation. The non-significant coefficient is deleted. This process of selection of new variables to enter is thus continued until all the variables are exhausted and are found significant, resulting in the final equation comprising only the significant independent variables.

**RESULTS AND DISCUSSION**

The data on the population of the mealybugs in relation to Total soluble solids (TSS) and weather factors under Bangalore conditions is presented in table 1. The mealybug population was found to be 25.30 / plant in December 2004. The population of mealybug began to increase from January and reached peak population of 522.9 mealybugs/plant during March 2005 at the time of harvesting. The data on the population of the mealybugs in relation to TSS and weather factor under Pune conditions is presented in Table 2. The number of mealybug colonies was found to be 0.80 / plant in December 2006. The population of mealybug began to increase from January and reached peak population of 7.40 colonies/plant during March 2005 at the time of harvesting.

Linear correlation coefficient among weekly mealy bug population with previous week weather parameters and TSS were worked out and presented in Table 3 & 4. Perusal of the results for Bangalore conditions indicated for the presence of highly significant positive correlation among mealy bug population with minimum temperature (r = 0.92), maximum temperature (r = 0.92), TSS (r = 0.94) and negatively with relative humidity (r = -0.82). Similarly, perusal of the results for Pune conditions indicated for the presence of highly significant positive correlation among mealy bug population with minimum temperature (r = 0.83), TSS ( r = 0.99) and negatively with relative humidity (r = -0.90). Similar kind of correlations were reported between weather factors and mealybug population (Mani and Thontadarya,1987) but the role of TSS in the buildup of mealybug population was not determined in that study.

In general, the mealybug population was considerably high during February- March. This is due to high temperature prevailing in this period which had helped in faster multiplication of the mealybugs. Maximum temperature showed positive and significant correlation with the mealybug population under Bangalore condition. At Pune, there was positive relationship between maximum

**Table 1:** Effect of weather factors and the TSS on the buildup of the grape mealybug population (Bangalore)

| Date | No.of mealybugs/ plant) | Min. temp. ($^0$C) | Max. temp. ($^0$C) | Relative humidity (%) | Rainfall (mm) | Total soluble solids |
|---|---|---|---|---|---|---|
| 16.12.04 | 25.3 | 15.1 | 27.7 | 61.0 | 4.9 | 2.8 |
| 2.1.05 | 33.3 | 16.2 | 27.8 | 61.2 | 0.0 | 4.8 |
| 16.1.05 | 47.6 | 16.4 | 30.0 | 61.5 | 2.8 | 5.2 |
| 2.2.05 | 56.4 | 16.9 | 31.5 | 55.4 | 0.0 | 8.8 |
| 16.2.05 | 127.5 | 17.6 | 33.4 | 48.5 | 0.0 | 14.5 |
| 2.3.05 | 416.4 | 18.5 | 38.8 | 48.0 | 0.0 | 19.4 |
| 16.3.05 | 522.9 | 19.7 | 37.5 | 47.0 | 20.7 | 22.6 |

**Table 2:** Effect of weather factors and the TSS on buildup of the grape mealybug population (Pune)

| Date | No.of mealybug colonies/plant | Min. temp. ( $^0$ C) | Max. temp. ( $^0$ C) | Relative humidity (%) | Rainfall (mm) | Total soluble solids |
|---|---|---|---|---|---|---|
| 14-12-06 | 0.8 | 6.6 | 30.9 | 70.6 | 2.0 | 2.6 |
| 31-12-06 | 1.6 | 4.4 | 32.6 | 68.5 | 0.6 | 4.4 |
| 14-1-07 | 1.8 | 6.7 | 32.7 | 64.5 | 0.4 | 5.6 |
| 03-2-07 | 2.0 | 7.7 | 37.6 | 60.5 | 0.8 | 8.4 |
| 17-2-07 | 2.4 | 13.8 | 38.7 | 56.0 | 1.2 | 15.5 |
| 02 –3-07 | 5.4 | 14.4. | 38.9 | 52.5 | 2.6 | 19.0 |
| 16-3-07 | 7.4 | 15.1 | 39.2 | 50.0 | 0.0 | 22.3 |

**Table 3:** Correlation coefficient ( r) values for grape mealybug population Vs weather factors and TSS Bangalore)

| Variable | Y | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ |
|---|---|---|---|---|---|---|
| Mealy bug (Y) | 1 | | | | | |
| Minimum temperature ($X_1$) | 0.92* | 1.00 | | | | |
| Max. temperature ($X_2$) | 0.90* | 0.93* | 1.00 | | | |
| Relative humidity ($X_3$) | -0.82* | -0.90* | -0.92* | 1.00 | | |
| Rainfall ($X_4$) | 0.65 | 0.56 | 0.38 | -0.35 | 1.00 | |
| TSS ($X_5$) | 0.94* | 0.98* | 0.97* | -0.96* | 0.51 | 1.00 |

*Significant at 5% level

**Table 4:** Correlation coefficient ( r) values for grape mealybug population Vs weather factors and TSS (Pune)

| Variable | Y | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ |
|---|---|---|---|---|---|---|
| Mealy bug (Y) | 1.00 | | | | | |
| Minimum temperature ($X_1$) | 0.83* | 1.00 | | | | |
| Max. temperature ($X_2$) | 0.74 | 0.89* | 1.00 | | | |
| Relative humidity ($X_3$) | -0.90* | -0.69 | -0.60 | 1.00 | | |
| Rainfall ($X_4$) | 0.13 | 0.36 | 0.37 | 0.31 | 1.00 | |
| TSS ($X_5$) | 0.99* | 0.89* | 0.76* | -0.89* | 0.14 | 1.00 |

*Significant at 5% level

**Table 5:** Results of statistical models along with goodness of fit statistics

| Model type | Statistical Model | $R^2$ | MSE |
|---|---|---|---|
| **Bangalore** | | | |
| Full regression model (All weather parameters & TSS) | Y =-682.72-137.32 $X_1$+4.101 $X_2$+39.76 X3+2.82 $X_4$+80.91 $X_5$<br>t-stat 3.13 0.3 4.13 1.09 3.85 | 99.8% | 632.8 |
| Optimised model I | Y=-102.33+24.92 $X_5$<br>t-stat 6.1 | 88.2% | 37.2 |
| Optimised model II | Y=-2039.72+50.09$X_5$+30.31$X_3$<br>t-stat 5.42 2.84 | 96.1% | 49.0 |
| Optimised model III | Y=-878.35+90.58 $X_5$+45.73 $X_3$-142.79 $X_1$<br>t-stat 8.1 7.2 3.9 | 99.4% | 155.8 |
| **Pune** | | | |
| Full regression model (All weather parameters) | Y =-4.71-0.258 $X_1$+0.164 $X_2$+0.5 $X_5$<br>t-stat 3.9 2.42 15.3 | 99.70 | 44.0 |
| Optimised model | Y=-0.425+0.395 $X_5$<br>t-stat 12.99 | 97.70 | 193.0 |

temperature and the number of mealybug colonies but was statistically non-significant. The population of *P.citri* was positively correlated with maximum temperature mandarin oranges at Shevroy hills of Tamil Nadu (Sridharan *et al*., 1989). Another study at Karnataka indicated that there was significant positive correlation between maximum temperature and *P.citri* on coffee (Kumar 1987). In both the locations, minimum temperature was significantly and positively correlated with the build- up of mealybug population in the present study. Similar kind of relationship was noticed between infestation of red scale *Aonidiella aurantii* and minimum temperature (Rao and Pathak, 2001).

The relative humidity showed negative and significant correlation with the mealybug population under both the locations. The trend of increasing mealybug population with decrease in humidity was evident both at Bangalore and Pune conditions.

There was significant negative correlation between relative humidity and *P.citri* on coffee at Karnataka (Kumar 1987). The negative relationship of relative humidity with the sucking pests like *Siphoninus phyllireae* Haliday (Mani and Krishnamoorthy, 2002) was earlier reported.

There was positive and significant relationship between TSS and the mealybug population buildup in both the locations. There was increase in TSS from 2.8 on 16 $^{th}$ December to 22.6 on 16 $^{th}$ March under Bangalore conditions. Similar results were obtained under Pune conditions. There was gradual increase in TSS and mealybug population up to mid February in both the locations. There was sudden spurt in the mealybug population coinciding with high increase in TSS by mid February. Manjunath (1985) also reported that *M. hirsutus* became active during January and the population reached peak in March on grapevine around Bangalore. Highest numbers of mealybugs were recorded at the time of harvesting in the vineyards (Mani, 1988). Similar observations were reported by Berlinger (1977) for *Planocoocus vitis* (Nied) on grapevine in Israel and by Charles (1982) for *Peudococcus longispinus* (Targioni-Tozzetti) in New Zealnd. Results clearly confirmed that the highest peak in the mealybug population was observed on the fruit bunches nearing maturity having highest TSS. Arora *et al.* (1999) reported that ber fruitfly *Carpomya vesuviana* infestatation was positively correlated with TSS .

As a next step, statistical models were developed by regressing weekly mealy bug population on all the weather parameters and TSS. Perusal of Table 5 indicates that about 99.8 per cent of the variation in mealy bug population (under Bangalore condition) has been collectively explained by all the weather parameters and TSS. Though the developed models resulted in considerably high $R^2$ values and lesser mean square error values, as described earlier, the statistical significance of the regression coefficients were worked out, tested and presented in Table 5. Further, only the regression coefficients corresponding to minimum temperature, humidity and TSS were significantly related to mealy bug population as indicated by the t-test statistic value (being greater than 2.0). Accordingly, step-wise regression models were developed to optimize the role of these factors and the results are presented in Table 5. Perusal of Table-5 indicates that only two variables, viz. relative humidity and TSS could they collectively explain about 96.1 percent of the variation in weekly mealybug population under Bangalore conditions. Similarly results presented for Pune data indicated that about 99.7 percent of the variation in mealy bug population has been collectively explained by all the weather parameters and TSS. Results of optimized models indicated that about 97.7 % of the variation in mealy bug population is due to TSS. Further, the regression coefficients corresponding to the variables in the final models were also statistically significant, as indicated by the t-statistic value, which in absolute value exceeded 2.0, the critical region value. This further strengthens the statistical validity of the optimized model.

It is concluded that the mealybug population buildup coincided with increase in temperature, decrease in the humidity and advancement in the berry development. The weather factors coupled with TSS influenced the buildup of mealybug population in the vineyards in both the locations. Two variables, viz. relative humidity and TSS contributed about 96.1-99.7 percent to the variation in population build up of the mealybug.

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# Influence of imidacloprid and thiamethoxam treated stored seeds on honey bee visitation in sunflower

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**ABSTRACT**

The field experiment carried out in factorial randomized block design, during *rabi* 2009-10, with six insecticidal treatments and six storage periods to evaluate eco-friendly approaches for the management of early season sucking pests in sunflower and their impact on honey bee visitation to sunflower field revealed that the honey bee visitation to sunflower field, both in the morning and afternoon hours during peak flowering period, was unaffected by seed treatment with imidacloprid 600 FS @ 10 ml/kg seeds, imidacloprid 70 WS @ 5 g/kg of seeds, thiamethoxam 70 WS @ 5 g/kg of seeds, thiamethoxam 35 FS @ 10 ml/ kg of seeds or spraying with imidacloprid 200 SL (0.25 ml/l) at 22 days after sowing and storage period of six months before sowing. Similarly, the head diameter was also not significantly influenced by the insecticidal treatments and storage periods. The higher seed yield (13.62 q/ha) was recorded from imidacloprid 600 FS @ 10 ml/kg seeds. The superiority of registering higher yield in other treatments was in the descending order i.e. thiamethoxam 70 WS @ 5 g/kg seeds, thiamethoxam 35 FS @ 10 ml/kg seeds and imidacloprid 70 WS @ 5 g/kg seeds with 12.76, 12.50 and 12.37 q/ha, respectively. Similarly, the storage periods significantly influenced the seed yield of sunflower, the highest (17.06 q/ha) being obtained from one month stored seeds.

**Key words:** Seed treatment, Head diameter, Seed yield, Bee visitation

Sunflower cultivars with any degree of self compatibility must have interplant movement of pollen for maximum seed set. It is generally accepted that wind is of little importance in interplant transfer of pollen and bees are the primary pollen movers (Mc Gregor, 1976). Most of the bees visiting sunflower are nectar collectors but a small percentage collect only pollen (Benedek and Manninger, 1972). Poor seed filling is often considered to be a major reason for low yield in sunflower. The sunflower cultivars, especially the open pollinated varieties, show poor seed setting in the central part of the head due to self incompatibility, absence of pollen carriers, insufficient nutrition, moisture stress, lack of growth regulators during seed formation and competition among seeds themselves (Sindagi, 1979). Ganeswara Rao *et al*. (1993) reported that seed yield from bee pollinated plots was significantly higher (1321 kg/ha) than the self pollinated plots (436 kg/ha) while the yields from hand pollinated plot was 1007 kg/ha. Similar observation on beneficial effect of honey bee pollination in sunflower was reported by Singh and Singh (1993). Large scale experiments in Russia indicated that fields supplied with bees produced 79 per cent more seed yield than field without bees (Ponomareva, 1958). Similarly, higher seed equivalent yield of sunflower was obtained by attracting more number of honey bees in the intercropping system of sunflower + niger in 12:3 row proportion as compared to sole sunflower (Gaddanakeri *et al*., 2008). Keeping these points in view, efforts were made to find out eco-friendly approaches for the management of early season sucking pests in sunflower and their impact on honey bee visitation to sunflower field.

## MATERIALS AND METHODS

The field experiment was carried out in factorial randomized block design during *rabi* 2009-10 at U.A.S., Dharwad with six insecticidal treatments and six storage periods, replicated twice. Sunflower hybrid (KBSH-41) seeds were hand dibbled in a plot size of 4.2×3.0 m following a spacing of 60 cm between rows and 30 cm between plants within a row according to the treatment combinations. All the seeds were treated with carboxyin (vitavax) @ 2 g/kg seeds before treating with insecticides. The insecticidal treatments included ($T_1$) imidacloprid 600 FS @ 10 ml/kg seeds, ($T_2$) imidacloprid 70 WS @ 5 g/ kg of seeds, ($T_3$) thiamethoxam 70 WS @ 5 g/ kg of seeds, ($T_4$) thiamethoxam 35 FS @ 10 ml/ kg of seeds, ($T_5$) treated check (imidacloprid 200 SL @ 0.25 ml/l) and ($T_6$) untreated check.

Freshly harvested sunflower seeds (100 g) were taken for each treatment. These were uniformly treated with

**Table 1:** Mean honey bee population during peak flowering period in sunflower as influenced by insecticidal seed treatments and storage periods

(Mean of 10 observations)

| Storage periods→ / Insecticides↓ | Honey bee visitation in sunflower at 10 -11 am (Number of honey bees/ 5 min/plot) | | | | | | | Honey bee visitation in sunflower at 3-4 pm (Number of honey bees/ 5 min/plot) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Months | | | | | | | Months | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | Mean | 1 | 2 | 3 | 4 | 5 | 6 | Mean |
| Imidacloprid 600 FS @ 10 ml/kg seed | 2.80 (1.82) | 2.85 (1.83) | 2.80 (1.82) | 2.80 (1.81) | 2.70 (1.78) | 2.65 (1.77) | **1.81 (2.80)** | 2.85 (1.83) | 2.90 (1.84) | 2.80 (1.81) | 2.90 (1.84) | 2.70 (1.79) | 2.70 (1.79) | **2.80 (1.82)** |
| Imidacloprid 70 WS @ 5 g/kg seed | 2.65 (1.77) | 2.70 (1.79) | 2.70 (1.79) | 2.90 (1.84) | 2.75 (1.80) | 2.85 (1.83) | **2.75 (1.80)** | 2.75 (1.80) | 3.00 (1.87) | 2.75 (1.80) | 2.65 (1.77) | 2.80 (1.81) | 2.85 (1.83) | **2.80 (1.82)** |
| Thiamethoxam 70 WS @ 5 g/kg seed | 2.70 (1.79) | 2.60 (1.76) | 2.90 (1.84) | 2.75 (1.80) | 2.85 (1.83) | 1.84 (2.90) | **2.80 (1.81)** | 2.70 (1.79) | 2.60 (1.76) | 2.80 (1.82) | 3.05 (1.88) | 2.90 (1.84) | 2.70 (1.79) | **2.80 (1.81)** |
| Thiamethoxam 35 FS @ 10 ml/kg seed | 2.65 (1.77) | 2.60 (1.76) | 2.65 (1.77) | 2.80 (1.81) | 2.80 (1.82) | 2.95 (1.86) | **2.75 (1.80)** | 2.65 (1.77) | 2.55 (1.75) | 1.79 (2.70) | 2.70 (1.79) | 2.70 (1.79) | 2.80 (1.81) | **2.70 (1.78)** |
| Treated check (Imidacloprid 200 SL @ 0.25 ml/l) | 2.70 (1.79) | 2.65 (1.77) | 2.70 (1.79) | 2.75 (1.80) | 2.85 (1.83) | 2.70 (1.79) | **2.70 (1.79)** | 2.55 (1.74) | 2.75 (1.80) | 2.70 (1.79) | 2.65 (1.77) | 2.85 (1.83) | 2.75 (1.80) | **2.70 (1.79)** |
| Untreated check | 2.75 (1.80) | 2.70 (1.90) | 2.80 (1.82) | 2.75 (1.80) | 2.80 (1.81) | 3.10 (1.90) | **2.80 (1.82)** | 2.70 (1.79) | 2.70 (1.79) | 2.75 (1.80) | 2.85 (1.83) | 2.80 (1.81) | 2.90 (1.84) | **2.80 (1.81)** |
| **Mean** | **2.70 (1.79)** | **2.75 (1.78)** | **2.75 (1.80)** | **2.80 (1.81)** | **2.80 (1.81)** | **2.85 (1.83)** | **2.80 (1.81)** | **2.70 (1.79)** | **2.75 (1.80)** | **2.75 (1.80)** | **2.80 (1.82)** | **2.80 (1.81)** | **2.80 (1.81)** | **2.80 (1.81)** |

| For comparing means of | 10 - 11 am | | 3 - 4 pm | |
|---|---|---|---|---|
| | S.Em.± | C.D. at 5% | S.Em.± | C.D. at 5% |
| Insecticides (A) | 0.03 | NS | 0.02 | NS |
| Storage Periods (B) | 0.03 | NS | 0.02 | NS |
| Interaction (A x B) | 0.01 | NS | 0.01 | NS |

Figures in parentheses indicate √x+0.5 transformed values

**Table 2.** Effect of seed treatment with different dosages of imidacloprid and thiamethoxam and storage periods on head diameter and seed yield of sunflower

| Storage periods→ / Insecticides↓ | Head diameter (cm) | | | | | | | Seed yield q/ha | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Months | | | | | | | Months | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | Mean | 1 | 2 | 3 | 4 | 5 | 6 | Mean |
| Imidacloprid 600 FS @ 10 ml/kg seed | 21.00 | 19.90 | 18.80 | 18.40 | 20.10 | 20.30 | **19.75** | 19.44 | 15.08 | 13.10 | 11.90 | 11.51 | 10.71 | **13.62** |
| Imidacloprid 70 WS @ 5 g/kg seed | 20.00 | 20.10 | 19.80 | 18.40 | 20.10 | 20.20 | **19.77** | 17.46 | 13.10 | 11.90 | 10.32 | 10.71 | 10.71 | **12.37** |
| Thiamethoxam 70 WS @ 5 g/kg seed | 20.60 | 19.50 | 18.40 | 20.20 | 19.10 | 19.30 | **19.52** | 15.87 | 14.68 | 13.89 | 9.13 | 11.51 | 11.51 | **12.76** |
| Thiamethoxam 35 FS @10 ml/kg seed | 20.10 | 20.20 | 19.10 | 20.10 | 19.50 | 19.40 | **19.73** | 18.65 | 13.89 | 11.51 | 11.51 | 9.92 | 9.52 | **12.50** |
| Treated check Imidacloprid 200 SL @ 0.25 ml/l) | 20.40 | 19.10 | 20.50 | 19.30 | 18.30 | 18.10 | **19.28** | 17.06 | 12.30 | 10.71 | 9.52 | 8.33 | 9.13 | **11.18** |
| Untreated check | 19.70 | 18.70 | 19.80 | 19.60 | 17.80 | 17.20 | **18.80** | 13.89 | 11.51 | 9.13 | 8.33 | 7.94 | 7.94 | **9.79** |
| **Mean** | **20.30** | **19.58** | **19.40** | **19.33** | **19.15** | **19.08** | **19.48** | **17.06** | **13.43** | **11.71** | **10.12** | **9.99** | **9.92** | **12.04** |

| For comparing means of | Head diameter (cm) | | Seed yield q/ha | |
|---|---|---|---|---|
| | S.Em.± | C.D. at 5% | S.Em.± | C.D. at 5% |
| Insecticides (A) | 0.28 | NS | 0.19 | 0.55 |
| Storage Periods (B) | 0.28 | NS | 0.19 | 0.55 |
| Interaction (A x B) | 0.11 | NS | 0.08 | 0.22 |

respective dosage of insecticides at monthly intervals up to six months and were dried under shade for 24 hours to bring to its original moisture content, packed in polythene bags of 700 gauge and stored at ambient condition. The sowing was done on the same day by using the treated seeds stored at monthly interval up to six months. In treated check, imidacloprid 200 SL (0.25 ml/l) was sprayed once at 22 days after sowing. Hexaconozoal 5 EC (1.0 ml /l) was sprayed once for control of alternaria leaf spot at 40 days after sowing. Spinosad 14.5 SC (0.1 ml/l) was sprayed once for control of lepidopteran pests at 58 days after sowing.

Observations on honeybee visitation in sunflower field was recorded by counting the number of honeybees visiting sunflower plots for every five minutes during peak bee activity *i.e.,* 10-11 am and at 3-4 pm for 10 days during peak flowering period and the mean was worked out. Diameter of 10 randomly selected heads per treatment was recorded and average head diameter was worked out. Seed yield per plot was recorded in quintals per hectare at harvest.

## RESULTS AND DISCUSSION

The data on honey bee visitation for 10 days during peak period of flowering stage in the morning and evening peak hours are presented in Table 1. Irrespective of storage periods, the insecticidal treatments did not influence the honey bee visitation during morning hours. All the treatments were on par with each other. The lowest mean of 2.70 honey bees/5 min/plot was recorded in treated check and highest of 2.80 honey bees /5 min/ plot in thiamethoxam 70 WS @ 5g/kg seeds and untreated check. Similarly, the visiting of honey bee to sunflower field with storage periods did not differ significantly. Lower numbers of honey bees were recorded (2.70 honey bees/5 min/plot) in one month stored seeds, followed by 2.75 honey bees /5 min/plot in two and three months stored seeds. The highest honey bee visitation (2.85 honey bees/5min/plot) was observed in six month stored seeds. Interaction effect was non-significant.

No significant difference among the treatments during afternoon peak hours (3-4 pm) was recorded. The lowest honey bee visitation of 2.70 honey bees/5 min/plot was observed in thiamethoxam 35 FS @ 10 ml/kg seeds and in treated check. The highest (2.80) was observed in imidacloprid 600 FS @ 10 ml/kg seeds, imidacloprid 70 WS @ 5 g /kg seeds, thiamethoxam 70 WS @ 5 g/kg seeds and untreated check. Similarly, the visitation of honey bee did not show any significant differences with respect to storage periods. However, the lowest honey bees were recorded (2.70 honey bees/5 min/plot) in one month stored seeds, followed by 2.75 honey bees/5 min/plot in two and three months stored seeds and highest of 2.80 honey bees/5 min/plot in four, five and six months stored seeds. Interaction effect was non-significant. Thus, the honey bee visitation to sunflower field was unaffected by seeds treated with insecticides and storage period of six months before sowing.

It is evident from the Table 2 that the head diameter was not significantly influenced by the insecticidal treatments. Among the treatments, higher head diameter was noticed in imidacloprid 70 WS @ 5 g/kg seeds (19.77 cm) followed by imidacloprid 600 FS @ 10 ml/kg seeds (19.75 cm) and lowest in untreated check (18.80 cm). The head diameter of the sunflower with different storage periods did not differ significantly.

All the treatments proved their superiority over the untreated check with regard to seed yield. Higher seed yield (13.62 q/ha) was recorded from imidacloprid 600 FS @ 10 ml/kg seeds. The superiority of registering the higher yield in other treatments in the descending order was thiamethoxam 70 WS @ 5 g/kg seeds, thiamethoxam 35 FS @ 10 ml/kg seeds and imidacloprid 70 WS @ 5 g/kg seeds with 12.76, 12.50 and 12.37 q/ha, respectively. Similarly, storage periods significantly influenced the seed yield of sunflower. Highest seed yield (17.06 q/ha) was obtained from one month stored seeds (Table 2). Sreelatha and Diwakar (1997) also reported that seeds treated with imidacloprid gave increased yield over control. Faqir and Gul (1998) also reported that the yield was highest in imidacloprid treated plot. This may be due to phytotonic effect of imidacloprid and less incidence of insect pests. Patil *et al.* (2002) reported imidacloprid as highly effective against jassids and gave highest yield of dry chilli. Kale *et al.* (2005) reported that seed treatment with thiamethoxam @ 5 g a.i./kg followed by alphamethrin @ 0.05 per cent spray was effective in obtaining higher yield in okra.

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# Search for alternatives to Sugar Syrup for off season apiculture

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**ABSTRACT**

In an effort to develop an efficient and economical alternative of sugar supplement for off-season feeding of honey bees (*Apis mellifera*) colonies, syrups prepared with four different sources viz., wheat bran, rice bran, maize and sugarcane juice were evaluated against the control (Sugar solution), and their impact on desirable attributes of bee colonies determined. The results indicated that none, other than the sugar syrup, proved effective in improving the honey bee colonies. However, feeding bees with these syrups reduced the cost of feeding.

**Key words** Apis mellifera, bran, maize, honey bee, sugar, sugar cane

Pollen, nectar and water are the usual nutritional requirements of bees. These are collected by field bees as per needs of its colony and their availability in field. However, during off floral season, sugar supplement forms the main food supplement necessary for the maintenance of their population. Feeding bees with sugar syrup during off season increase the number of bees and frames covered by bees, brood area and colony weight (Sahinler *et al.* 2003) However, the increasing price of sugar every year made the bee keepers less thoughtful for proper off season feeding of honey bees. Honeybees can utilize the complex carbohydrate (Harssnigg *et al.* 2003). Buckwheat powder, sweet pumpkin, turnip; malus fruits are reported to be used to feed bees during off-season in the higher hill of Nepal (Upadhyay, 2003). Therefore, feeding bees with fruits or vegetables or cereals rich in carbohydrates, proteins, minerals and fats can be the best alternative to replace expensive cane sugar. However, feeding bees with pollen substitute and sugar syrup increase the number of bees and frames covered by bees, brood area and colony weight than by feeding only the sugar syrup or pollen (Sahinler et al., 2003) Bees need more than just carbohydrates to survive besides they cannot live on protein alone. Keeping this in view, the present study was conducted. At Haldwani (Distt. Nainital) Uttarakhand, India.

## MATERIALS AND METHODS

The study was conducted during rainy season (June to October) in 2007 and 2008 at Haldwani (Distt. Nainital, Uttarakhand, India). Five treatments namely syrups made from wheat (*Triticum aestivum*) bran, Rice (*Oryza sativa*) bran, maize (*Zea mays*) and sugarcane (*Saccharum officinarum*) juice and the control with sugar syrup supplement were evaluated in Randomized Block Design in three replications.

Sugar syrup was prepared by dissolving crystal sugar in fresh water (i.e. 1:1 v/v ratio). The other syrups were prepared by adding 200 gram of maize (powder)/rice bran/ wheat bran or 200 ml of sugarcane juice to sufficient amount of fresh water. The mixture was blended properly with the help of wooden stirrer. To it, 100g of crystal sugar and 20g of honey was added and strained though a single layer of muslin cloth and the volume was made up to one liter by adding water. The syrup, kept in washed glass bottles was refrigerated (~ $8^0$C) until used. Syrups (@ 30 ml/frame) were placed inside the bee hive provided with a dry leaf twigs to prevent the bees being drawn in the syrup at an interval of 5 days.

Suitability of syrups was adjudged on the basis of quantity of syrup consumed in each colony by using the formula used by (Kencharaddi *et al*. 2003).

$$\text{Feeding preference} = \frac{\text{Initial volume of syrup} - \text{volume of left over syrup}}{\text{Initial volume of syrup}} \times 100$$

(Per cent)

Data on brood area ($cm^2$), honey and the pollen stored ($cm^2$) ( by measuring the total area covered by the brood, (sealed and unsealed), honey and pollen capped and uncapped using wire grid device (5 cm x 5 cm) and the

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**Table 1 :** Cost of syrups (prepared by different sources) and per cent reduction in cost

| Treatment | Required amount of raw material (g) for 1 lit. of syrups | | Cost of raw material (Rs./Kg) | Cost of raw material used in 1 liter of nectar substitute | Total Cost | Per cent reduction |
|---|---|---|---|---|---|---|
| | Material Required | Quantity in (g) | | | | |
| **Wheat bran** | Powder | 200 | Rs. 7 | Rs. 1.4 | Rs. 6.1 | 54.81 |
| | Sugar | 100 | Rs. 27 | Rs. 2.7 | | |
| | Honey | 20 | Rs. 100 | Rs. 2.0 | | |
| **Rice bran** | Powder | 200 | Rs. 40 | Rs. 8.0 | Rs. 12.7 | 5.93 |
| | Sugar | 100 | Rs. 27 | Rs. 2.7 | | |
| | Honey | 20 | Rs. 100 | Rs. 2.0 | | |
| **Maize** | Powder | 200 | Rs. 24 | Rs. 4.8 | Rs. 9.5 | 29.63 |
| | Sugar | 100 | Rs. 27 | Rs. 2.7 | | |
| | Honey | 20 | Rs. 100 | Rs. 2.0 | | |
| **Sugar cane** | Juice | 200[a] | Rs. 25[b] | Rs. 5.0 | Rs. 9.7 | 28.15 |
| | Sugar | 100 | Rs. 27 | Rs. 2.7 | | |
| | Honey | 20 | Rs. 100 | Rs. 2.0 | | |
| **Sugar (control)** | Sugar | 500 | Rs. 27 | Rs. 13.5 | Rs. 13.5 | 0 |

[a]Quantity in ml, [b]Cost of 1 liter sugarcane juice

activity of forager honeybees (estimated before and at 15 days interval by counting the number of workers going out from he entrance of the hive for a minute, after every two hours of interval from 10.00 AM to 4.00 PM were recorded (Srivastava *et al*. 2004).

Besides, the data on shelf life of the syrups, recorded at room and refrigeration temperature as also as the economics also formed the basis of syrup suitability.

Randomized Block Design (RBD) was used to compute the variance. After the determination of significance of difference between the treatments means at 0.05% probability, critical difference was calculated in order to compare the treatment means (Snedecor and Cochran, 1968).

## RESULTS AND DISCUSSIONS

### Feeding Preference

Utilization of syrups was significantly different with each other (Fig. 1A). Honey bees preferred sugar solution the most with maximum utilization (99.6%), followed by, syrups with sugar cane juice (69.9%), rice bran (46.6%), Wheat bran (41.9%) and maize (35.2%) as indicated by left over amount viz., 41 ml, 3900 ml, 6951 ml, 7528 ml and 8397 ml of syrups, respectively in 2007. Similar trend was also observed in 2008 (Fig. 1B).

### Effect of syrups feeding treatments on brood area

In 2007, highest number of brood area was observed on Sugar solution (435.667 sq cm) after 15 Days after treatment (DAT), followed by maize syrup (414.67 sq cm) and the lowest with wheat bran syrup (413. 333 sq cm) and sugar cane juice (418 . 667 sq cm). At the end of the off-season the sugar syrup also recorded the maximum of brood area in colonies (680.000 sq cm), followed by with sugar cane juice (428.000 sq cm), while the minimum was in maize (410.00 sq cm), wheat bran (418.667) and rice bran (421.000 sq cm) (Fig 2A) in 2007. Similar trend was observed during 2008 also (Fig 2B).

### Effect of syrup feeding treatments on honey store

During the year 2007, significant increase in honey store was observed in sugar soln. (799.000 sq cm), followed by sugar cane (436. 333 sq cm), rice bran (421.000 sq cm) and wheat bran (406. 333 sq cm). A sharp reduction was observed in with maize syrup (390.667 sq cm). (Fig. 3A). Similar trend was found during 2008 also (Fig. 3B).

### Effect of syrups feeding treatments on pollen store

In case of pollen stored the trend was found similar to brood area. The data (Fig 4) revealed that at the end of the experiment, pollen stored in the sugar syrup fed colonies was significantly maximum (298.667 sq cm) followed with

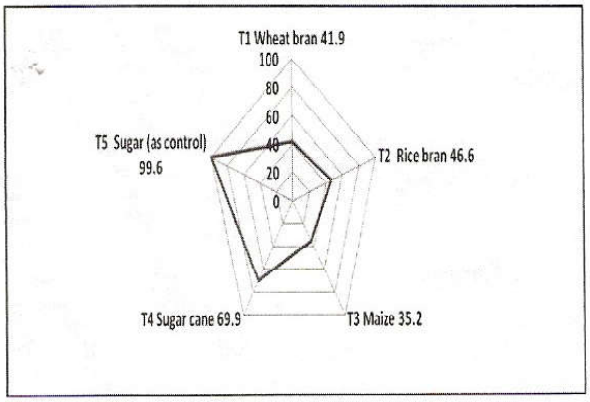


Fig 1a

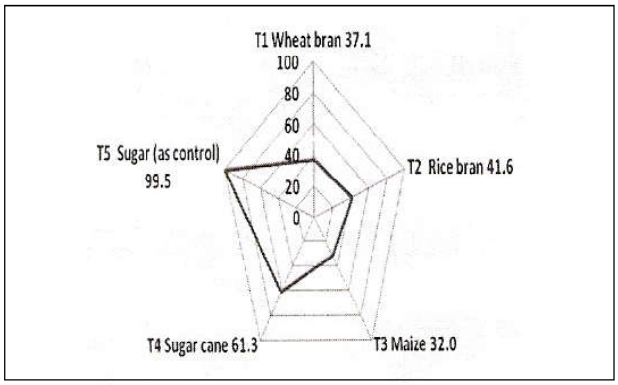


Fig 1b

**Fig 1 :** Feeding preference of different syrups (Dearth period, 2007 and 2008)

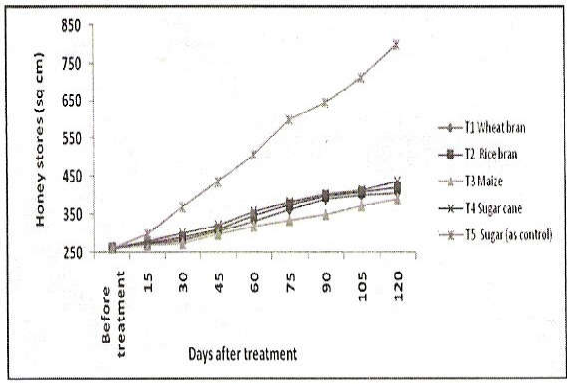


Fig 2a

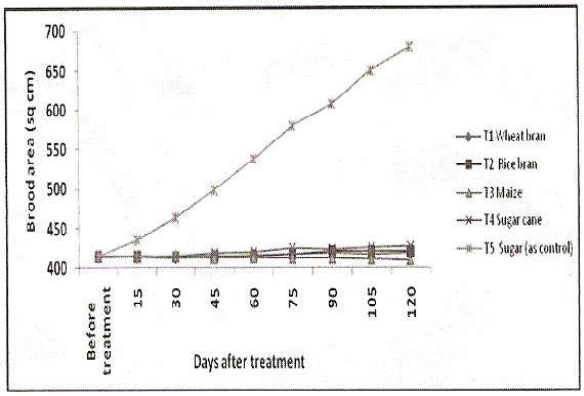


Fig 2b

**Fig 2 :** Brood area in different syrups fed colonies (Dearth period, 2007 and 2008)

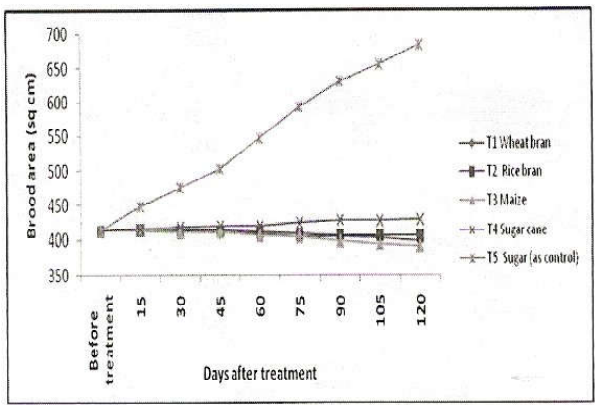


Fig 3a

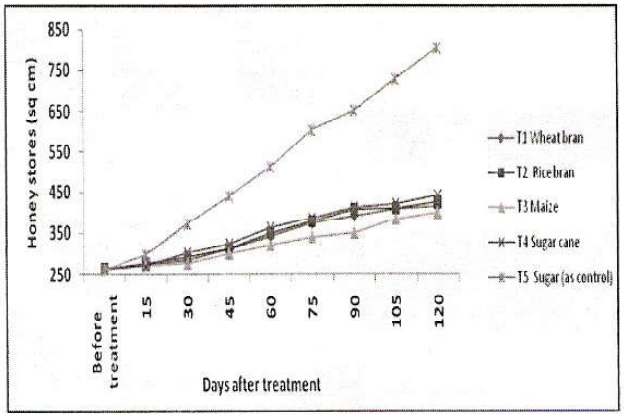


Fig 3b

**Fig 3 :** Honey stores in different syrups fed colonies (Dearth period, 2007 and 2008)

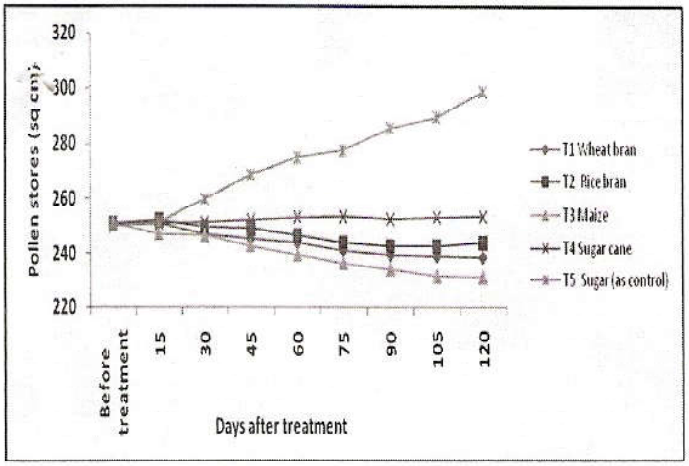


Fig 4a

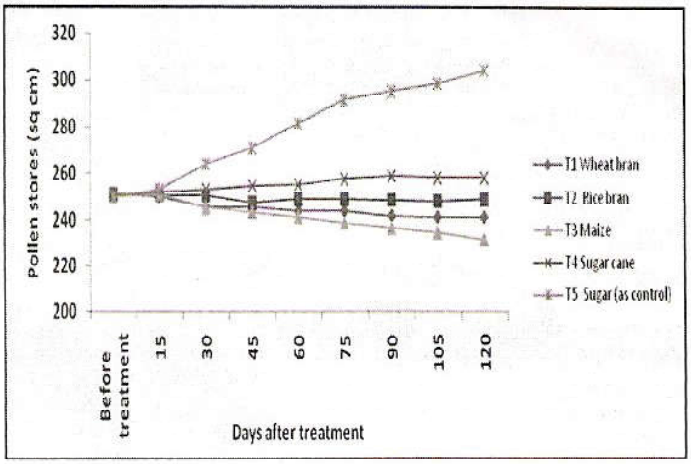


Fig 4b

**Fig 4 :** Pollen stores in different syrups fed colonies (dearth period, 2007 and 2008)

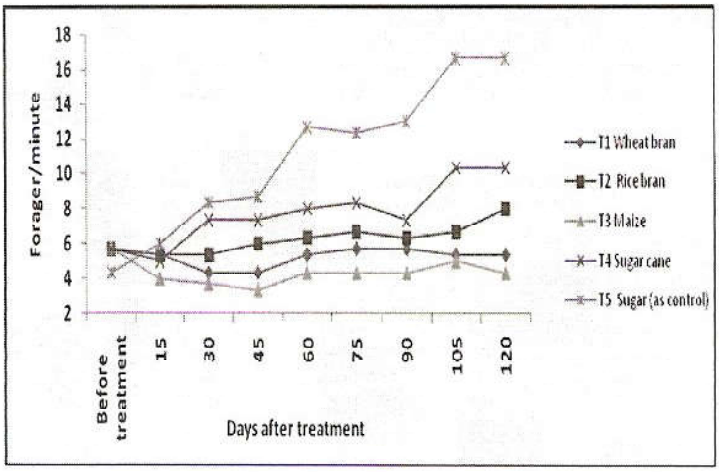


Fig 5a

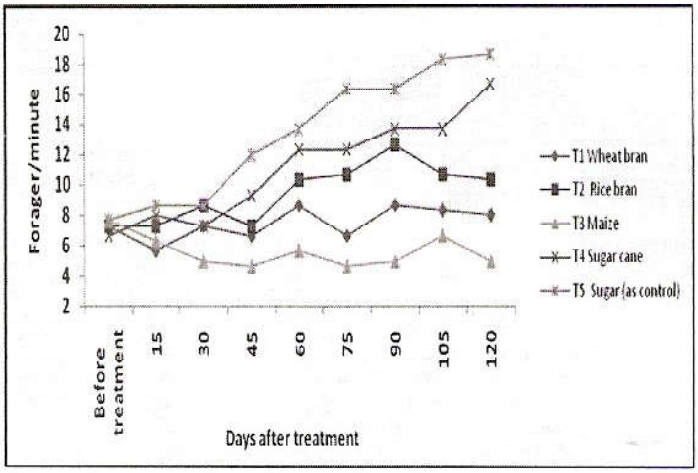


Fig 5b

**Fig 5 :** Forager/minute in different syrups fed colonies (dearth period, 2007 and 2008)

sugar cane (253.667 sq cm), rice bran (244.000 sq cm), wheat bran (238.667 sq cm) and maize (231.000 sq cm) (Fig. 4A). Similar trend was observed during the year 2008 (Fig. 4B) also .

*Effect of syrup feeding treatments on foraging activity*

During 2007, the forager/minute was highest with sugar syrup (16.667) followed by sugar cane juice (10.333), rice bran (8.000) and wheat bran (5.333). Least was found with maize syrup (4.333) (Fig. 5A.). Similar pattern was observed during the year 2008 (Fig. 5B).

**Cost of syrups and per cent reduction in cost.**

Cost of a liter of syrup (prepared from different sources) (Table.1) varied significantly. The highest was recorded for sugar syrup solution. (Rs. 13.5) followed by with rice bran (Rs. 12.7), sugar cane juice (Rs. 9.70), maize (Rs. 9.5) and wheat bran syrup (Rs. 6.1). It was noticed that the cost of sugar feeding during off-season can be reduced by 54.81 per cent by feeding bees with wheat bran syrup, 29.63 by maize syrup, 28.15 per cent cane juice syrup and 5.93 per cent by rice bran syrup.

Three days old syrups stored at room temperature were

not preferred by the honey bees. However, under refrigeration conditions ($8^0$C to $10^0$C), the shelf life and bee acceptance extended up to 5 days.

Reduced feeding preference exhibited by honey bees exposed to syrups other than the control (sugar solution) may be because of their consistency. The least preference towards maize syrup may be attributed to its high starch contents which can be toxic to bees (Somerville, 2005a). It is also reported that inverted syrup produced from wheat starch was detrimental to bees since it contained undigested polysaccharides, especially starch (Doull, 1974). Neupane and Thapa (2005) reported decrease number of brood cells on honeybees colonies fed with maize and rice bran syrup.

Increased foraging activity observed with different syrups treatment was as per the findings of Somerville (2005b) and Thapa and Pokhrel (2005). Highest foraging activity in sugar syrup may be due to increment of broods in the hive as reported by Vergheese and Prasad (1980). Although the cost of feeding syrups with wheat bran, rice barns, maize and sugarcane juice reduced from 5 per cent to more than 50 per cent and was opted as an alternative supplement for honey bees (Neupane and Thapa 2005). Its use to maintain bee colony during the off season proved unproductive. Record of three days shelf life at room temperature might be due to fermentation of the syrups by natural microbial flora and lower metabolic activity of microbes. Somerville (2005c) reported similar result on shelf life of syrups.

The study concludes that, sugar syrup maintains its superiority over other tested syrups during off-season apiculture.

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# Spinosad 45% SC - Natural insecticide for the management of thrips, *Scirtothrips dorsalis* in grape vineyards

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**ABSTRACT**

Spinosad 45% SC @ 25 ml/ 100 l water was found to be most effective dose in reducing thrips population as well as in obtaining the fruit yield but it was on par with higher dose of 30 ml / 100 l water. However both the doses were superior over its lower dose of 20 ml/ 100 l water. All the spinosad 45% SC doses were superior over standard checks, dimethoate, oxydemeton methyl and endosulfan at recommended doses both in reducing thrips population and in obtaining the fruit yield. None of the tested doses of Spinosad 45% SC found phytotoxic. Natural enemies activity was noticed in all the spinosad 45% SC treated plots along with untreated plots and it revealed that it did not adversely affect the natural enemy population at recommended doses. Spinosad 45% SC was found to be compatible with the commonly used pesticides in vineyards.

**Key Words:** Bio-efficacy, Compatibility , Grape, Phyto-toxicity, Spinosad 45% SC, Thrips

Grape is attacked by number of insect pests. Among these, the thrips, that cause considerable damage, are very important. Both adult and nymphs cause damage by rasping the lower surface of the leaves, blossoms and developing berries. In case of heavy incidence, the leaves may dry up and drop off the vine. When the attacked leaves begin to dry up, adults as well as nymphs migrate to healthy leaves. They are also considered to be responsible for the scab formation on the berries (Reddy, 1957 and FIP, Hydrabad,1982) The affected berries develop a corky layer and become brown. In the present experiment, a new generation insecticide Spinosad 45% SC (Tracer 45% SC) has been tested on thrips in grape.

Spinosad is a natural insecticide derived from an actinomycete bacterium species, *Saccharopolyspora spinosa*, that displays the efficacy of a synthetic insecticide. It consists of the two most active metabolites, designated spinosyn A and D. Both spinosyns are readily degraded in moist aerobic soil, and field dissipation, which is quite rapid (half-life, 0.3-0.5 d) can be attributed to photolysis or a combination of metabolism and photolysis. Spinosad causes neurological effects in insects that are consistent with the general activation of nicotinic acetylcholine receptors but by a mechanism that is novel among known insecticide compounds. Spinosad has a high level of efficacy for lepidopteran larvae, as well as some Diptera, Coleoptera, Thysanoptera, and Hymenoptera, but has limited to no activity to other insects and exhibits low toxicity to mammals and other wildlife. (Mayes, *et.al*., 2003)

## MATERIAL AND METHODS

The experiment was conducted in two consecutive fruiting seasons during October – April in 2005-06 and 2006-07 on Tas-A-Ganesh variety trained to 'Y' system at National Research Centre for Grapes, Pune. Experiment was laid out in a Randomised Block Design (RBD) with eight treatments replicated four times containing four vines each. The block was pruned during first fortnight of October in both the years.

### Treatments

T-1 : foliar sprays of spinosad 45% SC @ 20 ml/100 l; T-2 : foliar sprays of spinosad 45% SC @ 25 ml/100 l; T-3 : foliar sprays of spinosad 45% SC @ 30 ml/100 l; T-4 : foliar sprays of dimethoate @ 1ml/l; T-5 : foliar sprays of oxydemeton methyl @ 1ml/l; T-6: foliar sprays of endosulfan-2ml/l and T-7 : untreated check (water spray)

### Bio-efficacy in control of sucking pests

Bio-efficacy of spinosad 45% SC on thrips was studied during the experiment. Pre-count of thrips was taken before the start of an experiment and reduction in population was observed after 3,7 and 14 days after spray. For the management of thrips two sprays were given at the interval of 30 days. First spray was given on full bloom stage and second spray was given on berry setting stage . Yield was recorded at the time of harvest as Kg/vine.

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**Table 1:** Bio-efficacy of different Spinosad 45% SC doses for the management of Thrips (Pooled analysis of 2005-06 and 2006-07).

| Tr. No. | Pre count of thrips/ shoot/vine | Thrips/shoot/vine after I Spray | | | Thrips/shoot/vine after II Spray | | | Yield (Q/ha) |
|---|---|---|---|---|---|---|---|---|
| | | 3 DAS* | 7 DAS | 14 DAS | 3 DAS | 7 DAS | 14 DAS | |
| 1 | 8.90 | 6.53 | 6.20 | 6.00 | 5.87 | 4.20 | 2.33 | |
| | (3.14)** | (2.74) | (2.68) | (2.64) | (2.62) | (2.28) | (1.82) | 196.02 |
| 2 | 9.03 | 6.07 | 5.13 | 4.87 | 4.07 | 3.60 | 1.87 | |
| | (3.17) | (2.65) | (2.48) | (2.48) | (2.25) | (2.14) | (1.69) | 208.12 |
| 3 | 8.83 | 5.93 | 5.00 | 4.80 | 3.87 | 3.33 | 1.60 | |
| | (3.13) | (2.63) | (2.45) | (2.46) | (2.21) | (2.08) | (1.61) | 214.38 |
| 4 | 9.03 | 6.93 | 7.07 | 6.87 | 6.87 | 5.20 | 3.87 | |
| | (3.17) | (2.82) | (2.84) | (2.80) | (2.80) | (2.54) | (2.21) | 165.77 |
| 5 | 9.00 | 7.33 | 7.33 | 7.00 | 9.33 | 5.33 | 3.60 | |
| | (3.16) | (2.84) | (2.89) | (2.83) | (2.82) | (2.57) | (2.14) | 162.14 |
| 6 | 9.13 | 7.80 | 8.07 | 8.00 | 7.93 | 6.33 | 4.47 | |
| | (3.18) | (2.97) | (3.01) | (3.00) | (2.99) | (2.71) | (2.34) | 158.51 |
| 7 | 8.93 | 8.87 | 9.20 | 9.53 | 8.87 | 7.93 | 6.00 | |
| | (3.15) | (3.14) | (3.19) | (3.25) | (3.14) | (2.99) | (2.64) | 130.68 |
| SEm | 0.03 | 0.03 | 0.02 | 0.04 | 0.01 | 0.02 | 0.03 | 2.18 |
| CD at 5% | NS | 0.08 | 0.05 | 0.14 | 0.04 | 0.07 | 0.08 | 6.71 |

*=DAS-Days After spraying
**=Figures in the parantheses are X+1 transformed values.

**Phytotoxicity observations:**

Spinosad 45% SC treated plants were critically observed for presence of phytotoxic effects such as chlorosis, tip burning, necrosis on leaves and berries, epinasty, and russeting on berries during the observation period.

**Effect of spinosad 45% SC on natural enemies under field conditions**

Natural enemies like predatory beetles, *Cryptolaemus montrouzieri* activity was observed during the experimental period.

**Compatibility of spinosad 45% SC with commonly used pesticides in grapes.**

Compatibility of commonly used pesticides was done by testing bio-efficacy of spinosad 45% SC with commonly used pesticides under field conditions.

The treatment details are as below.

T-1: foliar sprays of spinosad 45% SC @ 25 ml/100 l + dimethoate 1 ml/l; T-2-foliar sprays of spinosad 45% SC @ 25 ml/100 l +mancozeb 2.5 g/l; T-3 foliar sprays of spinosad 45% SC @ 25 ml/100 l + 2% Urea; T-4 : foliar sprays of spinosad 45% SC @ 25 ml/100 l and T-5 : untreated check (water spray)

**RESULTS AND DISCUSSION**

**Bio-efficacy for the management of thrips**

Pre-count of thrips was taken before the start of the experiment and it was found that population was distributed uniformly in all the treatments. There was significant difference in treatments after 3,7 and 14 days of spraying. Reduction in population of thrips and yield (q/ha) are given in Table 1.

Reduction in thrips population was evident even after 3 days of spraying. However, there was a conspicuous reduction after 7 days after treatment and it was continued up to 14 days after spray. Similar trend was reflected in 2nd rounds of spray. Among two doses of spinosad 45% SC foliar spray, 25 ml/100 l water dose was significantly superior to lower dose of 20 ml/100 l but at par with higher dose of 30 ml/100 l and all three the doses were over superior to standard chemicals dimethoate @ 1ml/l and oxydemeton methyl @ 1ml/l, endosulfan @ 2 ml/l and untreated check. Highest yield of 214.38 q/ha was recorded in spinosad 45% SC @ 30 ml/ 100 l water treated plots and it was on par with spinosad 45% SC @ 25 ml/ 100 l water (208.12 Q/ha) but both the doses were significantly superior over lower dose of 20 ml/100 l water (196.02 Q/ha). All the doses of spinosad 45% SC were superior over standard insecticide checks as well as untreated check in obtaining the fruit yield. Bio-efficacy of spinosad on thrips was reported by several

**Table 2:** Compatibility of Spinosad 45% SC for the management of Thrips.

| Tr. No. | Pre count of thrips/ shoot/vine | Thrips/shoot/vine after I Spray | | | Thrips/shoot/vine after II Spray | | |
|---|---|---|---|---|---|---|---|
| | | 3 DAS | 7DAS | 14 DAS | 3 DAS | 7 DAS | 14 DAS |
| 1 | 8.97 | 6.50 | 5.10 | 4.30 | 3.95 | 2.20 | 1.00 |
| | (3.15) | (2.74) | (2.47) | (2.30) | (2.22) | (1.79) | (1.41) |
| 2 | 8.65 | 6.10 | 4.90 | 4.35 | 3.90 | 2.30 | 0.95 |
| | (3.11) | (2.66) | (2.43) | (2.31) | (2.21) | (1.81) | (1.39) |
| 3 | 8.97 | 6.25 | 5.15 | 4.60 | 4.05 | 2.40 | 1.10 |
| | (3.16) | (2.71) | (2.48) | (2.36) | (2.25) | (1.84) | (1.44) |
| 4 | 8.70 | 6.25 | 5.05 | 4.50 | 3.95 | 2.10 | 0.85 |
| | (3.11) | (2.69) | (2.46) | (2.34) | (2.22) | (1.76) | (1.36) |
| 5 | 8.90 | 8.70 | 8.95 | 8.75 | 8.65 | 6.85 | 4.35 |
| | (3.14) | (3.11) | (3.15) | (3.12) | (3.11) | (2.80) | (2.31) |
| SEm | 0.02 | 0.02 | 0.01 | 0.03 | 0.02 | 0.03 | 0.02 |
| CD at 5% | NS | 0.07 | 0.05 | 0.09 | 0.05 | 0.08 | 0.07 |

* DAS-Days After spraying

workers in the past by Dutton *et. al* (2003), Caputo *et. al.* (2005), Warnock and Cloyd (2005).

**Phytotoxicity observations:**

None of the treatments tried in the experiment showed any type of phytotoxic effect either on leaves or berries during the period of observation.

**Effect of spinosad 45% SC on natural enemies under field conditions**

Among different natural enemies, Coccinellid predator or ladybird as well *Chrysoperla carnea* population was noticed in all the spinosad 45% SC treated plots along with untreated plots and population ranged between 1-2 grubs or adults per vine. Safety of spinosad to natural enemies was reported by Williams *et.al.* (2003) and Medina *et.al* .(2003)

**Compatibility of spinosad 45% SC for the management of thrips**

Compatibility of commonly used pesticides was done by testing bioefficacy of spinosad 45% SC with commonly used pesticides under field conditions. The results are presented in Table 2.

Compatibility studies indicated that none of the pesticides tested adversely affected the bioefficacy of spinosad 45% SC during the experiment period and the same trend was reflected in obtaining the fruit yield. None of the mixture treatments in the compatibility evaluation trial showed any phyto-toxicity to grape vines. No sedimentation, separation and flocculation was observed on mixing of spinosad 45% SC with above mentioned fungicides, insecticides and urea.

It has been concluded that spinosad 45% SC @ 25 ml/ 100 l water was found to be most effective dose in reducing the sucking pests thrips as well as in obtaining the fruit yield but it was on par with higher dose of 30 ml / 100 l water. However, both the doses were superior over its lower dose of 20 ml/ 100 l water. All the spinosad 45% SC doses were superior over standard checks dimethoate , oxydemeton methyl and endosulfan at recommended doses both in reducing thrips population and in obtaining the fruit yield. None of the tested doses of spinosad 45% SC was found phytotoxic. Natural enemies activity was noticed in all the spinosad 45% SC treated plots along with untreated plots and it revealed that it did not adversely affect the natural enemy population at recommended doses. Spinosad 45% SC was found to be compatible with the commonly used pesticides in vineyards.

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# Response of *Trichoderma harzianum* in direct seeded rice under medium low land rainfed conditions

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**ABSTRACT**

A field experiment carried out during *kharif* 2010 at research farm of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, to assess response of *Trichoderma harzianum* in direct seeded rice under medium low land rainfed conditions, revealed that *T. harzianum* treated seed recorded significantly improved seed germination, plant height, panicle length, total number of tillers per plant, number of grains per panicle, test weight and grain yield per plant, while it significantly reduced the days to 50 per cent flowering, days to maturity and disease intensity as compared to their control. *Trichoderma* treated seeds of variety Sahbhagi Dhan (96.67%) showed maximum seed germination percentage followed by IR-64sub1 (95.0%) compared to untreated control 71.67% and 41.67%, respectively. It increased 25 to 53 % seed germination percentage while reduced days to 50 per cent flowering and maturity by 5 to 7 days. *T. harzianum* not only improved the grain yield and its contributing traits but also reduced the intensity of blast disease (*Magnaporthe grisea*). The *Trichoderma* treated seeds showed 23.30% to 30.55% disease intensity compared to untreated seed 40.50% to 48.09%, hence, it reduced approximately 10-25% disease intensity, suggesting that *T. harzianum* spp. may be used as bio-inoculants for controlling blast disease of rice besides increasing the grain yield.

**Keywords:** Biological control, blast, rice, *Trichoderma harzianum*

*Trichoderma* spp. is free living fungi that are highly interactive in root, soil and foliar environment. It has long been recognised as biological agent to control plant diseases and widely studied as potential biological agent for controlling many plant pathogens (Harman, 2011). Since it's first application in 1930s, *Trichoderma* spp. became popular biological agent to protect crops against plant pathogens all over the world. Several species of *Trichoderma* has been used for many years as antagonists in the biological control of fungal diseases (Elad *et al*., 1998, *Singh et al*., 2009, Singh, 2011 and Vinale *et al*., 2008). Probably the first successful use of a *Trichoderma* product to control fungal diseases of rice was demonstrated by researchers at the National Institute of Plant Protection in 1995. The use of a *Trichoderma* product has both, short term effects i.e., immediate control of diseases with growth enhancement of crops as well as long term effects, which are demonstrated by the decrease in fungal pathogen inoculums in the field (Harman, 2011). Presently, use of *Trichoderma harzianum* based products are considered as relatively novel biological control agents which can help farmers to reduced plant diseases and increase plant growth (Singh *et al*., 2009). The use of *Trichoderma* based products is not only safe for the farmers and consumers but it is also good for the environment (Lorito, 2010). The effective biological disease control depends not only on suitable bio-control organisms but also on methods and strategies for introducing and maintaining population levels and their activities. Regardless of the activity of the biocontrol agents, one of the popular methods of introducing bio-inoculants is seed treatment.

Seed, being an essential component of agriculture acts as a passive carrier of many diseases. Seed treatment with beneficial microorganisms is becoming increasingly important. Seed priming with biological agents along may serve as an important tool of managing many soil and seed borne diseases. It provides an alternative to the use of chemical pesticides. Successful application of selected microorganisms to seed in a commercially viable way is only the first step towards using beneficial microorganisms to improve crop health. It is equally important that the microorganisms remains viable and may colonise the developing roots and rhizosphere in order to continue improving plant growth and to potentially control disease. Seed-applied microorganisms have the potential to become established in the rhizosphere of plants, as they may transfer onto the developing roots as it emerges from the seed (Harman, 1991). *Trichoderma* spp. is well documented as effective biological control agent of plant diseases caused by soil borne fungi (Whipps and Lumsden, 2001; Pan and Bhagat, 2007).

## MATERIALS AND METHODS

The experiment consisted of eight rice germplasms viz. IR-64 sub-1, IR-64, HUBR-38B, Sahbhagi Dhan, MTU-7029, BD-2010, Swarna sub-1 and HUR-105. All these germplasms were direct seeded as treated and untreated in Randomised Block Designs with three replications each at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Seeds were surfaced sterilized with 0.1% sodium hypochlorite solution for 2 minutes then washed thoroughly with sterile water. After surface sterilization, the seeds were treated with dried powder spore mass of *Trichoderma harzianum* strain NBRI 1055 (cfu $2x10^6$ spores/g) @ 4 g/kg and mixed thoroughly to ensure uniform coating. The coated seeds were kept in refrigerator for 24 h and were sown in the field @ 150 seeds per plot the next day. Each plot consisted of 5 rows of 3 m length with spacing 15 cm X 10 cm. All the recommended package of practices was followed to raise a good crop. Observations on germination percentage, days to 50 per cent flowering, days to maturity, plant height, panicle length, number of tillers, grains per panicle, test weight, grain yield per plant and disease incidence were recorded on ten randomly selected plants from each entry for both treated and untreated plots in each replication, and their means were used for statistical analysis.

**Germination percentage:** This was calculated by counting the number of seeds sprout in each plot and total number of seeds sown in each plot.

Germination % = (number of seeds sprout per plot/ total number of seeds sown per plot) X 100

**Disease assessment:** For the assessment of rice blast, ten plants were selected randomly from both treated and untreated plots in each replication. Five leaves from top of each culm were taken for observation. The disease area was calculated according to the rating scale of 0-9 (Table 1) developed by International Rice Research Institute (IRRI, 1996) which was converted into per cent disease intensity by using the following formula.

Disease intensity (%) = (Area of disease score/ 9) X 100

Disease scoring area (%) = (Area of leaf affected / Total leaf area) X 100

**Table 1:** Scale for rating of blast disease (IRRI 1996)

| Scale | Description |
|---|---|
| 0 | No lesion observed |
| 1 | Small brown specks of pin point size |
| 2 | Larger brown specks |
| 3 | Small roundish to slightly elongated, necrotic gray spots, about1-2 mm in diameter, with a distinct brown margin |
| 4 | Typical blast lesions elliptical, 1-2 cm, usually confined to the area of the 2 main veins infecting <2% of the leaf area |
| 5 | Typical blast lesions, infecting <10% of the leaf area |
| 6 | Typical blast lesions, infecting 10-25% of the leaf area |
| 7 | Typical blast lesions, infecting 26-50% of the leaf area |
| 8 | Typical blast lesions, infecting 51-75% of the leaf area |
| 9 | All leaves dead |

## RESULTS AND DISCUSSION

The result revealed that *T. harzianum* showed significant and positive responses on seed germination, plant height, panicle length, total number of tillers per plant, number of grains per panicle, test weight and grain yield, while negative responses on days to 50 per cent flowering and days to maturity as compared to untreated control (Table 2). Similar result was reported by Mathivanan *et al.,* (2006). *Trichoderma* treated seed of rice germplasms showed range of mean performance of seed germination from 86.67 to 96.67 per cent, days to 50 per cent flowering from 90.33 to 112.66, days to maturity from 120.33 to 143.17, plant height from 52.73 to 85.76 cm, panicle length from 22.61 to 28.20 cm, total number of tillers per plant from 7.93 to 10.80, number of grains per panicle from 107.33 to 224.60, test weight from 20.20 to 26.43 g and grain yield from 26.52 to 41.57 g per plant while untreated control showed range of mean performance of seed germination from 41.67 to 71.67 per cent, days to 50 per cent flowering from 95.83 to 116.16, days to maturity from 125.83 to 149.33, plant height from 46.14 to 79.97 cm, panicle length from 20.25 to 25.80 cm, total number of tillers per plant from 7.53 to 10.50, number of grains per panicle from 100.27 to 213.07, test weight from 20.09 to 26.33 g and grain yield from 26.41 to 41.49 g per plant. *T. harzianum* enhanced seed germination percentage of all rice germplasms, highest for Sahbhagi (96.67%) followed by IR-64 sub-1 (95.0%) and lowest for MTU-7029 (86.67%) compared to untreated control Sahbhagi (71.67%), IR-64 sub-1 (41.67%) and MTU-7029 (58.33%), respectively. It increases approximately 25-53% seed germination of rice germplasm. These finding were in agreement with the earlier reports of Mishra *et al.,* (2000) and Ashraf *et al.,* (2005). *Trichoderma* treated seeds showed 5 to 7 days early flowering and maturity, increased plant height 3 to 17 cm, panicle length 1.4 to 2.8 cm, total number of tillers per plant 0.14 to 1.07, number of grains per panicle 5 to 17,

**Table 2:** Effect of *Trichoderma harzianum* on yield attributing traits of eight rice germplasms under medium low land rainfed conditions

| Germ-plasms | | Germi-nation (%) | Days to 50 per cent flowering | Days to maturity | Plant height (cm) | Panicle length (cm) | Number of tillers per plant | Number of grains per panicle | Test weight (g) | Grain yield per plant (g) | Disease Intensity (%) | Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IR-64 | Control | 41.67 | 97.50 | 127.50 | 64.98 | 21.65 | 9.47 | 107.0 | 26.23 | 38.333 | 48.09 | 7 |
| sub-1 | **Treated** | **95.00** | **91.66** | **121.50** | **67.91** | **23.10** | **10.20** | **116.80** | **26.43** | **38.48** | **23.30** | **6** |
| IR-64 | Control | 66.67 | 98.83 | 128.83 | 64.99 | 20.64 | 9.87 | 117.0 | 26.23 | 41.49 | 41.96 | 7 |
| | **Treated** | **95.00** | **93.16** | **123.67** | **73.49** | **22.61** | **10.20** | **124.27** | **26.31** | **41.57** | **25.67** | **7** |
| HUBR-38B | Control | 53.33 | 95.83 | 125.83 | 67.37 | 21.37 | 7.53 | 105.53 | 22.35 | 32.62 | 47.33 | 7 |
| | **Treated** | **93.33** | **90.33** | **120.33** | **79.69** | **22.75** | **8.60** | **122.60** | **22.53** | **32.87** | **30.55** | **7** |
| Sahbhagi Dhan | Control | 71.67 | 103.83 | 129.67 | 79.97 | 21.41 | 10.33 | 104.47 | 20.28 | 29.83 | 44.16 | 7 |
| | **Treated** | **96.67** | **98.50** | **128.17** | **85.76** | **23.96** | **10.47** | **119.20** | **20.53** | **29.87** | **27.77** | **7** |
| MTU-7029 | Control | 58.33 | 116.0 | 145.83 | 53.83 | 22.79 | 10.50 | 213.07 | 20.28 | 37.55 | 40.50 | 7 |
| | **Treated** | **86.67** | **109.50** | **138.67** | **69.55** | **24.55** | **10.80** | **224.60** | **20.43** | **38.26** | **30.25** | **7** |
| BD-2010 | Control | 50.00 | 116.16 | 148.0 | 46.14 | 20.25 | 7.53 | 100.27 | 21.31 | 33.57 | 46.67 | 7 |
| | **Treated** | **91.67** | **110.66** | **142.50** | **63.30** | **23.05** | **7.93** | **107.33** | **21.43** | **33.78** | **26.66** | **7** |
| Swarna sub-1 | Control | 53.33 | 116.16 | 148.33 | 49.61 | 22.55 | 7.87 | 103.40 | 20.09 | 29.32 | 43.67 | 7 |
| | **Treated** | **88.33** | **112.00** | **141.67** | **52.73** | **24.74** | **8.53** | **108.47** | **20.20** | **29.38** | **24.00** | **7** |
| HUR-105 | Control | 46.67 | 116.0 | 149.33 | 63.63 | 25.80 | 8.47 | 175.07 | 22.73 | 26.41 | 44.26 | 7 |
| | **Treated** | **91.67** | **112.66** | **143.17** | **70.60** | **28.20** | **9.40** | **181.13** | **22.83** | **26.52** | **25.35** | **7** |
| S.Em. ± | Control | 4.81 | 2.41 | 3.02 | 2.89 | 1.87 | 0.50 | 4.79 | 1.09 | 2.21 | 2.18 | |
| | **Treated** | **3.97** | **2.18** | **2.58** | **2.45** | **1.50** | **0.43** | **3.15** | **0.80** | **1.98** | **1.75** | |
| CD. (0.05) | Control | 10.31 | 5.18 | 6.48 | 6.20 | 4.02 | 1.07 | 10.28 | 2.35 | 4.74 | 4.67 | |
| | **Treated** | **8.52** | **4.69** | **5.54** | **5.27** | **3.22** | **0.91** | **6.76** | **1.71** | **4.24** | **3.76** | |

test weight 0.1 to 0.25 g and grain yield per plant 0.04 to 0.25 g compared to untreated control. Similar results were also reported by Mathivanan *et al.,* (2006). *T. harzianum* not only improved the grain yield and its contributing traits but also reduced the intensity of blast (*Magnaporthe grisea*) disease of rice. Maximum reduction in disease intensity was recorded in germplasm IR-64 sub 1 (25%) followed by BD-2010 (20%) and lowest for MTU-7029 (10%) compared to untreated control. However, *Trichoderma* treated seeds showed 23.30% to 30.55% disease intensity compared to untreated seed 40.50% to 48.09%, thereby reducing disease intensity by 10-25%. Similar results were reported by Hegde (2006); Khan and Sinha (2007); Chinmay *at el.,* (2010) and Tran (2010).

It was thus concluded that *T.harzianum* has positive response on the seed germination, grain yield and yield attributing traits which also reduced the intensity of blast disease of rice. Therefore, it can be used as effective bio-control measure for rice blast besides increasing the yield of rice crop.

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# Antagonistic activity of *Trichoderma viride* against fungal pathogens causing diseases in agriculture crops

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**ABSTRACT**

The antagonistic screening of *Trichoderma viride* against fungal pathogens, *Alternaria alternata, Fusarium solani, F. oxysporum, Mycosphaerella* spp and *Colletotrichum falcatum,* isolated from diseased cauliflower, pigeon pea, banana and sugarcane plants from Sultanpur, Uttar Pardesh, showed 72.50, 67.75, 70.45, 45.40 and 62.75 per cent growth inhibition suggesting for its commercial exploitation under localized climatic conditions.

**Key words:** Antagonistic activity, fungal pathogens, *Trichoderma viride,*

Fungal plant pathogens such as *Alternaria alternata, Fusarium solani, F. oxysporum, Mycosphaerella* spp. and *Colletotrichum falcatum* cause serious damage to the agricultural crops. Their management through the use of chemicals or through breeding varities for disease resistance being difficult, and thus led to indiscriminate use of synthetic pesticides posing for several social, environmental, health, economic and newer pest problems. Biological control has emerged as a promising tool to maintain current level of agricultural production while reducing the release of polluting chemical pesticides to the environment.

In recent years, considerable success has been achieved by the use of *Trichoderma* bioagent. The mycoparasite ability of *Trichoderma* species against some economically important aerial and soil borne plant pathogens (Papavizas, 1985; Elad *et al.*, 1993; Elad, 2000; Freeman *et al.*, 2004, Dubey *et al.*, 2007) and nematodes (Windham *et al.*, 1989; Sharon *et al.*, 2001) allows for the development of biocontrol strategies. Several *Trichoderma* species are known to reduce the incidence of soil borne plant pathogenic fungi under natural conditions (Sivan and Chet, 1986; Calvet *et al.*, 1990); however the efficacy of this depends largely on the physical, chemical and biological condition of soil.

Therefore, the present investigation to explore the antagonistic activity of *T. viride* against major disease causing fungi of agricultural crops was undertaken.

## MATERIAL AND METHODS

The investigation was conducted in the month of October, 2011at bio-control laboratory of Gama Organomed Plus (Pvt) Ltd, E-223, UPSIDC, Kursi Road, Lucknow (U.P.).

### Collection of Soil Sample

Five soils samples (50g each), four from corners and one in the center from top 2-5cm depth of soil, from the respective field were collected and brought to the bio-control laboratory from different localities of Sultanpur and adjoining areas. These were mixed to make a composite sample (250g) and kept separately in the labeled paper bag. Ten such samples from ten different locations were collected.

### Isolation and identification of *Trichoderma viride*

One gram of the composite soil sample was added to 1ml of sterilized distilled water to make a dilution of $10^{-1}$. This suspension was then subjected to serial dilutions and a dilution of $10^{-5}$ was attained. One milliliter of each dilution viz., $10^{-3}$ to $10^{-4}$ was poured on to *Trichoderma* specific medium (TSM) (Elad and Chat, 1983) and purified by single spore method. Morphological characters (Refai,1969) formed the basis of their identification. Cultures were identified according to conidiophore, shape of the phialides and emergence of phialophores and phialospores. The purified and identified cultures of *Trichoderma* spp. were maintained on potato dextrose agar (PDA) medium and stored at 4°C for further use.

### Collection of diseased specimens

The diseased samples were collected from different farmer's field at Sultanpur. Random sampling technique was applied in the fungal collection procedure. The diseased plants of potato, pigeonpea, banana and sugarcane were collected in polythene bags and brought to the laboratory for isolation of pathogens.

**Isolation and Identification of plant pathogens**

Identification of isolated micro-organisms was done at Gama Organomed Plus (Pvt) Ltd, E-223, UPSIDC, Kursi Road, Lucknow (U.P.). Test fungi were isolated on potato dextrose agar (PDA Synthetic HiMedia) medium from their respective hosts collected from farmer's field. The cultures were further purified by single spore isolation technique and maintained at 25±2$^{O}$C on PDA slants. 7-10 days old cultures were used in the experiment. The fungi included in the present study viz., *Alternaria alternata, Fusarium solani, F. oxysporum, Mycosphaerella* spp and *Colletotrichum falcatum,* were isolated from infested potato, pigeonpea, banana and sugarcane, respectively. The isolates were maintained on potato dextrose agar (PDA) medium and stored in refrigerator for further use.

**Antagonistic activity of *Trichoderma viride* against isolated pathogens**

*In vitro* antagonistic potential of the bio-control agent was evaluated against isolated pathogens through dual culture technique. The isolates were screened for their antagonistic potential against the pathogen on PDA by measuring the relative growth rates as a function of the incubation period. Five mm mycelial discs taken from the margin of young vigorously growing 4-day-old culture of the antagonists and the pathogen was inoculated at the margin of the petridish containing 20 ml sterilized PDA medium (opposite to each other). Observations were recorded up to 5 days of incubation (at 28±1oC). The treatments were replicated three times. The data on the growth of the pathogen and *T. viride* isolates were recorded regularly. Percentage of mycelial growth inhibition was calculated according to the formula:

$$MGI\% = (dc - dt) \times 100 / d$$

**RESULTS AND DISCUSSION**

**Isolation and Identification of *Trichoderma* isolates**

Out of eight isolates of *Trichoderma* spp., two were identified as *T. viride* on the basis of conidiophore, shape of the phialides and emergence of phialophores and phialospores. Colonies fast growing (5-9cm) conidiation forming compact tufts glacous to dark green (Fig.1). Reverse typically uncolored. Odour usually distinctly aromatic, as of coconut. Conidiophores usually not extensively branched and having a relatively loose arrangement, branches most often paired, or single or 3-verticillate, often appearing flexous. Phialides frequently paired, or arising singly or 3-verticillate, narrowly logeniform conidia were globose to ellipsoidal, usually consipicuosly warted, bluish-green to dark green (Rifai, 1969).

Fig 1: *Trichoderma* viride culture and spore suspension

**Identification of isolated pathogens**

During survey, banana plantation in nearby areas of Sultanpur were found extensively infected with leaf spot and wilt diseases (*Mycosphaerella* spp *and Fusarium spp*). Cauliflower plants were found infected with *Alternaria* leaf spot disease, pigeonpea with *Fusarium* wilt. Sugarcane plants were found infected with red rot of sugarcane disease (Table 1).

**Antagonistic activity of *Trichoderma viride* isolates using dual culture method against isolated fungal pathogens**

In dual culture, a clear zone of inhibition was observed exhibiting antibiosis between pathogen and antagonist. It was observed that *T. viride* reduced the growth of *A. alternata* by 72.50 percent, *F. solani* by 67.75 percent, *F. oxysporum* by 70.45 percent, *Mycosphaerella* spp by 45.40 percent and *C. falcatum* by 62.75 percent (Table:2, Fig:2,3 and 4). The presence of an inhibition zone in dual culture without the hyphae contact in the treatments suggests the secretion of diffusible non-volatile inhibitory substance by the *T. viride* isolates.

It is important to mention that *Trichoderma* spp. are known to produce a number of antibiotics, trichodernin, trichodermol, harzianum A and harzianolide (Dennis and Webster, 1971c; Kucuk and Kivanc, 2004) as well as some cell walls degrading enzymes such as chitinases, glucanases that break down polysaccharides, chitins and -glucanase, thereby destroying cell wall integrity (Elad, 2000). These may also play a major role in mycoparasitism because of changes in cell wall integrity prior to penetration.

It was established that *Trichoderma* have various strategies for fungal antagonism and indirect effects on plant health also vary. Several species of *Trichoderma* are well documented mycoparasite and have been used successfully against certain pathogenic fungi (*Bose et.al.*, 2005). *T. harizanum*, *T. viridae*, *T. virens*, *T. hamatum, T. roseum* and *T. koningii* are the species most often used for biological control of pathogens. Possible mechanisms of antagonism employed by *Trichoderma* spp. includes nutrient and niche competitions, antibiosis by producing volatile components

**Table 1:** Diseases identified in Laboratory

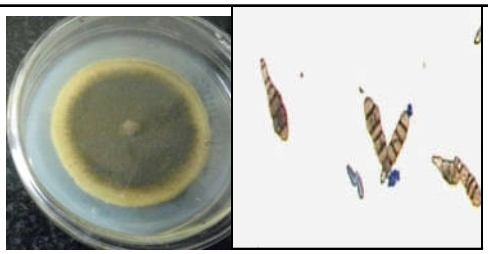
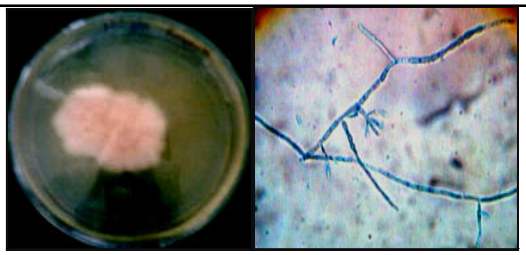
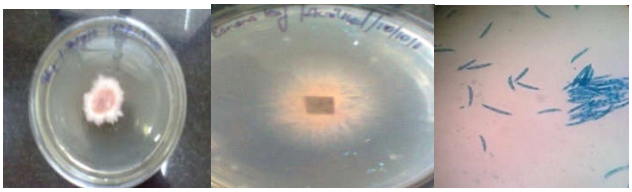
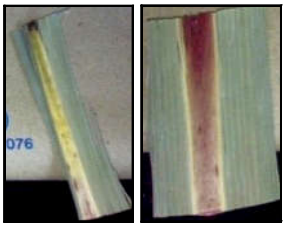
| *Host plant Sample name* | *Identified Causal organism* |
|---|---|
| Cauliflower: *Brassica oleracea var. botrytis*  |  **Leaf spot disease:** ***Alternaria alternata*** |
| Pigeonpea: *Cajanus cajan*  |  ***Fusarium*** **wilt:** ***Fusarium solani*** |
| Banana: *Musa acuminata*  |  ***Fusarium*** **disease:** ***Fusarium oxysporum*** |
| Banana: *Musa acuminata*  |  **Banana leaf spot:** ***Mycosphaerella*** **spp** |
| Sugarcane: *Saccharum officinarum*  |  **Red rot of sugarcane:** ***Colletotrichum falcatum*** |

**Table 2:** Antagonistic activity of various isolates of *Trichoderma viride* against different fungal plant pathogens

| Percent Growth inhibition of various fungal plant pathogens by dual culture method | | | | |
|---|---|---|---|---|
| *Alternaria alternata* | *Fusarium solani* | *Fusarium oxysporum* | *Mycosphaerella* spp | *Colletotrichum falcatum* |
| **72.50** | **67.75** | **70.45** | **45.40** | **62.75** |

Mean of three replicates

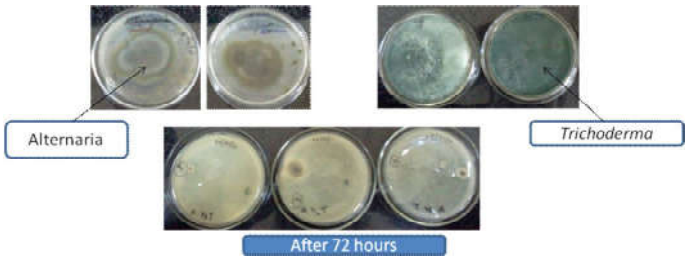



Fig 2: Antagonistic activity of *Trichoderma viridae* against *Alternaria alternata* from cauliflower

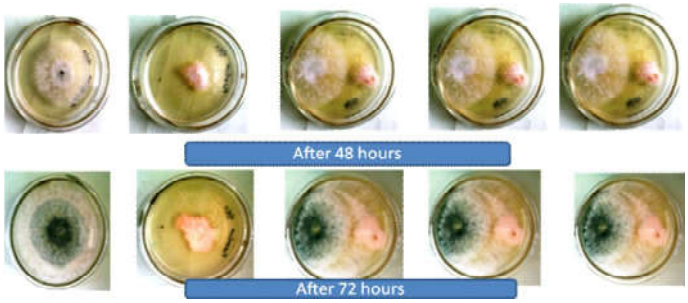



Fig 3: Antagonistic activity of *Trichoderma viridae* against *Fusarium solani from pigeonpea*


Fig 4: Antagonistic activity of *Trichoderma viridae* against *Colletotrichum falcatum* from sugarcane

and non-volatile antibiotics (Harman and Hadar, 1983) that are inhibitory against a range of soil borne fungi, as well as parasitism. Environmental parameters such as abiotic (soil type, soil temperature, soil pH, water potential and such like) and biotic (plant species and variety, microbial activity of the soil) factors as well as others such as method and timing of applications may have influence on the biological control efficacy of *Trichoderma* isolates. Therefore, it is important that before introducing *Trichoderma* isolates in field, the bio-control potential and environmental factors must be evaluated.

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# Effect of soil reaction on some fungal antagonists in suppressing white mould of french bean

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**ABSTRACT**

The effect of soil reaction on *Trichoderma koningii*, *Gliocladium virens* and *Aspergillus terreus* in suppressing *Sclerotinia sclerotiorum* (Lib.) de Bary, the cause of white mould of French bean, was studied under screen house condition. The results showed that soil application of *T. koningii*, *G. virens* and *A. terreus* significantly inhibited development of white mould incidence in all the soil reactions *viz*., acidic, neutral and alkaline. The highest per cent plant mortality was recorded in alkaline soil (24.32%) followed by neutral (23.11%) and acidic (19.32%) soils. Among the three soil reactions, maximum plant height (30.57cm), root length (11.88cm), dry weight of shoots (5.94g), dry weight of roots (3.20g) and pod yield (195.39g) was recorded in neutral soils. Maximum reduction in per cent plant mortality was observed with *T. koningii* (12.50%) in soils with acidic pH and *A. terreus* in neutral pH (9.12%) and alkaline pH (10.56%) soils, respectively.

**Key words:** French bean, *Sclerotinia sclerotiorum*, white mould, antagonists.

White mould, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most important diseases of french bean (*Phaseolus vulgaris* L.) leading to serious and unpredictable yield losses of as high as 100 per cent (Purdy, 1979; Tu, 1989). The pathogen is polyphagous and soil-borne, therefore, control through fungicides and host resistance is difficult. A few chemicals are known to give effective control against the disease (Hunter *et al*., 1978; Kerr *et al*., 1992), however, they are costly besides being toxic to both human and animal. In the light of these hazards, employing potential antagonists by manipulating the existing soil microflora to the disadvantage of the pathogen or by addition of non-resident antagonists offers an excellent alternative in combating the ravages of soil borne diseases. The present investigation reports the effect of soil reaction on *Trichoderma koningii*, *Gliocladium virens* and *Aspergillus terreus* in suppressing *S. sclerotiorum*, the cause of white mould of French bean.

## MATERIALS AND METHODS

The pathogen, *Sclerotinia sclerotiorum* (Lib.) de Bary, isolated from diseased tissues of infected standing plants in the field and further purified was maintained in potato dextrose agar (PDA) at 28 ± 1°C. Cultures of *Trichoderma koningii* (Oudem) Rifai, was obtained from the culture collection of the Department of Plant Pathology, Assam Agricultural University, Jorhat, while *Aspergillus terreus* Thom. and *Gliocladium virens* Miller, Giddens & Foster were collected from the Institute of Microbial Technology, Chandigarh. The cultures were maintained on PDA adjusted at pH 6.0 and sub culturing was done periodically to maintain the purity of the cultures. Interactions between the antagonists and the pathogen were studied in dual culture. Two mycelial culture discs (5 mm diam.) of each antagonist, *T. koningii*, *G. virens* and *A. terreus*, were placed one opposite the other in separate Petri dishes (90 mm diam.) near the periphery. In the centre of each petri dish, a disc of the pathogen, *S. Sclerotiorum,* was placed. A control having the test pathogen only was kept for comparison. The petri dishes were incubated at 28 ± 1°C maintaining five replications for each treatment. Observation on radial growth of *S. sclerotiorum* was recorded at 24 hr interval till the control dish was completely covered by *S. sclerotiorum*. The per cent growth inhibition of the test pathogen as compared to that of control was also calculated using the following formula-

$$PI = (A_1 - A_2)/A_1 \times 100$$

Where, PI = Per cent inhibition.

$A_1$ = Radial growth of *S. sclerotiorum* in control plates.

$A_2$ = Radial growth of *S. sclerotiorum* in treated plates.

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The mass inocula of the pathogen, *S. sclerotiorum* was prepared on 4.0 percent maize-meal sand medium (MSM) while the antagonists, *T. koningii*, *G. virens* and *A. terreus*, were raised in farmyard manure (FYM) medium. The media were inoculated aseptically with mycelia discs of the pathogen and the antagonists (5 mm diam.) taken from the margin of actively growing culture and incubated for 15 days at 28 ± 1°C.

The efficacy of soil reaction on the antagonists against the white mould pathogen was evaluated under screen house condition in earthen pots (30 cm diam.) containing 5 kg each of different soil reactions *viz*., acidic, neutral, and alkaline. Surface soils (0-15 cm) collected from the experimental field of the Department of Plant Pathology, Assam Agricultural University, Jorhat, was determined using Elico model glass electrodes pH meter (1:2.5 soil water suspension), following the method described by Jackson (1973). The soils analysed was found to have a pH value of 5.8. Agricultural lime was added to the soils by adopting the method of Shoemaker *et al.* (1961) to adjust the soil into three different reactions *viz*., acidic (pH 5.8), neutral (pH 7.2), and alkaline (pH 8.4). Treated soils were allowed to remain as such for 30 days with light watering at regular intervals. The pots were inoculated with the mass inocula of the pathogen (*S. sclerotiorum*) @ 10 g/kg soil (w/w). Mass inocula of *T. koningii*, *G. virens* and *A. terreus*, were then inoculated in each pot containing the different soil reactions with three different doses *viz*., 10, 20 and 30 g/kg soil (w/w) and kept for 5 days before sowing. One treatment inoculated with *S. sclerotiorum* alone and another treatment without *S. sclerotiorum* or the antagonists (*T. koningii*, *G. virens* and *A. terreus*) were kept as controls. Six seeds of susceptible french bean cv. 'Contender' were sown in each pot. The experiment was laid out in completely randomised block design (CRBD) and each treatment replicated five times. Observations on per cent plant mortality, pod yield (g) and growth parameters *viz*., plant height (cm), root length (cm), dry weight of shoot and root (g) of french bean plants were recorded at 15 days after sowing and the subsequent ones at 15 days interval till harvest.

## RESULTS AND DISCUSSION

The presence of *T. koningii*, *G. virens* and *A. terreus* in dual culture showed significant reduction on radial growth of *S. sclerotiorum* under *in vitro* condition. *G. virens* caused maximum inhibition of mycelia growth (66.33 %) followed by *T. koningii* (60.29 %) while *A. terreus* caused the least inhibition (50.79 %) (Table 1). *S. Sclerotiorum* was observed to grow until it came in contact with the leading edges of the antagonists. The antagonists either suppressed the colony of *S. sclerotiorum* or its hyphae got lysed on contact with the antagonists. Attempts to re-isolate the pathogen from such test assay dishes resulted in isolating only the antagonists, suggesting that the pathogen was completely destroyed. Microscopic examination revealed coiling around the hyphae of *T. koningii* and *A. terreus* while coagulation and disintegration of the hyphal cell wall of *S. sclerotiorum* in presence of *G. virens*. Formation of coiled structure around the hyphae of *S. sclerotiorum* by *Trichoderma* spp. was reported by Matroudi *et al*., (2009). Moreover, production of cell-wall degrading enzymes like chitinases and β-1,3 glucanases was demonstrated by many as an effective mechanisms of biocontrolling plant pathogens by *Trichoderma* spp. (Brimner & Boland 2003; Wang *et al*. 2003; Harighi *et al.* 2007). The inhibitory activity of the antagonists against *S. sclerotiorum* was probably due to direct mycoparasitism and/or antibiosis. Similar phenomenon of host-parasite interaction as observed

**Table 1:** Effect of antagonists on the radial growth and per cent inhibition of S. *sclerotiorum* in dual culture

| Treat ments | Radial growth (mm)* | | | Per cent inhibition (%) | | |
|---|---|---|---|---|---|---|
| | 24 hrs | 48 hrs | 72 hrs | 24 hrs | 48 hrs | 72 hrs |
| Ss + Tk | 14.00$^{b}$ | 24.00$^{c}$ | 41.00$^{c}$ | 12.27 (3.35)$^{ab}$ | 53.77 (7.40)$^{a}$ | 60.29 (7.82)$^{b}$ |
| Ss + At | 14.80$^{ab}$ | 29.80$^{b}$ | 45.50$^{b}$ | 7.41 (2.75)$^{b}$ | 43.25 (6.64)$^{b}$ | 50.79 (7.19)$^{c}$ |
| Ss + Gv | 12.90$^{c}$ | 23.20$^{c}$ | 32.50$^{d}$ | 19.41 (4.42)$^{a}$ | 55.20 (7.49)$^{a}$ | 66.33 (8.20)$^{a}$ |
| Control | 16.00$^{a}$ | 51.90$^{a}$ | 90.00$^{a}$ | - | - | - |
| CD (0.05) | 1.35 | 3.09 | 4.44 | 1.38 | 0.38 | 0.29 |

*Average of five replications

Figures in the parentheses represents square root transformed values. Any two mean values separated by DMRT at 5% level showing common letter are not significantly different.

Ss+Tk = *S. sclerotiorum* + *T. koningii*, Ss + At = *S. sclerotiorum* + *A. terreus*, Ss + Gv = *S. sclerotiorum* + *G. virens*.

**Table 2:** Per cent mortality of french bean in different soil inoculated with *S. sclerotiorum* and different doses of antagonists.

| Treatments | Plant Mortality % | | |
|---|---|---|---|
| | Acidic | Neutral | Alkaline |
| Ss+Tk @ 10+10g/kg soil | 29.17 (28.84) | 37.50 (33.68) | 40.00 (39.14) |
| Ss+Tk @ 10+20g/kg soil | 8.34 (12.65) | 20.84 (26.88) | 16.67 (18.24) |
| Ss+Tk @ 10+30g/kg soil | 0.00 (1.18) | 4.17 (6.92) | 9.17 (13.24) |
| Ss+At @ 10+10g/kg soil | 29.17 (28.84) | 25.00 (23.08) | 22.50 (21.63) |
| Ss+At @ 10+20g/kg soil | 16.67 (18.24) | 4.17 (6.92) | 9.17 (13.24) |
| Ss+At @ 10+30g/kg soil | 4.17 (6.92) | 0.00 (1.18) | 0.00 (1.18) |
| Ss+Gv @ 10+10g/kg soil | 26.67 (27.37) | 37.50 (33.68) | 44.17 (41.57) |
| Ss+Gv @ 10+20g/kg soil | 8.34 (12.65) | 20.84 (26.88) | 25.84 (29.93) |
| Ss+Gv @ 10+30g/kg soil | 0.00 (1.18) | 12.50 (18.36) | 10.00 (10.67) |
| Uninoculated control | 0.00 (1.18) | 0.00 (1.18) | 0.00 (1.18) |
| Inoculated control | 90.00 (79.33) | 91.67 (77.38) | 90.00 (79.33) |
| CD (0.05) | 18.76 | 20.86 | 20.00 |

Figures in the parentheses represents square root transformed values.

Uninoculated control = Without *S. sclerotiorum* or the antagonists, Inoculated control = *S. sclerotiorum* alone, Ss+Tk = *S. sclerotiorum* + *T. koningii*, Ss + At = *S. sclerotiorum* + *A. terreus*, Ss + Gv = *S. sclerotiorum* + *G. virens*.

in the present studies has been reported on *Rhizoctonia solani* by *G. virens* (Aluko and Hering, 1970), on *S. sclerotiorum* by *T. koningii* (Trutmann and Keanne, 1990) and on *R. solani* by *A. terreus* (Das, 1992). The mycoparasite, *Aspergillus terreus* was reported to sporulate abundantly on sclerotial surface of *S. sclerotiorum* and caused break down of inner rind cells through cell wall disruption (Melo *et al*., 2006). UV-produced mutant strain of *T. harzianum* was reported to be more effective than wild strain in reducing radial growth and arresting sclerotial production of *S. sclerotiorum*, the causal agent of white mold of french bean (Mech *et al*., 2006).

The results obtained from the pot trials under screen house condition (Table 2) showed that soil application of *T. koningii, G. virens* and *A. terreus* significantly inhibited development of white mould incidence in all the different soil reactions *viz*., acidic, neutral, and alkaline. Inoculated control recorded the maximum per cent mortality (90.00 %, 91.67 %, and 90.00 %). The lowest per cent mortality was recorded with *T. koningii* and *G. virens* in acidic soil and *A. terreus* in neutral and alkaline soils when applied @ 30 g/kg soil (w/w). Increased dose of antagonists was observed to be inversely proportional to the per cent plant mortality. Greater efficacy of the antagonists might be due to increase in their population enabling establishment in the rhizosphere and triggering the release of antibiotic substances. The antagonists might have become aggressive to parasitize sclerotia as well as vegetative hyphae of *S. Sclerotiorum* through antibiosis or through direct competition. Similar phenomenon on *S. sclerotiorum* has been earlier reported in relation to *T. koningii* (Lacicowa and Pieta, 1986) and with *Aspergillus* spp., *Gliocladium* spp., *T. harzianum*, *Mucor hiemalis* and *Penicillium* spp. (Carkaci and Maden, 1986). In a similar study, reduction in seedling mortality of french bean due to *S. sclerotiorum* in field condition by seed treatment with *T. harzianum* was reported by Ahangar *et al*. (2008). The highest per cent plant mortality was recorded in alkaline soil (24.32%) followed by neutral (23.11%) and acidic (19.32%) soils. Among the three soil reactions, maximum plant height (30.57 cm), root length (11.88 cm), dry weight of shoots (5.94g), dry weight of roots (3.20g) and pod yield (195.39g) was recorded in neutral soils (Table 3). Maximum reduction in per cent plant mortality with *T. koningii* was observed in soils with acidic pH (12.50%), with *A. terreus* in neutral pH (9.12%), while with *G. virens* maximum reduction in per cent plant mortality was recorded in soils with acidic pH (11.67%) (Table 4). Soil with pH 7.20 or 7.28 has been reported to be more potent in suppressing *R. solani* than soil having pH 5.2 or 5.5 with *A. terreus* (Gogoi and Roy, 1996).

**Table 3:** Effect of different soil reactions on per cent mortality and growth parameters in the presence of S. *sclerotiorum* and antagonists.

| Soil reaction | Mortality % | Plant height (cm) | Root length (cm) | Dry wt. of shoots (g) | Dry wt. Of roots (g) | Pod yield (g) |
|---|---|---|---|---|---|---|
| Acidic | 19.32 | 28.87 | 11.18 | 5.58 | 2.98 | 187.28 |
| Neutral | 23.11 | 30.57 | 11.88 | 5.94 | 3.20 | 195.38 |
| Alkaline | 24.32 | 29.21 | 10.21 | 5.48 | 2.66 | 178.58 |
| CD (0.05) | 4.86 | 0.59 | 0.32 | 0.13 | 0.14 | 2.25 |

**Table 4:** Per cent mortality of french bean in the presence of *S. sclerotiorum* and antagonists influenced by different soil reactions.

| Antagonists | Plant Mortality % | | |
|---|---|---|---|
| | Acidic | Neutral | Alkaline |
| *T. koningii* | 12.50 (14.22) | 20.84 (22.46)[a] | 21.95 (24.52)[a] |
| *A. terreus* | 16.67 (18.00) | 9.72 (10.37)[b] | 10.56 (12.02)[b] |
| *G. virens* | 11.67 (13.73) | 23.61 (26.31)[a] | 26.67 (27.39)[a] |
| CD (0.05) | NS | 8.64 | 8.75 |

NS = Non Significant

Figures in the parentheses represents square root transformed values. Any two mean values separated by DMRT at 5% level showing common letter are not significantly different.

Das *et al*. (1996) also observed higher antagonistic activity of *A. terreus* in neutral pH than on acidic pH, which resulted in lower disease incidence with higher grain yield. Maximum biomass production of *Trichoderma* spp. was reported to occur between pH 4.6 and 6.8 with a tendency for better growth towards acidic pH (Jackson *et al*., 1991) as also the sensitivity to fungistasis was more pronounced in neutral or alkaline soil than in acidic soil (Danielson and Davey, 1973; Papavizas, 1985). Seed treatment with *T. harzianum* and soil treatment with FYM which lowered soil pH also recorded best control of the white mold of french bean cv. Contender among other treatments using fungal agents *G. virens* and *Aspergillus flavus* (Das and Das, 2005). Saha and Pan (1995) reported maximum growth and germination of phialospores of *G. virens* in acidic soil with effect of soil fungistasis more pronounced in alkaline and neutral soil but decreased in acidic soil. On the contrary, Phillips (1986) reported that *G. virens* parasitized and decayed sclerotia of *S. sclerotiorum* causing reduction in their survival being active in soil over a broad range of soil moisture level and pH. In the experiment, temperature was reported to be the main limiting factor for its use as bioagent. These cumulative evidences indicate the influence of soil reaction on the efficacy of the antagonists and corroborate the results of the present investigation.

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# Management of white rot of pea

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**ABSTRACT**

Field trials were conducted during 2009-10 and 2010-11 seasons to manage white rot of pea caused by *Sclerotinia sclerotiorum* (Lib.) de Bary through bioagents and chemical treatments revealed that the most effective treatments for the control of white rot achieved was with Bavistin i.e. seed treatment + foliar spray (disease intensity 4.5%) followed by *Trichoderma harzianum* i.e. soil application + foliar spray (6.5%), *T. harzianum* i.e. seed treatment + foliar spray (6.75%) over untreated control (37.5%). The maximum yield was recorded by seed treatment + foliar spray with Bavistin (52.55 q $ha^{-1}$), followed by soil application + foliar spray with *T. harzianum* (51.18 q $ha^{-1}$), seed treatment + foliar spray with *T. harzianum* (50.5 q $ha^{-1}$) over the control (29.33 q $ha^{-1}$). Soil application alongwith foliar spray by *T. harzianum* reduced disease intensity, produced higher yield over the control and was economical than chemical.

**Keywords:** White rot, pea, *Sclerotnia sclerotiorum, Trichoderma harzianum*, Bavistin

White rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most destructive diseases of pea (*Pisum sativum* L.) in India. It appears at seedling and flowering stages affecting stem and leaves resulting in more than 50% reduction in yield (Sharma and Kanwar, 1989). The damage may be very high depending on susceptibility of the crop, weather conditions and nature of infection. On pea pods, the symptoms mainly consist of white mycelium sticking to pod surface, mostly at the basal and distal ends and the tissues show necrosis. Ultimately, the fungus enters the pods and the developing seeds rot. Partial or total wilt is the characteristic symptom of infection of stems and branches. The infection may occur at any part of the foliage, mainly the stems and branches. If the infection is at the base of the main stem, the entire plant wilts (Singh, 2005). Management of this disease through resistant varieties is limited as most of the commercially grown varieties are susceptible (Kapoor and Singh, 1992). Due to soil born nature of the pathogen and explosive pathogenic potential of the fungus under favourbale conditions, no single approach may effectively manage this disease. Hence, efforts to develop an integrated disease management practice, using fungicide and bioagents was made.

## MATERIALS AND METHODS

Experiments were conducted at Krishi Vigyan Kendra Farm, Jeolikote, district Nainital (altitude 1250 m above mean sea level) located in the hills of Uttarakhand state, during rabi season of 2009-10 and 2010-11. Bioagent (*Trichoderma harzianum*) was obtained from Bio-control Laboratory, G.B. Pant University of Agriculture and Technology, Pantnagar. Farm manure (FYM) @ 10 kg $plot^{-1}$ (5 × 2 $m^2$) was applied during field preparation. The pea (var. Arkle) was sown during the last week of October in plot of 5 × 2 $m^2$ with four replications each with treatments including, seed treatment with bavistin @ 2 g $kg^{-1}$ seed ($T_1$), seed treatment with bavistin @ 2 g $kg^{-1}$ seed + one foliar spray of bavistin @ 0.2% solution ($T_2$), seed treatment with *Trichoderma harzianum* @ 10 g $kg^1$ seed ($T_3$), seed treatment with *T. harzianum* @ 10 g $kg^{-1}$ seed + one foliar spray of *T. harzianum* @ 1% solution ($T_4$), soil application before sowing with *T. harzianum* @ 100 g $plot^{-1}$ + one foliar spray of *T. harzianum* @ 1% solution ($T_5$) and control, no treatment ($T_6$) in randomized block design. The sprays of bavistin and *T. harzianum* were given in the month of January at 50 percent flowering stages of the crop. The crop was grown as per recommended agronomic practices. The data on disease intensity as percentage of green area infected (Sharma, 1985) and green pod yield were recorded.

## RESULTS AND DISCUSSION

Effect of different treatments on disease intensity and yield are presented in Table 1. Intensity of white rot disease was minimum 4.5 per cent, using seed treatment @ 2 g $kg^{-1}$ + foliar spray @ 0.2% with bavistin and followed by soil application @ 100 g $plot^{-1}$ + foliar spray @ 1.0% with *T. harzianum* (6.5%). Both treatments did not differ significantly from each other. Disease intensity was 6.75, 12.75 and 17.5 per cent in plots where seed treatment (10 g $kg^{-1}$) + foliar spray (1.0%) with *T. harzianum*, seed treatment with bavistin (2 g $kg^{-1}$) and seed treatment (10 g $kg^{-1}$) with *T. harzianum*

Table 1 : Effect of different treatments on white rot of pea

| | Treatment | Disease intensity (%) | Pod yield (q/ha) | Per cent increase in yield |
|---|---|---|---|---|
| $T_1$ | Bavistin (seed treatment @ 2 g $kg^{-1}$) | 12.75 (20.68) | 44.25 | 50.87 |
| $T_2$ | Bavistin (seed treatment @ 2 g $kg^{-1}$ + foliar spray @ 0.2%) | 4.5 (10.56) | 52.55 | 79.17 |
| $T_3$ | *Trichoderma harzianum* (seed treatment @ 10 g $kg^{-1}$) | 17.5 (24.44) | 40.18 | 36.99 |
| $T_4$ | *T. harzianum* (seed treatment @ 10 g $kg^{-1}$ + foliar spray @ 1.0%) | 6.75 (12.90) | 50.50 | 72.19 |
| $T_5$ | *T. harzianum* (soil application @ 100 g $plot^{-1}$ + foliar spray @ 1.0%) | 6.5 (14.29) | 51.18 | 74.49 |
| $T_6$ | Control (no treatment) | 37.5 (37.71) | 29.33 | – |
| | CD at 5% | 8.27 | 6.76 | – |

Values given in parentheses are angular transformed value.

was done, respectively. The disease level remained significantly highest in control with no treatment (37.5%).

The present study supports the findings of Sugha (2001), who working with white rot of pea caused by *Sclerotinia sclerotiorum*, found 97.0 per cent control of the disease with integration of pre-sowing application of carbendazim (10 kg/ha), seed treatment (2.5 g/kg) and three sprays of 0.1% carbendazim starting with the initiation of flowering. Singh (2005) reported soil application and seed treatment with *T. harzianum* and *T. viride* showing encouraging results in managing white rot of pea. Sokhi (1994) also observed that the seed treatment with Bavistin reduced the incidence of white rot in pea. The efficacy of biocontrol agents (*T. harzianum, T. viride* and *Gliocladium virens*) against *S. sclerotiorum* has also been demonstrated (Sharma, 1994; Sharma *et al*., 1999).

Data presented in the table indicate that green pod yield of treated plots were significantly higher than control. The most effective treatment on yield was recorded with Bavistin (seed treatment + foliar spray) 52.55 q $ha^{-1}$ ($T_2$), followed by soil application + foliar spray with *T. harzianum* (51.18 q $ha^{-1}$; $T_5$), which do not differ significantly from each other. The results revealed that seed treatment + foliar spray with *T. harzianum*, seed treatment with Bavistin and seed treatment with *T. harzianum* produced 50.5 q $ha^{-1}$, 44.25 q $ha^{-1}$ and 40.18 q $ha^{-1}$ pod yield, respectively. However, 29.33 q $ha^{-1}$ yield was observed in control (without treatment). The results showed that $T_2$, $T_5$, $T_4$, $T_1$ and $T_3$ produced 79.17, 74.49, 72.19, 50.87 and 36.99 per cent higher yield over check, respectively. Hyseik *et al.* (2002) also reported that *T. harzianum* based commercial product applied at 0.5 g per kg of mineral fertilizers could suppress foliage diseases in various crops and increase the yields.

It can thus be concluded that the seed treatment (2 g $kg^{-1}$) + foliar spray (0.2%) with bavistin and soil application (100 g $plot^{-1}$) + foliar spray (1.0%) with *T. harzianum* proved to be the most effective treatments in reducing white rot infection and producing higher pod yield.

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# Effect of soil amendment with *Trichoderma atroviride* and prochloraz in presence of wilt pathogen on growth and yield of tomato in tropical agro ecosystems

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**ABSTRACT**

Efficacy of soil amendment with *Trichoderma atroviride* and prochloraz ($C_{15}H_{16}Cl_3N_3O_2$) against wilt pathogen was evaluated on tomato plants in tropical agro ecosystems. Both *Trichoderma atroviride* and the fungicide suppressed the wilt disease and enhanced tomato growth and the yield. Antagonistic activity of the bioagent against wilt pathogen and its growth promoting ability on tomato plants increased when applied in combination with prochloraz in the soil. Occuring abundantly in agricultural soils, the bioagent may be mass cultured and used as safe alternative to the hazardous chemicals for sustainable agriculture.

**Key words:** *Trichoderma atroviride,* prochloraz, *Fusarium* wilt

*Fusarium* wilt is a severe disease of tomato, occurring in many parts of the world including India, where it is found in every state. Prochloraz [N-Propyl-N-(2,4,6-trichlorophenoxy)ethyl-imidazole-1-carboxamide] ($C_{15}H_{16}Cl_3N_3O_2$) is a non-systemic imidazole fungicide that is used in agriculture to combat the wilt of tomato (Amini and Sidovich (2010). But this chemical fungicide has repercussion on environment and human health. Also progressive confrontation in a midst of pathogen resistance to accessible chemical plant protectants has engrossed the need of alternative methods of disease control (Kumar and Sharma, 2011). Fungi of the genus *Trichoderma* are important biocontrol agents of several soil borne phytopathogens (Benitez *et al*., 2004). Antagonistic strains of *Trichoderma* can act against the target pathogen through production of extracellular enzymes (Haran *et al*., 1996), antifungal antibiotics (Ghisalberti and Rowland, 1993), inducing resistance in plants (Meyer *et al*., 1998), competing to fungal pathogens (Simon and Sivasithamparan, 1989) and promoting plant growth (Inbar *et al*., 1994). For practical use to combat wilt pathogen and sustainable agriculture, antagonistic strains of *Trichoderma* spp. must be preceded by precise identification, adequate formulation and studies about the synergistic effects of their mechanisms of biocontrol (Hermosa *et al*., 2000). An important factor to be considered when screening new microbial isolates is their activity in the range of environments in which they would be expected to be used; in particular different soil types (Ross *et al*., 2000). For this reason it is important to study native strains isolated in the same region where they may be used as microbial inoculants. The efficacy of prochloraz ($C_{15}H_{16}Cl_3N_3O_2$) and the antagonistic activity of *T. atroviride* in checking *Fusarium* wilt and raising growth and yield of tomato in tropical agro ecosystems was therefore tested.

## MATERIALS AND METHODS

### Isolation of fungi from rhizosphere

Healthy and wilted tomato plants were collected at regular growth intervals i.e. seedling, vegetative, flowering and fruiting stages from tropical agro ecosystems in Indo-gangetic plains. Care was taken to dig out, as far as possible, the whole root system with a sterilized spatula. The root systems were then brought to the laboratory in separate polyethylene bags. The roots were given gentle taping to loosen-off the lightly adhering soil, in order to have just the rhizosphere soil attached to the root system. Small pieces of roots (2 cm) were cut with sterilized scissors under aseptic condition and 25 such root pieces for each sample were transferred to flasks (one for healthy and the other for diseased roots) containing 100 ml of sterilized distilled water. The flasks were shaken vigorously with the help of a shaker to get a homogenous suspension of the rhizosphere soil. Taking this as the stock solution, conventional soil dilution plate method (Warcup, 1950) was followed for isolation of the rhizosphere fungi. Dilutions of 1:100, 1:1000, and 1:10000

were prepared. Three replicates of sterilized petri plates were inoculated with one ml aliquots from all the diluted suspensions. To this, 20 ml melted and cooled (40° C) potato dextrose agar (PDA) medium was added and the plates were rotated slowly in clock-wise and anti-clock wise directions to disperse the soil solution uniformly in the culture medium. All the inoculated plates were then incubated at 25±2 °C. The plates were examined regularly and the fungal colonies appearing on the medium were transferred into fresh sterilized petri plates containing PDA medium to avoid over-running by the fast growing forms. The pure cultures of *Trichoderma atroviride and Fusarium oxysporum* f. sp. *lycopersici* thus isolated were preserved on PDA slants at 4°C.

**Preparation of mass culture**

The mass culturing of rhizosphere fungi was done on barley grains (Shivanna *et al,* 1994). Clean and intact barley grains were taken for this purpose. The grains were pre-wetted by boiling them in water for 20-30 minutes so as to raise the moisture content of the grains up to 40-50% and to make them soft enough for the profuse growth of the fungus. After boiling, the grains were spread on wire mesh to drain excess of water. Later, the grains were mixed with gypsum (calcium sulphate 2%) and chalk powder (calcium carbonate 0.5%) on dry weight basis to check pH of the medium and prevent grains from sticking with each other. Clean glucose were filled with such barley grains (100g each) were then steam sterilized for 1-2 hour. These were then allowed to cool at room temperature and inoculated with five agar blocks (5 mm diameter each) cut from the margin of actively growing culture of each fungus. The bottles were incubated at 25 ± 2°C for 10 days. The bottles were shaken once or twice daily for rapid and uniform colonization of the fungi. Barley grains colonized by the rhizosphere fungi were air dried and aseptically stored at 4°C for further use.

**Preparation of pots**

The soil sample was collected from tropical agro ecosystems in Indo-gangetic plains, India. The soil was air dried at room temperature and ground to fine powder form with the help of pestle and mortar. The pure inoculum of *T. atroviride*, which was prepared on barley grains, was mixed separately with sterilized natural soil (1% w/w). Chemical fungicide namely prochloraz ($C_{15}H_{16}Cl_3N_3O_2$) was mixed separately with sterilized natural soil samples at the rate of 0.24 $kgh^{-1}$ (w/w) (Hoeven and Bollen, 1980). The pure inoculum of the test pathogen (FOL) prepared separately on barley grains, was mixed with each sample of sterilized natural soil inoculated with the chemicals and pure inoculum of *T. atroviride* (1% w/w). The soil samples so prepared were separately filled in clay pots (15 ´ 25 cm). The pots were kept at room temperature for a week during which bioagent and the test pathogen developed and colonized the soil particles. Soil supplemented with barley grains without inocula was used as control. The moisture level of the soil (25-30%) was maintained by watering the pots from time to time. Twenty surface sterilized seeds of tomato variety H-24 were sown in each pot after 8 days of combined soil amendment with bioagent, fungicide and wilt pathogen. The experiments were set in replicates of three pots in a greenhouse. The observations for the combined effect of the bioagent, fungicide and wilt pathogen on growth and yield of tomato plants were made on plant height, branches/plant, fruits/plant and weight of 100 dry seeds at 65, 95 and 115 days after sowing (DAS). Ten plants were uprooted randomly from each treatment and the plant length above the ground was measured in cm and average height per plant was calculated. Branches or fruits on tomato plants formed and developed under each treatment were counted and average number of branches and/or fruits per plant was calculated. The seeds harvested from the ripened fruits separately from each treatment were air dried. One hundred dry seeds, selected randomly from triplicate sets from individual treatments, were weighed.

**RESULTS AND DISCUSSION**

The results clearly indicated that *T. atroviride* and the fungicide promoted the growth of tomato in presence of wilt pathogen FOL (Table 1). Out of all the treatments, *T. atroviride* + prochloraz was found to be the maximum growth promoter at 65 DAS. This was followed by *T. atroviride* and prochloraz. Plant growth promotion by the bioagent and the fungicide was significantly higher than the control ($P < 0.05$). In most of the treatments growth promotion of tomato increased with time up to 115 DAS. Plant growth at 65 DAS was significantly lower in the FOL inoculated compared to un-inoculated plants. Antagonistic activity of *T. atroviride* against the test pathogen and its growth promoting ability increased when used in combination with the fungicide.

Maximum number of branches/plant was recorded at 115 DAS with *T. atroviride* + prochloraz treatment followed by *T. atroviride* and prochloraz which were significantly higher than control ($P < 0.05$). In FOL inoculated plants, no branching was observed.

Weight of 100 dry seeds observed at 115 DAS, was maximum in *T. atroviride* + prochloraz treatment followed by *T. atroviride* and prochloraz which were significantly higher than control ($P < 0.05$).

Among all the treatments, *T. atroviride* + prochloraz was found the best (at 115 DAS) as plant growth promoter in

**Table 1.** Effect of soil amendment with *T. atroviride* and procloraz in presence of wilt pathogen on growth and yield of tomato grown in tropical agro-ecosystem

| Treatment | Days | Plant height (cm) | No. of branches plant$^{-1}$ | No. of fruits plant$^{-1}$* | Weight of 100 dry seeds (g) ** |
|---|---|---|---|---|---|
| *T. atroviride* | 65 | 15.00 ± 0.00 | 2.00 ± 0.00 | - | - |
| | 95 | 31.05 ± 0.00 | 5.00 ± 0.00 | 10 ± 0.0 | - |
| | 115 | 35.00 ± 0.00 | 6.00 ± 0.00 | 18 ± 0.0 | 0.80 ± 0.0 |
| Prochloraz | 65 | 14.00 ± 0.02 | 2.00 ± 0.00 | - | - |
| | 95 | 31.00 ± 0 00 | 4.00 ± 0.00 | 9 ± 0.0 | - |
| | 115 | 34.25 ± 0.00 | 5.25 ± 0.00 | 17 ± 0.0 | 0.79 ± 0.0 |
| *T. atroviride* + prochloraz | 65 | 16.00 ± 0.00 | 3.00 ± 0.00 | - | - |
| | 95 | 32.00 ± 0.00 | 6.00 ± 0.00 | 11 ± 0.0 | - |
| | 115 | 35.50 ± 0.00 | 7.00 ± 0.00 | 19 ± 0.0 | 0.81 ± 0.0 |
| FOL | 65 | 8.00 ± 0.00 | 0.00 ± 0.00 | - | - |
| | 95 | 12.00 ± 0.00 | 0.00 ± 0.00 | - | - |
| | 115 | 12.00 ± 0.00 | 0.00 ± 0.00 | - | - |
| Control treatment | 65 | 13.00 ± 0.00 | 3.00 ± 0.00 | - | - |
| | 95 | 22.10 ± 0.00 | 3.00 ± 0.00 | 8 ± 0.0 | - |
| | 115 | 27.00 ± 0.00 | 4.00 ± 0.00 | 13 ± 0.0 | 0.40 ± 0.0 |

±, Standard error of mean of three replicates (SEM); -, Not recorded; *Yield in terms of number of fruits only; **Weight taken when seeds were ready for harvesting; Data were statistically analyzed which were found to be significant ($P < 0.05$)

terms of all the four parameters viz, plant height, no. of branches/plant, no. of fruits/plant and weight of 100 dry seeds studied. An overall 2-folds increased fruit yield was observed under bioagent and fungicide treatments which were significantly higher than the control ($P < 0.05$). The FOL inoculated plants recorded high intensity of wilt, poor plant growth and no yield.

*Trichoderma* spp. are known for their antagonistic activity against the pathogenic soil microbes (Howell and Stipanovic, 1983). Inhibition of mycelial growth of *Fusarium oxysporum*, *Heterobasidion annosum* and *Phytophthora* spp. by non-volatile metabolites of *Trichoderma* spp. has been reported (Etebarian *et al*, 2000) and the reasons have been attributed to the production of substances such as antibiotics, toxins, etc. in the culture filtrates of the test microorganisms (Skidmore, 1976). It has been reported that *Trichoderma* spp. produce non-volatile substances such as Trichodermin which could be the cause of inhibition of the growth of FOL (Dennis and Webster, 1971). Vinale *et al*. (2006) reported that *T. harzianum* produced a metabolite, T22azaphilone(83), that inhibited the growth of *Rhizoctonia solani*, *Pythium ultimum* and *Gaeumannomyces graminis var. tritici*. *T. aggressivum* has been reported to produce an antifungal metabolite (3, 4-dihydro-8-hydroxy-3-methylisocoumarin) that inhibited the growth of *Agaricus bisporus* and other fungi (Krupke *et al*, 2003). John *et al*. (2004) studied the interaction between *T. harzianum* and *Eutypa lata*, the pathogen which causes dieback disease of grapevine and reported that the metabolites produced by *T. harzianum* reduced the growth of this test pathogen *in vitro*. Eziashi *et al.* (2006) tested the metabolites of *Trichoderma* spp. against *Ceratocystis paradoxa* and found them to be growth inhibitory. Narisawa *et al.* (2002) reported that *Verticillium dahliae* causing wilt disease of eggplant was suppressed by *Heterconium chaetospira, Phialocephala fortinii, Penicillium* sp. and *Trichoderma* sp. *T. harzianum, T. viride* and *T. virens* have been found to suppress the mycelial growth of *Fusarium oxysporum* f. sp. *ciceris* and enhance the growth and yield of this crop plant (Dubey *et al*, 2007).

Suppression of deleterious microorganisms by the fungicides and their stimulatory effect on the growth and yield of plants has been reported (Nasir, 2003; Trybom and Jeschke, 2008). Bharath *et al*. (2005) studied the effect of Topsin and dithane M-45 on the growth and yield of watermelon (*Citrullus lanatus*) and reported that these fungicides suppressed the fungal pathogens and significantly enhanced the growth and yield of watermelon. Azoxystrobin has been reported to enhance the growth and yield of rice (Groth, 2008). The present study reveals that the antagonistic activity

of *T. atroviride* against the test pathogen and its growth promoting ability increased when applied in combination with the fungicide. This might be due to combined effect of the treatments (Adhilakshmi *et al*. 2008). *T. atroviride* therefore, hold potential for use as an alternative to the hazardous chemicals for sustainable agriculture.

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# Compatibility of *Trichoderma* spp. isolates with plant extracts

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**ABSTRACT**

Five species of *Trichoderma* namely, *T. harzianum*, *T.viride*, *T. aureoviride*, *T.koningi* and *T. pseudokoningii* were tested for their compatibility against various plant extracts of onion, garlic and neem leaf extracts in 5, 10 and 15 per cent concentrations. T. harzianum isolates Th3 and Th1 were found the most compatible with onion extract at 5 and 10 percent, respectively. But isolates of T.harzianum Th6 and Th7, T.viride, T.koningii, T.aureoviride and T.pseudokoningii were found incompatible with 10 and 15 per cent concentration of onion extract. At 10 percent concentration, T. harzianum isolate Th 1 was more compatible with onion leaf extract followed by garlic and neem extract. The neem extract at all concentration levels was incompatible with all the species of Trichoderma.

**Key words**: *Trichoderma*, plant extract, onion, garlic, neem, compatibility

*Trichoderma* is an exceptionally good model of biocontrol agent as it is ubiquitous, easy to isolate and culture, multiply rapidly on many substrates. There are several mechanisms involved in *Trichoderma* antagonism namely antibiosis whereby the antagonistic fungus shows production of volatile metabolites including ethylene and acetone as well as diffusible antibiotics (Dennis and Webster, 1971), competition for nutrients, the ability to colonize the ecological niche favoured by the pathogen (Papavizas, 1985) and mycoparasitism, one of the major mechanisms involved in the antagonistic activity, able to degrade phytopathogens cell walls. The antagonistic *Trichoderma* induces the production of extracellular hydrolytic enzymes responsible for the direct attack against the pathogen (Lorito et al., 1993). This is known to produce relatively high concentrations of cell wall degrading enzymes as β-1-3 gluconase and different chitinolytic enzymes (Haran et al., 1996). Synthetic chemicals being hazardous to health the use of plant extracts along with *Trichoderma* spp. forms sustainable environment friendly methods for disease management. Therefore, there was need to look for compatible strains of *Trichoderma* spp. with plant extracts and exploit their bioactive potential. Antifungal activity of neem leaves, garlic and onion bulbs extracts at 5, 10 and 15 percent concentrations was studied against isolates of *Trichoderma* spp.

## MATERIALS AND METHODS

### Collection of soil and plant samples

Fifty soil and roots samples were collected from established castor field plots of different locations of Patan and Banaskantha districts where the castor is commonly cultivated (Table 1). Healthy plant of castor (Ricinus communis L.) of 60 to 75 days growth were carefully uprooted alongwith adhering soil and was carried to the laboratory in polythene bags. The soil particles loosely adhering to the roots were gently teasted out and used for isolation of rhizosphere fungi.

### Isolation of isolates of Trichoderma spp.

The isolation of *Trichoderma* spp. were made by soil dilution plate technique (Johnson and Curl, 1972). From each soil sample, 10 g of closely associated rhizosphere/ rhizoplane soil was mixed thoroughly with 90ml sterile distilled water to prepare stock solution and serially diluted up to $10^{-5}$. One ml suspension from the soil dilutions were plated on solidified *Trichoderma* selective medium (TSM) and gently shaken to spread evenly. These petriplates were incubated at $28^0 \pm 1^0$C temperature for one week with periodic observation for the development of colonies of *Trichoderma* spp. The early growing colonies with different morphology were critically examined, picked-up and transferred to potato dextrose agar slants. Finally, the cultures were purified and maintained on PDA slants at low temperature ($5^0$C) in refrigerator.

### Compatibility study of isolates of *Trichoderma* spp. with plant extracts

The effect of phytoextracts of three plant species belonging to different families was evalutated on the growth and sporulation of *Trichoderma* spp. in vitro by poison food technique. Healthy fresh leaves and bulbs were collected for evaluation of compatibility of *Trichoderma* spp. isolates at

desired concentration of 5.00, 10.00 and 15.00 per cent.

Fresh and healthy plant parts (100g) of each plant species were crushed in grinder by adding 100ml distilled water to obtain 1:1 extract. The material was homogenized for five minutes and filtered through double layer sterilized muslin cloth. The filtrates were centrifuged at 5000 rpm for 15 minutes. The clear supernatant collected was used as standard solution.

Desired concentrations (5.00, 10.00 and 15.00%) prepared by adding appropriate amount of standard solution of plant extract to 100ml PDA medium in 250 ml conical flasks was used for evaluation. 20ml extract mixed PDA medium were poured in sterilized petriplates.

Discs of 5 mm diameter were cut out from actively growing 3 day old isolates with the help of a sterile cork borer and transferred to the centre of petri plates containing the desired media inoculated with the different concentrations of plant extracts. The inoculated plates were incubated at 28 ± $1^0$C. Control plates without plant extracts were also inoculated and incubated simultaneously for comparison. Three replicates in PDA medium were maintained for each treatment including control.

The growth of colony in each treatment was measured in two directions at right angles to each other at 48h after treatment and per cent inhibition was calculated by using formula given by Sundar et al., 1995. Completely randomized and DMRT design were followed for the analysis of the data.

## RESULTS AND DISCUSSION

### Isolation of *Trichoderma* spp. isolates from rhizosphere

On the basis of early growing colonies of different morphology, twenty one isolates of *Trichoderma* spp. were obtained on *Trichoderma* Selective Medium (TSM) from the rhizosphere of castor plant by soil dilution plate technique (10-5 dilution) after incubation period of one week at $28^0$ ± $1^0$C. Out of 20 soil sample collected from ten villages of Patan district, 8 isolates of *Trichoderma* species were obtained. Whereas, 13 isolates were gained from 30 soil sample from seven villages of Banaskantha district (Table-1). Over all, 21 isolates were obtained from 50 soil samples. The results are in accordance with the methodology adopted by Sivan and Chet (1989) and Kapil and Kapoor (2005). Various species of *Trichoderma* were identified with the help of their morphology and microscopic structure. Out of twenty one isolates, seven isolates of *T. harzianum*, five of *T. viride*, four of *T. koningii*, three of *T. aureoviride* and two *T. pseudokoningii* were obtained.

**Table 1:** Designation of isolates of *Trichoderma* species.

| Place from isolate obtained | Isolates of | Isolate designated as |
|---|---|---|
| Patan | *T.harzianum* | $Th_1$ |
| Anavada | *T. harzianum* | $Th_2$ |
| Charup | *T. harzianum* | $Th_3$ |
| Sardarkrushinagar | *T. harzianum* | $Th_4$, $Th_5$ |
| Chandisar | *T. harzianum* | $Th_6$ |
| Deesa | *T. harzianum* | $Th_7$ |
| Khado | *T. viride* | $Tv_1$ |
| Sardarkrushinagar | *T. viride* | $Tv_2$ |
| Ganeshpura | *T. viride* | $Tv_3$ |
| Chandisar | *T. viride* | $Tv_4$ |
| Deesa | *T. viride* | $Tv_5$ |
| Matarvadi | *T. koningii* | $Tk_1$ |
| Sardarkrushinagar | *T. koningii* | $Tk_2$ |
| Rajpur | *T. koningii* | $Tk_3$ |
| Vaghrol | *T. koningii* | $Tk_4$ |
| Patan | *T. aureoviride* | $Ta_1$ |
| Chandisar | *T. aureoviride* | Ta2 |
| Ganeshpura | *T. aureoviride* | $Ta_3$ |
| Brahmanvada | *T. speudokoningii* | $Tpk_1$ |
| Sardarkrushinagar | *T. speudokoningii* | $Tpk_2$ |

### Compatibility study of isolates of Trichoderma spp. with plant extracts

The effect of phytoextracts on the growth of *Trichoderma* isolates are presented in table-2. The results revealed that neem leaves, garlic and onion bulb extracts significantly reduced the growth of isolates of *Trichoderma species*. When the lowest concentration (5%) of plant extract of onion, garlic and neem was tested against different isolates of *Trichoderma spp*. *T. harzianum* isolate Th3 showed maximum growth of 81.00 mm and 79.33 mm against onion and garlic extract with minimum inhibition of 7.95 percent and 9.51 percent, respectively. At the same concentration, neem extract inhibited growth of Th3 isolate by 23.88 percent. It shows that onion and garlic extract at 5 percent concentration are more compatible with *T.harzianum* isolate $Th_3$ than neem extract at same concentration. At the same time, onion and garlic extract at 5.0 per cent was incompatible with other isolates of *T.koningii, T.aureoviride*, *T. pseudokoningii*. At 10 percent concentration, *T. harzianum* isolate Th1, showed maximum compatibility with onion extract inhibiting growth of Th1 isolate by 21.13 and 31.58 per cent, respectively. Thus at 10% concentration, Th1 isolate showed maximum compatibility with onion extract. Isolates of *T.harzianum* Th6 and Th7, *T.viride, T.koningii, T.aureoviride* and *T. pseudokoningii* were found incompatible with 10 per cent

**Table 2:** Effect of plant extracts on *Trichoderma* isolates:

| Isolats | Onion | | | Garlic | | | Neem | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5% | 10% | 15% | 5% | 10% | 15% | 5% | 10% | 15% |
| $Th_1$ | *80.33$^{ab}$ | 75.57$^{a}$ | 59.67$^{a}$ | 78.33$^{a}$ | 69.67$^{abc}$ | 58.00$^{a}$ | 67.67$^{abcde}$ | 60.67$^{a}$ | 49.00$^{a}$ |
| | **(9.47) | (14.33) | (31.94) | (10.65) | (21.13) | (34.59) | (24.25) | (31.58) | (44.53) |
| $Th_2$ | 79.67$^{abc}$ | 71.67$^{bc}$ | 59.37$^{ab}$ | 73.67$^{bcdefg}$ | 68.67$^{abcd}$ | 52.33$^{bcde}$ | 70.67$^{a}$ | 60.00$^{ab}$ | 46.67$^{ab}$ |
| | (9.46) | (18.86) | (31.94) | (15.97) | (22.26) | (39.86) | (20.89) | (32.33) | (47.16) |
| $Th_3$ | 81.00$^{a}$ | 75.00$^{ab}$ | 60.00$^{a}$ | 79.33$^{a}$ | 66.33$^{abcdef}$ | 54.33$^{abc}$ | 68.00$^{abcde}$ | 59.67$^{ab}$ | 41.33$^{cd}$ |
| | (7.95) | (15.09) | (31.56) | (9.51) | (24.91) | (38.73) | (23.88) | (32.71) | (53.21) |
| $Th_4$ | 78.67$^{abcd}$ | 71.00$^{cd}$ | 59.33$^{ab}$ | 76.33$^{abcde}$ | 70.00$^{ab}$(20.7 | 55.33$^{ab}$ | 64.333$^{defgh}$ | 58.67$^{abc}$ | 42.67$^{bc}$ |
| | (10.60) | (19.62) | (32.33) | (12.93) | 5) | (37.60) | (27.99) | (33.83) | (51.69) |
| $Th_5$ | 79.00$^{abcd}$ | 70.00$^{cd}$ | 57.33$^{abc}$ | 72.67$^{defghi}$ | 71.00$^{a}$ | 54.33$^{abc}$ | 68.67$^{abc}$ | 56.67$^{vcd}$ | 42.67$^{bc}$ |
| | (10.23) | (20.75) | (34.61) | (17.11) | (19.62) | (38.73) | (23.13) | (36.09) | (51.69) |
| $Th_6$ | 77.67$^{abcde}$ | 69.67$^{cd}$ | 53.00$^{de}$ | 73.33$^{cdefgh}$ | 65.00$^{cdefg}$ | 55.33$^{ab}$ | 68.67$^{abc}$ | 53.33$^{defgh}$ | 39.67$^{cde}$ |
| | (11.74) | (21.13) | (39.55) | (16.36) | (26.41) | (37.60) | (23.13) | (39.86) | (55.09) |
| $Th_7$ | 77.00$^{abcdef}$ | 69.00$^{cde}$ | 53.33$^{cde}$ | 76.33$^{abcde}$ | 66.000$^{bcdef}$ | 53.67$^{abcd}$ | 68.00$^{abcde}$ | 60.00$^{ab}$ | 39.67$^{cde}$ |
| | (12.5) | (21.88) | (39.17) | (12.93) | (25.28) | (39.47) | (23.88) | (32.33) | (55.09) |
| $Tv_1$ | 76.33$^{bcdef}$ | 68.67$^{cde}$ | 52.67$^{de}$ | 78.00$^{ab}$ | 61.67$^{fgh}$ | 52.00$^{bcdef}$ | 46.67$^{cdefgh}$ | 54.33$^{def}$ | 38.67$^{cde}$ |
| | (13.26) | (22.26) | (39.92) | (11.03) | (30.18) | (41.36) | (27.61) | (38.73) | (56.22) |
| $Tv_2$ | 77.00$^{abcdef}$ | 68.33$^{cdef}$ | 52.67$^{de}$ | 76.00$^{abcde}$ | 64.67$^{defg}$ | 52.00$^{bcdef}$ | 69.67$^{ab}$ | 54.00$^{defg}$ | 40.33$^{cde}$ |
| | (12.5) | (22.64) | (39.92) | (13.31) | (26.79) | (41.36) | (22.01) | (39.10) | (54.34) |
| $Tv_3$ | 76.00$^{cdefg}$ | 68.33$^{cdef}$ | 53.00$^{de}$ | 77.00$^{abcd}$ | 67.33$^{abcde}$ | 52.00$^{bcdef}$ | 68.33$^{abcd}$ | 52.67$^{efgh}$ | 41.00$^{cd}$ |
| | (13.64) | (22.64) | (39.55) | (12.17) | (23.97) | (41.36) | (23.51) | (40.60) | (53.58) |
| $Tv_4$ | 75.33$^{defgh}$ | 69.33$^{cd}$ | 53.33$^{cde}$ | 77.67$^{abc}$ | 61.67$^{fgh}$ | 51.67$^{bcdef}$ | 64.67$^{cdefgh}$ | 55.67$^{cde}$ | 38.33$^{de}$ |
| | (14.40 | (21.51) | (39.17) | (11.41) | (30.18) | (41.73) | (27.61) | (37.22) | (56.61) |
| $Tv_5$ | 76.67b$^{cdef}$ | 69.33$^{cd}$ | 50.67$^{e}$ | 75.67$^{abcdef}$ | 65.67$^{bcdefg}$ | 50.33$^{cdef}$ | 67.00$^{abcdef}$ | 50.33$^{h}$ | 38.67$^{cde}$ |
| | (12.88) | (21.51) | (42.22) | (13.69) | (25.65) | (43.24) | (24.99) | (43.24) | (56.22) |
| $Tk_1$ | 76.00$^{cdefg}$ | 70.00$^{cd}$ | 55.33$^{bcd}$ | 72.00$^{efghi}$ | 62.67$^{efgh}$ | 49.00$^{efg}$ | 64.00$^{efgh}$ | 51.67$^{fgh}$ | 41.33$^{cd}$ |
| | (13.64) | (20.75) | (36.89) | (17.87) | (29.06) | (44.74) | (28.36) | (41.73) | (53.21) |
| $Tk_2$ | 72.33$^{ghi}$ | 67.67$^{def}$ | 53.00$^{de}$ | 70.33$^{ghi}$ | 62.00$^{fgh}$ | 49.33$^{defg}$ | 66.00$^{bcdefg}$ | 50.67$^{gh}$ | 36.33$^{ef}$ |
| | (17.81) | (23.39) | (39.55) | (19.78) | (29.81) | (44.37) | (26.12) | (42.86) | (58.87) |
| $Tk_3$ | 70.00$^{i}$ | 65.00$^{fg}$ | 54.00$^{cde}$ | 71.33$^{fghi}$ | 61.67$^{fgh}$ | 50.33$^{cdef}$ | 67.33$^{abcde}$ | 52.00$^{fgh}$ | 34.00$^{f}$ |
| | (20.45) | (26.41) | (38.41) | (18.64) | (30.18) | (43.24) | (24.63) | (41.36) | (61.51) |
| $Tk_4$ | 72.00$^{hi}$ | 65.67$^{efg}$ | 50.67$^{e}$ | 73.33c$^{defgh}$ | 62.67$^{efgh}$ | 50.67$^{cdef}$ | 68.67$^{abc}$ | 55.67$^{cde}$ | 40.000$^{cde}$ |
| | (18.18) | (25.65) | (42.22) | (16.36) | (29.06) | (42.86) | (23.13) | (37.22) | (54.72) |
| $Ta_1$ | 73.33$^{fghi}$ | 65.00$^{fg}$ | 53.67$^{cde}$ | 73.67$^{bcdefg}$ | 63.33efgh | 50.00$^{cdef}$ | 61.33$^{h}$ | 50.67$^{gh}$ | 40.00$^{cde}$ |
| | (16.67) | (26.41) | (38.78) | (15.97) | (28.30) | (43.61) | (31.34) | (42.86) | (54.72) |
| $Ta_2$ | 75.33$^{defgh}$ | 61.00$^{h}$ | 51.00$^{e}$ | 69.67$^{ghi}$ | 60.00$^{h}$ | 48.67$^{fg}$ | 62.67$^{gh}$ | 51.33$^{fgh}$ | 41.00$^{cd}$ |
| | (14.40) | (30.94) | (41.83) | (20.53) | (32.07) | (45.11) | (29.84) | (42.11) | (53.58) |
| $Ta_3$ | 73.67$^{efghi}$ | 62.67$^{gh}$ | 51.67$^{de}$ | 68.67$^{i}$ | 61.00$^{gh}$ | 48.33$^{fg}$ | 61.67$^{h}$ | 52.67$^{efgh}$ | 41.00$^{cd}$ |
| | (16.28) | (29.05) | (41.06) | (21.67) | (30.94) | (45.49) | (30.96) | (40.60) | (53.58) |
| $Tpk_1$ | 76.00$^{cdefg}$ | 64.33$^{g}$ | 53.67$^{cde}$ | 69.00$^{hi}$ | 61.33$^{gh}$ | 45.67$^{g}$ | 62.67$^{gh}$ | 51.33$^{fgh}$ | 38.00$^{de}$ |
| | (13.64) | (27.17) | (38.78) | (21.30) | (30.57) | (48.49) | (29.84) | (42.11) | (56.98) |
| $Tpk_2$ | 72.33$^{ghi}$ | 61.00$^{h}$ | 51.00$^{e}$ | 69.33$^{ghi}$ | 59.67$^{h}$ | 49.67$^{defg}$ | 63.00$^{fgh}$ | 51.33$^{fgh}$ | 36.67$^{ef}$ |
| | (17.81) | (30.94) | (41.83) | (20.92) | (32.45) | (43.98) | (29.47) | (42.11) | (58.49) |
| Control | 88.00$^{i}$ | 88.33$^{i}$ | 87.67$^{f}$ | 87.67$^{j}$ | 88.33$^{i}$ | 86.67$^{h}$ | 88.33$^{i}$ | 88.67$^{i}$ | 88.33$^{g}$ |

* Growth in mm
** % Inhibition

Treatments means with the letter/letters in commom are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance

concentration of onion extract. Similarly, onion extract at 15 percent concentration was most compatible with Th1 isolate. Observations showed that neem extract was most imcompatible with all the extracts as it did not allow much growth of any of the isolate at any concentration. This is in contrast with the results of Bagwan (2010) who observed that neem oil (5%) neem, leaves extract (10%), neem cake, castor cake and mustard cake extract (10%) enhanced the

*Trichoderma* growth. *T. harzianum* isolates Th3 and Th1 were found most compatible with onion extract at 5 per cent and 10 per cent, respectively. Similar findings were observed by Bourguignon, E. (2008) who reported that dry onion scale residues had no antifungal activity against *Trichoderma* or *Penicillium* and instead tended to promote their hyphal growth and sporulation. The average per cent inhibition in mycelial growth of isolated species indicated that all the five species were more or less similar regarding sensitivity towards tested plant extract. The results are in agreement with the reports related to screening and reviewing of plant extracts for antimicrobial activities (Kurucheve *et al*., 1997: Sharma, 1998; Sengupta *et al*., 2004 and Bohra *et al*., 2006).

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# Study of osmotolerant yeast isolted from spoiled aonla segments in syrup

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**ABSTRACT**

The osmophilic budding spoilage yeast (*Saccharomyces sp.*), isolated from spoiled commercial aonla segments in sugar syrup having TSS $70^0$B, was studied for sugar and heat tolerance, acid and preservative resistance and invertase production properties. The yeast exhibiting high invertase activity recorded sugar and acid tolerance by $70^o$B and 1.2 per cent, respectively and resistant to preservative up to 800 ppm KMS. The yeast could however be controlled by proper heat treatment (boiling for 10 minutes at $100^0$C) of spoiled aonla segments.

Aonla or Indian gooseberry (*Embellica officinalis* Gaertn), known for its medicinal and therapeutic properties in India since ancient times, is considered to be a wonder fruits for health conscious population. Its fruit is one of the richest known natural sources of vitamin C. While raw fruits are used for making pickles and preserves like murrabba, candy etc., the sugar syrup is very popular because of good taste and high neutraceutical value (Durrani and Verma, 2011).Since aonla segments have TSS as high at $55^0$ B and acidity around 1.5%, it is supposed to be unfavourable for the growth of most of microbes. However, these products some times get spoiled due to growth of osmophilic yeast and mould which deteriorate the market value of the product leading to economic loss to the processor. One such yeast, *Saccharomyces sp*., isolated from spoiled aonla segments in syrup was studied for its sugar and acid tolerance, preservative resistance and invertase production properties.

## MATERIALS AND METHODS

The osmophilic spoilage yeast was isolated from commercial sample of spoiled aonla segments in syrup. The cultures were isolated, purified and maintained on MY 40 (Malt Yeast Extract Agar with 40% Glucose) slants as per method described by Speck, 1985. It was identified on morphological, microscopic and biochemical basis (Rose and Harison, 1969).

**Sugar tolerance test** : Sugar solutions having final concentration of 20, 40, 60 and $70^0$ B having 0.5% acidity were prepared.

**Preparation of solutions for acid tolerance**: Sugar solution ($50^0$ B) was acidified with citric acid with final acidity 0.3, 0.5, 0.75, 1.0 and 1.25%.

**Preparation of solutions for preservative resistance**: To sugar solution ($50^0$B and 0.5% acidity, preservative was added @ 200, 400, 600 and 800 ppm concentration in the form of potassium meta bisulphite (KMS).

One hundred ml of said solutions taken in 250 ml capacity glass bottles were autoclaved in triplicate for each treatment along with control. The bottles were inoculated with $1.0 \times 10^6$ cfu (colony forming units) / ml of yeast isolate and kept for incubation at $35 \pm 2^0$C. After one and two months of storage, changes in pH, T.S.S, reducing sugar, non-reducing sugar, total sugar and acidity were observed as per method of AOAC (1984). Microbial growth was monitored visually in terms of turbidity and bubble formation.

**Determination of heat resistance:**

Preparation of sugar solutions – Glass tubes containing 5 ml of $50^0$B sucrose solution were inoculated with 0.1 ml of appropriately diluted yeast isolate and kept in a boiling water bath. Duplicate tubes were withdrawn at regular time intervals and plated on MY-40 agar media. The plates were incubated at $35 \pm 2^0$C for 72 hr and surviving yeast population were counted.

**Determination of invertase activity :**

100 ml sucrose solution $40^0$B and 0.5% acidity was inoculated with $1.0 \times 10^6$ cfu (colony forming units) / ml of yeast. Control was kept for each treatment. These bottles were

**Table 1:** Sugar tolerance study : Changes in biochemical constituents after two month of inoculation with *Saccharomyces sp*.

| Treatment | Initial TSS ($^0$B) | One month TSS($^0$B) | Two months TSS($^0$B) | Initial Acidity (%) | After two month Acidity (%) | After two months Acidity (%) | Initial R.S. g(%) | After One month R.S. g(%) | After Two months R.S. g(%) | Initial T.S. g(%) | After One month T.S. g(%) | After Two months T.S. g(%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T1 | 20 | 6.0 | 8.4 | 0.5 | 0.64 | 0.96 | 9.8 | 2.0 | 6.1 | 18.3 | 12.0 | 8.0 |
| T2 | 40 | 27 | 11 | 0.5 | 0.96 | 1.43 | 10.2 | 10.1 | 19.7 | 37.9 | 29.1 | 12.3 |
| T3 | 60 | 59 | 58.5 | 0.5 | 0.64 | 0.63 | 10.6 | 9.7 | 32.6 | 58 | 56.7 | 47.3 |
| T4 | 70 | 68 | 67.5 | 0.5 | 0.49 | 0.49 | 12.0 | 10.9 | 33.2 | 66.8 | 64.2 | 55.4 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CD at p = 0.01 | | | | CD at p = 0.01 | | | CD at p = 0.01 | | | CD at p = 0.01 | | |
| Due to treatments | = | 0.09 | | Due to treatments | = | 0.19 | Due to treatments | = | 6.6 | Due to treatments | = | 0.10 |
| Due to months | = | 0.08 | | Due to months | = | 0.16 | Due to months | = | 5.7 | Due to months | = | 0.08 |
| Due to (Treatments x Months) | = | 0.16 | | Due to (Treatments x Months) | = | NS | Due to (Treatments x Months) | = | NS | Due to (Treatments x Months) | = | 0.17 |

**Table 2:** Sugar tolerance study of spoilage yeast: Visual growth observations

| Time period after inoculation | 20$^0$B | 40$^0$B | 60$^0$B | 70$^0$B |
|---|---|---|---|---|
| **After 3 weeks** | +++ | ++ | - | - |
| **After 4 weeks** | +++ | +++ | + | - |
| **After 5 weeks** | +++ | +++ | + | - |
| **After 7 weeks** | +++ | +++ | + | - |

+++ very good growth
++ good growth
+ poor growth
- no visual growth symptoms
As indicated by turbidity and gas produced yeast.

kept for incubation under ambient condition. After ten days, the contents were filtered, centrifuged at 5000 RPM for 10 min and the supernatant was assayed for invertase activity.

**Characterization of Invertase :**

**Enzyme assay**

The soluble invertase was assayed in 1ml reaction volume containing 0.2 ml enzyme and 0.8ml of 2.5% sucrose in 0.1M sodium acetate buffer (pH-5.0). The amount of reducing sugars in the supernatant was estimated with dinitrosalycylic acid (Miller, 1972). For enzyme kinetic study, sucrose concentrations (0.1 – 1.0% w/v in 0.1 M Sod, acetate buffer, pH 5.0), pH (3.5 – 7.0), temperatures (30-70$^0$C), were used as variables. The reaction mixture was incubated for 30 min and then assayed for invertase activity. One unit of invertase is the amount of enzyme that produced one µmol of glucose $min^{-1}$ $ml^{-1}$ under assay conditions. Protein in the supernatant was measured by the method of Lowry *et al.* (1951). For partial purification, enzyme in the culture filtrate was precipitated by centrifugation at 14000 rpm using cold acetone and then dried under vacuum to eliminate acetone residue and then dissolved in acetate buffer (pH 5.0) for determining the enzyme activity.

**RESULTS AND DISCUSSION**

The isolated microbial isolate was found to be a budding yeast and identified as *Saccharomyces sp*. The results indicated that the isolated yeast is a potent spoilage micro-organism responsible for spoilage of anola segments. Analysis of syrup revealed production of 2 per cent alcohol in the spoiled fermented syrup. It is tolerant to sugar and acid and resistant to preservative. The sugar tolerance study showed TSS in all the treatments while the acidity increases (Table 1, 3 and 5). Visual growth observation showed that at 20$^0$ and 40$^0$ B and 60$^0$ B, after three weeks onward, there was poor growth (Table 2 and 4). However, at 70$^0$B upto seven weeks of observation, there was no visual growth. Plate 1 shows the spoiled commercial anola murabba samples showing frothing.

The yeast isolate was found to be preservative tolerant as it could grow at all tested preservation levels. Plate 2 depicts the heat resistance potential of yeast isolates. It was quite heat tolerant as it could survive boiling for more than 5 min. D100 value was found to be 5.4 min (Fig.1) Initially (upto 1.5 min). the heat killing was faster, which might be due to the fact that vegetative cells are more heat labile as compared to ascospores which survive higher temperature for longer time.

Splittstoesser *et al*. (1986) have reported that the ascospores of *Saccharomyces cerevisiae* are over 100-fold more resistant than vegetative cells of the same strain. Put and De jong (1982) reported that the vegetative cells of *Saccharomyces* sp. showed a low heat resistance, with a $D_{60}$ value of 0.1- 0.3 min. Ascospores were more resistant. For three isolates, the $D_{60}$ value for ascospores ranged from 8 to 14 min. Since the yeast isolate had shown higher heat resistance, the only way to control this yeast is by heat treatment up to 10 min. Scott and Bernard (2006) have reported the heat resistance of 10 strains of yeast strains. Three of these strains showed little

**Table 3:** Acid tolerance study: Changes in biochemical constituents after two month of inoculation with yeast.

| | 0.3% acidity | | 0.5% | | 0.75% | | 1% | | 1.25% | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Inoculated | control | Inoculated | Control | Inoculated | Control | Inoculated | Control | Inoculated |
| T.S.S ($^0$B) | 50 | 37.5 | 50 | 38.5 | 50 | 39.5 | 50 | 40 | 50 | 48 |
| pH | 3.37 | 3.30 | 3.18 | 3.17 | 3.06 | 3.17 | 2.96 | 2.98 | 2.90 | 2.90 |
| Acidity (%) | 0.3 | 0.70 | 0.5 | 0.89 | 0.75 | 1.47 | 1.0 | 1.21 | 1.25 | 1.85 |
| Reducing Sugar (%) | 25.28 | 19.21 | 25.35 | 21.54 | 25.58 | 21.21 | 23.78 | 20.31 | 23.41 | 20.68 |
| Total sugar (%) | 30.41 | 31.20 | 27.54 | 27.70 | 19.26 | 34.07 | 22.29 | 31.04 | 36.93 | 34.23 |

**Table 4 :** Acid tolerance study of spoilage yeast : Visual growth observations

| Time period after inoculation | 0.3% | 0.5% | 0.75% | 1.0% | 1.25% |
|---|---|---|---|---|---|
| **After 3 weeks** | ++ | + | + | + | - |
| **After 4 weeks** | ++ | ++ | + | + | - |
| **After 5 weeks** | +++ | +++ | ++ | + | - |
| **After 7 weeks** | +++ | +++ | ++ | ++ | - |

+++ very good growth
++ good growth
+ poor growth
- no visual growth symptoms
As indicated by turbidity and gas produced yeast.

**Table 5 :** Preservative resistance study : Changes in biochemical constituents and growth after two month of inoculation with osmophilic yeast.

| | Zero time | After two months | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 200 | 400 | 600 | 800 |
| **TSS** | 40 | 40 | 33 | 32 | 34 | 35 |
| **Acidity** | 0.5 | 0.96 | 0.53 | 0.63 | 0.63 | 0.65 |
| **Total sugar** | 36.56 | 29.56 | 33 | 32 | 34 | 35 |
| **pH** | 28 | 2.7 | 2.73 | 2.75 | 2.76 | 2.79 |
| **Visible growth** | - | +++ | + | + | + | + |

- No growth
+ Poor but visible growth
+++ Very good growth
As indicated by turbidity and gas produced yeast.

**Table 6:** Production of invertase

| Micro-organisms | Enzyme activity (uM/ml/min) | Protein (mg/ml) | Specific activity (U/g) |
|---|---|---|---|
| Yeast | 0.031617 | 3.75 | 8.4312 |
| Fungus | 0.002581 | 6.625 | 0.389585 |

**Plate 1 :** Commercial aonla murabba

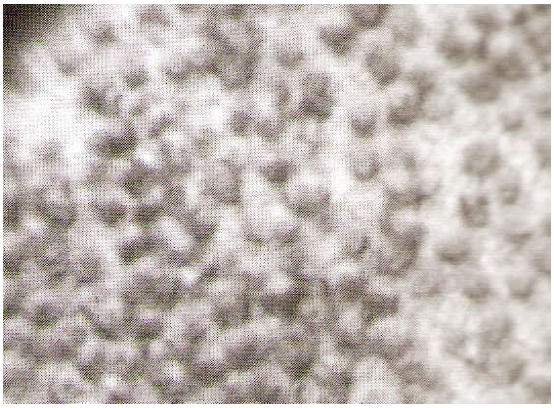

**Plate 2:** *Saccharomyces sp.*

survival at 110$^0$C, four other strains had Duo-c value between 1 and 4 min. *Torulopsis glabrata* had a $D_{126.7}{}^0$C of 0.78 min. *Saccharomyces* strains showed the highest dry heat resistance, with the most heat resistance strain tested having a $D_{126.7}{}^0$ of 5min. Partially purified enzyme extract obtained from culture filtrate of yeast isolate was found to have invertase activity (0.031617 uM/ml/min) and specific activity 8.4312 U/g. The temperature and pH for maximum enzyme activity were optimized. Maximum invertase activity (0.011564 uM/ml/min) was observed at 40$^0$C and pH 4.5 (0.023243 uM/ml/min). The $K_M$ and Vmax values for invertase were 5.472 mg/ml and 9.163103 uM/ml/min (Table 6), respectively.

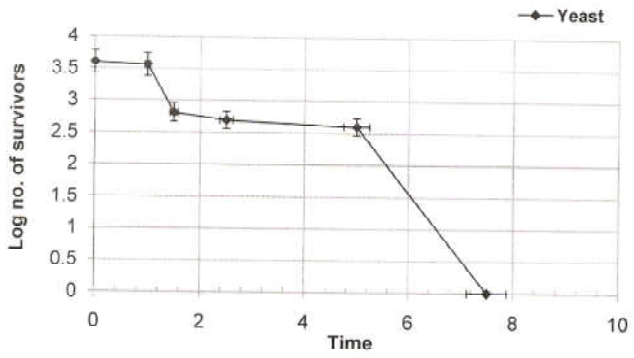


**Fig. 1 :** Survival of isolates at 100°C

The isolated osmophilic spoilage yeast *Saccharomyces sp.*, proved to be a potent spoilage micro-organism posing a potential threat to aonla segment processing industry being sugar and acid tolerant, preservative resistant and with invertase activity. The only way to control this yeast is by way of proper heat treatment up to 10 min at 100%.

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# Development and storage stability of whey blended papaya fruit beverage

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**ABSTRACT**

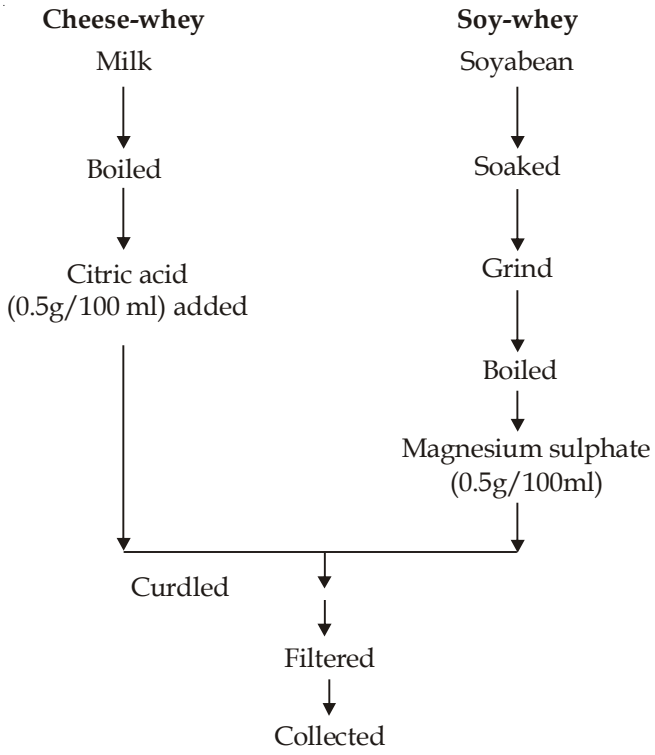
Papaya squash prepared by blending fruit pulp with cheese-whey and soy-whey (50 : 50) and stored at ambient temperature for a period of three months was tested for chemical and organoleptic changes during the storage period. Acidity and sugars increased with increase in storage period whereas, pH, TSS, ascorbic acid decreased. The soy-whey squash was found more acceptable by the consumer as compared to cheese whey.

India is the fourth largest producer of papaya (FAO, 1991) with an output of about 9.05 lakh tones (Chadha 1995). Though the fruit is nutritious, its products are not acceptable to many people due to odd flavour (Aruna 1955). The fresh papaya fruit is a good source of natural sugars, vitamin A, vitamin C besides having fair amount of calcium and phosphorous. It is, therefore, imperative to develop recipes to produce squash from ripe papaya fruit by addition of whey, instead of water, to further supplement the nutritional value.

The beverage industry is by far the largest outlet for fruit juice and concentrates absorbing over 80 per cent of production. In India, a little over 60 per cent of the fruit produced is used in fruit based beverages. Now-a-days there is increased passion for natural juices over the synthetic ones as they are the concentrated source of nutrients. Papaya (*Carica papaya* L.) belonging to the family caricaceae, is predominantly dioecious, and native of tropical America and all the other tropical regions of the world. India's climatic conditions are highly suitable for its cultivation well around the year. The world's annual production is 55 lakh tones while in India, it is estimated at about 4.5 lakh tones from an area of 40,000 hectares.

## MATERIALS AND METHODS

Efforts were made to standardize cheese-whey and soy-whey for which different coagulants were tried at different levels. These were obtained by curdling milk and soymilk by using citric acid and magnesium sulphate as per following flow chart:




Flowchart for the preparation of whey

Papaya squash was prepared as per following flow chart:

Sugar and 50 ml whey (cheese-whey/soy-whey)
↓
Heated for 2-3 minutes
↓
Stirred continuously
↓
Removed scum (with the help of ladle)
↓
Allowed to cool
↓
Added 50 ml fruit juice
↓
Added sodium benzoate (0.25 g/100ml)
↓
Transferred into sterilized bottles

Flow chart for the preparation of papaya squash

The beverage squash so prepared was evaluated for pH, TSS, acidity, ascorbic acid, total sugars, reducing sugars and non-reducing sugars.

## RESULTS AND DISCUSSION

### Chemical Parameters:

**pH**: The initial pH of cheese-whey (4.30) reduced to 4.25 after a gap of 90 days, whereas, in case of soy-whey squash it was 4.28, 4.26, 4.24 and 4.23 at 0, 30, 60 and 90 days, respectively (Table -1). The higher pH values obtained at 0 days, decreased significantly with storage periods. This could be attributed due to chemical reactions, such as Maillard's reaction, occuring during storage leading to the production of organic acids. This may be due to the breakdown of ascorbic acid into dehydro-ascrobic acid; $SO_2$ to sulphurous acid and pectin to pectic acid. Similar results are reported by Hassan and Ahmed 1998; Sogi and Singh 2001).

**TSS**: The TSS of cheese-whey squash was 43.50, 42.60, 41.40 and 40.47 $^0$Brix at 0, 30, 60 and 90 days, respectively, while these values in case of soy-whey squash were 42.67, 41.53, 40.47 and 39.53 $^0$Brix (Table – 1). The values of cheese-whey squash were more as compared to soy-whey squash. The decrease in TSS during storage might be due to solubilization of insoluble portion of the product (pulp) because of presence of acids (ascorbic and citric) during storage period as reported by Sirohi *et al.* (2005). There no report on effect of storage period on TSS in case of papaya. However, similar results have been reported by Sogi and Singh (2001) in case of kinnow beverage.

**Acidity :** The acidity in cheese-whey squash was 0.19, 0.25, 0.30 and 0.36 per cent, whereas, in soy-whey squash it was 0.17, 0.23, 0.28 and 0.33 per cent at 0, 30, 60 and 90$^{th}$ days of storage, respectively (Table-1). The acidity in cheese-whey was more in comparison to soy-whey at all the storage periods. The increase may be due to the conversion of lactose into lactic acid, formation of organic acids by ascorbic acid, $SO_2$ to sulphurous acid and breakdown of pectin to pectic acid inherently present in papaya pulp as reported by Sirohi *et al.* (2005). The increase in acidity during storage is also reported by Krishnaveni et al., 2001; Saravana and Manimegalai, 2005.

**Ascorbic acid :** At 0, 30, 60 and 90 days of storage its values were 50.18, 50.04, 49.97 and 49.91 and 49.07, 48.99, 48.97 and 48.90 per cent in cheese and soy-whey squashes, respectively. The ascorbic acid also showed declining trend during different storage intervals (Table-1). Ascorbic acid of the squashes followed a significant decreasing trend with the increase in the storage duration. The value of ascorbic acid in cheese-whey was more in comparison to soy-whey. The decrease in ascorbic acid during storage might be due to its breakdown to dehydro-ascorbic acid by the action of heat, air and light or its conversion to dehydro-ascorbic acid by its participation in browning. Another reason might be its more instability with increase in enxymatic and non-enzymatic oxidation which resulted in gradual but significant decrease in ascorbic acid content. Similar results were reported by Sood (2000) during storage of IMF fruits under different modes of packaging and Krishnaveni et al (2001) with the increase in storage intervals in jackfruit RTS.

### Nutritional Parameters

**Total Sugar**: Total sugar increased in the range of 37.15 to 40.45 per cent in cheese-whey and 26.64 to 29.54 per cent in case of soy-whey during storage period of 0 to 90 days (Table-2). The total sugar increased in cheese and soy-whey which might be due to the breakdown of polysaccharides like pectin and starch into simple sugars and higher fixed acidity might have been responsible for the purpose. No such reports are available in case of papaya. However, similar reports in case of bael fruit beverage by Singh and Nath, 2004 and in jackfruit beverage by Krishnaveni *et al* (2001) corroborate present findings.

**Reducing sugar**: Results showed gradual increase in reducing sugar from 20.46 to 22.03 per cent in cheese-whey and 20.48 to 22.03 per cent in soy-whey at 0, 30, 60 and 90$^{th}$ days of storage, respectively (Table-2). Reducing sugar also showed an increasing trend with the increase in storage duration. This might be due to the hydrolysis of non-reducing sugars that was positively correlated with the increase corresponded with the total sugars. No such work was done

**Table 1:** Effect of storage intervals on chemical parameters of papaya squash

| Treatments | Parameters | 0 days | 30 days | 60 days | 90 days |
|---|---|---|---|---|---|
| **Cheese whey** | pH | 4.30 | 4.28 | 4.26 | 4.25 |
| **Soy whey** | " | 4.28 | 4.26 | 4.24 | 4.23 |
| **Cheese whey** | TSS | 43.50 | 42.60 | 41.40 | 40.47 |
| **Soy whey** | " | 42.67 | 41.53 | 40.47 | 39.53 |
| **Cheese whey** | Acidity | 0.19 | 0.25 | 0.30 | 0.36 |
| **Soy whey** | " | 0.17 | 0.23 | 0.28 | 0.33 |
| **Cheese whey** | Ascorbic acid | 50.18 | 50.04 | 49.97 | 49.91 |
| **Soy whey** | " | 49.07 | 48.99 | 48.97 | 48.90 |

| CD at 5% | pH | TSS | Acidity | Ascorbic acid |
|---|---|---|---|---|
| **A** | 0.47 | 0.27 | 0.16 | 0.33 |
| **B** | 0.67 | 0.38 | 0.22 | 0.47 |
| **A X B** | 0.94 | 0.54 | 0.32 | 0.66 |

A : Cheese-whey; B: Soy-whey and A X B: Cheese-whey X Soy-whey

**Table 2 :** Effect of storage intervals on nutritional parameters of papaya squash

| Treatments | Parameters | 0 days | 30 days | 60 days | 90 days |
|---|---|---|---|---|---|
| **Cheese whey** | | 37.15 | 38.95 | 39.64 | 40.95 |
| **Soy whey** | Total sugars | 26.64 | 27.69 | 28.36 | 29.54 |
| **Cheese whey** | | 20.46 | 21.02 | 21.47 | 22.03 |
| **Soy whey** | Reducing sugars | 20.48 | 21.16 | 21.58 | 22.03 |
| **Cheese whey** | | 16.69 | 17.93 | 18.17 | 18.93 |
| **Soy whey** | Non-reducing sugars | 6.16 | 6.53 | 6.78 | 7.51 |

| CD at 5% | Total sugars | Reducing sugars | Non-reducing sugars |
|---|---|---|---|
| **A** | 0.11 | 0.53 | 0.10 |
| **B** | 0.15 | 0.75 | 0.15 |
| **A X B** | 0.21 | 0.10 | 0.21 |

A : Cheese-whey; B: Soy-whey and AXB: Cheese-whey X Soy-whey

earlier in case of papaya but reported in case of beverages of other fruits (Sethi, 1992, Krishnaveni et al., 2001: Bhatia and Chawla 2004)

**Non-reducing sugar**: The range of non-reducing sugar in cheese-whey based squash varied from 16.69 to 18.93 per cent, while in soy-whey squash it was in the range of 6.16 to 7.51 per cent at 0, 30, 60 and 90 days after storage (Table-2). The decrease in non-reducing sugars might be due to the inversion or hydrolysis of non-reducing sugars to reducing sugars. The same results are also reported by Sood (2000) and Rait (2003). All these observations are in perfect harmony to present findings.

**Organoleptic Scores:**

**Colour :** The colour score of soy-whey was better as compared to cheese-whey but decreased with the increase in storage period (Table-3). The cheese-whey scores were 6.70, 6.68, 6.65 and 6.64, whereas it was 7.77, 7.75, 7.74 and 7.72 in case of soy-whey at 0.03, 60 and 90$^{th}$ day of storage. The present data of papaya based squash obtained under different storage days might be attributed to the bleaching of papaya pulp colour resulting in non-appealing colour of the samples. The colour deteoration during storage may be because of losses in ascorbic acid, $SO_2$ and increase in tannins. The Mailard's reaction may also be possible for dark colouration. Similar to present findings are reported by Sood (2000); Riat (2003) and Sirohi *et al.* (2005) in case of other fruit beverages.

**Taste**: There was decreasing trend in both the types of papaya squashes which ranged from 7.53 to 7.20 in cheese-whey and 7.60 to 7.43 in soy whey in storage period of 0 to 90 days. The soy-whey showed better taste score as compared to cheese-whey. Declining trend in taste during storage has also been reported by Sirohi *et al.* (2005). The decrease in taste might be due to volatile flavouring components, reduced during storage and ultimately responsible for the decreased taste. However, the whey based papaya, squash had a highly acceptable aroma as reported by Sarvana and Manimegalai (2005).

**Table 3 :** Effect of storage period on organoleptic scores of papaya squash

| Treatments | Parameters | 0 days | 30 days | 60 days | 90 days | Mean |
|---|---|---|---|---|---|---|
| **Cheese whey** | Colour | 6.70 | 6.68 | 6.65 | 6.64 | 6.66 |
| **Soy whey** | Colour | 7.77 | 7.75 | 7.74 | 7.72 | 7.74 |
| **Mean** | | 7.23 | 7.21 | 7.19 | 7.18 | |
| **Cheese whey** | Taste | 7.53 | 7.37 | 7.27 | 7.20 | 7.34 |
| **Soy whey** | Taste | 7.60 | 7.57 | 7.50 | 7.43 | 7.52 |
| **Mean** | | 7.56 | 7.47 | 7.38 | 7.31 | |
| **Cheese whey** | Consistancy | 6.56 | 6.50 | 6.42 | 6.38 | 6.46 |
| **Soy whey** | Consistancy | 6.75 | 6.58 | 6.63 | 7.70 | 6.91 |
| **Mean** | | 6.65 | 6.64 | 6.52 | 6.48 | |
| **Cheese whey** | Acceptability | 6.93 | 6.58 | 6.78 | 6.74 | 6.82 |
| **Soy whey** | Acceptability | 7.37 | 7.30 | 7.29 | 7.61 | 7.39 |
| **Mean** | | 7.15 | 7.07 | 7.03 | 7.17 | |

| CD at 5% | Colour | Taste | Consistency | Overall acceptability |
|---|---|---|---|---|
| A | 0.34 | 0.40 | 0.47 | 0.40 |
| B | 0.47 | 0.56 | 0.67 | 0.56 |
| A X B | 0.67 | 0.79 | 0.94 | 0.80 |

A: Cheese-whey; B:Soy-whey and AXB: Cheese-whey X Soy-whey

**Consistency** : The consistency scores declined with the increase in storage period (0-90 days) with the score values of 6.56, 6.50, 6.42 and 6.38 in case of cheese-whey and 6.75, 6.58, 6.63 (0-60 days) but increased (7.70) soy-whey. The consistency scores decreased with increase in storage intervals in all squashes except after 90 days in soy-whey. This declining trend might be due to decrease in TSS with the increasing storage period affecting the consistency. Similar trend has also been reported by Hassan and Ahmed (1998).

**Acceptability** : The overall acceptability scores decreased with the storage period and a significant decrease was obtained from the first day to the last month in both the treatments (Table -3) Soy-whey showed better values as compared to cheese-whey. The scores obtained in case of soy-whey was 7.37, 7.30, 7.29 and 7.61, while in case of cheese-whey, these values were 6.93, 6.85 and 6.78 and 6.74 at 0, 30, 60 and 90 days of storage, respectively.

The study revealed that soy-whey squash was more acceptable by the consumer as compared to cheese whey squash.

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