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**Review article**

# Mango stem borers: new threat to mango in India

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**ABSTRACT**

Mango stem borer has been observed to cause an alarming situation in old and young orchards posing a great threat to mango cultivation in India during the last decade. Five species of *Batocera* namely *B.rufomacuata* de Geer, *B.rubus* Linnaus, *B.roylie* Hope, *B.numitor* Newmen and *B.titana* Thompson have been reported infesting mango trees. Among them, *B. rufomaculata*, is increasingly becoming a menace in mango orchards across the country. The infestation is reported to be in the range of one to eight per cent depending upon maintenance of the orchards. Affected trees gradually loose their vigour, manifest drying of branches to in severe cases and even death ensues. The grubs are the damaging stage, they bore and eat below the bark, making tunnels, subsequently entering into main stem following feeding on wood causing damage. The fross coming out of the entry point indicates the presence of trunk borer in the mango tree. The severe damage results in yellowing of branches followed by drying of terminal shoots and branches ultimately leading to the death of whole tree, if not managed properly. This paper presents a detailed review of informations on their symptoms of damage, distribution, varietal reactions, alternate hosts, life history and the control measures.

**Key words:** Mango stem borer, distribution, varietal reaction, life history and management

Mango (*Magnifera indica*) is a tropical fruiting tree of great economic importance. Besides its fruits, every other plant part viz., leaves, flowers, wood, bark and trunk are of significant importance. The leaves are fed to cattles during scaracity of fodder, dried flowers are used to cure dysentry, cattarah of bladder and the wasp string wood is used to prepare various type of furniture, high quality charcoal bark provides tannins used in taining hides, the mangifern, a bioactive ingradient having antioxidant quality, is used as an effective therapeutic agent against several disorders, the trunk produces resinous gums that help in curing cracks in the skin, the pulp used as laxative and the stone in the kernals are fed to pigs.

The mango fruit, one of the most delicious fruit in India, is famous as national fruit of India, Pakistan, Philippines and as a national tree in Bangladesh. It is a good source of Vitamin A, Mg, Na, K and iron etc. It is used at its all developmental stages. Raw mango is used in various ways ranging from chutnee, pickles and curries while ripe fruit is used in preparation of jams, jelly, syrup and juice. India, as per National Horticulture Database, published by National Board India, produced 90.2 million tonnes of fruit during 2015-16.

About 175 insect species have been reported damaging mango fruit and trees. Of these, the stem borer of genus *Batocera* are the most serious and destructive pest in India. Five species of *Batocera* namely *B. rufomacuata* de Geer, *B. rubus* Linnaus, *B. roylie* Hope, *B. numitor* Newmen and *B. titana* Thompson have been reported infesting mango trees. Among these, the *B. rufomacuata,* previously observed damaging old trees are now being observed even in 8-10 years old mango plants, calling for immediate attention. *Batocera rufomaculata* (De Geer, 1775) the Lamiinae beetle under the tribe Batocerini of the family Cerambycidae and commonly known as mango borer, fig borer and tropical fig borer is a serious pest of many economic plants species including mango plants. Besides, these *Stromatium barbatum* F. also damages the mango tree and reduces its vigor (Wadhi & Batra, 1964). Other long horned beetles attacking mango trees in India include *Acolestes haloserica* F., *Pachydissus velutinus* Thompson, *P.similus* Gahan, *Neocerambyx holoseriecus* Cotes, *Acanthophorus serraticornis* Olivier, *Anaplophora versteggi* Ritseema, *Epepeotes ficicolola* Fisher, *E.luscos* F., *Glenea multiguttata* Guerin Meneville, *Microtoma crenata* F., *Olenecamptus bilobus* F., *Pharsatia proxima* Gaham, *Plocaedrus obesus* Gahan, *P. pedestris* White, *Rhytidodera bowringi* White, *R.simulas* White and *Xylotrech smei* Laporte & Gory. However, these are not of major significance. Mango buprestids, *Balinota prasina* Thunberg, *Xyleborus affinis* Eichhoff, *Crossotarus saundersi* Chapius, and *Platypus solidus* Walker have also been reported feeding on the inner side of bark and boring inside the sapwood but causing negligible damage to mango trees.

Moreover, a large number of bostrychid beetles have been reported damaging mango trees. These include *Dinoderus distinctus* Lense, *Heterobastrychus aegualis* Waterhouse, *H.hamatipennis* Lense, *Parabostrychus elengota* Lense, *Schistoceros anobiodies* Waterhouse, *Sinoxylon anale* Lense, *S. conigerum* Lense, *S.dekhanense* Lense, *S. indicum* Lense, *S.oleare* Lense, *Xylodectus ornatus* Lense, *Xylothrips flavipes* Giliger, *Xylopsocus capunicus* Fabricius, *Micrapate simplicipennis* Lense, *Minthae sugicollis* Walker, and *Lyctorylon convixtor* Lense. In addition, *Lyctus africanus* Lense and *L.malayanus* Lense, two power dust beetles have also been reported from mango trees.

***Batocera rufomaculata* de Geer:**

**Distribution**

*Batocera rufomaculata* was described by De Geer in 1755. It is found in Burma, China (Hainan, Xizhang), Indonesia (Java, Sumatra), Myanmar, Malaysia, Nepal, Pakistan, Sri Lanka, Thailand, Tibet, Vietnam, Egypt (Sinai), Israël, Iraq, Jordan, Lebanon, Oman, Syria, Turkey, Yemen, Solomon Islands, Barbados, Br. Virgin Isl. Isl. St. Croix, Isl. S. John, Isl. St. Thomas, Puerto Rico ; Africa: Comores Isl., Réunion, Mauritius, Madagascar, Maldives, Rodriguez, Seychelles and Socotr (Mitra et al., 2016). In India it is recorded from Andaman and Nikobar, Arunachal Pradesh, Assam, Bihar, Jammu and Kashmir, Karnataka, Kerala, Manipur, Madhya Predesh, Maharashtra, Mizoram, Meghalaya, Nagaland, Punjab, Sikkim, Tamil Nadu, Uttarakhand, Uttar Pradesh, Chattis Garh and West Bengal. (Mitra, 2016; Kariyanna, 2016). Ballou (1916) reported *B.rufomaculata* from Verigin Isalands, Trinidad, Mauritius, Sri Lanka, India and Barneo (Fig 1). Hoffman (1935) recorded this pest from China (Kwangtung). In India this pest is more serious in south India (Ayyar, 1963). Surulivelu *et al*. (1978) studied the distribution of this pest in Tamil Nadu and found that stem borer infestation in North Arcot and Tirucharapalli was very high. Upadhyaya *et al.* (2013) reported mango stem borer is major pest of mango in Eastern tharai region of Nepal.

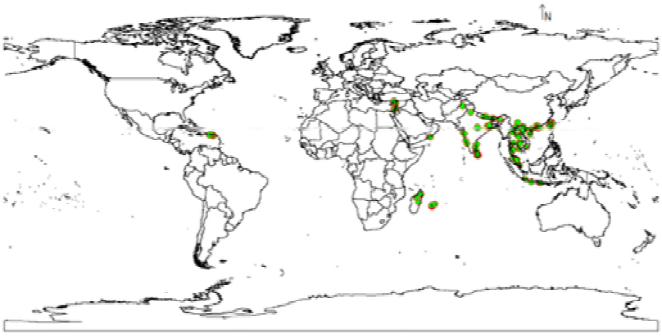

Fig. 1. Distrubution of *Batocera rufomaculata* in world.

**Host Plant Resistance (Varietal Evaluation):**

Surulivelu *et al.* (1976) evaluated 52 varieties in different districts of Tamil Nadu and found that the infestation was more than 20 per cent in the cultivars Amlet, Mulgoa, Alphanso (Gundu), Banganapalli, Jahangir and Rumani. The two varieties, Amlet and Mulgoa consistently recorded high infestation. Verghese *et al*. (2001) studied the varietal response of fig to *B.rufomaculata* and observed the infestation on five cultivars namely Deanna, Conandria, Excel, Zardzrinz, poona and Brown Turkey. Among all, Deanna recorded the highest infestation (68.75%) followed by poona (21.87% ) and Zardzrinz (13.75%). The other varieties were free from stem borer infestation.

**Alternate hosts:**

Singh (1960) recorded this pest on Avacodo (*Persea gratissima* Gaerten), Papaya (Carica papaya L.), banana (Musa spp.), *Ficus elestica* Roxb., hag plum (*Spondias* spp.) and *Ochroma lagopus* Sw. In Sri Lanka, It has been observed to attack hevea (*Hevea brasilensis* (Meuli) and Jack fruit (*Artocarpus heterophyllus*). Ayyar (1963) recorded this pest an jackfruit, rubber and fig trees. Kalainnan et al (1978) reported *Ficus tseila* Roxb, a new host for mango stem borer, *B.rufomaculata*. Butani (1963) reported *B.rufomaculata* and *B.rubus* on guava and Jackfruit. *B.rufomaculata* has also been reported on apple, gular, mulberry, pomegranate and walnut trees. Sundra Babu (1974-75) while studying the biology of the pest on different hosts viz, *Ficus bengalensis*, *Delonix regia*, *Azadirachata indica*, *Tamarindus indica*, *Glyricidia maculata*, *Peltophorum ferrugineum*, *Pythacolabium dulci*, *Moringa oleifera*, *Carica papaya*, *Mangifera indica* and *Bombax malabaricum* introduced grub of uniform age in the logs of the above host plants , and found that *F.bangalensis*, *C.papaya*, *M.oleifera*, *M.indica* and *B.malabaricum* were suitable for the development of grubs. The analysis of the chemical components of the suitable host material indicated that the development of the grubs was the quickest when the cellulose, hemicellulose and lignin contents were low and there was no development when these contents were high. Vasanti and Raviprasad (2015) reported that *B. rufomaculata* infest cashew (Anacardium occidentale L.) as a secondary borer.

**Symptomatology of damage:**

*B. rufomaculata* damaged stem or its branch shows exuding sap and masses of frass from the bored holes (Fig 2) The damage is confirmed by falling off of the leaves of the branches and sudden collapse of severely attacked branches resulting in death of the entire tree (Srivatsava, 1987). The damage is done either to the roots or the stems. The newly hatched first instar larva feed on the bark and makes irregular cavities producing harmonious sound by its mandibles. This



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is why the insect is popularly known as the 'Violin beetle'. It makes tunnels which may either be in the peripheral region or may go deep down into the heart of the tree.

**Semi-synthetic diet:**

Sundra Babu (1974-75) evolved a suitable semi-synthetic diet comprised of viz, Bengal gram powder (70g), Mango stem of alternate host stem powder (60g), Sucrose (50g), Agar-agar (15g), yeast (10g), Multivitamin (4ml), Ascorbic acid (4g), Paraben(2g), Formline 10% (2 g), Sorbic acid (1g), Cholestorin (1g), Casein (2g) and Aureomycin (1g) for the rearing of grubs in the laboratory. It was observed that with the host plant material, incubation period, larval and pupal period and total lifecycle lasts for 10.2, 145, 27 and 182.2 days respectively, while with the semi-synthetic diet, it lasts for 10.4, 90, 24.2 and 124.6 days, respectively. Gundappa et al. (2015) prepared artificial diet with gram powder, host stem powder and other nutritional components. The exponential increase was observed with the body weight and length of the grub in artificial diet at fortnightly intervals. Adults emerged 10 days after pupation and percent pupation was 83.3 % and adult emergence was 33.3% the adults were robust and active with a mean body length and width of 4.5 and 1.5 cm, respectively.

Reddy *et al.* (2016) reared *B. rufomaculata* on three hosts viz., drum stick, jack fruit and mango. Among them highest adult recovery (80%) was recorded on drumstick.

**Life history:**

*B. rufomaculata* adults are large prominent greyish brown beetle covered with grey or yellow grey pubescence. The colour in general resembles that of the mango bark. There is conspicuous white triangular patch at the junction of the elytra. The thorax has pair of raised horn like structures. The antenna is long and eleven segmented with two to three basal segments having row of small teeth on inner side. According to Palaniswamy et al. (1979), the average length and width of the male is 4.39 and 1.49 cm and that of female is 5.13 and 1.84cm, respectively. The adults mostly take rest in day time in their hiding places and rarely seen outside on the branches. During flight , they made much noise. The longevity of the adult males is 25.4 days and of females 26.8 days. In case of male, the fore tibia has serration while in female, there are no serrations. The last abdominal segment in male has no clefting on the dorsal side while the female has the clefting.

Copulation generally commence days after the adult emerged. The adults usually during this period fed well. The male mounts over the female either from the sides or from the back or from the front, turns its head towards the head of the female and start licking and patting with its palpi and mendibles of the elytra of the female. It then coveres the abdomen forward with the apex forming a scoop like right angle and attempt to insert its phallus which took 2-4 minutes. The copulation last for 60-75 seconds (Paliniswamy et al., 1977). The observation that there was a period of adult feeding between adult emergence and first mating confirms the report of Beesan and Bhatia (1938). The courtship and actual peocess of mating were reported by Chemsak (1966), Shirifi et al. (1970) and Chemsal & Powell (1971).

The pre-oviposition period ranges from 24-48 h with an average of 38.4h. The oviposition period ranges from 20-24 days with an average of 22 days. After mating the female feed for a while and on same or the next day move over the stem in search of a suitable site for oviposition. The female make semi lunar incisions on the bark with its powerful mandibles. The eggs were then laid parallel to the bark layers and protected by an exudation from the bark and also by a viscous fluid secreted by the beetle. Sudhi-Aroma Chal (2008) observed that female beetle chew small tunnel shaped depressions in the tree bark and insert ova under the bark. An egg niche measures 15-20 mm long with a single egg was laid in each niche. The eggs are laid at a depth of 2.5-4 mm on the bark. More number of eggs are laid on the trunk and primary branches than on secondary branches. The adult beetle lays eggs till its death during day and night.

The freshly laid eggs are white and shiny with oval, narrowly rounded ends. The color changes to dirty white or yellowish white later. The length of the egg ranges from 5.97 to 6.25 mm and the breadth from 1.96 to 2.58 mm and weight varies from 12.08 to 13.74 mg. The incubation period varies from 7 to 13 days. The viable eggs are shiny and oval white while non-viable ones are shrivelled and dull colored. Mir (2017) reported that females deposits eggs singly onto the bark, daily with mean fecundity of 1.27 and 148.66 eggs, respectively.

The freshly hatched first-instar larva is slender, pale white, turning creamy white with yellowish tinge on the thorax. Body bears hairs all over which are more prominent at posterior abdomen. The head capsule is dark brown. Antennae are very small and three segmented; spiracles are nine in number. It measured 0.92 cm and lived for 2.8 days. The second-instar larva is creamy white or yellow in color, body cylindrical with elongated head. Head capsule is strongly depressed. Each abdominal segment possesses dorsal and ventral ampullae. It measures 1.52 cm and lasted for 10.4 days. The third-, fourth-, fifth- and sixth-instar larvae measure 2.43, 4.24, 5.97 and 9.20 cm and last for 13.8, 11.0, 16.4 and 16.6 days, respectively. The last seventh-instar is a

full grown larva, yellowish creamy, shining, tapering towards eighth segment and then becoming cylindrical. The grub is fat, fleshy and creamy white (Singh, 1960). According to Palaniswamy et al. (1979), each abdominal segment possesses dorsal and ventral ampullae bearing two transverse furrows of tubercles. Thoracic legs are very small. This grub measures 8.20 cm and lasts for 19 days. According to Butani (1993), grubs remain active for 140-160 days.

The larva when fully fed shrinks and attains pre-pupal stage. It takes complete rest. The average length of the body is 5 cm while the width of the thorax and head capsule is measures 1.52 and 1.05 cm, respectively.

The pupa is ivory colored in the initial stage with small curved antennae, wing pads and legs turning yellowish brown to dark brown. Head is deflected inside with very long antennae. The hind legs are folded below the tips of elytral pads. The average length of the pupa is 5.27 cm, width of the thorax and head are 2.01 and 0.99 cm, respectively and the weight was 6.30 g. The fully grown larva excavats an oval elongated and slightly depressed pupal chamber in the host plant. The walls of the pupal chamber are coated with fine stem powder with oral secretions. The pupal period varies from 19-38 days with an average of 29.1 days. After the transformation from the pupa to adult, the adult remaine in the pupal chamber for 2 to 5 days.

The total life cycle of the beetle from egg to emergence of the adult was 182 days in host and 124 days in semi-synthetic diet (Palaniswamy et al., 1979) (Fig 3). According to Butani (1993), total life cycle occupies 170-190 days and adult longevity is 60-100 days. According to Palaniswamy et al. (1979) and Browne & Foenander (1937), the longevity of adult male was 25.4 days and of female 26.8 days.

***Batocera rubus* Linnaeus (*Cerambyx rubus*, *Batocera rubra*)**

The adult beetles are stout, 30-40 mm long and are dull yellow in color. The mandibles are powerful and long antennae are black and longer than the beetle in males while in females, they do not reach even the apex of elytra. The prothorax is ridged transversely on the anterior and posterior edges, at the sides the thorax is produced medianly into a sharp point. The life cycle resembles that of *B. rufomaculata*. Adults lay eggs in June and July and oviposition continues till end of August. Egg period lasts for 8-12 days and pupal period lasts for 3-4 months and the adult longevity is between 75 to 105 days (Fletcher, 1914).

***Batocera titana* Thompson:**

The adult beetle is large (57 to 75 mm long) and grayish yellow. The body is mottled with blackish markings and orange spots, antennae are brown. Adults mate from middle July to end of August and lay eggs. The pupal period and the resting stage of newly emerged beetle occupy 3 to 3.5 months (Stebbing, 1913).

***Acanthophorus serraticornis* Olivier:**

*A. Serraticornis* attacks *Bombax malabaricum*, *Mangifera indica*, *Morus alba* and *Shorea robusta*. The large prionine larva lives in decaying roots and stems of large trees and at intervals leaves the wood and travels through the soil alongside. The hibernation cell is usually formed in the soil. The beetle comes to light and is on the wing between April-September. Fruit trees such as *M. indica* and *M. alba* are seriously affected (Beesan, 1941). Beetles are long, measuring about 35 to 92 mm with head, basal half of the antennae and pronotum black, eyes large and not very widely separated. Antennae are 12 jointed. Elytra coriaceous and dull but sparsely punctured and glossy near the base (Gaha, 1906). Mating takes place in early June and egg laying takes place during July-August. The grub feeds from 9-10 months and pupates. The pupal period lasts for 3-5 weeks (Butani, 1993).

***Olenecamtus bilobus* Fabricius:**

This pest has been recorded on *Artocarpus blume*, *A. chaplasha*, *A. hirsulta*, *A. integrifolia*, *Bauhinia* sp., *Ficus bengalensis*, *F. carica*, *F. elastic*, *F. glomerata*, *F. infectoria*, *F. laccifera*, *F. religiosa*, *F. roxburghii*, *F. tjakela*, *Litsaea polyantha*, *Mangifera indica* and *Morus indica*. It is widely distributed in oriental region. The adult beetles are 10-15 mm long, brown with round white spots and with very large antennae. This is the main pest of *Ficus* spp. and has been recorded from all over India, Java and Sumatra (Fletcher, 1921). It mostly infests dead wood but also attacks living branch and small wood. The beetles are active in day and feed by gnawing the green bark of shoots or by eating in the blade of large leaves. The head and greater part of prothorax is having orange pubescence. Elytra are moderately shining, reddish brown with apical portion yellowish brown. In north India, the life cycle is annual with an extended emergence period from May to November. The details of the larva and pupa are described by Gardner (1927).

***Aeolesthes halosericea* Fabricius:**

The pest has been reported from Indian sub-continent, Malaysia, Thailand, Ceylon, Burma, the Andaman and Nicobar, etc. this is called as cherry stem borer but it is an polyphagous pest and has been reported on fruit trees viz. *Ficus bengalensis*, *Mangifera indica*, *Morus alba*, *Prunus communis*, *Psidium guajava* and *Pyrus communis*.

The adult beetles are black with a mottled yellowish or



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silvery short pubescence on the elytra. Pronotum intricately wrinkled, antennal segments without spines, in the male the antenna is about one and half times as long as the body and in the female, about as long as the body.

Eggs are laid in crevices in the bark of dead or felled trees. A female beetle lays about 200 eggs during May and October. The eggs are about 2.5 mm long whitish and oval with a short stout stalk at one end; the eggs hatch in 7-12 days and the larva bores down in the bark and then begins to make a tunnel. The tunnel is packed with wood and bark fragments and a considerable amount is thrown out through ejection hole. When mature, the larva is about 3 inches long; the larval stage lasts for 27 to 32 months. The pupal tunnel runs more or less horizontally for 2.5 to 3 inches from the wood, turns downwards and ends as a vertical chamber. Before pupation, the larva returns to the bark and bites and exit hole and clears out the woody debris from the gallery immediately around the entrance to the prepupal tunnel. The larva now appreciably shrunken, smooths off the roughly excavated wall of the pupal chamber and in the process accumulates quantity of fine fragments of wood quite distinct from the coarse fibrous strands cut out in the excavation of the prepupal gallery. The majority of larvae pupate in or about October and after a pupal period of three weeks change to beetle. The pupa is about 1.25 inches long. The prepupal stage lasts from 3 to 150 days and the pupal period occupies 40-100 days. The beetle emerging from pupae formed in October, remains inside the stem throughout the following winter and spring. While those that emerge from pupae formed in April, rest within the stem for six weeks. The single life cycle studies by Beesan lasts for 2 years while Rehman & Khan (1942) in Punjab found it to be completed in 31 to 36 months.

***Plocaederus obesus* Gahan:**

This pest infests *Bombax malabaricum,Buchanania latifolia*, *Butea frondosa*, *Cedrela toonga*, *Cordia myxa*, *Eriodendron anfractuosum*, *Garuga pinnata*, *Gmelina arborea*, *Kydia talycina*, *Lannea grandis*, *Mangifera indica*, *Protium seeratum*, *Shorea robusta*, *Spondias mangifera*, *Sterculia colorata* and *Terminalia tomentosa*.

It is found in North India, Andaman islands and Union of Myanmar. Earlier this species was identified as synonymous with *P. pedestris* White but Gahan (1906) found the two to be separate species. The adult beetles are chestnut brown with grayish pubescence and 27 to 45 mm in length. The pest is on the wing early in the year, i.e. March. The larva rapidly penetrates into the deeper layer and tunnels very irregularly. The full grown larva is 75 mm in length and the excavations in which it pupates are very wide galleries sometimes six inches deep from the surface. The larvae are yellowish brown with rounded segmented bodies. The calcareous cocoon is quite regular and unlined with any other secretion. The larva pupates as early as September or in early next spring and shortly turns into a beetle which may emerge in October-December. The majority, however, hibernate as immature beetles in the cocoons and emerge in March-April.

***Plocaederus pedestris* White:**

It is found infesting *Mangifera indica*. The beetle is 25 mm long, reddish brown to blackish, with a pale grey pubescence. The prothorax armed with a short conical spine on each side is strongly and irregularly corrugated (Butani, 1993). Elytra are slightly narrowed backwards finely and closely punctuate. The larva feeds between the bark and sapwood and later makes a small and wide oblique chamber like tunnel into the heart of the wood. Emergence occurs in June.

***Rhytidodera bowringi* White:**

This pest infests *Mangifera indica* trees, and has been reported from India (Bihar), China, Thailand and Malaysia. The pest prefers old trees and thick branches (Fletcher, 1930). The adult beetle is about 20 to 30 mm long; the antennae of males are as long as their body, while of female are short and robust. The prothorax is rugose rounded at sides, constricted and transversely grooved near the base and apex. Eggs are laid on living shoots and branches of mango trees over 8-10 years old and the larva bores down the centre of the branch making a tunnel. The life cycle is annual and the beetle emerges in May-July.

***Stromatium barbatum* Fabricius:**

This species is chiefly important as pest of packing cases, timber and furniture but it also attacks living stems and branches. It has been reported from Indian subcontinents, Madagascar and Mauritius on citrus and mango. The adult beetle is 12 to 30 mm long, reddish brown to almost black with a tawny pubescence closely punctuate. Beetles are inactive during day light hiding in dark shelters. The life of adult female beetles is 18 days while of male is 32 days.

The newly emerged female mates as early as the first day after emergence. Mating takes place at night. Egg laying begins immediately after the first mating. The average number of eggs laid by a single female is 100. The oviposition period is 5-16 days. The egg is 2.0-2.5 mm long and 0.8 mm wide. The larvae after hatching bore and make tunnels. They run irregularly in the trunks; the mature larva is 30-40 mm long.

The pupal period lasts for two weeks. The life cycle is completed in one year or several years. The beetles emerge during June-July.

***Glenea multiguttata* Guerin Meneville:**

It infests *Bombax malabaricum*, *Boswellia serrata*, *Buchanania latifolia*, *Garuga pinnata*, *Mangifera indica*, *Pterocarpus marsupium* and *Shorea robusta*. The beetles are 8-15 mm long. The head and prothorax are yellow having black spots. The elytra are orange to grayish brown spotted with black (Stebbing, 1913; Beesan, 1941). The adults emerge in April-June. The pupal chamber is at right angles to the pre-pupal tunnel.

***Xylotrechus smei* Laporte:**

This pest has been reported from north west and central India and Bhutan on *Adina cordifolia*, *Aegla marmelos*, *Anogeissus latifolia*, *Bauhinia retusa*, *Bombax malabaricum*, *Bridelia retusa*, *Buchanania latifolia*, *Butea frondosa*, *Calycopteris floribunda*, *Careya arborea*, *Cassia fistula*, *Cedrela toona*, *Chloroxylon swietenia*, *Dalbergia latitolia*, *D. paniculata*, *D. sissoo*, *Ehretia acuminate*, *Eugenia jambolana*, *Ficus gibbosa*, *Ficus religiosa*, *Garuga pinnata*, *Gmelina arborea*, *Grewia tiliaefolia*, *Grewia vestita*, *Holoplelea integrifolia*, *Hymenodictyon excelsum*, *Kydia calycina*, *Litsaea sebifera*, *Mahilus odoratissima*, *Mallotus philippinensis*, *Mangifera indica*, *Morus alba*, *Morus indica*, *Pterocarpus marsupium*, *Schrebera swietenioides*, *Shorea robusta*, *Strychnos nuxvomica*, *Tectona grandis*, *Terminalia tomentosa*, *Vengueria spinosa, Vitex altissima*, *Vitex pinnata*, *Vitis latifolia* and *Xylia dolabriformis.*

The adult beetle is 10-18 mm long, brown with a greyish or yellowish pubescence on head and prothorax and forming bands or spots on elytra. The prothorax may be reddish. The maximum number of eggs laid by a single female is 190. The oviposition period is 6 days in April. The eggs are laid in crevics and covered depressions on the surface of bark in large clusters. The egg hatches in 4-5 days. The larva lasts for 52 days and the pupa for 18-19 days.

***Belionata prasina* Thunberg:**

This pest has been reported on *Acrocarpus fraxinifolious*, *Anacardium occidentale*, *Anogeissus latifolia*, *Holigarna nraottiana*, *Hopea parviflora*, *Lannea grandis*, *Mangifera indica*, *M. xeylonica*, *Psidium guajava*, *Sapium sebiferum*, *Sonneratia apetala*, *Spondias mangifera*, *Terminalia belerica* and *T. paniculata*. The wing period is normally April-August. The life cycle lasts for one year or even three years. This pest has been reported from India, Bangladesh, Sri Lanka, Indonesia, Zanzibar, etc. Mango crop is the main host of this pest but it also infests citrus and guava seriously. The beetles are oblong elongated, 20-30 mm long (Fig 4).

**Management:**

**Biological** Sundra Babu (1974-75) observed a species of mite, *Proctolaclans bickleyi* on the body of grubs in the laboratory. He also observed the presence of a rhabditid nematode, *Brevibucca* sp. parasitic on the grub. Studies conducted on the gut microflora of healthy grubs revealed the presence of two species of fungi viz. *Aspergillus niger* and *A. flavus* and seven isolates of gram positive bacteria but he could not test the pathogenecity of these pathogens.

**Chemical** The control of this pest is very difficult as the larvae live inside the stem. Attempt have been made to control this pest, when the larva is inside the trunk but failed to achieve the desired control. Keeping the present scenario in view, it is of paramount importance for the workers to find out the peak period of egg laying, hatching of the eggs and controlling them before the larvae penetrate inside the stem.

Ayyar (1938) reported the removal and destruction of the infested branches of to control this pest. Beetles found in the garden should be collected and killed. He also found holes with powdery matter as such or in heaps below a tree. The grubs should be extracted through hooked wires or destroyed by injecting dilute kerosene oil, petrol or naphthalene, chlorosol or carbon disulphide or tar water and the holes be covered with wet clay soaked in tar.

Beesan (1941) stated that closing the stem with stout paper coated with coal tar or with wire gauze 1/16$^{th}$ inch mesh or by spraying with repellent like Bordeaux mixture, citronella oil, camphor oil or concentrated extract of pyrethrum in mineral oil are effective methods. In Israel, the control of this pest was achieved on fig (*Ficus carica*) with a plastic wax preparation of parathion.

In India so far mainly fumigation with chloroform, creosote mixture, petrol, EDCT mixture and paradichlorobenzene crystals has been recommended (Haq & Akhtar, 1960; Atwal, 1963; and Vevai, 1969).

Singh (1960) advocated that the beetles be collected and destroyed and the affected parts cut open and the grub destroyed, logs of wood should be placed in orchards to trap the beetles and after infestation, the logs may be cut open and grubs destroyed.

Wadhi & Batra (1964) advocated wrapping of the trunk with wire gauze (1/16$^{th}$ mesh) or coating with coal tar painted paper or even spraying with some repellent. The cutting and destruction of infested branches is also helpful. For controlling in its tunnels, injection of kerosene oil, creosole, crude oil, petrol, BHC (0.15%) or DDT emulsion or introduction of cotton wool soaked in carbondisulphide and chloroform (1:1) or putting inside the holes 2-5 g potassium



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cyanide and finally the holes may be sealed with mud. However, if the holes are found open next day, repeat the same process again.

Sharma & Tandon (1972) reported the insertion of cotton wick soaked in dimethoate (0.06%) or methyl demeton (0.05%) or phosphamidon (0.06%) or dichlorvos (0.1%) into the bore holes and covering with wet clay for the control of mango stem borer. Singh (1975) recommended injection of endrin or methyl parathion into the bore holes.

Palaniswami et al. (1977) conducted two trials one at Kanyakumari and the other at Tirunelveli in South India for the control of *B. rufomaculata* and found that swabbing with dieldrin or DDT (0.5%) or prophylactic spray or monocrotophos (0.1%) is very effective treatment. They also found that in the affected trees, effective control was achieved by using phostoxin half tablet per bore hole.

Fig. 2. Damage symptoms of *B.rufomaculata* on mango

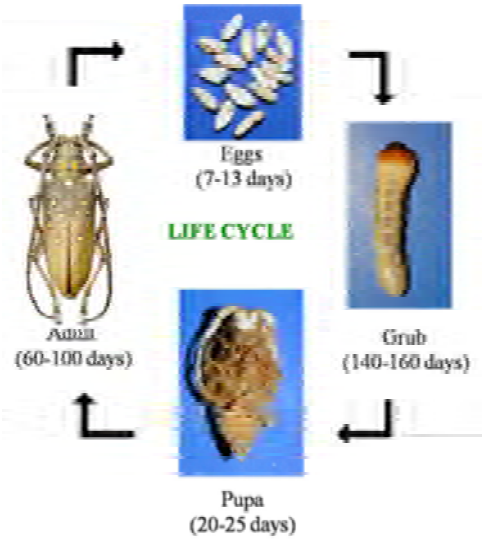


Fig. 3. Life cycle of *B.rufomaculata*.

Gopalkrishnan *et al.* (1979) used padding method of application for the control of the stem borer. They evaluated monocrotophos, phosphamidon, acephate and dimethoate. The bark in the main trunk was removed in an area of 7.5 $cm^2$ and on the exposed portion, a layer of sterilized absorbent cotton was placed and 10 ml of the insecticide was poured slowly so that the insecticide got absorbed by the cotton. Among different insecticides tried in the padding method, monocrotophos and dimethoate were found effective with 55 and 40 percent kill, respectively.

Kaliannan et al. (1979) did field experiments to control stem borer, *B. rufomaculata,* and found spraying of either monocrotophos (0.1%) or phosalone (0.1%) at fortnightly intervals as a prophylactic measure to be very effective. They also found that application of half a tablet of aluminium phosphide (1.5 g) or dichlorvos (0.1%) spray fluid at 10-20 ml/bore hole are effective treatments. The hole after treatment may be closed by wet soil. They also advocated that painting the tree trunk proved better than spraying. Painting was recommended by Bindra (1969) for the control of the citrus stem borer, *Stromatium barbatum* which is also a minor pest of mango.

Sharma and Tara (1986) studied the efficacy of 11 fumigants for the control of *B. rufomaculata* on mulberries in Jammu and Kashmir, India. Chloroform ethylacetate, Metasystox (demeton S. methyl) and a mixture of Petroleum and Kerosene oil (5 ml $bore^{-1}$) and ethylene bromide of (3 ml $bore^{-1}$) gave 100 per cent mortality of the larvae.

Fig. 4. Damage symptoms, Grub and adult of *B.prasina* on mango

Butani (1993) cutting and destruction of the infested stem or branches as the best method of control. The insect can also be controlled by injecting in the holes dichlorvos (0.02%) or endosulfan (0.05%) at the rate of 5 ml hole$^{-1}$ and seal the holes with mud. Singh (1993) advocated application of celphos (aluminium phosphide) 3 g table hole$^{-1}$ and carbofuran @ 0.1 g (20 ml bore$^{-1}$ hole) brought about cent percent kill of the grubs after 72 hours. Upadhyay et al. (2013) evaluated eight treatments viz., Nimex @5 ml/L of water, Sweet flag stolen extract @5% solution, Lantana camera leaf extracts @5% solution, bordeax-mixture @ 5:5:5 (cuso 4:lime:water), thiamethoxame 25% WG 1 gm/ L of water, imidacloprid 17.8 % SL 1 ml/L water and trizophos 40 per cent EC @ 2 ml L$^{-1}$ water against *B. rufomaculata* along with control**.** Among eight treatments, Imidacloprid 17.8% SL, Thiamethoxame 25 per cent WG and trizophos 40 per cent SL performed best in management of mango stem borer.

Ahmed et al. (2013) evaluated ten treatments viz, injection of petrol into the hole by using syringe + sealing of the hole withBordeaux paste, injection of petrol into the hole + sealing of the hole with cow dung, injection of kerosene into the hole + sealing of the hole with bordeaux paste, injection of kerosene into the hole + sealing of the hole with cow dung, placing aluminium phosphide into the hole + sealing of the hole with bordeaux paste, placing aluminium phosphide into the hole + sealing the hole with cow dung, injection of dursban 20 EC @ 2ml litre$^{-1}$ water into the hole, injection of cypermethrin (ripcord 10 EC) @ 1 ml liter$^{-1}$ water into the hole, inspection of orchard at 15 days interval + cutting open the tunnel with help of chisel and sharp haft knife + hooking the hole by sharp iron rod and untreated control against *B.rufomaculata* on jackfruit. Among them placing aluminium phosphide + sealed hole with bordeaux paste ensured the highest (83.33 %) control of infestation and the highest increase in yield (51.30%) over the control.

## CONCLUSION

The study concludes that the mango stem borers infestation in old orchards at the productive stage results in lowering the fruit yield. Although informations with reference to its symptoms, distribution, varietal reactions, alternate hosts, life history and control measures are available, it is felt that control measures adopted till date are insufficient to tackle the pest. Therefore, inclusion of bioagents and biopesticides while developing future control strategies against serious pest *B.rufomaculata* was recommended.

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# Isolation, characterization and evaluation of efficient rhizobia suitable for production of biofertilizers for important arid legumes

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**ABSTRACT**

The study carried out to isolate and evaluate efficient *Rhizbium* cultures from guar and moth bean plants grown at farmers' fields in arid districts of western Rajasthan showed that the GBR-2 and GBK-21-2 cultures from guar and MBR-8 and MBK-15 from moth bean grew fast and developed wet and gummy colonies on YEMA plates within 24-48 hrs. Cultures of GBR-2 and GBK-21-2 also exhibited better plant biomass in nursery and 15-18 per cent higher yield and nodulation over the uninoculated control under field conditions. MBR-8 and MBK-15 also exhibited higher plant biomass in nursery and higher yield (up to 19 %) and nodulation over the uninoculated control under field conditions. MBR-8 was used for studying the shelf life of the liquid bioformulations for later use as bioinoculant. The liquid bioformulations having polyethylene glycol (0.5%) or gum arabic (0.5%) as cell protectants or top layer of castor oil retained viable cell density (e" $10^{10}$ cfu $ml^{-1}$) up to 180d after storage.

**Key words:** Arid legumes, *Rhizobium,* cell protectant, liquid bioformulation

Biofertilizers or microbial inoculants are commercial preparations of microorganisms that when used enhance the crop production by enhancing micro and macronutrients' uptake in the soil. The beneficial micro-organisms, especially PGPR (Plant growth promoting rhizobacteria) are grown in the simple, low cost media and are mixed with the appropriate carriers to produce biofertilizers. PGPRs like *Rhizobium* fix nitrogen ($N_2$) in arid legume crops and help in saving 20-40 kg chemical nitrogen per hectare (Bahru, 2019). *Rhizobium* is a soil inhabiting gram-negative bacterium, that colonizes the legume roots by the formation of root nodules and fixes atmospheric $N_2$ symbiotically. In addition to fixing the atmospheric $N_2$ through nodulation, it imparts many characteristics with other PGPRs including hormone production, root expansion, improved uptake of plant nutrients, protection of plants from root diseases and improvement in biomass production etc (Gopalakrishnan *et al*, 2015). Clusterbean *or guar (Cyamopsis tetragonoloba L.)* and moth bean *(Vigna aconitifolia* (jacq.) Marechal) are two drought-hardy legume crops, commonly grown in arid and semi-arid regions of India. Clusterbean is famous for export value of the "guar gum" extracted from the endosperm (Ramachandra *et al*, 2015) and raw, green pods of clusterbean are used as delicious vegetable (Bhatt et al, 2015). Moth bean is known for its tolerance to high temperature and soil moisture deficit situations (Sadashivanagowda et al, 2017). It acts as a source of pasture, fodder and green manure and also used in a variety of local dishes and snacks. Although the production of cluster bean in Rajasthan is increasing probably due to increase in its area, the productivity of cluster bean does not show any specific trend and fluctuates considerably (Bhatt et al, 2015). Similarly, in case of moth bean, the area and production has been highest in Rajasthan (96.75% and 94.49%) followed by Gujarat (2.38% and 3.6%). However, the yield (292 kg $ha^{-1}$) in Rajasthan was below the national average yield (299 kg $ha^{-1}$). One way to enhance the productivity of these two important legume crops is by ensuring the availability of compatible rhizobia in the soil through application of nitrogenous biofertilizers (Kumar and Rodge, 2012; Pawar *et al*, 2014). Liquid biofertilizers are preferred over carrier-based ones owing to their higher shelf life, tolerance to higher temperatures, ultraviolet radiations and ease of handling (Santhosh, 2015). Therefore, the present study to isolate, characterize and evaluate efficient rhizobia from guar and moth bean and also to determine the shelf life of different formulations of liquid biofertilizer by adding cell protectants during the preparation of rhizobial inoculants was undertaken.

## MATERIAL AND METHODS

The root nodule samples were collected from guar and moth bean plants growing at farmers' fields from Barmer and Bikaner districts of arid Rajasthan. For isolation, the roots bearing nodules were washed thoroughly under tap water. The nodules were separated and surface sterilized with 70 per cent alcohol for 3 min, 0.1 per cent $HgCl_2$ for 2

min and rinsed thoroughly with sterile, distilled water (Setu et al, 2019). Each nodule was crushed in a drop of water under aseptic conditions with the help of a sterile rod. The whitish suspension was streaked on to yeast extract mannitol agar (YEMA) plates having congo red dye and incubated at 28°C for seven days. Whitish *Rhizobium* like colonies were streaked again on YEMA plates to get pure culture. Rhizobial isolates were maintained on YEMA medium slants in test tubes containing congored dye and stored at 4°C until used.

The time taken for the isolates to form colonies on YEMA plates at 28°C was followed for seven days and the isolates were classified as fast or slow growers. Colony morphology was scored as dry where the surface was smooth and firm, and wet where the surface was watery or slimy. Rhizobial isolates were also screened for K solubilization on aleksandrov agar medium having: glucose (0.5%), $MgSO_4$ $7H_2O$ (0.05%), iron chloride (0.0005%), $CaCO_3$ (0.01%), 0.2% calcium phosphate (0.2%), feldspar (0.2%) and agar (1.8%) (Prajapati and Modi, 2012). Screening for P solubilizing activity was done on pikovskayas agar medium (HiMedia) for detection of P-solubilizing rhizobia. The cultures were spot inoculated and incubated for 4-5 days at 28°C. A clear zone around the colony was indicative of K or P solubilization by the isolate. The isolates were also tested for their ability to grow on YEMA medium plates having pH range (4 to 8) adjusted by addition of acid or alkali during the preparation of the medium.

Seeds of Guar (RGC 936) and moth bean (CAZRI moth 2) were inoculated with their respective fast growing rhizobial cultures and sown in polythene bags containing sterile soil mixture (Sand:Soil:FYM::1:2:1). There were total four treatments including one commercial culture and one uninoculated control with five replications each. Data per plant was collected on plant fresh weight, nodule number, shoot length and root length in three-month old seedlings.

Guar and moth bean seeds were inoculated with rhizobial cultures before sowing in the field in kharif season of 2019, in Random Block Design. Plot size was 8x8 meters and distance between rows was 45cm in each plot. There were total four treatments including one commercial culture and one uninoculated control with three replications each. Data was collected on nodulation index and yield.

The *Rhizobium* culture tested for shelf life of liquid biofertilizer formulation was MBR-8 isolated from moth bean plants of Barmer region. Yeast extract mannitol (YEM) broth was used to grow *Rhizobium* culture. The sterilized YEM broth (50 ml) was inoculated with MBR-8 strain in 150 ml flask and incubated at 28±2°C on a rotary shaker for 24 hrs to prepare mother culture. Two ml of the mother culture was inoculated into each of 50 ml of YEM broth in 150 ml flasks containing one of the cell protectants viz., glycerol (GLY, 0.5%), polyvinyl pyrrolidone (PVP, 0.5%), polyethylene glycol (PEG, 0.5%), gum Arabic (GA, 0.5%) and castor oil as top layer in addition to GLY, 0.5%, added to the broth during the preparation of media. Incubation was done at 28±2 °C on a rotary shaker for three days to get luxuriant growth. There were a total of seven formulations, out of which, four (T2-T6) were prepared using cell protectants in optimum concentrations. Treatment T1 was only broth without any addition of any cell protectant. The talc based formulation (T7) was prepared by mixing the broth culture media with sterile talc powder at 1: 2.5 ratio, dried in shade to 8-10 % moisture.

Liquid inoculant formulations containing fully grown rhizobial cultures (~ $1x10^{10}$ cfu $ml^{-}$) were transferred to UV sterilized high density polyethylene bottles (100 ml capacity) and transferred to a BOD incubator at 28°C. One ml cell suspensions were withdrawn from each treatment at monthly intervals starting from zero days (0d) up to 180d after storage (DAS). Plate count was assessed after making suitable dilutions in sterile distilled water and plating on YEMA medium plates. Values obtained were means of five replications.

## RESULTS AND DISCUSSION

### Isolation and evaluation of rhizobial cultures

Examination of a total of 68 rhizobial isolations, 35 from cluster bean and 33 from moth bean plants, collected from different districts of hot, western Rajasthan, showed that fifty eight per cent of the isolated cultures were fast growers where colonies were formed within 72 hrs, three percent were very fast growers where colonies were formed in 24 -48 hrs, 37 per cent were slow growers with colonies appearing after 72 hrs on YEMA plates at 28°C and 2 per cent were very slow growing with colonies appearing after 5-7 days of incubation. Majority (68.5%) of the colonies were wet, gummy and compact (WGC), 20 per cent were wet, gummy and soft (WGS) as the colonies were merging with each other on slight tilting of the plate. Remaining 11.5 per cent were dry where the surface was smooth and firm. None of the rhizobial isolates solubilized K or P. We chose fast growing cultures GBR-2, GBK-21-2 for guar and MBR-8 and MBK-15 for moth bean for further studies. Fast-growing rhizobia have been found to outperform slow growers as far as nodule forming capability is concerned (Sanginga *et al*, 1989). Also the selected four cultures were wet and gummy in colony morphology based on the findings of Karthik et al (2017) who reported that higher production of exopolysaccharides (EPS) was indicative of versatility and

survivability of the rhizobial isolates against various physiological stresses present in the arid/semiarid climate.

**Nursery evaluation**

In guar, four rhizobial treatments were commercial strain (T1), GBK-21(2) (T-2) from Bikaner, GBR-2 (T-3) from Barmer and uninoculated control (T4). Fresh weight per plant was highest in GBR-2 (8.67g) followed by GBK-21(2) (8.33g), commercial (5.8g) and uninoculated control (5.73g). Nodule number/plant was also higher in GBK-21(2) (2.87) and GBR-2 (2.53) as compared to T1 (2.27) and T4 (1.53). Values of shoot length and root length per plant also showed similar trends (Fig.1a). In moth bean, fresh weights per plant were 3.53g in MBR-8 and 3.4g in MBK-15, followed by commercial (2.71g) and uninoculated control (2.13). Nodule number/ plant was also highest in MBR-8 followed by MBK-15, commercial and uninoculated control. Shoot length plant$^{-1}$ in cm was 15.77, 15.9, 15.0 and 14.33 in MBR-8, MBK-15, commercial and uninoculated control respectively. Similar trend was observed in root length plant (cm) in all the treatments (Fig.1b). Thus, the rhizobial inoculation with efficient rhizobial cultures was found to increase the growth and nodulation of guar and moth bean plants under nursery conditions in the present study. Similar results were reported by Sun et al (2020) where rhizobial inoculation was found to enhance biological $N_2$ fixation in *Robinia pseudoacacia* Seedlings.

**Field evaluation**

Under field evaluation also in guar, GBR-2 and GBK-21-2 outperformed as compared to commercial culture and uninoculated control for yield (kg ha$^{-1}$) and nodule index (Table 1). GBR-2 and GBK-21-2 gave 15 and 18 per cent higher yield, respectively than the control and 8 and 5 per cent higher yield than the commercial culture (Table 1). Fig. 2 shows lush green plot (8x8 meter) and root of guar plant inoculated with *Rhizobium* culture (GBR-2).

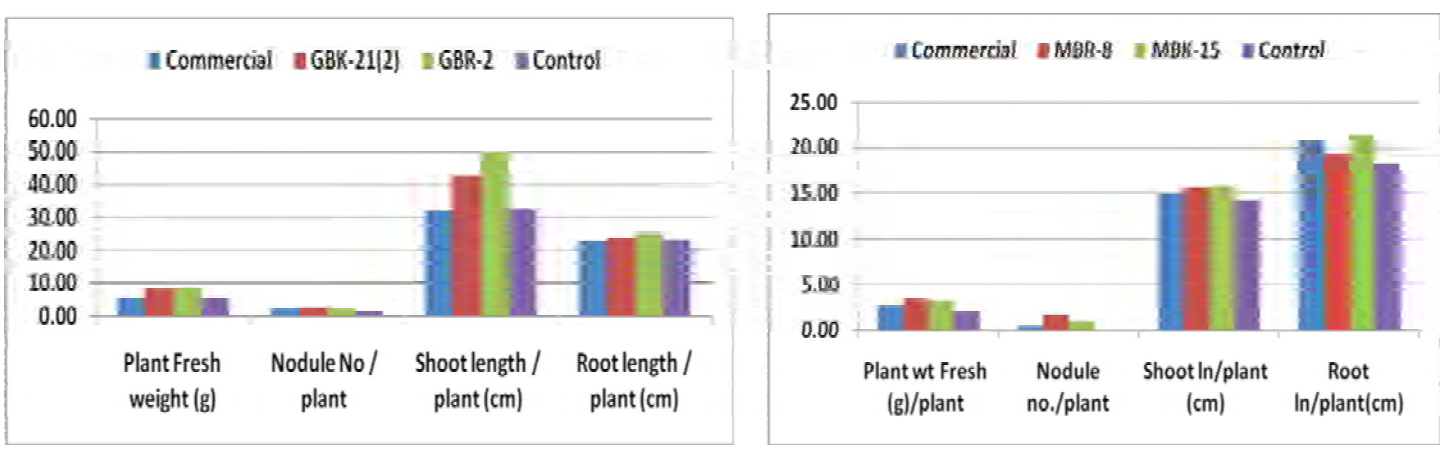
In case of moth bean, MBR-8 and MBK-15 outperformed commercial culture and uninoculated control for yield (kg ha$^{-1}$) and nodule Index (Table 2). MBR-8 and MBK-15 gave 19 and 9 per cent higher yield respectively than control and

(a) (b)

Fig. 2: GBR-2 inoculated plot of RGC 936(a) and inoculated root showing healthy nodules (b)

(a) (b)

Fig. 3: MBR-8 inoculated plot of CAZRI Moth 2 (a) and inoculated root showing healthy nodules (b)




(a) (b)

Fig. 1. Nursery evaluation of guar (RGC 936) and moth bean (CAZRI moth 2)



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Table 1: Effect of *Rhizobium* inoculation on yield and nodule index in guar (var. RGC 936) under field conditions

| Treatment | Strain | *Rhizobium* cultures | Isolated/ procured from | Guar yield (kg ha$^{-1}$) | Nodule index |
|---|---|---|---|---|---|
| T1 | GBR-2 | *Rhizobium pusense* | Barmer | 1146 | 15.3 |
| T2 | GBK-21-2 | *R. pusense* | Bikaner | 1116 | 12.2 |
| T3 | Commercial culture | *Rhizobium sp* | commercially | 1061 | 8.9 |
| T4 | Untreated Control | - | - | 971 | 5.8 |
| CD at 5% | | | | 151.9 | 1.7 |

Table 2: Effect of *Rhizobium* inoculation on yield and nodule index in moth bean (var. CAZRI Moth-2)

| Treatment | Strain | *Rhizobium* cultures | Isolated/ procured from | Moth bean yield (kg ha$^{-1}$) | Nodule Index |
|---|---|---|---|---|---|
| T1 | MBR-8 | *Sinorhizobium saheli* | Barmer | 378 | 13.3 |
| T2 | MBK-15 | *S. chiapanecum* | Bikaner | 343 | 10.7 |
| T3 | Commercial culture | *Rhizobium sp* | Commercially | 338 | 9.3 |
| T4 | Control | Untreated | - | 316 | 6.7 |
| CD at 5% | | | | 48.5 | 1.3 |

Table 3: Effect of different formulations on viable cell count of *Rhizobium* on YEMA plates

| Treatment | Days after storage (DAS) | Viable cell count (CFU ml$^{-1}$) of *Rhizobium* on YEMA plates (x10$^{8}$) * | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0d | 30d | 60d | 90d | 120d | 150d | 180d |
| T1 | YEM broth | 418 | 420 | 404 | 202 | 16 | - | - |
| T2 | YEM + 0.5 % glycerol | 412 | 419 | 406 | 303 | 85 | - | - |
| T3 | YEM + 0.5 % PVP | 431 | 411 | 360 | 266 | 97 | - | - |
| T4 | YEM + 0.5 % PEG | 445 | 439 | 441 | 435 | 425 | 343 | 322 |
| T5 | YEM + 0.5 % GA | 416 | 405 | 370 | 292 | 264 | 251 | 245 |
| T6 | YEM + Castor oil layer | 414 | 398 | 400 | 385 | 313 | 292 | 251 |
| T7 | YEM culture + Talc:: 1:2. | 321 | 266 | 152 | 110 | 58 | - | - |

*Values are means of five replications

up to 11 per cent higher yield than commercial culture. Fig. 3 shows healthy green plot (8x8 meter) and root of moth bean inoculated with *Rhizobium* culture (MBR-8). Similar beneficial effects of *Rhizobium* inoculation on growth and yield of chickpea (Cicer arietinum L.) were observed by Khaitov et al (2020).

### Shelf life of liquid biofertilizer

Treatments T1, T2, T3 and T7 showed decline in viable cell density at 120 DAS to considerable extent. Treatments T4, T5 and T6 more or less maintained initial cell density till 180d after storage (Table 3). The liquid bioformulation having PEG (0.5%) retained maximum number of colonies of *Rhizobium* culture MBR-8 followed by YEM+castor oil and YEM+GA (0.5%). Polyethylene glycol is a small molecular weight, water soluble compound with adhesive and sticky consistency. This property of PEG might be enhancing cell adherence to seed, and the viscous nature slows the drying process of the inoculant (Temperano *et al*., 2002) giving it advantage over other formulations. Jayasudha et al (2018) studied shelf life of liquid formulations of effective bacterial bio-agents using different oils and reported their beneficial effects on the survival of cultures.

## CONCLUSION

The study concluded that the liquid bioformulation containing only YEM broth gave very poor performance as compared to those having cell protectants such as PEG, GA or castor oil in addition to glycerol in appropriate amounts.

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# Systems approach in coconut for higher productivity and profitability in coastal ecosystem of Maharashtra

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**ABSTRACT**

The field experiment conducted on systems approach in coconut for higher productivity and profitability in coastal ecosystem at ICAR-AICRP on Palms, Regional coconut Research Station, Bhatye, Ratnagiri, (DBSKKV, Dapoli), Maharastra during the year 2013-14 to 2017-18, showed that the application of organic manures in combination with inorganic fertilizer either in 75 per cent of recommended NPK +25 per cent of N through organic recycling with vermi-compost or 50 per cent of RDF+50 per cent of N through organic recycling with vermi-compost +vermiwash application +bio-fertilizer application +*in situ* green manuring resulted in higher yield of 141.28 nuts palm$^{-1}$ year$^{-1}$ and 126.33 nuts palm$^{-1}$ year$^{-1}$, respectively. Soil organic carbon was higher, where 75 per cent of recommended NPK +25 per cent of N through organic recycling with vermi-compost ($T_1$) was added. Microbial population of fungi were higher where 100 per cent of N through organic recycling with vermi-compost +vermiwash application +bio-fertilizer application +*in situ* green manuring and green leaf manuring +composted coir pith, husk incorporation and mulching with coconut leaves ($T_3$) was added, whereas the bacteria and actinomycetes present in top soil were higher in the former treatment where 50 per cent RDF + 50 per cent N through organic reyching with vermicompost + vermiwash + bio-fertilizer + in situ, respectively was given. Earthworm population was highest in the treatment $T_3$ followed by the treatment $T_1$ and $T_2$. Highest (3.03) benefit:cost ratio was recorded in $T_1$ followed by $T_2$ (2.81) as against the control having monocrop of coconut with recommended NPK and organic manure.

**Key words:** *Coconut, INM, organic recycling, nutrient status, nut yield*

Coconut (*Cocus nucifera* L.) is widely grown in coastal sandy soils which occur around the coastal tract of West and East coast of Peninsular India. However, coconut productivity is very low in the coastal sandy soils due to an array of facts like poor water holding capacity, excessive infiltration, rapid leaching loss of nutrients resulting in low nutrient retentive capacity and low availability of major and micro nutrients, small specific surface area on account of low clay and organic matter content, low CEC and low organic carbon content (Ollangnier and Ochs, 1978). Moreover, the sole crop of coconut planted at a wider spacing of 7.5 m x 7.5 m is not able to fully utilize the available basic resources of crop production, *viz*. soil, solar energy, water and nutrients. Introduction of component crops, especially, adoption of multi-storeyed cropping system with compatible crops favours better utilization of resources for augmenting returns besides alleviating inherent soil limitations. Adoption of such systems can provide food security through food sufficiency, nutritional foods rich in vitamins and minerals, employment generation through farm diversification and ecological stability (Ramadasan and Lal, 1966). The soil resource base of the coastal sandy coconut zone is poor which needs to be upgraded to ensure timely and adequate availability of nutrients especially when multi-storeyed cropping system is adopted for various reasons. There is a need to consider the system as a unit especially with respect to supply of inputs like organic manures, fertilizers, herbicides, water and plant protection chemicals (Nampoothiri, 2001). Generally, lesser quantities of inputs are sufficient in cropping systems compared to sole cropping. The fertilizer doze for coconut is 2.25:3.0:2.0 kg urea, single super phosphate and muriate of potash per palm per year and continuous application of large quantities of fertilizers over a considerable period of time will definitely affect the physico-chemical and biological properties of soils turning the system unsustainable in all aspects. Sustainability can be in built in the system by introducing compatible crops as component crops and promoting positive interferences by judicious application of inputs especially fertilizers. In this context, an investigation was carried out to develop appropriate cost effective practices for enhancing nutrient use efficiency, productivity and profitability of coconut based

integrated nutrient management systems involving a combination of coconut, nutmeg, cinnamon banana and pineapple in a typical coastal sandy zone.

## MATERIALS AND METHOD

The field experiment was conducted at ICAR-AICRP on palms, Regional Coconut Research Station, Bhatye, Ratnagiri, (DBSKKV, Dapoli), (M.S) during the year 2013-14 to 2017-18. The experimental site is situated on the coast of Arabian Sea on western outskirts of village Bhatye and linked with the southern-borders of Ratnagiri city by the Bhatye Creek-Bridge on the mouth of river Kajali. Its height from mean sea level is 3.2 M and located at 16° 58'N Latitude and 73°17' E Longitude. The climate of experimental site is warm and humid tropical with mean annual rainfall of 3,500 mm and 120 rainy days. Mean temperature ranges from 21°C to 36°C. Average relative humidity varies between 60 to 95 per cent. Soil of experimental plot was sandy loam, well drained with medium fertility status. Experiment was laid out in 30-year-old coconut garden (cv. D x T – 'COD x West Coast Tall') which was planted at a distance of 7.5 m × 7.5 m in a square system. Each treatment consisted of 4 palms plot$^{-1}$, replicated 5 times in randomized block design. Treatments were $T_1$:75 per cent of recommended NPK + 25 per cent of N through organic recycling with vermicompost, $T_2$:50 per cent of RDF+50 per cent of N through organic recycling with vermicompost +vermiwash application + bio-fertilizer application + *in situ* green manuring, $T_3$: fully organic:100 per cent of N through organic recycling with vermicompost +vermiwash application +bio-fertilizer application +*in situ* green manuring and green leaf manuring (glyricidia leaves) +composted coir pith, husk incorporation (once in 3 years) and mulching with coconut leaves and $T_4$:control: mono-crop of coconut with recommended NPK and organic manure were imposed. The coconut palms along with component crops like nutmeg, cinnamon, banana and pineapple as a farming model. Experiment was conducted on 0.11 ha coconut garden that where inter planted with released varieties of the Maharashtra State (Table 1) as illustrated in fig. 1 and 2.

Quantity of NPK and vermicompost applied under different treatments has been described by Maheswarappa

Table 1: Plant population in coconut based integrated nutrient management system

| Crops | Varieties/Hybrids | Plants block$^{-1}$ | Plants acre$^{-1}$ |
|---|---|---|---|
| Coconut | D x T | 20 | 70 |
| Nutmeg | Konkan Swad | 12 | 54 |
| Cinnamon | Konkan Tej | 62 | 246 |
| Banana | Konkan Safed Velchi | 72 | 246 |
| Pineapple | Kew | 960 | 4320 |

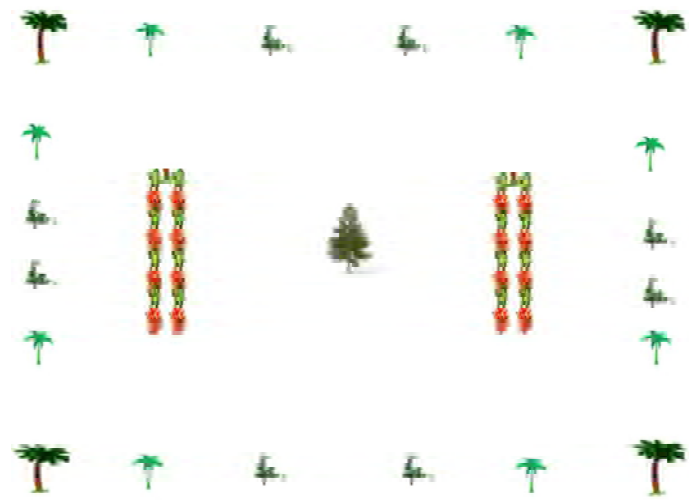

Fig. 1. Layout of single plot

*et al.* (2011). As per the treatments, vermicompost was applied during September–October and inorganic fertilizers in the form of urea, SSP and muriate of potash were applied in 2 equal splits during June-July and September–October. Vermicompost was obtained by decomposing coconut leaves as per the procedure explained by Prabhu *et al.* (1998). Biomass was recycled back into system after making vermicompost and recommended dose of fertilizers applied both for coconut and component crops as per treatment and farm waste utilization done effectively to meet nutrient requirement. Vermicompost was prepared and applied to meet requirement of nutrients. Vermiwash collected was drenched in basin of each crop by diluting it in ratio of 1:10 with water and applied twice in year for coconut @5 lits basin$^{-1}$, nutmeg@3 lits tree$^{-1}$, banana @2 lits plant$^{-1}$, pineapple 4 lits in a bed of 40 plants and cinnamon @2 lits plant$^{-1}$. In addition, glyricidia plants were grown as green manuring crop at a border of plot and green leaf manuring was done for coconut and intercrops in month of June, while application of fertilizer. Palms were irrigated with drip system while sprinkler irrigation was followed for irrigating different crops in system. During December to January, 27 litres water and 32 litres water during February to May per palm/day was applied. Husk burial was followed before planting perennial crops and husk burial in the trenches was followed in each set of four coconut palms (once in 3 year). Dried coconut leaves were used for mulching in summer months (February-May) in order to reduce the evaporation of moisture from soil.

Soil and leaf samples were collected from 3 palms in each plot. Soil samples were collected from 0–25 cm, 25-50 cm and 50-100 cm depth, 1 m away from the bole of the coconut using augur and soil properties were determined



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by adopting standard procedures. Leaf samples were collected from index leaf (4th leaf) of palm by using a specially designed knife, by cutting 4–5 leaflets from middle of frond on both sides. Leaf samples were washed with distilled water, oven-dried at 65°C for 72 hr and powdered using a Tecator Cyclotec sample mill. Powdered fraction (0.5 mm) of leaf sample was digested in di-acid mixture of HNO3: HClO4 (3:1) and analysed for phosphorus and potassium content. Nitrogen content in plant sample was estimated according to modified Kjeldahl procedure using Tecator Kjeltec Auto Analyser. Content of K was estimated in atomic absorption spectrophotometer.

Soil samples drawn from circular basins at 1.0 m away from bole, at 0–25 cm using a tube augur were used for microbial enumeration. Population of bacteria, fungi and actinomycetes were determined in collected soils. Samples were serially diluted in sterile water blanks to produce several dilutions and 1 ml aliquot was pour plated in different media. Three sets of samples were drawn from each treatment. Three replications for each group of microorganisms were maintained. Total numbers of culturable bacteria were counted on nutrient agar after incubating for 24–48 hr at 28°C, actinomycetes on Ken Knights and Munaier's agar (1) counted after 5–7 days incubated at 28°C, fungi on Martin's rose Bengal agar counted after 2–4 days incubated at 28°C. Results of the microbial analyses were given as CFU $g^{-1}$ of dry soil. Each CFU value was the average of 4 × 3 sample replicates. Annual leaf production was recorded from the selected palms by marking a newly emerged leaf and later counting number of leaves emerged above the marked leaves as leaf production/palm/year. Numbers of spadices and buttons were timely recorded. Nuts were harvested at maturity stage palm-wise and average for the year was worked out. Copra and oil yield were also calculated. Growth and yield characters of component crops were timely recorded. Earthworm population (Nos./$m^2$), biomass generation (kg) and light interception (%) were recorded and economics of coconut based integrated nutrient management system was worked out.

## RESULTS AND DISCUSSION

### Number of leaves on crown

Data (table 4) showed the highest number of functional leaves on crown in treatment $T_1$ (30.46), whereas lowest in control $T_4$ (29.20). Nath *et al*. (2012) reported increase in number of leaves due to INM in coconut. Results are in tune with observations of Maheswarappa *et al*. (2014) wherein application of different sources of organics and inorganics did not reduce or increase number of leaves on crown. Number of leaves present on crown also did not differ among the treatments. Being a perennial crop, effect of different treatments might not have any influenced on growth and development of palms.

### Rate of leaf production

Number of leaves produced per palm year$^{-1}$ did not significantly differ among treatments (Table 4). However, it was found that the leaf production was higher (11.87) in the integrated nutrient management treatments $T_1$ compared to other treatments. Nath *et al*. (2012) reported increase in leaf production owing to INM in coconut. Being a perennial crop, effect of different treatments might not have significantly influenced growth and development of palms.

### Number of spadices

Number of spadices produced per palm year$^{-1}$ did not significantly differ among treatments (Table 5). However, it was found that the spadices production were higher (11.52) in integrated nutrient management treatments $T_1$ followed by treatment $T_2$ (11.38) and $T_3$ (11.36) whereas lower spadices production were in treatment $T_4$ (11.31 nos.). Kalpana *et al*. (2008) reported increase in spadices production owing to INM in coconut and recorded maximum spadices per palm/year in the application of 100 per cent CCP and 50 per cent CCP + 50 per cent RDF.

### Number of buttons

Number of buttons produced per palm year$^{-1}$ did significantly differ among treatments (Table 5). However, it was found that the buttons production was higher (327.44) in the integrated nutrient management treatments $T_1$ followed by treatment $T_2$ and $T_3$ whereas lowest buttons production was in treatment $T_4$. Kalpana *et al*. (2008) reported increase in buttons production due to INM in coconut and recorded maximum female flowers per palm year$^{-1}$ in the application of 100 per cent CCP and 50 per cent CCP + 50 per cent RDF and Nath *et al*. (2012) reported increase in buttons production owing to INM in coconut.

### Coconut nut yield

Coconut yield recorded among the treatments over years and data are presented in Table 5. In general, there was an increase in yield of coconut and yield obtained in different treatments was higher over years than pre-treatment yields, which was mainly owing to effect of treatments and irrigation provided to the palms. During 2014-2017, application of 75 per cent of recommended NPK+25% of N through organic recycling with vermicompost ($T_1$) treatment recorded higher nut yield (141.28 s) and was at par with 50 per cent of RDF+50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer

Table 2: Soil nutrient status as influenced by coconut based INM system

| Soil depth | Parameter → / Treatment ↓ | 2013-2014 | | | | | | 2017-2018 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | EC (dS $m^{2-1}$) | N (kg $ha^{-1}$) | $P_2O_5$ (kg $ha^{-1}$) | $K_2O$ (kg $ha^{-1}$) | OC (%) | pH | EC (dS/$m^2$) | N (kg $ha^{-1}$) | $P_2O_5$ (kg $ha^{-1}$) | $K_2O$ (kg $ha^{-1}$) | OC (%) |
| 0-25 | $T_1$ | 7.5 | 0.183 | 253 | 17 | 301 | 0.42 | 7.55 | 0.190 | 277 | 21.5 | 309.3 | 1.26 |
| | $T_2$ | 7.1 | 0.149 | 248 | 18 | 289 | 0.54 | 7.24 | 0.158 | 262 | 25.6 | 293.1 | 1.24 |
| | $T_3$ | 6.8 | 0.168 | 220 | 16 | 278 | 0.39 | 6.82 | 0.176 | 243 | 19.3 | 284.0 | 1.20 |
| | $T_4$ | 6.7 | 0.186 | 265 | 17 | 312 | 0.30 | 6.71 | 0.196 | 284 | 20.3 | 318.0 | 0.89 |
| 25-50 | $T_1$ | 7.4 | 0.170 | 234 | 17 | 277 | 0.31 | 7.43 | 0.181 | 248 | 21.6 | 286.0 | 1.22 |
| | $T_2$ | 7.1 | 0.168 | 217 | 15 | 242 | 0.51 | 7.11 | 0.173 | 241 | 18.1 | 256.0 | 1.20 |
| | $T_3$ | 6.9 | 0.178 | 167 | 9 | 269 | 0.44 | 6.9 | 0.181 | 178 | 13.0 | 276.1 | 1.18 |
| | $T_4$ | 6.8 | 0.190 | 240 | 12 | 265 | 0.30 | 6.81 | 0.198 | 251 | 16.0 | 278.1 | 0.91 |
| 50-100 | $T_1$ | 7.3 | 0.211 | 191 | 13 | 212 | 0.38 | 7.22 | 0.221 | 202 | 16.1 | 218.3 | 1.19 |
| | $T_2$ | 7.0 | 0.151 | 143 | 9 | 228 | 0.43 | 7.10 | 0.168 | 151 | 13.0 | 233.1 | 1.18 |
| | $T_3$ | 6.8 | 0.201 | 152 | 9 | 261 | 0.41 | 6.79 | 0.206 | 158 | 12.2 | 273.0 | 1.16 |
| | $T_4$ | 6.7 | 0.192 | 210 | 10 | 215 | 0.32 | 6.41 | 0.198 | 221 | 13.4 | 226.1 | 0.78 |

Table 3: Leaf nutrient status and soil microbial population as influenced by coconut based INM system

| Treatment | Leaf nutrient status (%) | | | | | | Soil microbial population (CFU $g^{-1}$ dry soil) (2017-18) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2013-2014 | | | 2017-2018 | | | Bacteria ($10^5$ CFU/g soil) | Fungi ($10^4$ CFU/g soil) | Actinomycetes ($10^3$ CFU/g soil) |
| | N (%) | P (%) | K (%) | N (%) | P (%) | K (%) | | | |
| $T_1$ | 1.50 | 0.12 | 1.2 | 1.75 | 0.18 | 1.31 | 95.0 | 153.0 | 134.0 |
| $T_2$ | 1.40 | 0.14 | 1.0 | 1.71 | 0.16 | 1.26 | 89.0 | 148.0 | 136.0 |
| $T_3$ | 1.38 | 0.13 | 0.9 | 1.53 | 0.11 | 1.24 | 77.0 | 166.0 | 112.0 |
| $T_4$ | 1.48 | 0.14 | 1.1 | 1.49 | 0.10 | 1.20 | 42.0 | 59.0 | 67.0 |

Table 4: Influence of integrated nutrient management system on growth of coconut

| Treatment | Number of leaves on crown (Nos $palm^{-1}$) | | | | | | Rate of leaf production (Nos $palm^{-1}$ $year^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled |
| $T_1$ | 31.38 | 30.98 | 31.43 | 28.78 | 29.27 | 30.46 | 12.00 | 11.77 | 11.95 | 11.87 | 11.97 | 11.87 |
| $T_2$ | 30.00 | 30.05 | 30.80 | 29.60 | 29.62 | 30.07 | 11.65 | 11.37 | 11.55 | 11.60 | 11.53 | 11.57 |
| $T_3$ | 30.22 | 29.00 | 30.35 | 28.53 | 28.83 | 29.57 | 11.65 | 11.42 | 11.85 | 11.77 | 11.80 | 11.73 |
| $T_4$ | 29.48 | 28.95 | 29.92 | 28.45 | 28.65 | 29.20 | 11.58 | 11.40 | 12.05 | 11.75 | 11.90 | 11.69 |
| S.Em.± | 0.34 | 0.25 | 0.24 | 0.53 | 0.50 | 0.15 | 0.19 | 0.13 | 0.18 | 0.20 | 0.18 | 0.05 |
| CD (P=0.05) | 1.03 | 0.76 | 0.75 | NS | NS | 0.45 | NS | NS | NS | NS | NS | 0.14 |

Table 5: Influence of coconut based INM system on yield and yield attributing characters of coconut

| Treatment | Number of spadices (nos. $palm^{-1}$) | | | | | | Number of buttons (nos. $palm^{-1}$) | | | | | | Nut yield (nos. $palm^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled |
| $T_1$ | 11.95 | 11.35 | 11.62 | 11.87 | 11.97 | 11.52 | 323.97 | 330.42 | 335.22 | 332.17 | 331.75 | 327.44 | 126.03 | 178.25 | 129.75 | 154.58 | 143.30 | 141.28 |
| $T_2$ | 11.50 | 10.98 | 11.45 | 11.60 | 11.53 | 11.38 | 315.55 | 311.57 | 319.95 | 324.90 | 325.97 | 321.45 | 120.43 | 146.92 | 123.80 | 148.60 | 130.08 | 126.33 |
| $T_3$ | 11.28 | 11.07 | 11.57 | 11.72 | 11.80 | 11.36 | 314.53 | 306.17 | 325.47 | 323.00 | 329.00 | 320.43 | 102.40 | 125.40 | 113.87 | 134.33 | 119.12 | 120.01 |
| $T_4$ | 11.20 | 10.75 | 11.68 | 11.80 | 11.80 | 11.31 | 308.67 | 302.00 | 311.67 | 328.40 | 328.40 | 314.76 | 98.60 | 87.45 | 110.50 | 122.75 | 113.48 | 112.62 |
| S.Em.± | 0.20 | 0.19 | 0.14 | 0.21 | 0.18 | 0.10 | 5.90 | 3.82 | 4.77 | 3.14 | 2.85 | 2.92 | 6.25 | 8.03 | 9.79 | 11.05 | 6.25 | 6.42 |
| CD (P=0.05) | NS | NS | NS | NS | NS | 0.29 | NS | 11.78 | 14.69 | NS | NS | 8.81 | 19.25 | 24.75 | NS | NS | 19.27 | 19.35 |

Table 6: Influence of coconut based integrated nutrient management system on copra and oil yield of coconut

| Treatment | Copra yield (kg $palm^{-1}$) | | | | | | Oil yield (kg $palm^{-1}$) | | | | | | Oil yield (tonnes $ha^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled |
| $T_1$ | 21.43 | 30.30 | 22.06 | 26.28 | 20.25 | 23.18 | 14.57 | 20.61 | 14.99 | 17.86 | 13.77 | 15.75 | 2.55 | 3.61 | 2.62 | 3.12 | 2.40 | 2.80 |
| $T_2$ | 20.47 | 24.98 | 21.05 | 25.26 | 22.11 | 21.48 | 13.92 | 16.98 | 14.31 | 17.17 | 15.03 | 14.60 | 2.44 | 2.97 | 2.50 | 3.00 | 2.63 | 2.79 |
| $T_3$ | 17.41 | 21.32 | 19.36 | 25.28 | 19.29 | 20.60 | 11.84 | 14.50 | 13.16 | 15.53 | 13.12 | 13.73 | 2.07 | 2.54 | 2.30 | 2.71 | 2.29 | 2.40 |
| $T_4$ | 16.76 | 14.87 | 18.79 | 20.87 | 24.36 | 19.99 | 11.40 | 10.11 | 12.77 | 14.19 | 16.56 | 13.59 | 1.99 | 1.77 | 2.23 | 2.48 | 2.89 | 2.38 |
| S.Em.± | 1.06 | 1.37 | 1.66 | 2.27 | 1.06 | 1.27 | 0.72 | 0.93 | 1.13 | 1.28 | 0.72 | 0.85 | 0.13 | 0.16 | 0.20 | 0.22 | 0.13 | 0.20 |
| CD (P=0.05) | 3.27 | 4.21 | NS | NS | 3.28 | 3.84 | 2.22 | 2.86 | NS | NS | 2.23 | 2.58 | 0.39 | 0.50 | NS | NS | 0.39 | 0.61 |



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application + *in situ* green manuring ($T_2$) (126.33 s) and was significantly differed compared to other treatments. Pooled data on nut yield for 5 years (2013-14 to 2017-18) indicated the significant differences among the treatments (Table 5). Increase in yield under these treatments might be owing to better availability of required nutrients which resulted in improvement in yield. Srinivasa Reddy and Upadhyay (2002), Talashilkar *et al*. (2008) and Nath *et al*. (2012) reported increase in yield of coconut with the application of inorganic fertilizer (50%) and through vermicompost (50%) and the positive effect of integration of organic and inorganic fertilizer combination on coconut yield in different soil types also. An increase in annual productivity in coconut by following mix cropping has been reported by Shinde *et al*., 2019. Additional increased in yield of coconut with farming system component could be due to synergistic effect of crop combination and nutrient status maintained in the system. Application of vermicompost alone could not result in increase in yield of coconut, as it could not provide the required P and K and application of inorganic fertilizer alone could not provide the suitable soil environment for the growth and development of coconut (Shinde *et al*., 2019).

## Copra and oil yield

Copra and oil yield recorded among the treatments over the years and are presented in Table 6. In general, there was an increase in copra and oil yield of coconut and yield obtained in different treatments was higher over years than pre-treatment yields, which was mainly owing to effect of treatments and irrigation provided to the palms. During 2014-2017, application of 75 per cent of recommended NPK+25 per cent of N through organic recycling with vermicompost ($T_1$) treatment recorded higher copra (23.18 kg palm$^{-1}$) and oil yield (15.75 kg palm$^{-1}$) and was at par with 50 per cent of RDF+50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring treatment ($T_2$) (21.48 kg palm$^{-1}$) and (14.60 kg palm$^{-1}$) respectively and was differed compared to other treatments. Also the oil yield (tonnes/ha) obtained under 75 per cent of recommended NPK+25 per cent of N through organic recycling with vermicompost ($T_1$) treatment and 50 per cent of RDF+ 50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring ($T_2$) treatment was at par and ranged from 2.79 to 2.80 tonnes hectare$^{-1}$. Increase in copra and oil yield under these treatments might be owing to better availability of required nutrients which resulted in improvement in yield. Application of any single manure could not result in increase in copra and oil yield of coconut, as it could not provide the required P and K and application of inorganic fertilizer alone could not provide the suitable soil environment for the growth and development of coconut (Shinde *et al*., 2019). Results analogous to these finding were also recorded by Kalpana *et al*. (2008) in the coconut based INM system on nut yield and quality of coconut under coastal ecosystem.

Table 7: Influence of coconut based INM system on growth of component crops as an intercrops in coconut orchard

| Treatment | Height of nutmeg (cm) | | | | | | No. of branches in nutmeg | | | | | | Height of cinnamon (cm) | | | | | | No. of branches in cinnamon | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled |
| $T_1$ | 84.0 | 95.0 | 107.4 | 102 | 152 | 94.0 | 5.0 | 7.4 | 8.4 | 7.4 | 6.0 | 6.1 | 197.7 | 206.5 | 261.5 | 415.5 | 354.5 | 253.1 | 13.2 | 15.2 | 8.8 | 2.3 | 1.9 | 8.6 |
| $T_2$ | 76.4 | 84.4 | 92.8 | 120 | 264 | 112.8 | 3.4 | 5.8 | 7.6 | 8.2 | 18.2 | 7.6 | 178.8 | 189.5 | 231.5 | 399.0 | 305.5 | 226.5 | 10.6 | 12.3 | 11.7 | 2.7 | 1.8 | 7.4 |
| $T_3$ | 118.2 | 150.4 | 163.4 | 226 | 178 | 157.9 | 7.6 | 11.8 | 12.8 | 12.2 | 3.4 | 9.3 | 213.6 | 219.7 | 316.0 | 373.0 | 400.5 | 268.3 | 20.9 | 21.2 | 7.6 | 1.8 | 1.6 | 10.5 |
| $T_4$ (Mono) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S.Em.± | 22.31 | 24.67 | 25.88 | 38.64 | 36.05 | 9.94 | 1.81 | 2.19 | 2.57 | 1.53 | 2.90 | 0.75 | 9.88 | 10.72 | 18.92 | 33.34 | 28.73 | 6.94 | 1.16 | 0.75 | 0.79 | 0.21 | 0.12 | 1.24 |
| CD (P=0.05) | NS | NS | NS | NS | NS | 28.28 | NS | NS | NS | NS | 9.46 | 2.13 | NS | NS | 61.71 | NS | NS | 19.74 | 3.77 | 2.46 | 2.56 | 0.69 | NS | NS |

Table 8: Influence of coconut based INM system on yield of nutmeg and cinnamon as an intercrops in coconut orchard

| Treatment | Yield of component crops block$^{-1}$ | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of nuts in nutmeg | | | | | | Cinnamon bark (kg) | | | | | | Cinnamon leaves (kg) | | | | | |
| | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled |
| $T_1$ | 2.2 | 2.6 | 3.6 | 9.4 | 12.0 | 5.1 | 8.4 | 17.0 | 18.0 | 15.4 | 15.4 | 13.0 | 42 | 60 | 65 | 67 | 57 | 55.2 |
| $T_2$ | 2.0 | 2.0 | 3.8 | 8.0 | 8.0 | 4.2 | 8.0 | 8.0 | 8.0 | 17.5 | 17.5 | 10.7 | 40 | 40 | 40 | 45 | 50 | 44.2 |
| $T_3$ | 6.6 | 7.0 | 6.0 | 7.0 | 7.0 | 5.9 | 9.0 | 8.4 | 9.0 | 20.0 | 20.0 | 11.7 | 43 | 42 | 45 | 48 | 62 | 45.0 |
| $T_4$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S.Em.± | 1.90 | 1.87 | 1.49 | 1.58 | 0.48 | 0.58 | 0.37 | 0.62 | 0.58 | 0.81 | 0.81 | 1.34 | 1.26 | 1.85 | 2.56 | 1.68 | 1.28 | 3.25 |
| CD (P=0.05) | NS | NS | NS | NS | 1.58 | NS | NS | 2.03 | 1.88 | 2.65 | 2.65 | NS | NS | 6.03 | 8.35 | 5.49 | 4.17 | 10.23 |

### Growth and yield of components crops

Component crops growth as influenced by coconut based INM system in coconut is presented in Table 7. It was observed data that the height of nutmeg increases after the 4th year of treatment initiation and maximum height of nutmeg was 157 cm in $T_3$ whereas minimum was in treatment $T_1$ (94 cm). Maximum number of nutmeg branches was in treatment $T_3$ (9.30) whereas minimum in treatment $T_1$ (6.10 nos.). Maximum height of cinnamon was 268.3 cm in $T_3$ whereas minimum was in treatment $T_2$ (226.5 cm). Maximum number of cinnamon branches was in treatment $T_3$ (10.50) whereas minimum in treatment $T_2$ (7.44). According to the pooled yield data, the highest yield of component crops namely pineapple and banana were in the treatment $T_3$ such as 40.92 kg block$^{-1}$ and 401.67 kg block$^{-1}$, respectively whereas highest yield of component crops nutmeg nuts in $T_3$, cinnamon bark and cinnamon leaves in $T_1$ such as 5.9 nuts block$^{-1}$, 13 kg block$^{-1}$ and 55.2 kg block$^{-1}$ respectively. Similar results were also reported by Bavappa and Jacob (1982) in coconut farming system.

### Biomass generation, vermi-compost production and light interception

Biomass generation was highest in the treatment $T_3$ (5700 kg/ha/year) followed by $T_1$ (5670 kg ha$^{-1}$ year$^{-1}$), $T_2$ (5285 kg ha$^{-1}$ year$^{-1}$) and $T_4$ (3791 kg ha$^{-1}$ year$^{-1}$). Vermi-compost production was higher in the treatment $T_3$ (3875 kg ha$^{-1}$ year$^{-1}$) followed by the treatment $T_1$ (3700 kg ha$^{-1}$ year$^{-1}$), $T_2$ (3518 kg ha$^{-1}$ year$^{-1}$) and $T_4$ (2477 kg ha$^{-1}$ year$^{-1}$) (Table 12). Also the generated biomass and vermi-compost production from different component crops which can be recycled in the coconut based INM system. Percent of light intensity was highest in treatment $T_1$ (67.89 %) and $T_3$ (65.06 %). Additional yield obtained by different crops in the system and additional biomass generated on the same piece of land, and other resource like space, height, irrigation etc. could be benefited to obtain higher income. Similar results were also reported by Bavappa and Jacob (1982) in coconut farming system.

### Soil properties

The electrical conductivity of soil (at 0–25 cm depth) did change due to the INM practices in the basins of coconut, as seen during treatment initiation (2013-14) and mean of 4th years after treatment initiation (2014-15 to 2017-18) (Table 2). After 4th years of treatment initiation soil pH and organic carbon content differed among treatments. With the application of vermicompost, there was change in the pH of the soil, and the application of 75 per cent of recommended NPK + 25 per cent of N through organic recycling with vermicompost recorded higher pH (7.55) followed by the application of 50 % of RDF+ 50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring (7.24) as compared to the other two treatments. The soil organic carbon also higher with the application of 75 per cent of recommended NPK + 25 % of N through organic recycling with vermicompost (1.26 per cent) followed by the application of 50 per cent of RDF+ 50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring (1.24 %) as compared to the other two treatments. The soil nutrient content (NPK) was also highest with the application of 75 per cent of recommended NPK + 25 per cent of N through organic recycling with vermicompost

Table 9: Influence of coconut based INM system on yield of pineapple and banana as an intercrops in coconut orchard

| Treatment | Yield of component crops block$^{-1}$ | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pineapple (kg) | | | | | | Banana (kg) | | | | | |
| | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled |
| $T_1$ | 50 | 60 | 70 | 35 | 13 | 39.5 | 450 | 420 | 450 | 490 | 450 | 406.6 |
| $T_2$ | 45 | 50 | 60 | 30 | 20 | 36.0 | 400 | 360 | 370 | 430 | 420 | 362.3 |
| $T_3$ | 40 | 70 | 75 | 40 | 13 | 40.9 | 350 | 480 | 500 | 580 | 380 | 401.7 |
| $T_4$ | - | - | - | - | - | - | - | - | - | - | - | - |
| S.Em.± | 2.74 | 1.83 | 5.40 | 1.94 | 0.86 | 2.40 | 12.97 | 9.57 | 12.38 | 12.71 | 12.65 | 21.69 |
| CD (P=0.05) | NS | 5.95 | NS | 6.32 | 2.79 | NS | 42.31 | 31.22 | 40.38 | 41.47 | 41.25 | NS |

Table 10: Economics of coconut based integrated nutrient management system

| Treatment | Cost of production (Rs ha$^{-1}$) | | | | | | Gross return (Rs ha$^{-1}$) | | | | | | Net return (Rs ha$^{-1}$) | | | | | | B:C ratio | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Mean | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Mean | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Mean | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Mean |
| $T_1$ | 30852 | 30384 | 30432 | 33756 | 33754 | 31836 | 830650 | 1281300 | 1712550 | 2479897 | 686783 | 1398236 | 621954 | 1112629 | 1543885 | 2311117 | 518012 | 1221519 | 2.1 | 2.9 | 3.0 | 3.1 | 4.1 | 3.03 |
| $T_2$ | 41739 | 33734 | 33733 | 30450 | 30486 | 34028 | 784120 | 967800 | 1236750 | 1546836 | 665168 | 1040135 | 629860 | 815880 | 1084590 | 148743 | 512739 | 638362 | 1.9 | 2.1 | 2.8 | 2.9 | 4.4 | 2.81 |
| $T_3$ | 30458 | 30424 | 29749 | 29770 | 29734 | 30027 | 781650 | 900450 | 1388100 | 1495072 | 611146 | 1035284 | 504746 | 748328 | 1239357 | 1346222 | 462476 | 860226 | 1.6 | 1.9 | 2.1 | 2.4 | 4.1 | 2.42 |
| $T_4$ | 11400 | 11400 | 11400 | 11400 | 11400 | 11400 | 214200 | 189000 | 258300 | 311682 | 392280 | 273092 | 157200 | 132000 | 201300 | 254682 | 335280 | 216092 | 1.6 | 1.7 | 1.9 | 2.1 | 3.9 | 2.25 |



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followed by the application of 50 per cent of RDF+ 50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring as compared to the other two treatments (Table 2). Increase in N, P and K, content of coconut cropping system from 2013-14 to 2017-18 could be attributed to organic recycling of biomass glycricidia leaf lopping, vermiwash application in the system. Krishnakumar and Maheswarappa (2010), Shinde *et al.*, 2019, Srinivasa Reddy and Upadhyay (2002) and Maheswarappa *et al.* (2014) reported significant change in soil properties due to integrated nutrient management practices.

**Leaf-nutrient status**

Nutrient content in the index leaf in respect of N, P and K differed among the treatments (Table 3). After 4th years of treatment initiation, the mean N content was higher with the application of 75 per cent of recommended NPK + 25 per cent of N through organic recycling with vermicompost (1.75 per cent) followed by 50 per cent of RDF+50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring (1.71 %) as compared to the other two treatments. Also the P and K content were higher with treatment $T_1$. It was observed that, as the recommended NPK was reduced, the leaf N, K content also found to be decreased, mainly because of lower N and K supply through vermi-compost and reduced dose of recommended N. In general, it was found that, there was improvement in leaf nutrient status in respect of major and micronutrients due to different treatments compared to pre-experimental nutrient status. This is mainly attributed to timely application of nutrients and irrigation for the crop. It was observed from the data that N, P, K content of coconut leaf increased after four years from system. Results analogous to these finding were recorded by Maheswarappa *et al.* (1998) in mix cropping of coconut.

**Soil microbial population**

Population of bacteria, fungi and actinomycetes did differ among various treatments, when analysed at 0–25 cm soil depth (Table 3). Though the top soil (0–25 cm depth) is the zone of intensive microbial activity and therefore, should have reflected changes undergoing in microbial community structure in response to extraneous inputs, which in present study are organic and inorganic fertilizers. However, the population of fungi were, in general, more in treatments $T_3$ where 100 % of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring and green leaf manuring + composted coir pith, husk incorporation and mulching with coconut leaves was applied as compared to other treatments. Bacteria and actinomycetes present in top soil were higher in treatment $T_1$ and $T_2$ respectively. Also the earthworm population were highest in the treatment $T_3$ followed by the treatment $T_1$ and $T_2$ (Table 11). Results analogous to these finding were also recorded by Maheswarappa *et al.* (2014) in the coconut based INM with vermi-composted coconut leaves.

Table 11: Earthworm population (No's/m$^2$) as influenced by coconut based integrated nutrient management system

| Treatment | Soil depth | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-10 cm | | | | | | 10-20 cm | | | | | | 20-30 cm | | | | | |
| | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | Pooled |
| $T_1$ | 12.4 | 12.0 | 12.0 | 13.6 | 12.0 | 12.33 | 6.4 | 6.4 | 6.8 | 6.0 | 5.6 | 5.90 | 2.2 | 2.8 | 2.8 | 3.0 | 2.6 | 2.5 |
| $T_2$ | 8.6 | 9.0 | 10.0 | 10.2 | 9.6 | 9.23 | 2.8 | 3.2 | 3.4 | 4.8 | 5.0 | 3.53 | 1.6 | 1.8 | 2.0 | 2.8 | 2.0 | 1.9 |
| $T_3$ | 16.0 | 14.8 | 15.2 | 15.0 | 14.0 | 15.23 | 9.6 | 9.6 | 10.0 | 10.0 | 10.4 | 9.57 | 3.8 | 4.2 | 4.2 | 4.0 | 3.6 | 3.9 |
| $T_4$ | 11.6 | 7.2 | 7.6 | 7.8 | 8.0 | 7.70 | 2.0 | 2.2 | 2.2 | 3.8 | 4.0 | 2.67 | 0.8 | 1.4 | 1.6 | 2.8 | 2.6 | 1.7 |
| S.Em.± | 1.02 | 1.39 | 1.29 | 0.52 | 0.36 | 0.29 | 0.35 | 0.42 | 0.47 | 0.38 | 0.55 | 0.16 | 0.20 | 0.22 | 0.16 | 0.20 | 0.41 | 0.10 |
| CD (P=0.05) | 3.14 | 4.27 | 3.96 | 1.62 | 1.12 | 0.87 | 1.09 | 1.30 | 1.46 | 1.18 | 1.68 | 0.45 | 0.60 | 0.68 | 0.49 | 0.60 | NS | 0.31 |

Table 12: Biomass generation, vermi-compost production and light interception in coconut based integrated nutrient management system (Light intensity in Lux during 11.00 am to 1.00 pm) (2017-2018)

| Treatments | Biomass generation (Kg ha$^{-1}$/year$^{-1}$) | Vermi-compost production (Kg ha$^{-1}$/year$^{-1}$) | Light intensity (Lux) | Light interception (%) |
|---|---|---|---|---|
| Open field | - | - | 75850 | - |
| $T_1$ | 5670 | 3700 | 25350 | 67.89 |
| $T_2$ | 5285 | 3518 | 27500 | 63.74 |
| $T_3$ | 5700 | 3875 | 26500 | 65.06 |
| $T_4$ | 3791 | 2477 | 63500 | 16.28 |

### Economics

Economics of coconut based integrated nutrient management system revealed that the highest (3.03) benefit:cost ratio was recorded with the application of 75 per cent of recommended NPK + 25 per cent of N through organic recycling with vermicompost followed by 50 per cent of RDF+ 50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring ($T_2$) (2.81) as compared to the other two treatments.

## CONCLUSION

It was concluded that the application of organic manures in combination with inorganic fertilizer either in 75 per cent of recommended NPK+ 25 per cent of N through organic recycling with vermicompost or 50 per cent of RDF+50 per cent of N through organic recycling with vermicompost + vermiwash application + bio-fertilizer application + *in situ* green manuring is beneficial in maintaining nutritional status of coconut improving soil microbial population and producers higher coconut yield over a period of time.

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# Role of different cations and anions of HMW salt mixture in artificial diets on growth and development of *Bactrocera dorsalis* (Hendel)

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**ABSTRACT**

The effect of cations and anions of HMW salt mixture for their role on the growth and development of maggots of *Bactrocera dorsalis,* studied through deletion method, revealed that potassium among seven cations (Ca, Mg, Mn, Fe, Cu, K, and Na) and iodide, sulphate and chloride among anions ($CO_3$, $SO_4$, $PO_4$, I, F and Cl) were found as the most essential ingredients of an artificial diet for normal growth and development of *B. dorsalis*. A diet devoid of potassium showed prolonged maggot period (25.15 days), pupal period (16.10 days) and reduced pupal weight (6.92 mg), pupal formation (1.66%), nil adult emergence and lowest growth index (0.07) against the maximum (3.50) in the control. The low pupal period (8.62 days) in the diet without manganese and high pupal formation (50%) in the diet without calcium were observed. The diet devoid of chloride showed no maggot emergence, pupal formation and adult emergence while that without iodide showed pro-longed maggot period of 23.75 and 23.66 days, respectively. The control showed the shortest pupal period (9.75 days) and the highest pupal weight (12.08 mg). The pupal formation was high in the diet without $CO_3$ (61.70%) and $PO_4$ (61.70 %). The overall growth index was high in control (3.60) followed by the diet without $CO_3$.

**Key words:** Cation, anion, HMW salt mixture, artificial diet, *Bactrocera dorsalis*.

India ranking second in fruits and vegetables production in the world has witnessed voluminous increase in horticulture production over the last few years. Significant increase in the area (3.0%) and production (5.4%) per annum over the last one decade has been made. The production of about 295.2 million tonnes from an area of 24.9 million hectare horticulture crops during 2016-17, outpaced the food grain production since 2012-13.

As the demand for fruits and vegetables are increasing day by day, the development of technology for still higher production and productivity is the need of the day. In India, *Bactrocera dorsalis* (Hendel), commonly known as oriental fruit fly, is one of the most destructive fruit and vegetable pest in the world. They damages the crop in many ways: i) causing ovipositoinal injury by the female on fruits and vegetative parts leading to black or brown lesions, ii) larvae damaging ovaries and fruit pulp iii) decomposition of fly-damaging fruits by invading saprophytic microorganisms. The losses of fruit due to this pest ranges from 1 to 31 per cent with a mean of 16 per cent. In addition to the direct losses, its economic impacts results in the loss of export markets as well as costly requirement of quarantine restrictions and eradication measures (Badii *et al.* 2015).

The artificial diets are an essential component for conducting basic studies on toxicology, physiology, biological control and integrated pest management. Decades have passed but the commercial mass production of fruit fly species are yet being done on their natural host fruits, which is expensive and more labour-intensive requiring a lot of space. Moreover, fruits are impractical to handle as well as disposal, difficult to standardize with respect to larval density and prone to inconsistent rates of decomposition (Roeder *et al.* 2010). As early as 1953, Hagen developed a diet for adults of *B. dorsalis, B. cucurbitae and Ceratitis capitata* and showed that carbohydrate, amino acids, vitamins and certain minerals were essential for their survival and ovarian development. Insects like vertebrates, require a number of minerals for growth and development and those can neither be synthesized nor can be replaced by any other class of nutrients (Cohen, 2015). So, complex salt mixtures, as an essential components was included in insect diets (House, 1967; Blaine and McEwen's, 1984). The research work showed that mineral nutrition especially different cations and anions have their profound effect on the growth and development of the maggots of *B. dorsalis*. The knowledge on the role of mineral nutrition helps us to formulate a suitable artificial diet which will be useful for mass multiplication of flies in the laboratory. It is useful to manipulate the plant

resistance mechanism against insect pests and to apply mineral nutrition principle instead of hazardous chemicals in controlling or limiting the population of fruit flies (Anand and Sharma, 2005).

## MATERIALS AND METHODS

The culture of oriental fruit fly, *Bactrocera dorsalis* was maintained in the laboratory at 27°C ± 1°C and 75% R.H. on mango, guava and sapota throughout the year in insect physiology section, Indian Agricultural Research Institute, as described by Srivastava (1975) and was fed on artificial diet comprising of yeast hydrolysate enzymatic, sugar and water. Experiments were conducted to know the effect of different cation and anions of the Beta hydroxyl-beta methyl butyrate (HMW) salt mixture, which is considered to be the best salt mixtures for the growth and development of of *B. dorsalis* maggots. A casein based artificial diet formulated by Srivastava *et al.,* 1989 was used.

The efficiency of the diet was assessed on the basis of average pupal percentage, period taken by the maggots to become pupae, growth index, pupal weight and percentage emergence of adults. The data were expressed as an average of six in case of maggot diet and adult emergence in experiment of three replications. The data were analyzed in completely randomized block design by using AgRes software, 3.01 version and all values with percentage were arcsine transformed before statistical analysis.

## RESULTS AND DISCUSSION

Data on role of different cations of HMW salt mixture on growth and development of *B. dorsalis* maggots showed that the diet devoid of potessium (K) followed by that devoid of Ca and Mn recorded higher maggot period of 25.15, 21.39 and 21.25 days, respectively (Table 1). The diets without Fe, Na, Cu and Mg, recorded 21, 19, 18.51 and 17.5 days of maggot period, respectively. The control (with all cations) recorded the lowest maggot period (13.26 days). It showed that deletion of cations like K, Ca, Mn, Fe, Na, Cu and Mg prolonged the maggot period. The maggot period in control was significantly different from the diet without K. Silva et al., (2005) suggested that insects need considerable amounts of potassium, phosphorous and magnesium in their diets, where as little calcium, sodium and chloride are required.

No pupal formation was observed in the diet without K. The pupal period was higher in the diet without Cu (14.2 days) and Mg (14 days) followed by the diet without Na (13.75 days), Ca (11.4 days) and Fe (10 days). These were higher than the control, where the pupal period was 9.75 days. Pupal period in the diet without Mn was 8.62 days which was lower than the control.

The pupal weight in the diet without Mg, Na, Cu, Mn and K, was 8.95, 7.94, 7.55, 7.45 and 6.92 mg, respectively than against 12.8 mg in the control and 10.54 and 10.03 mg in the diet without Ca and Fe, respectively.

The pupal formation was the highest (45.01%) in the diet without Ca followed by control (43.05%). In the diet without Cu, the pupal formation was 24.09 per cent, while in the diet without Mn (19.97%), Na (16.74%), K (12.92%), Mg (10.47%) and Fe (7.4%) it was drastically reduced. Bhattacharya and Kaliwal (2005) studied the biochemical effects of potassium chloride on the silkworm. The supplementation of KCl increased the fat body glycogen, protein and haemolymph protein significantly.

The data showed higher adult emergence (26.56%) in the control. It was lower in the diets without Mn (12.92%), Ca (10.51%), Na (10.51%), Mg (7.40%), Fe (7.4%), Cu (7.4%) and nil in absence of K. The percentage of adult emergence was lower in all the treatments as compared to control, which was significantly different from others. McFarlane (1976) reported that the addition of copper to an artificial diet greatly improves the growth of the house cricket *Acheta domestica*. Adding zinc improves growth in the presence of added copper but not in its absence, indicating an interaction between two trace elements. Calcium and magnesium ions stimulated the amino peptidase N activity but copper ion was rather inhibitory Hua *et al.,* (1998). Fanson and Taylor (2012) found that the addition of minerals increased lifespan of both male and female flies housed in single sex cages by decreasing baseline mortality of adults emeged.

The diet with all cations (control) recorded highest growth index (3.50). The Ca deleted diet showed higher growth index (2.39) but only next to the control. It was lower in all other treatments. The growth and development of *B. dorsalis* was severely affected. Pant and Gupta (1984) worked on the role of various trace elements like Cu, Fe, Mn, Cu and Zn on the growth and survival of cotton jassid, *Amrasca devastans*. The survival of cotton jassid was high where N @ 90 kg and 120 kg $ha^{-1}$ was used alone or in combination with P. It was low where K alone or in combination with P was applied.

The data on effect of different anions of HMW salt mixture on the growth and development of *B. dorsalis* maggots (Table 1) recorded nil maggots emergence in the diet without C1. The maggot period was however higher (23.75 days) in the iodide deleted diet followed by the diet without $So_4$ (23.66 days), F (23.2 days) and $Co_3$ (22.36 days). In the diet without $Po_4$ the maggot period was of 18.43 days against the lowest in the Control (13.26 days).



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Table 1: Effect of different cations of HMW salt mixture on the growth and development of B. dorsalis maggots in the artificial diet under aseptic conditions by deletion experiment

| Treatments (cation deleted from salt) | Average | | | | | |
|---|---|---|---|---|---|---|
| | Maggot period (days) | Pupal period (days) | Pupal weight (mg) | Pupal formation (%) | Adult emergence (%) | Growth index |
| Ca | 21.39 | 11.40 | 10.54 | 45.01(50.00) | 10.51(3.33) | 2.39 |
| Ma | 17.50 | 14.00 | 8.95 | 10.47(3.33) | 7.40(1.66) | 0.19 |
| Mn | 21.25 | 8.62 | 7.45 | 19.97(11.66) | 12.92(5.00) | 0.60 |
| Fe | 21.00 | 10.00 | 10.03 | 12.92(5.00) | 7.40(1.66) | 0.15 |
| Cu | 18.51 | 14.02 | 7.55 | 24.09(16.66) | 7.40(1.66) | 0.90 |
| K | 25.15 | 16.10 | 6.92 | 7.4(1.66) | 0.95(0.00) | 0.07 |
| Na | 19 | 13.75 | 7.94 | 16.74(8.30) | 10.51(3.33) | 0.39 |
| Control | 13.26 | 9.75 | 12.08 | 43.05(45.00) | 26.56(21.33) | 3.503 |
| C D 5% | 9.71 | 5.49 | 5.50 | 13.64 | 10.61 | 0.83 |

Figures in the parenthesis are transformed to angle = Arc sin "percentage

Table 2: Effect of different anions of HMW on the growth and development of B. dorsalis maggots in the artificial diet under aseptic conditions by deletion experiment

| Treatments (anion deleted from salt) | Average | | | | | |
|---|---|---|---|---|---|---|
| | Maggot period (days) | Pupal period (days) | Pupal weight (mg) | Pupal formation (%) | Adult emergence (%) | Growth index |
| $CO_3$ | 22.36 | 11.00 | 7.70 | 51.7( 61.7) | 15.0(6.7) | 2.66 |
| $SO_4$ | 23.66 | 10.50 | 7.71 | 35.3(33.3) | 16.8(8.3) | 1.27 |
| $PO_4$ | 18.43 | 12.80 | 7.99 | 51.7(61.7) | 18.4(10.0) | 2.91 |
| I | 23.75 | 10.76 | 8.96 | 48.8(56.7) | 15.0(6.7) | 2.48 |
| F | 23.20 | 10.20 | 8.95 | 24.1(16.7) | 15.0(6.7) | 0.36 |
| CI | 0.00 | 0.00 | 0.00 | 0.4(0.0) | 0.4(0.0) | 0.00 |
| Control | 13.26 | 9.75 | 12.08 | 43.1(46.7) | 28.3(23.0) | 3.60 |
| C D 5% | 3.60 | 1.95 | 2.05 | 11.09 | 9.64 | 0.96 |

Figures in the parenthesis are transformed to angle = Arc sin "percentage

No maggot emergence and pupa formation was recorded in the diet without Cl. The pupal period was higher in the diet without $Po_4$ (12.8 days) followed by $Co_3$ (11.0 days). In the diet without iodide (10.76 days), $So_4$ (10.5 days) and F (10.2 days), the maggot period was slightly higher than the Control. The maggot period was lowest (8.95 days) in the diet without Mg. In control it was 9.75 days.

The pupal weight in the diet without iodide and F was 8.96 and 8.95 mg, respectively against 12.08 mg in the control. In the diet without $Po_4$ (7.99 mg), $So_4$ (7.71mg) and $Co_3$ (7.70 mg), the pupal weight was lower than the control and other treatments. The pupal weight in the control was significantly different from all other treatments. Magadum (1987) showed that the dietary supplementation of copper sulphate, nickel chloride and potassium iodide increased economic parameters of silkworm.

The percentage of pupa formation was higher in the diet without $Co_3$ and $Po_4$ (51.75%) followed by iodide (48.81%) it was 43.1 per cent in the control. In the $SO_4$, F deleted diets the percentage of pupal formation was 35.26 and 24.09 per cent respectively. In the Cl deleted diet no pupa formation was observed. Islam et al. (2004), observed that dietary supplementation of copper sulphate, nickel chloride and potassium iodide increased the economic parameters of silkworm.

The percentage of adult emergence in the control was highest (28.33%). If was 18.43 and 16.76 per cent, respectively in diets without $PO_4$ and $SO_4$. In the diets without $CO_3$, iodide, F the percentage adult emergence was 14.96 per cent.

The control recorded the highest growth index (3.6) followed by the diet without $PO_4$ (2.91), $CO_3$ (2.66) and I (2.48). In the diet without $SO_4$ (1.27) and F (0.36) the growth and development was drastically reduced. No growth observed in the diet without Cl showed its essentiality in the maggots growth and development. Blaine and McEwen's (1984) worked on nutrition of onion maggot, *Delia antiqua* on acidic diet and concluded that the complex salt mixture (Wesson's) normally used could be replaced by a solution of salts containing specific trace element ions (Fe, Mn, Cu, Zn) and two salts $CaHPO_4$, $H_2O$ and $KH_2PO_4$.

## CONCLUSION

The study concluded that K, Fe and Mg and Cl and F, being the most essential cations and anions required for proper growth and development of *B. dorsalis,* must form an important component of the artificial diet for successful mass multiplication of flies in the laboratory.

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# First record of *Pandora formicae* on ant, *Camponotus angusticollis* and *Batkoa amrascae* on white leaf hopper, *Cofana spectra* in rice agroecosystem of India

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**ABSTRACT**

Two entomophthoralean fungi, *Pandora formicae* and *Batkoa amrascae* were reported for the first time from India as a dominant biocontrol agents of *Camponotus angusticollis* and *Cofana spectra,* respectively in rice agroecosystem. The biocontrol potential of *B. amrascae* studied under field conditions during 2012-2016 showed its ability in drastically reducing the population of *C. spectra* in rice fields and the nursery and could be used as a potential biocontrol agent against white leaf hopper.

**Key words:** Entomophthoralean fungi, *Pandora formicae*, *Camponotus*, *Batkoa amrascae*, *Cofana spectra*

Entomophthoralean fungi require very high humidity for survival and spread. In Meghalaya, > 90 per cent humidity during rainy season, is quite favourable for fungal infection in insects. Entompothoralean fungi form vegetative structures (hyphal bodies) in the host haemocoel which are rod shaped to irregular rather than hypha-like (Humber, 1989). Entomophthoromycota has been demonstrated as monophyletic branch among early diverging terrestrial fungi (Gryganskyi *et al*., 2013). This phylum contains six families Ancylistaceae, Basidiobolaceae, Completoriaceae, Entomophthoraceae, Meristacraceae and Neozygitaceae (Humber, 1989). According to new phylogenetically based classification by Humber (2012), most of the entomopathogenic genera are in family entomophthoraceae. Gryganskyi *et al*. (2013) have reported five main phylogenetically identified lineages based on multigene analysis (LSU, SSU, RPB2 and mtSSU) in Entomophthoromycota, corresponding to the main genera *Basidiobolus*, *Conidiobolus*, *Batkoa*, *Entomophthora*. These fungi are considered very important because of their potential role in being used in microbial control of insect pests.

Ants are important, widespread and the most successful of all insect groups in tropical and temperate ecosystems. The genus *Camponotus* belongs to the subfamily Formicinae with 1087 extant species in the world. In India, the genus *Camponotus* is the most speciose ant genus with 83 named species (one tenth of the total known Indian species) (Bharti et al., 2016). Interestingly, all 83 species of genus *Camponotus* are found in northeast India and about 13 species are also reported from Meghalaya (Bharti et al., 2016). The Long-necked Sugar Ant, *Camponotus* (*Dinomyrmex*) *angusticollis* (Jerdon) (Hymenoptera: Formicidae) is a widely distributed ant species, commonly found in Indo-Australian Region (Borneo, Indonesia, Malaysia) and the Oriental Region (Bangladesh, Myanmar, India (type locality), Nepal) (Boltan, 1995). In India, it is distributed in several states including northeast India (Tiwari and Tiwari 2002; Tak, 2008). *Camponotus angusticollis* is one of important ant species, usually found foraging throughout the year, but more abundant in monsoon season and are often associated with aphids on trees (Vanitha *et al*., 2017). Summit disease (fungal disease) of ants was first described by P. I. Marikovsky, in 1962 on *Formica rufa*. The entomophthoralean fungus genus associated with the disease was *Pandora*. But there is a lot of confusion related to species epithet naming in this pathogen (Malagocka et al., 2017).

The white leafhopper (WLH), *Cofana spectra* (Distant) (Hemiptera: Cicadellide) is a widely distributed pest which is relatively large in size among all the leafhopper species found in rice ecosystem (Mitra *et al*., 2014). Host plants of the *C. spectra* includes, *Hordeum vulgare* (barley), *Pisum sativum* (pea), Poaceae (grasses), *Saccharum officinarum* (sugarcane), *Sorghum bicolor* (sorghum), *Triticum* (wheat) etc (http://www.plantwise.org/KnowledgeBank/Datasheet.aspx?dsid=14798). Adults of *C. spectra* are mostly confined to the lower surface of the leaves. Leaf tips of affected plants first dry up and later the leaf turns orange and curls and ultimately become yellow and stunted. White leafhopper is

also a vector of pathogenic virus like rice yellow mottle virus (RYMV) (Nwilene et al., 2009). *Cofana spectra* has also been reported on grasses found nearby the rice fields (Young, 1979).

Natural infection of *Pandora formicae* on ant, *Camponotus angusticollis* and *Batkoa amrascae* on white leaf hopper, *Cofana spectra* in rice agroecosystem of Meghalaya state of northeast India was recorded for the first time. In this study, efforts were made to characterize and determine their biotic potential in rice ecosystem.

## MATERIALS AND METHODS

Cadavers of ant (*C. angusticollis*) and white lead hopper, *C. Spectra* were refrigerated at 4°C to prevent deterioration and saprophytic growth. These were put in petridishes lined with wet blotting paper and kept in moisture chamber at 25±1°C for 12 hrs before examination. Microscopic observations were made using Olympus BX 53 microscope (equipped with Cell sens software) for identification of the entomopathogen. Further, detailed examination was carried out using Aceto orceum for nuclear starting as suggested by Humber (1981) who has clearly advocated the use of the important characters like number and nature of nuclei in primary spores and morphology of sporophores for delineating the genera in entomophthorales. He has also suggested that characters like the morphology of primary spores, types of secondary spores formed, presence or absence of rhizoids, presence or absence of cystidia and the mode of formation of resting spores should not be considered as important characters for delimiting the genera in entomophthorales. Specimen was dried and sputter coated in a JFC-1100 fine coat ion sputter device (JEOL, Tokyo, Japan) under vacuum and then mounted on stubs and observed in JEOL JSM 6360 scanning electron microscope (JEOL, Tokyo, Japan).

Natural incidence of *C. spectra* was also studied during 2012-16. Data on the presence (nos.) of *C. spectra* (in rice fields) in per $m^2$ area and also on number of parasitized *C. spectra* in the area was recorded.

## RESULTS AND DISCUSSION

### Entomophthoralean fungi on ant (*Camponotus angusticollis*)

While conducting surveys for exploring entomopathogenic fungi on rice plant at Nalapara (**Latitude:** N 25°55′13.67"; **Longitude:** E 91° 55′ 23.17" and altitude 676 meters MSL), Ri-Bhoi district Meghalaya, India an ant hanging on leaf blade was observed.

The cadaver (worker) was found below the tip of the rice plant at tillering stage (naturally grown in between the edges of fish ponds) at Nalapara. No other members of this species were found during that time on nearby rice and other plants growing in 25 $m^2$ habitat. Infected ants are easy prey for spiders and other predators hence they are removed and remain undetected in majority of the cases (Boer 2008). The same and nearby rice plants were also free from sucking pests including aphids. The habitat is having three fish ponds, duckery unit, rice fields (wet lands), several tree species, including guava, pear, banana, wild trees etc. The meteorological parameters are provided in the Table 1.

Observations using dissecting microscope further revealed intersegmental fungal growth and sporulation (Fig 1a). Prominent monohyphal rhizoids (hyphal width just above branching or holdfast was 8-11 µm) with branched endings were present which appeared to anchor the cadaver to the leaf surface (Fig 1b). Hyphal bodies were irregular rod shaped formed inside the host. Nuclear staining clearly revealed that the ovoid conidia (18-21 x 11-13 µm) were mononucleate with nuclear diameter of 5-6 µm.

Since the conidia were mononucleate it appeared to belong to Erynioideae. The subfamiliy, Erynioideae consists of six genera *Erynia*, *Furia*, *Orthomyces*, *Pandora*, *Strongwellsea* and *Zoophthora*. Since conidia were produced on the surface rather than abdomen hence *Strongwellsea* was ruled out. Hyphal bodies are Pandora type- irregular rod shaped to short hyphae like (Not like Erynia where hyphal bodies are mostly spherical to elongate subspherical). Conidia appear to be bitunicate, rhizoids monohyphal with branched endings (this type is found in some Pandora species). Bitunicate spores (as per Humber 1981)- All known bitunicate spores contain a single large nucleus). Capilliconidia were absent hence *Zoophthora* and *Neozygites* were ruled out. These observations clearly indicated the presence of *Pandora*. Two species of Pandora are reported on Ants, *Pandora myrmecophaga* and *P. formicae*. It has clearly been stated by Humber and Ben-Ze'ev (1981) that *Erynia formicae* (now *P. formicae*) has rhizoids but *Erynia myrmecophaga* (now *P. myrmecophaga*) lacks rhizoids. *P. formicae* has smooth walled resting spores whereas *P. myrmecophaga* has rough walled resting spores (but resting spores were not detected in our sample) (Humber and Ben-Ze'ev 1981, Ma³agocka *et al*., 2017). There has been a lot of confusion related to name of the entomophthoralean fungi on ants (Ma³agocka et al., 2017) but they also preferred to refer the rhizoid producing fungus on ants as *P. formicae*. Hence, we conclude that the entomophthorelean fungus on ant in our sample was *P. formicae*. This pathogen has not been reported on ant genus *Camponotus* worldwide.



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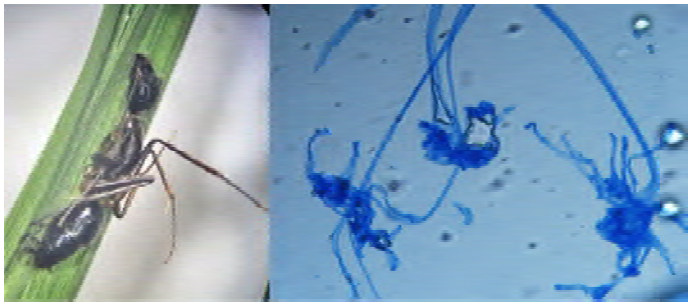

**Fig. 1** **A.** Infected ant attached to leaf blade **1B.** Rhizoids with holdfast

Table 1: Weather parameters at Umiam, Meghalaya*

| | Months (2017) | | | | |
|---|---|---|---|---|---|
| | July | Aug | Sept | Oct | Nov |
| Rainy Days (No.) | 20 | 28 | 19 | 13 | 3 |
| Total Rainfall (mm) | 381.2 | 752.7 | 348.4 | 254.1 | 20.8 |
| Temperature (°C) | | | | | |
| Maximum | 28.4 | 28 | 27.9 | 26.7 | 24.8 |
| Minimum | 20.4 | 20.6 | 20.2 | 16.9 | 11.3 |
| Relative Humidity (%) | | | | | |
| Morning | 88.9 | 90.5 | 88.9 | 87.2 | 84.6 |
| Evening | 74 | 76.3 | 76.5 | 71.6 | 54.1 |

*Observatory is around 40 kms from the site of collection

**Entomophthoralean fungi on white leaf hopper (*Cofana spectra*)**

Many moribund white leafhoppers (*C. spectra*) were spotted in the rice fields (tillering stages) and also to a lesser extent in rice nursery in Umiam, Meghalaya, India (Latitude 25°30′N, Longitude 91°51′E, Elevation ~1010 msl). Infected cadavers (adults) remained attached to the leaves and showed external mycoses. External mycoses was also confirmed using Scanning electron microscope (Fig. 2a). Microscopic observations revealed the presence of a fungus

Table 2. Species of *Batkoa* described on various hosts

| Species | Rhizoids/ holdfast | Primary conidia size (µm) | Nuclear number in conidia | Size of the nucleus (conidia/ conidiophore) (µm) | Host | Stain with aceto orcein | Primary conidia/ secondary conidia almost same | References |
|---|---|---|---|---|---|---|---|---|
| *Batkoa apiculata* | +/* | 35.5-38.5 x 29-32 | - | 2.6-2.7 | Homopteran, flies, Polyphagous | weakly | + | Keller, 2006 |
| *B. major* | +/+ | 40-45 x 33.5-38.5 | - | 2.5 | Polyhagous | weakly | + | Humber, 1989 |
| *B. gigantea* | Absent | 87-98.5 x 70.5-84.5 | 50-100 | 5 | *Tipula paludosa* Meig. (Diptera, Tipulidae | + | + | Humber, 1989 |
| *B. papillata* | +/- (rhizoid present but holdfast absent) | 47-52.5 x 32.5-35 | 50-80 | 3-3.5 | Midges, Diptera, Nematocera: Chironomidae, Simuliidae | weakly | + | Humber, 1989 |
| *B. limoniae* | +/* | 48-52 x 40-47 | 20-39 | - | Diptera, Limoniidae | + | + | Niell and Santamaría, 2001 |
| *B. hydrophila* | +/- | 30.4-34.6 x 22.5-26.4 | * | * | *Leuctra* sp. (Plecoptera, Leuctridae) | + | + | Keller, 2007 |
| *B. pseudoapiculata* | + | 35-39 x 29-32 | - | 2.5 | Diptera | + | + | Huang et al., 2007 |
| *B. cercopidis* | + | 40-45 x 33-39 | - | 2.6 | Homoptera: Cercopidae | + | + | Huang et al., 2007 |
| *B. amrascae* | +/+ | 22.6-26.4 x 18.4-23.1 | 6-13 | 4.5-4.6 | Homoptera: Cicadellidae | + | + | Villacarlos and Keller, 1997 |
| *Batkoa* sp. (from India) | * | 30 x 27 (on wings), smaller inside body (24 x 20) | 7-17 (av 10) | 4.5 | *Pyrilla perpusilla* (Homoptera, Fulgoridae) | + | + | Keller and Yubak Dhoi, 2007 |
| *Batkoa* sp. | +/+ | Primary 23.5 - 28.5-x 22.5-24.3- secondary 21.5 - 24.5 x 20.5- 21.5- | 8-10 | 3.5- 4.0 | *Cofana spectra* (Hemiptera: Cicadellide) | + | + | This study |

*Information not available

with hyphal bodies (elongated) and conidia. These characters clearly pointed out the presence of a fungus belonging to entomophthorales. Even rhizoids were visible using dissecting microscope which was further examined using Olympus BX 53. Microscopic examination using cotton blue revealed the presence of simple conidiophores with a narrow neck between the conidia and conidiogenous cells and almost globose primary conidia (23.5-28.5-x 22.5-24.3 µm) on insect body, primary conidia on wings were slightly bigger and measured 30.5-33.5 x 27.5- 29.5 µm (n=50) (Fig. 2b). Primary conidia and secondary conidia were almost similar in shape but secondary conidia were smaller in size (21.5 -24.5 x 20.5- 21.5 µm). Papilla was rounded or conical. As suggested by Humber (1981) aceto-orcein was used for staining the conidia and the nuclei got stained with granular contents and were clearly visible (av. number in conidia- 8-10 nuclei, size 3.5-4.0 µm). Monohyphal rhizoids (~ 18 µm) with terminal disc-like holdfast were also detected. Germination of primary conidia (formation of secondary conidium on primary conidia) was also observed.

Presence of simple conidiophores pointed out the possibility of *Entomophaga*, *Entomophthora*, *Batkoa* and *Conidiobolus*. In case of *Entomophaga* conidial shape is pyriform to ovoid but in our case globose, in case of *Entomophthora* conidia are also surrounded by halo like droplet but in our case conidia were not surrounded by halo like droplet. *Conidiobolus* was ruled out since nuclei in our case got stained with aceto orcein. *Batkoa* species have discoid terminal holdfast resembling *Pandora* sp. but in case of *Pandora* conidiophores are branched. *Batkoa* was separated from genus *Entomophaga* on the basis of formation of globose to subglobose conidia, narrowed extension of conidiogenous cell before conidia formation and presence of discoid terminal holdfasts (Humber, 1989).

Based on characters such as conidial dimension, presence of rhizoids and holdfast, host family and size of nucleus (Table. 2) we were able to confirm that the species involved is *Batkoa amrascae* Keller & Villacarlos on *C. spectra*.

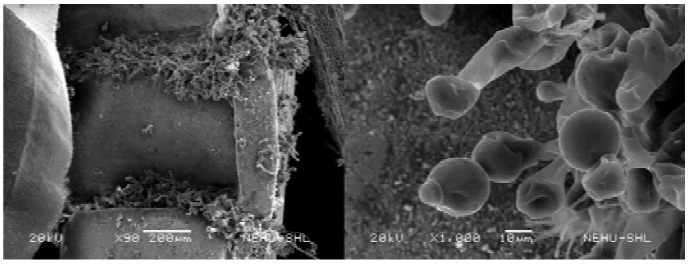

**Fig. 2A.** Mycoses on *Cofana spectra*

**Fig. 2B.** Condia and conidiophores of *Batkoa amarascae*

**Natural infection of *B. amrascae* on leaf hopper *Cofana spectra***

It is evident from the data that the population of *C. spectra* starts building up from June and continues upto July and then starts declining (Fig. 3). Parasitization also peaks during June and continues upto August and then starts declining (Fig. 4). Over the years the data shows that from 2012 to 2015 the population has been declining and parasitization has been increasing but in the year 2016 population as well as parasitization increased (Fig. 5). All these observations were taken from the rice fields where no pesticides were used. This trend shows that this entomopathogen has the capacity to check the population level of this insect pest and over the years the population level might have been under control because of infection by this pathogen.

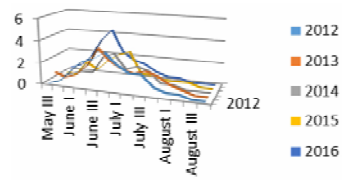


**Fig. 3.** Leaf hoppers (*Cofana spectra*)/m$^2$

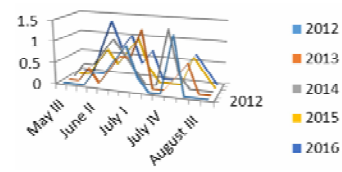


**Fig. 4.** Infected Leaf hoppers (*Cofana spectra*)/m$^2$

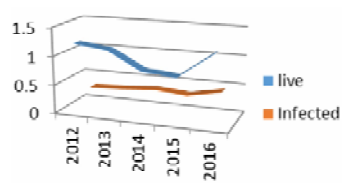


**Fig. 5.** Live and infected *Cofana spectra*

## CONCLUSION

It was concluded that the *C. spectra* is fairly susceptible to *B. amrascae* in Nalapara, Meghalaya. Infection due to *B. amrascae* might be the reason behind low population levels of *C. spectra* in this region over the years. The entomopathogen can therefore be utilised as a potential biocontrol agents for managing *C. spectra*.

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# Management of san jose scale, *Quadraspidiotus perniciosus* Comstock in apple orchards of Kashmir through horticultural mineral oils

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**ABSTRACT**

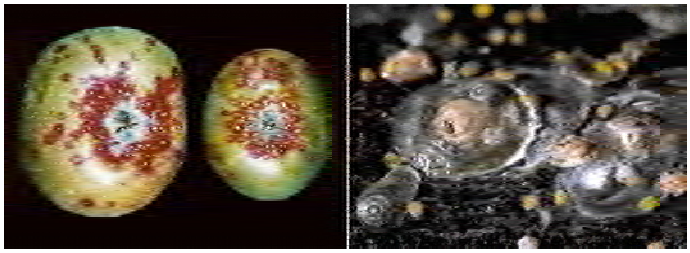
The field experiment, conducted with the objective of examining the efficacy of different concentrations of two oil dormant sprays of Arbofine extra along with standard check (ATSO) to manage san jose scale (*Quadraspidiotus Perniciosus* Comstock) in delayed dormant season of 2015-16, revealed highest comulative mean mortality of SJS (82.69%) with the application of 2.5 per cent of Arbofine extra followed by 80.71 per cent mortality of SJS with 2.0 per cent of Code-204 as against the standard check (ATSO) applied at 2.5 per cent concentration (80.12%) and the lowest (76.47 per cent) at 1.5 per cent concentration. Two parasitoids, *Encarsia pernicious* and *Aphytis proclia* and one predator *Chilocorus infernalis* were found on SJS twigs from the treated area. Arbofine extra @ 2.5 per cent concentration recorded mean yield (10.0 boxes) of 'A' grade followed by 8.33 boxes @ 2.0 per cent concentration.

**Key words:** Delayed dormant spray, horticulture mineral oil, san jose scale, apple, Kashmir

Horticulture sector, the back bone of Jammu and Kashmir economy, where apple, a highly remunerative crop, plays pivotal role, faces many challenges. The one and the major among these is the San Jose scale, *Quadraspidiotus perniciosus* (Comstock) (Hemiptera: Diaspididae), key pest of apple in certain hilly tracts of India. It injures the fruit directly and reduces tree vigor by removing sap and eventually killing the tree. Introduced into the United States from China in 1870 (Muneer 2008), this scale insect continues to be a major pest in apple-growing regions of North America (Pfeiffer, 1985, Rice and Jones, 1988; Rice *et al*., 1979). Apple (*Malus domestica* Borkh) is a native of southwestern Asia, Europe and is grown in all temperate regions of the world. In Kashmir, it is the most important fruit crop among all the cultivated fruits. However, the production and quality of apple there is low because of several factors including insect pests. The major insect pests attacking this crop are San Jose scale, european red mite, two spotted mite and apple wooly aphid. San Jose scale and european red mite are the most destructive and widely distributed all over the world. These pests have been accidentally introduced to many countries and in most regions of the world where deciduous fruits are grown. The damage is caused by nymphs and the female scales, which suck the sap from branches, twigs and fruits, weakens the plant and leaves and render the fruit unacceptable and unmarketable. Keeping in view the severity of the pest information this experiment to test the bio efficacy of horticultural mineral oils (HMO's), its impact on quality of apple yield and effect on natural enemies in the apple ecosystem of Kashmir Valley was undertaken.

## MATERIAL AND METHODS

Field trials were laid out at two different locations of district Bandipora of Kashmir at Shelwat and Shadipora of Sumbal during 2015-16. Horticulture mineral oils Arbofine Extra, standard check ATSO oil @ 1.5, 2.0 and 2.5 concentration and diesel oil in ratio of 1:10 were evaluated against SJS as delayed dormant spray on 18-25 years old red delicious plant at Shelwat and Shadipora (Sumbal) of district Bandipora. Different oils were sprayed with the help of motorized sprayer. Live *Q. perniciosus* population were counted on four randomly selected twigs on four spots of 1cm$^2$ area under binocular microscope, Live count a day of before treatment and at the subsequent intervals of post treatment were recorded. There were three replications of each concentration in each treatment. Percent mortality was worked out by computing the difference between pre and post treatment population applying Abbot's Formula (1925). The bio-efficacy of these newer horticulture mineral oils viz a viz impact on natural enemy complex as well as yield parameters were studied. The data were subjected to analysis of variance at 5 per cent level of significance.



San Jose Scale damage

## RESULTS AND DISCUSSION

Bio-efficacy of different horticulture mineral oils against the standard check and water as control were evaluated against SJS at Shelawat and Shadipera during 2015 and 2016. Data recorded at Shelawat in 2015 revealed that arbofine extra applied @ 2.5 per cent resulted in highest mean mortality of SJS (80.64%) followed by ATSO oil (80.57%) at same concentration, the standard check (diesel oil emulsions 1:10) recorded mean mortality to the extent of (76.82%). The arbofine extra at 2.0 per cent concentration exhibited 80.64 per cent mean mortality as against 77.59 per cent in the standard check ATSO at the same concentration. All the treatments were statistically different from control (Table-1). Perusal of data at shadipora in 2015 revealed that arbofine extra @ 2.5 per cent concentration recorded highest mean mortality (85.36%) of SJS which was statistically at par with arbofine extra @ 2.0 per cent concentration with 81.97 per cent mean mortality. The standards check (ATSO) and diesel oil emulsion sprayed at 2.5 per cent concentration and 10 in 1:10 ratio, respectively recorded mean mortality of 83.23 per cent and 79.25 per cent, respectively. All the treatments were statistically different from the control.

The pooled data for both the locations in 2015 revealed highest mean mortality (83.00%) with arbofine extra applied @ 2.5 per cent concentration followed by 81.90 per cent in ATSO at same concentration. The data on SJS mortality at both the locations during 2016 was almost identical. Arbofine extra at 2.5 concentration at Shelwal Sumbal and Shadipora was 80.66 and 84.12 per cent, respectively during 2016. ATSO at 2.5 per cent concentration recorded 82.24 and 80.86 per cent mean mortality, respectively. It was almost at per with diesel oil emulsion at both the locations in 2016.

The pooled data for both the locations in 2016 revealed highest mean mortality (82.39%) with arbofine extra applied at 2.5 per cent concentration followed by 81.55 per cent with standard check (ATSO) at same concentration. Arbofine extra exhibited 80.24 per cent mean mortality of SJS at 2.0 per cent concentration and lowest (76.47%) with 1.5 per cent concentration. The cumulative mean of both the locations for 2015-16 revealed highest mortality (82.69%) with arbofine extra applied @ 2.5 per cent concentration followed by 80.71 per cent with its 2.0 per cent concentration. The standard check (ATSO) at 2.5 per cent exhibited 80.12 per cent cumulative mean mortality of SJS, while arbofine extra at 1.5 per cent exhibited the lowest (76.476%) in both the years.

Two parasitoids, *Encarsia pernicious* and *Aphytis proclia* and one predator *Chilocorus infernalis* were found on SJS intested twigs. The data (table 2) on their mortality percent revealed that arbofine extra at 0.50 per cent concentration recorded the least cumulative mean mortality (26.46%) of natural enemies. All the treatments were statistically different from the control.

## CONCLUSION

The perusal of data (fig. 1) revealed that arbofine extra applied @ 2.5 per cent concentration recorded mean yield (10.0 boxes) of 'A' grade followed by 8.33 boxes at 2.0 per cent concentration. Its 1.5 per cent concentration recorded the least number of 'A' grade boxes (4.00). The standard

Table 1. Comparative bio-efficiency of different horticultural mineral oils (HMO'S) against san jose scale (*Q perniciusus*) in red delicious cv. of apple

| Treatment | Concentration (%) | (%) Mean mortality of SJS in 2015 at | | Pooled mean | (%) Mean mortality of SJS in 2016 at | | Pooled mean | Cumulative Mean |
|---|---|---|---|---|---|---|---|---|
| | | Shelwat Sumbal | Shadipora | | Shelwat Sumbal | Shadipora | | |
| | 1.5 | 76.69 | 77.42 | 77.05 | 74.69 | 78.26 | 76.47 | 76.76 |
| **Arbofin extra** | 2.0 | 80.40 | 81.97 | 81.18 | 79.59 | 80.90 | 80.24 | 80.71 |
| | 2.5 | 80.64 | 85.36 | 83.00 | 80.66 | 84.12 | 82.39 | 82.69 |
| **ATSO** | 1.5 | 76.67 | 79.55 | 78.11 | 77.84 | 78.48 | 78.16 | 78.13 |
| | 2.0 | 77.59 | 79.81 | 78.70 | 78.25 | 79.91 | 79.08 | 78.59 |
| | 2.5 | 80.57 | 83.23 | 81.90 | 82.24 | 80.86 | 81.55 | 80.12 |
| **Diesel oil emulsion** | 1:10 | 76.82 | 79.25 | 78.03 | 81.06 | 79.56 | 80.31 | 79.17 |
| **Check (Water)** | - | 3.48 | 4.30 | 7.78 | 3.48 | 4.30 | 3.89 | 5.83 |

Table 2. Toxicity of Code-204 against natural enemies of san jose scale (*Q. perniciosus)* on apple cv red delicious

| Treatments | Conc. (%) | Pre-treatment count | | Post treatment count (Percent mean mortality of natural enemies (DAT) population of | | | | | | Pooled mean mortality (%) | | Cumulative mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Predator | Parasitoids | Predator | | | Parasitoids | | | | | |
| | | | | 1st | 3rd | 7th | 1st | 3rd | 7th | Predator | parasitoid | |
| Arbofine extra | 0.50 | 3.33 | 1.83 | 20.12 | 30.03 | 31.04 | 13.66 | 22.95 | 40.98 | 27.06 | 25.86 | 26.46 |
| | 0.75 | 3.33 | 1.91 | 20.12 | 39.93 | 49.15 | 13.08 | 26.17 | 43.45 | 36.40 | 27.56 | 31.98 |
| | 1.00 | 4.85 | 2.08 | 25.00 | 45.75 | 49.55 | 15.86 | 27.80 | 48.07 | 38.76 | 30.57 | 34.66 |
| ATSO | 0.50 | 3.76 | 1.91 | 15.47 | 18.03 | 28.03 | 13.08 | 30.36 | 58.11 | 20.51 | 33.85 | 27.18 |
| | 0.75 | 4.33 | 1.66 | 15.47 | 23.09 | 30.71 | 15.06 | 34.93 | 65.06 | 23.09 | 38.35 | 30.72 |
| | 1.00 | 5.00 | 1.75 | 20.00 | 40.09 | 45.61 | 14.28 | 28.52 | 66.85 | 35.23 | 36.55 | 35.89 |
| Check | Water | 4.21 | 1.91 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C.D(P≤0.05) | | | | 2.45 | 2.56 | 2.98 | 5.47 | 3.07 | 4.78 | | | |

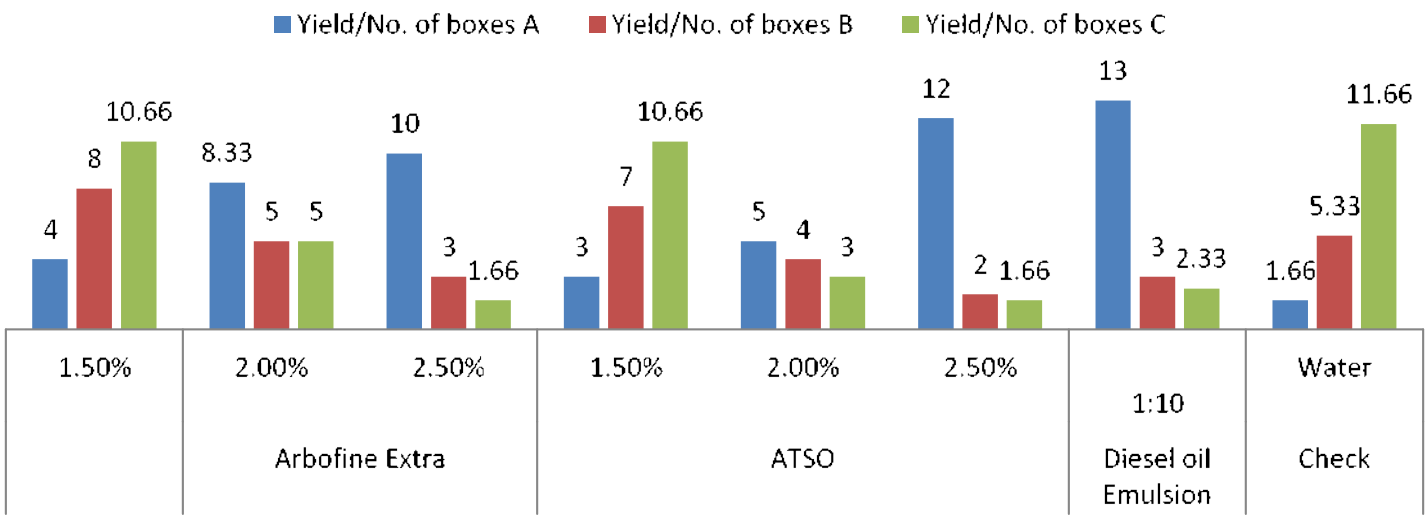



Fig. 1: Effect of different horticulture mineral oils(HMO'S) as delayed dormant spray on yield of apple

check (ATSO) at 2.5 per cent concentration yielded 12.0 'A' grade boxes. All the treatments were statistically different from the control.

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# Association of causative agents of root rot complex of soybean in northern Karnataka

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**ABSTRACT**

Examination of pure culture of the pathogens collected from the root samples of soybean plants showing typical wilting symptoms revealed that the major pathogens involved in causing root rot complex are *Sclerotium rolfsii* Sacc. *Rhizoctonia bataticola* and *Fusarium* sp. in northern Karnataka. The pathogenicity studies on susceptible cultivar JS 335 inoculated with all the pathogens revealed maximum percent disease incidence in case of dual inoculation of *S. rolfsii* + *R. bataticola* (83.33%) and mixed inoculation of *S. rolfsii* + *R. bataticola* + *Fusarium* sp. (88.50%) followed by dual inoculation of *Fusarium* sp. + *R. bataticola* (75%) and *S. rolfsii* + *Fusarium* sp. (72.34%).

**Key words:** Association, root rot complex, soybean, stem fly

Soybean *Glycine max* (L.) Merr. is the number one oil seed crop of India. This is grown in an area of 11.07 m ha with a production of 8.64 m t and productivity of 781 kg ha$^{-1}$ (Anon., 2015). Six major states growing soybean in India are Madhya Pradesh, Uttar Pradesh, Maharashtra, Rajasthan, Karnataka and Telangana. The area in the state of Karnataka is 2.90 lakh ha with a production of 1.81 lakh tonnes and a productivity of about 615 kg ha$^{-1}$ (Anon., 2015).

The soybean crop is attacked by more than 100 pathogens (Sinclair and Shurtleff, 1975). The major economically important are rust, wilts, leaf spots, rots, powdery mildew, bacterial and viral diseases. Among the soil-borne diseases the root rot complex caused by *Sclerotium rolfsii* Sacc., *Rhizoctonia bataticola* (Taub.) Butler (= *Sclerotium batati-cola* Taub.) (Pycnidial stage: *Macrophomina phaseolina*) and *Fusarium oxysporum* [(Schlet) Emed. Snyd and Hans] are gaining more importance. This disease is distributed throughout the world and is prevalent in areas that experience a warm climate and causes significant yield losses in monoculture or short rotation of soybean (Aken and Dashiell, 1991). All three pathogens in the root rot complex are soil inhabitants and polyphagous facultative parasites. These exhibit variations in their morphological, biological and pathogenic characters. Hence, understanding their structural and molecular variation may be useful in devising novel management strategies. Information in this aspect is very much limited and soybean being an important pulse crop is receiving wider attention in India as well as in Karnataka.

Root rot complex has become an important production constraint in northern Karnataka for the last few years. Sangeetha and Shamarao Jahagirdar (2013) reported the association of *S. rolfsii, R. bataticola* and *Fusarium* sp. in causing root rot complex of soybean in northern Karnataka. *R. bataticola* was found predominately associated in all the areas surveyed and the degree of pathogenic variability of these pathogens varied from one region to another. There was also increased severity of stem fly at all the stages of and crop growth making the plants more vulnerable to secondary infection of these pathogens. Considering these points in view, the present investigation was carried out.

## MATERIALS AND METHODS

An intensive roving survey was conducted to record the incidence of root rot in major soybean growing areas of northern Karnataka *viz*., Belagavi, Dharwad and Haveri districts. Five fields from each village were randomly selected. The observations on root rot disease incidence and other details like genotypes, cropping system, type of soil, stage of crop, area and pathogens associated with the disease were recorded and furnished in Tables 1 and 2.

The root samples of soybean plants severely infected by *S. rolfsii*, *Fusarium* sp. and *R. bataticola* showing typical wilting symptoms were collected. The infected root portion was used for isolation. The infected specimen was cut into small bits, washed in running water, surface sterilized with 1 per cent sodium hypochlorite solution for a minute, washed thoroughly with sterile distilled water to remove the traces

of sodium hypochlorite and aseptically transferred to petriplates containing the sterilized PDA medium. The plates were incubated at 27 ± 1° C for 3 days for *Sclerotium* and *Rhizoctonia* and 8 days for *Fusarium*. The fungal growth which agarose through the infected tissue was taken by inoculation loop and transferred aseptically to petriplates containing the sterilized potato dextrose agar medium. The pure culture of the fungus was maintained by further growing the culture and following the hyphal tip culture method under aseptic conditions (Rangaswami, 1972).

This method was followed for obtaining the pure culture of the pathogen. Hyphal tip isolation was done on water agar plates. Spore suspension of the pathogen was prepared in sterilized distilled water and diluted so as to get eight to ten spores per ml from 10 days old culture. One ml of such suspension was spread uniformly on 2 per cent solidified water agar plates and observed for spores under the microscope. A single spore was marked with a marker on the backside of the petriplate and it was allowed to germinate. Such plates were periodically observed for spore germination under a microscope. The hyphae growing from each cell of the single spore was traced and marked with a marker. The tip of the hyphae was cut carefully and transferred to PDA plates and incubated at 28 ± 2 °C for 10 days. Later, mycelial bits of the fungus were transferred in the center of petriplates containing PDA and incubated at 28 ± 2 °C for 10 days. Sectoring was observed in the culture to confirm the pure culture of the fungus. The identification of *Fusarium* sp. was done based on the spore morphology and colony characters of the fungi as described by Ainsworth (1971); Barnett and Hunter (1972); Leslie and Summerell (2006). The morphology, formation of sclerotia and branching of mycelium were the principal characters considered for the identification of pure culture isolates of *R. bataticola* and *S. rolfsii*. The hyphal tip cultures of the fungus were sub-cultured on potato dextrose agar slants and kept in the laboratory at 28 ± 2 °C for 10 days. Such mother culture slants were preserved at 4 °C in the refrigerator. Further, these cultures were sub-cultured once in a month and used for future studies.

Sand and cornmeal (90:10) was used as a substrate for gaint culture preparation in a conical flask and sterilized alternatively for two consecutive days. Fresh culture of 7 days old *Fusarium* sp. of 5 mm disc was inoculated to the flask and incubated for 20 days for full growth of the fungus, during incubation the culture was mixed thoroughly to get uniform growth. After full growth, it was used for further studies. The same procedure was used for *R. bataticola* and *S. rolfsii*.

Sterilized soil was taken in earthen pots. Three to five healthy soybean seeds were sown in pots filled with sterilized soil. Giant culture of *S. rolfsii, R. bataticola* and *Fusarium* sp. of Dharwad isolate was mixed thoroughly with soil at the rate of 10 g per 100 g of soil at 2-3 leaf stage of seedlings combinally (15 days old) around the root zone (Sangeetha, 2011). Seeds sown in pots without inoculums served as control. Observations were taken at regular intervals for symptoms development. When the plants showed wilt symptoms, such plants were uprooted and the pathogens were reisolated by the standard tissue isolation method. The pathogens were compared with the original culture for proving pathogenicity. The treatments included were *Sclerotium rolfsii* ($T_1$), *Rhizoctonia bataticola* ($T_2$), *Fusarium* sp. ($T_3$), *Sclerotium rolfsii* + *Rhizoctonia bataticola* ($T_4$), *Rhizoctonia bataticola* + *Fusarium* sp. ($T_5$), *Sclerotium rolfsii* + *Fusarium* sp. ($T_6$), *Sclerotium rolfsii* + *Rhizoctonia bataticola* + *Fusarium* sp. ($T_7$) and uninoculated control ($T_8$).

## RESULTS AND DISCUSSION

The percent root rot disease incidence in the surveyed areas ranged from 2.17 to 36.54 per cent. Among the villages surveyed, the mean maximum disease incidence (36.54%) was recorded from Ugarbudruk followed by Budarkatti (22.60%) of Belagavi district. Among the taluks, the maximum disease incidence (19.22%) was noticed in Athani taluk of Belagavi. Among the districts surveyed, the mean maximum incidence (11.04 %) was noticed in Belagavi district followed by Dharwad district (5.53 %) and the mean least incidence (4.66 %) was noticed in Haveri district. In Dharwad district, the maximum disease was recorded at Kavalgiri (12.56 %) followed by Murakatti (8.34 %), while in Khalghatagi the maximum incidence was recorded in Dummawad (4.84 %) followed by Devikoppa (3.17 %) and in Hubballi maximum incidence was recorded in Rayapur (10.56 %) followed by Gabbur (5.80 %) and Bammagatti (3.29 %). In Haveri district, the maximum incidence was recorded in Haveri (7.80 %) followed by Devagiri (4.60 %) and Motebennur (3.90 %). In Hirekerur taluk, maximum disease incidence was found in Koda (4.84 %) followed by Rattihalli (2.97 %). In Athani taluk maximum incidence found in Ugarbudruk (36.54%) followed by Ugarkhurd (15.48 %) and Maheshwadagi (5.64 %). In Baihongal taluk maximum incidence was recorded in Budarakatti (22.60 %) followed by Ankalagi (12.67 %). Whereas in Raibag taluk maximum disease incidence was recorded in Satti (4.45 %) followed by Kudachi (3.46 %). In Gokak taluk maximum disease was recorded in Musguppi (4.56 %) followed by Vaderhatti (2.17%) (Tables 1 and 2).

A total of three most commonly varieties, JS 335, JS 93-05 and DSb 21, grown in the surveyed areas were observed

Table 1: Survey for root rot complex incidence of soybean in Dharwad district of northern Karnataka during *kharif* 2015

| District | Taluk | Location | Variety | Incidence of root rot complex | | | Per cent incidence/infestation Root rot complex |
|---|---|---|---|---|---|---|---|
| | | | | *R.* | *S.* | *F.* | |
| Dharwad | Dharwad | Narendra | DSb 21 | + | + | - | 3.47 |
| | | Murakatti | JS 93-05 | + | - | + | 8.34 |
| | | MARS Dharwad | JS 335 | + | - | + | 4.89 |
| | | Navalur | JS 335 | + | + | - | 5.46 |
| | | Kavalgeri | JS 93-05 | + | + | + | 12.56 |
| | | Lokur | JS 335 | + | + | - | 4.24 |
| | | Garag | JS 335 | + | - | - | 3.17 |
| | | | | **Mean** | | | **6.01** |
| | Kalagatagi | Devikoppa | DSb 21 | + | - | + | 3.17 |
| | | Dummavad | JS 335 | + | - | + | 4.84 |
| | | | | **Mean** | | | **4.05** |
| | Hubballi | Bammagatti | DSb 21 | - | - | + | 3.29 |
| | | Gabbur | JS 335 | - | + | + | 5.80 |
| | | Rayapur | JS 335 | + | + | + | 10.56 |
| | | | | **Mean** | | | **6.55** |

+: Associated, - : Not associated, R. - *Rhizoctonia bataticola, S. - Sclerotium rolfsii, F.- Fusarium* sp.

Table 2: Survey for root rot complex incidence of soybean in Haveri and Belagavi districts of northern Karnataka during *kharif* 2015

| District | Taluk | Location | Variety | Incidence of root rot complex | | | Per cent incidence Root rot complex |
|---|---|---|---|---|---|---|---|
| | | | | *R.* | *S.* | *F.* | |
| Haveri | Haveri | Haveri | JS 93-05 | + | + | + | 7.80 |
| | | Motebennur | JS 335 | - | - | + | 3.90 |
| | | Devagiri | JS 335 | + | + | - | 4.60 |
| | | | | **Mean** | | | **5.43** |
| | Hirekerur | Rattihalli | DSb 21 | + | - | - | 2.97 |
| | | Koda | JS 335 | + | + | + | 4.84 |
| | | | | **Mean** | | | **3.90** |
| Belagavi | Athani | Maheshwadagi | DSb 21 | + | + | - | 5.64 |
| | | Ugarkhurd | JS 335 | + | + | + | 15.48 |
| | | Ugarbudruk | JS 335 | + | + | + | 36.54 |
| | | | | **Mean** | | | **19.22** |
| | Bailhongal | Budarakatti | JS 335 | + | + | + | 22.60 |
| | | Ankalagi | JS 93-05 | + | + | + | 12.67 |
| | | | | **Mean** | | | **17.63** |
| | Raibag | Satti | JS 335 | + | + | - | 4.45 |
| | | Kudachi | JS 335 | + | - | + | 3.46 |
| | | | | **Mean** | | | **3.95** |
| | Gokak | Musguppi | JS 335 | + | - | + | 4.56 |
| | | Vaderhatti | JS 335 | - | + | - | 2.17 |
| | | | | **Mean** | | | **3.36** |

+: Associated, - : Not associated, R. - *Rhizoctonia bataticola, S. - Sclerotium rolfsii, F.- Fusarium* sp.

for disease and pest incidence. The disease and pest severity varied from genotype to genotype in different geographical regions. The maximum mean disease incidence was recorded in in JS 93-05 (10.34 %) and the mean least (3.70 %) was noticed in DSb 21 genotype (Table 3). The higher incidence was recorded in soybean grown as sole crop cropping system. In different cropping situation, the higher disease was noticed in irrigated situation (14.28 %) followed by rainfed (6.06 %) (Table 3). The higher mean disease was noticed during vegetative stage (9.17 %) followed by pod filling (8.15 %) and flowering stage (5.37 %) of the crop (Table 4). Out of three districts, 26 villages were surveyed and diseased specimen were collected and were subjected to isolation. The results revealed that *Fusarium* was found in



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Table 3: Severity of root rot in different cropping situation and variety during *kharif* 2015

| | | Cropping situation | | Variety | | |
|---|---|---|---|---|---|---|
| | | Rainfed | Irrigated | JS 335 | JS 93-05 | DSb 21 |
| | | R | R | R | R | R |
| Mean PDI | | 6.06 | 14.18 | 7.97 | 10.34 | 3.70 |
| Range | Max. | 22.60 | 36.54 | 36.54 | 12.67 | 5.64 |
| | Min. | 2.17 | 3.46 | 2.17 | 7.80 | 3.17 |

R- Root rot, PDI- Percent disease incidence

Table 4: Severity of root rot in black soil and different crop stages during *kharif* 2015

| | | Soil type (Black soil) | Crop stage | | |
|---|---|---|---|---|---|
| | | R | Vegetative | Flowering | Pod filling |
| | | | R | R | R |
| Mean PDI | | 7.06 | 9.17 | 5.37 | 8.15 |
| Range | Max. | 36.54 | 22.60 | 15.48 | 36.54 |
| | Min. | 2.17 | 3.47 | 2.17 | 3.17 |

R- Root rot, PDI- Percent disease incidence

two places *viz*., Bammagatti of Dharwad district and Motebennur of Haveri district. *Rhizoctonia bataticola* was found in the two places *viz*., Garag of Dharwad district and Rattihalli of Haveri district and *S. rolfsii* was found in one place *viz*., Vaderhatti of Belagavi district. Association of *Fusarium* sp. and *R. bataticola* in combination were found in the six places surveyed. Association of *Fusarium* sp. and *S. rolfsii* in combination were found in one place *i.e.,* Gabbur village of Dharwad district and *R. bataticola* and *S. rolfsii* in combination were found in six places. The association of all the pathogens *R. bataticola* + *S. rolfsii* + *Fusarium* sp. was noticed in eight places (Table 5).

The first external symptom noticed was necrosis or yellowing of older leaves followed by discolouration. The infected plants at the early stage showed seedling blight symptom, while the infected plant at the later stage showed general yellowing followed by defoliation of leaves and general wilting. Such wilted plants showed whitish mycelial growth with sclerotial bodies resembled mustard seeds on the collar region and also roots. When the infected plants uprooted the cross-section of the infected roots revealed brownish-black discolouration of vascular bundles. In addition, the extensive sloughing off of affected bark and shredding of roots was also observed. In the advanced stage, the aerial portion of the plants decayed completely. The repeated isolation of fungi associated with root rot complex samples collected from different soybean growing areas which yielded three major species of *Fusarium*, *Sclerotium* and *Rhizoctonia* (Fig. 1 & 2). Description of all these fungi agreed with the description of *Fusarium* sp. by Booth (1971), *S. rolfsii* by Mundkur (1934) and *R. bataticola* by Taub Butler (Pycnidial stage of *Macrophomina phaseolina* by Ashby (1927) (Fig. 1 & 2). Pathogenicity tests for root rot complex were carried out by using Dharwad isolates of *Fusarium* sp., *R. bataticola* and *S. rolfsii*. Observation for symptom expression showed that *Fusarium* sp. infected plants exhibited foliar yellowing. The affected plants wilted and dried up, but didn't fall on the ground. The days taken for symptom expression varied in different pathogens alone or combination. The affected plants showed a brown discoloration of the vascular system. The pathogenicity test for *R. bataticola* showed infected plants exhibited foliar yellowing. The bark of such infected plants came out very easily and root discoloration. Pathogenicity test for *S. rolfsii* was carried out and symptoms such as seedling mortality, foliar yellowing and white mycelial growth on the roots were observed.

Table 5: Association of the pathogens in root rot complex of soybean

| Pathogens isolated | No. of field surveyed *kharif* 2015 | |
|---|---|---|
| | No. of locations | Per cent mean |
| *Sclerotium rolfsii* | 1 | 2.17 |
| *Fusarium* sp. | 2 | 3.59 |
| *Rhizoctonia bataticola* | 2 | 3.07 |
| *Sclerotium rolfsii* + *Fusarium* sp. | 1 | 5.80 |
| *Fusarium* sp. + *Rhizoctonia bataticola* | 6 | 4.88 |
| *Sclerotium rolfsii* + *Rhizoctonia bataticola* | 6 | 4.64 |
| *Sclerotium rolfsii* + *Fuarium* sp. + *Rhizoctonia bataticola* | 8 | 15.38 |

The pathogenicity studies were conducted in a glasshouse on susceptible cultivar JS 335. The data on the percentage of diseased plants inoculated with all the pathogens are presented in Table 6. After 20 days of inoculation of the giant culture of the different pathogens alone and in combination, revealed that maximum percent disease incidence in case of mixed inoculation of *S. rolfsii* + *R. bataticola* + *Fusarium* sp. (88.50 %) followed by dual inoculation of *S. rolfsii* + *R. bataticola* (83.33 %) and inoculation of *Fusarium* sp.+ *R. bataticola* (75 %) and *S. rolfsii* + *Fusarium* (72.34 %). The pathogens alone also recorded more than 50 per cent disease incidence. Maximum percent incidence in single inoculation of *R. bataticola* with 66.66 per cent followed by *S. rolfsii* (58.33 %). The least disease incidence was recorded in *Fusarium* sp. (54.33 %). In the case of root rot index, the maximum of 5 cm root rot index was recorded in dual inoculation of *S. rolfsii* + *R. bataticola* as well as triple inoculation of *S. rolfsii* + *R. bataticola* + *Fusarium* followed by

*R. bataticola* (4.87 cm), stem fly infected leaves (4.45 cm), *S. rolfsii*+ *Fusarium* sp. (4.15 cm) and *S. rolfsii* (3.87 cm). However, the least incidence was recorded in *Fusarium* sp. (3.08 cm) (Table 6). Soybean is the wonder crop of 21st century which is highly important owing to its high protein and oil content. The yield levels have been reduced drastically because of abiotic and biotic factors. The key economically important diseases of soybean are rust, root rot and MYMV. The root rot complex of soybean has been reported in India as the most common disease (Wrather *et al*., 1997). Losses in yield upto 50 per cent have been experienced (Sinclair and Shurtleff, 1975). In India, 70% loss caused by root rot complex has been reported. The disease is common in MP, Maharashtra, Rajasthan, Uttaranchal, Karnataka, Punjab and Delhi. The pathogens have a very broad host range of 500 crops spread over different puleses, oil seeds and cereal crops. *Sclerotium rolfsii* is a well-known polyphagous, non-target and root pathogen of many cultivated economic crops. The pathogen is also a well-established causal agent of stem rot or southern blight of soybean (Aken and Dashiella, 1991; Ansari, 2005). *Rhizoctonia bataticola* (Taub.) Butler (= *Sclerotium batati-cola* Taub.) (Pycnidial stage: *Macrophomina phaseolina*) is a diverse omnipresent soil-borne fungal pathogen, in-fecting more than 500 plant species. The pathogen causes different types of diseases *viz*., seedling blight, root rot, charcoal rot, wilt, stalk rot, stem blight, fruit rot, seedling decay and leaf blight in crop plants (Dhingra and Sinclair, 1978). *Fusarium* species infect many crops around the globe either as parasite or pathogen (Alabouvelte *et al*., 1998) The seed-borne nature of *Fusarium oxysporum*


Fig. 1: Pathogens associated with root rot complex of soybean

Table 6: Interaction effect of *Sclerotium rolfsii, Rhizoctonia bataticola* and *Fusarium* sp. on soybean seedlings

| Treatment | Per cent disease incidence | Root rot length (cm) |
|---|---|---|
| *Sclerotium rolfsii* | 58.33 | 3.87 |
| *Fusarium* sp. | 54.43 | 3.08 |
| *Rhizoctonia bataticola* | 66.66 | 4.87 |
| *Sclerotium rolfsii* + *Fusarium* sp. | 72.34 | 4.15 |
| *Fusarium* sp. + *Rhizoctonia bataticola* | 75.00 | 4.33 |
| *Sclerotium rolfsii* + *Rhizoctonia bataticola* | 83.33 | 5.00 |
| *Sclerotium rolfsii* + *Fusarium* sp. + *Rhizoctonia bataticola* | 88.50 | 5.00 |
| Uninoculated control | 0.00 | 0.00 |
| **S.Em.±** | 0.58 | - |
| **C.D. at 1%** | 2.38 | - |

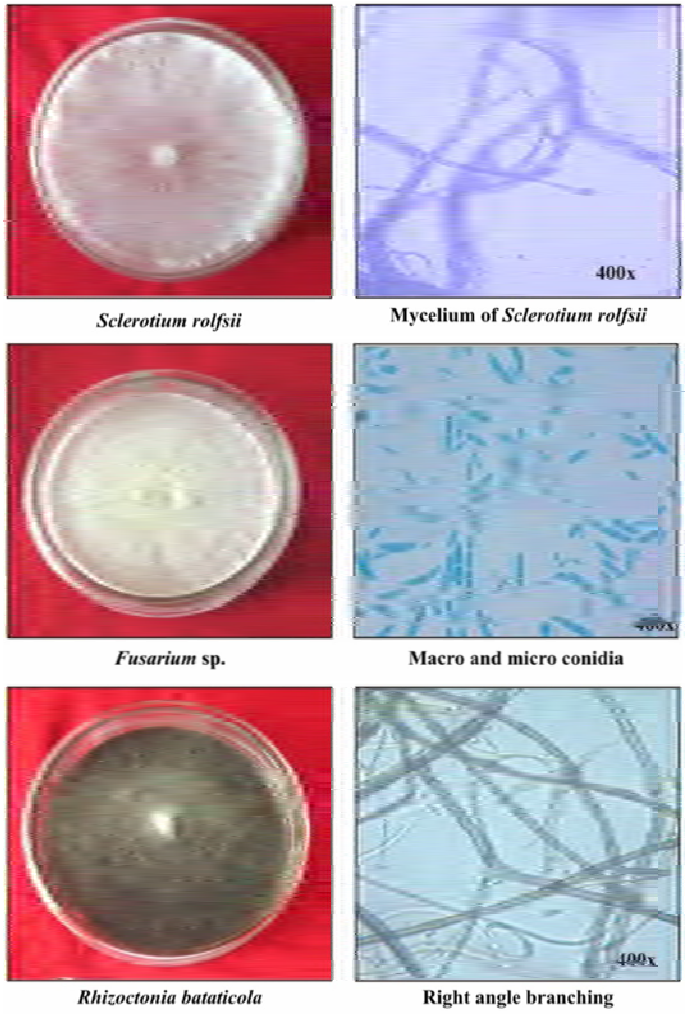


Fig. 2: Pure cultures of root rot pathogen complex of soybean



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f.sp. *glycine* [(Schlet) Emed. Snyd and Hans] have been often reported in seed samples (Nasir, 2003; Agarwal *et al*., 2006). The infection ranged from symptoms such as root rot to wilt (Akinsanmil and Adenkunle, 2003).

The root rot infection started at the collar region of plants as water-soaked areas and tissues soon turned into a soft, watery mass. Later spread to the roots of the plant and caused decay, which ultimately toppled and collapsed. These infected plants can be easily pulled off from the soil and exhibited brown discoloration of roots followed by rotting of roots. In addition, the extensive sloughing off of affected bark and shredding of roots was also observed. In the advanced stage, the aerial portion of the plants decayed completely (Ramprasad, 2005 and Sachidananda, 2005). In the present study, the infected soybean root rot samples from northern Karnataka and also soybean growing areas of India were collected. The pathogen was isolated by standard tissue isolation procedure. The pure culture thus obtained was subcultured on potato dextrose agar medium slants and kept in a refrigerator for further studies. Prabhu and Patil (2004) isolated *S. rolfsii* from soybean, Konde *et al*. (2008), isolated *S. rolfsii* and *Rhizoctonia* sp. from soybean and Nasir (2003) and Agarwal *et al*. (2006) isolated *Fusarium* sp. from soybean and Sangeetha (2013) isolated all the three associated pathogens *S. rolfsii, R. bataticola* and *Fusarium* sp. soybean growing areas of northern Karnataka. Among the districts surveyed, the mean maximum incidence (11.04 %) was noticed in Belagavi district followed by Dharwad district (5.53 %). Among the taluks, the maximum disease incidence (19.22 %) was noticed in Athani taluk of Belagavi district and the least incidence (3.36 %) was recorded in Gokak taluk of Belagavi district. Therefore, these places can be considered as 'hot spots' of soybean root rot complex disease. The disease incidence varied from locality to locality, because of cropping pattern, environmental conditions and buildup of inoculum. The higher disease incidence may be attributed to the soil type, environmental condition monocropping system which aggravated the disease situation. Such variations in soybean root rot complex incidence and widespread nature have been reported by earlier researchers (Harlapur, 1988; Kulkarni, 1992; Nargund, 1981; Prabhu and Patil 2004; Rani *et al*., 2014 and Sangeetha, 2011).

The disease severity was very high in areas coming under the black cotton soil and the farmers are growing the soybean extensively in these areas year after year. This monocropping has led to the buildup of the inoculum of the pathogen in the soil over the seasons and whenever there is an optimum soil temperature & moisture and caused more damage to the crop. These observations are in agreement with the earlier descriptions given by Booth (1971). In the areas where the disease incidence was higher, they have noticed the association of *Fusarium*, *Sclerotium* and *Rhizoctonia* (Arya *et al*., 2004; Ramprasad, 2005 and Sangeetha, 2011). Maximum stem fly infestation (33.74 %) was recorded from MARS Dharwad. Among the districts surveyed, the mean maximum infestation (26.06 %) was noticed in Belagavi district followed by Dharwad district (20.02 %). Similar observations were also made by Kavita, 2006; Patil and Kulkarni, 2004; Pradhan *et al*., 2000; Sangeetha, 2011 and Sharma *et al*., 1994 in recording severity of root rot of soybean in different locations. Intensive cultivation of soybean crop year after year, non-adoption of disease management practices, favourable weather condition and also the cultivation of highly susceptible varieties of soybean could be the reason for higher pest infestation in different locations of northern Karnataka (Kamala, 2000; Kujur, 2011; Singh *et al*., 2000; Vinodkumar *et al*., 2014; Virakar, 2004).

Major pathogens associated with disease are *Sclerotium rolfsii* + *Fuarium* sp. + *Rhizoctonia* sp. was major pathogen complex (15.38 %) followed by *Fusarium* sp. + *R. bataticola* (5.80 %) and *Fusarium* sp.+ *Rhizoctonia* sp. (4.88 %). These results of pathogens involved in root rot complex were similar to the findings of Khan (1979) reported the association of the fungi with wilt affected soybean plants included *Fusarium* sp. (60 %), *R. bataticola* (12 %), *S. rolfsii* (2 %) and *R. solani* (5 %) and also Sangeetha (2011) reported association of all the three pathogens at Ugarkhurd and many other areas surveyed. The root rot symptom was initially started at the collar region of the plants as water-soaked areas and the affected tissues soon turned into the soft, black and watery mass. Later the infection spread to the roots and caused decay. This ultimately caused toppling and collapse of the infected plants, similar finding was also observed by Dhingra and Sinclair 1973; Sangeetha, 2011. The pathogen was recovered from the collar portion by standard tissue isolation method the fungus produced white, dense, radiating mycelia. In the early stages, the fungus produced silky white mycelium and gradually lost its luster. Initiations of sclerotial bodies were obtained from the fifth day after inoculation. In the beginning, the sclerotial bodies were white but gradually turned to buff-brown colour and then to chocolate brown, at maturity, as described by Barnett and Hunter (1972) and pathogenicity was proved on susceptible cultivar JS 335.

## CONCLUSION

The study concluded predominance of *R. bataticola* over two other root rot pathogens, *S. rolfsii* and *Fusarium* sp. prevalent in Northern Kanbalka. Genotype DSb 21 and JS 335 with minimum (3.2%) and maximum (10.74%) incidence

of *R. bataticola* were graded as highly tolerant and high susceptible, respectively.

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# Impact of extreme weather conditions on plant genetic resources and livelihood in central Himalayan Region

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**ABSTRACT**

Unpredictable extreme weather, climate events and natural disasters have the potential to upset the normal life processes of plants, animals and human being. Central Himalayan Region (CHR) is known for its biological richness as well as vulnerability to natural disasters. Focus areas of natural disaster studies are damage to human lives, live stock and property. Plant genetic resources hardly get place in natural disaster management. Keeping the glacial lake outburst flood (GLOF) during June 2013 in Kedarnath region of the Uttarakhand province of India in view, the present study was conducted with the aim of assessing the damage to livelihood and plant genetic resources and find out possible solution to conserve the available genetic diversity and minimize the damage to livelihood.

**Key words:** Climate change; precipitation; germplasm; gene bank; livelihood.

Climate in Central Himalayan Region (CHR) is characterized by vagaries of weather. Unpredictable extreme weather and natural disasters have the potential to upset the normal life processes of plants and cause great uncertainty in animal life and livelihood of human beings. This region is known for its biological richness and has always been botanist's paradise. Its diversified landforms, relief and environmental conditions support wide range of vegetations. The Central Himalayan Region (CHR) of India, support a human population of 10.1 million and prone to number of natural hazards. People living in this terrain have often experienced wrath of these (Rautela, 2015). This region has been experiencing heavy rainfall, mostly restricted to monsoon period. Localized abnormally heavy precipitation (some times cloud burst) has been common in the region often resulting in debris flow, landslides and flash floods (Rautela, 2015). The region is witnessing effects of climate change at an unprecedented rate (Jianchu *et al*., 2009). Physiographic conditions, land characteristics and climatic conditions make the region vulnerable to vagaries of nature (Jianchu *et al*., 2009).

Some effects of climate change are warming in Himalayan Region about 2-3 times higher than global average of 0.74°C during the last 100 years (Du *et al*., 2004; IPCC 2007) which is adversely affecting agro-biodiversity. In addition, total annual precipitation and extreme precipitation events have increased during the previous century, especially during the past two decades (Karl 1998; Milly *et al*., 2002), often resulting in large crop losses and other flood-related damages (Chagnon *et al*., 1997; Pielke and Downtown 2002). Under current climatic conditions, damage to agricultural production due to excess precipitation has been substantial (Rosenzweig *et al*., 2002). The catastrophe that struck Kedarnath valley was on account of a glacial lake outburst flood (GLOF) (Srinivasan 2013). Glacial lake outburst floods (GLOFs) are natural hazards caused by indirect factors of climate change and are characterized by their sudden occurrence, high flood peak, large discharge, strong destructiveness and short duration with widespread range (Wang and Zhou, 2017). The effects of GLOF disasters are difficult to predict and seriously affect the lives and properties of residents in the downstream communities, transportation, infrastructure and agriculture (Wang and Zhou, 2017).

In ecologically fragile area like CHR natural replenishment of flora is unable to keep pace with accelerated anthropogenic exploitation of natural resources. This causes loss of genetic variability which is a precondition for food security. Food security cannot be assured if crop diversity is not conserved (Alcazar, 2005). The broader the genetic base on which our civilization relies on, better equipped it will be to adapt to changing climatic conditions and guarantee

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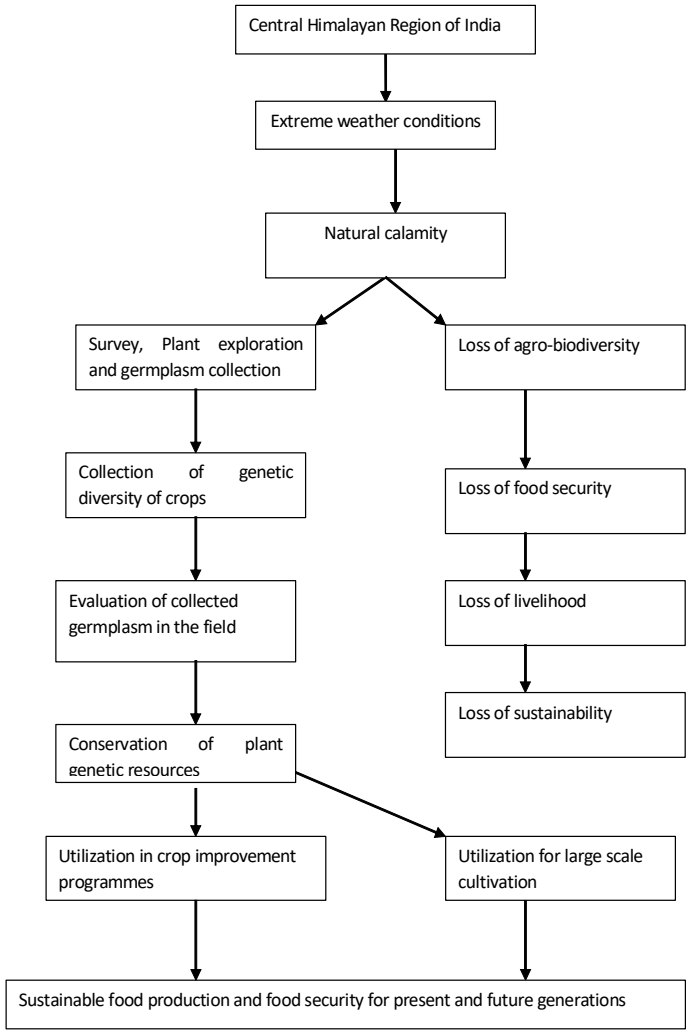
global food security (Boyer, 1982). Population density of CHR is approximately half that of the national average. Hence, in spite of low productivity as compared to national average, this region has potential to provide food security to sparsely populated inhabitants. Tourism is an important source of income for local residents as four tourist destinations - Yamunotri, Gangotri, Kedarnath and Badrinath (the Chardham) attract large number of tourists and pilgrims from within the country and abroad. In order to attract tourists, unplanned construction near river banks is rapidly increasing, which creates obstruction to the flow of small rivulets at several places. This causes diversion of water stream, as well as, floods in down stream in the river basin. Topography of the region limits the use of land for agricultural and horticultural enterprise. Farmers have to utilize small piece of land (terraces) for cultivation and livelihood support (Panth and Gautam 1990; Tang *et al*., 2013). Unplanned and ill regulated anthropogenic intervention in this fragile ecosystem is a matter of serious concern. It induces high risk for human and animal life and causes severe damage to natural resources. Keeping these facts in view, the impact of extreme weather condition on plant genetic resources and livelihood of inhabitants, was assessed. Concurrently, the germplasm of plants that were damaged due to extreme weather and natural calamity were collected.

## MATERIAL AND METHODS

After glacial lake outburst flood (GLOF) disaster in Kedarnath region of the Uttarakhand province of India during June 2013, exploration expeditions were conducted in CHR (Figure 1). Post disaster survey was conducted to record the perception of local inhabitants regarding change in rainfall, food security, loss of livelihood and perception of climate change. A proforma was provided to the local inhabitants to assess their perception regarding immediately preceding extreme weather conditions. A total of 86 germplasm samples having unique traits of agricultural importance were collected from disaster affected areas along with passport information. Damage sites were visited along with local folks to assess the actual loss and severity of damage (Photoplate 1-6). About 4 or 5 farmers from each village that was visited were contacted with a formal questionnaire as well as informal talks, to gather relevant information.

The survey included field research, household survey and participatory rural appraisal (PRA) sessions with the communities (Rademacher-Schulz *et al*., 2012). There were separate PRA sessions for women, men and persons of different age groups. Full efforts were made not to exclude respondents of any particular caste residing in the village.




**Graphical Abstract**

The survey included questions directly or indirectly related to the three main variables of the study, namely rainfall variability, food security and plant genetic resources. Meteorological data recorded at ICAR-National Bureau of Plant Genetic Resources, Regional Station Bhowali, Nainital (Uttarakhand) during last three decades were considered for drawing the inferences.

## RESULTS AND DISCUSSIONS

Extreme weather conditions and natural disasters caused havoc to human being, live stock, property as well as plant genetic resources. However, priority of disaster risk reduction plans remained limited to human lives, live stock and property. So far plant genetic resources could not get

proper place in disaster management plans. In view of vital role of plant genetic resources for food security, it has been emphasized in convention on biological diversity that any living material of present and potential value for humans is plant genetic resource (CBD, 1992). If a plant genetic resource is lost, it is lost for ever. Plant genetic resources of different locations and elevations have unique characteristics due to ample climatic variability particularly in Himalayan region. However, during rainy season, torrential rains, flash floods, cloud bursts, land slides pose a threat to people, livestock and property as well as plant genetic resources. In addition, over 2000 glacial lakes are available across the Himalaya (Fujita, *et al*. 2013). Glacial lake outburst floods (GLOFs) predominate during the peak monsoon months (Allen *et al*., 2015). In Kedarnath region also Chorabari lake breach took place in rainy season (June, 2013) when *Kharif* season crops viz., paddy (*Oryza sativa*), soybean (black bhat) (*Glycine max*), finger millet (*Eleucine coracana*), barnyard millet (*Echinocloa frumentaceae*) etc. were in the field. Hence, maximum loss of plant genetic resources (germplasm) was recorded in these crops in decreasing order (Table 1). Trait specific germplasm of 16 species representing 5 families was found to be radically affected; it was collected and conserved (Table 1).

The precipitation pattern of this region during last three decades was found to be erratic (Figure 2). Plant genetic resources surviving in such environmental conditions may be environmental/ abiotic stress tolerant and useful for crop improvement as donor parent. Plant responses to environmental/ abiotic stresses are dynamic and complex

Table 1. Germplasm samples damaged and on the verge of extinction in CHR

| S. No. | Genera | Species | Family | Number of accessions/ samples |
|---|---|---|---|---|
| 1 | *Abelmoschus* | *esculanta* | Malvaceae | 1 |
| 2 | *Amaranthus* | *virdis* | Amaranthaceae | 2 |
| 3 | *Brassica* | *juncea* | Cruciferaceae | 3 |
| 4 | *Echinochloa* | *frumentacea* | Graminaceae | 6 |
| 5 | *Eleusine* | *coracana* | Cruciferaceae | 8 |
| 6 | *Glycine* | *max* | Leguminosae | 10 |
| 7 | *Hordeum* | *vulgare* | Graminaceae | 4 |
| 8 | *Lens* | *culinaris* | Leguminosae | 2 |
| 9 | *Macrotyloma* | *uniflorum* | Leguminosae | 1 |
| 10 | *Oryza* | *sativa* | Graminaceae | 33 |
| 11 | *Phaseolus* | *vulgaris* | Leguminosae | 4 |
| 12 | *Sesamum* | *idicum* | Pedaliaceae | 2 |
| 13 | *Setaria* | *italica* | Graminaceae | 1 |
| 14 | *Triticum* | *aestivum* | Graminaceae | 1 |
| 15 | *Vigna* | *mungo* | Leguminosae | 3 |
| 16 | *Vigna* | *unguiculata* | Leguminosae | 1 |
| 17 | *Zea* | *mays* | Graminaceae | 4 |
| **Total** | **16** | **16** | **5** | **86** |

(Skirycz and Inze, 2010; Cramer, 2010); they are both elastic (reversible) and plastic (irreversible). Environmental factors may limit crop production by as much as 70 per cent (Boyer 1982). But it is difficult to get accurate estimates of the effects on crop production (Cramer et al., 2011). The erratic precipitation pattern, accepted by 31 per cent of inhabitants (Table 2), has affected seasonal water supply. Impact of climate change is more on seasonal water supplies than annual water supply (Singh and Bengtsson, 2004; Singh and Bengtsson, 2005). In recent year's significant increasing trend in stream flow, both annual and wet season, without any significant difference in precipitation has been found (Zhang et al., 2011) which in turn caused uncertainty in availability of water to crops and change in micro climate of different areas. In erratic environment conditions, the level and duration of stress (acute vs chronic) can have significant effect on the complexity of the response (Tattersall *et al*., 2007; Pinheiro and Chaves 2011). Natural disasters cause irretrievable damage to plant genetic resources. To cope with

Table 2. Perception of residents regarding precipitation pattern in CHR during last three decades

| Rainfall change | Response of residents (Yes) |
|---|---|
| Longer rainy seasons | 24 (8%) |
| Shorter rainy seasons | 42 (14 %) |
| More rain | 45 (15%) |
| Longer dry spells | 48 (16%) |
| Shorter dry spells | 21 (7%) |
| More dry spells | 27 (9%) |
| Erratic rainy season | 93 (31%) |
| Total | 300 (100%) |

Digits in the table are number of respondents and digits within parenthesis are percent value.

Table 3. Perception of climatic change by local residents in CHR

| Category | Response of residents | | | |
|---|---|---|---|---|
| | Yes | No | Same as before | Don't know |
| More drought/dry spells | 132 (44%) | 63 (21%) | 81 (27%) | 24 (8%) |
| Less drought/dry spells | 117 (39%) | 63 (21%) | 108 (36%) | 12 (4%) |
| More rains | 108 (30%) | 66 (22%) | 54 (18%) | 72 (24%) |
| Less rains | 78 (26%) | 114 (38%) | 99 (33%) | 18 (6%) |
| More flood | 159 (53%) | 54 (18%) | 69 (23%) | 18 (6%) |
| Less flood | 66 (22%) | 147 (49%) | 78 (26%) | 9 (3%) |

Digits in the table are number of respondents and digits within parenthesis are percent value.



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Photo Plates: Areas of CHR affected by extreme weather conditions

Photo Plate 1: Landslide affected agricultural field near Kapkot, Bageshwar

Photo Plate 2: Landslide affected agricultural field near Balighat, Bageshwar

Photo Plate 3: Damage of agricultural fields and farmers houses near Nainipatal, Pithoragarh

Photo Plate 4: Damage of agricultural fields near Nainipatal, Pithoragarh

Photo Plate 5: Loss of crops, agricultural field near Modiyani, Champawat

Photo Plate 6: Damage of farmers houses by river Kali near Sathgarh, Pithoragarh

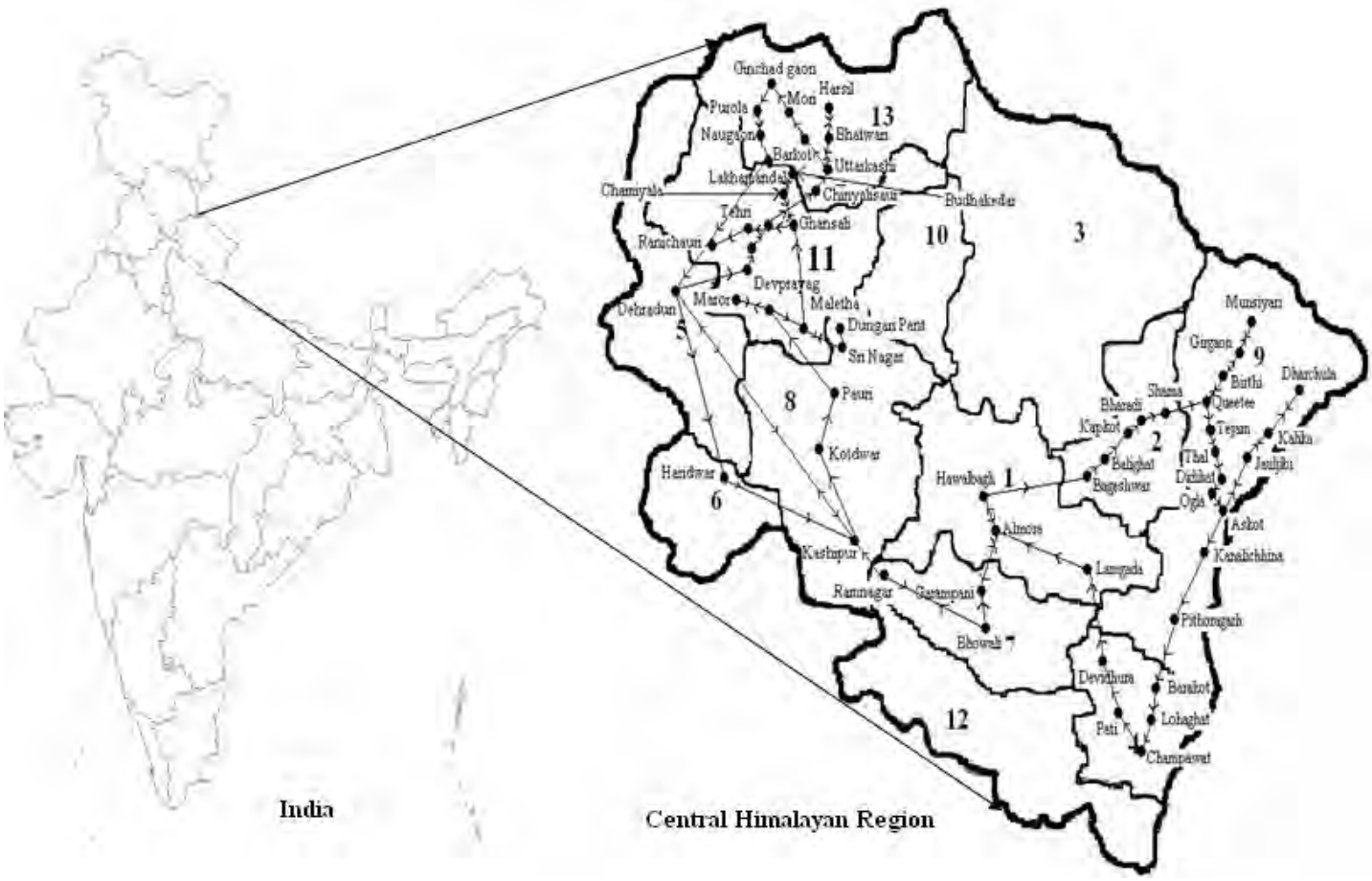


Figure 1. Route map of survey of the areas affected by extreme weather conditions

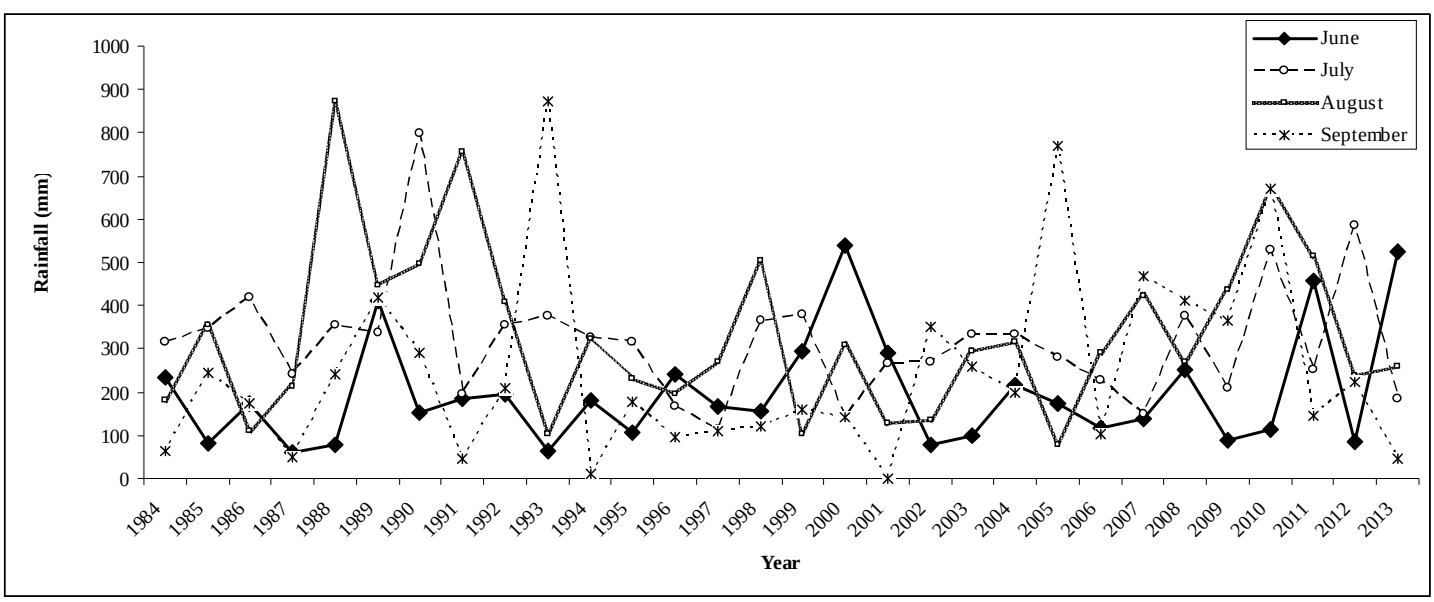


Figure 2. Mean precipitation during June – September (1984-2013).



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the situation, it is essential to collect, characterize and conserve untapped genetic diversity.

Extreme weather conditions/ natural disasters create potentially damaging variations in the immediate environment of the plants. Rapid perception of these changes and appropriate phenotypic adjustments in response to environmental fluctuations is critical for plants in view of their sessile nature. In recent decades flood risk under climate change has increased dramatically (Hirabayashi *et al*., 2013). In the last 50 years, increasingly frequent and severe flooding events have negatively impacted terrestrial plant life (Sasidharan *et al*., 2018). Many wild plant species and nearly all crops are intolerant to floods and thus excessive water will affect the natural patterns of plant distribution and biodiversity (Silvertown *et al*., 1999) and have a devastating impact on crop growth and survival and thus on food production (Normile, 2008). Even different accessions of same species show natural variation in flooding tolerance (Vashisht *et al*., 2011). Precious plant genetic resources are available in CHR, hence, collection and conservation of germplasm from disaster affected areas like Kedarnath region is inevitable for future food security. Even though, there is relatively limited evidence for current extinctions caused by climate change, studies suggest that climate change related habitat destruction may be the greatest global threat to biodiversity during the coming decades (Leadley *et al*., 2010). Therefore, study and prediction of response of biodiversity to climate change has become an extremely active field of research (Dillon *et al*., 2010; Pereira *et al*., 2010; Salamain *et al*., 2010; Beaumont *et al*., 2008; Dawson *et al*., 2011; McMahon *et al*., 2011). Due to severe effects of climate change, it is a crucial question whether species will be able to adapt fast enough to keep up with the rapid change of climate (Salamin et al., 2010; Lavergne *et al*., 2010). Failing to adapt, population or species will go extinct locally or globally (Bellard et al., 2012). In the current scenario, most serious issue is to quantitatively assess the prospects for biological diversity in the face of global climate change (Lepetz et al., 2009). Although several methods exist to draw inferences, starting with existing paleontological or recent data, experiments, observations, and meta-analyses (Lepetz *et al*., 2009), it is uncertain as to which type of germplasm will survive and which will be wiped out under fast pace of changing climate. This emphasizes the need to collect, characterize and conserve the available plant genetic resources.

The detailed analysis of census 2011 data reveal that, over all population growth of Uttarakhand province was 18.81 per cent in this decade (2001-2011) [which include negative growth rate recorded in two districts viz., Pauri Garhwal (-1.41%) and Almora (-1.28%)] compared to past decade (1991-2001). Therefore, to cope with the increasing demand of food for growing population, collection, conservation and utilization of diversity is essential as frequent droughts, floods and erratic weather patterns (Table 3) adversely affect food security and agro-biodiversity (Table 4).

Table 4. Opinion of local residents for food security in CHR

| Category | More | Less | Do not know |
|---|---|---|---|
| Agro-biodiversity (As compared to 30 year ago) | 126 (42%) | 153 (51%) | 21 (7%) |
| Food security for round the year | 183 (61%) | 81 (27%) | 36 (12%) |
| Food grain quality | 162 (54%) | 111 (37%) | 27 (9%) |
| Availability of fruits | 177 (59%) | 84 (28%) | 39 (13%) |

Digits in the table are number of respondents and digits within parenthesis are percent value.

Table 5. Opinion of local residents for loss of livelihood

| Reason of livelihood loss | Response of local residents |
|---|---|
| Loss of perennial fruit trees | 43 (14.33) |
| Loss of seed material of annual crops | 22 (7.33) |
| Damaged roads | 62 (20.67) |
| Loss of animals due to calamities | 47 (15.67) |
| Loss of arable land due to calamities | 83 (27.67) |
| Loss of job due to effect of calamities on tourism | 29 (9.67) |
| Death of earning member of the family due to calamities | 14 (4.67) |

Digits in the table are number of respondents and digits within parenthesis are percent value.

Due to damage of arable land, the source of income after the incidence of Chorabari GLOF in Kedarnath region was found to be uncertain. Extent of damage to house/ property was found to vary 5-100 per cent. Similarly, cultivable area affected was also found in the range 5-100 per cent. As a consequence of disaster, local inhabitants became deprived of livelihood. Loss of arable land, perennial fruit trees and seed material of annual crops were found to be main reasons (Table 5). Above and beyond limited education, healthcare and transport facilities in the remote and inaccessible areas of the region; economic growth was found to be higher than national average. Proportion of population below poverty line was found to be approximately half the national average (Table 6) which is an indication of the potential available in the region.

Table 6. Geographical and development indicators of the region (state) compared to other parts of India

| Indicators | India | CHR (Uttarakhand) | Source |
|---|---|---|---|
| Population density (Persons per km² area) | 382 | 189 | Census of India, 2011, (www.censusindia.gov.in) |
| Cultivable land (% Geographical area) | 55.5 | 28.2 | Directorate of Economics and Statistics, Ministry of Agriculture, Govt. of India |
| Per capita net sown area (hectares) | 0.12 | 0.07 | Directorate of Economics and Statistics, Ministry of Agriculture, Govt. of India |
| Area under forests (% of Geographical area) | 21.05 | 45.8 | India: State of the Forest Report 2011 |
| Percentage of Population below Poverty Line (Tendulkar Method) (2011-12) | 21.92 | 11.26 | Planning Commission, Government of India, Press Note on Poverty Estimates 2011-12,, July 2013 |
| Transport Density as on March 31, 2009 [Railway Density (kms. of railway line per 100 km² of area)] | 19.47 | 6.45 | India Economic Portal |
| Transport Density as on March 31, 2011 (Road Density (kms. of road length per 100 km² of area)] | 142.68 | 92.14 | India Economic Portal |
| Growth Rates of GSDP (% per annum) (2004-05/2013-14) | 9.23 | 11.63 | CSO, www.mospi.gov.in |
| Per Capita Gross State Domestic Product (GSDP) (at current prices) (2013-14) (Rs.) | 74920 | 112478 | Economic Survey, Government of India |
| Relative Position of State Per Capita GSDP in Relation to Indian Average (2013-14) | 100 | 150.41 | Economic Survey, Government of India |

## CONCLUSION

For sustainable and eco-friendly development anthropogenic interventions in the fragile ecosystems of mountain regions need to be minimized and properly regulated. Employment opportunities and options of livelihood with minimal disturbance to natural resources ought to be created, to face the challenge of frequent natural calamities. To strengthen crop improvement programmes/ food security in the climate change scenario, available plant genetic resources should be collected, characterized and conserved. In this as well as such other agro-biodiversity rich regions community level gene banks should be established, so that available diversity of plant genetic resources may be conserved at local level, which will be easily accessible to breeders, researchers, farmers and remain conserved for future generations.

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# Foresight vision on exogenous applications of *in vitro* derived dsRNAs in Agriculture

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**ABSTRACT**

RNA interference (RNAi) is a gene regulatory mechanism that limits the transcript level by either suppressing transcription (transcriptional gene silencing [TGS]) or by activating a sequence-specific RNA degradation process (posttranscriptional gene silencing [PTGS]). The discovery of RNA silencing via RNAi has facilitated major recent breakthroughs in medicine, veterinary, agriculture, and environmental sciences. As such, the use of RNAi in agricultural fields presents an environmental friendly approach to generate pest- and pathogen-resistant crops. Spray-induced gene silencing (SIGS) provides an intelligent method of using double-stranded (ds)RNA as a trigger to silence target genes in pests and pathogens without side-effects like in the case of chemical pesticide use. This review examines the risks associated with accumulation of dsRNA and small interfering RNA (siRNA) in plants and invasive targeted organisms and environmental contamination, transgeneratioal gene silencing, dsRNA fate and future implementation of SIGS.

**Key words:** RNAi, siRNA, dsRNA, gene silencing, environmental risks

Plant pests are a major limitation to crop production worldwide and their effective control remains a challenge in various cropping systems. While plants are naturally immune to infections of most potential bacterial, viral, and fungal pathogens, the few that manage to establish disease can be extremely problematic to control, causing economic losses. Conventional crop breeding methods have been used to develop cultivars resistant to various diseases. However, this process is time-consuming because of the limited availability of genetic resources for most of the crops. The appearance of new virulent strains of disease-causing microorganisms attacking existing resistant cultivars illustrates the urgent need for development of novel approaches to combat these highly variable crop pests.

Spray-Induced Gene Silencing (SIGS) is the most recent application of RNAi in agriculture. It has the potential to serve as a smart solution for the public's concerns about food safety issues including genetically modified organisms (GMOs), off-targeting risks associated with pesticide residues in crop plants, spraying of dsRNA on plant leaves, stems, flowers and fruits. The targeted pathogens uptake the dsRNA molecule and then use its RNAi machinery to amplify, process and move on the silencing signal. Given the low cost, specific targeting, and applicability to several plant pests, the use of SIGS offers unique potential as a friendly agroecosystem protection strategy.

RNA interference (RNAi) is a cellular mechanism activated by double-stranded RNA (dsRNA) shared by most eukaryotic organisms with apparent roles in gene regulation and defense against viral infection (Baulcombe, 2004). RNAi works by expression of a dsRNA homologous to a target gene to silence expression of that gene. The technology has been used to study functional genomics of non-transformable species such as insects and nematodes, and the first step involves design of the dsRNA with a strand complementary to a fragment of the target gene into cells. After checking that target gene mRNA levels have been down regulated, the study of the phenotype illuminates the corresponding functions (Belles, 2010). An example is the screen for tyrosine kinase receptor regulators in *Drosophila melanogaster* cells (Friedman and Perrimon, 2006).

RNAi technology has also been used commercially to engineer virus resistance in plants by expression of viral sequences as transgenes. For example, Papaya expressed Coat Protein from Papaya ring spot virus (PRSV CP) suppressed the virus growth (Frizzi and Huang 2010). Silencing has been demonstrated for several insects and nematodes that have directly fed on diets containing the dsRNA of the target gene (Huvenne and Smagghe, 2010; Bhatia *et al*. 2012). Host-induced gene silencing (HIGS), also described as host-induced RNAi by expressing dsRNA in plants has shown promise for insect, fungi, parasitic plants

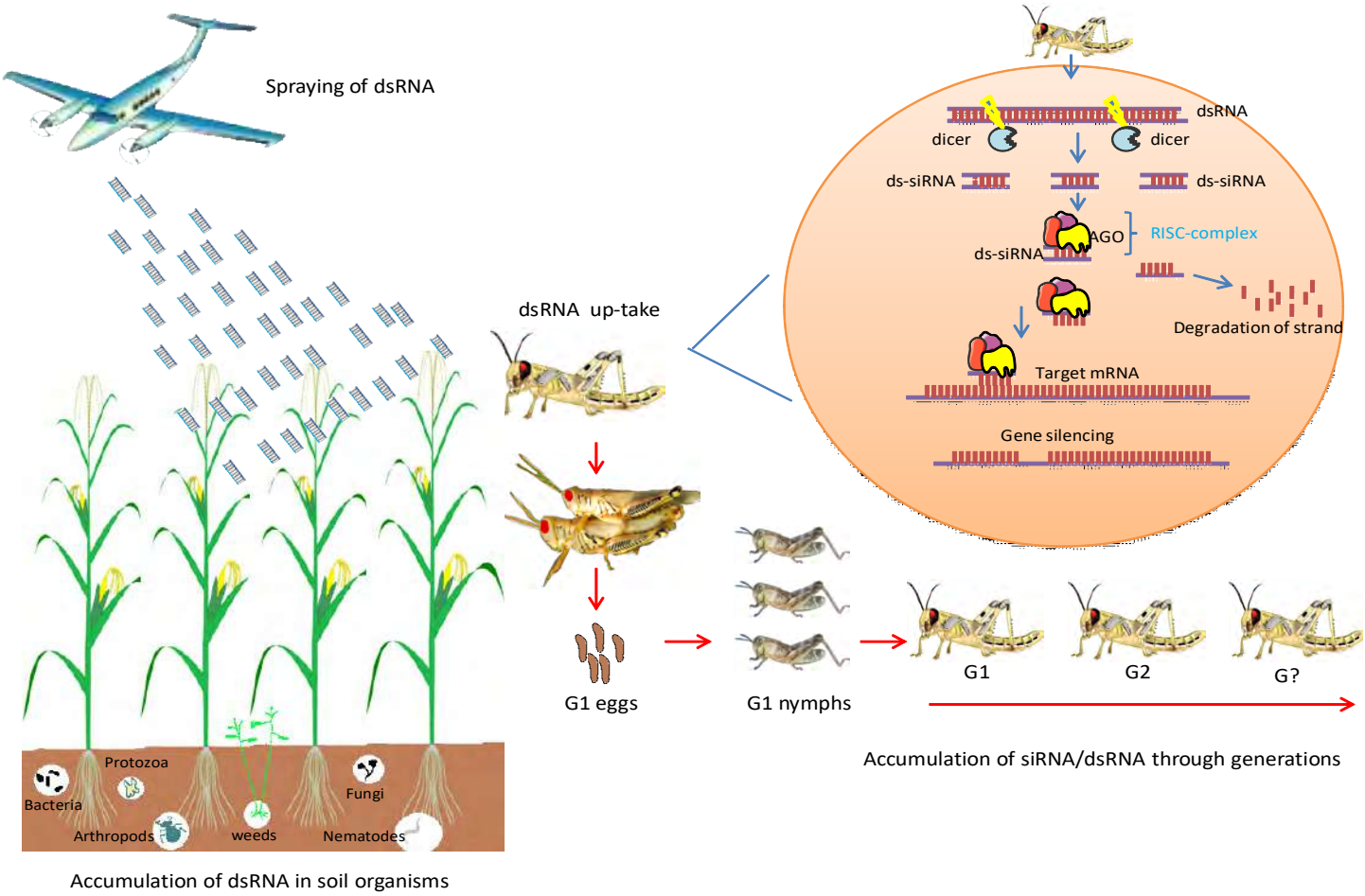
and nematode resistance (Huang *et al*. 2006; Yadav *et al*. 2006; Baum *et al*. 2007; Sindhu *et al*. 2009; Nowara *et al*. 2010; Koch *et al*. 2013; Abdellatef *et al*. 2015). However, HIGS technology has limited uses due to debate in using the transgenic plants in many countries, insufficient transformation protocols in many crop species (Wang *et al*. 2016). Recent studies have shown that Spray-induced gene silencing (SIGS) methods are very effective against insects, viruses and fungi, and they provide solid evidence that the fungi can devour external sRNAs/dsRNA. Foliar application of dsRNA under greenhouse conditions in potato *Solanum tuberosum* L., against the Colorado potato beetle acting gene provided increased resistance against this pest and the resistance lasted for almost a month (San Miguel and Scott 2016). Wang *et al*. 2016 showed that applying sRNAs or dsRNAs that target the necrotrophic fungus, *Botrytis cinerea Dicer-Like1* and *Dicer-like2* genes on the surface of fruits, vegetables and flowers significantly inhibited growth of grey mould disease. This study confirmed that the fungal pathogen *B. cinerea* was capable of taking up external sRNAs and long dsRNAs. In another study using tobacco, *Nicotiana tabacum* L. cv. Xanthi, foliar application of dsRNA targeting tobacco mosaic virus (TMV) p126 (silencing suppressor) and coat protein genes, resulted in 50–65 percent resistance to this virus (Konakalla et al. 2016). Also, Koch *et al*. 2016 demonstrated that a foliar spray of dsRNA (791 nt *CYP3*) on barley leaves, targeting the *Fusarium graminearum* ergosterol biosynthesis genes (*CYP51A*, *CYP51B*, *CYP51C*), inhibited *F. graminearum* growth in the local (directly sprayed) as well as distal the (non-sprayed) parts of barley detached leaves. Unexpectedly, efficient spray-induced control of fungal infections in the distal tissue involved passage of *CYP3*-dsRNA via the plant vascular system and processing into small interfering (si)RNAs by fungal DICER-LIKE 1 (*FgDCL-1*) after uptake by the pathogen.

**Vision 1. Fate of siRNA/dsRNA in soil and soil living organisms**

Implementation of dsRNA spraying in fields can lead to accumulation of s dsRNA in soil as well as uptake by soil organisms such as nematode, fungi and bacteria. Dubelman *et al*. (2014) reported results from a laboratory soil degradation study to determine the biodegradation potential of a DvSnf7 dsRNA transcript derived from a Monsanto genetically modified (GM) maize product that confers resistance to corn rootworm (CRW; Diabrotica spp.). Results from this study indicated that DvSnf7 RNA was degraded and biological activity was undetectable within approximately 2 days after application to soil, regardless of texture, pH, clay content and other soil differences. Furthermore, soil-incorporated DvSnf7 RNA was non-detectable in soil after 48 h, as measured by QuantiGene, at levels ranging more than two orders of magnitude (0.3, 1.5, 7.5 and 37.5 mg RNA/g soil). Results from this study indicated that the DvSnf7 dsRNA was unlikely to persist or accumulate in the soil. However, the accumulation of dsRNA in soil microorganisms is unknown, despite the results of a recent study showing that fungi are capable of taking up external sRNAs and long dsRNAs (Wang *et al*. 2016). Additional studies have reported that the nematode, *Caenorhabditis elegans*, can take up dsRNAs from the environment (Feinberg and Hunter 2003; Winston *et al*. 2007; Whangbo and Hunter 2008; McEwan *et al*. 2012) as well as some herbivores with longer than 50–60 bp, but not sRNA (Bolognesi *et al*. 2012; Ivashuta *et al*. 2015), deep studies must be conducted to follow the dsRNA uptake by soil organisms and investigate the genomic modifications that may occur (Scheme1). Such studies can be carried out using g a green fluorescent protein (GFP)-derived dsRNA (dsRNA-GFP) as control dsRNA to guide experimental filed to examine the permanence of dsRNA in living organisms such nematode, fungi, bacteria and parasitic weeds, as well as investigating the long term rotation of dsRNA between plants and pathogens, corresponding with theories of plant-pathogen interaction on level of miRNAs and siRNAs. Exposure to different dsRNA types in same the generation must be well described.

**Vision 2. Long lasting gene silencing**

The uptake of several dsRNAs targeting different genes of diverse sequence and processing into 21 nt siRNAs by insects RNAi machinery raises serious questions as to whether these thousands of siRNAs are capable of regulating gene expression in insects. A previous study reported the trangenerational silencing phenomena in grain aphid *Sitobion avenae* when it fed on transgenic barley expressing salivary sheath protein (*shp*-dsRNA) correlated with morphological and physiological aberrations such as winged adults and delayed maturation which maintained over seven aphid generations feeding on wild type plants. (Abdellatef *et al*. 2015). In the same study, permanent silencing has been reported and *shp* expression remained low when aphids were transferred from transgenic plants and fed for 1 or 2 weeks on wild-type plants, confirming that silencing had a prolonged impact (Abdellatef *et al*. 2015). Similar results were found by Coleman *et al*. 2015, who reported the impact of RNAi on over three generations of green peach aphids *Myzus persicae*. This study revealed that aphids reared on dsMpC002 transgenic plants experienced a 60% decline in aphid reproduction. Since transgenerational gene silencing in some insects may cause DNA sequence change (mutation), more studies are required to figure out the accumulation of dsRNA/siRNA through generations in




Scheme 1: Predictable effect of dsRNA spraying on the long-term-insect transcriptome and soil living organisms' behavior.

The invasive insects take up the dsRNA directly or the plant-derived long dsRNA by sucking or chewing. In the insects cells dsRNA process into 21 nt long siRNAs by the dicer (RNAse III enzyme). Produced-siRNAs binds the RNAi inducing silencing complex (RISC). The guide strand of siRNAs helps RISC to target the corresponding mRNA, leading to gene silencing and no protein expression. The siRNA silencing signals can remain for several generations.

different insect species as well as exposure of these insects to different dsRNA packages through their life cycle. Also, to date there are no reports indicating the accumulation of dsRNA/siRNA in fungi spores and parasitic plants (Scheme 1). Additionally, precautionary studies are needed to be conducted to follow-up on dsRNA/siRNA accumulation in insects and predators such as ladybird beetles, spiders and lacewings.

**Vision 3. Accumulation of siRNA/dsRNA in seeds and fruits**

RNA uptake through diet and its possible impact on the consuming animals is an intriguing phenomenon and highly discussed topic of interaction with the environment (Witwer and Hirschi 2014). A pilot study demonstrated that plant small RNAs acquired orally through food intake directly influences gene expression in animals after migration through the plasma and delivery to specific organs. (Vaucheret and Chupeau, 2012).

Several studies have elucidated systemic spread of siRNA signals in plants. Six-year-old citrus trees and grapevines trees were exposed to the dsRNA either by root drenching, foliar spray and trunk injections. The dsRNA could be detected for 7 weeks post a single exposure using 2 g of dsRNA in 15 L of water (Hunter et al. 2012). This study also showed that the Asian citrus psyllid, *Diaphorina citri*, potato psyllid, *Bactericera cockerelli* and the glassy-winged sharpshooter, *Homalodisca vitripennis*, took up the dsRNA after feeding on citrus trees previously treated with dsRNA.



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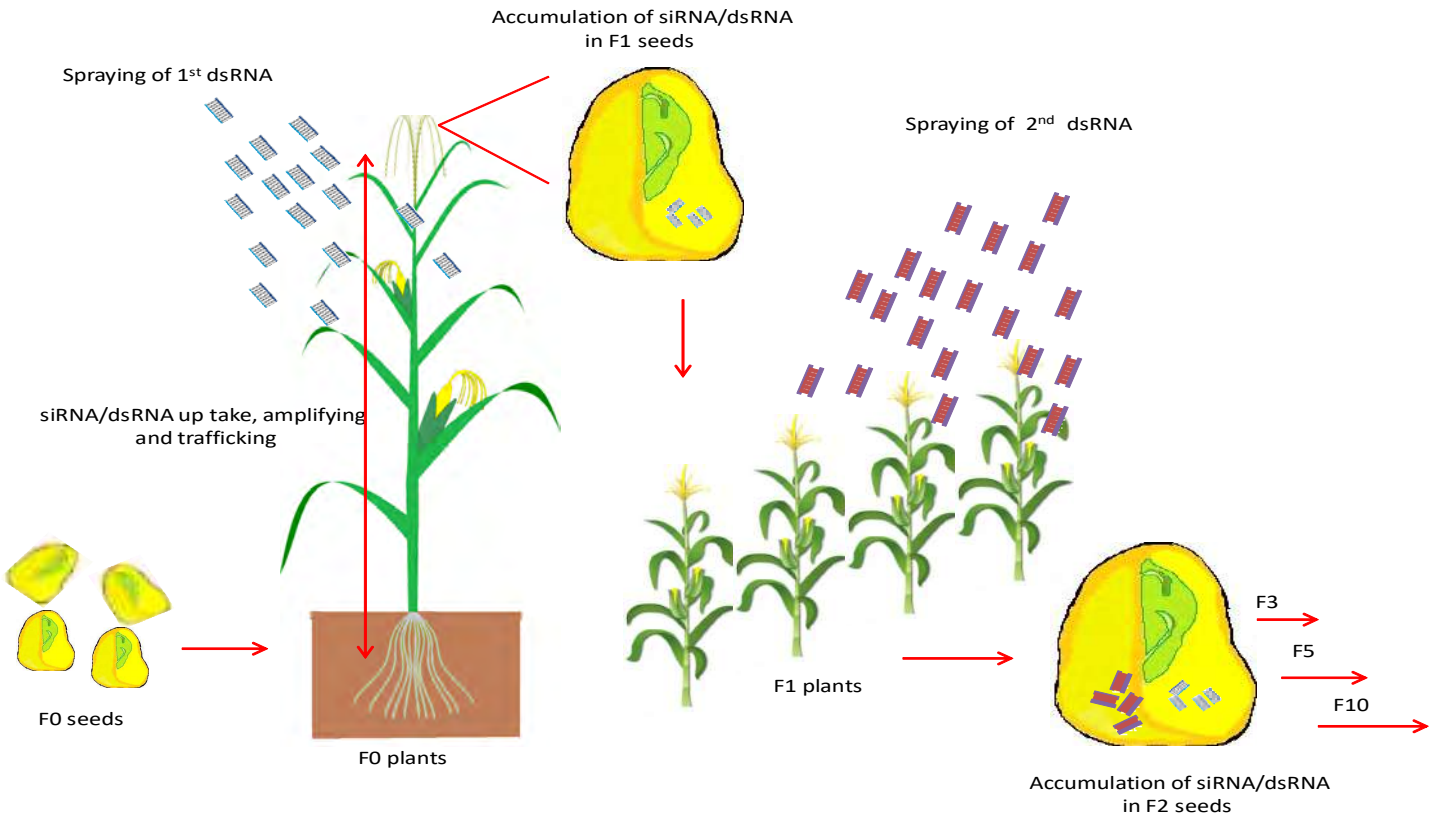


Scheme 2: Predictable model illustrating effect of dsRNA spraying on seeds genomic modifications.

After foliar application of dsRNA, plants accumulate the Ex-dsRNA and the dsRNA/siRNA signals in plants can spread systemically through the vascular tissue system. Accumulation of siRNA due to applicable different dsRNA may lead to phenotypic variation in plants from generation to other generation, expansion of siRNA could produce preferable mutations or modify the genes functions as the epigenetic modifications in plants can be directed and mediated by sRNAs.

The dsRNA moved through the vascular system of the citrus trees and the dsRNA was taken up, for instance by *psyllids* which fed on the phloem. Moreover, the dsRNA was identified in *psyllids* and leafhoppers 5-8 days post ingestion from plants, while in treated citrus tissues, dsRNA was found up to 57 days post treatment (Hunter *et al*. 2012). Microinjection studies in pumpkin *Cucurbita maxima* provided direct evidence that phloem small RNA binding protein1 (CmPSRP1) could mediate the trafficking among cells of 25 nt single stranded RNA (siRNA), but not double stranded RNA (dsRNA) (Yoo *et al*. 2004). Systemic spread of the silencing signal to the adjacent leaves within an hour and the presence of dsRNAs up to 9 days post-application have been documented (Konakalla *et al*. 2016). Also efficient spray-induced control of *Fusarium graminearum* in the distal tissue involved transport of CYP3-dsRNA via the plant vascular system and processing into siRNAs by FgDCL-1 after uptake by the fungi have been described (Koch *et al*. 2016).

sRNAs are now known to be a core component of a signaling network that mediates epigenetic modifications in plants (Simon and Meyers 2010). Epigenetic regulation can be mediated through a dynamic interplay between sRNAs, DNA methylation, and histone modifications, which together modulate transcriptional silencing of DNA. Regulatory sRNAs are short (20–24), noncoding RNAs produced through the RNA interference (RNAi) pathway that involves the plant specific DNA-dependent RNA polymerases Pol IV and Pol V (Zhang *et al*. 2007; Wierzbicki *et al*. 2008). Overall, the examples illustrated above suggest that siRNA/dsRNA could accumulate in the seeds, and exposure to different dsRNA doses may lead to genomic modifications in seed as well as the plant, implying that

long term experiments are needed to investigate the remains of siRNA/dsRNA in seeds for several generations (Scheme2).

## CONCLUSION

Application of SIGS has been presented as a powerful technique to replace GM crops. Implementing this method will be accompanied by environmental risk assessments which will consider the potential for harmful impacts to non target organisms; particularly the fate of sprayed dsRNA in the environment have also been questioned. Our current knowledge of the sensibility of organisms to exposure to various doses of dsRNA as well as the circumstance which affect the probability of off-target gene effects, are not completely understood. Additional research directing these areas is required to improve the certainty associated with risk assessments of dsRNA applications. In this vision statement, the possible incidence of dsRNA in the environment health will certainly become an explosive field of investigation. This vision implies precise awareness from biotechnologists who intend to make use of dsRNA, especially in the field of plant protection against pests.

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# Influence of stages of fruit maturity on germination, growth and vigour of papaya (cv. Solo) seedlings

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**ABSTRACT**

Investigations carried out at Department of Horticulture, College of Agriculture, Dharwad during the year 2015-16, to assess the influence of different stages of fruit maturity on germination, growth and vigour of papaya (cv. Solo) seedling, revealed that the harvesting of fruit at 3/4 ripe stage significantly increased the germination percentage (48.00%), dry weight of seedling (8.50 mg) and vigour index (1179.55) as compared to fruit harvested at other stages of maturity. Fruit at 3/4 ripe stage also recorded highest 100 seed weight.

**Key words:** *Papaya, stage of maturity, germination, vigour index*

Papaya (*Carica papaya* L.) belonging to the family Caricaceae is native to Mexico in tropical America. It is an evergreen commercial fruit crop of tropical and subtropical regions. The genus *Carica* has about 48 species but only *Carica papaya* is grown for its edible fruits. It is one among the fruits that has attained a great popularity in recent years because of its easy cultivation, quick returns, adoptability to diverse soil and climatic conditions and above all its attractive delicious wholesome fruits having multifarious uses. The ripe fruits of papaya are used for table purpose, raw fruits are cooked and used as vegetable and immature fruits are used for extraction of papain. Papaya has occupied a unique place in the diet of people worldwide because of its striking nutritional and medicinal values.

Propagation by seed is the universally followed method of its multiplication. Though this method does not safeguard the purity of the progeny, it is inevitable because of the absence of any commercially feasible vegetative propagation techniques. With the commercialization of papaya cultivation, the demand for quality seeds of well established varieties has increased. Few attempts have been made in the past to develop effective methods of producing quality seeds. The level of fruit maturity determines the physiological maturity and affects both germination and vigour of the seed. Proper seed germination and seedling growth are most important considerations in successful production under nursery technique of papaya cultivation. With this in view, the influence of stages of fruit maturity on germination, growth and vigour of papaya seedling was assessed.

## MATERIAL AND METHODS

The investigation was carried out at Department of Horticulture, College of Agriculture, Dharwad during the year 2015-16. The experiment carried out in completely randomised design with four replications had five treatments i.e., $T_1$ : Mature green, $T_2$ : 1/4 ripe (1/4 of skin turn yellow), $T_3$ : 1/2 ripe (1/2 of skin turn yellow), $T_4$ : 3/4 ripe (3/4 of skin turn yellow) and $T_5$ : full ripe (skin completely turned yellow). Each treatment in every replication consisted of 50 seeds. Robust trees were selected from established orchard and flower were tagged on the day of fertilization (fruit set). Fruits of uniform size were randomly harvested at different maturity levels to get mature green and different ripening stages as suggested by Hittalmani (1986). Three fruits of uniform shape and size were harvested in each maturity stage. The fruits were cut into two halves longitudinally. Seeds were scooped out of the fruit using a stainless steel spoon. These were subjected to fermentation for one day by adding little amount of water. The sarcotesta was removed by rubbing the seeds with coarse cotton cloth and later washed thoroughly in running water. The chaffy seeds floating in water were removed and the sinkers were dried in shade by spreading over nylon net in thin layer till they attained 8-10 per cent moisture content (Yogeesha *et al*., 2013). Standard germination test was conducted with 50 seeds for each treatment and in each replication by using the Between paper (BP) method at a constant temperature of 25°C. The first count on 20$^{th}$ and final count on 30$^{th}$ day were recorded for normal seedling. The total germination was calculated

and expressed in percentage as prescribed by ISTA Rules (Anon, 1999). The shoot and root length were measured. The seedling dry weight was also recorded. The seedling vigour index was calculated. The data were analysed by following the procedure outlined by Panse and Sukhatme (1967).

## RESULTS AND DISCUSSION

The investigation reveals a significant influence of stage of fruit maturity on seed quality parameters of papaya such as per cent germination (Table 1), root length, seedling dry weight and vigour index (Table 2).

The data on to 100 seed weight as influenced by different stages of fruit maturity are presented in Table 1. No significant difference was found among the treatments. However, highest 100 seed weight was recorded in $T_4$ (1.85 g) followed by $T_5$ (1.68 g) and lowest was in $T_1$ (1.58 g). This indicated that seeds attained the maximum dry matter at 3/4 ripe stage and remain constant thereafter.

The perusal of data (Table 1) reveals that influence of stage of fruit maturity on per cent germination was significant. In case of first count, highest germination percentage was recorded in $T_4$ (32.00%), which was significantly superior over others treatments followed by $T_3$ (22.00%) and lowest in $T_1$ (6.50). There was linear increase in the germination percentage among the all treatments towards the final count. In case final count, higher values for germination percentage was recorded in $T_4$ (48.00%), which was significantly superior over other treatments followed by $T_3$ (34.00%) and least was observed in $T_1$ (10.50%). The highest germination percentage was recorded in 3/4 ripe fruit as compared to mature fruit this may be due to more accumulation of dry matter in seeds of 3/4 ripe fruit.

Table 1. Influence of stage of fruit maturity on seed weight and germination in papaya (cv. Solo)

| Treatment | 100 Seed weight (g) (Test weight) | Germination (%) | |
|---|---|---|---|
| | | First count | Final count |
| $T_1$ : Mature green | 1.58 | 6.50 (14.74)* | 10.50 (18.89)* |
| $T_2$ : ¼ Ripe | 1.64 | 15.00 (22.63) | 22.00 (27.80) |
| $T_3$ : ½ Ripe | 1.68 | 22.00 (27.90) | 34.00 (35.63) |
| $T_4$ : ¾ Ripe | 1.85 | 32.00 (34.42) | 48.00 (43.83) |
| $T_5$ : Full Ripe | 1.68 | 15.50 (23.15) | 24.50 (29.63) |
| S.E.m± | 0.14 | 1.10 | 1.23 |
| CD (1%) | NS | 4.60 | 5.12 |

* Values in the parenthesis indicate arcsine transformed value
NS – Non significant

Table 2. Influence of stage of fruit maturity on seedling growth in papaya (cv. Solo)

| Treatment | Shoot length (cm) | Root length (cm) | Dry weight of seedling (mg) | Vigour index |
|---|---|---|---|---|
| $T_1$ : Mature green | 13.00 | 9.90 | 6.98 | 240.75 |
| $T_2$ : ¼ Ripe | 13.73 | 11.88 | 7.65 | 558.60 |
| $T_3$ : ½ Ripe | 13.83 | 9.78 | 7.88 | 797.85 |
| $T_4$ : ¾ Ripe | 13.85 | 10.73 | 8.50 | 1179.55 |
| $T_5$ : Full Ripe | 14.38 | 9.70 | 7.58 | 588.25 |
| S.E.m± | 0.38 | 0.40 | 0.24 | 40.45 |
| CD (1%) | NS | 1.68 | 1.02 | 168.56 |

The data pertaining to shoot length as influenced by different stages of fruit maturity are presented in Table 2. No significant difference among the treatments was found. However, the highest shoot length was recorded in $T_5$ (14.38 cm) followed by $T_4$ (13.85 cm) and the lowest was recorded in $T_1$ (13.00 cm). The data regarding the root length as affected by different stages of fruit maturity are found to be significant. The highest root length was recorded in $T_2$ (11.88 cm), which was significantly superior over other treatments and lowest was recorded in $T_5$ (9.70cm) which was on par with $T_1$ (9.90 cm), $T_3$ (9.78 cm) and $T_4$ (10.73 cm).

The data regarding the seedling dry weight as influenced by different stages of fruit maturity (Table 2) was found to be significant. The highest seedling dry weight was recorded in $T_4$ (8.50 mg) which was on par with $T_2$ (7.65 mg), $T_3$ (7.88 mg) and $T_5$ (7.58 mg) and lowest in $T_1$ (6.98 mg).

The data pertaining to vigour index as influenced by different stages of fruit maturity presented (Table 2) was found significant. The highest vigour index was recorded in $T_4$ (1179.55), which was significantly superior over the other treatments followed by $T_3$ (797.85) and lowest was recorded in $T_1$ (240.75).

The appropriate stage of fruit harvest based on the physiological maturity of fruit may enable the seed to produce vigorous seedlings as those seed might have sufficient food material. These corroborate the findings of Sangakkara (1995) and Yogeesha *et al*. (2013) in Papaya.

## CONCLUSION

This study showed the importance of stage of fruit maturity for obtaining good quality of papaya seeds. The seeds attaining maximum seed weight at 3/4 ripe stage could be considered as the stage of physiological maturity and produce vigorous seedlings.

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# Response of mesta (*Hibiscus* spp.) genotypes to stages of harvesting under rainfed conditions

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**ABSTRACT**

The field experiment, conducted to find out the optimum stage of harvesting of different mesta varieties for obtaining higher yield of quality fibre for three consecutive rainy seasons at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, showed that mesta crop harvested at maturity gave significantly higher fibre and seed yields compared to those harvested at 20, 30 and 40 days of flowering. For only the fibre purpose, the crop can be harvested at 50 per cent flowering stage. This however gave lower fibre yield than that harvested at maturity. It is advised to harvest the crop at 120-130 days after sowing when, the pod development sets in to get higher fibre yield and fibre equivalent yield.

**Key words:** Fibre yield, Genotypes, Mesta, Stage of harvest

Mesta (*Hibiscus* spp.), an important annual fibre crops next to jute has long been recognized as a source of cordage fibre used in making rope, twine, carpet backing and burlap. In India, it presently plays an important role in supplementing the short supply of jute in jute industry to manufacture various jute goods. The productivity of this crop is high in jute belt of northeastern states compared to non-traditional areas. The major constraints in productivity of mesta appear to be non-availability of improved varieties and lack of suitable crop management practices. Though the crop is well suited and adapted to the northeastern parts of the country, it is capable of growing under much wider range of soil and climate conditions due to its hardiness and less exacting nature in the edaphic requirements. Hence, there is a scope to extend its cultivation commercially in the non-traditional areas having much drier climatic conditions. The productive efficiency of a crop though depends on its genetic potential; its yield could be improved to a perceptible magnitude through suitable agronomic practices. Keeping this in view, the study to find out the optimum stage of harvest for higher fibre yield and quality of mesta under rainfed conditions was undertaken.

## MATERIALS AND METHODS

The field experiment was conducted for three consecutive rainy seasons at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The experiment comprised of 12 treatment combinations having four stages of harvest (20 days after flowering, 30 days after flowering, 40 days after flowering and at maturity) and three genotypes *viz*., AS 73-CP-560, AMV-3 and AMV-4 belonging to *Hibiscus sabdariffa*. The experiment was laid out in split-plot design with the stage of harvesting as main plots and genotypes as subplots with three replications. The gross and net plot sizes of the experiment were 5.0 x 3.0 m and 5.0 x 2.4 m, respectively. The crop was sown at a spacing of 30 x 10 cm and fertilized with 40:40:20 kg N:$P_2O_5$:$K_2O$/ha. All the cultural and plant protection measures were adopted as per the state recommendations. The soil of the experimental site was medium-deep black with a soil pH of 7.8 and organic carbon content of 0.56 per cent. The available nitrogen, $P_2O_5$ and $K_2O$ were 219, 22 and 308 kg/ha, respectively. The soil was having 9.1 per cent fine sand, 15.0 per cent silt and 40.0 per cent clay with 14.8 % permanent wilting point, 33.8 per cent field capacity and 1.46 g $cc^{-1}$ bulk density. A total rainfall 269.6, 394.1 and 178.0 mm was received from January to December in 29, 29 and 23-rainy days during the first, second and third year, respectively as against the normal rainfall of 713.9 mm of last 52 years.

## RESULTS AND DISCUSSION

### Varietal performance

The pooled data showed that variety AS-73-CP-560 recorded significantly higher fibre yield (992 kg $ha^{-1}$) as compared to AMV-3 (892 kg $ha^{-1}$), however, it was on par

with AMV-4 (961 kg ha$^{-1}$). AMV-4, in turn, did not differ significantly with AMV-3 (Table 4). The results corroborate the findings of Guggari and Sheelavantar (2004b) who also noticed significantly higher yield of AS-73-CP-560 over AMV-3 (23.1%) and local cultivar (67.0%) under dryland conditions. Higher fibre yield of AS-73-CP-560 is attributed to its significantly higher plant height and fresh weight of stem compared to AMV-3 and AMV-4.

The plant height in AS-73-CP-560 was significantly higher (168.0 cm) as compared to AMV-3 (161.3 cm) and AMV-4 (163.4 m). Further, AMV-4 and AMV-3 were on par with each other (Table 1). A similar trend was observed during the first and third year, while during the second year all the varieties were on par with each other. Similarly, AS-73-CP-560 recorded significantly higher fresh weight of stem (20.50 t ha$^{-1}$) as compared to AMV-3 (18.23 t ha$^{-1}$) and AMV-4 (18.68 t ha$^{-1}$) genotypes, however, the later genotypes were on par with each other. The individual year's data indicated a similar trend except during the first year wherein AMV-4 was on par with AS-73-CP-560 but significantly superior over AMV-3 during the third year.

Varieties differed with respect to growth parameters and total dry matter production under rainfed conditions at MARS, UAS, Bangaluru. Higher dry matter production and accumulation in reproductive parts was observed in HS-108 variety as compared to AMV 4 (Pushpa *et.al*., 2013). Similarly, according to Mehadi Hassan (2018), the Tossa Jute variety BJC-7370 had more efficient on the whole growth, yield and yield attributing traits. BJC-7370 or Kenaf variety HC-3 would be most suitable variety under the Agroecological Zone-13 or the climatic or soil (regional) condition of Southern Part of Bangladesh.

The stem diameter and dry weight of stem (Table 2) did not differ significantly with respect to varieties with pooled and as well as individual years data of second and third year. However, during second year, AS-73-CP-560 recorded significantly higher dry weight of stem as compared to AMV-3 and it was on par with AMV-4.

The pooled data showed that the variety AS-73-CP-560 recorded significantly higher seed yield (553 kg ha$^{-1}$) as compared to AMV-3 (452 kg ha$^{-1}$) and was on par with AMV-4 (520 kg ha$^{-1}$). A similar trend was observed during the first and third year except during second year where in both AS-73-CP-560 and AMV-4 were superior over AMV-3 (Table 4). Significantly higher seed yield of AS-73-CP-560 may be attributed higher of capsules per plant and 1000-seed weight

Table 1. Plant height and stem diameter of mesta as influenced by varieties and stage of harvesting

| Treatment details | Plant height at harvest (cm) | | | | Stem diameter (cm) at harvest | | | |
|---|---|---|---|---|---|---|---|---|
| | First year | Second year | Third year | Pooled | First year | Second year | Third year | Pooled |
| **Varieties (V)** | | | | | | | | |
| AS-73-CP-560 ($V_1$) | 198.4 | 186.9 | 118.8 | 168.0 | 1.58 | 1.38 | 0.86 | 1.27 |
| AMV-3 ($V_2$) | 190.7 | 181.5 | 111.7 | 161.3 | 1.57 | 1.34 | 0.83 | 1.24 |
| AMV-4 ($V_3$) | 192.5 | 183.5 | 114.1 | 163.4 | 1.57 | 1.37 | 0.85 | 1.26 |
| **LSD (0.05)** | **2.09** | **NS** | **2.69** | **4.07** | **NS** | **NS** | **NS** | **NS** |
| **Stage of harvesting (S)** | | | | | | | | |
| 20days after flowering ($S_1$) | 185.9 | 178.4 | 108.5 | 157.6 | 1.55 | 1.35 | 0.82 | 1.24 |
| 30days after flowering($S_2$) | 195.7 | 181.9 | 114.1 | 163.9 | 1.57 | 1.36 | 0.83 | 1.25 |
| 40days after flowering ($S_3$) | 196.5 | 186.3 | 117.3 | 166.7 | 1.58 | 1.37 | 0.87 | 1.27 |
| At maturity ($S_4$) | 197.3 | 189.2 | 119.5 | 168.7 | 1.59 | 1.38 | 0.87 | 1.28 |
| **LSD (0.05)** | **5.78** | **4.32** | **5.28** | **4.70** | **NS** | **NS** | **NS** | **NS** |
| **Interaction (VxS)** | | | | | | | | |
| $V_1S_1$ | 189.7 | 181.9 | 116.0 | 162.5 | 1.57 | 1.35 | 0.84 | 1.25 |
| $V_1S_2$ | 199.7 | 184.4 | 118.5 | 167.5 | 1.57 | 1.38 | 0.85 | 1.26 |
| $V_1S_3$ | 200.9 | 188.5 | 120.1 | 159.8 | 1.59\ | 1.38\ | 0.88 | 1.28 |
| $V_1S_4$ | 203.0 | 192.7 | 120.6 | 172.1 | 1.60 | 1.40 | 0.89 | 1.29 |
| $V_2S_1$ | 183.1 | 175.5 | 103.5 | 154.0 | 1.55 | 1.33 | 0.80 | 1.22 |
| $V_2S_2$ | 192.4 | 179.3 | 110.8 | 160.0 | 1.57 | 1.33 | 0.82 | 1.24 |
| $V_2S_3$ | 193.4 | 184.1 | 114.1 | 163.9 | 1.58 | 1.34 | 0.85 | 1.25 |
| $V_2S_4$ | 193.8 | 186.9 | 118.3 | 166.3 | 1.56 | 1.34 | 0.86 | 1.25 |
| $V_3S_1$ | 184.9 | 177.8 | 106.1 | 156.3 | 1.54 | 1.36 | 0.82 | 1.24 |
| $V_3S_2$ | 195.0 | 181.8 | 113.1 | 163.3 | 1.57 | 1.36 | 0.84 | 1.25 |
| $V_3S_3$ | 195.1 | 186.3 | 117.6 | 166.3 | 1.58 | 1.38 | 0.88 | 1.28 |
| $V_3S_4$ | 195.1 | 188.1 | 119.7 | 167.7 | 1.60 | 1.39 | 0.88 | 1.29 |
| **LSD (0.05)** | **NS** | **1.84** | **9.15** | **8.14** | **NS** | **NS** | **NS** | **NS** |

NS: Non-significant



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over AMV-3 and AMV-4. The pooled data showed that AS-73-CP-560 recorded significantly higher number of capsules per plant (16.66) over AMV-3 (14.82) and AMV-4 (15.61) and the later genotypes were on par with each other (Table 3). Similarly, the variety AS-73-CP-560 recorded significantly higher 1000- seed weight (22.13 g) over AMV-3 (21.00 g) and was on par with AMV-4 (21.56 g) with pooled data.

The data showed that varieties did not differ significantly with respect to fibre equivalent yield. However, the individual year's data showed that AS-73-CP-560 recorded significantly higher fibre equivalent yield as compared to AMV-3, however it was par with AMV-4 during the first and third year. Similarly, varieties differed with respect to fiber yield and quality (Pushpa, 2009). During the second year, varieties did not differ significantly with each other (Table 5).

**Stage of harvesting**

Pooled data of three years (Table 4) showed that fibre yield was significantly higher when the crop was harvested at maturity (1139 kg ha$^{-1}$) as compared to harvesting at 20 days (741 kg ha$^{-1}$) and 30 days after flowering (881 kg ha$^{-1}$), however, it was on par with crop harvested at 40 days after flowering (1039 kg ha$^{-1}$). Higher fibre yield of mesta from the crop harvested at maturity or 40 days after flowering is attributed to significantly higher plant height and fresh weight of stem. Guggari and Sheelavantar (2004a) also reported a close and linear relationship between the total fresh weight of stem and fibre yield (r=+0.99**). Results also corroborate the findings of Pathak *et al.* (1989), Krishnamurthy *et. al.* (1994) and Naidu *et. al.* (1996b). The plant height was significantly higher when the crop was harvested at maturity (168.7 cm) as compared to harvesting at 20 days after flowering (157.6 cm), however, it was on par with harvesting at 30 and 40 days after flowering (163.9 and 166.7 cm, respectively). Whereas, significantly lower plant height was noticed when the crop was harvested at 20 days after flowering. With respect to fresh weight of stem, pooled data showed that harvesting at 40 days after flowering (17.55 t ha$^{-1}$) and maturity (17.44 t ha$^{-1}$) were on par recorded significantly lower fresh weight of stem as compared to harvesting at 20 and 30 days after flowering (21.25 and 20.30 t ha$^{-1}$, respectively) which were on par with each other. Similar results were reported by Naidu *et al.* (1996a) who reported significantly higher green matter yield when the crop was harvested at 50 per cent flowering. Higher fresh weight of stem when the crop was harvested at 20 and 30 days after flowering did not bring significant improvement

Table 2. Fresh and dry weight of mesta varieties as influenced by stage of harvesting

| Treatment details | Fresh weight of stem (t/ha) | | | | Dry weight of stem (t/ha) | | | |
|---|---|---|---|---|---|---|---|---|
| | First year | Second year | Third year | Pooled | First year | Second year | Third year | Pooled |
| **Varieties (V)** | | | | | | | | |
| AS-73-CP-560 ($V_1$) | 22.5 | 31.9 | 7.02 | 20.50 | 4.39 | 7.48 | 1.73 | 4.53 |
| AMV-3 ($V_2$) | 19.6 | 29.5 | 5.60 | 18.23 | 3.92 | 7.23 | 1.62 | 4.25 |
| AMV-4 ($V_3$) | 21.5 | 28.5 | 6.06 | 18.68 | 4.19 | 7.47 | 1.73 | 4.46 |
| **LSD (0.05)** | **1.32** | **2.64** | **0.43** | **1.48** | **0.34** | **NS** | **NS** | **NS** |
| **Stage of harvesting (S)** | | | | | | | | |
| 20 days after flowering ($S_1$) | 19.1 | 37.0 | 7.62 | 21.25 | 3.22 | 7.67 | 1.90 | 4.26 |
| 30 days after flowering ($S_2$) | 21.0 | 33.5 | 6.35 | 20.30 | 3.71 | 7.52 | 1.63 | 4.29 |
| 40 days after flowering ($S_3$) | 21.7 | 25.1 | 5.88 | 17.55 | 4.66 | 7.50 | 1.64 | 4.59 |
| At maturity ($S_4$) | 23.0 | 24.3 | 5.05 | 17.44 | 5.08 | 6.88 | 1.59 | 4.51 |
| **LSD (0.05)** | **1.02** | **2.93** | **0.89** | **1.71** | **0.29** | **NS** | **NS** | **NS** |
| **Interaction (VxS)** | | | | | | | | |
| $V_1S_1$ | 20.0 | 40.4 | 8.26 | 22.87 | 3.37 | 7.78 | 1.98 | 4.37 |
| $V_1S_2$ | 22.3 | 37.0 | 6.93 | 22.09 | 3.97 | 7.59 | 1.66 | 4.41 |
| $V_1S_3$ | 23.2 | 25.9 | 6.63 | 18.59 | 4.93 | 7.51 | 1.66 | 4.70 |
| $V_1S_4$ | 24.6 | 24.4 | 6.27 | 18.43 | 5.27 | 7.04 | 1.61 | 4.64 |
| $V_2S_1$ | 18.1 | 37.4 | 7.12 | 20.87 | 3.01 | 7.59 | 1.77 | 4.12 |
| $V_2S_2$ | 19.1 | 31.9 | 5.85 | 18.95 | 3.45 | 7.41 | 1.62 | 4.16 |
| $V_2S_3$ | 19.9 | 24.5 | 5.08 | 16.49 | 4.27 | 7.33 | 1.59 | 4.39 |
| $V_2S_4$ | 21.4 | 24.1 | 4.37 | 16.60 | 4.95 | 6.59 | 1.51 | 4.35 |
| $V_3S_1$ | 19.2 | 33.3 | 7.50 | 20.02 | 3.27 | 7.64 | 1.95 | 4.28 |
| $V_3S_2$ | 21.7 | 31.5 | 6.29 | 19.85 | 3.71 | 7.57 | 1.65 | 4.31 |
| $V_3S_3$ | 22.0 | 24.8 | 5.92 | 17.58 | 4.77 | 7.67 | 1.65 | 4.69 |
| $V_3S_4$ | 23.0 | 24.3 | 4.51 | 17.27 | 5.02 | 7.01 | 1.66 | 4.56 |
| **LSD (0.05)** | **1.77** | **5.07** | **1.54** | **2.96** | **0.51** | **NS** | **NS** | **NS** |

NS: Non-significant

in fibre yield per hectare. It might be due to a greater amount of moisture content in the plant material and this resulted in a greater loss in weight after drying compared to the crop harvested at maturity. The studies carried out by Das and Maiti (1998) revealed that the best time of harvesting is the small pod stage for *H. cannabinus*, whereas for *H sabdariffa* it is at 50 per cent flowering stage.

Stem diameter did not differ significantly due to stages of harvest during the individual years as well as with pooled data (Table 2). Similarly, pooled data showed that the dry weight of stem did not differ significantly due to stages harvesting. A similar trend was observed during individual years except during the first year wherein harvesting at maturity recorded was significantly higher dry weight of stem.

Harvesting at maturity recorded significantly higher seed yield (1102 kg ha$^{-1}$) as compared to successive early stages of harvesting at 40, 30 and 20 days after flowering (990, 891 and 721 kg ha$^{-1}$, respectively) which noticed a significant reduction in seed yield. A similar trend was observed during individual year's data except during the first and third year wherein harvesting at 40 days after flowering and maturity were on par in seed yield. Significantly higher seed yield of mesta when the crop was harvested at maturity is attributed to a significantly higher number of capsules per plant and higher test weight. Pooled data showed that harvesting at maturity (18.03) and 40 days after flowering (16.94) were on par and recorded a significantly higher number of capsules per plant over harvesting at 20 and 30 days after flowering (12.92 and 14.90, respectively). A similar trend was observed with individual years data except during second year. wherein, harvesting at maturity recorded a significantly higher number of capsules per plant over the rest of the stages. The test weight did not differ significantly among the stage of harvesting at 30 and 40 days after flowering (21.49 and 21.99 g, respectively) and at maturity (22.46 g) whereas harvesting at 20 days after flowering noticed significantly lower test weight (20.30 g).

The pooled data showed that fibre equivalent yield (Table 5) was significantly higher when the crop was harvested at 40 days after flowering (1952 kg ha$^{-1}$) and attaining maturity (2148 kg ha$^{-1}$) as compared to harvesting at 20 and 30 days after flowering (741.4 and 881.1 kg ha$^{-1}$, respectively). A similar trend was observed with individual year's data except during second year wherein harvesting

Table 3. Number of capsules and 1000-seed weight of mesta varieties as influenced by stage of harvesting

| Treatment details | No. of capsules per plant at harvest | | | | 1000-seed weight (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | First year | Second year | Third year | Pooled | First year | Second year | Third year | Pooled |
| **Varieties (V)** | | | | | | | | |
| AS-73-CP-560 ($V_1$) | 32.9 | 11.7 | 5.43 | 16.66 | 22.1 | 22.0 | 22.26 | 22.13 |
| AMV-3 ($V_2$) | 29.7 | 9.8 | 4.90 | 14.82 | 20.8 | 20.8 | 21.44 | 21.00 |
| AMV-4 ($V_3$) | 31.5 | 10.3 | 5.06 | 15.61 | 21.6 | 21.2 | 21.92 | 21.56 |
| **LSD (0.05)** | **2.13** | **0.91** | **0.33** | **0.98** | **0.96** | **0.55** | **NS** | **0.91** |
| **Stage of harvesting (S)** | | | | | | | | |
| 20 days after flowering ($S_1$) | 24.4 | 10.1 | 4.26 | 12.92 | 19.6 | 20.5 | 20.82 | 20.30 |
| 30 days after flowering ($S_2$) | 29.8 | 10.0 | 4.87 | 14.90 | 21.9 | 21.3 | 21.31 | 21.49 |
| 40 days after flowering ($S_3$) | 34.8 | 10.6 | 5.43 | 16.94 | 22.2 | 21.7 | 22.14 | 21.99 |
| At maturity ($S_4$) | 36.5 | 11.6 | 5.95 | 18.03 | 22.3 | 21.8 | 23.21 | 22.46 |
| **LSD (0.05)** | **1.67** | **0.99** | **0.76** | **1.14** | **1.24** | **0.92** | **1.19** | **1.05** |
| **Interaction (VxS)** | | | | | | | | |
| $V_1S_1$ | 25.5 | 11.4 | 4.70 | 13.87 | 19.7 | 20.8 | 21.37 | 20.65 |
| $V_1S_2$ | 31.9 | 11.1 | 5.13 | 16.02 | 22.8 | 21.9 | 21.97 | 22.23 |
| $V_1S_3$ | 36.5 | 11.5 | 5.93 | 17.96 | 22.9 | 22.6 | 22.33 | 22.62 |
| $V_1S_4$ | 37.9 | 12.5 | 5.96 | 18.81 | 22.9 | 22.7 | 23.37 | 23.01 |
| $V_2S_1$ | 23.5 | 9.3 | 3.90 | 12.22 | 19.3 | 20.0 | 20.03 | 19.77 |
| $V_2S_2$ | 27.6 | 9.4 | 4.70 | 13.89 | 20.6 | 20.9 | 20.77 | 20.75 |
| $V_2S_3$ | 32.9 | 10.0 | 5.10 | 16.03 | 21.3 | 21.1 | 22.00 | 21.48 |
| $V_2S_4$ | 35.0 | 10.5 | 5.90 | 17.13 | 21.8 | 21.2 | 22.97 | 21.99 |
| $V_3S_1$ | 24.3 | 9.5 | 4.20 | 12.68 | 19.7 | 20.7 | 21.07 | 20.49 |
| $V_3S_2$ | 30.0 | 9.5 | 4.80 | 14.78 | 22.1 | 21.1 | 21.20 | 21.50 |
| $V_3S_3$ | 34.9 | 10.3 | 5.26 | 16.85 | 22.3 | 21.3 | 22.10 | 21.88 |
| $V_3S_4$ | 36.5 | 11.9 | 6.00 | 18.13 | 22.2 | 21.6 | 23.30 | 22.37 |
| **LSD (0.05)** | **4.16** | **1.72** | **1.32** | **1.97** | **2.14** | **1.60** | **2.07** | **1.82** |

NS: Non-significant



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at maturity was significantly superior over harvesting at 40 days after flowering. Significantly higher fibre equivalent yield of mesta when the crop was harvested at maturity is attributed to significantly higher fibre and seed yields.

**Interaction of varieties and stage of harvesting**

The interactions of varieties and stage of harvesting showed that AS-73-CP-560 and AMV-4 harvested at maturity recorded significantly higher fibre yield compared to other combinations except AS-73-CP-560 and AMV-4 harvested at 40 days after flowering and AMV-3 harvested at maturity were on par. All the varieties harvested at 20 and 30 days after flowering noticed significantly lower fibre yield. Higher fibre yield of AS-73-CP-560 harvested at maturity or 40 days after flowering may be attributed to higher plant height and dry weight of stem compared to other stages of harvesting (Table 4).

AS-73-CP-560 harvested at maturity recorded significantly higher plant height over the other combinations and all the varieties harvested at 20 days after flowering. This shows that the plant height irrespective of varieties, did not differ significantly with the stage of harvesting up to 30 days after flowering. Earlier to this the plant height decreased significantly. Similarly, the treatment combinations of AS-73-CP-560 and AMV-4 with harvesting at 40 days after flowering and maturity and AMV-3 with harvesting at maturity noticed significantly higher dry weight of stem as compared to other combinations.

AS-73-CP-560 harvested at 20 days after flowering recorded significantly higher fresh weight of stem as compared to all the varieties harvested at 40 days after flowering and maturity. All the varieties harvested at 20 and 30 days after flowering produced a significantly higher fresh weight of stem and were on par with each other except AMV-3 harvested at 30 days after flowering noticed the significantly lower fresh weight of stem.

The interaction of varieties and the stage of harvesting was significant. The interactions of AS-73-CP-560 variety harvested at maturity recorded significantly higher seed yield as compared to other combinations except AMV-4 and AMV-3 harvested at maturity and AS-73-CP-560 harvested at 40 days after flowering were on par with each other. This may be attributed to a higher number of capsules per plant and higher test weight of AS-73-CP-560 harvested at maturity or 40 days after flowering. The interaction of AS-73-CP-560 harvested at maturity recorded a significantly higher number of capsules per plant (18.81) over other combinations except

Table 4. Fibre and seed yield of mesta varieties as influenced by stage of harvesting

| Treatment details | Seed yield (kg ha$^{-1}$) | | | | Fibre yield (kg ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | First year | Second year | Third year | Pooled | First year | Second year | Third year | Pooled |
| **Varieties (V)** | | | | | | | | |
| AS-73-CP-560 ($V_1$) | 870 | 1517 | 553 | 980 | 1021 | 1636 | 319 | 992 |
| AMV-3 ($V_2$) | 764 | 1383 | 465 | 871 | 964 | 1441 | 272 | 892 |
| AMV-4 ($V_3$) | 827 | 1436 | 520 | 928 | 989 | 1585 | 308 | 961 |
| **LSD (0.05)** | **40** | **NS** | **65** | **74** | **38** | **161** | **NS** | **91** |
| **Stage of harvesting (S)** | | | | | | | | |
| 20 days after flowering ($S_1$) | 628 | 1063 | 470 | 721 | 778 | 1181 | 265 | 741 |
| 30 days after flowering ($S_2$) | 851 | 1329 | 493 | 891 | 992 | 1376 | 275 | 881 |
| 40 days after flowering ($S_3$) | 877 | 1563 | 530 | 990 | 1083 | 1715 | 320 | 1039 |
| At maturity ($S_4$) | 924 | 1825 | 557 | 1102 | 1111 | 1944 | 338 | 1139 |
| **LSD (0.05)** | **49.8** | **127** | **54** | **85** | **56.5** | **189** | **34** | **106** |
| **Interaction (VxS)** | | | | | | | | |
| $V_1S_1$ | 644 | 1094 | 485 | 741 | 818 | 1233 | 279 | 777 |
| $V_1S_2$ | 902 | 1384 | 516 | 934 | 1023 | 1453 | 285 | 920 |
| $V_1S_3$ | 938 | 1740 | 590 | 1089 | 1106 | 1889 | 345 | 1113 |
| $V_1S_4$ | 994 | 1850 | 621 | 1155 | 1136 | 1968 | 366 | 1157 |
| $V_2S_1$ | 614 | 1004 | 448 | 689 | 750 | 1089 | 256 | 698 |
| $V_2S_2$ | 774 | 1300 | 460 | 845 | 962 | 1311 | 261 | 845 |
| $V_2S_3$ | 803 | 1417 | 467 | 896 | 1061 | 1478 | 281 | 940 |
| $V_2S_4$ | 864 | 1809 | 486 | 1053 | 1083 | 1887 | 291 | 1087 |
| $V_3S_1$ | 627 | 1090 | 478 | 732 | 765 | 1222 | 261 | 750 |
| $V_3S_2$ | 877 | 1304 | 504 | 895 | 992 | 1363 | 280 | 878 |
| $V_3S_3$ | 891 | 1531 | 533 | 985 | 1084 | 1778 | 333 | 1065 |
| $V_3S_4$ | 914 | 1817 | 564 | 1098 | 1114 | 1977 | 357 | 1149 |
| **LSD (0.05)** | **87** | **219** | **94** | **147** | **98** | **327** | **59** | **183** |

NS: Non-significant

for the combinations of AS-73-CP-560 harvested at 40 days after flowering, AMV-3 harvested at maturity and AMV-4 harvested at maturity and at 40 days after flowering were on par. Similarly, AS-73-CP-560 harvesting at maturity recorded significantly higher test weight (23.01 g) over the combinations of all varieties with harvesting stage at 20 days after flowering but did not differ significantly with remaining treatment combinations except AMV-3 with harvesting at 30 days after flowering (Table 4).

The pooled data of interactions showed that variety AS-73-CP-560 and AMV-4 harvested at maturity recorded significantly higher fibre equivalent yield as compared to all the varieties in combination with harvesting at 20 days after flowering and were on par with other interactions. Higher fibre equivalent yield of AS-73-CP-560 and AMV-4 harvested at maturity is attributed to higher fibre yield and seed yields compared to other stages of harvest.

Table 5. Fibre equivalent yield of mesta varieties as influenced by stage of harvesting

| Treatment details | Fibre equivalent yield (kg ha$^{-1}$) | | | |
|---|---|---|---|---|
| | First year | Second year | Third year | Pooled |
| **Varieties(V)** | | | | |
| AS-73-CP-560 ($V_1$) | 1839 | 3063 | 780 | 1894 |
| AMV-3 ($V_2$) | 1683 | 2743 | 660 | 1595 |
| AMV-4 ($V_3$) | 1767 | 2936 | 741 | 1815 |
| **LSD (0.05)** | **114** | **NS** | **72** | **NS** |
| **Stage of harvesting (S)** | | | | |
| 20 days after flowering ($S_1$) | 1369 | 2182 | 657 | 1403 |
| 30 days after flowering ($S_2$) | 1793 | 2627 | 687 | 1702 |
| 40 days after flowering ($S_3$) | 1909 | 3186 | 761 | 1952 |
| At maturity ($S_4$) | 1980 | 3662 | 803 | 2148 |
| **LSD (0.05)** | **131** | **254** | **45** | **341** |
| **Interaction (VxS)** | | | | |
| $V_1S_1$ | 1425 | 2263 | 683 | 1457 |
| $V_1S_2$ | 1872 | 2755 | 715 | 1781 |
| $V_1S_3$ | 1989 | 3526 | 837 | 2118 |
| $V_1S_4$ | 2072 | 3709 | 883 | 2221 |
| $V_2S_1$ | 1328 | 2034 | 629 | 1330 |
| $V_2S_2$ | 1690 | 2535 | 644 | 1623 |
| $V_2S_3$ | 1817 | 2811 | 670 | 1766 |
| $V_2S_4$ | 1896 | 3590 | 697 | 2061 |
| $V_3S_1$ | 1355 | 2248 | 660 | 1421 |
| $V_3S_2$ | 1818 | 2590 | 701 | 1703 |
| $V_3S_3$ | 1921 | 3219 | 777 | 1973 |
| $V_3S_4$ | 1974 | 3687 | 827 | 2163 |
| **LSD (0.05)** | **227** | **440** | **77** | **590** |

NS: Non-significant

## CONCLUSION

It can be concluded that AS 73-CP-560 variety of mesta produced significantly higher fiber yield as compared to other varieties. Harvesting mesta crop at physiological maturity produced significantly higher fibre and seed yields compared to other stages of harvest. It was advised to harvest the crop at 120-130 days after sowing as it gave higher yield of good quality fibre and fibre equivalent yield.

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# Conservation of plants in home gardens of Tiruchirappalli district of Tamil Nadu

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**ABSTRACT**

The ethnobotanical survey conducted in Tiruchirapalli, Tamil Nadu during April to May 2020 showed that the area segregated for home gardens were slowly occupied by the buildings. Cultural beliefs and vasthusastra, believed in protecting human being from evil eyes, played a major role in the conservation of plants in the home gardens. Besides growing plants for religious offerings, culinary, medicinal, health, and of aesthetic values were also reported. Home gardening during Covid-19 lock down worked as a stress buster in men, women and children.

**Key words:** Home gardens, evil eye, vasthusastra, stress buster

Iterative domestication processes such as selection, translocation and cultivation are known to affect the morphological and genetic diversity of plant species (Wiehle, et al., 2014). Historical events along the time line of agriculture and technology development, altered the size and plant species occuring in home gardens. Home garden can be defined as a farming system that combines different physical, social, and economic functions in the area in and around home with no restriction on the size of the garden. In cities, it generally offers a very small portion of supplementary fresh fruit and vegetable needs. On the contrary, the Kani tribes of Kanyakumari wildlife sanctuary documented home gardens contributing food, medicine, timber, firewood and as a good source of income (Mary Suba, et al., 2014). Globally, old buildings were demolished along with the home gardens and new multi-storeyed buildings arised with a change in the scenario and removing the rare plants. According to FAO, agroecology seeks to optimize the interactions between plants, animals, humans and the environment while taking into consideration the social aspects that need to be addressed for a sustainable and fair food system (FAO). Therefore, a study to document the cultural beliefs in conserving the plants found in the home gardens in Tiruchirappalli district of Tamil Nadu, India was aimed at.

## MATERIALS AND METHODS

Ethnobotanical surveys were conducted around K Abishekapuram panchayat area in Tiruchirappalli west districts of Tamil Nadu. A total of 158 home gardens of 33 families were surveyed from April to May, 2020. The occupants were enquired about the plants they grow. Out of 158 informants, 69.62 per cent were females of 19 to 83 age and the rests were males of 40 to 67 years.

## RESULTS AND DISCUSSIONS

The survey indicated predominance of 63 plant species in the area (Fig. 1). These were grown for fresh fruits, leaves roots and flowers according to individual preferences. Overlapping of usages were reported for many plants, for example tulsi used as medicine, for religious purposes evil eye, protactant and vasthusastra.

Majority of residents grew flowers for religious offerings throughout the year. They considered the home gardens as sacred grooves and protected them with care. Flowers of *Aegle marmelos*, *Artabotryshexapetalus*, *Calotropis gigantea*, *Clitoriaternatea*, *Chrysanthemum morifolium*, *Hibiscus rosa-*

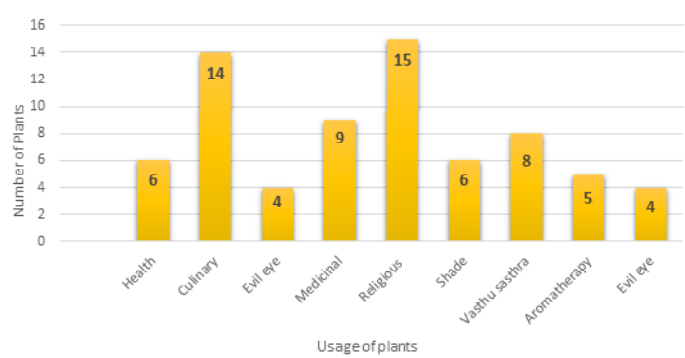


Fig. 1. Usage of plants in the study area

*sinensis* (Fig. 2), *Jasminum adenophyllum*, *Jasminum polyanthum*, *Jasminum grandiflorum*, *Neriumodorum*, *Nyctanthes Arbor Tristis*, *Rosa gallica* and *Tabernaemontana divaricate* were offered as garlands or as single flowers to God. In addition, leaves of *Ocimum sanctum* (Fig. 3) and *Piper betle* (Fig. 4) were offered to God.

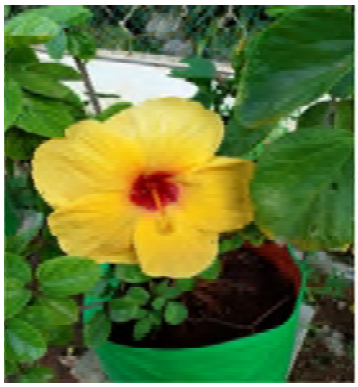

Fig. 2. *Hibiscus rosa-sinensis*

Fig. 3. *Ocimum sanctum* and

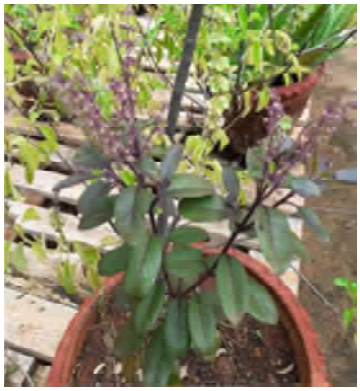

Fig. 4. *Piper betle*

The survey revealed growing of 21 plants for fresh fruits and culinary purposes. These were *Annona reticulata*, *Carica papaya*, *Mangifera indica*, *Manilkara zapota*, *Psidium guajava*and *Punicagranatum*. *Abelmoschus esculentus*, *Capsicum annuum* L., *Cissus quadrangularis*, *Cocos nucifera*, *Dolichos lablab*, *Mentha spicata*. L, *Moringa oleifera*, *Murrayakoenigii*, *Solanum lycopersicum*, *Solanum melongena*, *Solanum nigrum*, *Solanum torvum* and *Spinacia oleracea* were grown for culinary uses. *Musa sapientum* was commonly found in more than half of the houses, which they grew for leaf, flower, fruit and stem. During fasting days, the inhabitants eat these in breakfast, lunch and dinner on the banana leaves, generally grown for their leaves rather than for its fruits. Banana flower and stem is loaded with high fiber content. *Cassia fistula*, *Leucaena leucocephala*, *Millettia pinnata*, *Muntingia calabura*, *Prunus amygdalus* and *Tectona grandis* were grown for shade especially in front of the house.

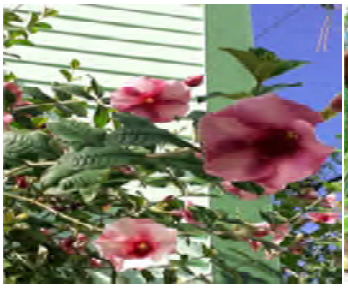

Fig. 5. *Allamanda blanchetii*

Fig. 6. *Cissus quadrangularis*

Inhabitants also grew ornamental plants like *Allamanda blanchetii*, (Fig. 5). *Bambusa arundinacea*, *Bougainvillea glabra*, *Crossandra infundibuliformis*, *Chrysanthemum morifolium*, *Epipremnum aureum*, *Euphorbia pulcherrima* and *Ixora coccinea* were grown for their aesthetic value. They also grew plants like *Azadirachta indica*, *Aloevera*, *Cissus quadrangularis* (Fig. 6), *Morinda citrifolia* (Fig. 7), *Ocimum basilicum*, *Phyllanthusniruri* and *Plectranthus amboinicus* (Fig. 8), *Solanum nigrum*, and *Solanum torvum* having high medicinal values. Tea prepared with Neem leaves, tulsi, ginger, pepper and pink salt was reported as a home remedy to cure respiratory infections and as immunity booster. Fresh *Aloe vera* gel was reported as a cure to skin infections, dandruff and stomach ulcer. *Solanum torvum* fruits are used as vegetables and traditionally it was considered to expel intestinal worms. *Solanum nigrum* leaves were boiled with onion, pepper, cumin, and fresh coconut milk was consumed to control body heat and to cure ulcers. *Morinda citrifolia* (Fig. 7) is a rare plant in home gardens. The fruit was locally called as noni and considered to have anti-cancer properties, However, its consumption was not very attractive and mostly wasted due to its bitter taste. *Cissus quadrangularis* was believed to contain high percentage of fibre and was used to cure hemorrhoid and calcium to heal bone fracture.

Fig. 7. *Morinda citrifolia*

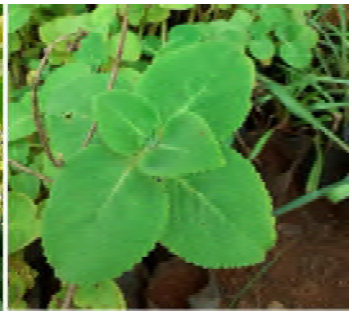

Fig. 8. *Plectranthus amboinicus*



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Women inhabitants used home garden flowers like *Chrysanthemum morifolium, Jasminum adenophyllum*, *Jasminum polyanthum*, *Jasminum grandiflorum* and *Rosa gallica*in on their hair as aromatherapy. The inhabitants believed that the fragrance of these flowers make them forget their worries and give them satisfaction beyond explanation. When they offer the flowers to god, a mixed fragrance spread throughout the house offering a peaceful environment.

Growing plants according to Vasthusastra is common among the inhabitants of hindu religion. Plants such as Aloe, Banana, Bamboo, Chrysanthemum, Coconut, Mango, Money plant, Papaya and Tulasi, were planted at specific locations of the home gardens to bring good luck and prosperity. The inhabitants also believed in horoscope and grew plants according to their horoscope to bring luck.

A trend of growing *Aloe vera*, *Azadirachta indica, Holy basil* and *Lawsonia inermis* in front of their houses to ward off bad things and protect them from evil eye was observed among many inhabitants of the area. *Lawsonia inermis* was found in 22.15 per cent of home gardens. The inhabitants traditionally apply leaf paste of *L. inermis* in on their nails and fingers believing that these cure deformation of nails and keep the body cool. Tomato, chilli, pumpkin and brinjal was grown on kitchen wastes.

The diversity of home gardens was determined by the geographical area, seed dispersal and individual preferences. It varied with the succeeding generations. Elderly people in the study area informed that the home gardens in their times, occupied medicinal plants. Recently people received rare plants from garment stores and as seed ball packs in marriages and functions as gifts.

Around 62.02 per cent of the informants dump kitchen wastes at the bottom of the trees and shrubs in their garden, while 12.02 per cent of them prepare compost using the wastes generated in their homes. About 18.98 per cent do not apply additional nutrients apart from watering, while 6.96 per cent of the informants buy organic fertilizers and 3.16 per cent use chemical fertilizers to boost the flower production at home gardens. Waste water after washing rice, fish, vegetables and left-over foods are applied to the plants exclusively to rose plants to boost flowering. The inhabitants used turmeric solution to control diseases and pests.

People from all walks of life were stressed during COVID-19 pandemic because of the pressure from work at home. Attending office, cooking, managing house chores, teaching children, continuous work in systems and sharing the computer/laptop and internet created a new atmosphere and stress at home. Women, men and children involved in gardening reported gardening as stress reliever. Even one single flower or a vegetable from their home garden excited them in contributing to their psychological well being.

The choice of plant species and planting techniques reflects the acquired wisdom and insights of people who have interacted with the environment for generations and made the home gardens a principal hub of crop evolution and diversity. The composition of plants in home gardens greatly varied according to individual's need and their tradition to some extent. Generally, friends, relatives, neighbours, workers and visitors also contribute plant species as gifts or give them in exchange for other species (Kumar, 2007).

Globally, home gardens have been documented as an important supplemental source contributing to food and nutritional security and livelihoods. According to Revitalizing Rainfed Agriculture Network (RRAN), home gardens are for seed conservation and food security. Developing countries around the world consider home gardens as an integral part of local food systems (Galhena et al., 2013). These can be described as a mixed cropping system that encompasses vegetables, fruits, plantation crops, spices, herbs, ornamental and medicinal plants as well as livestock that can serve as a supplementary source of food and income (Galhena et al., 2013 Vijayakumari et al., 2019). Rapid industrialization has led to an uncontrolled increase in human interference to play a spoil sport, which has resulted in a considerably decline of the habitat (Mazid et al., 2012) Documentation of the plants in home gardens has been done in pockets around the world and in India. The accumulation of knowledge and species diversity in each locality may provide an insight to include the plant species in the conservation sites and botanical gardens. For example, *Moringa oleifera,* a common tree in home gardens of Tamil Nadu, has gained considerable attention in India and across the world due to the belief that the leaf can cure anaemia and cancer. Experiments conducted in Tanzania confirmed that the Moringa leaf powder significantly reduced the prevalence of anaemia cases by half and worked better in moderate anaemia cases. (Angela E.S, 2019). Inhabitants of the study area reported regular use of leaves and fruits of drumstick tree. Similarly, research evidences proved that *Cissus quadrangularis* can cure bone fractures (Brahmkshatriya et al., 2015) and anti-arthritic (Kirthika and Janci Rani, 2019) properties. It was also mentioned in Ayurvedic literature as a general tonic and analgesic, with specific bone fracture healing properties (Kumar, 2019). Current study found that usage and purpose in the city has changed and still it is considered as an important culinary

plant used in the preparation of chutney.

Household gardens tend to be located close to dwelling for security, convenience, and special care. Gonzalez et al., (2009) discovered that engaging with a garden distracts them from their worries and stops them from obsessing. The current study confirmed that the COVID-19 stress was well handled by the home gardening activities. Psycological wellness and wearing jasmine flowers and offering to God might be due to methyl jasmonate (MJ), a bioactive compound isolated from *Jasminum grandiflorum* with proven anti-depressant activity (Solomon et al., 2011). Hindus traditionally use a large number of plant species for worshiping different gods and goddesses (Jintu and Ashalata, 2015). Certain plants an associated with Vastu shastra and are believed to be auspicious or inauspicious near dwellings or in particular locations (Jain and Kapoor, 2007). The study found that the plants grown according to vasthusastra protect the people from evil eye and has overlapping utilities such as health, medicinal, culinary and ornamental values. Therefore, it occupies a key role in conservation of plants in the home gardens of Tiruchirapalli district.

## CONCLUSION

Growing plants in the home garden as an evil eye protectaetant and religious offerings played a key role in conservation of plants in home gardens. This also relieved online work stress among men, women, and children during COVID-19 lockdown.

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# Influence of girdling on stimulation of flowering and yield in mango (cv. Alphonso) in hard lateritic rocky areas

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**ABSTRACT**

The investigations, conducted during the year 2018-19 at Mango Research Sub-Centre, Rameshwar, Tal. Deogad, Dist. Sindhudurg (Maharashtra), located on hard lateritic rocky area, with five girdling time treatments viz., girdling in second fortnight of August ($T_1$), girdling in first fortnight of September ($T_2$), girdling in second fortnight of September ($T_3$), girdling in first fortnight of October ($T_4$) and control (no girdling) ($T_5$), showed early initiation of flowering at 43rd standard meteorological week with maximum flowering intensity (63.75%) and highest hermaphrodite flower (9.40%) when girdling was done in first fortnight of September ($T_2$). This treatment also recorded highest number of fruits (149.25 fruits tree$^{-1}$), yield kg tree$^{-1}$ (32.68) and t/ha$^{-1}$ (3.27) than against lowest of 101.25, 22.31 and 2.23 of the respective parameters in the control.

**Key words:** *Mango, girdling, flowering, yield*

Mango occupying an important place in horticulture is the main commercial fruit crop of India. It is the third widely produced fruit crops of the tropics after banana and citrus. India has rich wealth of mango germplasm with more than 1000 varieties growing throughout the country. Alphonso among them tops the list and is grown along the west coast of India, Maharashtra, Goa, Karnataka and Gujarat which is acclaimed as one of the best Indian mango variety. Konkan is the major and famous mango producing region on the west coast of Maharashtra where 90 per cent area under mango is occupied by single cultivar "Alphonso", locally called "*Hapus*". It is specially preferred for export because of its delicious taste exceeding acceptable flavor, attractive colour and exemplary nutritive value. It is also best for table and processing purpose (Cheema and Dhani, 1934). The warm and humid climate throughout the year and rain free season from November to May prevalent in Konkan region is most ideal for mango cultivation in general and Alphonso in particular.

The mango grown under hard lateritic rocky conditions are distinctive where fruits mature early and fetch high rates in the market. Though, the mango cultivation in this region is renowned, the productivity is very low compared to national productivity. In the recent years, weather vagaries affected the crop phenology especially the reproductive phases disturbing crop yield and productivity in Alphonso production (Malshe *et al*., 2016).

The improvement in productivity in modern agriculture system is increasingly dependent on manipulation of the physiological activities of the crop by chemical means. In commercial mango plantations, it is desirable to control the vegetative growth to get uniform and regular flowering. However in the recent years, the climatic aberration at the time of induction of the flowering altered the reproductive phase of mango trees. To defeat such problem, the adoption of precise management practice is the needed. The girdling is one of the horticultural practice that helps in induction of flowering by barricading the movement of assimilates which ultimately results in accumulation of carbohydrates above girdle portion. The standardization of girdling time is also essential for a particular cultivar of mango. Considering this, the present investigation on effect of girdling practice on stimulation of flowering and yield in mango (cv. Alphonso) in hard lateritic rocky area was undertaken.

## MATERIAL AND METHODS

The investigation was conducted on 22 years old Alphonso trees at Mango Research Sub-Centre, Rameshwar, Tal. Deogad, Dist. Sindhudurg located between 15$^0$30′ and 18$^0$15′ N latitude and 72$^0$45′ and 74$^0$50′ E longitude on west

coast of Maharashtra on the bank of Vijaydurg creek during the year 2018-19. Uniformly grown trees selected for the purpose were applied with the recommended cultural practices. The experiment was laid out in randomized block design comprising four replications and five times of girdling treatments viz., girdling in second fortnight of August ($T_1$), girdling in first fortnight of September ($T_2$), girdling in second fortnight of September ($T_3$), girdling in first fortnight of October ($T_4$) and control (no girdling) ($T_5$). Each treatment was given for two trees per replication. The girdling was done on tertiary branches of experimental mango trees by giving circular deep cut with the help of sharp knife as per the schedule time. The data on induction of flowering, flowering phases, intensity of flowering, hermaphrodite flower percentage in panicle, days required for harvesting from fruit set, yield per tree were recorded. The data was analyzed by the using statistical methods suggested by Panse and Sukhatme (1967).

## RESULTS AND DISCUSSION

The data on effect of time of girdling on flowering presented in Table 1 indicate that the girdling significantly influenced the flowering parameters in mango (cv. Alphonso). The early flowering was noticed in 43rd standard meteorological week (22-28, October) when girdling was done in first fortnight of September ($T_2$). It was followed by $T_3$ (44th SMW), $T_1$ (45th SMW). The flowering initiation was delayed in control ($T_5$) and observed in 52nd SMW (last week of December). This indicates that the girdling in first fortnight of September induced early flowering by 9 days than control. The intensity of the flowering varied significantly due to girdling practices. The maximum flowering intensity (63.75%) was recorded in $T_2$ (girdling in first fortnight of September) and it was at par with rest of the girdling treatments. The lowest intensity of flowering (42.50%) was observed in control ($T_5$). The earliness in flowering and maximum flowering in girdling treatments might be caused due to interruption in the phloem pathway and altered the C : ratio resulting into stimulation of flowering. Urban et al. (2009) concluded that the girdling improved the earliness and intensity of flowering in mango cv. Cogshall.

The highest hermaphrodite flower percentage (9.40%) was recorded on the trees where the girdling was done in first fortnight of September ($T_2$). It was closely followed by T3 and T4 treatments. While $T_1$ (girdling in second fortnight of August) and $T_5$ (Control) registered the lowest hermaphrodite flower percentage (7.78). The improved percentage of hermaphrodite flower is a decisive factor of yield in mango and it is apparent that the girdling done in the month of September and first week of October improved the sex ratio. This might be due to physiological role of girdling causing accumulation of carbohydrates in upper part producing productive panicle. The results are analougus with the earlier finding of Shinde *et al*. (2014).

The number of flowering flushes was in the range of 3 to 6 (Table 1). This indicated that girdling practices induced the flowering in minimum phases which is beneficial for fruit development and harvesting with synchronized period. Haldankar *et al.* (2014) noticed three flowering flushes of mango cv. Alphonso under normal conditions. The flowering phases was governed by several factors like temperature fluctuation during flowering period and the maturity of shoots, etc. The sex ratio might also be changed in every flowering phase.

Table 1. Effect of time of girdling on flowering in mango (cv. Alphonso) planted on hard lateritic rocky area

| | Treatment | Initiation of flowering (SMW)* | Earliness in flowering over control (Weeks) | Intensity of flowering (%) | Hermaphrodite flower (%) | Number of flowering phases |
|---|---|---|---|---|---|---|
| $T_1$ | Girdling in Second fortnight of August | 45 | 7 | 52.50 (46.43)# | 7.78 | 4 |
| $T_2$ | Girdling in First fortnight of September | 43 | 9 | 63.75 (52.98) | 9.40 | 3 |
| $T_3$ | Girdling in Second fortnight of September | 44 | 8 | 58.75 (50.04) | 8.73 | 3 |
| $T_4$ | Girdling in First fortnight of October | 48 | 4 | 61.25 (51.50) | 8.11 | 4 |
| $T_5$ | Control (No girdling) | 52 | - | 42.50 (40.69) | 7.78 | 6 |
| | S.E.m ± | - | - | 2.35 | 0.39 | - |
| | CD (at 5%) | - | - | 10.16 | 1.66 | - |
| | C. V. (%) | - | - | 9.72 | 9.21 | - |

*SMW : Standard meteorological week #Figures in parenthesis indicate arcsine transformed value



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Table 2. Effect of time of girdling on fruit yield in mango (cv. Alphonso) planted on hard lateritic rocky area

| Treatment | Days required for fruit set to harvest | Number of fruits tree$^{-1}$ | Yield (kg tree$^{-1}$) | Yield (T ha$^{-1}$) |
|---|---|---|---|---|
| $T_1$ | 98 | 120.50 | 26.35 | 2.64 |
| $T_2$ | 93 | 149.25 | 32.68 | 3.27 |
| $T_3$ | 95 | 141.25 | 30.31 | 3.03 |
| $T_4$ | 99 | 131.00 | 28.50 | 2.85 |
| $T_5$ | 106 | 101.25 | 22.31 | 2.23 |
| S.E.m ± | - | 10.3 | 1.98 | 0.02 |
| CD (at 5%) | - | 31.73 | 6.11 | 0.06 |
| C. V. (%) | - | 16.01 | 14.14 | 14.15 |

The days required to attain maturity for harvest also varied due to girdling treatments and ranged between 93 to 99 days whereas, in control it was 106 days (Table 2). The maturity of the fruits depends on the fulfilment of heat units and period of flowering and fruit set. Burondkar *et al*. (2000) stated that the mango grown on the red lateritic rocky hills matures much earlier than those commonly grown on plain area of the region.

The data presented in Table 2 revealed significant increase in mango (cv. Alphonso) yield due to girdling treatments. The highest number of trees fruits tree$^{-1}$ (149.25), yield in kg tree$^{-1}$ (32.68) and t/ha$^{-1}$ (3.27) was achieved in $T_2$ treatment (girdling in first fortnight of September). It was on par with the $T_3$ and $T_4$ treatments. The control ($T_5$) recorded the lowest yield (101.25) fruits tree$^{-1}$, 22.31 yield kg tree$^{-1}$ (22.3) and t/ha$^{-1}$ 2.23. The control ($T_5$) was observed having not much effect in yield improvement. The increased number of fruits in girdling treatments may be due to higher percentage of hermaphrodite flowers. Beside this, the girdling improves the availability of the carbohydrates to fruits which lead to increase in fruit set and fruit retention. The girdling in first fortnight of September (T2) was found superior. That may be due to girdling time treatment in which accumulation of carbohydrate was higher than other treatments. The enhancement of carbohydrate availability has been associated with fruit set and number of fruits per shoots which leads to increased yield attributes. The results are analogous with the earlier findings of Rakshe *et al*. (2013) and Ghadage *et al*. (2017) in mango.

## CONCLUSION

It was concluded that girdling of mango trees done in the first fortnight of September stimulated early and more flowering and produced higher mango (cv. Alphonso) yield in hard lateritic rocky areas. This was, therefore recommended to be adopted as a promising practice to induce flowering and fruiting in mango.

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# Enzyme production from different fruit and vegetable waste using lactic acid fermentation

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**ABSTRACT**

Laboratory experiment conducted with the objective of utilizing fruit and vegetable wastes and by-products *viz.,* mango stone, citrus and mixed fruit peels, mosambi peel, banana pseudo-stem cuttings and cauliflower leaves, left over in the open in the fruit and vegetable mandies and processing industries or thrown in rivers for enzyme production showed highest pectinase, cellulose and amylase activity of 0.7605, 021465 and 0.3174 U µmol m1$^{-1}$ min$^{-1}$ in mosambi peel on 7$^{th}$ day of *Laetobacillus plantarum* inculation and 05289 & 0.5218, 0.4543 & 0.4314 and 0.4424 & 0.3626 U m$^{-1}$ min$^{-1}$ in the mango waste after 13 and 28 days of *L. plantarum* inoculation, respectively. The *Lactobacillus* counts were highest (>10$^{8}$) in animal feed from mixed fruit pomace and mango stone waste till 13 days of incubation.

**Key words:** Enzyme, cellulase, pectinase, amylase, fruits, vegetables, waste

A large quantity of fruit and vegetable wastes, in generated vegetable mandies and fruit juice vender's shops in the country almost everyday. These is usually dumped out in land fills in the open or thrown in the nearby ponds or rivers, leads to the environmental pollution. According to a report by ICAR-CIPHET, the horticulture produce sector (fruits and vegetables) incurs the highest economic loss of about 34 per cent due to their soft texture, high water content and perishable nature (Jha *et al.,* 2015). Therefore, post-harvest management of fruits and vegetables need immediate attention. According to Indian Agricultural Research Data Book, the losses in fruits and vegetables are to the tune of 30 per cent. India, According to a report of Central Institute of Post-Harvest Engineering and Technology, Ludhiyana Rs. 13,300 crore per year gets wasted due to lack of adequate cold storage facilities, (Jha *et al.,* 2015). These also create problems in municipal landfills due to their high biodegradability. Though, technology for making biogas from these wastes are available (Deressa *et al.,* 2015), it is not in use at most of the places because of technical problems in the maintenance units.

Fruit and vegetable wastes are rich source of carbohydrates, proteins, vitamins and minerals (Coman *et al.,* 2019). These may be used as substrates for various value added products including biofuel, photochemicals, enzymes etc. Latter are proteins, which act as catalysts and lower the energy required for a reaction to occur without being used up in the reaction. Many industries utilize enzymes to save energy, chemicals and/or water compared to conventional processes in the generation of their products.

Enzymes from microbial sources are preferred compared to plant or animal sources because of its being relatively cheaper and controllable (Singh *et al.,* 2016). The enzyme production cost can further be reduced if negative value or cheap value substrates such as fruit processing industry waste are used. This will also help in solving fruit industry waste problem to some extent and will reduce the pollution levels. Lactic acid bacteria are the most prominent probiotic bacteria which exert beneficial effects in the host gastro intestinal tract in addition to production of enzymes. Tevea *et al.,* 2016 reported highly thermostable á-amylase enzyme from *Lactobacillus fermentum* 04BBA19. Keeping in view the objective of making the best use, the different fruit and vegetable wastes were evaluated for production of cellulases, pectinases and amylase enzymes using lactic acid bacteria.

## MATERIALS AND METHODS

### Substrate

Mango stone waste, mixed fruit pomace, mosambi peel, banana pseudo-stem cuttings and cauliflower leaves were collected from fruit and vegetable whole sale market and fruit juice vendors. The micro-organisms, *Lactobacillus plantarum* ATCC 8014TM used in the experiment was maintained on MRS agar medium. Each fruit and vegetable wastes sample was thoroughly washed with tap water, chopped into small pieces and later filled in separate glass jars. Water in 1:2 ratio was added and the contents so obtained were inoculated separately with *Lactobacillus plantarum* under the anaerobic condition at 35°C for 7, 13

and 28 days. The contents were filtered with fine muslin cloth and the filtrate was used as liquid crude enzyme extract. The residue was mixed with wheat husk in 0.5 ratio, air dried and packed.

Each sample was added with cold acetone mixture in 1:4 ratio, kept at -20°C for 20 minutes and centrifuged for 15 minutes at 4°C at 10,000 rpm. The supernatant was discarded and pellets was re-suspended in 0.2mM acetate buffer pH at S.S solution and used for estimation of cellulase, pectinase, and amylase using carboxy methyl cellulose, pectin and starch as substrates, as per method described by Miller (1972), Garg and Ashfaque (2010) and Wood and Bhat, 1988, respectively. The enzyme activity was expressed as units of sugar released ml $min^{-1}$ of incubation. The residue left after filtration was mixed with wheat straw and water in 2:1:1 ratio. The mixture was manually shaped into the feed pellet (6.5 × 3 × 2 inch). The dried product was analysed for *Lactobacillus* counts (CFU/g) using MRS agar growth medium (Süle *et al.*, 2014).

## RESULTS AND DISCUSSION

Lactic acid bacteria are the most prominent probiotic bacteria known to produce lactic acid and enzymes such as proteases, peptidases, polysaccharide degrading enzymes, ureases, lipases, amylases, esterases and phenoloxidases which can enhance nutrient digestion in the gastrointestinal tract. The probiotic *Lactobacillus* species includes *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, *L.paracasei*, *L.casei*, *L. gasseri* etc. (Walter, 2008).

The results revealed that mosambi peel showed highest pectinase, cellulase and amylase activities of 0.7605, 0.21465 and 0.3174, U $m^{-1}/min^{-1}$, respectively after 7 days of fermentation, while the mango stone waste substrate recorded 0.5289 and 0.5218, 0.4543 and 0.4314 and 0.4424 and 0.3626 U $\mu mo^{-1}$/ml $min^{-1}$, respectively after 13 and 28 days.

Ravindran *et al.* (2018) reviewed research work on production of different industrially important enzymes viz. alpha -amylase, cellulase, protease, lipase, pectinase, phytase, catalase, insulinase, laccase etc. using lignocellulosic agricultural residues. Lactic acid bacteria are used in food industry, pharma and chemical industry. Animal feed are often enriched by lactic acid bacteria . Padmavathi *et al.* (2018) reported *Lactobacillus* sp. G3-4-1TO2 as potential amylase producer. Jawad *et al.* (2018) reported production of lactic acid from mango peels. Dange and Harke (2018) used mosambi & lemon peel and wheat bran as substrate for production and purification of pectinase using *Aspergillus oryzae*. Priya and Sashi (2014) reported pectinase production by *Penicillium sp*. and *Aspergillus sp.* on the medium plates containing commercial citrus pectin as sole carbon source. A *Lactobacillus acidophilus* strain isolated from fermented ragi was reported to produce lactase enzyme, even when the lactose content in the media was reduced to 0.75 per cent, supplemented with 1 per cent ragi (Akolkar *et al.*, 2005).

The *Lactobacillus* counts (Table 2) were the highest ($6.64*10^8$ CFU $g^{-1}$) in mango stone waste followed by mix fruit waste ($2.4*10^8$ CFU $g^{-1}$) and mosambi peel waste ($2.7*10^7$ CFU $g^{-1}$). The counts in banana pseudo stem were higher than cauliflower waste.

The crude liquid enzyme extract as well as solid animal feed fed to cows from Gopeshwar Gaushala, Malihabad showed positive results on their digestive system and general health.

Table 2: *Lactobacillus* counts (CFU $g^{-1}$) in solid fruit and vegetable waste residue after different incubation period

| Days of incubation | Mixed Fruit pomace | Cauliflower leaves | Mango stone waste | Mosambi peel | Banana pseudo stem |
|---|---|---|---|---|---|
| 7 | $2.41X10^8$ | $2.6X10^6$ | $6.64X10^8$ | $2.71X10^7$ | $7.1X10^6$ |
| 13 | $2.31X10^8$ | $5.2X10^6$ | $7.46X10^8$ | $2.32X10^7$ | $8.2X10^6$ |
| 28 | $9.4X10^5$ | $1.2X10^5$ | $6.8X10^7$ | $2.92X10^6$ | $7.3X10^6$ |

Table 1: Enzyme activity of different interval from fruit and vegetable waste

| Days of incubation | Enzyme | Enzyme activity (Unit $ml^{-1}$ $min^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | Mixed fruit pomace | Cauliflower leaves | Mango stone waste | Mosambi peel | Banana pseudo stem |
| 7 | Pectinase | 0.0992 | 0.2591 | 0.0462 | 0.7605 | 0.1575 |
| | Cellulase | 0.0384 | 0.0927 | 0.0210 | 0.2146 | 0.0246 |
| | Amylase | 0.0522 | 0.0486 | 0.0573 | 0.3174 | 0.0768 |
| 13 Day | Pectinase | 0.0098 | 0.0439 | 0.5289 | 0.000 | 0.0151 |
| | Cellulase | 0.0807 | 0.0881 | 0.4543 | 0.0119 | 0.0265 |
| | Amylase | 0.0632 | 0.0155 | 0.4424 | 0.000 | 0.0146 |
| 28 Day | Pectinase | 0.000 | 0.1378 | 0.5218 | 0.000 | 0.0143 |
| | Cellulase | 0.0294 | 0.1404 | 0.4314 | 0.0183 | 0.0321 |
| | Amylase | 0.000 | 0.0422 | 0.3626 | 0.000 | 0.000 |

## CONCLUSION

The study concluded that mango stone waste and mosambi wastes can be utilized as the potential substrate for production of enzymes using *Lactobacillus plantarum*.

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# Phenolic components, ascorbic acid and organic acids profiling of six guava varieties grown under subtropical region of Lucknow, Uttar Pradesh

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**ABSTRACT**

Guava (*Psidium guajava* L.) is a well known source of ascorbic acid, phenolic compounds and organic acids. Both ascorbic acid and phenolic components are powerful antioxidants and organic acids impart flavour in fruits. Gallic acid, chlorogenic acid, catechin, epicatechin, caffeic acid, ellagic acid and *p*-coumaric acid as phenolic components and ascorbic acid, oxalic acid, citric acid, tartaric acid and malic acid as organic acids were identified in fruits of six guava varieties at edible ripe stage using HPLC-PDA. Maximum amount of ascorbic acid (234.80 mg $100g^{-1}$) was recorded in Dhawal followed by Allahabad Safeda (198.80 mg $100g^{-1}$). Among the phenolic compounds, gallic acid, chlorogenic acid and catechin were the predominant ones. Maximum amounts of gallic acid (8.00 mg $100g^{-1}$), chlorogenic acid (9.00 mg $100g^{-1}$) and epicatechin (6.00 mg $100g^{-1}$) were detected in Shweta. Lalit had maximum amounts of catechin, ellagic acid, caffeic acid and *p*-coumaric acid (6.00, 5.93, 2.46 and 0.90 mg $100g^{-1}$, respectively). Citric acid was the major organic acid noticed in guava fruits distantly followed by tartaric acid. Lalima contained maximum amounts of citric acid (460.00 mg $100g^{-1}$) and malic acid (2.50 mg $100g^{-1}$). The genotype Sardar possessed maximum amount of tartaric acid (71.00 mg $100g^{-1}$) and Dhawal that of oxalic acid (18.50 mg $100g^{-1}$). In terms of ascorbic acid, Dhawal and Allahabad safeda were the best varieties for consumption, while Shweta and Lalit were the best varieties to consume having anticancer antioxidant phenolic components.

**Key words:** Guava, ascorbic acid, organic acids, phenolic components, HPLC-PDA estimation

Now-a-days health awareness and changes in lifestyle lead consumers to invest in the consumption of functional foods that can contribute to the disease resistance. A functional food is a food material which when consumed as part of the usual diet imparts metabolic / physiological / beneficial health effects besides the nutritional functions and must be safe for consumption without any medical supervision. Functional foods contain nutraceuticals which are products identified in foods or biological materials with proven physiologically protective action. Nutraceuticals are bioactive phytochemicals like dietary fiber, proteins, peptides, amino acids, polyunsaturated fatty acids, vitamins and antioxidant phenolic compounds. Many tropical and subtropical fruits possess various nutraceutical components which can be used in food supplements, as food additives, in phyto-pharmaceutical industries as well as can help in increasing growers' income by encouraging consumption.

Guava (*Psidium guajava* L.) belonging to the family Myrtaceae originated from Mexico or Central America. It is now cultivated in many subtropical and tropical countries of Asia, North America, South America, Africa and Oceania (Jimenez-Escrig *et al*., 2001). India is leading in the world production of guava (46.5 million tonnes) with 41 per cent share followed by China (10%) and Thailand (7%) during 2019 (Tridge, 2019). The fruit is a very good source of ascorbic acid or vitamin C (about 4 times higher than orange), niacin or vitamin B3, potassium, phosphorus, magnesium and calcium. Besides vitamins and minerals, guava fruits are also rich in antioxidant phenolic compounds, dietary fibre, triterpenes, sugars and organic acids (Mccook-Russell *et al*., 2012). Pink fleshed guava is also rich in lycopene, a precursor of vitamin A. Guava plant parts are commonly used as folk medicine in numerous parts of the world (Mexico, Central America, Africa and Asia) for the cure of ailments like diarrhea, fever, caries, dysentery, diabetes, gastroenteritis, hypertension, pain relief and wounds (Anand *et al*., 2016; Naseer *et al*., 2018). The anticancer and antioxidant activities of guava fruit is believed to be due to the presence of phenolic compounds like gallic acid, galangin, kaempferol, homogentisic acid and cyanidine-3-glucoside in peel, seeds and pulp (Chen *et al*., 2015). Fleuriet and Macheix (2003) have observed that polyphenols and ascorbic acid are the most abundant antioxidants present in fruits while polyphenols are mostly flavonoids and are present in

glycoside and ester forms. Organic acids not only strongly influence the organoleptic properties of fruits and vegetables particularly flavor, color and aroma but also are responsible for fruit sourness or acidity (Kader, 2008). Ascorbic acid can also be considered as an organic acid because of its mild acidic nature.

ICAR-CISH, Lucknow has recently released two promising guava varieties Dhawal and Lalima besides releasing two other varieties earlier (Lalit and Shweta) which are commercially cultivated throughout India. Profiling of ascorbic acid, phenolic components and organic acids is important for characterization of different varieties and when studying the influence of maturity or agroclimatic factors on fruit quality. Determination of phenolic compounds in guava varieties in some countries have been conducted (Jimenez-Escrig *et al*., 2001; Mahattanatawee *et al*., 2006; Chen *et al*., 2015; Fu *et al*., 2016; dos Santos *et al*., 2017), though the literature on profiling of organic acids in guava is very few (Chan Jr. *et al*., 1971; Wilson *et al*., 1982). In India, estimation of ascorbic acid in several guava varieties is plenty (Tandon *et al*., 1983; Chauhan *et al*., 1986; Reddy *et al*., 1999; Ghosh *et al*., 2013), but no literature is available regarding estimation of individual phenolic compounds and organic acids in fruits. The current study was undertaken to evaluate six popular guava varieties grown in India under subtropical conditions for their contents of phenolic compounds and organic acids including ascorbic acid using HPLC-PAD which can add valuable data in scientific literature as well as help consumers and processors to choose suitable variety for particular purposes.

## MATERIALS AND METHODS

Uniform sized, blemish less and physiologically mature guava fruits from six varieties (Allahabad Safeda, Sardar, Lalit, Shweta, Dhawal and Lalima) were harvested from the Institute farm located at Rehmankhera, Lucknow. Fruits were chopped in small pieces, macerated in a mixer-grinder and required amount of sample was collected using quartering method for the determination of phenolic compounds and organic acids including ascorbic acid.

Reference standards of phenolic compounds like gallic acid, chlorogenic acid, (+) catechin, (–) epicatechin, caffeic acid, ellagic acid and *p*-coumaric acid and organic acids like oxalic acid, citric acid, malic acid, tartaric acid and L-ascorbic acid were procured from Sigma-Aldrich India branch, Mumbai. Solvents such as ethanol (AR grade), methanol, acetonitrile, water (all HPLC grade) and reagents like potassium dihydrogen orthophosphate (AR grade) were procured from the local market. Stock solutions of 1000 ìg/mL were prepared for each reference standard in phosphate buffer solution for organic acids including ascorbic acid and in methanol for phenolic compounds. Working solutions of required lower concentrations of each reference standard were prepared by subsequent dilution in respective solvents. Mixed standard solutions for phenolic components and organic acids were also prepared.

Phenolic compounds were extracted from 5 g fruit sample (in triplicate) with 25 mL of 80 per cent methanol through centrifugation at 10000 rpm for 10 min. Ascorbic acid and organic acids were extracted similarly from 5 g fruit samples (4 replicates) with 25 mL of 20 per cent ethanol followed by centrifugation at 10000 rpm for 10 min. The process was repeated twice and 20 ìL of the collected supernatant was injected to HPLC after filtering through nylon membrane filter (Axiva, 13 mm diameter, thickness 0.45 ì).

All the nutraceuticals were estimated using a Shimadzu make HPLC (model SCL 10 AVP) coupled with photodiode array (PDA) detector and rheodyne injector. The stationary phase for these two groups of nutraceuticals were same – reverse phase C18 column (250 mm X 4.6 mm, 5 ì film thickness, 100 Å porosity). However, the mobile phase for the determination of phenolic compounds was 0.01 M phosphate buffer with pH 3.05 (A) and acetonitrile-water (75:25, v/v) (B) at a ratio of 80:20 (v/v) (Bhattacherjee *et al*., 2011), while the same for ascorbic acid and organic acids was 50 mM phosphate buffer (pH 2.8) (Nour *et al*., 2010). Ascorbic acid was estimated along with organic acids. Flow-rate for both the chemical group was maintained at 1.0 mL/min. Detector wavelengths for phenolic compounds and organic acids including ascorbic acid were set at 280 and 214 nm, respectively. Total amount of identified phe-nolic compounds were calculated by adding the amounts of individual phenolic compounds detected by HPLC.

The statistical analysis was carried out using web agri stat package (WASP) version 2.0 software developed at ICAR Research Complex for Goa, Goa. Analysis of variance (ANOVA) was performed through student's t-test (LSD) at *p* d" 0.05 using completely randomized design (CRD) with 3 replications for phenolic compounds and 4 replications for organic acids.

## RESULTS AND DISCUSSION

### Characterization of phenolic compounds

Seven phenolic compounds, *viz*. gallic acid, chlorogenic acid, catechin, epicatechin, caffeic acid, ellagic acid and *p*-coumaric acid were detected and quantified in six guava varieties by RP-HPLC. Except Shweta and Lalima, none of the other variety contained all the phenolic components.



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Chlorogenic acid and ellagic acid were not detected in Dhawal, epicatechin was not found in Allahabad Safeda and Lalit, whereas caffeic acid was not quantified in Sardar. Gallic acid, catechin and *p*-coumaric acid were detected in all six varieties. Shweta was found as the richest source of antioxidant phenolic components followed by Lalit and Lalima. Gallic acid (8.00 mg$100^{-1}$ g), chlorogenic acid (9.00 mg $100^{-1}$ g) and epicatechin (6.00 mg $100^{-1}$ g) were quantified maximum in Shweta, while catechin (6.00 mg $100^{-1}$g), ellagic acid (5.93 mg $100^{-1}$ g), caffeic acid (2.46 mg $100^{-1}$ g) and *p*-coumaric acid (0.90 mg $100^{-1}$ g) were recorded maximum in Lalit (Table 1). Total of identified phenolic compounds were found maximum (29.06 mg $100^{-1}$ g) in Shweta followed by Lalit (23.82 mg $100^{-1}$ g) and Lalima (20.12 mg $100^{-1}$ g). Gallic acid, chlorogenic acid and catechin were the most predominant phenolic compounds identified in guava varieties. The concentrations of all seven identified phenolic compounds varied significantly among the varieties. Other good sources of gallic acid were Lalit (6.53 mg $100^{-1}$ g) and Dhawal (6.17 mg $100^{-1}$ g). Similarly, Lalima (8.00 mg $100^{-1}$ g) and Allahabad Safeda (7.00 mg $100^{-1}$ g) were other good sources of chlorogenic acid, while Dhawal and Allahabad Safeda were also rich in caffeic acid (2.31 and 2.13 mg $100^{-1}$ g, respectively).

Antioxidant phenolic compounds are recently characterized in many fruits. Substantial *in vitro* evidences have suggested that they have the ability to alter numerous cellular processes like gene expression, apoptosis, platelet aggregation, intercellular signaling with anti-carcinogenic and anti-atherogenic implications (Duthie *et al*., 2003). Several phenolic components like gallic acid, ellagic acid, catechin and epicatechin showed anti-inflammatory, antimutagenic, antimicrobial, anticancer and antiviral properties as well as efficacy against congestive heart failure, myoglobinuric acute renal failure and myocardial ischemia (Madsen and Bertelsen, 1995; Mandal *et al*., 1988; Augustyniak *et al*., 2005). The phenolic compounds in guava cure cancerous cells and prevent skin aging before time (Naseer *et al*., 2018). In a study, it has been observed that guava has high contents of protocatechuic acid, quercetin, ferulic acid, gallic acid and caffeic acid as important phenolic components (Jiminez-Escrig *et al*., 2001), two of them (gallic acid and caffeic acid) were also detected in the present study. Red guava fruit was found to have ellagic acid conjugates, flavones glycosides and gallic acid conjugates as phenolic compounds (Mahattanatawee *et al*., 2006). Fu *et al*. (2016) have identified six phenolic compounds, *viz*. gallic acid, (+) catechin, (–) epicatechin, quercetin, luteolin and kaempferol in guava fruit collected from Guangzhou market in China among which (+) catechin (391.93 mg $kg^{-1}$) was the most abundant one. Flores *et al*. (2015) have detected ellagic acid, quercetin, myricetin, isorhamnetin, quercitrin and 1-*O*-trans-cinnamoyl-â-D-glucopyranose as phenolic components in guava. In another study, Flores *et al*. (2013) have determined the amounts of ellagic acid and quercetin as 10.50 and 9.20 $mg^{-1}$ g dry extract, respectively, by HPLC in Costa Rican guava fruit. Simultaneous determination of thirteen bioactive phenolic compounds (gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ellagic acid, catechin, syringic acid, vanilic acid, ferulic acid, trans-cinnamic acid, rutin, quercetin and kaempferol) by HPLC-PAD in guava fruit has been achieved by dos Santos *et al*. (2017). Among them, first six phenolic compounds were identified in our varieties also. The highest concentrations were obtained for ellagic acid (5.72 to 30.60 mg $100^{-1}$ g DW), gallic acid (1.27 to 5.62 mg/100g DW), rutin (5.02 to 45.02 mg $100^{-1}$ g DW) and catechin (1.89 to 13.07 mg $100g^{-1}$ DW). The values for gallic acid and catechin are in between and that of ellagic acid is lower in the present investigation. Using HPLC coupled with tandem mass spectrometry (HPLC-MS/MS), Chiari-Andréo *et al.* (2017) have identified 10 phenolic compounds (most of them are in ester form) in Brazilian guava. HPLC coupled with MS or mass spectrometry has recently been employed to detect 69 phenolic compounds in peel, flesh and seed of guava by Liu *et al.* (2018) where flavonoids, hydrolysable tannins, phenolic acid derivatives and benzophenones were

Table 1. The amounts of various phenolic components in six guava varieties

| **Varieties** | **Phenolic components (mg 100 $g^{-1}$)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gallic acid | Chlorogenic acid | Catechin | Epicatechin | Caffeic acid | Ellagic acid | *p*-Coumaric acid |
| Allahabad Safeda | 2.53 | 7.00 | 4.40 | ND | 2.13 | 1.42 | 0.16 |
| Sardar | 5.37 | 1.27 | 3.47 | 3.10 | ND | 2.88 | 0.37 |
| Shweta | 8.03 | 9.00 | 3.10 | 6.00 | 1.33 | 1.46 | 0.14 |
| Dhawal | 6.17 | ND | 3.02 | 3.70 | 2.31 | ND | 0.43 |
| Lalima | 3.70 | 8.00 | 2.57 | 2.20 | 1.62 | 1.33 | 0.70 |
| Lalit | 6.53 | 2.00 | 6.00 | ND | 2.46 | 5.93 | 0.90 |
| LSD ($p \leq 0.05$) | 0.771 | 0.819 | 1.408 | 1.318 | NS | 0.973 | 0.218 |
| CV | 8.041 | 8.255 | 21.118 | 18.792 | 43.891 | 20.552 | 27.217 |

ND = Not detected; NS = Non-significant; CV = Coefficient of variation

the predominant phenolic compounds. The concentrations of bioactive phenolic components in fruits depend on various factors like degree of maturity, variety, climate, soil composition, geographic location, etc. which explains the discrepancy between data from different studies for the same fruit.

**Profiling of organic acids including ascorbic acid**

As ascorbic acid can also be considered as an organic acid, a simultaneous estimation procedure was standardized by RP-HPLC for this and other organic acids like oxalic, citric, tartaric and malic acids. For organic acids, the highest concentrations (mg $100g^{-1}$) were observed for citric acid (192.50 to 460.00) followed by ascorbic acid (146.00 to 234.80), while the lowest for malic acid (1.55 to 2.50) (Table 2). All five organic acids were detected in all six guava varieties. The content of ascorbic acid was noticed maximum (234.80 mg $100g^{-1}$) in Dhawal followed by Allahabad Safeda (198.80 mg $100g^{-1}$) and Shweta (171.80 mg $100g^{-1}$). Similarly, the concentrations of citric acid and malic acid were recorded maximum in Lalima (460.00 and 2.50 mg $100g^{-1}$ respectively), whilst that of tartaric acid (71.00 mg $100g^{-1}$) and oxalic acid (18.50 mg $100g^{-1}$) were found maximum in Sardar and Dhawal, respectively. All the guava varieties were found good to moderate source of organic acids and ascorbic acid. The concentrations of various organic acids vary significantly among the varieties. Dhawal is the richest source of organic acids with very good amounts of citric and ascorbic acids followed by Sardar and Lalima, while Allahabad Safeda is the poorest one. The total concentrations of all identified organic acids are almost at par in Shweta and Lalit. It was noticed in the present investigation that white-fleshed guava fruits (Dhawal, Allahabad Safeda, Shweta and Sardar) possessed higher ascorbic acid contents than pink-fleshed guava fruits (Lalit and Lalima) (Table 2).

Table 2. The concentrations of different organic acids including ascorbic acid in guava varieties

| Varieties | Organic acids (mg/100g$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Oxalic acid | Citric acid | Tartaric acid | Malic acid | Ascorbic acid |
| Allahabad Safeda | 14.75 | 192.50 | 60.00 | 1.75 | 198.80 |
| Sardar | 10.25 | 445.00 | 71.00 | 1.85 | 155.30 |
| Shweta | 14.00 | 422.50 | 64.25 | 1.78 | 171.80 |
| Dhawal | 18.50 | 440.00 | 62.00 | 1.55 | 234.80 |
| Lalima | 18.00 | 460.00 | 52.50 | 2.50 | 154.00 |
| Lalit | 12.25 | 432.50 | 61.75 | 2.30 | 146.00 |
| LSD ($p \leq 0.05$) | 3.489 | 55.061 | NS | NS | 17.415 |
| CV | 16.056 | 9.925 | 16.838 | 26.770 | 6.632 |

NS = Non-significant; CV = Coefficient of variation

Very few studies have been conducted on determination of organic acids in guava. Six non-volatile organic acids in guava fruits (lactic, malic, citric, ascorbic, galacturonic and succinic acids) were isolated and identified using TLC and GLC (Chan *et al*., 1971). The authors have reported that in cultivated guava citric and malic acids present in almost equal amounts and lactic acid in much lesser amount, however in wild guavas, citric acid was the predominant acid with lesser amounts of malic and lactic acids. HPLC analysis revealed the presence of glycolic, malic, ascorbic and citric acids in five Florida grown guava cultivars where citric being the major acid in all five cultivars and traces of fumaric acid was also detected in guava fruits (Wilson *et al*., 1982). This result is at par with the present investigation where citric acid was also found as the major organic acid. The authors have also reported that ascorbic acid content varied more than tenfold (0.04 – 0.44%) among the cultivars. Tandon *et al*. (1983) have reported that among the varieties evaluated, Behat Coconut was found to be richest in ascorbic acid content (295.70 mg/100g) followed by Karela (267.50 mg $100g^{-1}$) and Sardar (254.70 mg $100g^{-1}$), while Gunees was the poorest source (58.20 mg $100g^{-1}$). Ascorbic acid content in guava varies significantly between the cropping seasons and cultivars and winter season fruits have considerably higher amounts of ascorbic acid than rainy season fruits (Chauhan *et al*., 1986). It was observed that Allahabad Safeda possessed maximum ascorbic acid (329 mg $100g^{-1}$) during winter season and Sardar (128 mg $100g^{-1}$) during rainy season among various cultivars tested. Ascorbic acid content was found maximum in Chittidar (234 mg $100g^{-1}$ pulp) followed closely by Allahabad Safeda (232 mg $100g^{-1}$ pulp), Red Flesh (226 mg $100g^{-1}$ pulp), Apple Colour (220 mg $100g^{-1}$ pulp) and Arka Mridula (216 mg $100g^{-1}$ pulp) and minimum in Sardar (192 mg $100g^{-1}$ pulp) guavas grown under rainfed sub-humid region of Chhotanagpur plateau, India (Reddy *et al*., 1999). In a performance study of 21 guava cultivars in red and laterite soil of West Bengal under irrigated condition, ascorbic acid content in winter season fruits was found much higher than in rainy season fruits and cv. Supreme contained maximum ascorbic acid in both winter and rainy seasons (183 and 129 mg $100g^{-1}$ pulp, respectively) distantly followed by cv. Florida Seedlings (89 and 61 mg $100g^{-1}$ pulp, respectively), while minimum ascorbic acid content was recorded in cv. Chittidar (33 and 21 mg $100g^{-1}$ pulp, respectively) (Ghosh *et al*., 2013). Similar to our findings, white fleshed guavas contained higher ascorbic acid (84.50 mg $100g^{-1}$) than pink fleshed guavas (70.00 mg $100g^{-1}$) grown in Sudan (Bashir and Abu-Goukh, 2003). Dina *et al*. (2014) have also reported that ascorbic acid content in white guava varieties grown in Sudan was higher (250.80 mg $100g^{-1}$) than in pink guava varieties (190.70 mg



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$100g^{-1}$). Similarly in Florida, USA the red fleshed guava was found to have lower ascorbic acid content (122.30 mg 100 $g^{-1}$ puree) as compared to white fleshed guava (201.00 mg $100g^{-1}$ puree) (Baldwin *et al*., 2008). Ascorbic acid contents were estimated as130 and 112 mg $100g^{-1}$ FW in white (cv. Pansithong) and red guava (cv. Samsi), respectively, in Thailand (Thuaytong and Anprung, 2011).

## CONCLUSION

It was concluded that Dhawal and Allahabad Safeda were the best of guava for consumption in terms of ascorbic acid, while Shweta and Lalit for the presence of anticancer antioxidant phenolic components.

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# Development of value added products from subtropical peach

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**ABSTRACT**

The laboratory experiment conducted with the aim of developing value added products from Peach (*Prunus persica*), a soft, juicy and fleshy stone fruit, generally consumed as fresh, resulted in development of a schematic model for processing the fruit in preparing a variety of food products. Fresh fruit slices subjected to lactic acid fermentation in brine solution using *Lactobacillus* sp for two days led to production of probiotic drink having $1.37 \times 10^5$ CFU $ml^{-1}$ living population of *Lactobacillus* Sp. The left over slices were utilized in the preparation of sweet candy by dipping these in the increasing concentrations of sugar syrup and later drying to an intermediate moisture level of 12 per cent. The excessive softened slices were utilized in preparation of chutney using various spice ingredients. The sugar syrup left was utilized in the preparation of squash, wine and vinegar. Peach probiotic drink so prepared possessed 0.63 per cent lactic acid and 31.5 mg 100 $ml^{-1}$ phenolics, The wine contained 9.73 per cent ethanol, 10.2 °B TSS, 1.38 per cent acidity and 143.5 mg 100 $ml^{-1}$ phenolics, 5.41 per cent acetic acid and 96.8 mg 100 $ml^{-1}$ phenolics. Peach chutney possessed 78.4 mg 100 g $phenolics^{-1}$. All the products showed good organoleptic acceptability scoring above 7.0 out of total 9.0. The seeds were used for sowing and raising the fruit crop further.

**Key words:** Peach, probiotic drink, candy, chutney, *Lactobacillus*

Peach (*Prunus persica* L.) is a small, soft, juicy drupe or stone fruit, related to plum, apricot cherry and almond, with a sweet white or yellow flesh The fruit bears an attractive color ranging from red and white to purple with pleasant aroma and tangy taste. Different peach varieties contain 86-89 per cent water, 7.5-8.4 per cent sugars, 0.6-1.2 per cent protein, 0.3 per cent fat and 1.2 per cent fibre. The fruit possesses 1-27 mg 100 $g^{-1}$ vitamin-C, 0.8 per cent minerals and 30-140 mg 100 $g^{-1}$ phenolics, while white and yellow flesh varieties contains 0.19-0.79 per cent total carotenoids and 20-178 mg 100 $g^{-1}$ leucoanthocyanins (Joshi and Bhutani, 1995). It also possesses many essential nutrients but relatively in low proportion. The vitamins, fibre, phenolics and pigments present in it help in maintaining balanced diet. These elements also have high level anti-oxidants that is effective at protecting oxidative damages.

Peaches are generally consumed a fresh or can be added to a variety of dishes. It is also widely used in cosmetic industries for making creams, moisturizer, scrub and face wash. Efforts of processing this fruit in India is minimal. Sortino *et al.* (2017) studied shelf life of fresh cut peach cv. 'Ruby Rich'. Sharma *et al.* (2002) worked on canning of peach-halves in fruit pulp and juice. Arora and Aggarwal (2009) prepared carbonated and non-carbonated beverages from peach pulp. Similarly, Saleem *et al.* (2011) processed peaches in to squash. Frozen and dehydrated peaches and peach juice sometimes seen in the market. The present papers deals with development of a schematic model for production of processed products viz. probiotic drink, candy, squash, chutney, vinegar and wine from single raw material in a step-wise manner.

## MATERIAL AND METHODS

Healthy, mature peach fruits of cv. Sharbati harvested from ICAR-CISH, Lucknow experimental orchard, were washed thoroughly under tap water in the laboratory, cut into slices, seeds removed and later used for preparation of different peach products as per the method illustrated in Fig. 1.

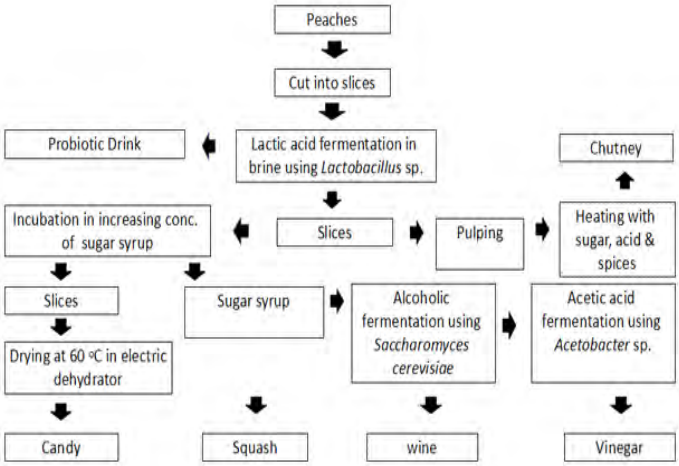


Fig. 1: Flow diagram for preparation of products from peach fruits

*Probiotic drink* was prepared as per the method described by Garg *et al.* (2015) for preparation of cucumber probiotic drink. Slices were subjected to lactic acid fermentation in brine solution under ambient conditions using *Lactobacillus* sp. maintained in Microbiology Laboratory. Two days after fermentation, when desired level of lactic acid is achieved, the slices were separated out and the probiotic drink was collected, filled in clean dry glass bottles, sealed and later stored at room temperature.

The left over slices were divided in to two batches. One batch with firm slices was used in preparation of candy by the method developed by Tandon *et al.* (2004). Slices were dipped in 50° B sugar syrup containing 0.5 per cent citric acid overnight. These were separated from the syrup the next day. The syrup was reconstituted by heating and addition of extra sugar where the slices were dipped again and incubated for overnight. Finally, slices were separated, rinsed in warm water and kept for drying in electric dehydrator at 60°C till the attainment of an intermediate moisture level.

The squash and chutney were prepared as per the method described by Ravani and Joshi (2013) with modifications. The second batch comprising of loose slices were homogenized in to pulp, heated and added with salt, sugar, onion, garlic and Indian spices to form chutney. The left over sugar syrup from candy preparation was utilized for preparation of squash and wine. The squash was prepared by heating and adjusting sugar and acid contents to 50° B and 0.9 per cent, respectively. Wine was prepared through alcoholic fermentation of sugar syrup using *Saccharomyces cerevisiae* cultured in the Microbiology Laboratory. The protocol of Yadav *et al*. (2017) was adopted for preparation of wine. A portion of peach wine was further processed to vinegar through acetic acid fermentation using immobilized *Acetobacter* sp. bacteria on bamboo pieces as per the method developed by Garg *et al*. (2014).

The peach products were analyzed for physico-chemical attributes. Total soluble solids (TSS), titratable acidity and vitamin-C were done as per the methods described by Ranganna (2000). TSS was recorded by using hand refractometer (Erma, Japan) while acidity was done by titrating the sample solution against 0.1N NaOH solution using phenolphthalein as indicator. The acidity was represented in term of lactic acid in case of probiotic drink, as acetic acid in vinegar and as citric acid in other products. Vitamin-C content was estimated using titration of sample solutions against 2, 6-dichlorophenol indophenol dye solution. Total phenolic content was estimated by using method evolved by Folin and Ciocalteu (1927). The concentration of ethanol in peach wine was measured spectrophotometrically as per the method of Caputi *et al*. (1968). The sensory evaluation of products was carried out by a panel of semi-skilled judges on composite scoring (Amerine *et al*., 1965) based on colour, aroma and taste of samples. The microbial counts for bacteria, yeast and mould in products were observed as per Speck (1985) method.

The samples were tested in three replicates and data was analyzed statistically for mean value and standard deviation using microsoft excel.

## RESULTS AND DISCUSSION

The probiotic drink was light pink in colour due to extraction of some anthocyanins from slices into the drink. The *Lactobacillus* bacteria count in the product was found to be $1.37 \times 10^5$ CFU $ml^{-1}$. The TSS content was found to be 3.8 °B while acidity was 0.63 per cent (Table 1). The drink contained very low vitamin-C content (1.6 mg 100 $ml^{-1}$). The total phenolics was found to be 31.5 mg 100 $ml^{-1}$ (Fig. 2). With mild acidic taste, the product scored 8.0 out of 9 during sensory evaluation by a panel of 7 semi-skilled judges (Fig. 3). Physical observation of candy prepared from left over slices of peach revealed its soft texture with moisture content of 12.3 per cent. Bhattacherjee et al. (2013) prepared aonla candy having moisture content of around 11 per cent. The chemical analysis of all the products revealed that TSS content was highest in candy (69 °B), followed by squash (52 °B) and chutney (40 °B). The titratable acidity was highest in vinegar (5.41%), then chutney (2.55%) and wine (1.38%). The vitamin-C contents of peach products other than probiotic drink was in negligible amounts. It may be due to reason that fruit contains low amount of vitamin-C and most of it extracted into brine during fermentation process of probiotic drink preparation. Heating process involved in preparation of other products further destroyed its content to negligible limit. El-Ishaq and Obirinakem (2015) also reported reduction in vitamin-C of fruit juices during heating. Candy contained maximum phenolic content (182 mg 100 $g^{-1}$) followed by wine

Table 1: Chemical characteristics of different peach products

| Product | Total soluble solids (°B) | Titratable acidity (%) | Vitamin-C (mg 100 $g^{-1}$ or ml) |
|---|---|---|---|
| Probiotic drink | 3.8±0.00 | 0.63±0.01 | 1.6±0.51 |
| Candy | 69.0±0.60 | 0.94±0.03 | Trace |
| Squash | 52.0±0.20 | 0.96±0.00 | Trace |
| Vinegar | 7.6±0.00 | 5.41±0.22 | Trace |
| Chutney | 40.0±0.40 | 2.55±0.18 | Trace |
| Wine | 10.2±0.00 | 1.38±0.02 | Trace |

Mean values ± standard deviation



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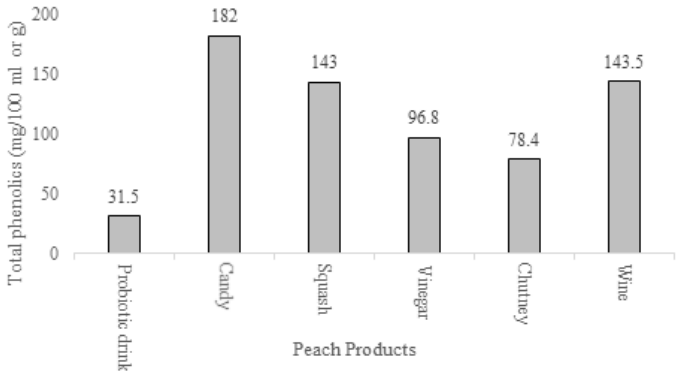
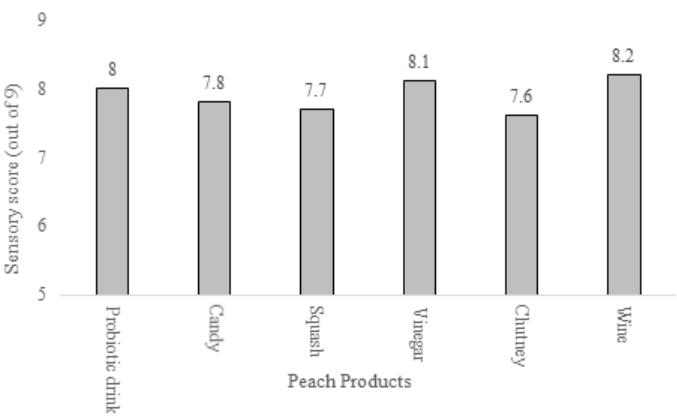



[Standard Deviation : Prob. drink ± 0.5; Candy ± 5.0; Squash ± 2.0; Vinegar ± 3.7; Chutney ± 1.7; Wine ± 0.8]

Fig. 2: Total phenolic contents of peach products




[Standard Deviation : Prob. drink ± 0.52; Candy ± 0.38; Squash ± 0.2; Vinegar ± 0.45; Chutney ± 0.1; Wine ± 0.4]

Fig. 3: Sensory evaluation of peach products

(143.5 mg 100 $ml^{-1}$). Cakar et al. (2016) reported total phenolics content (TPC) range of 584-2414 mg GAE/L for different berry fruits. Peach wine was clear brownish in colour and astringent in taste owing to presence of 9.73 per cent ethanol content besides fair amount of phenolics. Lee (2015) prepared peach wine containing up to 9.2 per cent alcohol. All peach products was found to have good acceptability obtaining sensory scores above 7.0.

## CONCLUSION

A schematic model developed for processing of peach fruit to prepare various peach food products viz., probiotic drink, squash, candy, chutney, vinegar and wine, from a single raw material, leaving no by-product unutilized, was recommended to be adopted by the processing industries.

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**Short communication**

# Impact of cement factories in saffron cultivation Pulwama district of Kashmir

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**ABSTRACT**

Investigations on the impact of cement factories on saffron farming in Kashmir valley, carried out through group discussions among 30 respondents of three saffron growing villages, Khrew, Khanmoh, and Wuyan, revealed that the pollution caused by cement factories immensely affected its corm, stigma and petals resulted into sharp decline in saffron production, productivity, marketing and the industry in particular. The study suggested strict enforcement of environmental policies and procedures by the government pollution management agencies of the area.

**Key words:** Impact, cemnt factories, saffron

Saffron (*Crocus sativus* L.), the world's most expensive perennial flower cash crop (Hill, 2004), is cultivated in Iran, Spain, India, Greece, Italy, Turkey, France, Israel, Pakistan, Azerbaijan, Switzerland, China, Egypt, Japan, Afghanistan, Iraq, UAE and Australia (Nehvi et al., 2007). The purple coloured flowers containing six petals, three stamens, and three red stigmas are predominantly used as species and a colouring as well as flavouring agent. Saffron export is a regular article of International trade (Wani, et al., 2008; Yasmin and Nehvi, 2013). Iran producing 89 per cent of the world's production and 90 per cent of the export yearly dominates both in terms of area and production, while Jammu & Kashmir's contribution is just 5 per cent (15 MT). Spain is the main market for Kashmir saffron which is followed by France, USA, UK, UAE, Israel, etc. The decline in saffron production, productivity, area and exports in the past many years and with every passing day around cement factories in Jammu and Kashmir Valley necessiated studies on the impact of cement factories and the problems in the saffron industry with regard to its industrialization in the area. It is the main source of environmentally unfriendly practices and environmental pollutions. Their aerial discharge comprises of particulate matter, Nitrogen Oxides, and Sulphur Dioxide which bring into being continuous and clear clouds that at last decompose in the soil. The full biotic life everywhere is affected thereby unpredictably exploiting the whole ecosystem around the cement factory.

Unplanned discharges of atmospheric pollutants, mounting population and urbanization that are influenced by strong industrialization result in injury and damage to the environment. The intensity and nature of the damage is a function of the concentration of the pollutant and the duration of exposure (dose). This has immediate (acute) and long-lasting (chronic) impact on the quantity and quality of agricultural crops causing significant changes in agricultural output. The first and foremost argument advanced by the cement industry entrepreneurs is that the industry did not emit any pollutants at all and that what is added to the environment is only dust and nothing beyond that. The truth outrageously is otherwise as confirmed from the findings of the study. All the manufacturing units of cement factory including a kiln, raw mill, cement mill, and coal mill are root causes of pollution. In addition, some other activities associated with post-manufacturing stages like open-air handling, loading and unloading etc. also release dust into the environment which is called fugitive emissions. On similar grounds the cement factories in the sample areas affected the saffron cultivation and community at large.

The present study is based on primary data. It adopted *Purposive sampling* for the selection of respondents. Thirty respondents, 10 each from three of 29 villages, namely Khrew, Khanmoh and Wuyan of Pampore tehsil of district Pulwama, located in the vicinity of cement factories, were selected. These villages produce more than 80 per cent of the total production of saffron in Kashmir vallage. Three informers having full knowledge in the village environments and nitty-gritty of the problems from each of the village were identified. Group discussions on problems associated with the setting up of factories in saffron growing areas of Khrew, Khanmoh and Wuyan were held.

Major problems identified through discussions were emission of a large amount of lethal fumes and particulate matter per day from the cement factories, inefficient pollution control devices being adopted in the factories, lack of *in-situ* monitoring devices, development of tremors when machinery is in operation and during blasting in quarries and damage to the landscape from quarrying. The cement industry ranked 17th in the pollution. Khrew area was reported to release one lack kilograms of lethal fumes per day thereby wrapping the neighbourhood villages in a toxic grey blanket. Ecologists reported that concentrations of fine dust, aerosol, and soot particles of less than 10 microns in diameter contributing to bad are in the Knew and other factory areas of the major pollutant. Some experts and locals revealed that the production of saffron per Kanal has reduced from the normal 150 gram to 50 grams. The respondents of the area reported that Khrew area of Pampore town has very high capability to grow saffron per year (as much as 250 Kg) but with the onset of cement factories in Khrew which are in close proximity with saffron villages like Pakhribal and Nagadore, the production declined to a level of 50 kg. There are a considerable amount of metals like nickel, chromium, lead, cobalt, and mercury in the cement dust that are hazardous to the environments, predominantly biotic environs with very high proportional change in animal life and plant life due to proportional change in pollution (Darley, 1996). Emissions of Carbon dioxide take place during cement manufacturing due to decarbonisation of Calcium carbonate and Magnesium carbonate and burning of fossil fuels. Oxidation of sulphur in fuel generates sulphur dioxides and combination of oxygen and nitrogen at high temperature in the burning zone generates Nitrogen oxides. The cement factories, therefore, result in the emission of a large number of hazardous pollutants that retard agricultural production and affect the quality of life of the community residing there in the area. In addition, there is a sharp decline in saffron production and productivity with the onset of factories, saffron adulteration, large intermediaries in the saffron industry, low education of saffron growers, and asymmetric market information. In fact, all these factors are interrelated in the sense that the establishment of factories generate pollution that decline production and productivity of saffron which in turn trigger adulteration and large chain of intermediaries alongside exploiting the growers to a great extent.

## RECOMMENDATIONS

1) Management of pollution in general and air pollution in particular, being the need of the hour, be taken up by all the government and non-government agencies to manage the menance.

2) Saffron being an expensive crop and highly prone to pollution, the growers around cement factories be enlightened to check the saffron quality as per International Organization for Standardization (ISO) criteria and sell only the un-adultered product.

3) Traders in the saffron growing areas must be provided with better market knowledge for taking the best business.

## CONCLUSION

The study concluded that the cement factories adopting inadequate pollution contral measures surrounding Khew, Khonmoh and Wuyan villages of Pampore tehsil of Pulwama district in Kashmir affected saffron corn, stigma and petals leading to drastic reduction in saffron agronomy in general and its production, productivity and marketing in particular. The study recommended that government pollution management agencies must ensure strict enforcement of eco-friendly and sustainable laws concerning the production of cement dirt at administrative levels.

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