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Antifungal activity of *Datura stramonium*, *Calotropis gigantea* and *Azadirachta indica* against *Fusarium mangiferae* and floral malformation in mango

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Abstract Floral malformation caused by *Fusarium mangiferae* is a serious threat to mango cultivation in various countries. Different long-term measures suggested to control it were found to be unsuccessful. Present studies clearly showed strong antifungal activity of a concoction brewed from *Datura stramonium*, *Calotropis gigantea, Azadirachta indica* (neem) and cow manure (T_1) followed by methanol-water (70/30 v/v) extracts of *Datura stramonium*, *Calotropis gigantea* and *Azadirachta indica* (T_2) against *Fusarium mangiferae*. Optimal control of floral malformation was found in trees sprayed with T_1 followed by T_2 at bud break stage and again at fruit set stage when compared with the control. All the malformed buds or panicles completely dried two days after foliar spray with T_1 or T_2. In the trees treated with T_1 at fruit set stage, flower abscission was observed from the fourth day after spraying and all flowers dropped by the ninth day without requiring any manual de-blossoming, whereas in the control, the malformed panicles remained green and competed with the growing fruits for plant nutrients. *In vitro* culture of fresh malformed tissues in MS media along with T_1 or T_2 showed no growth of any fungus in the media. However, *in vitro* culture of the completely dry malformed tissues in MS media after foliar treatment with T_1 or T_2 revealed growth of *F. mangiferae* on the twenty fifth day indicating that the concoction-brewed compost (T_1) or methanol-water (70/30 v/v) extracts (T_2) could not completely eliminate the pathogen but helped in controlling malformation by suppressing the activity of *F. mangiferae*. Mango trees sprayed with T_1 and T_2 revealed significant differences in percent fruit set and retention when compared with the control. This could be due to observed higher levels of nitrogen, phosphorus, potassium, calcium, magnesium, copper, zinc, iron and manganese in T_1, followed by T_2 when compared with T_3 (control). Among the different fruit quality parameters analysed, the total flavonoids were found to be significantly higher in T_1 and T_2 when compared with T_3. The study proved that the concoction-brewed compost (T_1) is effective, inexpensive, easy to prepare and constitutes a sustainable and eco-friendly approach to control floral malformation in mango when it is sprayed at bud break stage and again at fruit set stage.

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In this present study, exogenous treatment of emerging buds with (T_c) further proved that with increase in the number of malformed panicles/tree the number of buds developing into healthy panicles/tree decrease.

Keywords Benzyl adenine · Calotropis · Datura · Floral malformation · Fusarium mangiferae · Mango · Neem · Paclobutrazol

Introduction

Floral malformation is a serious threat to mango cultivation in various countries. Economic losses of up to 60% have been reported in different commercial varieties of mango in India. Although malformation was first reported in India in 1891 (Kumar and Beniwal 1992), it has subsequently been reported from Egypt, South Africa, Sudan, Swaziland, Brazil, Central America, Mexico, USA, Israel, Pakistan and Malaysia (Manicom 1989; Ploetz 2001). Malformation causes gross deformation of vegetative and floral tissues in mango (Ploetz 2001). Affected flowers are either sterile or abort shortly after fruit have set; as a consequence fruit yields are usually significantly reduced (Zheng and Ploetz 2002).

The etiology of malformation has been a contentious issue, and a wide range of biotic and abiotic factors have been reported to cause the disease, including viruses, mites and nutritional deficiencies (Ploetz 2001). Convincing evidence that a fungus causes malformation has been in the literature for decades (Crookes and Rijkenberg 1985; Chakrabarti and Ghosal 1989; Usha *et al*. 1994, 1997; Steenkamp *et al*. 2000; Ploetz 2001). In India, researchers were the first to report that *Fusarium moniliforme* (recognized later as *F. subglutinans*) was the cause of the floral (Summonwar *et al*. 1966) and vegetative (Varma *et al.* 1974) forms of the disease. The taxonomy and nomenclature of *F. subglutinans* has undergone considerable change since the monograph of Nelson *et al.* (1983) was published. Morphological features that had been used to identify '*F. subglutinans*' in the past clearly do not distinguish the different phylogenetic species of which it is comprised. Using the phylogenetic species concept, O'Donnell *et al*. (1998, 2000) identified many new species within the *G. fujikuroi* species complex. Several of the new species were previously named *F. subglutinans*, including one isolate of the mango malformation pathogen from India. Steenkamp *et al*. (1999, 2000) examined phylogenetic relationships in the malformation pathogen with ß-tubulin and histone H3 gene sequences. They indicated that a group of isolates from Florida, India, Israel and South Africa were closely related and were conspecific with isolates of '*F. subglutinans'* that had previously been shown to cause mango malformation worldwide (Ploetz and Gregory 1993; Freeman *et al*. 1999). Recently, these isolates were described as members of a new species, *Fusarium mangiferae* Britz, Wingfield and Marasas sp. Nov. (Britz *et al*. 2002). This new species was established based on nuclear and mitochondrial DNA sequences; it included strains of *F. subglutinans* from Egypt, Florida, Israel, Malaysia, and South Africa, some of which had been shown to cause mango malformation disease by artificial inoculation (Britz *et al*. 2002; Marasas *et al*. 2006). *Fusarium mangiferae* proved to be the dominant fungus in Punjab, with 100% of malformed samples infected with this fungus (Zafar *et al*. 2006). Frequent recovery of *F. mangiferae* from malformed trees grown in different ecological zones of the world has been shown (Britz *et al*. 2002; Marasas *et al*. 2006; Youssaf *et al.* 2006; Zafar *et al*. 2006). As malformed shoots show elevated levels of infection compared with non-malformed ones, it is suggested that symptom manifestation occurs only after massive colonisation by the fungus *F. mangiferae* (Ploetz and Gregory 1993; Zafar *et al*. 2006).

Different measures suggested to control *F. moniliforme* var. *subglutinans* (for example, the application of growth regulators, nutrients, acaricides fungicides and pruning the infected tissues) have met with little success (Kumar *et al*. 1993). The effectiveness of using composts for disease control, particularly against fungal pathogens, has been studied extensively (Weltzein 1991; Elad and Shtienberg 1994; Cronin *et al*. 1996; Hoitink *et al*. 1997). Composts of various kinds have been used to reduce the incidence of *Pythium and Rhizoctonia* (Weltzien and Ketterer 1986) in different fruits and vegetables. Stindt and Weltzien (1988) at the University of Bonn achieved effective control of *Botrytis cinerea* in strawberries as well as blight in potatoes. Powdery mildew and root rot were significantly reduced in peas and beets in other trials in Germany (Thom and Moller 1988).

The alkaloids and terpenoids of *Datura* and *Calotropis* are well known for their medicinal

properties in ayurveda (Duke 1985). Products based on *Azadirachta indica* (neem) are also known for their antifungal and pest control properties. (Singh 2003). Hence present investigations using concoction brewed from datura, calotropis, neem, cow manure and solvent extracts of datura, calotropis and neem were initiated to see their effect on *F. mangiferae* and floral malformation, when sprayed at three different periods. The study was further extended to see their effect on fruit set, retention and fruit quality parameters measured at maturity. Attempts were also made to artificially induce the symptoms of malformation under field conditions.

Materials and methods

Plant material

Mango cv. Amrapali (Dashehari x Neelam) which is a regular bearing, short-stature tree suitable for high density planting and highly susceptible to floral malformation was selected for this study. The experiment was conducted on twelve year-old mango trees planted at a distance of 3.0×3.0 m and having uniform growth and vigour with a canopy height and spread of 3.4 and 3.2 m respectively in the experimental orchard, Division of Fruit and Horticultural Technology, Indian Agricultural Research Institute, New Delhi, India for two consecutive years (2005 to 2006). Ten trees were randomly selected for each spray treatment and each treatment was sprayed at three different periods (n=30). A randomised block design was adopted and the data was statistically analysed employing analysis of variance (ANOVA). Duncan's multiple range test was used to differentiate means Duncan (1955). To avoid the residual effect of the treatment in the following season, the same trees in each treatment were used in the following year of the experiment.

In our preliminary trials (2001–2004), aerial parts of datura, calotropis and neem (5 kg, 2.5 kg, 1 kg and 0.5 kg fw) combined with cow manure were fermented for 30 days in 10 l of water, under both aerobic and anerobic conditions. In the absence of adequate oxygen supply, a foul-smelling, anaerobic soup was formed. One kg and 0.5 kg fw of the aerial parts of datura, calotropis and neem, brewed under aerobic conditions took less time for fermentation and were found to be equally effective in controlling mango malformation when compared with other plant weights used. The aerial parts (leaves, stem, flower, fruit and seed) of datura, calotropis and neem when used separately, were not effective in controlling mango malformation when compared with their combined use *in vivo*. Hence further studies were repeated for the next two years (2005–2006) using 0.5 kg fw of selected aerial parts of datura, calotropis and neem as mentioned below.

Preparation of solvent extracts

To isolate the biologically-active part of plants, different aerial parts of datura (100 g leaf, 100 g stem, 100 g flower, 100 g fruit and 100 g seed fw), calotropis (100 g leaf, 100 g stem, 100 g flower, 100 g fruit and 100 mg seed fw) and neem (100 g leaf, 100 g stem, 100 g flower, 100 g fruit and 100 g seed fw) were collected separately and ground into a fine paste in a mortar with hexane, methanol or methanol-water (70/30 v/v). The extracts were then centrifuged at 10,000 *g* for 20 min and the solvents hexane and methanol removed by evaporation at room temperature. The residue was re-extracted twice with hexane, methanol or methanol-water (70/30 v/v) and supernatants were pooled and the volume made up to 1 l with distilled water. The volume of the aqueous phase was used for *in vitro* culture as mentioned below. Solvent extracts found most promising to suppress the growth of *F. mangifera* from *in vitro* culture studies were selected for field trials.

Preparation of brewed compost concoction (T_1)

The selected aerial parts of datura, calotropis and neem from results of *in vitro* culture were collected from the wild and ground into a fine paste in a mortar. A slurry was prepared by mixing 500 g of well-rotted cow manure and fresh parts of datura (500 g leaves, 500 g fruits, 500 g seeds), calotropis (500 g leaves) and neem (500 g leaves) in 10 l of water. The slurry was allowed to brew for 15 days under continuous aeration using an air pump and then filtered using muslin cloth. The pH of the filtrate was found to be 6.4. Continuous supply of air helped to enrich the oxygen supply for better decomposition and aerobic microbial population growth (Weltzein 1991). The filtrate which was richly-coloured and odourless was used immediately for spraying without further dilution.

Extent of malformation

Visual comparisons were made on the extent of floral malformation in the selected trees before spray treatment and daily after treatment. The values were expressed as percent floral malformation per tree. All the fully dried malformed panicles were counted separately from each tree after spray treatment. Number of flowers/malformed panicle varied with the size (>900–1,000).Observations on the extent of flower abscission from the dry malformed panicles [complete abscission (>900 flowers) and partial abscission (<400 flowers)] were recorded daily for ten days. In control trees, the malformed panicles which appear during February-March flowering season, continue to remain hanging on the trees till next flowering season**.** Hence to record changes in their development into healthy or malformed panicles during next flowering season in 2006, the malformed panicles were not manually de-blossomed from either treated or control trees after fruit harvest in June 2005.

In vitro culture of malformed tissues

To isolate the biologically-active part of plants, different aerial parts of datura, calotropis and neem were extracted with hexane, methanol or methanol-water (70/30 v/v) as mentioned earlier. Malformed panicles were randomly collected from mango trees and cut into small pieces of 1–2 cm. The tissue segments were first surface-sterilised with 0.1% $HgCl_2$ for 2 min and then washed three times with sterile water to remove any traces of $HgCl_2$. The explants were then aseptically cultured in Murashige and Skoog medium (Usha *et al*. 1997) in sterile bottles with hexane, methanol or methanol-water (70:30 v/v) extracts (5,000 ppm and 10,000 ppm). Malformed tissues were also aseptically cultured in MS medium along with T_1 and T_2 (methanol-water extracts). Explants aseptically cultured in MS medium were treated as the control. Observations on the capacity of different plant extracts and treatments (T_1 and T_2) to suppress the growth of *F. mangiferae in vitro* were recorded daily for 60 days.

Observations on the differential growth of *F. mangiferae* with the increase in concentration of the concoction-brewed compost was recorded by aseptically culturing malformed tissues in MS medium along with T_1 (autoclaved 10%, 20% and 30% concoction-brewed compost) and (10%, 20% and 30% concoction-brewed compost which was not autoclaved before use) for comparison. Each treatment (extract concentration) was replicated ten times and all cultures were randomly incubated at 28°C, with a 12 h photoperiod. Progressive inhibition in the growth of *F. mangiferae* was measured by counting the number of macro and microconidia from randomly collected samples using a haemocytometer (Mather and Roberts 1998).

Similarly malformed tissues collected from mango trees before and after treatment with T_1 and T_2 were also cultured in MS medium. Observations were recorded daily for white cottony mycelial growth of the fungus for 60 days. The fungus was sub-cultured in PDA media for single-spore isolations and was identified as *F. mangiferae* at the Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India.

Foliar spray

Widely accepted regarding the epidemiology of the pathogen is that prior bud colonisation is a prerequisite for infection (Freeman *et al*. 1999; Ploetz and Gregory 1993; Usha *et al*. 1994, 1997; Usha 2005; Zafar *et al.* 2006). Since the pathogen is harboured within the bud and only at the bud break stage will the pathogen reveal itself by inducing the symptoms of malformation, the concoction-brewed compost (T_1) and solvent extracts (T_2) were sprayed on the mango trees at a rate of 3 l/tree (calculated based on the canopy size of tree) at three different stages (a) at flower bud break stage in January, (b) flowering period (February–March) and (c) at fruit set stage in April using a manually-operated foot sprayer, to see its effect on floral malformation. Full details of T_1, T_2 and T_3 are:

T1: Concoction (30%) brewed from *Datura stramonium*, (500 g leaves, 500 g fruit, 500 g seeds), *Calotropis gigantea* (500 g leaves), *Azadirachta indica* (neem) (500 g leaves) and cow manure (500 g) in 10 l water.

T2: Methanol-water (70/30 v/v) extracts of *Datura stramonium* (500 g leaves, 500 g fruit, 500 g seed), *Calotropis gigantea* (500 g leaves) and *Azadirachta indica* (neem) (500 g leaves) in 10 l water.

T3: Water spray (control)

Leaf nutrient status

Nutrient status of 'Amrapali' mango leaves was analysed by collecting four to six month-old leaves from the middle portion of the shoot, 30 days after fruit set. Leaf samples were decontaminated using 0.2% teepol solution, 0.1 N HCl, distilled water and double-distilled water in a series. The samples were dried in a forced-draft hot air oven at 70°C±1°C until a constant weight was reached. The samples were then ground and passed through a 1 mm sieve and stored in air-tight containers, and then analysed for macro and micronutrient content. Total nitrogen was estimated using an auto analyser (Issac and Johnson 1976), phosphorus by the vanadate phosphomolybdate method (Jackson 1958), and potassium using flame photometry (Chapman and Pratt 1961). Calcium, magnesium, manganese, zinc, copper and iron were measured using an atomic absorption spectrophotometer (Jones 1985).

Fruit yield and quality parameters

Ten trees from each treatment were randomly tagged and observations recorded daily on the number of fruits set and retained until harvest. Ten fruits were randomly collected from each treatment for fruit quality analysis at the maturity stage (July). Total soluble solids (TSS) in the freshly extracted juice of randomly selected fruits were measured with the help of a hand refracto meter and expressed as °Brix at 20°C (Ranganna 1986). Total titratable acidity was estimated by taking 10 ml of juice and titrating it against 0.1 N NaOH using a phenolphthalein indicator (Ranganna 1986). The results are expressed as percent acidity in terms of tartaric acid. The total sugar content (%) of the pulp was determined by the Lane and Eynon method (Ranganna 1986).

Total phenols

Total phenolics were determined using the Folin–Ciocalteau reagent (Singleton and Rossi 1965). Fruit samples (2 g) were homogenised in 80% aqueous ethanol at room temperature and centrifuged in the cold at 10,000 *g* for 15 min and the supernatant saved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated at room temperature. The residue was dissolved in 5 ml distilled water. One-hundred µl of this extract was diluted to 3 ml with water and 0.5 ml Folin–Ciocalteau reagent added. After 3 min, 2 ml 20% sodium carbonate was added and the contents mixed thoroughly. The colour was developed and absorbance measured at 750 nm in a Bausch and Lomb spectronic-21 UVD spectrometer after 60 min using Gallic acid as a standard. The results were expressed as mg of Gallic acid 100 g^{-1} fresh weight.

Flavonoids

The flavonoid content in the sample was estimated by spectrophotometric measurement of the colour at 360 nm from an 80% aqueous ethanol extract of the sample (modified method of Mazza *et al*. 1999). A known weight (2 g) of the sample was extracted with 80% aqueous ethanol (of known volume) using a pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant saved; 5 ml of this supernatant was taken in an evaporating dish and evaporated to dryness at room temperature. The residue was dissolved in 250 µl of 0.1% HCl in 95% ethanol and 4.55 ml of 2% HCl. The solution was mixed and allowed to settle for approximately 15 min. Quercetin in 95% ethanol was taken as the standard and absorbance was measured at 360 nm. Flavonoid content was expressed as mg 100 g^{-1} fresh weight.

Total carotenoids

Total carotenoids were determined by a modified method of Ranganna (1986). A known weight of the sample was ground with acetone with a pestle and mortar. The extract was decanted into a conical flask and continued until the residue was colourless. Collected extracts were then transferred into a separating funnel and 25 to 30 ml of stabilised petroleum ether containing 0.1% butylated hydroxytoluene (BHT) was added with 5-10 ml of 5% sodium sulphate. Petroleum ether was repeatedly used until all the colour was transferred into a petroleum ether layer; this was transferred into a volumetric flask and the volume made up to 50 ml with petroleum ether. Absorbance was measured at 450 nm using petroleum ether as the blank and the total carotenoid content was expressed as 100 g^{-1} fresh weight.

ß-carotene

ß-carotene was separated by column chromatography as described by the Carotene Panel of the Sub-committee on Vitamin estimation (1955), with suitable modifications. A known weight of the sample was repeatedly extracted with acetone using a pestle and mortar until the residue was colourless. The pooled acetone extracts were transferred into a separating funnel and about 20 ml of stabilised petroleum ether containing 0.1% BHT was added and mixed gently. About 20 ml of 5% sodium sulphate solution was added and the separating funnel shaken gently. The two phases were separated and the aqueous phase was re-extracted with petroleum ether until colourless. The pooled extracts were evaporated in vacuum at 30°C and taken in petroleum ether. The extract was passed on a 10 cm×1 cm column of alumina with 1 cm layer of sodium sulphate at the top. ß-carotene was eluted with 2 parts of acetone in hexane, measured at 450 nm and values were expressed as mg 100 g^{-1} fresh weight.

Ascorbic acid

Ascorbic acid was determined by titrating a known weight of sample with 2, 6-dichlorophenol indophenol dye using metaphosphoric acid as a stabilising agent (Albrecht 1993). Known weight of the sample was ground using a pestle and mortar with 3% metaphosphoric acid. Volume was made up to 100 ml and filtered. Ten ml of the aliquot was titrated against 2,6-dichlorophenol indophenol dye. The dye factor was calculated by titrating standard ascorbic acid solution against dye, and ascorbic acid content of the sample was expressed as 100 g^{-1} fresh weight.

Development of emerging buds into healthy or malformed panicles under field conditions

In the present study, we observed a decrease in the number of healthy panicles with an increase in the number of malformed panicles per tree. In order to understand this phenomenon the following experiment was carried out.

Ten Amrapali mango trees of uniform growth were randomly selected and the emerging buds (50/tree) were randomly tagged. Observations were recorded on the development of buds into healthy panicles or malformed panicles. Attempts were also made to artificially induce the symptoms of malformation under field conditions.

Artificial induction of mango malformation under field conditions

One year-old mango shoots (20 cm length) were randomly tagged in selected mango trees. The selected shoots were defoliated and the shoot apex was decapitated to induce outgrowth of axillary buds. The selected shoots were sprayed with bavistin (1%) for two weeks on alternate days to prevent fungal infection before treatment.

The number of emerging buds after decapitation of the shoot apex varied from 4–11/shoot (rarely >20). The emerging buds in the selected shoots were treated with (T_a) N-6 benzyl adenine (300 ppm), (T_b) paclobutrazol (5% active ingredient), (T_c) N-6 benzyl adenine (300 ppm) + paclobutrazol (5% active ingredient), (T_d) *F. mangiferae*, (T_e) distilled water (control) to record changes in their development into healthy or malformed panicles. Ten shoots were randomly selected for each treatment (n=50).

The malformed panicles developed 30 days after treatment with T_c, T_d and T_e were exogenously treated with GA_3 (300 ppm) to record changes in panicle length.

Results

In vitro culture of malformed tissues

Different aerial parts (leaf, stem, flower, fruits and seeds) of datura, calotropis and neem extracted with hexane, methanol or methanol-water (70/30 v/v) revealed differential capacity to suppress the growth of *F. mangiferae* (Table 1). *In vitro* culture of the malformed tissues in MS media (control) revealed white cottony mycelial growth of *F. mangiferae* on the second day from all tissues cultured in the media (Table 1). In treatments where 10,000 ppm of methanol-water (70/30 v/v) extracts of datura (leaves, fruits, seeds) and calotropis (leaves) were used in the media separately, the white cottony mycelial growth of *F. mangiferae* was first seen 21–25 days after incubation at 28°C, whereas in neem (leaves and seeds) the mycelial growth of *F. mangiferae* was observed on the ninth day. In all other treatments the

Table 1 Mean capacity of different plant extracts using three different solvents to suppress the growth of *Fusarium mangiferae* in vitro

S.No	Plant part	Number of days taken for appearance of white mycelial growth of *F. mangiferae*					
		Hexane		Methanol		Methanol-water 70/30 v/v)	
		(5,000 ppm)	(10,000 ppm)	(5,000 ppm)	(10,000 ppm)	(5,000 ppm)	(10,000 ppm)
1	Datura (leaves)	2±0.3a	2±0.5a	14±3.5a	21±3.2a	19±2.3a	21±2.1a
2	Datura (stems)	2±0.8a	2±0.3a	4±0.8b	9±2.1b	4±1.1bc	7±1.5b
3	Datura (flowers)	2±0.1a	2±0.1a	4±0.5b	4±2.2b	4±1.6bc	4±1.5b
4	Datura (fruits)	2±0.5a	2±0.5a	17±1.5a	21±1.9a	19±2.4a	21±2.9a
5	Datura (seeds)	2±0.5a	2±0.5a	19±1.5a	21±2.3a	21±2.1a	25±2.1a
6	Calotropis (leaves)	3±1.2a	2±0.2a	7±2.1b	21±2.6a	11±3.4c	19±3.1a
7	Calotropis (stems)	2±1.5a	2±.0.9a	4±1.9b	7±0.5b	6±1.1bc	6±1.5b
8	Calotropis (flowers)	2±0.9a	2±0.2a	4±2.4b	5±0.5b	3±1.2b	4±1.5b
9	Calotropis (fruits)	2±0.4a	2±0.5a	3±0.7b	3±1.1b	3±1.1b	3±1.4b
10	Calotropis (seeds)	2±0.5a	2±0.5a	3±1.1b	3±1.2b	3±1.2b	3±1.1b
11	Neem (leaves)	2±0.3a	2±0.9a	3±1.0b	7±1.1b	3±1.1b	9±2.1b
12	Neem (stems)	2±0.9a	2±0.1a	3±1.5b	3±1.2b	3±1.6b	4±1.4b
13	Neem (flowers)	2±0.5a	2±0.5a	3±1.0b	4±1.5b	3±1.1b	4±1.2b
14	Neem (fruits)	2±0.6a	2±0.6a	3±1,5b	3±1.1b	3±1,1b	4±1.2b
15	Neem (seeds)	2±0.5a	2±0.5a	4±1.1b	7±2.1b	4±1.7b	9±2.1b
16	Control	2±1.1a	2±1.2a	2±0.6b	2±1.0b	2±1.1b	2±1.0b

Means with the same letter in each column are not significantly different at $P<0.05$) (Duncan test) ($n=10$)

Values expressed are mean ± Standard deviation

mycelial growth of *F. mangiferae* appeared after 3–6 days of incubation at 28°C (Table 1).

Progressive inhibition in the growth of *F. mangiferae* was observed with increase in the concentration of T_1 in the media. (Fig. 1, Table 2). In treatment T_1 the mycelial growth was restricted to the surface of the explant only, and no fungal growth was observed in the media (Fig. 1).The number of macro and micro-conidia significantly declined with increase in the concentration of the treatments (Table 2). Similar observations were recorded when malformed tissues were cultured in MS media along with T_1 which was not autoclaved before use (Table 2).

Field experiment

Foliar spray with T_1 and T_2 at the bud break stage resulted in the development of the majority of buds into healthy panicles (Table 3). The best results were observed in trees sprayed with T_1 followed by T_2 at fruit set stage, where all the malformed panicles completely dried two days after the foliar spray (Table 4).

The extent of flowering and floral malformation was significantly different between the two years under study. In control trees, the extent of floral malformation was significantly higher in 2005 (63.98%) when compared with 2006 (33.12%) (Table 3). However, this annual variation in the number of malformed panicles per tree was not significant. We observed two types of flowering in the selected trees of mango cv. Amrapali. Bud break was either simultaneous in all the buds, in the month of January or bud break was continuous throughout the flowering season from January to March. During the bud break stage both healthy and malformed buds were found on the trees which could be differentiated from their morphological appearance (Fig. 2).

In mango trees where bud break was simultaneous in the majority of buds in January, foliar spray with T_1 at the bud break stage resulted in the development of all buds into healthy panicles (Table 3). No drying or

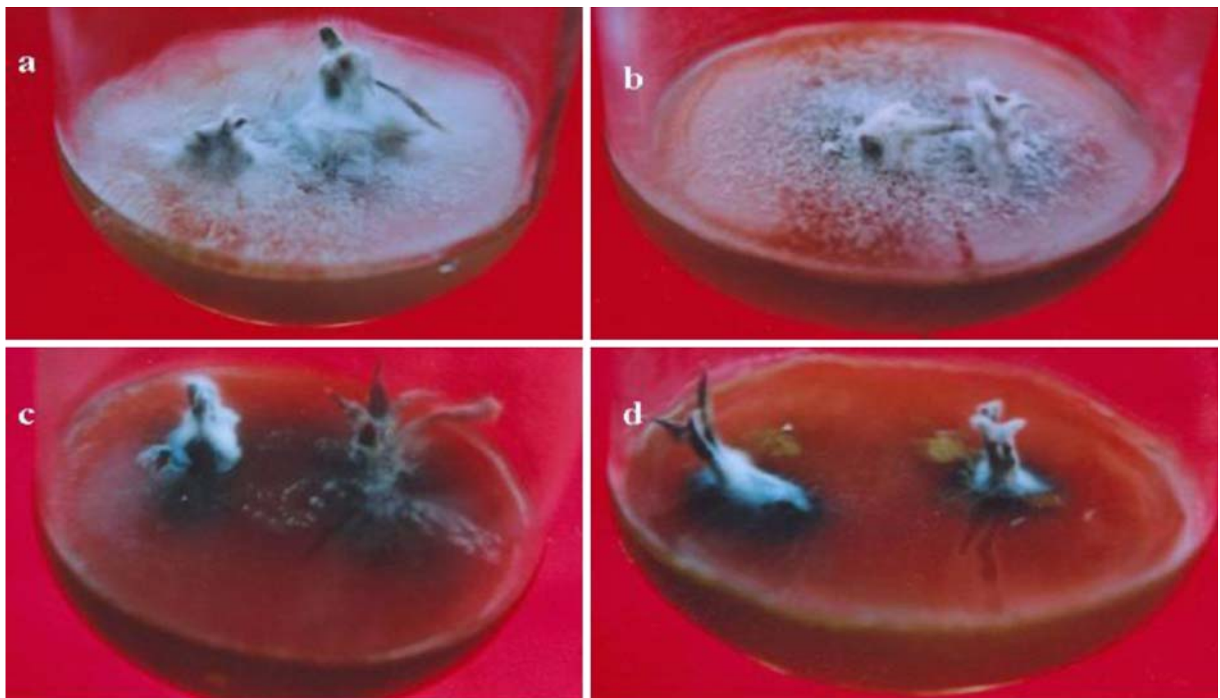

Fig. 1 Progressive inhibition in the growth of *Fusarium mangiferae* with the increase in concentration of brewed tea in the media. (a) malformed tissues cultured in MS media (control), (b) malformed tissues cultured in MS media +10% brewed extract, (c) malformed tissues cultured in MS media +20% brewed extract (d) malformed tissues cultured in MS media +30% brewed tea extract

scorching symptoms were seen on the healthy buds or other foliar parts of the tree. In these trees an average of only one malformed panicle was found which developed at a later stage (0.3% malformation). Similar significant decreases in the extent of floral malformation were observed when T_2 was sprayed onto mango trees at the bud break stage when compared with the control (Table 3). In trees where bud break continued throughout the flowering season, emerging buds (malformed and healthy), developing panicles (healthy and malformed) and fully developed panicles (healthy and malformed) were found growing simultaneously on the same tree (Fig. 3). Foliar spray with T_1 or T_2 in February–March affected the pollinator activity and damaged the developing and fully developed healthy panicles (Fig. 3).

Table 2 Mean number of macro and microconidia of *Fusarium mangiferae* in vitro measured in different treatments

S.No	Treatment	Macroconidia ml^{-1}	Microconidia ml^{-1}
1	Autoclaved MS + Autoclaved concoction-brewed compost T_1 (10%)	1×10^4a	2×10^4a
2	Autoclaved MS + Autoclaved concoction-brewed compost T_1 (20%)	0.001×10^4b	0.01×10^4 b
3	Autoclaved MS + Autoclaved concoction-brewed compost T_1 (30%)	0	0
4	Autoclaved MS + [a]concoction-brewed compost T_1(10%)	2×10^4a	3×10^4 a
5	Autoclaved MS + [a]concoction-brewed compost T_1 (20%)	0.001×10^4 b	0.01×10^4b
6	Autoclaved MS + [a]concoction-brewed compost T_1 (30%)	0	0
7	Autoclaved MS + Autoclaved concoction-brewed compost (T_1)	0	0
8	Autoclaved MS + Autoclaved Methanol-water extract (T_2)	0	0
	Autoclaved MS media (control)	3×10^4ac	7×10^4 c

Means with the same letter in each column are not significantly different at $P<0.05$ (Duncan test) ($n=10$)

[a] (not autoclaved)

Table 3 Effect of foliar spray at bud break stage on floral malformation in mango

S.No	Treatment	2005			2006		
		Healthy panicles/tree	Malformed panicles/tree	Malformation %	Healthy panicles/tree	Malformed panicles/tree	Malformation %
1	T_1	258±27a	3±1a	1.18±0.11a	325±11a	1±0.5a	0.31±0.15a
2	T_2	267±19a	4±1.5a	1.48±0.05a	278±21a	5±1.4a	1.41±0.06a
3	T_3	58±11b	103±7b	63.98±9b	218±28b	108±6b	33.12±1.9b

Means with the same letter in each column are not significantly different at $P<0.05$) (Duncan test) ($n=10$)

Values expressed are mean ± Standard deviation

The effect of foliar spray with T_1 and T_2 at the fruit set stage showed significant differences in the number of completely dried malformed panicles between the two years under study when compared with the control (10 days after the spray treatment). In trees treated with T_1, the percentage of malformed panicles where complete abscission of flowers was observed differed significantly between the two years of study, whereas in trees treated with T_2, the percentage of malformed panicles, where only partial abscission of flowers was observed, differed significantly (Table 4). The best results (Table 4) were observed in trees sprayed with T_1 followed by T_2 at the fruit set stage, where all the malformed panicles completely dried two days after the foliar spray. In trees treated with T_1, flower abscission was observed from the fourth day after spraying and all flowers dropped by the ninth day without requiring any manual de-blossoming. In control trees, the malformed panicles remained green on the trees and competed with the growing fruits for plant nutrients (Fig. 4).

Effect of foliar spray on the extent of malformation

In trees treated with T_1 or T_2 at the fruit set stage, 20% of fully-dried panicles recovered to develop into healthy panicles in the same flowering season (Fig. 5). In control trees, the malformed panicles which appeared during the February–March flowering season, remained green throughout the fruit-growing season (April–May), partially dried during the severe hot period (May–June), and developed back into fresh malformed panicles in July–August (Fig. 8). All these malformed panicles again completely dried during the severe cold period (November–December) when the temperatures were <6°C (Fig. 8); 89% of these fully-dried malformed panicles (November, 2005) developed into fresh malformed panicles during the next flowering season in March, 2006 (Figs. 5, 8). In trees treated with T_1, only 0.4% and 2.9% of the dry malformed panicles in April 2005 developed into fresh malformed panicles during the next flowering season in 2006.

Table 4 Effect of foliar spray at fruit set stage on floral malformation in mango cv. Amrapali

S.No	Treatment	[a]Malformed panicles (Number/tree)					
		2005			2006		
		Completely dried	Partial abscission of flowers	Complete abscission of flowers	Completely dried	Partial abscission of flowers	Complete abscission of flowers
1	T_1	109±4a	6±2a (5.5%)	97±1a (88.99%)	56±7a	4±2a (7.14%)	52±4a (92.86%)
2	T_2	97±6a	68±4b (70.10%)	4±2b (4.12%)	42±6a	40±4b (95.23%)	2±1b (4.76%)
3	T_3	0.0b	4±0.9a (3.88%)	0.0b	0.0b	2±1a (3.45%)	0.0b

Means with the same letter in each column are not significantly different at $P<0.05$) (Duncan test) ($n=10$)

Values expressed are mean ± Standard deviation

[a] Observations recorded ten days after spray

Fig. 2 Effect of brewed extract when sprayed in January on mango trees (bud break was simultaneous in majority of buds). (a) healthy bud, (b) malformed bud, (c) healthy panicles

In trees treated with T_1 or T_2 at the fruit set stage, 20% of fully-dried panicles recovered to develop into healthy panicles in the same flowering season (Fig. 5). *In vitro* culture of these dry malformed tissues in MS media did not show any fungal growth. *In vitro* culture of the completely dry malformed tissues collected from other samples in MS media revealed white cottony mycelial growth of *F. mangiferae* on the twenty-fifth day from the surface of explants.

Fig. 3 Effect of brewed extract when sprayed on mango trees in February–March (bud break was continuous throughout the flowering season from January–March). (a) developing healthy panicle, (b) developing malformed panicles, (c) fully developed healthy panicle, (d) damaged healthy panicle after spray with brewed extract

Fig. 4 Effect of the brewed extract when sprayed at fruit set stage on treated and control trees. (a) drying malformed bud 1 day after spray (b) fully dried malformed bud and developing malformed panicle, (c) drying malformed panicle 1 day after spray, (d) flower abscission in dry malformed panicle after spray, (7) 7th day after spray treatment (f) control

Fruit yield

Mango trees sprayed with T_1 and T_2 revealed significantly higher percentage fruit retention when compared with T_3 (Table 5). The percentage fruit drop was significantly lower in mango trees sprayed with T_1 followed by T_2 when compared with T_3 (Table 5).

In control trees, the extent of fruit set was significantly lower in 2005 (174±19) when compared with 2006 (454±8) (Table 5). Significantly higher fruit set was observed in trees sprayed with T_1 and T_2 when compared with T_3 in 2005. However no significant differences were observed in the extent of fruit set in trees sprayed with T_1, T_2 or T_3 in 2006 (Table 5).

Nutrient status of Amrapali mango leaves

Tissue nutrient analysis of Amrapali mango leaves after treatment with T_1, T_2 or T_3 revealed significantly higher levels of nitrogen, phosphorus, potassium, calcium, magnesium, copper, zinc, iron and manganese in T_1 when compared with T_2 and T_3 (Table 6).

Fruit quality parameters

Since the foliar spray at the fruit set stage can affect the fruit quality in mango, different fruit quality parameters were analysed. The total phenols, total carotenoids, ß-carotene, total sugars and TSS measured did not differ significantly in different treatments (T_1, T_2 or T_3) in both the years under study. Although the ascorbic acid content was significantly higher in T_1 when compared with T_2 and T_3 in 2005, it did not differ significantly between T_1, T_2 or T_3 in 2006. However, the total titratable acidity was significantly higher in T_1 when compared with T_2 or T_3, in both years and the total flavonoids were significantly higher in T_1 and T_2 when compared with T_3 in both years under study (Table 7).

Development of emerging buds into healthy or malformed panicles under field conditions

Results from the randomly tagged buds in the selected mango trees under field conditions, revealed that 97% of the single buds developed into healthy panicles while 100% of multiple buds developed into malformed panicles. Progressive development of single

Fig. 5 Development of fully dry malformed panicle into healthy or malformed panicle after brewed extract spray. (a) Sprouting buds from fully dry malformed panicles after brewed extract spray, (b) development of bud into healthy panicle (c) development of dry malformed panicle into fresh malformed panicle in control

buds into healthy panicles and multiple buds into malformed panicles is shown in Fig. 6a and b.

Effect of N-6 benzyl adenine, paclobutrazol and *F. mangiferae* on development of buds into healthy or malformed panicles under field conditions

Observations made on the shoots that were defoliated and decapitated revealed an average of 7.9 buds emerging/shoot. Exogenous treatment of emerging buds with T_c and T_d revealed that each malformed panicle developed from 4 to 11 buds and rarely from > 20 buds (mean 7.9) (Fig. 7). Buds treated with T_c and buds treated with T_d mimicked the symptoms of malformed panicles in Te. Data in Table 8 shows no significant differences in the size and percentage of male and hermaphrodite flowers in the malformed panicles developed from treatments T_c,T_d and T_e Significant differences in the size and percentage of male and hermaphrodite flowers were observed between malformed panicles in T_c,T_d and T_e and healthy panicles in T_e (Table 8, Fig. 6a- 4).

The progressive development of buds treated with T_c into malformed panicles is shown in Fig. 7. The observed swelling of buds (Fig. 7-1) by the end of the first week after treatment with T_c (2.1 cm length) and bunching of the swollen buds (Fig. 7-2) by the end of second week (2.1 cm length) is shown in Fig. 7. By the end of third week cell division continued but cell elongation was reduced (Fig. 7-3) in the treated buds in T_c giving a bunchy appearance (Fig. 7-4). By the end of fourth week, the buds treated with T_c

Table 5 Effect of foliar spray at fruit set stage on fruit set, retention and fruit drop in mango cv. Amrapali

S.No	Stage of fruit development	T_1		T_2		T_3	
		2005	2006	2005	2006	2005	2006
1	No. of fruits set/tree (at marble stage)	584±22a	597±43a	571±36a	568±22a	174±19b	454±8a
2.	No. of fruits harvested / tree (at maturity)	224±24a	266±22a	214±14a	228±9a	56±11b	98±14b
3	Fruit drop (%)	61.64±4a	55.44±6a	62.52±9a	59.56±7a	67.18±5b	78.41±9b

Means with the same letter in each row are not significantly different at $P<0.05$) (Duncan test) ($n=10$)

Values expressed are mean ± Standard deviation

Table 6 Mineral nutrient composition of 'Amrapali' mango leaves after treatment with concoction-brewed compost (T_1) solvent extract (T_2) and water spray (T_3)

S.No	Mineral Nutrient	Composition/ml		
		T_1	T_2	T_3
1.	Nitrogen (%)	2.35a	1.15b	0.72b
2.	Phosphorus (%)	0.43a	0.11b	0.006b
3.	Potassium (ppm)	91.5a	51.5b	29.4b
4.	Calcium (ppm)	84.4a	66.6b	64.1b
5.	Magnesium (%)	0.87a	0.42b	0.15b
6.	Copper (ppm)	0.02a	0.01b	0.01b
b7.	Zinc (ppm)	1.73a	1.67b	1.29c
8.	Iron (ppm)	98.99a	61.76b	31.07c
9.	Manganese (ppm)	1.90a	1.10b	1.60c

Means with the same letter in each row are not significantly different at $P<0.05$ (Duncan test) ($n=10$)

developed into thick and shortened malformed panicles (9.6 cm in length, 23.1 cm width) with largely male flowers (Fig. 7, Table 8). While buds treated only with T_b developed into panicles (4.1 cm in length, 2.3 cm width) with no bud opening (Table 8), buds treated with T_a developed into panicles (10.2 cm in length, 7.1 cm in width) with largely sterile flowers (Table 8). In buds treated with T_e, the healthy panicles (22.6 cm in length, 12.4 cm width) had male and bisexual flowers, while the malformed panicles (10.2 cm in length, 23.4 cm width) had largely male flowers (Table 8).

The malformed panicles did not show any increase in panicle length when treated with GA_3 (300 ppm). No flower bud opening was observed in the malformed panicles treated with GA_3 (300 ppm) when compared with the control (-GA) (Fig. 7).

Discussion

Mango malformation is a serious disease in many areas where this important crop is grown. Despite this fact, relatively little is known about the disease. The recent discovery that several *Fusarium* spp. are associated with mango malformation is intriguing. However, only *F. mangiferae* has been shown to cause mango malformation (Britz *et al*. 2002). Several studies now support the involvement of the fungus *F. mangiferae* (Ploetz *et al*. 2002; Zheng and Ploetz 2002; Freeman *et al*. 2004; Usha 2005; Youssaf *et al*. 2006; Marasas *et al*. 2006; Zafar *et al*. 2006). Previous reports indicate that the disease moves slowly in infected orchards (Kumar and Beniwal 1992). Isolates of *F. mangiferae* from mango transformed with the GUS reporter gene (ß-glucoronidase), and consequently used to artificially inoculate mango, verified that bud and flower tissues of the host are primary infection sites, and that wounds may provide sites of entry for the pathogen (Freeman *et al*. 1999). Similar records proving that meristematic tissue is the primary site of infection were reported earlier (Usha *et al*. 1994, 1997).

Fusicoccin, when exogenously applied on fully swollen buds, induced typical symptoms of malformed inflorescences, proving that the toxins released by the fungus *Fusarium*, are indeed

Table 7 Effect of foliar spray at fruit set stage on fruit quality in mango cv. Amrapali

S.No	Fruit quality parameters	2005*			2006*		
		T_1	T_2	T_3	T_1	T_2	T_3
1.	Total phenols (mg 100^{-1}g) as gallic acid equivalents	58.5a	57.1a	57.4a	56.1ab	55.2ab	56.4ab
2.	Total flavonoids (mg 100^{-1}g) as catechin	77.9a	74.8b	72.4b	76.1ab	74.5bc	71.9bd
3.	Total carotenoids (mg 100^{-1}g)	7.1a	6.9a	7.0a	7.4a	6.2a	6.9.a
4.	ß-carotene (mg 100^{-1}g)	4.2a	3.6a	3.2a	4.1a	3.2a	3.1a
5.	Ascorbic acid (mg 100^{-1}g)	88a	83b	80b	86ab	84bc	81b
6.	Total titratable acidity (%)	1.4a	0.9b	0.9b	1.4a	0.9b	0.9b
7.	Total sugars (%)	17.1a	17.2a	17.3a	17.2a	17.2a	17.3a
8.	TSS ° brix	21.5a	21.4a	22.1a	21.5a	21.1a	22.0a

Means with the same letter in each row are not significantly different at $P<0.05$ (Duncan test) ($n=10$)

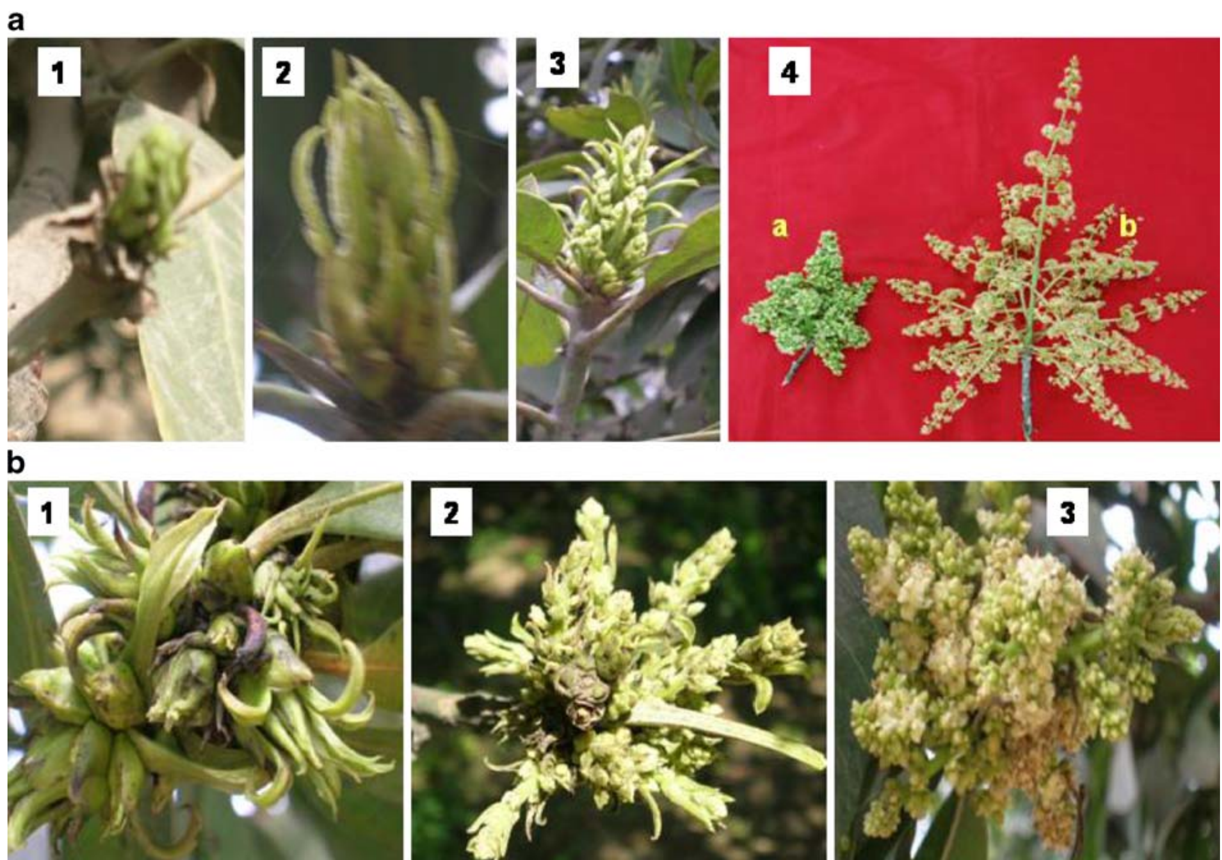

Fig. 6 **a** Progress of development of single bud into healthy panicle. **b** Progress of development of multiple buds into malformed panicle (a) malformed panicle (b) healthy panicle. (1, 2, 3, 4 are different stages)

responsible for the induction of malformation in mango (Usha and Singh 2000). Based on specific-PCR diagnosis, inoculum of the pathogen infected seedlings from the meristem, with colonisation descending from the top to lower stem sections and roots, further indicating that the pathogen is not seed-borne or translocated systematically from roots to meristem (Freeman *et al*. 2004). This is in agreement with previous studies that have stated that *F .mangiferae* is not seed-borne and that seedlings cultivated in a disease-free environment should be disease-free (Saeed and Schlosser 1972; Ploetz 2001). Therefore, the high incidence of seedling malformation in Egypt can be attributed to the location of the seedlings, whereby the nurseries that are situated under mature infected trees for shading and other purposes are being infected by the release of high levels of inoculum from the diseased panicles overhead (Ploetz *et al*. 2002). Furthermore, it has been reported on a number of occasions that *F. mangiferae* is infrequently isolated from old branch tissue and when isolated may be derived from remnant apical infections, left behind as the shoot developed (Darvas 1987; Ploetz 2001). This information further indicated that the pathogen does not infect systematically and that the inoculum does not originate from seed, although minor infections may occur from infected debris buried in the soil (Youssaf *et al*. 2006). Macro and microconidia of *F. mangiferae* are the infective propagules and form profusely on various malformed tissues (Freeman *et al*. 2004). *Fusarium mangiferae* may be windborne, being distributed in infected orchards only when sufficient inoculum is available, or spread with the aid of the mango bud mite *Aceria mangiferae* (Youssaf *et al.* 2006).

In this study, we isolated *F. mangiferae* from malformed tissues, and the inoculation tests on healthy buds under field conditions have been positive for this pathogen (Usha 2005). This agrees with other previous findings (Britz *et al*. 2002; Youssaf *et al*. 2006;

Fig. 7 The progressive development of buds treated with [N-6 Benzyl adenine (300 ppm) + Paclobutrazol (5% active ingredient)] into malformed panicles (1, 2, 3, 4, 5 weeks after treatment)

Marasas *et al*. 2006; Zafar *et al*. 2006). The observed development of buds treated with T_c and T_d into malformed panicles, prove that *F. mangiferae* probably induces the symptoms of malformation by altering the endogenous (↓)GA and (↑)cytokinin levels in the infected buds, thus resulting in the formation of multiple buds, thick and shortened malformed panicles with largely male flowers. It is assumed that probably *F. mangiferae* alters the C:N ratio of the infected buds, by feeding on carbohydrates and sugars for its survival. Addition of nitrogen sources to both GA_3-producing cultures and resting cell systems shut off GA_3 formation (Rybakov and Bourd. 1991). Since *Fusarium* is known to synthesise gibberrellic acid (Bruckner and

Table 8 Effect of N-6 benzyl adenine, paclobutrazol and *Fusarium mangiferae* on development of buds into healthy or malformed panicles

S.No	Treatment	Length of the panicle (cm)	Width of the panicle	Percentage of flowers/panicle			
				♂	Imperfect ⚥	Hermaphrodite	Sterile
1	N-6 benzyl adenine (300 ppm)	10.2 a	7.1a	0.02a	–	–	99.98a
2.	Paclobutrazol (5% active ingredient	4.1b	2.3b	–	–	–	–
3	N-6 benzyl adenine (300 ppm) + Paclobutrazol (5% active ingredient)	9.6a	23.1c	95.8b	–	4.2a	–
4	*Fusarium mangiferae*	11.2a	24.8 c	97.7b	–	2.3a	–
5	water spray (Healthy panicle)	22.6c	12.4d	78.8c	9.6a	11.6b	–
6	Water spray (Malformed panicle)	10.2a	23.4	96.1b	–	3.9a	–

Means with the same letter in each row are not significantly different at $P<0.05$) (Duncan test) ($n=10$)

Blechschmidt 1991) it is also assumed that it may be blocking the synthesis of gibberellins in the infected buds by competing with the gibberellin precursor ent-kaurene. It is also possible that the metabolites released by *F. mangiferae* may cause such endogenous hormonal change in the infected buds. The exact mechanism resulting in such changes is yet to be studied.

Data in Table 8 and Fig. 6a-4 reveal significant differences in the size of malformed panicles in T_c, T_d and T_e and healthy panicles in T_e. One of the main roles of gibberellins in trees is the stimulation of cell elongation (Richards *et al*. 2001; Matsuoka 2003).When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate. The result is shoots with the same numbers of leaves and internodes are compressed into a shorter length. The observed decrease in panicle length in the malformed panicle when compared with the healthy panicle (Table 8, Fig. 6a-4) may be due to the effect of paclobutrazol which blocks the biosynthesis of the active gibberellin GA_1 (Zeevaart *et al*. 1993). Suppression of growth by paclobutrazol occurs because the compound blocks the three oxidative steps of the gibberellin precursor *ent*-kaurene to *ent*kaurenoic acid (Hedden and Graebe 1985). Paclobutrazol is a triazole-type plant growth retardant that blocks gibberellin biosynthesis and is involved in reducing abscisic acid, ethylene and indole-3-acetic acid while increasing cytokinin levels. The unique structure of paclobutrazol allows it to bind to an iron atom in the enzymes, essential for the production of gibberellins, and has the capacity to bind to enzymes necessary for the production of steroids in fungi as well as those that promote the destruction of abscisic acid. (http://www.sepro.com/documents/Profile_article.pdf). Similar reduction in internode length in response to paclobutrazol has been reported by several workers for other trees and herbaceous plants (Quinlan 1981).

The best results on the antifungal activity of *F. mangiferae* and control of floral malformation were observed with T_1 followed by T_2 compared with T_3. The aerial parts of datura (leaves, fruits, seeds), calotropis (leaves) and neem (leaves and seeds) showed superiority over other plant parts in suppressing the activity of *F. mangiferae*. Hence these parts were selected for preparing T_1 and T_2.

We observed two types of flowering processes in the selected trees of mango cv. Amrapali. Bud break was either simultaneous in all the buds, in the month of January or bud break was continuous throughout the flowering season from January to March. This is possible because the flowering process in mango is regulated by several interacting pathways and factors, such as photoperiod, temperature, carbon to nitrogen ratio, and endogenous hormone levels, which are all closely associated with genes (Chacko 1991; Kulkarni 1991; Ram 1999; Shü 1999).

Results from the randomly tagged buds under field conditions, revealed that 97% of the single buds developed into healthy panicles while 100% of multiple buds developed into malformed panicles (Fig. 6a, b). Observations made on the shoots that were defoliated and decapitated revealed an average of 7.9 buds emerging per shoot. The decapitated shoots prevented apical dominance and thus probably increased the endogenous cytokinin levels. The stimulatory effects of cytokinins on the growth of lateral buds are well known (Chunjian and Fritz 2003; Schmulling 2002). In this present study, exogenous treatment of emerging buds with T_c revealed that each malformed panicle developed from 4 to 11 buds (sometimes from > 20 buds). All these single buds which had the capacity to develop into healthy panicles, resulted in the development of malformed panicles because cell division in each treated bud continued, but cell elongation was absent (Fig. 7). This proves that with increase in the number of malformed panicles/tree the number of buds developing into healthy panicles/tree decreases. The observed significant differences in the number of healthy panicles in control trees between the two years under study (Table 3) further proves this assumption. The number of buds emerging/ tree is again regulated by several interacting pathways and factors such as the carbon to nitrogen ratio and endogenous hormone levels, which are all again closely associated with genes (Chacko 1991; Kulkarni 1991; Ram 1999; Shü 1999).

During the bud break stage both healthy and malformed buds were found on all treated and untreated trees which could be differentiated from their morphological appearance (Fig. 2). The development of all buds into healthy panicles in trees treated with T_1 and T_2 at the bud break stage (Table 2, Fig. 2) could possibly be due to two reasons, (1) complete drying of the developed malformed buds after spraying, thus preventing the development of malformed buds into malformed panicles and (2) suppression of the activity of the pathogen by preventing spore formation. *In vitro* culture of malformed tissues in MS media along with T_1 or T_2 did not show growth of any macro or

microconidia in the media, thus supporting this assumption. However, further *in vitro* culture of the completely dry malformed tissues collected from trees treated with T_1 or T_2 revealed white cottony mycelial growth of *F. mangiferae* on the twenty-fifth day from the surface of explants, indicating that T_1 or T_2 could not completely eliminate the pathogen, but helped in controlling malformation by suppressing the activity of *F. mangiferae*.

The development of the dry malformed panicles (in April 2005) after spray treatment with T_1 and T_2 into fresh malformed panicles (0.4%, 2.9%, respectively) during the next flowering season in 2006, when compared with T_3 (89%) (Fig. 8) suggests that the high temperatures in May–June (>40°C) following spray treatment, probably contributed to the elimination of the pathogen from infected malformed panicles. Similar low inoculum counts of *F. mangiferae* during the summer months when compared with winter and controlled conditions were reported by Youssaf *et al.* (2006).

In control trees, the malformed panicles which appeared during the February–March flowering season**,** remained green throughout the fruit-growing season (April–May), partially dried during the severe hot period (May–June), and developed back into fresh malformed panicles in the July–August–September months (Fig. 8). All these malformed panicles completely dried again during the severe cold period (November–December) probably due to frost and low temperatures (<6°C). Further development of 89% of these fully-dried malformed panicles (November, 2005) into fresh malformed panicles during the following flowering season in February–March, 2006 (Fig. 8), supports the theory that the pathogen survived within the infected tissue even under the very low winter temperatures. Similar high inoculum counts of *F. mangiferae* during the winter months were reported by Youssaf et al. (2006).

In mango trees where bud break was continuous throughout the flowering season, T_1 or T_2 during February–March affected the pollinator activity and damaged the developing and fully developed healthy panicles, and thus drastically reduced fruit productivity. In these trees the best alternative found was to spray at the fruit set stage to prevent the damage of healthy panicles.

The control of floral malformation was best in trees sprayed with T_1 followed by T_2 at the bud break stage and again at the fruit set stage, when compared with T_3, which was ineffective against malformation. An increasing body of experimental evidence indicates that plant disease can be suppressed by treating plant surfaces with a variety of water-based compost preparations, known as compost extracts or compost teas. A variety of foliar diseases have been suppressed by applications of compost teas, including powdery mildew on roses and apples, grey mold on green beans, strawberries, grapes and geraniums, root rot on potatoes, tomatoes and grapes, *Fusarium* wilt of peppers and cucumbers and damping-off of pea seedlings (Scheuerell and Mahaffee 2002).

Datura is an important weed in the subtropics that contains alkaloids such as scopolamine and hyoscyamins. These alkaloids are well known for their medicinal properties. Calotropis is another common waste land and bund weed in India whose allelopathic effects on germination and seedling vigour is well known. This wild-growing plant has been reported to possess a number of medicinal properties (Kartikar and Basu 1935; Duke 1985). Some of the bioactive constituents from calotropis have been identified as triterpenoids, flavonoids, calotropins D1 and D2, and oxiopregnane-oligoglycosides calotropis A and B. The structure of these compounds was elucidated by chemical and spectroscopy methods (Pal and Sinha 1980; Kitagawa *et al*. 1992). The cardenoloids glycosides, calotropin frugoside and 4-O-Beta-D-glucopyranosyl frugoside were identified as the cytotoxic principles in the roots of calotropis (Kiuchi *et al*. 1998). Products based on *Azadirachta indica* are also known for their antifungal and pest control properties (Singh 2003). The well known biological constituents used in this study, are alkaloids in datura, azadirachtin in neem, sugars (De 1996), flavonoids (Rahman and Wilcock 1991), flavonol glycosides (Sen and Sahu 1992) and oxypregnane-oligoglycosides in calotropis (Shibuya and Zhang 1992; Gupta and Ali 2000). Although their mechanism of action is not known, these constituents are probably released during brewing (T_1) and solvent extraction (T_2) and might have mediated in suppressing floral malformation in mango.

Considering the diverse microbial community in concoction-brewed compost (due to use of cow manure which is rich in beneficial microorganisms), it is likely that multiple modes of activity associated with microbial antagonists are involved in disease suppression. Several studies indicate that applying the microbial component of

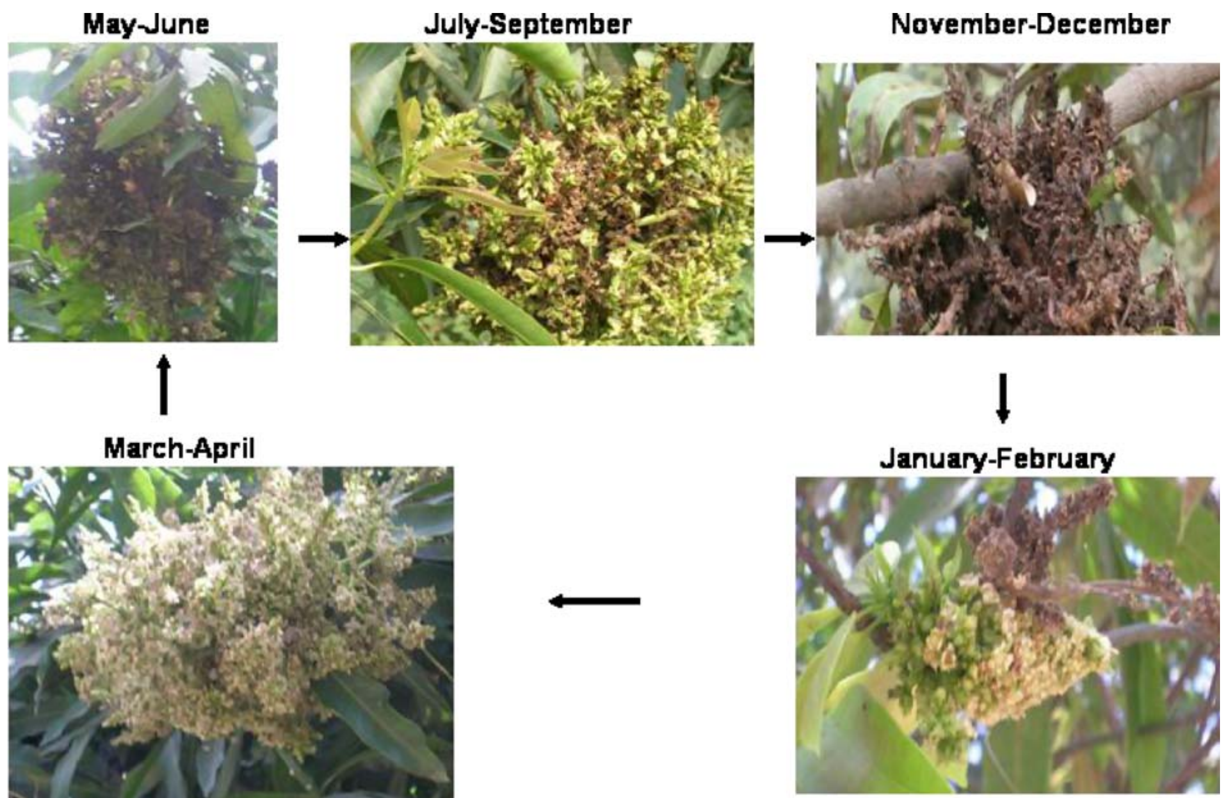

Fig. 8 Sequence of events occurring in a malformed panicle in control trees during different periods of the year

compost tea is necessary for disease suppression. However, it is not clear whether pathogen inhibition is due to parasitism, competition for nutrients and colonisation sites, or if applied organisms produce antibiotics *in situ* once established on plant surfaces. Regardless of the mode of action or source/type of microorganisms, preventative application before pathogen infection appears necessary for optimal control through all known modes of action (Scheuerell and Mahaffee 2002). The observed complete abscission of flowers from the dry malformed panicles in response to foliar spray with T_1 when compared with T_2 suggests that possibly the presence of beneficial microorganisms in the cow manure used for the preparation of T_1 not only assists in the fermentation process but also allows the release of certain compounds during the process of brewing which might cause flower abscission.

The present *in vitro* culture of malformed tissues in MS media along with unsterilised concoction-brewed compost (T_1) showed progressive decrease in the growth of *F. mangiferae* with increase in concentration, but did not show the growth of any other microorganisms in the media, suggesting that the activity of a diverse microbial community initially found in the cow manure at the start of brewing, was suppressed by the end of brewing, possibly due to the release of biologically-active ingredients from datura, calotropis and neem.

Mango trees sprayed with T_1 and T_2 revealed significant differences in percent fruit set and retention when compared with T_3. The percent fruit drop was significantly lower in mango trees sprayed with T_1 followed by T_2 when compared with T_3. This may be due to the fact that the dry malformed panicles do not compete with the growing fruits for plant nutrients, whereas in control plants the malformed panicles that remained green until fruits are harvested competed with the growing fruits for plant nutrients. The tissue nitrogen, phosphorus, potassium, calcium, magnesium, copper, zinc, iron and manganese levels were found to be significantly higher in T_1 when compared with T_2 and T_3 (Table 6). The rich mineral nutrient composition of T_1 when compared with T_2 and T_3 might have also assisted in better fruit growth and retention in trees treated with T_1.

Among the different fruit quality parameters analysed, only the total flavonoids were significantly higher in T_1 followed by T_2 when compared with T_3 in both years under study (Table 7). The presence of flavonoids and flavonol glycosides in calotropis has been previously reported (Rahman and Wilcock 1991; Sen and Sahu 1992). This could be one of the reasons for the observed higher levels of flavonoids in fruits collected from trees treated with T_1 and T_2 when compared with T_3. The observed non-significant differences in total phenols, total carotenoids, ß-carotene, total sugars, TSS and acidity in different treatments (T_1, T_2 or T_3) in both the years under study, suggest that the spray solution had less effect on changes in fruit quality parameters at maturity (90 days after the spray treatment).

The aerial parts of datura, calotropis, neem and cow manure used for the preparation of T_1 are naturally available and involve no additional cost when compared to conventional pesticides. Furthermore, the concoction is effective, inexpensive, biodegradable, easy to prepare with minimal risk to environmental pollution and constitutes a sustainable and eco-friendly approach to control floral malformation in mango when it is sprayed at the bud break stage and again at the fruit set stage. Since malformation was found to re-occur again during the July–August–September months in the control trees, repeating the foliar spray during this period could possibly reduce the extent of malformation in the next flowering season. Further studies on the isolation and structural elucidation of the most effective component of the concoction-brewed extract are required.

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