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Management of guava wilt in tarai regions of Uttarakhand

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Abstract
In guava plant, wilt caused by *Fusarium oxysporum* f.sp. *Psidii* is the most destructive disease in India and losses due to this disease are substantial. Therefore, the present communication, deals with the management of guava wilt in Tarai regions of Uttarakhand. Among the four fungicides bioassayed *in vitro*, Propiconazole + Carbendazim and Carbendazim at 75 and 100 µg ml^{-1} and Carbendazim + Mancozeb at 100 µg ml^{-1}, respectively, were most effective in inhibiting the mycelial growth (100%) followed by Carbendazim + Mancozeb causing 85 per cent mycelia growth at 75 µg ml^{-1}, while Propiconazole was comparatively found less effective at all the concentrations. Under field conditions *Bacillus amyloliquefaciens* (10kg/l) as an individual treatment showed the best management of guava wilt (96.9% reduction over check) followed by Pant bioagent-3 (10kg/l) (94.9% reduction over check) and compost tea (1kg/l) (94.6% reduction over check). In combination treatments, systemic fungicides Propiconazole + Carbendazim (2g/l) found to be the best management against guava wilt (98.4%). So it is recommended to apply an integrated approach by using antagonists, bioagents, bioagent consortium and fungicides and their combinations to manage the wilt disease of guava.

Keywords: ROC, percent disease incidence, inhibition, antagonist, bioagent

Introduction
Guava (*Psidium guajava* L.) is an important fruit crop and has spread to most countries of the world having subtropical and tropical climates. Guava is quite hardy and prolific bearer and is cultivated successfully even in neglected soils and is attacked by a large number of pathogens, of fungal origin, bacterial, algal and nematodes, are found to cause various type of disease. Wilt is the most destructive disease for guava plant in India and losses due to this disease are substantial (Prakash and Mishra, 1993; Prakash and Pandey, 2005; Prakash and Pandey, 2007) [15, 13, 14]. *Psidium guajava* wilt is known to occur from India, Latin America, Malaysia, Pakistan, South Africa and Taiwan. Wilt is predominantly caused by the species of *Fusarium*, of which *Fusarium oxysporum* is generally the main cause and its incidence in Uttarakhand, Uttar Pradesh and Bihar crosses the economic threshold more often due to the favourable climatic conditions causing 5-60% loss in guava production in India (Misra, 2006) [9]. The disease is soil borne and is difficult to control. It causes monitory as well as nutritional loss (Singh and Lal, 1953) [17].
Very little work has been done on epidemiology and management of the wilt disease in guava, therefore, in the present study, attention has been focused on the management of guava wilt in laboratory, glasshouse and orchard conditions of Tarai regions of Uttarakhand.

Materials and Method
Small pieces (2-3 mm) of the infected roots were cut with the help of sterilized scalpel to isolate the pathogen, surface sterilized with (0.1%) mercuric chloride solution for 10-20 sec, repeatedly washed in sterilized distilled water and transferred to PDA medium (200 g Potato, 20 g of Agar-Agar powder, 20 g dextrose per litre of distilled water) under aseptic conditions and incubated at 25 ± 2 ºC. The isolates were purified by hyphal tip or single spore method. The fungus obtained having similar growth pattern in majority of PDA slants was therefore maintained by sub-culturing on fresh PDA slants at every 15 days interval and stored in a refrigerator for further studies.
Pathogenicity test was proved under glasshouse conditions by two techniques: 1. Stem inoculation and 2. Soil inoculation.

In vitro assay of fungicides
Efficacy of four fungicides, viz., Carbendazim (Bavistin), Carbendazim + Mancozeb (Bendaco), Propiconazole (Spectator) and Propiconazole + Carbendazim

(Propazole Plus 250 SC) were determined against *Fusarium oxysporum* by poisoned food technique on PDA (Grover and Moore, 1962; and Thapliyal, 1993) [3, 12].

In this technique, double strength Potato Dextrose Agar Medium was prepared (all ingredients except water were doubled to that of normal PDA). Thereafter, 25 ml sterilized PDA was poured in sterilized conical flask of 100 ml capacity. Stock solution of 10000 ppm for all the above fungicides were prepared in sterilized distilled water in separate 250 ml Erlenmeyer flasks. Stock solution of each chemical was prepared by dissolving weighed quantity of fungicides in a measured volume of sterilized distilled water and added to double strength PDA medium. The amount of stock solution to be added to PDA was calculated by following formula:

$$C_1V_1 = C_2V_2$$

Where,

C_1 = concentration of stock solution (µg/ml)

C_2 = desired concentration (µg/ml)

V_1 = volume (ml) of the stock solution to be added

V_2 = measured volume (ml) of the PDA

Different concentrations i.e. 25, 50, 75, 100 µg/ml of each fungicide were prepared on active ingredient basis from above said stock solution of each fungicide. Required volume of the fungicides solution was mixed with sterilized PDA medium and the poisoned medium was poured into Petridishes (90 mm diameter). The Petridishes were inoculated by placing a mycelia disc (5 mm diameter) with the help of a sterilized cork borer of the actively growing test fungus in the centre on agar surface. The experiment was replicated thrice for each test concentration and incubated at 25 ± 2 ºC. Un-amended PDA plates were also inoculated with test fungus which was served as check.

The radial colony growth of the test fungus was recorded at every 24 hour interval till the check Petri plates were fully covered with the growth of the fungus mycelium. Percent inhibition in colony growth was calculated by using the standard formula: (Vincent, 1947) [19] as below:

$$\text{Percent inhibition of growth} = \frac{C-T}{C} \times 100$$

Where, I = Inhibition percentage

C = Colony diameter in check (mm)

T = Colony diameter in treatments (mm)

Statistical analysis of the data was done by using SPSS with simple CRD and two factorial CRD. Critical difference (CD) at 5 per cent level of significance was used for comparison of difference between the treatment means.

Evaluation of Antagonists, soil amendments and fungicides

Evaluation of antagonists, soil amendments and fungicides against *Fusarium oxysporum* was carried out *in vivo* conditions to select the most effective treatment for field experiments. The field experiments were conducted at Horticulture Research Centre (H.R.C.), Patthatchatta, Pantnagar during 2014 on cultivar Pant Prabhat. Integration of soil amendments, antagonists and fungicides were tested on newly transplanted guava seedling. Before transplantation the pit walls were sprayed with formaline-water solution of ratio 1:9, in a layered manner for soil sterilization. Different treatments were then applied to the soil pit and then after a week's time one year old guava plants from nursery were planted into the pits.

The most effective treatments among organic amendments, chemicals and antagonists were integrated to see their individual as well as combined effect on disease control and plant health in naturally infested soil in guava orchards in HRC, Pattharchatta. When the initiation of wilt started in guava orchards then in first week of July first treatment was given followed by second and third treatment at 25 days interval by soil drenching method. The observation on the wilt disease intensity were recorded for four years since 2014 to 2017 at three different months viz., September, October, and November from 10 randomly selected trees from the orchard using 0-5 scale as 0 = Healthy plants, 1 = Plants showing only yellowing of leaves, 2 = Plants with 25 per cent wilting, 3 = Plants with 50 per cent wilting, 4 = Plants with 75 per cent wilting, 5 = Plants with 100 per cent wilting. Based on these observations, percent disease index (PDI) was recorded. Disease incidence and reduction over check (ROC) is calculated to see the effect of treatment with respect to check.

Statistical analysis of the data was done by using SPSS for field experiment with RBD. Critical difference (CD) at 5 per cent level of significance was used for comparison of difference between the treatment means.

Result and Discussion

***In vitro* evaluation for fungicides**

All the fungicides at the test concentrations caused significant inhibition of mycelial growth of the pathogen over untreated control (Table 1). With an increase in concentration of fungicides corresponding decrease was noticed in mycelial growth of the pathogen. Among the systemic and non- systemic fungicides tested, Propiconazole + Carbendazim followed by Carbendazim was found more effective than the other fungicides. Propiconazole + Carbendazim and Carbendazim at 75 and 100 µg ml^{-1} and Carbendazim + Mancozeb at 100 µg ml^{-1}, respectively caused complete inhibition of mycelial growth of the pathogen. Carbendazim + Mancozeb could cause 85 per cent mycelial growth at 75 µg ml^{-1}. Propiconazole was found comparatively less effective at all the comparable levels in mycelial growth. Interaction among fungicides and concentrations were found significant. The correlation between the mycelial growth of *Fusarium* wilt and concentration of fungicides after 7^{th} days of inoculation is presented in Figure 1. The higher value of Propiconazole + Carbendazim (R^2 = 86.7) showed that this metal has highest potential to inhibit the mycelial growth of *Fusarium oxysporum* followed by Carbendazim (R^2 = 82.9). The lower value was observed in case of Propiconazole (R^2 = 63.1) which indicates that it has least potential to inhibit the growth among all studied fungicides. Similar results were also obtained by Leu *et al.* (1979) [7] who revealed that Carbendazim, Captafol and Thiabendazole proved effective against wilt pathogen under laboratory experiments. Joubert and Frean (1993) [5] reported that tebuconazole, propiconazole, prochloraz, triforine and carbendazim + flusilazole had been found effective against guava wilt pathogen *in vitro* evaluation. Srivastava *et al.* (2011) [18] also reported that radial growth of *Fusarium oxysporum* f.sp. *Psidii* was significantly less at high concentration of carbendazim. The present investigation has demonstrated that Propiconazole + Carbendazim @ 50 µg ml^{-1} is the most promising fungicides in the lab condition and this can be recommended for the field evaluation for the control of wilt of guava.

Table 1: Effect of fungicides at different concentrations on radial growth of *Fusarium oxysporum* f. sp. *Psidii*

S. No.	Fungicides	Concentration (μg ml^{-1})	Mycelial growth (mm)	Inhibition over control (%)
1.	Carbendazim	25	25.0 ± 0.03	72.22
		50	17.0 ± 0.09	81.11
		75	0.00	100.00
		100	0.00	100.00
2.	Carbendazim + Mancozeb	25	19.0 ± 0.13	78.89
		50	16.5 ± 0.13	81.67
		75	13.5 ± 0.09	85.00
		100	0.00	100.00
3.	Propiconazole	25	35.0 ± 0.18	61.11
		50	30.0 ± 0.09	66.67
		75	27.5 ± 0.13	69.44
		100	25.0 ± 0.14	72.22
4.	Propiconazole + Carbendazim	25	14.5 ± 0.09	83.89
		50	0.00	100.00
		75	0.00	100.00
		100	0.00	100.00
5.	Control		90.0 ± 0.0	0.00
	CD at 5%			
	Fungicides (a)		0.15	
	Concentration (b)		0.13	
	Interaction (a×b)		0.29	
	CV		0.62	

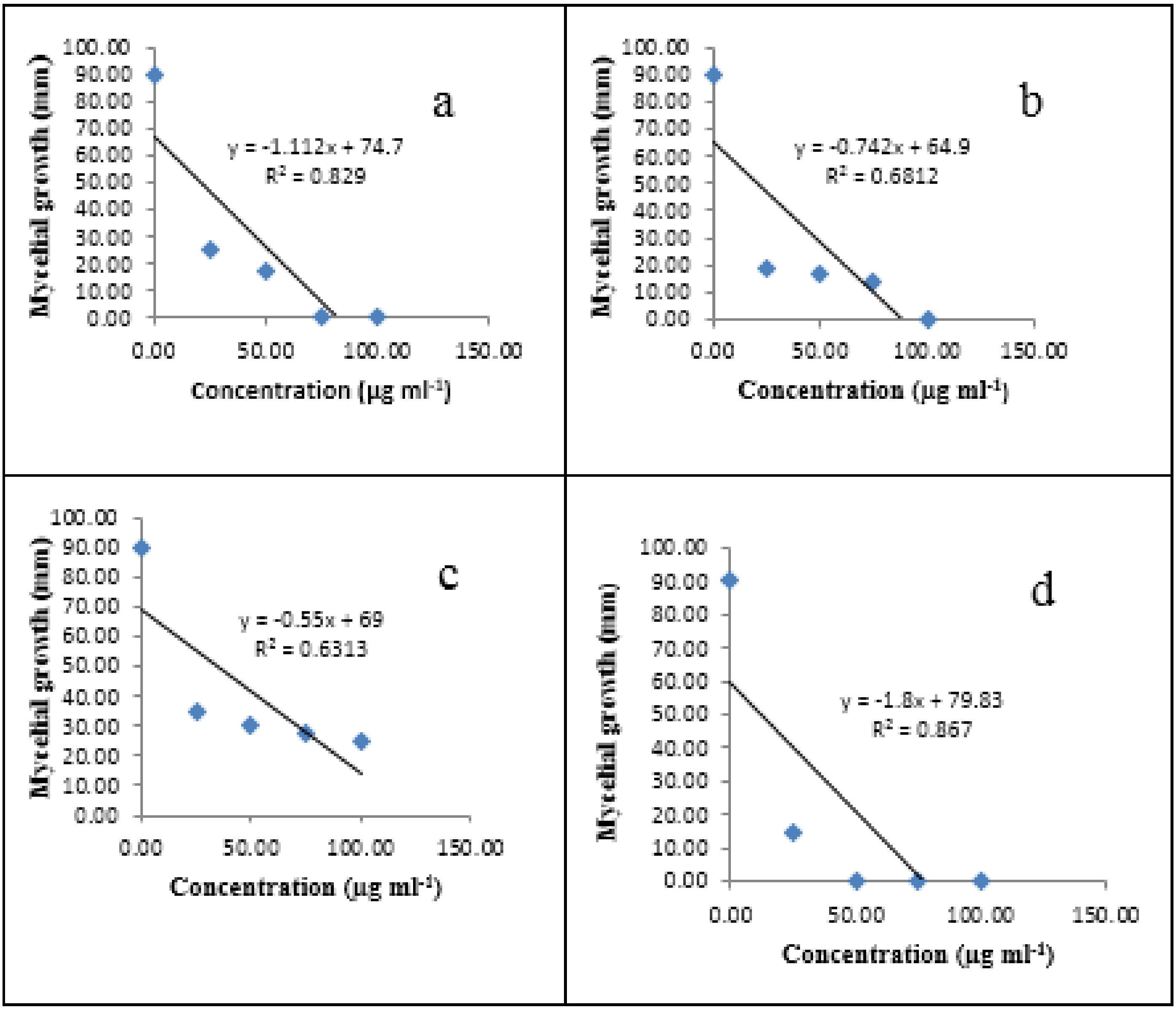

Fig. 1: Correlation between mycelial growth of *Fusarium oxysporum* and different concentrations of fungicides; (a) Carbendazim, (b) Carbendazim + Mancozeb, (c) Propiconazole, (d) Propiconazole + Carbendazim.

Integrated Disease Management

Organic amendments, antagonist and fungicides were integrated to see their individual as well as combined effect on wilt disease incidence on plants of guava at HRC, Patthatchatta, Pantnagar since 2014 to 2017. Observations of percent disease incidence of guava wilt during 2017 had been recorded during the months of September, October and November which are considered as highly favourable months for wilt incidence (fig.2). The data (Table 2, fig.3) revealed that the majority of individual components were found to be superior over interaction treatments. Among individuals the antagonists- *Bacillus amyloliquefaciens* was found to be the best effective treatment as significantly minimum percent wilt incidence (1.2 and 3.1) was recorded at 75 days after treatment in guava plants in 2016 and 2017. The next effective treatment was Pant bioagent-3 (*Trichoderma harzianum* + *Pseudomonas fluorescens*). Wilt disease in guava was effectively controlled by the application of *Bacillus amyloliquefaciens*, Compost and Pant bioagent-3 (*Trichoderma harzianum* + *Pseudomonas fluorescens*) in place of systemic fungicides like Carbendazim, Mancozeb and Propiconazole. In case of systemic fungicides, Propiconazole + Carbendazim showed maximum (98.4%) reduction over check in case of Guava wilt incidence. Significant reduction in disease intensity due to the application of the antagonists also entailed a concomitant increase in plant growth yield in the present investigation. These finding are in agreement with the work of Misra (2006) [9] and Gupta *et al*. (2009) [4] who reported complete inhibition of *F. oxysporum* causing wilt in guava with antagonists like *Aspergillus niger*, *Trichoderma sp.*, *Penicillium citrinum* and some bio – dynamic antagonists. Dwivedi *et al.* (2002) [2] reported that out of three bioagents *Trichoderma harzianum*, *T. Viride* and *Gliocladium virens*, *T. Viride* is best for the control of wilt. The reduction in pathogen population and wilt incidence might be due to fact that *Bacillus sp.* produces growth promoting metabolities such as hormones, e.g. cytokinins (Kampert and Strzelczuk, 1984) [6] and quantiatavely more important auxins (Brown, 1972; Selvodurai *et al.*, 1991) [1, 16]. Some plant related bacteria produce the siderophore pseudobactin, which can efficiently complex iron in soil making it unavailable to pathogen, thus inhibiting its growth. The present investigations revealed the potential of *Bacillus sp.* as one of the components in the integrated management besides its direct and indirect role in controlling *Fusarium* wilt of guava.

Management of *Fusarium* wilt is not possible when we go for a single control measure. So, an integrated approach based on biocontrol agents, soil amendments and cultural method are being suggested by Misra *et al.* (2003c, 2004b) [11, 10] and Misra (2005) [8] for the successful management of

Table 2: Effect of Fungicides, bioagents and soil amendments against wilt (*Fusarium oxysporum*) disease in Guava plants

S. No.	Treatments	Concentration	Incidence				
				2015	2016	2017	ROC
1	Carbendazim	2g/l	0.00	0.00	5.7	10.6	89.4
2	Carbendazim+Mancozeb	3g/l	0.00	0.00	4.9	8.6	91.4
3	Propiconazole	1ml/l	0.00	0.00	2.0	5.6	94.4
4	Propiconazole+Carbendazim	2g/l	0.00	0.00	0.2	1.6	98.4
5	Bacillus amyloliquefaciens	10g/l	0.00	0.00	1.2	3.1	96.9
6	Bacillus subtilis	10g/l	0.00	0.00	2.2	5.9	94.1
7	Pant Bioaget- 3	10g/l	0.00	0.00	1.8	5.1	94.9
8	Compost	5kg	0.00	0.51	4.5	7.9	92.1
9	Compost Tea	1kg/l	0.00	0.00	2.3	5.4	94.6
10	Vermicompost	5kg	0.00	0.00	4.0	6.9	93.1
	Control		14.133	57.7	100.0	100.0	0.0
	CD at 5 %		0.8	2.8	0.5	0.8	

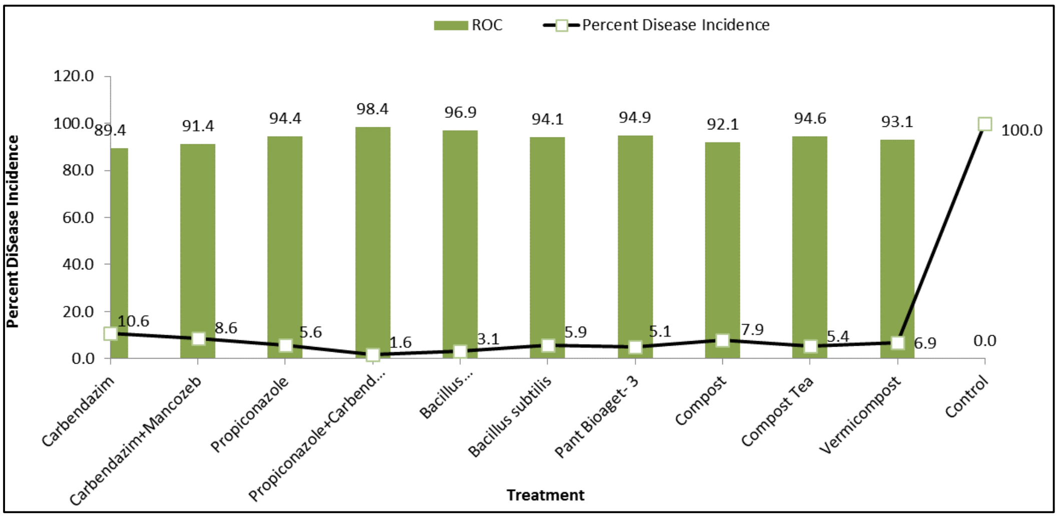

Fig 2: Effect of different treatments on *Fusarium* wilt incidence of Guava plants

Fusarium wilt. Thus, antagonists of *B. Amyloliquefaciens* (10g/l), Pant bioagent-3 (*Trichoderma harzianum* + *Pseudomonas fluorescens*) (10g/l) and soil amendment of compost tea (1kg/l) resulted in maximum reduction (96.9, 94.9, 94.6%) in incidence of *Fusarium* wilt and also enhanced plant growth and quality in guava wilt. This individual / integrated management practice should be evaluated on a large scale for making its recommendation for effective management of *Fusarium* wilt of guava.

Conclusion

Wilt is a soil borne disease and therefore not very easy to control consequently, fruit crops like guava are to be protected from the time of their sowing and the management practices are also initiated at the beginning. To manage the wilt of guava an integrated disease management approach is fruitful. Not only the fungicides but the antagonists and other soil amendment practices give satisfactory results in case of guava wilt management. Integrated practices of management are not only environment friendly but they also sustain the tree for future. The results of the above experiment show that by applying antagonists and systemic fungicides in an integrated manner, incidence of wilt of guava goes on decreasing in subsequent years also since their transplanting. So, it can be recommended to use antagonists, bio-agents and systemic fungicides in an integrated way so as to minimize the wilt incidence to threshold levels.

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