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Evaluation of bioagents and chemical treatments for the management of guava decline disease complex

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Abstract
Guava (*Psidium guajava* L.) decline, a disease complex involving the wilt fungus *Fusarium oxysporum* f. sp. *psidii* and root-knot nematode *Meloidogyne enterolobii*, is a serious soil-borne constraint to guava production. The present study isolated the wilt pathogen from diseased guava roots at the AICRP on Fruits, MPKV, Rahuri, identified it as *F. oxysporum* f. sp. *psidii* on the basis of symptomatology and morpho-cultural characters and confirmed its pathogenicity by fulfilling Koch's postulates. Potato dextrose agar supported the maximum growth (90.00 mm) of the pathogen among seven media tested. Under *in vitro* dual culture assay, *Trichoderma harzianum* (87.78%) and *T. viride* (86.67%) were the most inhibitory bioagents, whereas *Bacillus subtilis* was the least effective (30.00%), while among five fungicides screened by the poison food technique at 1000 ppm (0.1%), carbendazim 12% + mancozeb 63% WP, tebuconazole 25.9% EC and carbendazim 50% WP completely inhibited mycelial growth. In a pot culture experiment against the *Meloidogyne–Fusarium* complex, carbendazim 12% + mancozeb 63% WP + fluopyram 34.48% SC (T_1) and the biological combination *T. harzianum* + *Purpureocillium lilacinum* (T_4) were statistically at par and the best treatments, recording the lowest per cent disease index (12.50 and 15.00%), the highest disease reduction over control (86.11 and 83.33%), the lowest root-knot index (3.00 and 3.25) and the highest plant height and root and shoot biomass. The *T. harzianum* + *P. lilacinum* combination is therefore a promising biological alternative to chemical treatment for the management of guava decline and merits validation under field conditions.

Keywords: Guava decline, Bioagents, Fungicides

1. Introduction

Guava (*Psidium guajava* L.), the "apple of the tropics", is one of the most important tropical and subtropical fruit crops of India ranking, after mango, banana and citrus in importance. The fruit is rich in vitamins, minerals, fibre and antioxidants and the leaves and bark have traditional medicinal uses.

Wilt is the most destructive disease of guava. It is soil-borne and difficult to manage, with losses of up to 80% in severely affected orchards (Misra *et al.*, 2002) [1]. *Fusarium oxysporum* f. sp. *psidii* has long been regarded as the principal pathogen. Wilting is now increasingly attributed to a disease complex termed guava decline, in which root-knot nematodes (*Meloidogyne* spp.) predispose the roots to *Fusarium* infection (Gomes *et al.*, 2011) [2].

Chemical control is of limited effectiveness against soil-borne pathogens in perennial orchards, so the emphasis is shifting towards sustainable, bioagent-based approaches. *Trichoderma* spp. and *Purpureocillium lilacinum* have suppressed *Fusarium*–root-knot nematode complexes in other crops (Khan *et al.*, 2001) [3], but information on their performance against guava decline is limited. The present investigation was therefore undertaken to isolate and characterise the pathogen and to evaluate bioagents and fungicides against it *in vitro* and in pot culture experiment for the management of guava decline.

2. Materials and methods

The study was conducted during 2024–2026 in the Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra.

2.1 Isolation, identification and pathogenicity

Wilted guava plants with root galling and vascular discolouration were sampled in 2025 from the AICRP on Fruits field, MPKV, Rahuri. Pathogen was identified based on symptomatology as well as its morphological and cultural characteristics (spore and colony characters). Pathogenicity was tested on six-month-old guava seedlings by the stem cut-end wound-hole inoculation technique (Misra *et al.*, 2000) [4], with uninoculated seedlings as control; the fungus was re-isolated from wilted plants to fulfil Koch's postulates.

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2.2 Cultural characteristics

Growth of the pathogen was compared on seven media: Sabouraud's agar (SDA), nutrient agar (NA), rose bengal agar (RBA), czapek's dox agar (CDA), corn meal agar (CMA), malt extract agar (MEA) and potato dextrose agar (PDA). Colony diameter, colony characters and sporulation (conidia per microscopic field at 400×; + scanty to ++++ abundant) were recorded after one week.

2.3 In vitro evaluation of bioagents and fungicides

Trichoderma viride, *T. harzianum*, *T. koningii*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against the pathogen by the dual culture technique (Dennis *et al.*, 1971) [5] on PDA. Five fungicides, *viz*. carbendazim 12% + mancozeb 63% WP, copper oxychloride 50% WP, tebuconazole 25.9% EC, thiophanate methyl 70% WP and carbendazim 50% WP, were tested at 0.1% (1000 ppm) by the poisoned food technique (Nene *et al.*, 1979) [6].

2.4 In vivo evaluation of bioagents and fungicides against pathogens in pot culture experiment

The pot culture experiment was conducted at Department of Plant Pathology and Microbiology, Rahuri to evaluate the efficacy of bioagents and fungicides against guava decline. The experiment was led out in CRD design with four replication and seven treatments viz., T_1 (carbendazim 12% + mancozeb 63% WP + fluopyram 34.48% SC), T_2 (tebuconazole 25.9% EC + fluopyram 34.48% SC), T_3 (fluopyram 34.48% SC), T_4 (*T. harzianum* + *P. lilacinum)*, T_5 (*P. fluorescens* + *P. lilacinum),* T_6 (*P. lilacinum)*. Healthy seedlings of guava cv. Lucknow-49 were grown in earthen pots, two plants per pot were maintained. Bioagents and fungicides found effective in *In vitro* studies were applied to soil seven days before transplanting and repeated three months after transplanting. Fifteen days after transplanting, each pot was inoculated with about 5000 second-stage juveniles of *Meloidogyne enterolobii* and 5×10^6 cfu of *F. oxysporum* f. sp. *psidii*. The control pots were supplied with water only, without any treatment. Plant height, root and shoot fresh and dry weights, per cent disease index (PDI) and root-knot index were recorded. The disease severity index was calculated by using 0-5 scale given by (Misra *et al.*, 2000) [4]. Galls and egg masses (stained with 1% trypan blue) were counted per 5 g of root and rated on a 1–5 gall index (Johnson *et al.*, 1980) [7].

3. Results and discussion

3.1 Effect of culture media on growth of the pathogen

All seven media supported growth, with significant differences. PDA supported the maximum colony diameter (90.00 mm), followed by CDA (80.00 mm) and SDA (76.00 mm), while RBA was the most restrictive (32.00 mm). Abundant sporulation was recorded on PDA and CDA and scanty sporulation on NA and RBA. The superiority of PDA agrees with Gupta *et al.* (2010) [8], who recorded the best growth of *F. oxysporum* f. sp. *psidii* on PDA.

3.2 In vitro efficacy of bioagents

The results presented in Table 1, visually depicted in Plate 1 revealed that all bioagents significantly inhibited the mycelial growth of the pathogen compared with the control (90.00 mm). *T. harzianum* exhibited the highest inhibition (87.78%; 11.00 mm), followed by *T. viride* (86.67%) and *T. koningii* (83.33%), whereas *P. fluorescens* (53.33%) and *B. subtilis* (30.00%) were less effective. The superior efficacy of *Trichoderma* spp. is consistent with the findings of Kisana *et al.* (2025) [9] on guava wilt.

Table 1. *In vitro* efficacy of bioagents against *F. oxysporum* f. sp. *psidii*

Tr No.	Bioagents	MCD (mm)*	Inhibition (%)
T_1	*Trichoderma viride*	12.00	86.67
T_2	*Trichoderma harzianum*	11.00	87.78
T_3	*Trichoderma koningii*	15.00	83.33
T_4	*Pseudomonas fluorescens*	42.00	53.33
T_5	*Bacillus subtilis*	63.00	30
T_6	Control	90.00	-
	S.E. ±	0.12	
	CD at 1%	0.35	

*Average of four replications MCD: Mean Colony Diameter

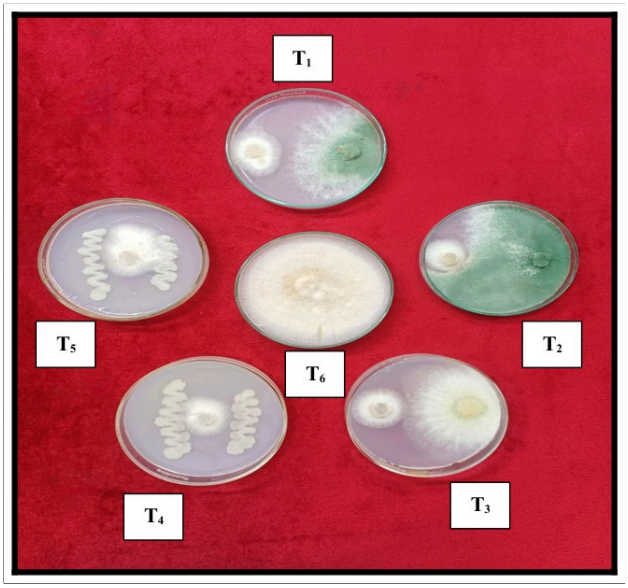


Plate 1. *In vitro* efficacy of bioagents against *F. oxysporum* f. sp. *psidii*

3.4 In vitro efficacy of fungicides

The results presented in Table 2, visually depicted in Plate 2 revealed that all fungicides significantly inhibited the mycelial growth of the pathogen. Carbendazim 12% + mancozeb 63% WP, tebuconazole 25.9% EC and carbendazim 50% WP exhibited complete (100%) inhibition, followed by thiophanate methyl 70% WP (73.33%), whereas copper oxychloride 50% WP was the least effective (22.22%). The complete inhibition observed with carbendazim-based and triazole fungicides is consistent with the findings of Sangeetha *et al.* (2019) [10] on the guava wilt pathogen.

Table 2. *In vitro* efficacy of fungicides against *F. oxysporum* f. sp. *psidii*

Tr No.	Fungicides	Concentration (%)	MCD (mm)*	Inhibition (%)
T_1	Carbendazim 12 % + Mancozeb 63 % WP	0.1	0.00	100
T_2	Copper oxychloride 50% WP	0.1	70.00	22.22
T_3	Tebuconazole 25.9% EC	0.1	0.00	100
T_4	Thiophanate methyl 70% WP	0.1	24.00	73.33

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Tr No.	Fungicides	Concentration (%)	MCD (mm)*	Inhibition (%)
T_5	Carbendazim 50% WP	0.1	0.00	100
T_6	Control	-	90.00	-
		S.E. ±	0.09	
		CD at 1%	0.27	

*Average of four replications MCD: Mean Colony Diameter

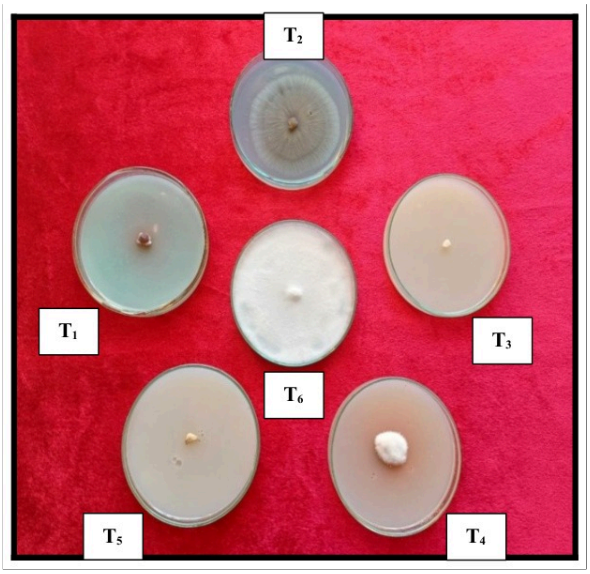


Plate 2. *In vitro* efficacy of fungicides against *F. oxysporum* f. sp. *psidii*

3.5 In vivo evaluation of bioagents and fungicides against pathogens in pot culture experiment

Plant growth parameters: The data presented in Table 3 revealed that all treatments significantly improved plant height and root and shoot biomass compared with the inoculated control (T_7). T_1(carbendazim 12% + mancozeb 63% WP + fluopyram 34.48% SC) recorded the highest plant height (84.73 cm), root fresh weight (26.43 g), root dry weight (15.80 g), shoot fresh weight (61.53 g) and shoot dry weight (28.30 g) and was statistically at par with T_4 (*T. harzianum* + *P. lilacinum*), which recorded 84.33 cm, 26.13 g, 15.60 g, 60.85 g and 27.90 g, respectively. T_2 (tebuconazole + fluopyram) and T_5 (*P. fluorescens* + *P. lilacinum*) showed intermediate performance, whereas T_3 (fluopyram alone) and T_6 (*P. lilacinum* alone) were less effective than the combinations and control recorded the lowest values (69.58 cm; 18.03, 10.03, 43.60 and 17.95 g respectively).

The superior plant growth observed in T_1 may be attributed to the combined fungicidal and nematicidal activity of fluopyram, which is consistent with the reported improvements in plant height and root and shoot fresh weight in nematode-infested tomato (Li *et al*., 2021) [11].

Table 3. Effect of bioagents and fungicides on plant growth in the pot culture experiment

Tr. No.	Treatment	Plant height (cm)*	Root fresh wt (g)*	Root dry wt (g)*	Shoot fresh wt (g)*	Shoot dry wt (g)*
T_1	Carbendazim 12% + mancozeb 63% WP + fluopyram 34.48% SC	84.73	26.43	15.80	61.53	28.30
T_2	Tebuconazole 25.9% EC + fluopyram 34.48% SC	81.45	24.35	14.43	57.50	26.05
T_3	Fluopyram 34.48% SC	75.48	21.13	12.33	50.60	21.95
T_4	*T. harzianum* + *P. lilacinum*	84.33	26.13	15.60	60.85	27.90
T_5	*P. fluorescens* + *P. lilacinum*	78.38	22.73	13.33	53.90	23.95
T_6	*P. lilacinum*	72.68	19.53	11.23	47.10	19.95
T_7	Control (inoculated)	69.58	18.03	10.03	43.60	17.95
	S.E. ±	0.23	0.18	0.16	0.27	0.20
	CD at 1%	0.68	0.52	0.48	0.81	0.59

*Average of four replications.

Disease index and root galling: The data presented in Table 4 revealed that all treatments significantly differed. T_1 (carbendazim 12% + mancozeb 63% WP + fluopyram 34.48% SC) recorded the lowest percent disease incidence (PDI) (12.50%) and the highest disease reduction (86.11%), followed by T_4 (*Trichoderma harzianum* + *Purpureocillium lilacinum*), with a PDI of 15.00% and disease reduction of 83.33%; both treatments were statistically at par. T_2 (tebuconazole 25.9% EC + fluopyram 34.48% SC) and T_5 (*Pseudomonas fluorescens* + *P. lilacinum*) showed intermediate effectiveness, whereas T_3 (fluopyram 34.48% SC alone) and T_6 (*P. lilacinum* alone) were less effective. Root galling and egg mass production were also lowest in T_1 (19.63) and T_4 (22.83) per 5 g of root, with corresponding root-knot index values of 3.00 and 3.25, respectively. The untreated control (T_7) recorded the highest PDI (90.00%), the maximum number of galls and egg masses (115.60 per 5 g of root) and the highest root-knot index (5.00). These findings are consistent with Khan *et al.* (2001) [3], who reported improved suppression of root-knot nematodes in tomato using *P. lilacinum* in combination with *T. harzianum*

Table 4. Effect of bioagents and fungicides on plant growth in the pot culture experiment

Tr. No.	Treatment	PDI (%)*	Disease reduction (%)	Galls/egg masses per 5 g root*	Root knot index*
T_1	Carbendazim 12% + mancozeb 63% WP + fluopyram 34.48% SC	12.50 (20.47)	86.11	19.63 (4.49)	3.00

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Tr. No.	Treatment	PDI (%)*	Disease reduction (%)	Galls/egg masses per 5 g root*	Root knot index*
T_2	Tebuconazole 25.9% EC + fluopyram 34.48% SC	25.00 (29.89)	72.22	36.68 (6.09)	3.75
T_3	Fluopyram 34.48% SC	40.00 (39.23)	55.56	86.33 (9.32)	4.00
T_4	*T. harzianum* + *P. lilacinum*	15.00 (22.50)	83.33	22.83 (4.80)	3.25
T_5	*P. fluorescens* + *P. lilacinum*	32.50 (34.72)	63.89	54.65 (7.43)	4.00
T_6	*P. lilacinum*	52.50 (46.44)	41.67	90.20 (9.52)	4.00
T_7	Control (inoculated)	90.00 (74.14)	–	115.60 (10.77)	5.00
	S.E. ±	2.73		0.13	0.13
	CD at 1%	8.02		0.38	0.39

*Average of four replications (Figures in parentheses are angular transformed values)

4. Conclusion

The wilt pathogen associated with guava decline was identified as *Fusarium oxysporum* f. sp. *psidii* and proved pathogenic on guava seedlings. *In vitro*, *T. harzianum* and *T. viride* were the most effective bioagents and carbendazim 12% + mancozeb 63% WP, tebuconazole 25.9% EC and carbendazim 50% WP completely inhibited the pathogen. In the pot culture experiment against the *Meloidogyne–Fusarium* complex, carbendazim + mancozeb + fluopyram (T_1) gave the lowest PDI (12.50%) and root-knot index (3.00), along with the highest plant height and root and shoot biomass. The combination of *T. harzianum* + *P. lilacinum* (T_4) was statistically at par with T_1 with a PDI of 15.00%, a root-knot index of 3.25 and 83.33% of disease reduction. Combination treatments were consistently superior to their single components. The *T. harzianum* + *P. lilacinum* combination is thus a promising and sustainable approach for the biological management of guava decline.

5. Limitations

The pathogen was identified on morphological and cultural characters and pathogenicity was demonstrated for *F. oxysporum* f. sp. *psidii*. The disease-management results were obtained in pot culture on a single cultivar with artificial inoculation; field validation across locations and seasons is needed before recommendation.

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