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In vitro Evaluation of Carbendazim 50% WP, Antagonists and Botanicals Against *Fusarium oxysporum* f. sp. *psidii* Associated with Rhizosphere Soil of Guava

[1]S. Srivastava, [2]V.P. Singh, [1]R. Kumar, [3]M. Srivastava, [1]A. Sinha and [4]S. Simon
[1]Department of Mycology and Plant Pathology, Institute of Agriculture Sciences, B.H.U., Varanasi-221 005, India
[2]Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250 110, India
[3]Department of Botany, Harish Chandra P.G. College, Varanasi-221 001, India
[4]Department of Plant Protection, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Former Allahabad Agricultural Institute-Deemed University), Allahabad-211 007, India

Corresponding Author: Seweta Srivastava, Department of Mycology and Plant Pathology, Institute of Agriculture Sciences, B.H.U., Varanasi-221 005, India Tel: 0091584222548 Fax: 00919453288623

ABSTRACT

The aim of the present study was to check the efficacy of carbendazim 50% WP, antagonists and botanicals against *Fusarium oxysporum* f. sp. *psidii* associated with rhizosphere soil of guava. Guava (*Psidium guajava* L.) the apple of tropics is one of the most important fruit in India. The fruit of guava is a good source of vitamin C and pectin. Guava wilt is a serious disease and it recognized as a main causal organism. Isolation and identification of pathogen (*Fusarium oxysporum* f. sp. *psidii*) was carried out in the Department of Plant Protection, Allahabad Agricultural institute Deemed University, Allahabad. The radial growth of *Fusarium cxysporum* f. sp. *psidii* was fully inhibited at high concentrations like 100, 1000 and 10,000 ppm of cardendazim 50% WP whereas antagonists like *Trichoderma* spp. Produced maximum inhibition zone (61.91%) followed by *Aspergillus niger* (61.12%). The radial growth of *Fusarium cxysporum* f. sp. *psidii* was also significantly less in neem leaf extract treatment followed by *Lantana* leaf extract. Management of guava wilt by chemical (systemic fungicide) can be spectacular but this is relatively short-term measure. Eco-friendly management practices, i.e., use of bio-control agents and botanicals was studied *in vitro* which gave better results and these practices can be economical, long lasting and free from residual side effects.

Key words: Guava, *Fusarium oxysporum* f. sp. *psidii*, carbendazim 50% WP, antagonists, botanicals

INTRODUCTION

Wilt disease is a major limiting factor for the productivity and production of guava. The exact cause of disease is not fully understood and it is recognized as disease complex (Suhag, 1976) caused by more than one organism and possibilities not to be ruled out. All the strains of *Fusarium* exist saprophytically, but some are well known for inducing wilt (O'Donnell *et al*., 2000; Jurgenson *et al*., 2002; Schroers *et al*., 2005). Forma specials of *Fusarium oxysporum* causing wilt disease in *Psidium guajava* L. is designated as *Fusarium oxysporum* f. sp. *psidii* (Prasad *et al*.,

1952; Gupta, 2010). *Fusarium oxysporum* f. sp. *psidii* mainly present in the rhizosphere soil and in vascular region of roots. Rhizosphere soils contain a wide variety of microbes viz. fungi, viruses, nematodes and bacteria, etc. Different type of interaction may takes place in the rhizosphere, which include effect of microflora on plant growth and different type of interactions like symbiosis, antagonism, synergism between the rhizosphere microfloras. Rhizosphere and Rhizoplane microbial population can influence the various aspect of plant growth and development like root morphology, root to shoot weight ratio, physiological process and crop yield, etc. Rhizosphere microorganisms, directly or indirectly effects the plant growth by making the nutrients available to the plants and by being antagonistic to phytopathogens. They influence the growth of plants and cause death in severe cases of infection. Edward (1960b, c) explained that *Fusarium oxysporum* f. sp. *psidii* penetrate either directly through the root piliferous layer of the guava seedlings or through openings caused by secondary roots. First external symptom of the guava wilt is the appearance of yellow colouration with slight curling of the leaves on terminal branches. Subsequently, browning, drooping and premature shedding of the leaves occur. Infected guava plants start showing sign of wilting right with onset of rainy season in August with maximum number dying in September and October (Das Gupta and Rai, 1947; Edward, 1960a; Suhag, 1976). As the wilt progresses, the fruits of affected branches remain under developed, hard and stony. Later on, the entire plant becomes defoliated and eventually dies, but hard, stony and dark brown and/or black fruits hang on the branches for sometime. Usually, fifteen days are requiring for the complete wilting, but some trees take even upto one year. The finer roots show black streaks, which become prominent on removing the bark (Das Gupta and Rai, 1947). The roots also show rotting at the basal region and the bark is easily detachable from the cortex. The cortical regions of the stem and root show distinct discolouration and damage. Light brown discolouration is also noticed in the vascular tissues (Chattopadhyay and Bhattacharjya, 1968). In general, above ten year old plants are more prone to the wilt incidence. Partial wilting is also common, when one side of the few branches wilt during the first year and in the next year full plant dies. Suhag (1976) observed that it is possible to regenerate even the worst affected trees by severe pruning followed by drenching with 0.2% either benlate or bavistin four times in a year and spray in twice with metasystox and zinc sulphate. Spread of the wilt could be checked by judicious amendments of N and Zn (Suhag and Khera, 1986). Pesticides are applied to plant and soil to control plant parasites and weed; they may also affect soil properties, micro-organisms and hosts (Altman and Campbell, 1977). Farmers are using very toxic pesticides, for the controlling of soil borne diseases as with caused by *Fusarium* sp. which are very hazardous to our flora and fauna. Biocontrol agents *Trichoderma* sp. and *Streptomyces chibaensis* have been suggested for the control of wilt pathogens (Dwivedi, 1992). *Trichoderma* sp. and *Aspergillus niger* were isolated from the rhizosphere of healthy guava plants and tested against the wilt pathogen in the laboratory (Singh *et al*., 2003). In addition to these control measures, use of many plant extracts and botanical fungicides have been found to be effective and gaining importance in crop production in the view of their selective action, low cost, environment friendly, long lasting effect, etc. to control of many plant diseases (Oros and Ujvary, 1999; Mamatha and Rai, 2004). The present study has been undertaken to evaluate the effects of some ecofriendly compounds against *Fusarium oxysporum* f. sp. *psidii*.

MATERIALS AND METHODS

Survey was conducted in 2005-2006 in five different places of Allahabad, district to collect the soil. One hundred soil samples were collected from the rhizosphere zone of wilted guava tree.

Isolation of *Fusarium oxysporum* f. sp. *psidii* was carried out with the help of serial dilution technique in PDA (Aneja, 2005). One milliliter of suspension (from rhizosphere soil) from dilution

(10^{-2}) to (10^{-5}) was transferred in sterilized plates. Ten milliliter of PDA medium was poured in each plate and gently rotated to ensure uniform distribution of inoculums. After solidification, the plates were incubated at 28°C for 24 h in inverted position so that the condensed vapour may not hamper the growth of the fungus to be isolated. The pathogens were identified on the basis of colony characters, microscopic examination and Camera Lucida drawings. The culture of *Fusarium oxysporum* f. sp. *psidii* was purified from isolated dishes and maintained by periodic sub-culturing in PDA slants after every 15 days (Aneja, 2005).

Effect of carbendazim 50% WP on *Fusarium oxysporum* f. sp. *psidii* was done by poisoned food technique was followed at different ppm (Nene and Thapliyal, 2000). The principle involved in this technique is to supplement the nutrient medium with a toxic chemical and then allowing a test fungus to grow on such a medium and evaluated the effect of such chemical by measuring the radial growth of the fungus. The required dilutions of chemical (10,000, 1000, 100, 10 and 0 ppm) were prepared. Stock solution of 10,000 ppm, 0.2 g of active ingredient for respective chemical was added to 100 mL of sterilized PDA medium. Five treatments including control were applied in experiment and each treatment was replicated three times.

Bio-efficacy test of antagonists has been done against *Fusarium oxysporum* f. sp. *psidii* with the help of dual culture (Aneja, 2005). The effects of antagonists were studied by Zone of Inhibition technique (*in vitro*). The three antagonists were *Aspergillus niger*, *Penicillium* sp. and *Trichoderma* sp.

Formula to calculate I.O.C. % is:

$$\text{I.O.C. (\%)} = \frac{\text{Radial growth in control plate} - \text{Radial growth in treated plate}}{\text{Radial growth in control plate}} \times 100$$

Four treatments including control were applied in experiment and each treatment was replicated three times.

Treatment	Symbol
Aspergillus niger + *Fusarium* sp.	T_1
Penicillium sp. + *Fusarium* sp.	T_2
Trichoderma sp. + *Fusarium* sp.	T_3
Control	T_0

Effect of neem leaf extract (*Azadirachta indica*), *Datura* leaf extract (*Datura stramonium*) and *Lantana* leaf extract (*Lantana camera*) at control, 5, 10 and 15% has been done against *Fusarium oxysporum* f. sp. *psidii* in three replications with the help of poisoned food technique (Nene and Thapliyal, 2000). Fifteen mililiter of extract was added in 85 mL of PDA medium for obtaining 15% concentration. Similarly 10 and 5% concentrations were prepared.

Inoculation of test organism: Measured quality of extract was added in sterilized Petri plates in three replications with treatments T_1, T_2 and T_3. No extract was added to Petri plate with treatment T_0 (control). Prepared medium was poured and spread uniformly in the Petri plates. After sometimes media was solidified, then with the help of sterilized cork borer, discs of 5 mm. diameter were cut from actively growing fungal cultures and then placed in centre of Petri plate under aseptic conditions containing the poisoned food. The inoculated Petri plates were incubated at 25°C and the radial growth of colony in mm. was measured after 2, 4 and 6 days of incubation.

In the present experiments, R.B.D. was applied. The analysis of variance technique was applied for drawing conclusions from the data. The calculated value was compared with tabulated value at 5% level of probability for the appropriate degree of freedom (Fischer and Yates, 1968).

RESULTS

Data presented in Table 1 reveals that different ppm of carbendazim 50% WP with pathogen *Fusarium oxysporum* f. sp. *psidii* was found to be significant. On 2nd, 4th and 6th days, radial growths of colony were not significantly different between treatments T_2, T_3 and T_4. The maximum radial growth of *Fusarium oxysporum* f. sp. *psidii* was found in T_0 (control) followed by T_1 (10 ppm carbendazim 50% WP). The radial growth of *Fusarium oxysporum* f. sp. *psidii* was fully inhibited at 100 ppm (T_2), 1000 ppm (T_3) and 10,000 ppm (T_4) of carbendazim 50% WP.

Data presented in Table 2 reveals that in treatments *viz.,* T_1 (*Aspergillus niger* + *Fusarium oxysporum* f. sp. *psidii*), T_2 (*Penicillium* sp. + *Fusarium oxysporum* f. sp. *psidii*) and T_3 (*Trichoderma* sp. + *Fusarium oxysporum* f. sp. *psidii*), growth of *Fusarium oxysporum* f. sp. *psidii* was significantly inhibited as compared to T_0 (control). After 24 h, inhibited over control percentage of treatments T_1 (19.22) and T_3 (19.22) was similar followed by T_2 (09.99). After 48 h, inhibition over control percentage of treatment T_3 (44.07) was maximum followed by T_1 (43.22) and T_2 (24.70). After 72 h, the maximum inhibition was observed in T_3 (61.91) followed by T_1 (61.12). The maximum inhibition over control percentage was in T_2 (49.22).

Table 1: Effect of carbendazim 50% WP on the radial growth of *Fusarium oxysporum* f. sp. *psidii* at different ppm

		Mean of radial growth (mm)		
Treatments	Concentrations (ppm)	2 days	4 days	6 days
T_0	0	31.50	66.30	90.00
T_1	10	16.16	19.30	24.33
T_2	100	12.00	12.00	12.00
T_3	1000	12.00	12.00	12.00
T_4	10,000	12.00	12.00	12.00
Statistical analysis				
F-test		S	S	S
SEM		0.35	0.58	0.67
CD at 5%		0.80	1.34	1.55

S: Significant, SEM: Standard error mean

Table 2: Effect of *Aspergillus niger*, *Penicillium* sp. and *Trichoderma* sp. on the growth of *Fusarium oxysporum* f. sp. *psidii* at different time intervals

	D1			D2			D3		
	Growth (mm)			Growth (mm)			Growth (mm)		
Treatment	A	P	I.O.C.%	A	P	I.O.C.%	A	P	I.O.C.%
T_1	33.33	14.00	19.22	50.33	15.33	43.22	73.67	16.33	61.12
T_2	16.00	15.60	09.99	40.00	20.33	24.70	68.67	21.33	49.22
T_3	33.00	14.00	19.22	60.33	15.00	44.07	74.00	16.00	61.91
T_0		17.33			27.00			42.00	

A: Antagonist, P: Pathogen, Each value is mean of 3 replicates. I.O.C.: Inhibition over control. D1: After 24 h of incubation, D2: After 48 h of incubation and D3: After 72 h of incubation

Table 3: Effect of neem leaf extract on the radial growth (mm) of *Fusarium oxysporum* f. sp. *psidii* at different concentrations

		Mean of radial growth (mm)		
Treatments	Concentrations (%)	2 days	4 days	6 days
T_0	C	31.67	66.33	90.00
T_1	5	7.50	11.00	22.33
T_2	10	6.17	6.83	7.83
T_3	15	5.33	6.33	7.33
Statistical analysis				
F-test		S	S	S
SEM		0.46	0.75	0.32
CD at 5%		1.14	1.83	0.783

S: Significant. SEM: Standard error mean

Table 4: Effect of *Datura* leaf extract on the radial growth (mm) of *Fusarium oxysporum* f. sp. *psidii* at different concentrations

		Mean of radial growth (mm)		
Treatments	Concentrations (%)	2 days	4 days	6 days
T_0	C	31.50	66.33	90.00
T_1	5	07.50	17.33	39.33
T_2	10	07.30	09.00	26.30
T_3	15	05.83	07.50	18.67
Statistical analysis				
F-test		S	S	S
SEM		04.16	0.75	0.60
CD at 5%		10.17	07.85	01.47

S: Significant. SEM: Standard error mean

Table 5: Effect of *Lantana* leaf extract on the radial growth (mm) of *Fusarium oxysporum* f. sp. *psidii* at different concentrations

		Mean of radial growth (mm)		
Treatments	Concentrations (%)	2 days	4 days	6 days
T_0	C	31.67	66.33	90.00
T_1	5	07.00	12.33	25.33
T_2	10	06.33	09.00	17.83
T_3	15	05.67	07.33	14.67
Statistical analysis				
F-test		S	S	S
SEM		0.22	0.66	0.59
CD at 5%		0.54	01.61	01.44

S: Significant. SEM: Standard error mean

Data presented in Table 3 reveals that neem leaf extract at different concentrations was found to be significant to manage the growth of *Fusarium oxysporum* f. sp. *psidii*. The radial growths of colony on 2nd and 4th day were not significantly different from T_2 and T_3 and significantly different from T_0 and T_1. On 6th day, the radial growth was not significantly different between T_2 (07.83) and T_3 (07.33) as compared to T_1 (22.33). Data presented in Table 4 reveals that radial growth of colony on 2nd day were not significantly different between T_1 (07.50), T_2 (07.30) and T_3 (05.83). On 4th day, the radial growth was not significantly different between T_2 (09.00) and T_3 (07.50). On 6th day, the radial growth was significantly different in T_0 (90.00), T_1 (39.33), T_2 (26.33) and T_3 (18.67). Data presented in Table 5 reveals that radial growth of colony on 2nd day

were significantly different in T_0 (31.67), T_1 (07.00), T_2 (06.33) and T_3(05.67). On 4th day, the radial growth was significantly different in T_0 (66.33), T_1 (12.33), T_2 (09.00) and T_3 (07.33). On 6th day, the radial growth was significantly different in T_0 (90.00), T_1 (25.33), T_2 (17.83) and T_3 (14.67).

DISCUSSION

According to Table 1, the radial growth of *Fusarium oxysporum* f. sp. *psidii* was inhibited at high concentrations of carbendazim 50% WP and at low concentration of carbendazim 50% WP the radial growth of *Fusarium oxysporum* f. sp. *psidii* was significantly less. This might be due to carbendazim 50% WP is a systemic fungicide which is effective against *Fusarium oxysporum* f. sp. *psidii*. Suhag, 1976 reported control of wilt by severe pruning and then drenching with 0.2% either benlate or bavistin 4 times in a year and spraying twice with metasystox and zinc sulphate. Mishra and Pandey (1999) reported that though different fungicides viz., bavistin, topsin M, indofil M-45, thiram, blitox check the various wilt pathogens increases its aggressiveness with profuse spore mass production in the soil, once the effect of these fungicides diminishes in soil.

According to Table 2 observations, *Trichoderma* sp. produced maximum inhibition zone followed by *Aspergillus niger* and might be due to *Penicillium* sp. against *Fusarium oxysporum* f. sp. *psidii*. This *Trichoderma* sp. produced toxic diffusates, gliotoxin and because of its fast growing nature it inhibits the growth of the pathogen. Singh *et al.* (2003), Misra (2006) and Gupta *et al.* (2009) reported that bioagents like *Aspergillus niger*, *Trichoderma* sp. and *Penicillium citrinum* and some bio-dynamic antagonists have shown their effectiveness towards the control of wilt pathogens of guava. Misra *et al.* (2004) was also tested these fungi for the control of wilt pathogen in laboratory conditions, these were found quite effective. When relative growth of the three bioagents was studied by Misra and Prasad (2003) then they were found that *Aspergillus niger* was fastest growing and most effective.

The radial growth of *Fusarium oxysporum* f. sp. *psidii* was significantly less in neem leaf extract treatment followed by *Lantana* leaf extract treatments. *Datura* leaf extract was not much effective against *Fusarium oxysporum* f. sp. *psidii*. Singh *et al.* (1993), Shivpuri *et al.* (1996), Bansal and Gupta (2000) and Dwivedi and Shukla (2000) studied and reported that aqueous leaf extracts of *Azadirachta indica*, *Lantana camera*, *Ocimum sanctum*, *Datura fastosa*, *Ficus religiosa*, *Vitea megendo*, *Atropa belladonna*, *Calotropis procera*, *Eucalyptus amygdalina*, *Alianthus excels*, *Vinca rosea*, etc. were tested against *Fusarium oxysporum*. Among these leaf extracts of neem was highly toxic to *Fusarium oxysporum* showing complete inhibition of mycelial growth and spore germination at 100%. *Lantana camera* also inhibited mycelial growth and spore germination at different concentrations although they did not exhibit complete inhibition even at 100% concentration.

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