Journal of
Plant Pathology & Microbiology
ISSN: 2157-7471

Kumari et al., J Plant Pathol Microb 2012, 3:5
DOI: 10.4172/2157-7471.1000136

Research Article Open Access

Integrated Management against Root-rot of Mungbean [*Vigna radiata* (L.) Wilczek] incited by *Macrophomina phaseolina*

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Abstract

Root rot is an important disease of mungbean [*Vigna radiata* (L.) Wilczek] caused by *Macrophomina phaseolina* (Tassi) Goid was observed in farmers field of Rajasthan. For the integrated management of the disease, biocontrol agents, fungicides, herbal oils, plant extracts and organic manure as well as their combinations. Among the tested biocontrol agents against *Macrophomina phaseolina*, *T. harzianum* was found the most effective against the fungus under *in vitro* and in pots conditions followed by *T. viride and T. polysporum. P. fluorescens* was the least effective in reducing root rot incidence. All the five herbal oils and three plant products tested *in vitro* by poisoned food technique, inhibited the growth of fungus. All herbal oils gave complete inhibition of mycelial growth of pathogen at 2% concentrations. Asafoetida was found least effective. The relative efficacy of herbal oils and plant products under pots house condition exhibited palmarosa oil to be the most effective as seed dresser in reducing root rot incidence. All the seven fungicides were tested *in vitro* by poisoned food technique and in pots (*in vivo*), bavistin was considered to be the most effective to inhibit mycelial growth of pathogen as well as reducing root rot incidence, followed by captan or thiram, indofil M-45 and vitavax or raxil, while copper sulphate was the least effective treatment in both conditions. In the case of organic manures, vermicompost was the most effective in reducing the root rot incidence under pots conditions. FYM and goat manure was found moderately effective in controlling root rot incidence. Integrated management approach showed that vermicompost and bavistin in combination was more effective in reducing the root rot incidence in pots conditions.

Keywords: Mungbean; Integrated management; Vermicompost; *Macrophomina phaseolina*; Root rot

Introduction

Mungbean/green gram [*Vigna radiata* (L.) Wilczek] is one of the most important pulse crops. It is grown in almost all parts of the country and belongs to family *leguminosae*. Mung bean is an excellent source of high quality protein. It is consumed in different ways as dal, halwa, snack and so many other preparations. Ascorbic acid (Vitamin-C) is synthesized in sprouted seeds of mung bean. The leguminous crops have the capacity to fix-atmospheric nitrogen through symbiotic nitrogen fixation. It is also used as green manure crop. It is grown in summer and kharif season in northern India and in southern India [1]. In India, it is the third important pulse crop after chickpea and pigeonpea. The major fungal diseases which infect the mungbean are root rot (*Macrophomina phaseolina* (Tassi) Goid), web blight, *Rhizoctonia solani* Khun (*Thanatephorus cucumeris*), powdery mildew (*Erysiphe polygoni* DC), *Cercospora* leaf spot (*Cercospora canescens* Ellis and Martin) and anthracnose [*Colletotrichum dematium* and *C. lindemuthianum*) [2]. *Macrophomina phaseolina* (Tassi) Goid is one of the most virulent and destructive pathogen which incite diseases in wide range of hosts, while the symptoms produced were seedling rot, collar rot, leaf blight and pod rot in mothbean [3]. Root rot incited by *Macrophomina phaseolina* (Tassi) Goid has been rated as most devasting disease of mungbean. The pathogen attacks on all parts of plant i.e. root, stem, branches, petiols, leaves, pods and seeds. Moreover, seed infection of *Rhizoctonia bataticola* (*M. phaseolina*) ranges from 2.2-15.7% which causes 10.8% in grain yield and 12.3% in protein content of seed in mungbean [4]. The infected seeds act as an important source of primary inoculum for new areas [3]. Soil and seed borne nature of the disease possesses problems for an effective disease management. Therefore, an attempt has been made to integrate management of root rot disease on mung bean [*Vigna radiata* (L.) Wilczek] incited by *Macrophomina phaseolina* (Tassi) Goid which have become a serious problem in hampering the production of the mungbean in all growing areas of India.

Materials and Methods

The roots of infested mung bean plants were collected from different farmer's fields in Rajasthan and their isolation, purification were done. For the integrated management of disease the following tests performed.

The efficacy of bioagents

The efficacy of biocontrol agents against *Macrophomina phaseolina in vitro*: *In vitro* efficacy of four resident bio-control agents, *Trichoderma harzianum* (ITCC No. 6390-06), *T. viride* (ITCC No. 6384-06), *T. polysporum* (ITCC No. 7768-02) and *Pseudomonas fluroescens* (ITCC No. 113126) was tested using dual culture plate method and paper disc method [5,6]. Autoclaved PDA was poured in each Petri plate and allowed to solidify then the plates were inoculated with 2 mm dia mycelial bit was taken from 7 day old culture of *M. phaseolina* and antagonistic agents both were placed separately at equal

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Received August 20, 2012; **Accepted** September 10, 2012; **Published** September 15, 2012

Citation: Kumari R, Shekhawat KS, Gupta R, Khokhar MK (2012) Integrated Management against Root-rot of Mungbean [*Vigna radiata* (L.) Wilczek] incited by *Macrophomina phaseolina*. J Plant Pathol Microb 3:136. doi:10.4172/2157-7471.1000136

distance on the periphery of petriplates. PDA petriplates inoculated with pathogen alone served as check. Inoculated petriplates were incubated at 25 ± 1°C in BOD incubator for 7 days. The linear growth of pathogen as well as biocontrol agent was measured and per cent growth inhibition was recorded after 7 days of incubation. Regarding the efficacy of bacterial bioagent (*Pseudomonas fluroescens*) the sterilized Petri plates containing 20 ml PDA were first inoculated with 7 days old culture of *M. phaseolina* and incubated at 25 ± 1°C for 24 hrs, then the plates were inoculated with 2 mm disc of four sterilized filter paper dipped in suspension of *Pseudomonas fluroescens*. Four such discs were placed at equal distance and incubated at 25 ± 1°C for 7 days. The per cent growth inhibition was measured after 7 days incubation. For each treatment three replications were taken.

The activity of biocontrol agent against *M. phaseolina* under pot conditions *in vivo:* Apparently healthy surface sterilize seeds of mung bean were coated with *T. harzianum, T. viride, T. polysporum* @ 4 kg/ kg seed and *Pseudomonas fluroescens* @ 8 gm / kg seed separately. Ten coated seeds were sown in each pot filled with sterilized soil and inoculated with pathogen grown on sorghum grain medium. Fungus inoculated pots without treatment served as check. Each treatment was replicated three times. The pots were watered as an when required. Observation on root rot incidence on 7 days (pre and post emergence mortality) was recorded. Per cent root rot of incidence was calculated by following formula:

$$\% \text{ root rot incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

The efficacy of herbal oils and plant products on the root rot disease

The efficacy of herbal oils and plant products against Macrophomina phaseolina *in vitro*: The efficacy of eight different herbal oils and plants products were estimated against mycelial growth of *M. phaseolina* in vitro. The oils were extracted from five medicinal plants by hydro distillation using clevanger's- apparatus [7].

The oils and plant products thus obtained were evaluated with recommended concentrations (oils 2% and plant products 5%) by poisoned food techniques [8]. Each oil was mixed with PDA to get the required concentration and approximately 20 ml PDA was poured in each sterilized petriplate. After 3 hours of pouring each plate was inoculated with 2 mm dia mycelial bit which taken from the periphery of 7 day old culture of *M. phaseolina* growing on PDA. Petri plates were inoculated with pathogen alone served as check, then the inoculated Petri plates incubated at 25+1°C. For each treatment three replications were taken. The colony diameter (mean of two diagonals) was measured after 7 days of incubation. Per cent growth inhibition was calculated by Vincent's [9] formula, which is as follows:

Whereas,

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

C =Diameter of the colony in check (coverage of both diagonals)

T = Diameter of colony in treatment (average of both diagonals)

The effectiveness of herbal oils and plant products against root rot disease *in vivo*: The mungbean seeds were surface sterilized and then soaked in herbal oil and coated with plant products @ 2%. Ten treated seeds were sown in each pot that filled with sterilized soil and inoculated with pathogen grown on sorghum grain medium. Fungus inoculated pots without treatment served as check. Each treatment was replicated thrice. The pots were watered when required. Furthermore, the root rot incidence (pre and post emergence mortality) of mungbean was observed and recorded on seedlings.

The efficacy of fungicides

The effect of systemic and non-systemic fungicides against *Macrophomina phaseolina in vitro:* Efficacy of seven systemic and non-systemic fungicides was tested against mycelial growth of *M. phaseolina* by poisoned food technique. Required quantity of each fungicide added aseptically to 100 ml of sterilized PDA medium in 150 ml erlenmeyer flask separate so as to get concentration of 100, 200, 500 and 1000 ppm. The flasks were shaken several times to ensure proper and uniform distribution of the fungicides, then poured separately in sterilized petriplates and allowed to solidity.

The medium without fungicides served as check. Each plate was inoculated with 2 m dia mycelium bit of the fungus and incubated at 25 ± 1°C for 7 days. The linear growth of tested fungus was recorded and percent of growth inhibition was calculated by Vincent's formula (1947).

***In vivo* efficacy of the fungicides against pre and post emergence mortality under pots conditions:** In this experiment, autoclaved soil (soil: FYM, 3:1 autoclaved at 1.045 kg/cm^2 for 2 hours on three consecutive days) was filled in 9x12 inches sterilized earthen pots. These pots were containing soil which mixed with inoculum of the pathogen on sorghum medium @ 8 per cent (w/w) in the upper 5 cm layer of soil of each pot. The pots were covered with polythene begs and kept for 24 hours in cage house. The surface of seeds was sterilized with $HgCl_2$ solution at 0.1 % for one minute followed by 3 consecutive washing in sterilized water then sowing. The seeds were treated separately with fungicide @ 2 gm/kg seeds, and then sown in pots @ 10 seeds/pot. The experiment was conducted in CRD with three replications. Surface sterilized seeds without fungicide sown in inoculated sterilized soil served as check. The pots were watered when required. All the pots were maintained under identical condition. The root rot incidence (pre and post emergence mortality) was recorded.

Efficacy of organic manure against root rot disease under pots conditions

The experiment was carried out in earthen pots 9x12 inches which contains organic manure (FYM, goat manure, poultry manure and vermicompost) that mixed thoroughly, 5 to 7 cm depth as recommended dose of nitrogen supplied by each manure in each pot @ 10% (w/w) of soil before 1 month of sowing. In addition the inoculum was added @ 8% (w/w) of soil in each pot. In each pot 10 surface sterilized seeds were sown. Surface sterilized seed sown without organic manure with inoculated pots served a check. Each treatment was replicated thrice. Light watering was given at regular interval in each pot to maintain proper moisture levels. The observations on pre and post emergence mortality were recorded.

Integrated Disease Management

The combination of vermicompost, fungicides (bavistin) as seed treatment, herbal oil (palmarosa) and bioagent (*Trichoderma harzianum*) were evaluated against the root rot incidence. The

vermicompost was mixed with unsterilized soil contained in earthern pots were allowed decomposed for a week. The pots were then inoculated with *M. phaseolina* @ 10 g/pot and under polythene cover for few days. After 7 day of inoculation, seed of mungbean dressed with requisite quantity of fungicide, bioagent and herbal oil were sown in pots. Three replications for each treatment were maintained. A check treatment without any amendments and sown with untreated seeds was also kept for comparison.

The pots were watered when required. All the pots were maintained under identical condition. The root rot incidence (pre and post emergence mortality) of mungbean seedlings was recorded.

Results and Discussion

In the present study *Trichoderma harzianum, Trichoderma viride*, *Trichoderma polysporum* and *Pseudomonas fluroescens* were tested in *in vitro* and in pots conditions. *Trichoderma harzianum* was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (23.20%) of *Macrophomina phaseolina* followed by *Trichoderma viride*, whilst *Pseudomonas fluroescens* was the least effective in growth inhibition of the fungus. Under pot conditions, *Trichoderma harzianum* was found most effective in reducing pre and post emergence mortality (13.50 and 9.09 per cent) (Table 1). *Trichoderma viridae* was moderately effective, while *Pseudomonas fluroescens* was the least effective in reducing pre and post emergence mortality. These findings are in confirmity of earlier findings of Deshmukh and Raut [10] reported. *T. harzianum* and *T. viridae* were effective inhibiting the mycelial growth of *M. phaseolina* and reducing the disease incidence in pot experiment. Hussain et al. [11] observed that *T. harizianum* and G. virens were effective in controlling the infection of *M. phaseolina* in mungbean. Manczinger et al. [12] reported that *Trichoderma harzianum*, *T. viride* and *T. polysporum* have a strong antagonistic against soil borne pathogens.

The extracted oil from five medicinal plant namely palmarosa, lemongrass, citronella, mentha and tulsi were tested under *in vitro* and *in vivo* conditions for their antifungal properties. All the five herbal oils in *in vitro* study exhibited 100% inhibition of mycelial growth of *Macrophomina phaseolina* at 2% concentration. In pots conditions palmarosa oil was the superior treatment that showed the minimum

S.No.	Bioagents	*In Vitro* Condition	*In Vivo* Condition		
			Root rot incidence*		
		Per cent inhibition of mycelial growth*	Dose g kg^{-1}	Pre-emergence	Post-emergence
1.	*P. fluorescens*	15.80 (23.44)	6 g	22.30 (28.11)	21.67 (27.69)
2.	*T. harzianum*	23.20 (28.79)	4 g	15.00 (22.79)	10.10 (18.53)
3.	*T. polysporum*	18.30 (26.02)	4 g	20.00 (26.56)	14.80 (22.63)
4.	*T. viride*	19.70 (26.30)	4 g	16.67 (24.04)	12.00 (20.27)
5.	Check	0.00 (0.00)	-	31.60 (34.20)	26.30 (30.85)
	S.Em ±	0.33		0.36	0.41
	C.D. at 5%	1.03		1.13	1.29

*Average of three replication
Figure in parentheses are angular transformed values

Table 1: *In vitro* and *in vivo* efficacy of bio-agents as seed treatment against root rot of mungbean.

S.No	Herbal oils / Plant products	*In Vitro* Condition	*In vivo* condition		
		Per cent inhibition of mycelial growth*	Dose g kg^{-1}	Root rot incidence*	
				Pre-emergence mortality (%)	Post-emergence mortality (%)
1.	*Assafetida*	37.60	2	26.60	16.70
		(37.81)		(30.66)	(24.12)
2.	Citronella	100.00	2	18.60	12.40
		(90.00)		(25.55)	(20.62)
3.	Lemon grass	100.00	2	17.60	10.30
		(90.00)		(24.80)	(18.72)
4.	Mentha	100.00	2	18.00	10.70
		(90.00)		(25.10)	(19.09)
5.	NSKE	61.00	2	21.30	13.80
		(51.34)		(27.49)	(21.81)
6.	Palmarosa	100.00	2	14.30	7.22
		(90.00)		(22.22)	(15.56)
7.	Tulsi	100.00	2	16.30	11.00
		(90.00)		(23.81)	(19.37)
8.	Turmeric	58.90	2	24.00	15.20
		(50.10)		(29.33)	(22.95)
9.	Check	0.00	-	31.33	26.10
		(0.00)		(34.02)	(30.72)
S.Em+		0.49		0.39	0.34
C.D. at 5%		1.45		1.17	1.02

*Mean average of three replication
Figure in parentheses are angular transformed values

Table 2: *In vitro* and *In vivo* efficacy of herbal oils and plant products as seed treatment against root rot of mungbean.

S.No.	Name of fungicides	Percent inhibition of mycelial growth*				
		100	200	500	1000	Mean
1.	*Bavistin*	98.80	99.20	99.80	100.00	99.60
		(83.71)	(84.87)	(88.01)	(90.00)	(86.66)
2.	Captan	99.00	99.20	99.60	100.00	99.50
		(84.26)	(84.87)	(86.66)	(90.00)	(86.62)
3.	Copper sulphate	61.80	96.80	97.50	99.20	92.80
		(51.83)	(79.69)	(80.90)	(84.90)	(74.33)
4.	Indofil M-45	89.60	99.00	99.60	99.80	98.40
		(71.19)	(84.26)	(86.66)	(88.01)	(82.53)
5.	Rexil	92.10	94.40	95.50	97.40	95.10
		(73.68)	(76.31)	(77.75)	(80.72)	(77.12)
6.	Thiram	98.30	99.40	99.60	99.70	99.40
		(82.51)	(85.71)	(86.66)	(87.25)	(85.53)
7.	Vitavax	63.50	98.70	99.00	99.40	94.60
		(52.83)	(83.20)	(84.26)	(85.71)	(76.50)
8.	Check	0.00	0.00	0.00	0.00	0.00
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
	Mean	78.80	90.90	92.30	94.00	89.60
		(62.58)	(72.38)	(73.86)	(75.82)	(71.16)
		S.Em ±	C.D. at 5%			
	F	0.98	2.75			
	C	0.56	1.59			
	F x C	1.60	4.50			

*Mean average of three replication
Figure in parentheses are angular transformed values

Table 3a: *In vitro* efficacy of fungicides against inhibition of radial mycelial growth of *Macrophomina phaseolina*.

pre and post emergence morality in mungbean with 12.87 and 6.50%, respectively, followed by tulsi (Table 2). Our findings are in conformity of earlier studies with Thakare et al. [7] who reported that lemongrass, palmarosa, citronella, mentha and tulsi exhibited 100% inhibition of *M. phaseolina* at 2% concentration in *in vitro* test. Ahmed and Srivastava (2002) reported that seed treatment with palmarosa oil gave best control of dry root rot of chickpea cause by *M. phaseolina* in pot condition.

In *in vitro* test, all the fungicides namely bavistin, captan, thiram, indofil-M-45, vitavax, raxil and copper sulphate tested at 100, 200, 500 and 1000 ppm concentration inhibited the mycelial growth of *M. phaseolina* in petriplates. Bavistin and captan were the most effective fungicides in reducing the mycelial growth of *M. phaseolina* and gave complete inhibition of mycelial growth at 1000 ppm concentrations (Table 3a).

Captan, bavistin, thiram and rexil effectively inhibited growth of the fungus at 100 ppm concentration by 99, 98.80, 98.30 and 92.10%, respectively.

On the other hand, Vitavax and copper sulphate were the least effective treatments that inhibit mycelial growth with values of 63.50 and 61.80%, consecutively. Furthermore, the suggested action of these chemicals were inhibits the germination, growth and multiplication of the pathogen or are directly toxic. Our findings are in confirmity to Sinha and Khare [13] who found carbendazim and thiram effective against *M. phaseolina* in lab and field test. Ramadoss and Sivapraskasam [14] reported that sclerotial production of *M. phaseolina* was completely inhibited by carbendazim and thiram.

Results of pots experiment indicated that all the tested fungicides significantly reduced the mungbean root rot disease incidence. Bavistin was found the most effective treatment when used as seed dresser @ 2 g/kg seeds as compared to the other used fungicides as well as exhibiting the minimum mortality pre and post emergence (13.50 and 8.82 %) compared to check (29.39 and 26.10%) (Table 3b). Vitavax, raxil and copper sulphate were the least effective in reducing root rot

S.No.	Fungicides	Dose g kg^{-1}	Root rot incidence*	
			Pre-emergence mortality	Post-emergence mortality
1.	*Bavistin*	2.0	15.00	9.80
			(22.79)	(18.24)
2.	Captan	2.0	16.60	10.60
			(24.04)	(19.00)
3.	Copper sulphate	2.0	25.30	21.60
			(30.20)	(27.69)
4.	Indofil M-45	2.0	19.30	14.90
			(26.06)	(22.71)
5.	Rexil	2.0	23.30	20.60
			(28.86)	(26.99)
6.	Thiram	2.0	17.30	12.80
			(24.58)	(20.96)
7.	Vitavax	2.0	21.70	16.60
			(27.76)	(24.04)
8.	Check	-	32.66	29.00
			(34.82)	(32.58)
	S.Em+		0.43	0.40
	C.D. at 5%		1.29	1.20

*Mean average of three replication
Figure in parentheses are angular transformed values

Table 3b: *In vivo* efficacy of fungicides as seed dresser against root rot of mungbean.

S.No.	Organic manure	Dose g kg^{-1}	Root rot incidence*	
			Pre-emergence	Post-emergence
1.	FYM	10	20.00	15.00
			(26.56)	(22.79)
2.	Goat manure	10	21.60	18.70
			(27.69)	(25.62)
3.	Poultry manure	10	24.60	20.00
			(29.73)	(26.56)
4.	Vermicompost	10	18.30	12.00
			(25.53)	(20.27)
5.	Check	-	31.60	27.30
			(34.20)	(31.50)
	S.Em +		0.57	0.50
	C.D. at 5%		1.78	1.56

*Average of three replication
Figure in parentheses are angular transformed values

Table 4: *In vivo* efficacy of organic manures against root rot of mungbean.

S.No	Treatments	Root rot incidence*	
		Pre-emergence	Post-emergence
1.	Vermicompost+seed treatment with *T. harzianum*	8.60	8.40
		(17.05)	(16.80)
2.	Vermicompost+seed treatment with palmarosa oil	12.50	9.00
		(20.70)	(17.46)
3.	Vermicompost+bavistin	6.40	5.60
		(14.65)	(13.69)
4.	Vermicompost+seed treatment with bavistin+*T. harzianum*	2.33	0.00
		(8.72)	(0.00)
5.	Check	30.33	27.60
		(33.40)	(31.69)
	S.Em ±	0.34	0.32
	C.D. at 5%	1.07	1.00

*Mean average of three replication
Figure in parentheses are angular transformed values

Table 5: Integrated efficacy of organic manure, bioagent, herbal oil and fungicide on the incidence of root rot of mungbean.

incidence (pre and post emergence mortality) 14.94 and 9.54, 15.57 and 11.52, 17.37 and 13.41, 19.53 and 14.94, 20.97 and 18.54 and 22.77 and 19.44%, respectively. Rathore [15] found that seed treatment with carbendazim @ 2 g/kg seeds was the most effective in reducing root rot incidence caused by *M. phaseolina* in green gram (vigna radiata), and these findings are in conformity of our study.

Addition of organic manures into the soil improves structure and texture of the soil. They influence by changing aeration, porosity temperature and water holding capacity of the soil which results in rapid root extension, balance availability of nutrients and better plant vigour. All these changes indirectly reduce the incidence of root rot disease. Their effect may include germination of pathogen propagate followed by starvation, microbial lysis and increase general fungistatis. Organic manures namely FYM, goat manure, poultry manure and vermicompost were tested against root rot incidence under pot conditions. Our results of pot experiment indicated that all the organic manure reduced root rot incidence (pre and post emergence mortality) significantly over check (Table 4). Vermicompost was the most superior treatment which reduced the root rot incidence by 16.47 and

10.80% as compared to check 28.44 and 24.57% followed by FYM and goat manure exhibited 18.00 and 13.50% and 19.44 and 16.83% root rot incidence.

Poultry manure (chicken manure) was the less effective treatment. Latha and Rajappan [16] reported that root rot incidence was significantly reduced by FYM in blackgram caused by *M. phaseolina*. Mathur et al. [17] observed that vermicompost was significantly superior in reducing incidence of *F. oxysporum* causing wilt of fenugreek in field conditions.

Root rot of mungbean caused by *M. phaseolina* and considered a soil borne disease. The current approach to control this disease is to use chemicals which in addition to being costly it is disturbs the soil ecology. An experiment was conducted to determine the compatibility of biocontrol agent, herbal oil with commercially effective fungicides which were found effective under *in vivo* conditions at different combinations. The obtained results of pots experiment indicated that vermicompost (10%) + bavistin (0.1%) was the most effective against the root rot incidence in mungbean giving minimum Pre and post emergence mortality (5.74 and 5.04%), followed by vermicompost (10%) + bavistin (0.1%) and *T. harizianum* (4%) exhibiting (5.76 and 5.04%) pre and post emergence mortality (Table 5). The ineffectiveness of the combination between bavistin with *T. harizianum* may be due to the sensitivity of *T. harizianum* to the bavistin fungicide. Our findings are in agreement with those obtained by Upmanu et al. [18] who found that soil amendment + seed treatment with bavisitn was highly effective in reducing pre and post emergence mortality in french bean. Besides, observed that *T. harizianum* was sensitive to bavistin. Gupta and Sharma [19] reported that bavistin (0.1 or 0.05%) in combination with different antagonistic viz., *T. viride* and *T. harizianum* were found effective against white root rot of apple.

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