academicJournals

Vol. 8(30), pp. 2835-2848, 23 July, 2014
DOI: 10.5897/AJMR2013.6336
Article Number: 95891EB46292
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Effect of fungicides, plant extracts / botanicals and bioagents against damping off in brinjal

V. M. Gholve*, V. R. Tatikundalwar, A. P. Suryawanshi and Utpal Dey

Department of Plant Pathology, V. N. Marathwada Krishi Vidyapeeth, Parbhani – 431 402, maharashtra, India.

Received 8 September, 2013; Accepted 7 July, 2014

Brinjal damping off incited by *Pythium ultimum* Trow. is one of the most important and destructive disease of brinjal, which can cause mortality upto 90% (pre -emergence seed rot and post emergence seedling mortality). Being soil borne, *P. ultimum* is very difficult to manage with fungicides alone and also uneconomical. Therefore, the present *in vitro* studies were undertaken to test bioefficacy of the nine fungicides (each at 500, 1000 and 1500 ppm), ten plant extracts / botanicals (each at 10, 15 and 20 %) and seven bioagents against *P. ultimum.* The experiments were designed with CRD and all the treatments replicated thrice. Results reveal that all the fungicides, botanicals and bioagents tested were found effective and were fungistatic against the test pathogen and significantly inhibited its growth over untreated control. Of the fungicides tested, Metalaxyl was found most effective and recorded 84.22% mean growth inhibition of the test pathogen. The second and third best fungicides found were Captan + metalaxyl and carbendazim + Mancozeb with mean growth inhibition of 82.42 and 62.88%, respectively. The rest of the fungicides tested recorded mean growth inhibition in the range of 24.50 to 52.79%. Of the botanicals evaluated, garlic was found most effective and recorded significantly the highest mean mycelial growth inhibition (94.83%). The second and third best botanicals found effective were Adulsa (75.53 %) and Datura (60.65 %). The rest of the botanicals tested recorded mean growth inhibition in the range of 20.82 to 56.83%. Of the bio-agents evaluated, *Trichoderma viride* was found most effective and recorded significantly highest mean mycelial growth inhibition (69.44%). The second and third best bioagents found effective were *Trichoderma koningii* (67.32%) and *Trichoderma hamatum* (63.99%); the rest of the bioagents also recorded significant inhibition of the test pathogen. Results reveals that seed treatment of captan (at 1.5 g/kg) + metalaxyl (at 3g/kg seed)+ garlic extract (at 100ml/kg soil) +soil application of *T. viride* (at 25g/kg soil) was the most effective treatment which could be practiced on large scale for management of damping off disease in brinjal and other solanaceous vegetable crops.

Key words: *Pythium ultimum,* plant extracts/ botanicals, bioagents, fungicides, Brinjal.

INTRODUCTION

Brinjal or egg plant (*Solanum melongena* L.) is an important Solanaceous vegetable crop of sub tropics and tropics, and supposed to have originated in India. Brinjal occupies second position among the vegetable. Brinjal is

*Corresponding author. E-mail: vikramgholve@rediffmail.com, vgvikram29@gmail.com. Tel: 09922969885,07588082912.

Plate 1. Brinjal seedlings showing damping off (*P. ultimum).*

known to have ayurvedic medicinal properties and good for diabetic patients. It has also been recommended as an excellent remedy for those suffering from liver complaints (Shukla and Naik, 1993). For brinjal, the following diseases have been reported this includes fungal diseases *viz.,* damping off, Phomopsis, blight and wilt, viral diseases *viz.,* mosaic and mottled dwarf and mycoplasma of leaf. Many fungi prevalent in soils can cause damping-off*. Fursarium* spp., *Pythium* spp., and *Phytophthora* spp. are most active in cool, wet soils whereas, *Cylindrocladium* spp. and *Rhizoctonia* spp. are more common in warm, wet soils.

The infected tissue become soft and water soaked, the collar portion rots and the seedlings ultimately collapse and die. The guaranteed supply of quality seedlings in required quantities is a major pre requisite for stabilized production of Brinjal. While raising seedlings in beds, the farmers face major problem of damping off incited by *Pythium* spp.

The damping off in brinjal is caused by *Pythium* spp., including *P. aphanidermatum, P.irregulare* and *P. ultimum*Trow, which can cause pre -emergence damping off and results in seed rot before the plants emerge out of the soil. The post emergence damping off phase is characterized by infection of the young tissues of the collar at the ground level. The pathogen is a soil borne with wide host range and almost worldwide distribution. The disease seems very difficult to control by conventional chemical means, due to its wide host range. Further, the use of chemicals/fungicides alone for the control of P*. ultimum* has been found to be impracticable and uneconomical. Therefore, an integrated disease management approach that encompass the use of chemicals, biocontrol agent, and plant extract could be the most economical and effective strategy for controlling the damping off and other soil borne plant diseases. Considering economic importance of the crop and losses caused by disease damping off in Brinjal, the present investigation was undertaken.

MATERIALS AND METHODS

The pathogen was isolated from the damping-off brinjal plants collected in nursery beds in *Kharif*, from the Department of Horticulture, VNMKV, Parbhani, Maharashtra, India in 2011. The Brinjal seedlings on nursery beds showing the symptoms of damping off (Plate 1) were collected in the polythene bags, labelled and brought to the laboratory. These samples were processed after surface sterilization (0.1% $HgCl_2$) for isolation of *P. ultimum* Trow. The isolate of the test pathogen were purified, numbered and maintained on potato dextrose agar (PDA) slants and stored at 8 to 10°C in a refrigerator.

Efficacy of fungicides

The efficacy of fungicides against *P. ultimum*Trow. was evaluated in three concentrations (500,1000 and1500 ppm) *in vitro* by applying poisoned food technique (Nene and Thapliyal, 1993) and using PDA as basal medium. The experiment was conducted by Completely Randomized Design with ten treatments and three replications.

100 ml PDA medium was poured in 250 ml capacity sterile glass conical flask and sterilized at 15 lbs pressure for 15 min. Required quantity of test fungicides for 500, 1000 and 1500 ppm was calculated and added in the sterilized PDA medium separately and mixed thoroughly.

This fungicide amended PDA medium with different concentrations of the test fungicides was poured (20 ml/plate) in sterilized glass Petri dish (90 mm.dia) and allowed to solidify at room temperature. The plates were inoculated by pure culture of *P. ultimum* Trow. For this purpose, 5 mm disc of one week old culture was cut with a sterilized cork borer. The disc was lifted and transferred aseptically in the centre of Petri plates containing the medium with test fungicides. Three plates per treatment per replication were maintained. The PDA plates without fungicides were also inoculated with the test pathogen and maintained as a uninoculated control. All the plates were incubated at 26 + 2°C.

The observations on colony diameter were recorded after a week of incubation. Per cent inhibition of the test pathogen was calculated by using the formula of Vincent (1927) and the data was statistically analysed:

$$PI = [(C-T)/C] \times 100$$

Where, PI= percent of inhibition, C= growth in control plates, T= growth in plates treated with fungicides.

Efficacy of plant extracts

Ten botanicals were evaluated *in vitro* at 10, 15 and 20% each for their fungistatic against *P. ultimum* Trow. by poisoned food technique (Nene and Thapliyal, 1993). The experiment was conducted by completely randomized design with 11 treatments and three replications.

Leaves/ rhizomes of the test botanicals were washed first in tap water, then in distilled water. Then 100 g of plant tissues + 100 ml distilled water were crushed (1:1 w/v) in mortar and pestle. The extract was filtered through double layered muslin cloth. The filtrate thus obtained was centrifuged at 5000 rpm for 15 min. The supernatant was collected and pellet was discarded. The supernatant obtained was strained through whatman No.1 filter paper and filtrate thus obtained was used as stock solution (100% conc.).

Aqueous plant extract (100%) were poured at 10, 15 and 20 ml each and separated into 100 ml autoclaved and cooled PDA in conical flask. The plant extract amended PDA was poured (each 20

Plate 2. Mass multiplication of *P. ultimum* (B) on sand : maize medium.

ml/plate) in sterile glass Petri plates (90 mm dia.) and allowed to cool. Five mm disc of *P. ultimum* Trow. was placed on the center of the solidified PDA plate under aseptic conditions. The PDA plates without plant extract and inoculated with the test pathogen served as untreated control.

These Petri plates were incubated at 26±2°C till the growth of the test pathogen in control plate was fully covered. The radial mycelial growth in all the plates was recorded and percent inhibition of mycelial growth over control was calculated by applying the formula (Vicent, 1927):

$$\text{Percent Inhibition (I)} = \frac{C-T \times 100}{C}$$

Where, C= growth of test fungus in control plates and T= growth of test fungus in treatment plates.

Efficacy of bioagents

The antagonistic potential of seven bioagents *viz*, *T. viride, T. harzianum, T. koningii, T. hamatum, Gliocladium virens, Bacilius subtilis* and *Pseudomonas fluorescence* against *P. ultimum* Trow. was evaluated *in vitro* by Dual culture technique (Stack et al., 1986) on PDA medium. The experiment was conducted by completely randomized design with eight treatments and three replications.

Autoclaved and cooled PDA medium was poured at 20 ml/plate in Petri plates (90 mm) and allowed to solidify. The plates were inoculated with 5 mm disc of 7 days old culture of biocontrol agents as well as 5 mm disc of 7 days old culture of *P. ultimum* Trow. at equidistance and exactly opposite with each other on PDA in plates. For bacteria, antagonist were streaked with the help of sterilized inoculating needle at one end of the PDA Petri plate. After 24 h of incubation, just opposite to the bacterial streak, a 5 mm disc of the test pathogen was placed. The PDA plates inoculated in center with the disc of the culture of the test pathogen only served as control. A triplicate set of inoculated PDA plates per treatment per replication was maintained and all the treatments were replicated thrice.

All these plates were incubated at 26±2° C in incubator. Observations on radial mycelial growth of the fungal pathogen and biocontrol agents was measured and per cent inhibition of the test fungus (*Pythium ultimum* Trow.) was calculated by applying formula given by Arora and Upaddhyay (1978) as follows:

$$\text{Percent Inhibition (PI)} = \frac{\text{Colony growth in Control Plate - Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Integrated evaluation of fungicides, botanicals, and bioagents in pot culture

Pot culture experiment was conducted to integrate the effective fungicides, bioagents and botanicals for integrated management of damping off (*Pythium ultimum*) disease in brinjal Cv.Hadgaon local. The experiment was conducted by Completely Randomized Design with eleven treatments and three replications.

Pathogen multiplied on sand: maize medium (Plate 2) was mixed with autoclaved (30 lbs for 30 min) potting mixture soil: sand: FYM (2:1:1) (at 25 g / kg potting mixture) and filled into pots, (disinfected with 5 % solution of copper sulphate) watered lightly and incubated at room temperature for two weeks in screen house. Within this period test pathogen multiplied in the pots. Those fungicides, plant extract /botanicals and bioagents found effective in *in vitro* studies were used for integrated disease management (IDM) experiment. The effective test fungicides *viz*., Metalaxyl (alone) and Captan (in combinations) were applied as seed treatment as detailed above. The 20% aqueous crude extract of garlic bulk was applied (alone and in combination) as soil drenching (100 ml/kg soil). The carrier based preparation of bioagent *T. viride* was applied (alone and in combination) in the soil at 25 g/kg soil, as detailed above. The fungicide treated seed of brinjal Cv. Hadgaon local were sown (6 seeds /pot) as per the treatment details. For T3, T4 and control treatments, the surface sterilized (0.1%$HgCl_2$) seed of brinjal Cv. Hadgaon local were sown (6 seeds/pot).The suitable untreated control with soil and surface sterilized seed sown of brinjal Cv. Hadgaon local was maintained. Three pots per treatments per replication were maintained and all the treatments replicated thrice. All these pots were watered regularly and maintained in screen house at the Department of Plant Pathology, Parbhani.

Observations on pre-emergence seed rot were recorded at one week after sowing and on post -emergence seedling mortality recorded at interval of 7 days and counted till 35 DAS and averaged finally. Observations were recorded for pre-emergence seed rot, post-emergence seedling mortality and per cent pre-emergence seed rot and per cent post-emergence seedling mortality were calculated by formula devised by Kataria and Grover (1967).

$$\%\text{PESR} = \frac{\text{Number of seeds rot per pot}}{\text{Total number of seeds per pot}} \times 100$$

$$\%\text{POESM} = \frac{\text{Number of seedlings affected per pot}}{\text{Total number of seeds per pot}} \times 100$$

PESR is pre emergence seedling mortality and POESM is post emergence seedling mortality.

RESULTS AND DISCUSSION

The efficacy of fungicides under *in vitro* tests

The results obtained on *in vitro* bio-efficacy of the nine fungicides (alone and combination) *viz.,* Captan, Thiram, Carbendazim,, Mancozeb, Carbendazim + Mancozeb, Metalaxyl, Benomyl, Carbendazim + Thiram, Captan + Metalaxyl against *P. ultimum* of the present study are presented in Table 1, Plates 3, 4 and 5 and Figure 1.

Radial mycelial growth

Result (Table 1) revealed that all the fungicides tested

Table 1. Efficacy of the fungicides against *Pythium ultimum* Trow.

Treatment	Fungicides	Colony diameter (mm)*			Average Mean col. dia. (mm)	Inhibition %			Average Inhibition (%)
		500 ppm	1000 ppm	1500 ppm		500 ppm	1000 ppm	1500 ppm	
T_1	Captan	71.86	40.06	35.20	49.04	20.14 (26.63)	55.48 (48.14)	60.88 (51.28)	45.50
T_2	Thiram	63.26	56.13	49.60	56.33	29.69 (33.01)	37.62 (37.82)	44.88 (42.05)	37.39
T_3	Carbendazim	60.30	58.70	52.21	57.07	32.96 (35.03)	34.77 (36.12)	41.98 (40.38)	36.57
T_4	Mancozeb	71.86	70.03	61.93	67.94	20.14 (26.63)	22.18 (28.07)	31.18 (33.86)	24.50
T_5	Carbendazim + Mancozeb	35.20	33.56	31.43	33.33	60.88 (51.28)	62.70 (52.35)	65.07 (53.76)	62.88
T_6	Metalaxyl	31.33	11.26	00.00	14.19	65.18 (53.83)	87.48 (69.28)	100.00 (89.98)	84.22
T_7	Benomyl	45.06	42.90	39.46	42.47	49.92 (44.95)	52.32 (46.32)	56.14 (48.52)	52.79
T_8	Carbendazim + Thiram	66.99	62.30	57.20	62.16	25.66 (30.42)	30.77 (33.68)	36.44 (37.12)	30.95
T_9	Captan + Metalaxyl	27.83	10.43	09.16	15.80	69.07 (56.20)	88.40 (70.08)	89.81 (71.38)	82.42
T_{10}	Control	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	SE±	0.83	0.88	1.12		0.58	0.61	0.77	
	CD	2.44	2.59	3.32		1.71	1.79	2.29	
	CV %	2.68	3.21	4.58		2.66	2.50	2.88	

*Mean of three replications; figure in parenthesis are angular transformed values.

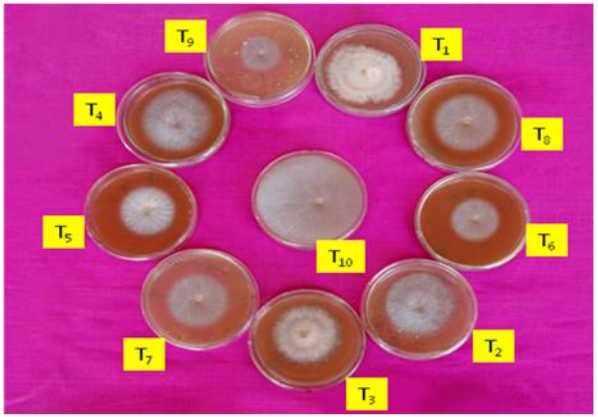


Plate 3. *In vitro* efficacy of fungicides at 500 ppm concentration on radial growth of *P. ultimum.*

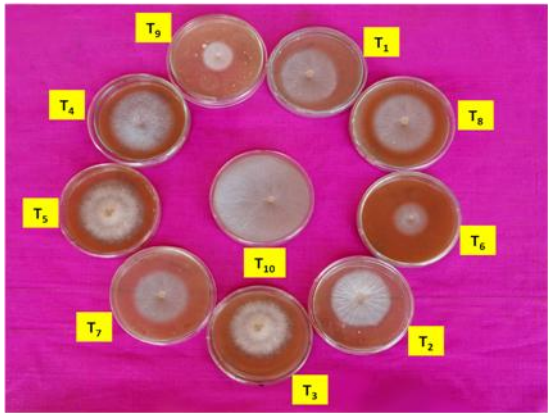
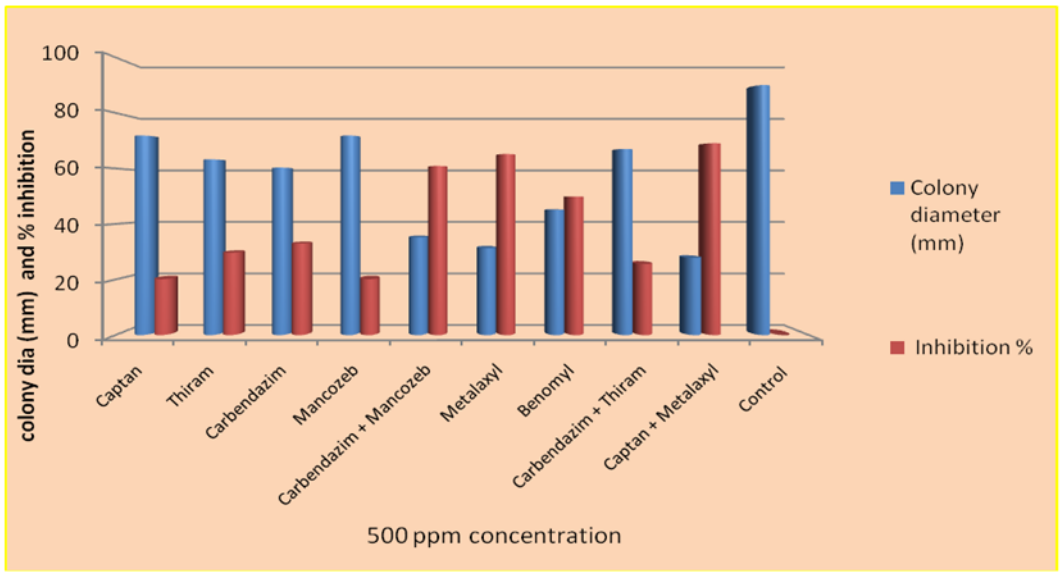
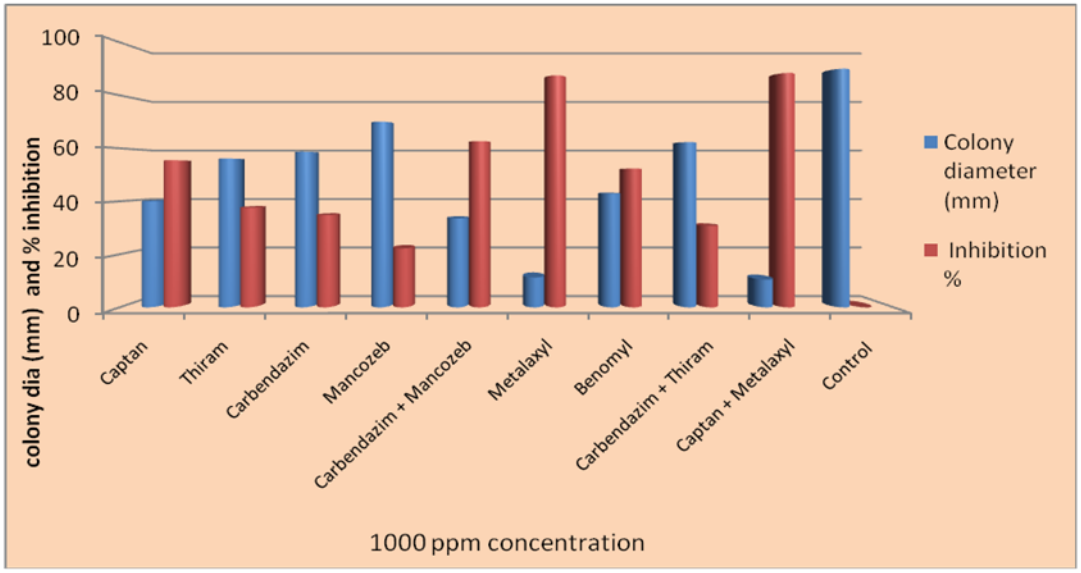


Plate 4. *In vitro* efficacy of fungicides at 1000 ppm concentration on radial growth of *P. ultimum.*

Plate 5. *In vitro* efficacy of fungicides at 1000 ppm concentration on radial growth of *P. ultimum.*





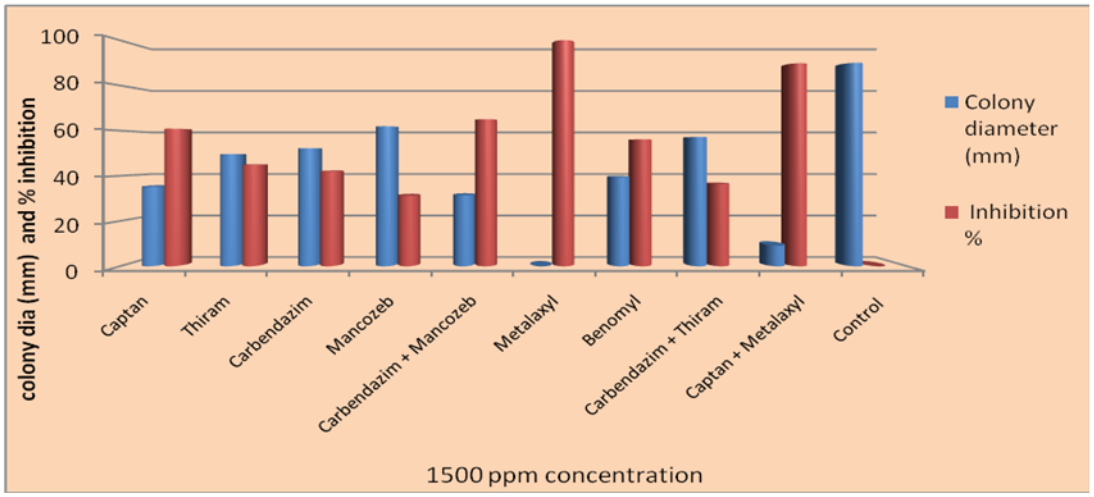


Figure 1. *In vitro* efficacy of fungicides at 500, 1000, 1500 ppm concentrations on radial growth of *P. ultimum* Trow.

recorded a wide range of radial mycelial growth of the test pathogen and it was varied with concentrations used of the test fungicides.

At 500 ppm, concentration (Plate 3) and Figure 1. radial Mycelial growth of the test pathogen was ranged from 27.83 (Captan + Metalaxyl) to 71.86 mm (Captan) and (Mancozeb). However, it was maximum with Captan (71.86 mm) and Mancozeb (71.86 mm) each both of which were on par. This was followed by Carbendazim + Thiram (66.99 mm), Thiram (63.26), and Carbendazim (60.30mm).Significantlyleast mycelial growth was recorded with the treatment Captan + Metalaxyl (27.83mm); followed by Metalaxyl (31.33 mm), Carbendazim + Mancozeb (35.20 mm), Metalaxyl (31.33 mm), captan + metalaxyl (27.83 mm) and Benomyl (45.06 mm).

At 1000 ppm concentration, (Plate 4 and Figure 1) radial mycelial growth of the test pathogen was ranged from 10.43 (captan + metalaxyl) to 70.03 mm (mancozeb). All the fungicide tested exhibited similar trend of radial mycelial growth as that of 500 ppm. However, maximum radial mycelial growth was recorded with mancozeb (70.03 mm) and was followed by carbendazim + thiram (62.30 mm), carbendazim (58.70 mm) and thiram (56.13 mm). Significantly least mycelial growth was recorded with Captan + Metalaxyl (10.43 mm); followed by Metalaxyl (11.26 mm), carbendazim + mancozeb (33.56 mm) captan (40.06 mm) and benomyl (42.90 mm).

At 1500 ppm concentration (Plate 5 and Figure 1), radial mycelial growth ranged from 00.00 (metalaxyl) to 61.93 (mancozeb). However, it was maximum with, mancozeb (61.93 mm) and this followed by carbendazim + thiam (57.20 mm) carbendazim (52.21 mm) and thiram (49.60 mm). Significantly least mycelial growth was recorded with captan + metalaxyl (09.16 mm), followed by carbendazim+mancozeb (31.43 mm), captan (35.20 mm), benomyl (39.46 mm) and fungicide metalaxyl recorded nil (00.00) growth of the test pathogen.

Average radial mycelial growth recorded with all the fungicides tested (at 500, 100, 1500 ppm) ranged from 14.19 (Metalaxyl) to 67.94 mm (mancozeb). However, highest mean radial mycelial growth was recorded with mancozeb (67.94 mm) which was followed by Carbendazim + Thiram (62.16 mm), Carbendazim (57.07 mm), Thiram (56.33 mm), and Captan (49.04 mm). Significantly least mean mycelial growth was recorded with metalaxyl (14.19 mm), followed by Captan + Metalaxyl (15.80 mm), Carbendazim + Mancozeb (33.3 mm) and Benomyl (42.47 mm).

Mycelial inhibition

Results (Table 1) revealed that all the fungicides at 500, 1000 and 1500 ppm significantly inhibited mycelial growth of the test fungus over untreated control. Further, it was found that per cent mycelial inhibition was increased with the increase in the fungicides concentrations.

At 500 ppm concentration, (Table 1) per cent mycelial growth inhibition ranged from 20.14 (Mancozeb) to 69.07% (Captan + Metalaxyl). However highest mycelial inhibition was recorded with Captan + Metalaxyl (69.07%). This was followed by the fungicides, Metalaxyl (65.18%), Carbendazim + Mancozeb (60.88%), Benomyl (49.92%), Carbendazim (32.96%), Carbendazim + Thiram (25.66%), Captan (20.14%) and Mancozeb (20.14%).

At 1000 ppm concentration, (Table 1) similar trend of mycelial growth inhibition with the test fungicides was recorded and it ranged from 22.18 (Mancozeb) to 88.40% (Captan + Metalaxyl). However, highest percentage mycelial inhibition was recorded with Captan + Metalaxyl (88.40%), this was followed by Metalaxyl (87.48%), Carbendazim + Mancozeb (62.70%), Captan (55.48%), Benomyl (52.32%), Thiram (37.62%), Carbendazim (34.77%), Carbendazim + Thiram (30.77%), and Mancozeb (22.18%).

At 1500 ppm concentration, (Table 1) the percentage of mycelial inhibition ranged from 31.18 (Mancozeb) to 100% (Metalaxyl). However, percent mycelial inhibition (100%) was recorded with Metalaxyl. This was followed by the fungicides, Captan + Metalaxyl (89.81%), Carbendazim + Mancozeb (65.07%), Captan (60.88%), Benomyl (56.14%), Thiram (44.88%), Carbendazim (41.98%), Carbendazim + Thiram (36.44%), and Mancozeb (31.18%).

Mean percentage mycelial inhibition of all the fungicides at 500, 1000 and 1500 ppm ranged from 24.50 (Mancozeb) to 84.22% (Metalaxyl). However, Metalaxyl was found to be most fungistatic with significantly highest mean mycelial inhibition of 84.22%. This was followed by Captan + Metalaxyl (82.42%), Carbendazim + Mancozeb (62.88%), Benomyl (52.79%), and the fungicides *viz.,* Mancozeb, Carbendazim + Thiram were found least effective against the test pathogen with the mean mycelial inhibition 24.50, 30.95 and 37.39%,respectively. Similar *in vitro* fungistatic effects of the test fungicides against *P. ultimum* infecting brinjal and other *Pythium* spp. infecting many other crops were reported earlier by several workers (Satija and Hooda, 1987; Sawant and Mukhopadhyay, 1990; Nene and Thapliyal, 1993; Ayub et al., 1998; Taylor et al., 2002; Jiskani et al., 2007).

In vitro bioefficacy of plant extracts/botanicals

Bioefficacy of ten botanicals/plant extracts *viz.,* Mehandi (*Lawsonia innermis*), Ginger (*Zingiber afficinale*), Datura (*Datura metal*), Tulsi (*Oscimum sanctum*), Parthenium (*Parthenium hysteriphorus*), Neem (*Azardirachta indica*), Garlic (*Allium sativum*), Turmeric (*Curcuma longa*), Satawari (*Asparugus racemosus*) was evaluated at 10, 15, and 20% *in vitro* against *Pythium ultimum* applying Poisoned food technique and using PDA as a basal medium, and the results obtained are presented in the Table 2 and depicted in Plates 6, 7, 8 and Figure 2.

Table 2. Efficacy of plant extracts/botanicals against *Pythium ultimum* Trow.

Botanicals	Colony diameter mm)*			Average Col. Diameter	Per cent inhibition* at			Average Inhibition (%)
	10%	15%	20%		10	15	20	
Mehandi	35.56	38.61	35.13	36.43	60.47 (51.04)	49.06 (57.08)	60.96 (51.32)	56.83
Ginger	47.46	44.69	41.52	44.55	47.25 (43.42)	50.33 (45.18)	53.85 (47.20)	50.47
Datura	37.43	35.43	33.34	35.40	58.40 (49.83)	60.62 (51.12)	62.94 (52.49)	60.65
Tulsi	67.90	64.36	61.37	64.54	24.55 (29.55)	38.47 (38.10)	31.80 (34.32)	31.60
Parthenium	47.50	44.43	41.44	44.45	47.22 (43.40)	50.62 (45.35)	53.95 (47.26)	50.59
Neem	66.06	63.73	60.46	63.41	26.58 (31.03)	29.18 (32.69)	32.81 (34.94)	29.52
Garlic	13.93	00.00	00.00	04.64	84.51 (66.81)	100.00 (89.98)	100.00 (89.98)	94.83
Turmeric	73.86	71.56	68.33	71.25	17.92 (25.04)	20.47 (26.89)	24.07 (29.37)	20.82
Adulsa	25.30	23.06	17.66	22.00	71.88 (57.97)	74.36 (59.57)	80.36 (63.69)	75.53
Shatawari	66.96	63.73	58.63	63.10	25.58 (30.37)	29.18 (32.68)	34.84 (36.16)	29.86
Control	90	90	90	90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
SE±	1.23	0.47	0.58		0.88	1.88	0.39	
CD	3.60	1.37	1.70		2.59	5.51	1.16	

* Mean of three replications. (Figure in parenthesis are angular transformed values).

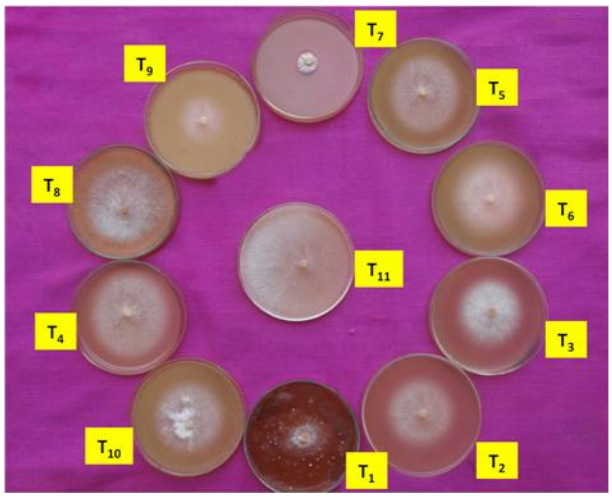


Plate 6. *In vitro* efficacy of plant extract/ botanicals at 10% concentration on radial growth of *P. ultimum.*

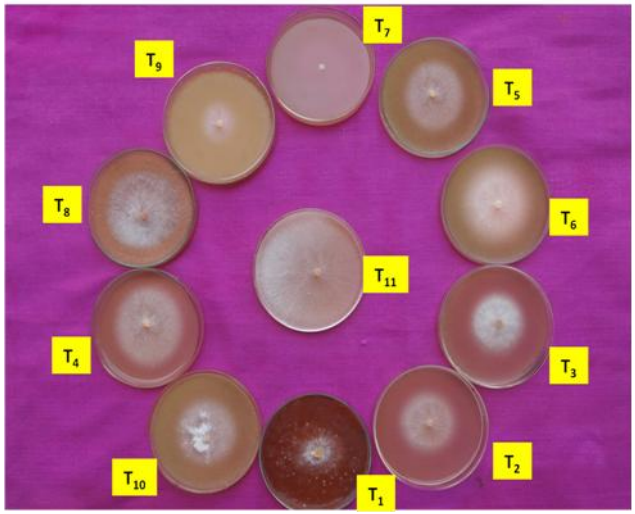


Plate 7. *In vitro* efficacy of plant extract/ botanicals at 15% concentration on radial growth of *P. ultimum.*

Radial mycelial growth

Results (Table 2) reveal that all the botanicals/plant extracts tested exhibited a wide range of radial mycelial growth of the test pathogen and it was varied with their concentrations used.

At 10%, (Plate 6) radial mycelial growth of the test pathogen ranged from 13.93 (Garlic) to 73.86 mm Turmeric). However, it was maximum with turmeric 73.86 mm). This was followed by Tulsi (67.90 mm), Shatawari (66.96 mm), Neem (66.06 mm), ginger (47.46 mm), Parthenium (47.50 mm) both of which were at par, (Datura (37.43 mm) and Mehandi (35.56 mm).

Comparatively, least growth was recorded with Adulsa (25.30 mm) and Garlic (13.93 mm).

At 15% (Plate 7), radial mycelial growth of the test pathogen ranged from 00.00 (Garlic) to 71.56 mm (Turmeric). The 10 and 15% plant extracts exhibited similar trend of radial mycelial growth. However, maximium radial mycelial growth was recorded with Turmeric (71.56 mm) followed by Tulsi (64.36 mm), Neem (63.73 mm) and Shatawari. (63.73 mm); all three were at par; Ginger (44.69 mm) and Parthenium (44.43 mm) were at par; for Mehandi (38.61 mm) and Datura (35.43 mm) significantly least mean mycelial growth was recorded with Adulsa (23.06 mm) and Garlic (00.00 mm). At 20 per cent, (Plate 8) radial mycelial growth was ranged

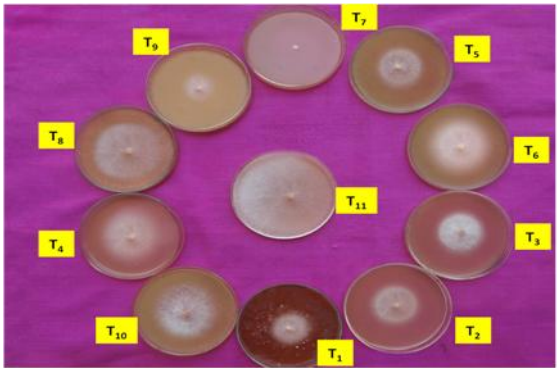


Plate 8. *In vitro* efficacy of plant extract/ botanicals at 20% concentration on radial growth of *P. ultimum.*

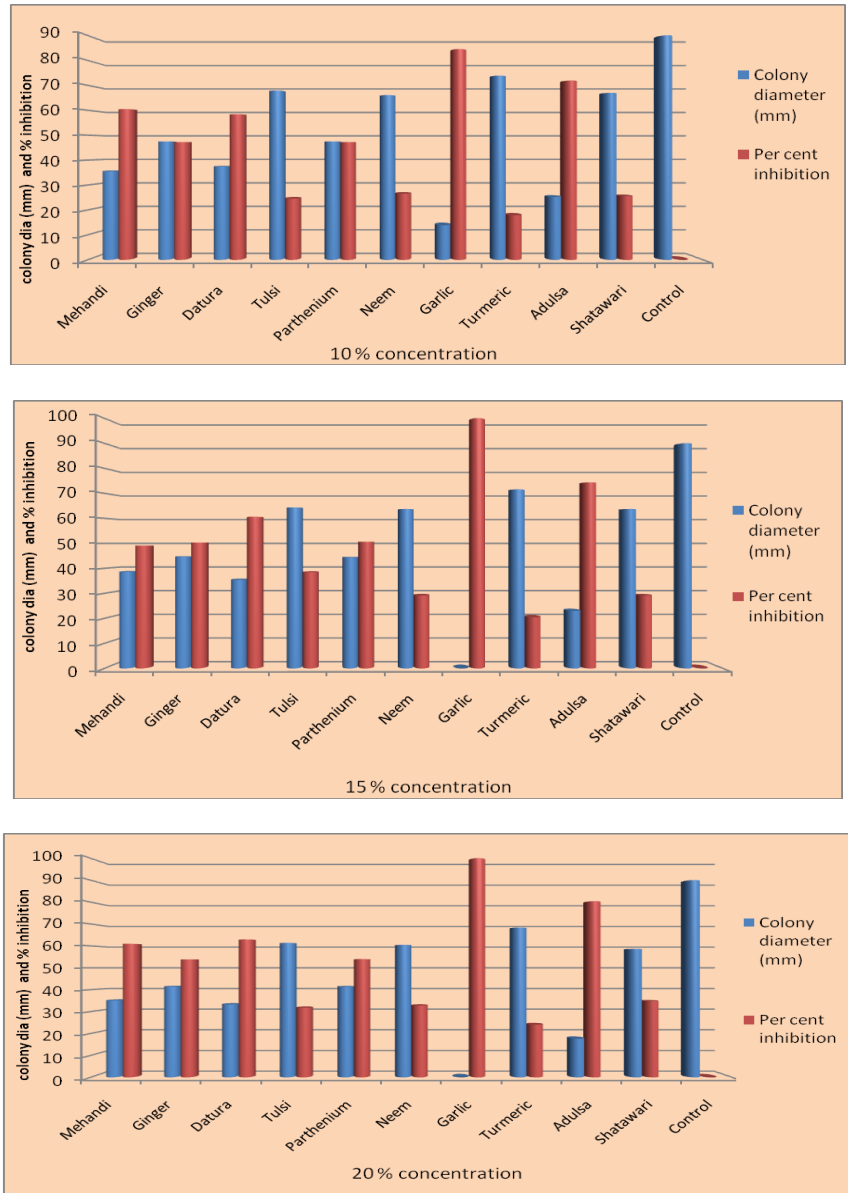


Figure 2. ***I****n vitro* efficacy of plant extract/ botanicals at 10, 15, 20 % concentration on radial growth of *P. ultimum* Trow.

from 00.00 mm (Garlic) to 68.33mm (Turmeric). However, significantly highest mycelial growth was recorded with turmeric (68.33mm), This was followed by Tulsi (61.37mm),Neem (60.46mm)both of which were on par and Shatawari (58.63mm) significantly least growth was recorded with Garlic (00.00mm)and this was followed by Adulsa (17.66mm) Datura (33.34mm) Mehandi (35.13mm) Ginger and Parthenium (41.52 and 41.44) both of which were on par.

Average radial myceilial growth (Table 2) recorded with all the plant extract tested (at 10, 15, and 20%) ranged from 04.64 (Garlic) to 71.25 mm (Turmeric). However, highest mean radial mycelial growth was recorded with Turmeric (71.25 mm) and was followed by Tulsi (64.54 mm), Neem (63.41 mm), Shatawari (63.10 mm). Ginger (44.55 mm) and Parthenium (44.45 mm) were at par, and Mehandi (36.43 mm), and Datura (35.40 mm). Significantly least mean mycelial growth was recorded with Garlic (00.00 mm) and Adulsa (22.00 mm).

Mycelial inhibition

Result (Table 2) reveal that all the plant extracts tested (at 10, 15 and 20 per cent), significantly inhibited mycelial growth of the test pathogen over untreated control and per cent mycelial inhibition was increased with increase in concentrations of the botanicals tested.

At 10%, mycelial growth inhibition ranged from 17.92 (Turmeric) to 84.51% (Garlic). However, significantly highest mycelial inhibition was recorded with Garlic (84.51%) and was followed by Adulsa (71.88%), Mehandi (60.47%), Datura (58.40%) both of which were on par; Ginger and Parthenium (47.25 and 47.22%) each were on Par, Neem (26.58%), Shatawari (25.58%), Tulsi(24.55%) and all three were on par and Turmeric (17.92%).

At 15% similar trend of mycelial inhibition as that of 10% was recorded and it ranged from 20.47 (Turmeric) to 100% (Garlic). However, significantly highest mycelial inhibition was recorded with Garlic (100%), and was followed by Adulsa (74.36%), Datura (60.62%), Parthenium (50.62%) and Ginger (50.33%); both were on par, Mehandi (49.06%), Tulsi (38.47%) Neem and Shatawari (each 29.18%); which are on par with and Turmeric (20.47%).

At 20%, the percentage mycelial inhibition ranged from 24.07 (Turmeric) and 100% (Garlic). However, significantly highest mycelial inhibition was recorded with Garlic (100%) and was followed by Adulsa (80.36%), Datura (62.94%), Mehandi (60.96%), Ginger and Parthenium (each 53.95%), (both were on par), Shatarwari (34.84%), Neem (32.81%), Tulsi (31.80%) and Turmeric (24.07%).

Average percentage mycelial inhibition (Table 2) recorded with all the test botanicals was from 20.82% for Turmeric to 94.83% for Garlic. However, Garlic was found to be most fungistatic which recorded significantly highest mean mycelial inhibition (94.83%). This was followed by Adulsa (75.53%), Datura (60.65%), Mehandi (56.83%), Parthenium and Ginger (each 50.47%), which were on par, Tulsi (31.60%), Satawari (29.86%) and Neem (29.52%) both were on par and Turmeric (20.82%).

Thus, all the plant extracts tested were found as fungistatic/antifungal against *P. ultimum* and significantly inhibited mycelial growth of the test pathogen over untreated control However, Garlic recorded highest mean mycelial inhibition (94.83%) followed by Adulsa (75.53%) and Datura (60.65%). Result of the present study are in conformity with those reported earlier by several workers (Bhat and Shrivastava, 2003; Bhora et al., 2006; Muthukumar et al., 2010 Ambikapathy et al., 2011).

Efficacy of bioagents

Seven fungal antagonists *viz., T. viride, T. harzianum, T. hamatum, T. koningii, Gliocladium virens, Pseudomonas flueroscens,* and *Bacillus subtilis*were evaluated *in vitro* against *P. ultimum* Trow. applying dual culture technique (Stack et al.,1986) using PDA as basal medium and result obtained are presented in Table 3 (Plate 9) and Figure 3.

Result (Table 3) reveals that all the bioagents evaluated exhibited fungistatic /antifungal activity against *P. ythium ultimum* Trow. and significantly inhibited its mycelial growth over untreated control (Plate 9). Of the antagonist tested, *T. viride* was found most effective and recorded significantly least mycelial growth (27.49 mm) with highest mycelial inhibition (69.44%) of the test pathogen over untreated control (00.00%). The second and third best antagonists found were *T. koningii* and *T. hamatum* which recorded mycelial growth of 29.40 and 32.40 mm, respectively and inhibition respectively of 67.32 and 63.99%. This was followed by *Bacillus subtilis* and *P. flueroscens* with colony growth respectively of 36.25 and 39.35 mm and corresponding growth inhibition of 59.71 and 56.27%. *G. virens* recorded mycelial growth of 41. and corresponding growth inhibition of 54.14%. *T. harzianum* was found relatively less effective with 45.35 mm colony diameter and 49.60% inhibition of the test pathogen.

Thus all the fungal and bacterial antagonists/bioagents evaluated *in vitro* were found fungistatic /antifungal against *P. ultimum* and caused significant reduction in the linear mycelial growth of the test pathogen over untreated control.

The inhibition effects of *Trichoderma* spp., *P. fluorescens* and *B. subtilis* against *P. ultimum* may be attributed to the mechanisms *viz,.* antibiosis, lysis, mycoparasitism, competition and production of volatile substances, by the test bioagents/antagonists.

Results of the present study on inhibitory effects of the test antagonists: *Trichoderma* spp., *B. subtilis,* and

Table 3. Efficacy of the bioagents/antagonists against *P.ultimum* Trow.

Treatment number	Treatments	Mean colony diameter(mm)*	Per cent inhibition
T_1	*Trichoderma viride*	27.49	69.44 (56.43)
T_2	*T. harzianum*	45.35	49.60 (44.76)
T_3	*T. koingii*	29.40	67.32 (55.13)
T_4	*T. hamatum*	32.40	63.99 (53.12)
T_5	*Gliocladium virens*	41.27	54.14 (47.36)
T_6	*Pseudomonas fluorescens*	39.35	56.27 (48.59)
T_7	*Bacillus subtilis*	36.25	59.71 (50.59)
T_8	Control(untreated)	90.00	00.00 (00.00)
SE±	--	0.55	0.36
CD	--	1.66	1.09

*Mean of three replications. Figure in parenthesis are angular transformed values.

Plate 9. *In vitro efficacy of the bioagents against P. ultimum Trow.*

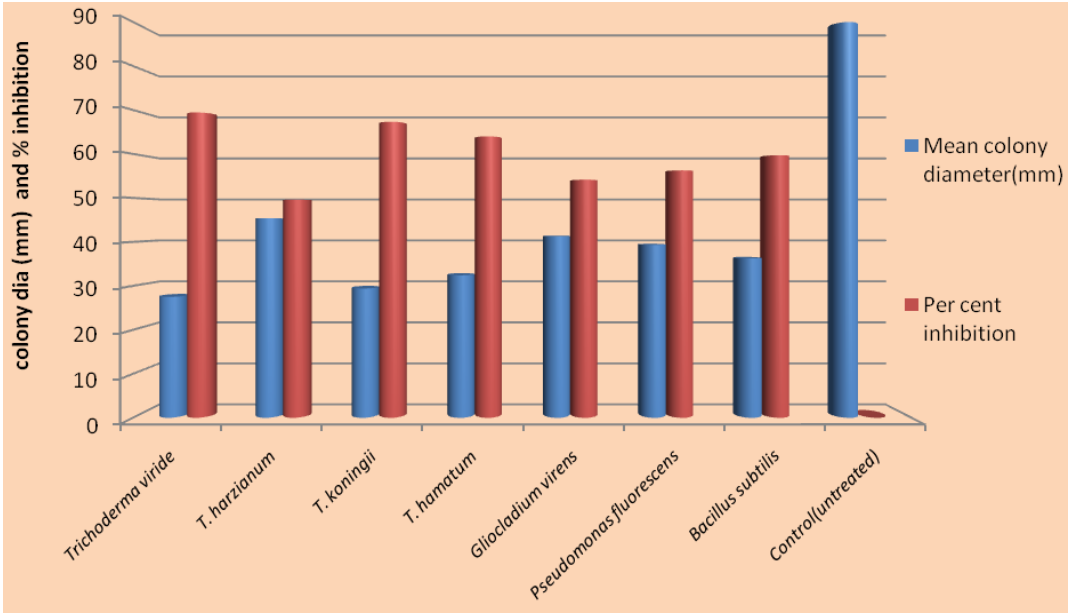


Figure 3. *In vitro* efficacy of bioagents against *P. ultimum* Trow.

Table 4. Integrated disease management with effective fungicides, botanicals and bioagents in pot culture.

Treatment number	Treatment	Rate of application	Pre emergence Damping Off (%)	Post emergence Damping Off (%)	Average mortality (%)
T1	Metalaxyl	6 g/kg seed	38.88(38.50)	36.11(36.91)	37.49
T2	Captan+ Metalaxyl	1.5g+3 g/kg seed	33.33(34.78)	26.11(30.60)	29.72
T3	Garlic	100 ml/kg soil	44.44(41.74)	41.66(40.18)	43.05
T4	*T.viride*	25 g/kg soil	55.55(48.23)	49.99(44.99)	52.77
T5	Metalaxyl + Garlic	6 g/kg seed + 100 ml/kg soil	33.33(34.78)	26.11(30.60)	29.72
T6	Metalaxyl + *T.viride*	6 g/kg seed + 25 g/kg soil	38.88(38.50)	36.11(36.91)	37.49
T7	Captan + Metalaxyl + Garlic	1.5 g + 3 g/kg seed + 100 ml/kg soil	22.21(27.81)	21.66(27.70)	21.93
T8	Captan + Metalaxyl + *T.viride*	1.5 g + 3 g/kg seed + 25 g/kg soil	33.33(34.78)	26.11(30.60)	29.72
T9	Metalaxyl + Garlic + *T.viride*	3 g/kg seed + 100 ml/kg soil + 25 g/ kg soil	33.33(34.78)	41.66(40.18)	37.49
T10	Captan + Metalaxyl + Garlic + *T.viride*	1.5 g + 3 g/kg seed + 100 ml/kg soil+25 g/kg soil	16.66(24.08)	20.00(26.56)	18.33
T11	Control	-	72.21(58.44)	66.66(54.77)	69.43
SE±	-	-	4.15	1.91	-
CD	-	-	12.16	5.62	-

Figure in parenthesis are angular transformed values.

P. fluorescens are in conformity with those reported earlier by several workers (Manoranjitham et al., 2000; Chakrabarti et al., 2005; Pandey and Pandey, 2005; Valerie et al., 2005, Abeysinghe, 2009).

Integrated disease management strategies

The results obtained on IDM *in vitro* studies (pot culture) of 11 treatments against *P. ultimum* of present study are presented in The Table 4 and depicted in Plate 10 and Figure 4.

Pre-emergence damping off

Results (Table 4) reveals that the percent pre-emergence damping off recorded with all the treatment ranged from 16.66 to 55.55% as against 72.21% in control. The least pre-emergence damping off percentage was recorded with the treatment (T_{10}) Captan + Metalaxyl + Garlic + *T. viride* (16.66%), followed by the treatment (T_7): Captan + Metalaxyl + Garlic (22.21%). The treatments T_2, T_5, T_8, T_9 were found on par with T_{10} and T_7.

The maximum pre-emergence damping off was recorded with the treatment (T_4, *T. viride)* (55.55%), followed by the treatment (T_3, Garlic) (44.44%) and depicted in Plate 10 and Figure 4.

Post emergence damping off

Results (Table 4) revealed that the percent post emergence damping off recorded with all the treatments ranged from 20% to T_4 as against 66.66% in control.

The similar pattern of result was recorded in post emergence damping off as observed in pre-emergence damping off. The least post emergence damping off was recorded with the treatment Captan + Metalaxyl + Garlic + *T. viride* (T_{10}) (20%) followed by Captan + Metalaxyl + Garlic (T_7) (21.66%). The treatments T_2, T_5 and T_8 were found on par with T_{10} and T_7. The maximum post emergence damping off, was recorded with the treatment T_4: *T. viride* (49.99%), followed by the treatment T_3: Garlic (41.66%) and treatment T_9: Metalaxyl + Garlic + *T. viride* (41.66%) and depicted in Plate 10 and Figure 4.

Average percent mortality

Results (Table 4) reveal that the average percent mortality of pre and post emergence damping off with all the treatments ranged from 18.33 to 52.77% as against 69.43% in control.

The least average per cent mortality was recorded with the treatment T_{10}: Captan + Metalaxyl + Garlic + *T. viride* (18.33%), followed by the treatment T_7: Captan + Metalaxyl (21.93%). The treatments T_2, T_5 and T_8 (29.72%) where found on par with T_{10} and T_7.

Comparatively maximum average mortality was recorded with the treatment T_4: *T.viride* (52.77%), followed by the treatment T_3: garlic (43.05%), T_1, T_6 and T_9 (37.49%).

Reduction in damping off

The results obtained on integrated disease management *in vitro* studies (pot culture) of 11 treatments against *P. ultimum* are presented in Table 4.

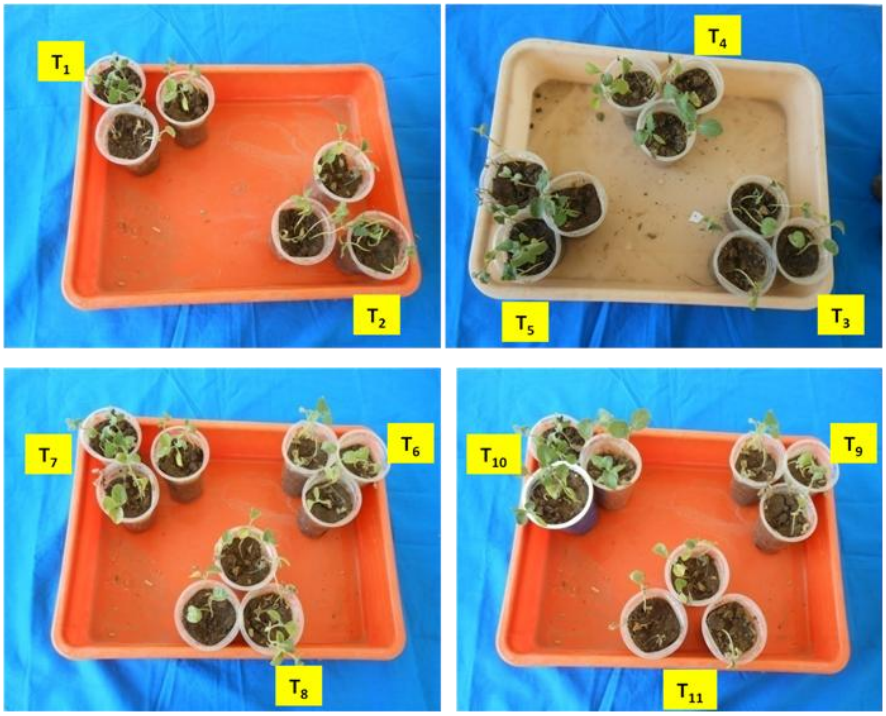


Plate 10. Experiment (pot culture) on integrated management of damping off in Brinjal Cv. Hadgaon local

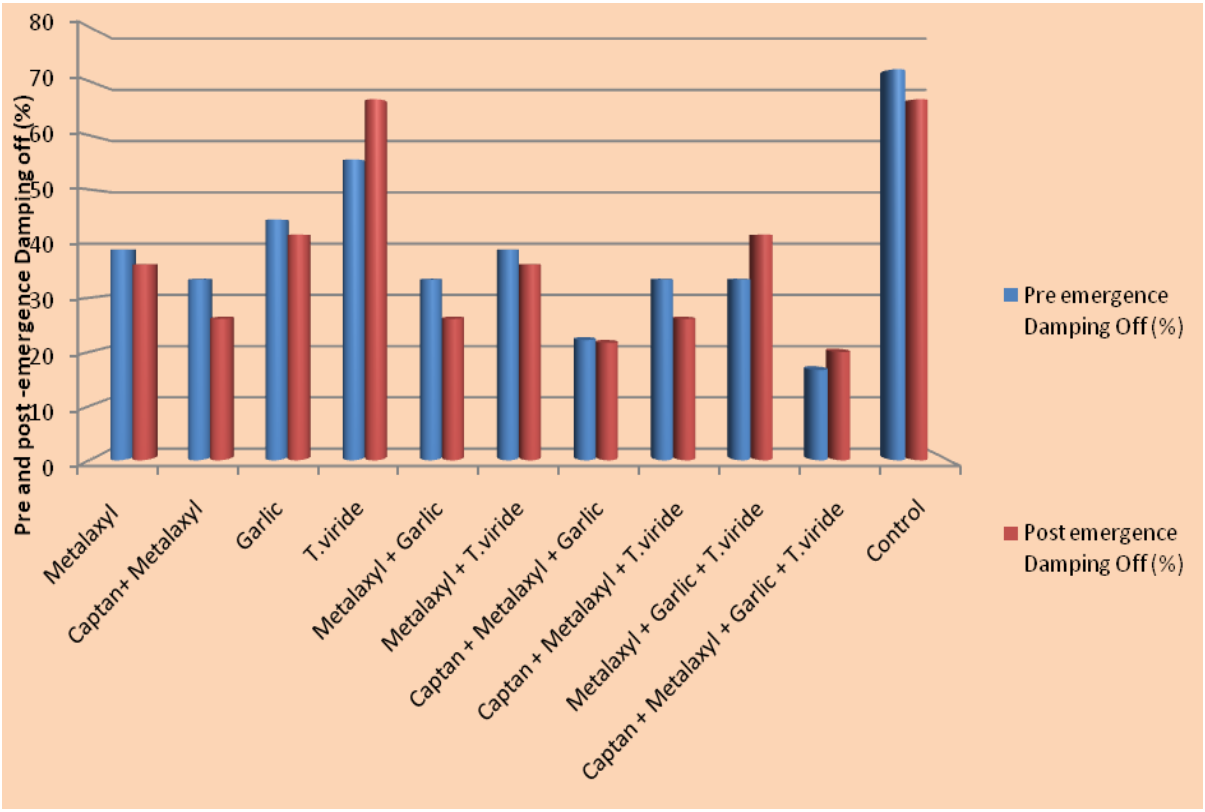


Figure 4. IDM (pot culture) of *P. ultimum* Trow. with effective Fungicides, botanicals and bioagents on brinjal cv. Hadgaon local.

Table 5. Efficacy of fungicides, bioagents and botanicals in reduction damping off in brinjal cv. Hadgoan local

T. No.	Treatments	Rate of application	Percent reduction in damping off		Average reduction (%)
			Pre emergence	Post emergence	
T_1	Metalaxyl	6 g/kg seed	46.15 (42.79)	45.82 (42.60)	45.98 (42.69)
T_2	Captan+ Metalaxyl	1.5 g + 3g/kg seed	53.84 (47.20)	60.83 (51.25)	57.33(49.21)
T_3	Garlic	100 ml/kg soil	38.45 (38.32)	37.50 (37.76)	33.97 (35.65)
T_4	*T.viride*	25 g/kg soil	23.07 (28.70)	25.50 (30.32)	24.28 (29.52)
T_5	Metalaxyl+ Garlic	6 g/kg seed + 100ml/kg soil	53.84 (47.20)	60.83 (51.25)	57.33 (49.21)
T_6	Metalaxyl+ *T.viride*	6 g/kg seed + 25g/kg soil	46.15 (42.79)	45.82 (42.60)	45.98 (42.69)
T_7	Captan+ Metalaxyl+Garlic	1.5 g + 3 g/kg seed + 100 ml/kg soil	69.24 (56.31)	65.50 (54.02)	67.37 (55.16)
T_8	Captan+ Metalaxyl+ *T.viride*	1.5 g + 3g/kg seed + 25 g/kg soil	53.84 (47.20)	60.83(51.25)	57.33 (49.21)
T_9	Metalaxyl + 0 Garlic + *T.viride*	3 g/kg seed + 100 ml/kg soil + 25 g/kg soil	53.84 (47.20)	37.50 (37.76)	45.67 (42.51)
T_{10}	Captan + Metalaxyl + Garlic + *T.viride*	1.5 g + 3 g/kg seed + 100ml /kg soil + 25 g/kg soil	76.92 (61.28)	69.99 (56.78)	73.45 (58.98)
T_{11}	Control (untreated)		00.00 (00.00)	00.00 (00.00)	00.00 (00.00)

Figure in parenthesis are angular transformed values.

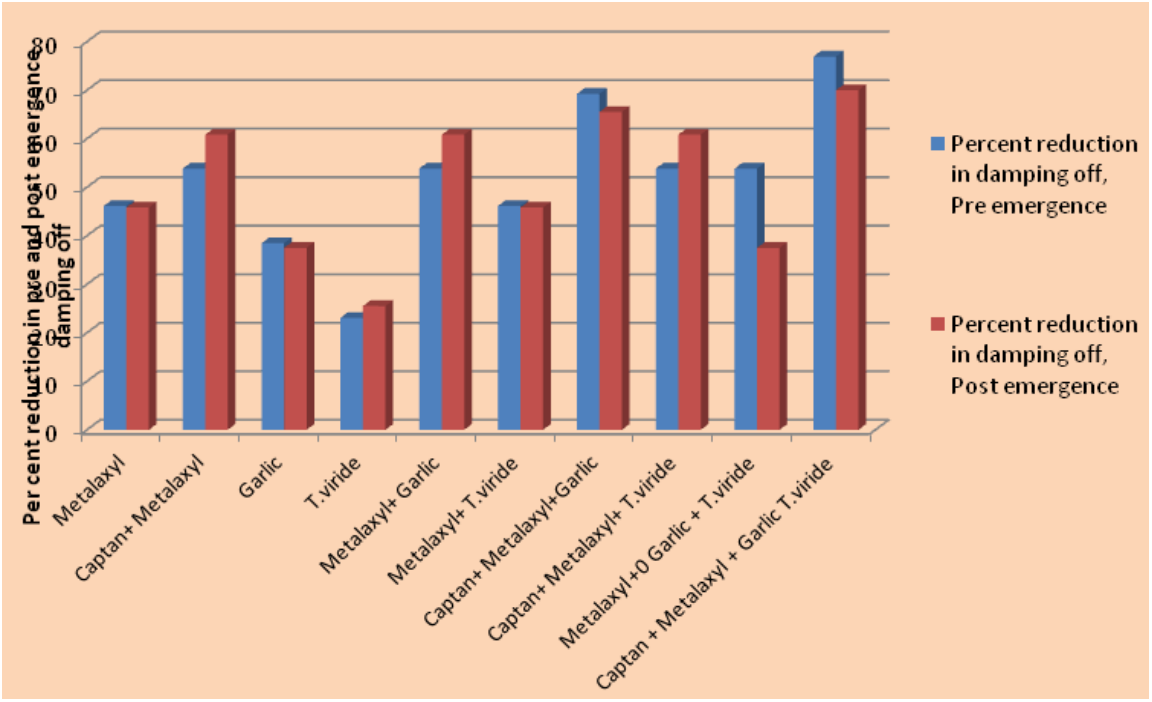


Figure 5. Efficacy of fungicides, bioagents and botanicals in reducing Pre and post emergence damping of in brinjal cv. Hadgaon local.

Percent reduction in pre-emergence damping off

Results (Table 5 and Figure 5) reveal that the percent reduction in pre-emergence damping off recorded with all the treatments ranged from 23.07 to 76.92% as against the control.

The maximum percent reduction in pre-emergence damping off was recorded with the treatment T_{10}:

Captan + Metalaxyl + Garlic + *T. viride*) (76.92%), followed by the treatment T_7: Captan + Metalaxyl + Garlic (69.24%). The treatment T_2, T_5, T_8 and T_9 were found on par with T_{10} and T_7.

The minimum percent reduction in pre-emergence damping off was recorded with the treatment T_4: *T. viride* (23.07%), followed by the treatment T_3: Garlic (38.45%), T_1: Metalaxyl (46.15%) and (T_6) Metalaxyl + *T. viride* (46.11%).

Percent reduction in post emergence damping off

Results (Table 5 and Figure 5) revealed that the percent reduction in post emergence damping off recorded with all the treatments ranged from 25.50 to 69.99%.

The maximum percent reduction in post emergence damping off was recorded with treatment T_{10}: Captan + Metalaxyl + Garlic + *T. viride* (69.99%) followed by the treatment T_7: Captan + Metalaxyl + Garlic (65.50%). The treatments T_2, T_5 and T_8 (60.83%) were found on par with T_{10} and T_7.

The minimum percent reduction in post emergence damping off was recorded with the treatment (T_4) *T. viride* (25.50%), followed by treatment T_3: Garlic (37.50%), T_9: Metalaxyl + Garlic (37.50%), $T_{1:}$ Metalaxyl (45.82%) and T_6 Metalaxyl + *T. viride* (45.82%).

Average percent reduction in damping off

Results (Table 5) reveals that the averaged percent reduction in pre and post emergence damping off with all the treatments ranged from 24.28 to 73.45%.

The maximum percent reduction in damping off was recorded with the treatment T_{10}: Captan + Metalaxyl + Garlic + *T. viride* (73.45%), followed by the treatment T_7: Captan + Metalaxyl + Garlic (67.37%). The treatment T_2, T_5 and T_8 (57.33%) were found at par with treatment T_{10} and T_7.

Comparatively minimum percent reduction in damping off was recorded with the treatment T_4: *T. viride* (24.28%) followed by the treatments T_3: Garlic (33.97%), T_9: Metalaxyl + Garlic + *T. viride* (45.67%), T_1: Metalaxyl (45.98%) and T_6: Metalaxyl + *T. viride* (45.98%). The result of the present study obtained in respect of IDM of *P. ultimum* Trow. with the fungicides, botanicals and plant extracts are in conformity with those reported earlier by several workers (Arya, 2004; Rakesh and Hooda, 2007; Muthukumar et al., 2010).

Conflict of Interests

The authors have not declared any conflict of interest.

REFERENCES

Abeysinghe S (2009). Effect of combined use of *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01 on *Rhizoctonia solani* on *Solanum melongena* and *Capsicum annum.* Pak. J. Nutr. 8:9-16.

Ambikapathy V, Gomathi S, Panneerselva MA (2011).Effect of antifungal activity of some medicinal plants against *Pythium debaryanum* (Hesse). Asian J. Plant Sci. Res. 1(3):131-134.

Arya S (2004). Evaluation of tomato seed treatment with biocontrol agents and soil amendment with neem cake against damping off. Plant Dis. Res. 20(20:187-189.

Ayub M, Ichan M, Khan A, Amin M (1998). Efficacy of some fungicides for managing pre emergence damping off in tomato and their effect on seedling vigour. Sarhad J. Agric. 14(5):475-481.

Bhat NM, Srivastava LS (2003). Evaluation of some fungicides and neem formulation some fungicides and neem formulation against six soil borne pathogens and three *Trichoderma* spp. *in vitro*. Plant Dis. Res. 18(1):56-59.

Bhora BB, Vyas N, Mistry KB (2006). Biocontrol agents and neem formulation for management of damping off in brinjal and chilli. Indian Phytopath. 59(2):223-226.

Chakrabarti A, Das S, Basu A, Raj SK (2005). Bio antagonist combinations against *Pythium* damping off of brinjal. J. Mycol. Plant Pathol. 35(3): 537.

Jiskani MM, Pathan MA, Wangan KH, Imran M, Abro H (2007). Different fungicide such as Benlate topsin – M. derosal and Copper oxychloride were applied as soil drench. Pak. J. Bot. 39(7):2749-2754.

Kataria HR, Grover RK (1967). Some factors affecting the control of *Rhizoctonia solani* by systemic and non-systemic fungicides. Ann. Appl. Biol. 82:267-278.

Manoranjitham SK, Praksam V, Amutha G (2000). Effect of two antagonists on damping off disease of tomato. Indian Phytopath. 533(4):44-443.

Muthukumar A, Eswaran A, Nakkeeran S and Sangeetha G (2010). Efficacy of plant extracts and biocontrol agents against *Pythium aphanidermatum* inciting chilli damping off. Crop Prot. 29:1483-1488.

Nene YL, Thapliyal PN (1993). Fungicides in plant disease control. Oxford and IBH, Pub. Col. New Delhi. pp. 1-501.

Pandey KK, Pandey PK (2005). Differential response of biocontrol agents against soil pathogens on tomato, chiil and brinjal. Indian Phytopath. 58 (3): 329-331.

Rakesh K, Hooda I (2007). Integrated management of damping off of tomato caused by *Pythium aphanidermatum*. J. Mycol. Plant Pathol. 37(2): 259-262.

Satija DV, Hooda I (1987). Influence of farmyard manure in the efficacy of fungicides in controlling damping off tomato and chilli. Veg. Sci. 14: 58-64.

Sawant IS, Mukhopadhyaya AN (1990). Integration of metalaxyl with *T. harzianum* for the control of *Pythium damping* of in sugar beet. Indian Phytopath. 43: 535-541.

Shukla V, Naik LB (1993). Agro techniques of Solanacious vegetables, in Advance in Horticulture vol5,vegetable crops,part1 CK.L.Chadha and G.kallo eds, Malhotra Pub. House, New Delhi. p. 365.

Stack JP, Kenerley CM, Pettit RE (1986). Application of biological control agents. In : Biological Control of PIant Diseases. Butterworth, London. 71 p.

Taylor RJ, Salas GA, Rivera V, Gudmestad NC (2002). Sensitivity of north American isolates of *Phytophthora crythrospeptica* and *Pythium ultimum* to mefenoxam (Metalaxyl). Plant Dis. (St. Poul. Min. American Phytopathological Society). 86:797-802.

Valerie Gravel, Martinez C, Antoun H, and Russell J Tweddel (2005). Antagonist microorganisms with ability to control *Pythium* damping off tomato seeds in rockwool. Biocontrol 50:771-786.

Vincent JM (1927). Distortion of fungal hyphae in presence of certain inhibitors. Nature 159:850.