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International Journal of Current Research
Vol. 4, Issue, 05, pp.156-161, May, 2012

INTERNATIONAL JOURNAL
OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

APPLICATION OF FUNGICIDES SUPPRESSED THE ROOT ROT DISEASE INCIDENCE AND IMPROVED THE BIOMETRIC PARAMETERS AND YIELD IN COLEUS

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ARTICLE INFO

Article History:
Received 18th February, 2012
Received in revised form
14th March, 2012
Accepted 08th April, 2012
Published online 30th May, 2012

Key words:
Fungicides,
Coleus and *M. phaseolina*

ABSTRACT

Nine fungicides at two different concentrations were tested for their efficacy in inhibiting growth of *M. phaseolina* through poisoned food technique. One effective concentration of all fungicides was tested under glass house and field conditions. Among all fungicides, carbendazim at 0.1 per cent was superior in arresting growth of test fungus under *in vitro* conditions. Further, the same fungicide reduced the disease incidence under glass house and field conditions apart from improving the biological parameters and yield.

INTRODUCTION

Coleus (*Coleus forskohlii* Briq.), is a subtropical and warm temperate species belonging to the family Labiatae naturally growing at 600 to 1800 m elevation. The crop is recently being grown over a large area owing to its immense medicinal value. An important drug called forskolin extracted from this is used in the treatment of glaucoma, asthma, hypertension, congestive cardiomyopathy and certain types of cancers (Shah *et al*., 1980). The novel feature of forskolin is its unique mechanism of generating cyclic adenosine monophosphate (AMP) in the cells through the direct activation of the catalytic unit of adenylate cyclase enzyme, which made the pharmaceutical industry to recognize the plant as most important (DeSouza and Shah, 1988). In the light of this intensive cultivation of this unique medicinal plant is gaining importance. The crop production is being challenged by a number of opportunistic pathogens. Among all *Macrophomina phaseolina* incitant of root rot disease is a major limiting factor inflicting heavy losses. Recently, Kamalakannan *et al*. (2005) for the first time reported the occurrence of root rot of coleus caused by *Macrophomina phaseolina* and *Rhizoctonia solani* in Tamil Nadu. Senthamarai *et al*. (2006) observed nematode fungal disease complex involving *Meloidogyne incognita* and *Macrophomina phaseolina* on coleus. Of the various methods adopted for the control of plant diseases, fungicides are undoubtedly the major and the most practical means of effective and reliable control of plant diseases. A very few reports are available on the management root rot disease in coleus through use of chemicals. Malathi and Sabitha (2003) screened three fungicides *viz.,* capton, carbendazim and thiram at four different concentrations for their inhibitory effect on growth and sclerotial production of *M. phaseolina* and reported that carbendazim at 100 µg/ml completely inhibited the mycelial growth. Suryawanshi *et al*. (2008) screened the fungicides against *M. phaseonia* and reported that carbendazim and thiram were most effective in arresting growth of fungus. This paper reports an evaluation of fungicide effectiveness against *M. phaseolina* of coleus plants and also its impact on biological and yield parameters.

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MATERIALS AND METHODS

Fungal isolation and culture

Coleus plants showing the typical symptoms of root rot disease caused by *M. phaseolina* were collected from Agriculture College and Research Institute, Madurai. The pathogen was isolated from the roots of these infected plants by tissue segment method (Rangaswami, 1972) on potato dextrose agar (PDA) medium. Diseased root bits were cut into small bits of three mm size by means of a sterilized scalpel, surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec; washed in repeated changes of sterile distilled water and three such bits were inoculated into previously sterilized, melted and solidified PDA medium contained in sterilized Petri dishes by means of a sterilized forceps, under aseptic conditions. The inoculated plates were incubated at room temperature (28 ± 2 °C) for seven days to obtain good growth of the fungus. The pathogen was purified by single hyphal tip method (Ricker and Ricker, 1936) and the auxenic cultures were maintained on PDA slants for further studies.

Fungicides

Fungicides used in the *in vitro* experiments were commercial formulations. All the nine fungicides were purchased from pesticides market, Madurai. The detail of fungicides tested is presented below.

S. No.	Fungicide	Chemical group	Group name	FRAC code	Concentration (%)
1.	Thiram	Dithiocarbamates and relatives	Dithio-carbamates and relatives	M3	0.05 and 0.1
2.	Captan	Phthalimides	Phthalimides	M4	0.05 and 0.1
3.	Copper oxy chloride	Copper fungicides	-	-	0.1 and 0.25
4.	Methoxy methyl mercuric chloride	Mercury fungicides	-	-	0.025 and 0.05
5.	Thiophenate methyl-M	-	-	-	0.05 and 0.1
6.	Propiconazole	DMI-fungicides	Triazoles	3	0.05 and 0.1
7.	Mancozeb	Dithiocarbamates and relatives	Dithio-carbamates and relatives	M3	0.05 and 0.1
8.	Metalaxyl	Phenylamides	Acylalanines	4	0.05 and 0.1
9.	Carbendazim	MBC fungicides	Benzimidazoles	1	0.05 and 0.1

Efficacy of fungicides on mycelial growth of *M. phaseolina*

The fungicides were assessed for their efficacy in inhibiting the growth of *M. phaseolina* by poisoned food technique at two levels of concentrations mentioned. The fungicides were mixed with PDA before inoculation. Twenty ml of media were added in each Petri plate and the Petri plates were inoculated with nine mm mycelial disc and incubated for four days at room temperature (28 $\pm$ 2 ^{o}C). The treatments were replicated thrice and suitable controls were maintained. The linear mycelial growth was recorded after four days and the per cent inhibition in mycelial growth was calculated using the formula suggested by Pandey *et al*. (2000).

$$\text{Per cent Inhibition} = \frac{Dc - Dt}{Dc} \times 100$$

Dc : Average diameter of fungal growth in control
Dt : Average diameter of fungal growth in treatment

Efficacy of fungicides on coleus root rot under glass house conditions

A pot culture experiment was laid out in completely randomized design to test the efficacy of fungicides against root rot of coleus. Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for one hour for two consecutive days. The virulent strain of *M. phaseolina* mass multiplied in sand maize medium (sand and maize powder at the ratio of 19: 1) was incorporated in the soil at the rate of 50 g per kg of soil. The coleus cuttings were planted in the inoculated pots. The treatments consist of soil drenching of fungicides in one effective concentration as revealed by poison food technique. The treatments were T1 - Thiram 0.1 per cent, T2 - Captan 0.1 per cent, T3 - Copper oxy chloride 0.25 per cent, T4 - Methoxy ethyl mercuric chloride 0.5 per cent, T5 - Thiophenate methyl-M 0.1 per cent, T6 - Propiconazole 0.1 per cent, T7 - Mancozeb 0.1 per cent, T8 - Metalaxyl 0.1 per cent, T9 - Carbendazim 0.1 per cent and T10 - Control. Three replications (10 pots per replication) were maintained and the pots were arranged in a randomized manner. The root rot incidence of *M. phaseolina* was recorded on 45 days after planting and expressed as percentage of disease incidence. Observations on shoot length, tuber length, shoot weight, tuber weight, were also recorded and vigour index was calculated.

Field studies

A field experiment was conducted during September, 2010 to February, 2011 for assessing the efficacy of fungicides for management of root rot disease. The experiment was conducted in a randomized block design replicated thrice with a plot size of 2.4 x 2 .25 sq.m. The medicinal plant *C. forskohlii* was used. The treatments were as in glass house conditions. The observation on the cuttings sprouting was made 30 DAP and expressed as per cent sprouting of cutting. The other biometric observation *viz*., shoot length, tuber length, shoot weight and tuber weight were made at the time of harvest. For the biometric observations 20 plants were selected at random and mean data were calculated. The tuber yield ha^{-1} was also recorded. The incidence of root rot disease was recorded at 30, 60, 90,120 and 150 DAP and expressed as per cent root rot incidence.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Prior to statistical ANOVA the percentage values of the disease index were arcsine transformed. Data were subjected to ANOVA at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's multiple range test (DMRT).

RESULTS

Influence of fungicides on mycelia growth of *M. phaseolina*

Nine fungicides at two different concentrations were screened for their inhibitory effect on the growth of *M. phaseolina* under *in vitro* through poisoned food technique. In general all fungicides were effective in inhibiting test fungus and the inhibition increased with the increase in concentration (Table 1). Among all fungicides, carbendazim 0.1 per cent concentration was found to be the superior in arresting growth with least mycelial growth of 9.2 mm accounting 89.77 per cent inhibition. It was followed by copper oxy chloride (0.25 %) with 11.49 mm mycelial growth and 87.25 per cent inhibition. The minimum inhibition of 33.11 per cent was noticed in thiram (0.05 %).

Effect of fungicides on root rot incidence under glass house conditions

The effective concentration of each fungicide was selected based on the results of *in vitro* studies and these were evaluated under glass house conditions against root rot disease incidence. Among the fungicides tested, carbendazim at 0.1 per cent was found to highly effective with least PDI of 15.92 accounting to 72.65 per cent disease reduction over control. It was followed by copper oxy chloride at 0.25 per cent (17.52 PDI) with 69.90 per cent reduction over control. The least

Table 1. *In vitro* evaluation of fungicides against growth of *M. phaseolina*

S. No	Fungicide	Concentration (%)	*Mycelial growth (mm)	Per cent inhibition
1.	Thiram	0.05	60.20	33.11
2.	Thiram	0.1	18.20	79.78
3.	Captan	0.05	30.30	66.33
4.	Captan	0.1	16.20	82.00
5.	Methoxy ethyl mercuric chloride	0.025	30.80	65.77
6.	Methoxy ethyl mercuric chloride	0.05	19.50	78.33
7.	Carbendazim	0.05	17.6	80.44
8.	Carbendazim	0.1	9.20	89.77
9.	Copper oxy chloride	0.01	49.60	44.88
10.	Copper oxy chloride	0.25	11.49	87.25
11.	Thiophenate methyl-M	0.05	48.70	45.88
12.	Thiophenate methyl-M	0.1	16.50	81.66
13.	Propiconazole	0.05	18.00	80.00
14.	Propiconazole	0.1	14.50	83.88
15.	Mancozeb	0.05	45.85	49.05
16.	Mancozeb	0.1	30.50	66.11
17.	Metalaxyl	0.05	28.50	68.33
18.	Metalaxyl	0.1	19.56	78.33
19.	Control	-	90.00	-
	CD (P= 0.05)		01.26	

*Mean of three replications

Table 2. Efficacy of fungicides on root rot incidence under glass house conditions

S. No	Treatment	*Disease incidence (%)	Per cent reduction over control
1.	Thiram 0.1 per cent (SD)	28.34 (32.16)	51.31
2.	Captan 0.1 per cent (SD)	20.34 (26.80)	65.65
3.	Copper oxy chloride 0.25 per cent (SD)	17.52 (24.75)	69.90
4.	Methoxy ethyl mercuric chloride 0.05 per cent (SD)	27.61 (31.69)	52.56
5.	Thiophenate methyl-M 0.1 per cent (SD)	29.06 (32.62)	50.07
6.	Propiconazole 0.1 per cent (SD)	21.47 (27.60)	63.11
7.	Mancozeb 0.1 per cent (SD)	30.13 (33.29)	48.23
8.	Metalaxyl 0.1 per cent (SD)	25.28 (30.18)	56.57
9.	Carbendazim 0.1 per cent (SD)	15.92 (23.51)	72.65
10.	Control	58.21 (49.72)	-
	CD (P=0.05)	2.17	

*Mean of three replications; Figures in parantheses are arcsine – transformed values; SD- Soil drenching

Table 3. Effect of fungicides on plant growth parameters under glass house conditions

S. No	Treatment	*Sprouting (%)	*Shoot length (cm)	*Tuber length (cm)	*Shoot weight (g)	*Tuber weight (g)	Vigour index
1.	Thiram 0.1 per cent (SD)	82.45 (65.23)	38.71	21.34	82.43	41.34	4951.12
2.	Captan 0.1 per cent (SD)	85.27 (67.43)	41.65	24.08	87.54	46.92	5604.79
3.	Copper oxy chloride 0.25 per cent (SD)	87.94 (69.68)	43.17	25.62	88.15	47.41	6049.39
4.	Methoxy ethyl mercuric chloride 0.05 per cent (SD)	80.46 (63.76)	37.91	20.31	82.63	43.26	4684.38
5.	Thiophenate methyl-M 0.1 per cent (SD)	78.19 (62.16)	36.04	19.42	81.07	42.75	4336.41
6.	Propiconazole 0.1 per cent (SD)	83.52 (66.05)	40.85	22.16	85.31	45.08	5262.59
7.	Mancozeb 0.1 per cent (SD)	77.06 (61.38)	35.24	17.59	80.92	40.83	4071.07
8.	Metalaxyl 0.1 per cent (SD)	81.48 (64.51)	38.3	21.47	83.74	44.12	4870.05
9.	Carbendazim 0.1 per cent (SD)	89.63 (72.21)	45.13	26.24	90.32	49.65	6396.89
10.	Control	49.34 (44.62)	20.72	11.59	51.63	16.81	1594.17
	CD (P=0.05)	1.43	1.44	1.77	1.34	0.82	-

*Mean of three replications; Figures in parantheses are arcsine – transformed values; SD- Soil Drenching

disease reduction of 48.23 was noticed in case of mancozeb 0.1 per cent with 30.13 PDI. From the results it was very clear that cabendazim at 0.1 per cent was the most effective fungicide in managing the disease under glass house conditions (Table 2). In the same study further, effect of fungicides on plant growth parameters *viz.,* sprouting, shoot length, tuber length shoot weight and tuber weight was documented and vigour index. The result clearly depicted that

Table 4. Influence of fungicides on root rot disease incidence under field conditions

S. No	Treatment	*Disease incidence (%)	Per cent reduction over control
1.	Thiram 0.1 per cent (SD)	25.14 (30.09)	52.38
2.	Captan 0.1 per cent (SD)	18.34 (25.35)	65.65
3.	Copper oxy chloride 0.25 per cent (SD)	16.81 (24.20)	69.90
4.	Methoxy ethyl mercuric chloride 0.05 per cent (SD)	24.51 (29.67)	52.56
5.	Thiophenate methyl-M 0.1 per cent (SD)	26.00 (30.65)	50.07
6.	Propiconazole 0.1 per cent (SD)	19.28 (26.04)	63.11
7.	Mancozeb 0.1 per cent (SD)	28.00 (31.94)	48.23
8.	Metalaxyl 0.1 per cent (SD)	22.00 (27.97)	56.57
9.	Carbendazim 0.1 per cent (SD)	13.10 (21.21)	72.65
10.	Control	52.80 (46.60)	-
	CD (P=0.05)	1.10	

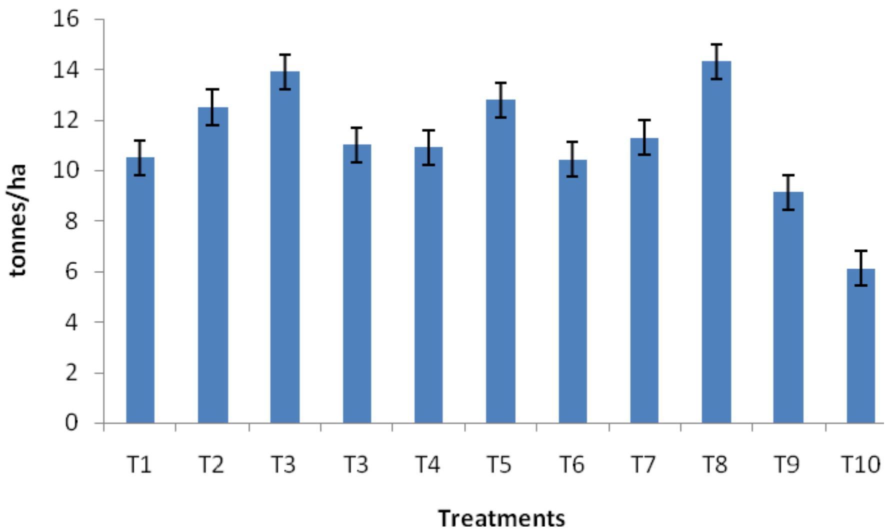
*Mean of three replications; Figures in parantheses are arcsine transformed values; SD- Soil Drenching

Table 5. Influence of fungicides on plant growth parameters under field conditions

S. No	Treatment	*Sprouting (%)	*Shoot length (cm)	*Tuber length (cm)	*Shoot weight (g)	*Tuber weight (g)	Vigour index
1.	Thiram 0.1 per cent (SD)	79.13 (62.81)	35.00	19.80	80.00	42.00	4336.32
2.	Captan 0.1 per cent (SD)	78.27 (62.20)	40.85	21.08	84.35	45.98	4847.26
3.	Copper oxy chloride 0.25 per cent (SD)	81.60 (64.60)	41.17	24.00	86.45	43.00	5317.87
4.	Methoxy ethyl mercuric chloride 0.05 per cent (SD)	78.00 (62.02)	35.00	18.75	79.63	41.00	4192.50
5.	Thiophenate methyl-M 0.1 per cent (SI	74.19 (59.46)	34.05	18.00	79.13	39.00	3861.58
6.	Propiconazole 0.1 per cent (SD)	80.12 (63.52)	39.67	21.00	83.00	43.00	4860.88
7.	Mancozeb 0.1 per cent (SD)	74.00 (59.34)	34.00	15.00	79.92	38.00	3626.00
8.	Metalaxyl 0.1 per cent (SD)	75.00 (60.00)	37.20	20.30	81.00	41.00	4312.50
9.	Carbendazim 0.1 per cent (SD)	83.00 (65.65)	44.20	25.00	88.00	44.65	5743.60
10.	Control	43.80 (41.43)	25.00	10.25	49.30	15.81	1543.95
	CD (P=0.05)	1.08	1.38	1.18	1.08	1.77	

*Mean of three replications; Figures in parantheses are arcsine transformed values; SD- Soil Drenching

Fig 1. Effect of fungicides on yield of coleus under field conditions



Vertical bar represents standard error of three replications
T1 - Thiram 0.1 per cent (SD)
T2 - Captan 0.1 per cent (SD)
T3 - Copper oxy chloride 0.25 per cent (SD)
T4 - Methoxy ethyl mercuric chloride 0.05 per cent (SD)
T5 - Thiophenate methyl-M 0.1 per cent (SD)
T6 - Propiconazole 0.1 per cent (SD)
T7 - Mancozeb 0.1 per cent (SD)
T8 - Metalaxyl 0.1 per cent (SD)
T9 - Carbendazim 0.1 per cent (SD)
T10 - Control

fungicides in addition to disease reduction had a pronounced effect on plant growth parameters. Among the treatments carbendazim (0.1 %) recorded the highest sprouting of cuttings (89.63 %) followed by copper oxy chloride recording 87.94 per cent sprouting as against 49.34 per cent in control.

In addition to this the plants grown from the same treatment *viz*., carbendazim (0.1 %) recorded maximum shoot length (45.13 cm), tuber length (26.24 cm), shoot weight (90.32 g), tuber weight (49.65 g) and vigour index (6396.89) and was followed by copper oxy chloride recording shoot length of

43.17 cm, tuber length of 25.62 cm, shoot weight (88.15 g) and tuber weight (47.41 g) and vigour index of 6049.39 when compared to other treatments (Table 3). There was pronounced reduction in all biometric parameters incase of control.

Field studies

The efficacy of fungicides on incidence of root rot disease was further validated under field conditions. The results of field studies were more consistent with that of glass house experiments. Among the treatments, carbendazim (0.1%) recorded least disease incidence (13.10 PDI) accounting 72.65 per cent reduction over control. It was followed by copper oxy chloride with 16.81 PDI documenting 69.90 per cent reduction over control. The least reduction of disease was noticed in case of mancozeb (0.1 %) with 48.23 PDI accounting 48.23 per cent reduction over control (Table 4). In a field study conducted to evaluate the efficacy of fungicides various plant growth parameters were documented and presented in table 5. The result indicated that there exists a marked variation among the treatments with respect to sprouting, shoot length, tuber length, shoot weight, tuber weight and vigour index. Among the various treatments, carbendazim recorded maximum sprouting (83.00 %), shoot length (44.20 cm), tuber length (25.00 cm), shoot weight (88.00 g), tuber weight (44.65 g) and vigour index (5743.60). This was followed by copper oxy chloride with 81.60 per cent of sprouting, shoot length of 4.17 cm, tuber length of 24.00 cm, shoot weight of 86.45 g, tuber weight of 43.00 g and vigour index of 5317.87. From the results it was very clear that carbendazim (0.1 %) was apart from reducing the disease incidence also increasing the plant growth parameters. Regarding the tuber yield, all the treatments were significantly superior to the control. The treatments carbendazim (0.1 %) recorded the maximum tuber yield of 14.30 t ha^{-1} accounting to the 3.15 per cent increase in yield as against the minimum of 9.13 t ha^{-1} in the control. It was on par with copper oxy chloride (0.25 %) recording 13.90 t ha^{-1} accounting 34.31 per cent increase over control. The least yield of 10.43 t ha^{-1} was recorded in mancozeb (0.1 %) accounting 12.46 per cent increase in yield (Fig.1).

DISCUSSION

Results obtained from *in vitro* fungicide testing are rapid and relatively easy to obtain. Neely (1969) states that *in vitro* fungicide testing often correlates well with results of *in vivo* trials, with provision that a number of *in vitro* trials should be performed. In the present study, nine fungicides were tested for there efficacy against mycelial growth under *in vitro* and among them carbendazim at 0.1 per cent was found to best in arresting growth. The results of the experiments were in agreement with findings of earlier workers. Mukherjee and Tripathi (2000) stated that carbendazim, propiconazole and hexaconazole at 10 µg ml^{-1} concentration completely inhibited the mycelial growth of *R. solani* under *in vitro* conditions. Similarly, Kanakamahalakshmi *et al*. (1998) stated that carbendazim, thiram, thiophanate-methyl and captan were equally and highly effective at 1500 ppm in inhibiting the mycelial growth of *M. phaseolina* completely under *in vitro* conditions. Similar results were reported by several authors in various crops (Anitha and Tripathi, 2001; Prasanthi *et al*., 2000; Malathi and Sabitha, 2003). The effectiveness of carbendazim against *M. phaseolina* could be attributed to interference with energy production and cell wall synthesis of fungi (Nene and Thapliyal, 1973).

The chemical fungicides are effective in controlling the root rot incidence of various crops. The inhibitory effect of fungicides against different isolates of *M. phaseolina* was reviewed by Ramadoss and Sivaprakasam (1987), Patel and Patel (1990) and Malathi (1996). In our study the results of glass house and field conditions were in agreement with that of *in vitro* findings. The evaluation of effective concentration of fungicides under glass house and field conditions revealed that carbendazim 0.1 per cent suppressed the disease incidence effectively in both conditions. The results of the experiment were in agreement with findings of Kamalakannan (2004) who reported carbendazim and propiconazole at 0.1 per cent concentration reduced the coleus root rot caused by *M. phaseolina* and *R. solani.* Kulkarni *et al*. (1992) reported seed treatment with carbendazim at 3 g/kg seeds and soil drench at 0.2 per cent effectively controlled the root rot of cotton caused by *R. bataticola.* In a similar way Peshney *et al*. (1992) reported that growth and sclerotial germination of *R. bataticola* were effectively controlled by thiram (0.2 %), captan (0.2 %), mancozeb (0.2 %), iprodione (0.2 %), carbendazim (0.2 %) and tridemorph (0.7 %). Further, Prashanthi (1994) tested six fungicides as soil drenching treatment against *R. bataticola* causing root rot of safflower. Among them, carbendazim (0.01%), propiconazole (0.05%), captan (0.2%) and chlorothalonil (0.2%) were found to be effective. An effective management practice should not only target the reduction in the disease incidence but also improve the other biological parameters and ultimately economic yield. In the light of this the findings made in present investigation were helpful in improving biological parameters in coleus apart from reducing disease incidence. Soil drenching of carbendazim 0.1 per cent improved the biological parameters like sprouting, shoot length, tuber length, shoot weight, tuber weight and vigour index when compared to other fungicides under investigation. Further, the same treatment significantly enhanced the yield of coleus plants when compared to other treatment. The results were in agreement with findings of Kamalakannan (2004) who reported the improvement of biological parameters and yield in case of coleus plant treated with carbendazim 0.1 per cent. The improvement in the other biological parameters and yield obtained in the carbendazim treated plants could be because of reduced incidence of root rot disease and also induction of systemic resistance which could possibly avoid other saprophytes or nectrotrophic pathogen infecting the plants in this treatment.

Conclusion

In conclusion, carbendazim 0.1 per cent protects coleus plants against root rot disease incidence as determined from results of *in vitro* and *in vivo* testing. In addition the same fungicides also improved biological parameters and yield.

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