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Research Article

Use of Plant Products, Bio Control Agents, Chemicals and Organic Amendments for Integrated Management of *Rhizoctonia solani* in Cowpea

Tribikram Sahoo[1], Abhisek Tripathy[2*], Shriram Ratan Pradhan[3] and Anirudha Tarai[4]

College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar-751003

*Corresponding Author E-mail: abhi.plantpathology@gmail.com

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ABSTRACT

For integrated disease management of root and stem rot of cowpea caused by Rhizoctonia solani experiments were conducted using different plant products, bio control agents, chemicals and organic amendments during Kharif of 2014-15. Out of seven phytoextracts tested against Rhizoctonia solani, the maximum inhibition was recorded in garlic(100%) followed by turmeric(34.08%) at 10 % concentration, whereas maximum inhibition 100% was recorded in both 15% and 20% concentration in garlic and calotropis respectively. Out of four biological agents were tested against Rhizoctonia solani, Bacillus subtilis was recorded maximum growth inhibition of 72.04% as compared to Trichoderma hamatum (49.78%) and Trichoderma harzianum (46.00%). In vitro studies of 13 fungicides against Rhizoctonia solani revealed that Carboxin 37.5% + Thiram 37.5%, Hexaconazole 5%, Difenoconazole 25%, Tebuconazole 25% and Tebuconazole 50% + Trifloxystrobin 25% recorded maximum growth inhibition (100%). In vivo studies of 13 fungicides maximum mortality was recorded from Carbendazim 50% followed by Propineb 70% (82% and 65.97%) respectively. Among the organic products tested against Rhizoctonia solani, the minimum mortality was recorded from spent mushroom substrate + Cowdung + Vermicompost followed by Vermicompost with 10% and 15% mortality respectively. The maximum mortality was recorded from Poultry manure (66.67%) as compared to rest of the treatments and control.

Keywords: *Phytoextract, Rhizoctonia solani, Biological agents, Bacillus subtilis, Cowpea*

INTRODUCTION

Cowpea diseases induced by different pathogens belonging to various pathogenic groups constitute one of the most constraints to profitable cowpea production in all agro-ecological zones where the crop is cultivated. Root and stem rot caused by (*Rhizoctonia solani*) is one of the most important diseases which cause considerable loss in the yield.

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The losses in green fodder and seed yield were estimated to be about 28.8 and 39.7 per cent, respectively (Ram & Gupta, 1988).

Root and stem rot of cowpea caused by *R. solani* is an important soil borne disease in worldwide. Although it has a wide range of hosts, its main targets are herbaceous plants. *R. solani* would be considered a basidiomycetous fungus if the teleomorph stage were more abundant. Therefore it attacks seeds of plants present below the soil surface. It can also infect pods, leaves and stems of the host plants. The most common symptom of *Rhizoctonia* is the failure of infected seeds to germinate. *R. solani* may invade the seed before it has germinated to cause pre-emergence mortality or death to very young seedlings soon after they emerge from the soil. Seeds that do germinate before being killed by the fungus have reddish-brown lesions and cankers on stems and roots. The pathogen prefers warm and wet climatic condition for successful infection and growth. *Rhizoctonia solani* can survive in the soil for many years in the form of sclerotia. Sclerotia of *Rhizoctonia* have thick outer layers to allow for survival and they function as the overwintering structure for the pathogen. In some rare cases (in the teleomorphic stage) the pathogen may also take on the form of mycelium that resides in the soil as well.

Based on this back ground, quite a number of experiments were designed intensively adopting several methodologies in order to propose the management measures of the root and stem rot disease in cowpea cultivated in the country in general and the state of Odisha in particular for agro climatic relevance. The results as conceived might of course, in one hand help to eradicate the disease while ensuring a bumper productivity in the other hand.

MATERIALS AND METHODS

The research was carried out both in the laboratory and field during 2014-16.The field and laboratory experiment was conducted at the AICRP on vegetable crops, Orissa University of Agriculture and Technology, Bhubaneswar and Research facilities of Department of Plant Pathology, OUAT, Bhubaneswar.

***In vitro* evaluation of plant extracts for fungal growth inhibition by poison food technique-**

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1973) *in vitro*. Different plant extracts used in these experiments are enlisted below.

Table 1: List of plant extracts used in *In vitro* evaluation against *R. solani*

Sl. No	Common name	Botanical name	Plant part used
1	Neem	*Azadirachta indica* A.	Leaf
2	Garlic	*Allium sataivum* L.	Clove
3	Turmeric	*Curcuma longa*	Rhizome
4	Karanja	*Pongamia glabra*	Leaf
5	Begunia	*Begunia nirgundi*	Leaf
6	Datura	*Datura stramonium*	Leaf
7	Milkweed	*Calotropis spp.*	Leaf

The per cent inhibition over control was calculated according to formula given by Vincent (1947) as follows.

$$I = \frac{(C-T)}{C} x100$$

I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

***In vitro* evaluation of bioagents by Dual culture technique**

The antagonistic microorganisms like *Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum* and *Bacillus subtilis* were maintained in medium potato dextrose agar and evaluated for their antagonistic effect under *in vitro* conditions against *Rhizoctonia solani* by dual culture technique. In dual culture technique, twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri dishes and allowed to solidfy. Fungal antagonistics inoculating the pathogen at one side of Petridish and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing cultures were used. Each treatment was replicated 2 times. After required period of incubation i.e. after control plate reached growth of 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to formulae given by Vincent (1947).

The fungicides were tested initially under *in vitro* condition by poison food technique (Nene & Thapliyal,1973) at desired concentration in *in vivo* condition by pot culture method. Per cent inhibition of mycelial growth over control was calculated by using the formulae given below by Vincent (1947).

***In vivo* evaluation of fungicides**

Soil was collected from the research plot of Department of Plant Pathology. The soil was sterilized in autoclave at 121^0C at 15 psi for 2 hours. Sterilized soils were put into polythene bag mixing with inoculum of actively growing culture of *R. solani*. Then it was incubated for 10days. After that seeds of cowpea at the rate of ten seeds per pot were sown. After thirteen days, thinning was done. Then the required concentrations of chemicals were prepared and incorporated into pot. Three replications were maintained for each treatment.

Table 2: List of different fungicides tested for efficacy in *R.solani*

Sl. No	Common names	Chemical name	Conc. (%)
1	Bavistin	Carbendazim 50%WP	0.2
2	Vitavax power	Carboxin 37.5% + Thiram 37.5%	0.2
3	Sheathmar 3L	Validmycin 3%L	0.1
4	Onestar	Azoxystrobin 23% SC	0.1
5	Sixer	Mancozeb 63%WP + Carbendazim 12% WP	0.2
6	Curzate	Cymoxanil 8% WP+Mancozeb 64% WP	0.15
7	Roko	Thiophanate Methyl 70% WP	0.15
8	Antracol	Propineb 70% WP	0.2
9	Contaf	Hexaconazole 5% EC	0.05
10	Score	Difenconazole 25% EC	0.05
11	Folicur	Tebuconazole 25%EC	0.05
12	Tilt	Propiconazole 25% EC	0.05
13	Nativo	Tebuconazole50%WP+ Trifloxystrobin25%WP	0.05

In vivo* efficacy of organic products against *R. solani

Different organic products were taken to evaluate their efficacy in field condition for controlling the *Rhizoctonia solani* infecting cowpea. Organic products were selected for experimental purpose as per their local availability viz. Mustard oil cake, Karanj oil cake, Neem oil cake, Mahua oil cake, Poultry manure, Goat manure, VAM, Vermicompost, Spent Musroom Substrate and fresh cow dung. Each treatment was replicated thrice. Soil was

sterilized at 15 psi at temperature of 121^{0}C for 20 minutes.The soil was inoculated with the culture of *Rhizoctonia solani*. The poly pots were closed with rubber bands and kept under shade. The organic products were amended thoroughly with previously incubated soil at the rate of 10gms per kg of soil. After mixing the organic products, the poly pots were closed with rubber bands and kept for seven days. Ten cowpea seeds were sown in each pot and watering was carried out thrice a week to promote germination and growth of the plants. The regular observation was recorded with respect to mortality percentage and plant height.

Table 3: List of organic products used against *Rhizoctonia soalni*

Sl. No	Name	Quantity(gm)/Kg of soil
1	Mustard oil cake	10
2	Karanja oil cake	10
3	Poultry manure	10
4	Goat manure	10
5	Vescicular arbuschular mycorrihiza(VAM)	10
6	Vermi compost	10
7	Spent mushroom substrate (SMS)	10
8	SMS + Cow dung	10
9	SMS + Vermi compost	10
10	SMS + Vermicompost +Cow dung	10
11	Neem oil cake	10
12	Mahua oil cake	10

Statistical analysis was carried out by following the standard procedures (Panse & Sukhatme, 1967). Data in percentage were transformed to angular values before analysis.

RESULTS

Efficacy of plant extracts in inhibiting the growth of the fungus

Seven plant extracts, which are easily available in the locality were evaluated for their fungitoxicity activity against the growth of *Rhizoctonia solani* at 10,15 and 20% concentration as per the procedure described above. The radial growth of the fungal colony on plant extract mixed medium were measured after seven days of incubation and the data obtained were presented in Table 4.

All the plant extracts under study inhibited mycelial growth of *Rhizoctonia solani* at 10, 15 and 20 per cent concentrations and were significantly superior over control. The results indicated that, the maximum inhibition was recorded in Garlic (100%) followed by Turmeric (34.08) at 10% concentration. At 15% concentration, a total inhibition was noticed in Garlic and Calotropis (100%) followed by Turmeric (41.11%). At 20% concentration, a total inhibition was noticed in Garlic and Calotropis (100%) followed by Turmeric (68.33%). The least reduction of growth was observed in Begunia (0.38%) at 10% concentration and in Karanja 5.56% and 9.82% at 15% and 20% concentration respectively. In general all the leaf extracts tested, significantly inhibited the mycelia growth of *Rhizoctonia solani* above 1% at 10% concentration, 5% at 15% concentration and 9% at 20% concentration except Begunia and Karanja.

Table 4: Efficacy of plant extracts against *Rhizoctonia solani* in *in vitro*

Sl. No.	Common name	% inhibition at 10% conc.	% inhibition at 15% conc.	% inhibition at 20% conc.
1	Garlic (*Allium sataivum* L.)	*100 (90.02)	100 (90.02)**	100 (90.02)
2	Neem *(Azadirachta indica* A.)	1.11 (6.05)	17.41 (24.67)	21.11 (27.36)
3	Begunia (*Vitex nirgundi*)	0.38 (3.52)	6.67 (14.97)	20.87 (27.20)
4	Datura (*Datura stramonium*)	30.93 (33.80)	33.33 (35.27)	41.67 (40.21)
5	Karanja (*Pongamia glabra*)	9.82 (18.27)	5.56 (13.64)	9.82 (18.27)
6	Turmeric (*Curcuma longa*)	34.08 (35.72)	41.11 (39.89)	68.33 (55.77)
7	Milkweed (*Calotropis spp*.)	7.78 (16.20)	100 (90.02)	100 (90.02)
8	Control	0	0	0
	SE(m)±	0.79	0.66	0.55
	CD(0.05)	2.36	1.88	1.65

** Figures in parenthesis are angular transformed value

Effect of Bio control Agents on Mycelial Growth of *Rhizoctonia solani.*

An experiment was conducted to explore the capabilities of four bio-agents against the test pathogen. The antagonistic nature of these bioagents against the test fungus was studied adopting dual culture technique and data obtained are presented in Table 5.

Antagonistic studies revealed that, growth of pathogen was significantly checked over control by the antagonistic nature of all the antagonists tested. The antagonists also restricted the growth of the pathogen and didn't allow it to grow further. The lowest growth of the pathogen was observed in *Bacillus subtilis* (25.16mm) followed by *Trichoderma hamatum* (45.20mm), *Trichoderma harzianum* (48.60mm), *Trichoderma viride* (50.60mm). However, the growth of *Rhizoctonia solani* in *Trichoderma viride* was maximum.

Regarding the degree of growth inhibition, *Bacillus subtilis* inhibited the maximum growth of the pathogen (72.04%) followed by *Trichoderma hamatum*, *Trichoderma harzianum* but such inhibition of the growth of the pathogen with *Trichoderma viride* was relatively least among all the antagonists. The range of growth inhibition was from 43.78% to 72.04% which undoubtly positively establishes a check in the growth of pathogen without treatment with fungicides.

Table 5: *In vitro* bio assay of bio control agent

Sl.no.	Name of antagonist	Mean diameter of *Rhizoctonia solani* (mm)	*Mean diameter in dual culture (mm)	Growth inhibition (%)
1	*Trichoderma hamatum*	90	45.20	49.78
2	*Trichoderma viride*	90	50.60	43.78
3	*Trichoderma harzianum*	90	48.60	46.00
4	*Bacillus subtilis*	90	25.16	72.04
5	Control	90	90.00	0.00

Efficacy of fungicides on growth of *Rhizoctonia solani* in *in vitro*

In order to evaluate the efficacy of some selected fungicides on growth of *Rhizoctonia solani*, the experiment was conducted as per the procedure mentioned earlier following posion food technique. The colony diameter and per cent growth inhibition have been presented in Table 6. It was observed from the data that, all the fungicides tested in solid medium significantly reduced the fungal colony in comparision to control.

It was revealed that fungicides namely Carboxin 37.5% + Thiram 37.5% along with Hexaconazole 5% EC, Difenoconazole 25% EC, Tebuconazole 25% EC and Tebuconazole 50% + Trifloxystrobin 25% proved efficacious in inhibiting the mycelial growth with 100 per cent inhibition. The next effective fungicides were Cymoxanil 8% WP+Mancozeb 64% WP and Propiconazole 25% EC which recorded 79.81 and 77.57 per cent inhibition respectively. However Validamycin, Mancozeb 63%WP + Carbendazim 12% WP Azoxystrobin 23% SC and Propineb 70 WP registered 54.63, 52.78 and 5.07 respectively.

Table 6: Efficacy of fungicides on growth of *Rhizoctonia solani* in *in-vitro*

Sl. No.	Chemical name	Conc.(%)	% growth inhibition
1	Carbendazim 50%WP	0.2	0.00* (0)
2	Carboxin 37.5% WP + Thiram 37.5% WP	0.2	100 (90.02)
3	Validamycin 3%L	0.1	54.63 (47.67)
4	Azoxystrobin 23% SC	0.1	8.37 (16.82)
5	Mancozeb 63%WP + Carbendazim 12% WP	0.2	52.78 (46.60)
6	Cymoxanil 8% WP+ Mancozeb 64% WP	0.15	79.81 (63.31)**
7	Thiophanate Methyl 70% WP	0.15	1.26 (6.44)
8	Propineb 70% WP	0.2	5.07 (13.02)
9	Hexaconazole 5% EC	0.05	100 (90.02)
10	Difenoconazole 25% EC	0.05	100 (90.02)
11	Tebuconazole 25% EC	0.05	100 (90.02)
12	Propiconazole 25% EC	0.05	77.57 (61.74)
13	Tebuconazole50% WP+ Trifloxystrobin25%WP	0.05	100 (90.02)
	SE (m)±		0.90
	CD(0.05)		2.71

**Figures in parenthesis are angular transformed value

Table 7: Efficacy of fungicides on *Rhizoctonia solani* in *in-vivo*

Sl. No.	Chemical name	Conc. (%)	*Mortality (%)
1	Carbendazim 50%WP	0.2	82.00*
2	Carboxin 37.5%WP + Thiram 37.5%WP	0.2	2.60
3	Validamycin 3%L	0.1	8.93
4	Azoxystrobin 23% SC	0.1	48.77
5	Mancozeb 63%WP + Carbendazim 12% WP	0.2	7.73
6	Cymoxanil 8% WP+ Mancozeb 64% WP	0.15	1.83
7	Thiophanate Methyl 70% WP	0.15	60.07
8	Propineb 70% WP	0.2	65.97
9	Hexaconazole 5% EC	0.05	2.67
10	Difenoconazole 25% EC	0.05	2.00
11	Tebuconazole 25% EC	0.05	12.63
12	Propiconazole 25% EC	0.05	12.10
13	Tebuconazole50%WP+ Trifloxystrobin25%WP	0.05	3.47
14	Control		99.07
	SE (m)±		1.51
	CD(0.05)		4.54

Efficacy of fungicides on growth of *R. solani* in *in-vivo*

The fungicides which were studied in laboratory, they were further studied under pot culture experiment as per the procedure described under 'Materials and Methods' and presented in table 7.

This study reaveled that least mortality was recorded from Cymoxanil 8% WP+mancozeb 64% WP (1.83%) followed by Difenoconazole 25% EC (2.00%) and Carboxin 37.5% + Thiram 37.5% (2.60%). The maximum mortality was recorded in Carbendazim 50%WP(82.00%) followed by Propineb 70% WP (65.97%)and Azoxystrobin 23% SC (60.07%). However the control pots recorded as high as 99.07% mortality.

Effect of organic amendment on growth of *Rhizoctonia solani*

This study revealed that, least mortality was recorded from spent mushroom substrate+ cowdung+ vermicompost (10.00%) followed by Vermicompost (15.00%). The mortality was recorded in poultry manure amendment(66.67%) followed by mustard oil cake (58.33%). Fifty per cent mortality was recorded in Spent mushroom substrate +vermicompost, Karanja oil cake and Mahua oil cake amended treatments. However control pots recorded as high as 91.66% mortality. So far as plant height was concerned the result was obtained in the same trend. Spent mushroom substrate + cowdung +vermicompost has recorded the maximum plant height (89.33cm) followed by vermicompost (81.67cm). However control pot recorded only (11.00cm).

Table 8: *In vivo* efficacy of organic products against *Rhizoctonia solani*

Sl.No.	Treatment	Mortality %	Plant Height(cm)
1	Control	91.66*	11.00*
2	Mustard oil cake	58.33	3.33
3	Karanja oil cake	50.00	58.33
4	Poultry manure	66.67	47.33
5	Goat manure	41.67	47.00
6	Vescicular arbuschular mycorrhiza (VAM)	41.67	58.33
7	Vermicompost	16.00	81.67
8	Spent mushroom substrate (SMS)	50.00	51.67
9	SMS + Cowdung	41.67	79.00
10	SMS + vermicompost	50.00	64.33
11	SMS+Cowdung+Vermicompost	10.00	89.33
12	Neem oil cake	41.67	31.33
13	Mahua oil cake	50.00	37.33
	SE(m)±	9.67	11.67
	CD(0.05)	28.40	34.27

DISCUSSIONS

All the phytoextracts under study inhibited mycelial growth of *Rhizoctonia solani* at 10%, 15% and 20% concentration as compared to control. The maximum inhibition was recorded in garlic (100%) at 10% concentration. Garlic and calotropis were recorded the maximum per cent growth inhibition (100%) in both 15% and 20% concentration respectively. Garlic, a potential phytoextract effective against a wide range of diseases including *R. solani* has been reported earlier by Shashidhara et al. (2008) and Sehajpal et al. (2009) which supports our findings. Dawar et al. (2010) reported potentiality of *Datura alba* against *R.solani* as efficacious one which also corroborating the present investigation.

In the present study the antifungal characterstics of bio-agents *viz. Trichoderma viride, Trichoderma hamatum, Trichoderma harzianum* and *Bacillus subtilis* were tested *in vitro* to study the effectiveness against *R.solani* employing dual culture technique. Maximum growth inhibition was found in *Bacillus subtilis* (72.04%) followed by *Trichoderma hamatum* (49.78%) and *Trichoderma harzianum* (46.00%). The effectiveness of *Trichoderma spp.* in field of plant disease management was confirmed earlier by various workers like Elad et al. (1983), Chang and Choi (1990), Nagarajkumar et al. (2004), Sharma et al. (2009), Bhat et al. (2009) and Panwar et al. (2012). The antagonistic nature of *Bacillus subtilis* also supports the findings of Schmiedeknecht (1993), who reported that *B. subtilis* is a suitable antagonists for biological control of *Rhizoctonia* disease on potato.

In vitro bio assay of fungicides against *Rhizoctonia solani* revealed that, Carboxin 37.5% + Thiram 37.5% @ 0.2%, Hexaconazole 5% @ 0.05%, Difenoconazole 25% @ 0.05%, Tebuconazole 25% @ 0.05% and Tebuconazole 50% + Trifloxystrobin 25% @ 0.05% recorded maximum growth inhibition however Validamycin 3% @ 0.1%, Cymoxanil 8% + Mancozeb 64% @ 0.15%, Mancozeb 63% + Carbendazim 12% @ 0.2%, Propiconazole 25% @ 0.05% concentration also checked the growth of *Rhizoctonia solani* ranging 52.78% to 91.81% inhibition. It was supported by earlier report of Sahu (1986), who reported that Carboxin performed best against *R.solani* in Rapeseed and mustard.

In *in vivo* study with chemicals in pot culture experiment, the minimum mortality

was recorded in Cymoxanil 8% + Mancozeb 64% @ 0.15% followed by Difenoconazole 25% @ 0.05% and Carboxin 37.5% + Thiram 37.5% @ 0.2 % concentration registered the mortality as 1.83%, 2.0% and 2.60% respectively. The maximum mortality was recorded in Carbendazim 50% @ 0.2 % followed by Propineb 70% @ 0.2% recording mortality as with 82.0% and 65.97% respectively. The effectiveness of Carbendazim was found to be less against *R.solani*, but Sunder et al. (2009) reported that Carbendazim was proved effective against *R. solani* in mung bean, which contradicts the present findings.

In pot culture experiment, the effectiveness of organic products were explored on the growth of *Rhizoctonia solani* which revealed that, the minimum mortality was recorded from Spent mushroom substrate + Cowdung + Vermicompost followed by Vermicompost with 10% and 15% respectively. The maximum mortality was recorded from Poultry manure 66.67% as compared to rest of the treatments including control. With regard to the plant height the result was also obtained in same trend. Spent mushroom substrate + Cowdung + Vermicompost recorded maximum plant height followed by Vermicompost with 89.33cm and 81.67cm respectively. Such findings are also in agreement with the findings of El-Mohamedy et al. (2006), who amended soil with *Trichoderma hazianum*. Ashlesha et al. (2009) amended soil with dry powder of cowdung and fresh cowdung urine and milk and reported effective against soil borne *Rhizoctonia* and *Fusarium* pathogens. The efficacy of Vermicompost against *R.solani* has been reported earlier by Sinha et al. (2010) which is also in agreement with the present finding.

CONCLUSION

Therefore the phytoextracts and organic products may be included in integrated disease management strategy against *Rhizoctonia* root and stem rot management in cowpea to the farming community of Odisha. Use of phyto extracts as a means of non-chemical crop disease management is found imperative in view of its eco-friendly nature. However use of chemicals in integrated disease management schedule has been the last resort, so far as crop disease management is concerned. But exploration of antagonists and organic products found to be efficacious and economic and non-hazardous to environment. The farming community of Odisha may be educated to use indigenous products like botanicals and organic products in crop disease management programme.

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