INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

Volume-1, Issue-2 :2011 *www.ijpaes.com* *ISSN 2231-4490*

INTEGRATED DISEASE MANAGEMENT OF DRY ROOT ROT OF CHILLI INCITED BY *SCLEROTIUM ROLFSII* (SACC.)

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ABSTRACT : Chillies is an important commercial crop of Andhra Pradesh. Recently the crop is affected by dry root rot disease caused by *Sclerotium rolfsii* (Sacc.) under rainfed conditions. An attempt was made to manage the disease by holistic approach. *In vitro* evaluation of nine fungicides by poison food technique showed that tebuconazole and combination of carbendazim+mancozeb were effective in inhibiting the mycelial growth (94.1%) followed by difenconazole (93.3%). *In vivo* soil drenching with same fungicides proved effective in controlling the pathogen at 1000, 2000 and 3000 ppm. Integration of different treatments including seedling dip with carbendazim+mancozeb, addition of vermicompost, drenching with fungicide and application of *Trichoderma harzianum* (7%) were found to be effective in management of disease in comparison with individual treatments.
Keywords: Chilli, dry root rot, *Sclerotium rolfsii*, fungicides, *Trichoderma viride*, soil amendments

Chilli (*Capsicum annum* L.) is mainly cultivated for its vegetable green fruits and for the dry chilli as the spice of commerce. It is a rich source of Vitamin C, A and B.In India it is an important cash crop, which is grown for the both domestic and export market.. India is the largest producer of chillies in the world (8.5 lakh tones) followed by China (4 lakh tonnes), Pakistan (3 lakh tonnes) and Mexico (3 lakh tonnes). Andhra Pradesh ranks first in India both in area and production with 2.04 lakh hectares producing 323 thousand tones (Anonymous, 2010). Chilli crop suffers with many fungal, bacterial and viral diseases resulting in huge yield losses. Among the fungal diseases, in recent years dry root rot of chilli caused by *Sclerotium rolfsii* is of major concern and causing the economic losses in chilli (Kalmesh and Gurjar 2001). In the year 2001 root rot of chilli caused by *S. rolfsii* was first time reported from Rajasthan near Jaipur chilli growing areas, where the sever mortality of chilli plants during March-April was observed (Kalmesh and Gurjar (2001).

Survey of the disease over a period of time revealed that the it affects the yield severely whenever it occur at any stage of the crop. Inspite chemical measures like drenching copper oxy chloride @3 g/L is recommended based on the previous studies on *Sclerotium* spp., the new fungicides along with earlier proven fungicides need to be evaluated to find out the effective and economic fungitoxicants with which the disease can be controlled. Presently, greater emphasis should be placed on biological control of soil borne pathogens, in order to reduce the cost of cultivation, environmental hazards and to avoid the development of resistant strains. . Hence, a holistic approach is needed for the effective management of root rot disease.

MATERIALS AND METHODS

Isolation of fungus

The dry root rot fungus was isolated from the roots of the chillies variety LCA 334 grown at RARS, Lam by standard isolation method under aseptic conditions. The infected tissues of the roots were cut in to small pieces of 1-2 mm size and surface sterilized with 1% mercuric chloride solution for one minute and washed repeatedly thrice in sterile distilled water and placed in petri plates containing sterilized PDA and incubated at 28± ºC. The culture thus obtained was purified by single spore isolation and identified as *Sclerotium rolfsii* (Plate-1) based on the morphological description given by Barnett (1960).

In vitro evaluation of the fungicides

Five systemic (hexaconazole 0.2%, propiconazole 0.1%, , tebuconazole 0.15%, difenconazole 0.5% and Benomyl 0.1%) and three contact (Pencycuron 0.1%, copper oxy chloride 0.3% and propineb 0.2%) and one combination of systemic and contact (carbendazim+ mancozeb 0.2%) fungicides were evaluated against *S. rolfsii* the casual organism of chillie root rot.

Poisoned food technique

Recommended dose of fungicide was added to the melted PDA just before pouring in to the plate. Twenty ml of medium with desired concentration of fungicide was poured in each sterilized petriplate. Suitable checks were kept for comparison. Five mm mycelial disc of *S. rolfsi* was taken from the periphery of 10 days old culture and sclerotia taken from one month culture were placed at the centre of the separate plates and incubated at 28± ºC. Growth of the fungus was measured by taking the diameter in two directions and the average was recorded. Final growth reading was recorded when the growth of the fungus in control plate was full. Per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

Soil used

For all pot culture experiments black cotton soil sterilized with 5% formaldehyde solution was used for raising chillies plants. One chillie plant was raised individually in 2 kg capacity plastic bags. Three replications, each comprised of 20 plants were maintained for each treatment.

Evaluation of fungicide by soil drenching

Sterilized soil was filled in polythene covers of size 7.5 cm width and 20 cm length. To evaluate 9 fungicides viz., hexaconazole 0.2%, propiconazole 0.1%, , tebuconazole 0.15%, difenconazole 0.5% , Benomyl 0.1%, Pencycuron 0.1%, copper oxy chloride 0.3% , propineb 0.2%) and one combination of systemic and contact carbendazim+ mancozeb by soil drenching. *S. rolfsii* was mass multiplied on sorghum grain and the culture was placed at the root zone of chilie plant at two depths of 10 cm and 15 cm. All the test fungicides were drenched and allowed to percolate to the corresponding depths and their efficacy in inhibiting the fungus was evaluated by recovering the fungus from the soil of rhizosphere.

Evaluation of *Trichoderma* species

Four isolates of *Trichoderma viride* and one isolate of *Pseudomonas fluroscens* from different chillie growing areas of Andhra Pradesh were collected and categorized as I, II, III and IV and evaluated under *in vitro* conditions for the antagonistic activity against the wilt pathogen by dual culture technique (Huang and Hoes, 1976). Mycelial discs of 5mm diameter each of bioagents and the pathogen were taken from the margins of their actively growing cultures and transferred to potato dextrose agar medium in the petri plates on the opposite sides. The Petri plates were subsequently incubated at 28±1 ºC. Colony diameter of the test fungus up to the zone of inhibition was recorded in case of each bioagent and per cent growth inhibition of the test pathogen was calculated.

Mass multiplication of Bio control agent *Trichoderma viride.*

The effective isolate of *T.viride* identified in *in vitro* studies was mass multiplied and further used in the pot culture experiments.

Evaluation of integrated disease management module for root rot disease.

A pot culture experiment was conducted in factorial randomized block design during 2008-09 and 2009-10 crop season at Regional Agricultural Research Station, Lam, Guntur.

Different treatments including T1- seedling dip in fungicide, T2-soil amendment with vermicompost, T3- T1+T2, T4-addition of biocontrol agent *T. viride*, T-5 drenching of effective fungicide and T-6-combination of all these treatments were evaluated to develop a reliable, ecofriendly integrated disease management approach.

Vermicompost was taken as soil amendment based on reports of significant superiority in reducing the *Fusarium* wilt disease incidence in fenugreek (Kamlesh Mathur *et al.,*2006)

Best isolate of *T. harzianume* isolated was applied at the rate of 10g/pot at the time of inoculation of *S rolfsii..*

Similarly the fungicides which proved effective in *in vitro* studies were used for seedling dip for 30 minutes before sowing and also for drenching treatments.

Incidence of root rot was recorded after 10 days of inoculation.

RESULTS AND DISCUSSION

In vitro evaluation of fungicides

Efficacy of nine fungicides was tested at recommended concentrations by poisoned food technique. The results (Table-1 & 2) revealed that there is significant difference in per cent inhibition of mycelial growth and sclerotial germination of *Sclerotium rolfsii* with all the tested fungicides.The percent inhibition of mycelial growth of *Sclerotium rolfsii* was found highest (94.1%) in both the treatments of Tebuconazole at 0.15 per cent concentration and Carbendazim+Dithane M-45-0.2% followed by difenconazole (93.3%) and propineb (92.6%) respectively. Hexaconazole (84.4%) and propiconazole (82.5%) treatments were also found effective where as benomyl is least effective (5%) but pencycuran and copper oxychlore have no effect on growth.inhibition.

Table-1. Inhibition of mycelial growth of *Sclerotium rolfsii* by different fungicides.

S. No	Treatment	Mean radial growth of the fungus (cm)	Per cent growth inhibition
1	Hexaconazole- 0.2%	1.40	84.4
2	Propiconazole- 0.1%	1.56	82.5
3	Tebuconazole- 0.15%	0.53	94.1
4	Difenconazole – 0.5%	0.60	93.3
5	Pencycuron-0.1%	9.00	0.00
6	Copper Oxy Chloride- 0.3%	9.00	0.00
7	Carbendazim+Dithane M-45-0.2%	0.53	94.0
8	Benomyl-0.1%	8.50	5.00
9	Propineb- 0.2%	0.63	92.60
10	Check	8.96	
	SEM±	0.05	
	SED	0.07	
	C.D	0.15	
	CV%	2.1	

Table-2. Inhibition of sclerotial germination and growth of *Sclerotium rolfsii* by different fungicides.

S. No	Treatment	Mean radial growth of the fungus (cm)	Per cent growth inhibition
1	Hexaconazole- 0.2%	0.76	91.5
2	Propiconazole- 0.1%	0.53	94.1
3	Tebuconazole- 0.15%	0.53	94.1
4	Difenconazole – 0.5%	0.80	91.1
5	Pencycuron-0.1%	8.36	7.01
6	Copper Oxy Chloride- 0.3%	8.86	1.50
7	Carbendazim+Dithane M-45-0.2%	0.56	93.7
8	Benomyl-0.1%	4.26	52.6
9	Propineb- 0.2%	1.03	88.5
10	Check	8.93	
	SEM±	0.071	
	SED	0.101	
	C.D	0.212	
	CV%	3.60	

The results are in agreement with the earlier report of Vinod Dange (2006) as carboxin (0.05%), propicanazole (0.10%) and hexaconazole (0.1%) were found effective against *S. rolfsii in vitro.* Carboxin, epoxiconazole, hexaconazole, propiconazole and tridemifon completely arrested the growth of *S.rolfsii* followed by tebuconazole and benomyl is not effective against *S.rolfsii* (Tiwari and ashok singh 2004). Similarly, Waterfield and Siler, 1990 and Hagans *et al*., 1992 reported that, propiconazole was found highly effective in inhibiting the growth of *Sclerotium rolfsii*.

In vitro evaluation of bio-agents against *S. rolfsii*

In vitro studies on antagonistic activities of five bio-agents against *S. rolfsii* revealed that, there is significant difference in per cent inhibition of mycelial growth of *S. rolfsii* by all the bioagents tested. Per cent mycelial inhibition of *Sclerotium rolfsii* was found highest (57.5%) with *Trichoderma harzianum* (plate-2, table-3) was significantly superior over the rest of bioagents tested. This was followed by *Trichoderma viride* I (55.8%) followed *Trichoderma viride* II (53.63 %).*T. hamatum* caused 44.46% inhibition and 40.7 % inhibition was recorded with *Pseudomonas fluorescens*.

Theese results were conformity of the observations of Virupaksha Prabhu *et al*. (1997) while working with collar rot of cotton caused by *S. rolfsii*. The maximum inhibition zone was recorded with *T. harzianum* and *T. viride*. This might be due to the production of antibiotics, which diffused, air filled pores, which are detrimental to the growth of *S. rolfsii*. (Bhagwat, 1997 and Vinod dangae 2006).

Pot culture experiment

In soil drenching all the systemic fungicides were found effective at three concentrations viz., 1000, 2000, 3000 ppm and at two (10 and 15 cm) depths of inoculum. Among them hexaconazole, propiconazole, difenconazole recorded 100 percent inhibition of mycelial growth at 1000, 2000 and 3000 ppm at both depths followed by carbendazim+mancozeb, propineb and benomyl benomyl recorded 100 percent inhibition with 2000 and 3000 ppm at 10 cm&15cm depths (plate -3&4, Table-4). The results are in conformity with the findings of previous workers in case of *F. solani* causing chili wilt (Verma and Vyas,1977, Haware *eta*l., 1978, Hiremath *etal*,1981 and Nene *etal*., 1991).

Plate1. *Sclerotium rolfsii* pure

Plate 2.Dual culture of *Sclerotium rolfsi* with *Trichoderma harzianum and T. viride* I isolates.

Plate 3. Chilli plants artificially inoculated with *S. rolfsii*

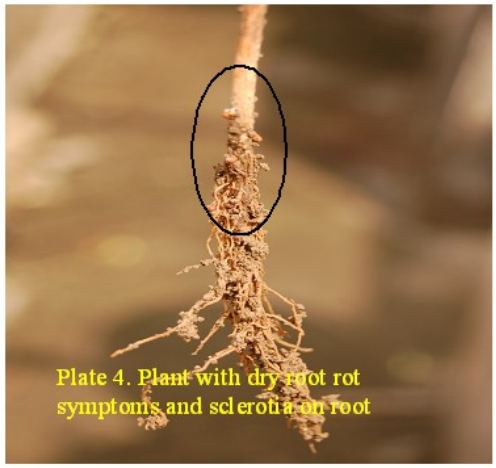

Plate 4. Plant with dry root rot symptoms and sclerotia on root

Table-3. Inhibition of mycelial growth of *S. rolfsii* by different bioagents

S.No.	Fungal Isolate	Radial growth (cm)	Per cent inhibition of growth
1	*Trichoderma viride-I*	3.95	55.8
2	*Trichoderma hamatum*	4.97	44.46
3	*Trichoderma harziaum*	3.80	57.5
4	*Trichoderma viride-II*	4.15	53.63
5.	*Pseudomonas fluroscens*	5.30	40.7
6	Control (*S. rolfsii* alone)	8.95	
	SEM	0.049	
	SED	0.069	
	CD	0.147	
	CV%	1.9	

Table:4. Effect of drenching of different fungicides on inhibition of mycelial growth of *S. rolfsii* at different depths.

Chemical	Depth (cm)	Percent inhibition of growth concentration		
		1000	2000	3000
Hexaconazole- 0.2%	10 15	100 100	100 100	100 100
Propiconazole- 0.1%	10 15	100 100	100 100	100 100
Tebuconazole- 0.15%	10 15	90 90	100 100	100 100
Difenconazole – 0.5%	10 15	100 100	100 100	100 100
Pencycuron-0.1%	10 15	00 00	00 00	50 50
Copper Oxy Chloride- 0.3%	10 15	00 00	00 00	00 00
Carbendazim+Dithane M-45- 0.2%	10 15	80 80	100 100	100 100
Benomyl-0.1%	10 15	50 50	100 100	100 100
Propineb- 0.2%	10 15	80 80	100 100	100 100
Check (water)	10 15	00 00	00 00	00 00

Integrated disease management of chillies dry root rot.

Of all the treatments integration of seedling root dip with carbendazim+ mancozeb , addition of vermicompost, drenching of fungicide carbendazim+diathane M-45 and soil application of *T.viride I* was found to be effective with minimum 7 % (Table-5) of dead plants. The present findings are in conformity with the earlier reports, integration of disease management practices are more effective in controlling Fusarial wilt in gladiolus (Suneel Anand and Harender Raj Gautam, 2006), collar and root rot in strawberry (Bhardwaj and Gautam, 2004) and soil borne diseases in vegetable nurseries (Steven *et al*., 2003).

Table 5. Efficacy of different treatment combinations in the management of dry root rot disease of chillies caused by *S. rolfsii* in pot culture during 2009 and 2010

S.No	Treatment	Percent of plants died 2008-2009	Percent of plants died 2009-2010	Percent of plants died Pooled (2009 &2010)
1	T1- Seedling dip- carbendazim @0.1%	35.0	21.6	28.3
2	T2- Vermicompost- 100g/kg soil	50.3	42.0	46.2
3	T3- T1+T2	35.3	29.3	32.3
4	T4- Drenching with fungicide	25.3	22.6	24.0
5	T5- Application of *Trichoderma viridei*@10 g/pot	15.6	28.6	22.2
6	T6- T3+T4+T5	0.0	14.0	7.0
7	T7- Un Inoculated Check	0.0	0.0	0.0
8	T8- Inoculated check	99.0	96.0	98.5
	SEM	1.09	1.60	1.23
	SED	1.54	2.27	1.74
	CD	3.32	4.87	3.74
	CV%	5.8	8.8	6.6

The results are in conformity with the earlier report of Saini et al (2005) as the disease could be managed effectively by incorporating FYM + Sorghum leaf + *T. harzianum* + Carboxin combination which recorded the least of 19.00 percent disease incidence. Similarly, FYM application to the soil + tuber treatment with *T. harzianum* prior to planting helped in reducing the sclerotial wilt of potato in field as well as in storage has been reported by Anahosur (2001). conducted a study to investigate efficacy of biological control agents and organic amendments in controlling while a combination of *Trichoderma viride* + FYM + dry adathada leaf powder was found effective against collar rot of brinjal caused by *Sclerotium rolfsii* (Vanith and Suresh 2002)

Based on the investigation drenching of systemic fungicide such as Carbendazim+dithane M-45 can be recommended as immediate control measure to protect against root rot. Finally adoption of integrated disease management was suggested for effective control of dry root rot disease.

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