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Research Article

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Laboratory management of *Sclerotium rolfsii* pathogen by different test to check the efficacy of plant products, biocontrol agents and fungicides.

Kamthe H. J[1], Ghante P.H[2], Hingole D. G[1] and Khaire P. B[3*]
[1]*Division of Plant Pathology, College of Agriculture, Badnapur 431202 (M.S), India*
[2]*Agricultural Research Station, Badnapur 431202 (M.S), India*
[3]*Department of Plant Pathology and Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri 413722 (M.S), India*
Corresponding author e-mail: pravinkhaire26893@gmail.com
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ABSTRACT
Eight bioagents and eight botanicals eleven treatment of each of them were evaluated in a laboratory environment and the results showed that all of the fungicides strongly suppressed *S. rolfsii* mycelia growth compared to the untreated control. Azoxystrobin, Hexaconazole, Penconazole, Propiconazole, and Carbendazim+Mancozeb showed the highest mycelia growth suppression (100%) and were followed by Carbendazim (96.60%). In case of bioagents, *Trichoderma viride* had the highest mycelial growth inhibition in bioagents (69.62%), followed by *T. harzianum* (66.66) and in terms of botanicals, *Zingiber offcinalis* (83.34%) was the botanical that considerably inhibited mycelial growth mostly, followed by *Allium sativum* (85.64).
Keywords: Propaconazole, *T. viride*, Botanicals, Efficacy test, *Cicer arientum*.

INTRODUCTION

One of the most significant pulses (Rabi) crops in India rainfed farming method is gram cultivation (*Cicer arietinum* L.). Both human intake and animal nutrition involve its utilisation. It is eaten fried, boiled, or both cooked and eaten. Husks and leftover dal pieces make excellent livestock feed. As a vegetable, fresh green leaves are utilised. For the preparation of various desserts, gramme flour is employed. It has therapeutic value as well. In addition to being rich in Ca, Fe, and Nicin, it has 21.1 percent protein, 61.5 percent carbs, and 4.5 percent fat. Its leaves exude malic acid, which is found in amounts ranging from 90 to 95 percent, along with oxalic acid, which is found in amounts ranging from 5 to 10 percent. The grains are available in markets in developed nations either dry or tinned for everyday use in soups, vegetable medleys, or as a component of fresh salads. Moreover, grains are utilised as vegetables. Besan, also known as dehusked gramme flour, is frequently used to make pakodas, kadhi, namkeens, and other snack foods. The chickpea is cultivated all throughout the world. Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, Rajasthan, Andhra Pradesh, Karnataka, and Maharashtra are among the Indian states where it is widely grown. It is primarily grown on a variety of soils with varied levels of residual moisture under rainfed conditions. More than 50 diseases can affect the chickpea crop (Nene et al. 1981). In addition to other diseases, the wilt (*Fusarium oxysporum*) f.sp *ciceri*, root rot [RR] (*Rhizoctonia bataticola*), and collar rot [CR] (*Sclerotium rolfsii*) that appear annually in the Marathwada region of Maharashtra have also become a limiting factor for the cultivation of chickpeas in many states of the nation. Farming faces a serious difficulty caused by fungi that cause Chickpea wilt complex (CWC). Researchers have noticed the CWC, which causes mortality rates ranging from 0% to 100%.

For the control of diseases, new fungicide chemical compounds are required. Contrarily, using biological management measures to manage soil-dwelling fungi is more cost-efficient, safer, and effective. Given the significance of the CWC, the current investigations were conducted to determine the effectiveness of novel chemical fungicides and other biocontrol agents for the management of CWC (wilt, RR, and CR). Particularly in the wet environment of the Indian subcontinent, chickpea is a significant pulse crop of the semi-arid tropics. This crop has seen an export-driven expansion in recent years in new riches like Australia and Canada. It is grown in India on an area of roughly 10.22 million hectares, producing 9.88 million tonnes at a yield of 967 kg per hectare. In Maharashtra, chickpea production in 2013–14 total 16.22 L tonnes, with an area under cultivation of 18.19 L ha. In Marathwada, the area planted with chickpeas in 2013–14 was 6.58 L.ha, and the crop produced 6.64 L. tonnes with a productivity of 918 kg/ha (Research work report on pulses of Agricultural Research Station, Badnapur 2014-15).

Wheat, mustard, lentils, and other crops are produced alongside or in addition to chickpeas. A number of air, soil, and seed-borne illnesses have an impact on it and lower its output. Some of these illnesses are brought on by soil-borne infections such *R. bataticola*, F. *oxysporum* f.sp *ciceri*, and *S. rolfsii* (CR). CR is one of these diseases that is growing more dangerous at the seedling stage, particularly in areas where paddy or soybean-based cropping systems are practised. Plant mortality in India ranges from 54.7% to 95%. The pathogen significantly reduced the population of plants with a diverse host range (Aycock, Punja, 1988). The pathogen *S. rolfsii* (Sacc.), which causes CR, is widely known for being polyphagous, widespread, and non-target. It is one of the most harmful soil-dwelling pathogens and causes significant crop loss (Azhar Hussain et al., 2006). The damaged area of potato plants and tubers displayed the distinctive white, radiating, and profuse mycelial development of *S. rolfsii*. On the mycelial mat, a significant number of sclerotial initiations were seen. Sclerotia, which appeared on the affected portions and initially had a white colour before turning chocolate brown, were spherical or ellipsoidal in shape and ranged in diameter from 0.5 mm to 2.5 mm. The disease known as CR, which is brought on by *S. rolfsii* Sacc., is one of the biotic reasons causing reduced chickpea yield. Good soil moisture, high soil temperature (25–30ºC), and little organic matter in the soil all favour the illness (Mathur & Sinha, 1968). Between 55 and 95 percent of chickpea seedlings can die from CR (Gurha & Dubey 1982). The existence of vulnerable hosts, a sufficient inoculum of virulent pathogen isolates, and climatic circumstances that favour disease development over time are some of the variables that contribute to a severe outbreak of the disease. The pathogen *S. rolfsii* has a wide host range, prolific proliferation, and the capacity to create chronic sclerotia that cause significant economic losses (Mordue, 1974). There may be free *S. rolfsii* Sacc. sclerotia in the soil or sclerotia associated with plant waste (Aycock and Punja, 1985). Sclerotia spreads through human activity (dirty tools and soil), infected transplant seedlings, water (through irrigation), wind, and perhaps seeds. Like other fungi that produce sclerotia, this one produce distinct sclerotia and sterile mycelia. Sclerotium was the name given to those with small, internally distinct, spherical sclerotia that ranged in colour from tan to dark brown to black (Punja and Rahe 1992). The teleomorphic condition, however, was later found (Punja 1988), proving that the fungus is a member of the basidiomycete class.

The best and most practical way to control the disease is to cultivate resistant cultivars, and sources of resistance to this disease have been found in many different nations (Sugha et al., 1991; Gurha et al). The frequency of virulent *S. rolfsii* isolates prevented the development of stable resistance. Previous researchers had shown that *S. rolfsii* populations varied geographically (Harlton et al., Nalim et al., 1995; Okabe et al., 1998). Understanding sclerotium development, mycelial compatibility, and sensitivity were the studies main goals. Hence, there is great potential to increase chickpea yield by reducing losses brought on by biotic factors like CWC. The study was conducted with the aim of managing the disease by biological agents and fungicides, taking into consideration the relevance of the illness, the socioeconomic position of the crop, and the insufficient scientific work carried out on the disease in the state.

MATERIALS AND METHODS

Laboratory test of fungicides

By employing the Poisoned food technique [PFT] (Nene and Thapliyal, 1993) and utilizing potato dextrose agar (PDA) as the primary culture medium, the effectiveness of 11 fungicides against *S. rolfsii* was assessed in lab at various three replications. To achieve the desired replication, the necessary quantity of each test fungicide was determined based on active component and well mixed with autoclaved and cooled (40ºC) PDA medium in conical flasks (250 ml/cap). After that, 20 ml of fungicide-added PDA medium was aseptically placed into Petri plates (90 mm in diameter) and left to solidify. A triple set of Petri plates, treatments, and replications were kept for each test fungicide and its test replication. Just after medium had solidified, each plate was individually injected aseptically with a 5 mm culture disc taken from a week-old, actively growing pure culture of *S. rolfsii* using a cork borer. The Petri plate was incubated at 27 + 1 ºC with the culture disc positioned in the centre of PDA in a reversed configuration. Petri dishes containing plain PDA (with no fungicide) was separately inoculated with the *S. rolfsii* culture disc and kept as control treatment.

Treatment details

Employing the PFT (Nene and Thapliyal, 1993) utilising PDA as the basal medium, it will be investigated how different fungicides affect the growth of *S. rolfsii* with treatment details as Design: CRD, Three replications, twelve treatments. The eight treatments were used when fungal antagonists were tested *in vitro* against *S. rolfsii* using the dual culture technique [DCT] (Dennis and Webster, 1971). The test pathogens (*S. rolfsii*) and test bioagents were grown in cultures for the investigation using material that was seven days old. The fungus culture development and bioagents were taken out of PDA discs (5 mm in diameter) using a sterilised cork borer. The test pathogen and bioagent were then deposited in pairs on two culture discs, one of each, and aseptically positioned exactly opposite of one another on solidified PDA media in Petri plates. The plates were then incubated at 27 + 1 ºC. Petri plates, treatments, and replications were all kept in triplicate. PDA plates with simply the test pathogen culture disc as an inoculum were kept as the untreated control. Whereas in the case of botanicals, plant species believed to have potential antifungal and therapeutic effects (Alice, 1984) on fungal pathogens and to be available locally were

gathered from the College of Agriculture, Badnapur farms and nearby areas. Eight plant species and botanicals that were locally accessible were employed in the trials. Table 2 lists all of the treatments in order as fungicides, bioagents, and botanicals, respectively. Using Vincent (1927) formula, the percentage of mycelia inhibition of growth of the test pathogen over the untreated control was computed. The per cent Inhibition (I) is equal to C-T divided by C X 100. Where, C is the growth (mm) of test fungus in untreated control plate and T is the growth (mm) of test fungus in treated plates.

RESULTS AND DISCUSSION

Evaluation of several fungicides *in vitro*

Observations on the effect of various eleven fungicides on the inhibition of pathogen mycelial development on PDA are shown in Table 1, which showed that *S. rolfsii* growth varied greatly as a result of the use of various fungicides. Azoxystrobin, Hexaconazole, Penconazole, Propiconazole, and Carbendazim + Mancozeb all showed the greatest mycelia growth suppression (100%); Carbendazim came in second (96.60%). Metalaxyl showed the highest level of inhibition (93.20%), followed by Mancozeb (69.56%), Copper oxychloride (56.85%), and Captan (44.87%). Thiram lowest percent of inhibition was 37.12%, which was likewise noticeably better than control. An earlier study found that fungicides had comparable fungistatic effects against *S. rolfsii*, which causes collar rot in numerous crops including chickpea (Sahu et al., 1990; Tiwari et al., 1995; Alam et al., 2004; Tripathi and Khare et al., 2006; Banyal et al., 2008 and Gour and Sharma 2010).

Evaluation of several bioagents *in vitro*

Table 1 shows the findings on the mycelial growth and inhibition of *S. rolfsii* using 7 fungal and 1 bacterial antagonist. The test pathogen showed the least linear mycelial growth (27.33 mm) and the highest mycelial inhibition (69.62%) of the eight bioagents examined, with *T. viride* being the most effective. *T. harzianum* and *T. hamatum*, which respectively recorded mycelial development of 30 mm and 36.67 mm of the test pathogen with inhibition of 66.66 and 59.25 percent, were the second and third best antagonists. *T. koningi* (col. dia.: 40 mm of test pathogen and inhibition: 55.55%) and *T. longibrachiatum* (col. dia. 44.33 mm of test pathogen and inhibition: 50.73%) came in second and third, respectively. The bacterial antagonist *P. fluorescens* was shown to be least effective, inhibiting mycelial growth of *S. rolfsii* by 28.88% while only causing a 26 mm colony growth of the latter.

The current study findings on the effect of bioagents on *S. rolfsii* mycelial growth are consistent with those previously reported by a number of researchers (Prasad and Rangeshwaran, 2001; Singh et al., 2003; Sharma et al., 2007; Kumar et al., 2008 and Reddy et al. 2011).

Evaluation of several botanicals *in vitro*

As shown in Table 1, *S. rolfsii* mycelial growth inhibition at 10% ranged from 22.34% (*Eucalyptus* sp.) to 85.64% (*Allium sativum*). *A. sativum*, however, significantly inhibited mycelial growth the most (85.64%), followed by *Zingiber offcinalis* (83.34%), *Curcuma longa*, *Azadiracta indica*, *Ocimum sanctum*, *Parthenium histeropous*, and *Lantana camera*, while *Eucalyptus* spp., among plant extract treatments, showed the least amount of inhibition (22.34%).

Several workers have previously reported that the test botanicals had a similar impact against S. rolfsii infecting chickpea and *S. rolfsii* infecting several other crops. Several researchers have previously reported that certain plants, including ginger, garlic, neem, turmeric, parthenium weed, nilgiri, and ghaneri, significantly inhibited the mycelial growth of *S. rolfsii* (Shivapuri et al., 1997; Seshakiran, 2002; Khanzada et al., 2006; Kulkarni, 2007; Singh et al., 2007; Suryawanshi et al., 2007; Patil and Raut, 2008; Mundhe et al., 2009; Farooq et al., 2010; Sultana et al., 2012).

Table 1. *In vitro* effect of fungicides, bioagents and botanicals on mycelial growth of *S. rolfsii*

Fungicides

Sr. No	Treatment	Conc. (ppm)	Colony Dia. of test pathogen (mm)	Per cent inhibition
T_1	Captan (50 %WP)	2500	49.00	44.87 (26.68)
T_2	Copperoxy chloride (50% WP)	2500	38.33	56.85 (34.71)
T_3	Mancozeb (75 %WP)	2500	27.00	69.56 (44.32)
T_4	Metalaxyl (25% WP)	2500	06.00	93.20 (69.39)
T_5	Thiram (75% WP)	2500	46.00	37.12 (21.98)
T_6	Azoxystrobin (23% SC)	1000	00.00	100 (89.98)
T_7	Carbendezim (50 %WP)	1000	03.00	96.60 (75.40)
T_8	Hexaconazole (5% EC)	1000	00.00	100 (89.98)
T_9	Penconazole (10 %EC)	1000	00.00	100 (89.98)
T_{10}	Propiconazole (25% EC)	1000	00.00	100 (89.98)
T_{11}	Carbendezim + Mancozeb (75% WP)	1000	00.00	100 (89.98)
T_{12}	Control	--	89.00	00.00 (0.00)
	S.E. $\pm$		1.83	2.49
	C.D. at 1%		5.35	7.26

Bioagents

Tr. No.	Treatment	Colony Dia. of bioagent * (mm)	Colony Dia. of test pathogen * (mm)	Per cent inhibition
T_1	*Aspergillus flavus*	39.00	51.00	43.33 (25.67)
T_2	*Aspergillus niger*	42.00	48.00	46.66 (27.82)
T_3	*Pseudomonas fluroscence*	26.00	64.00	28.88 (16.79)
T_4	*T. hamatum*	53.33	36.67	59.25 (36.34)
T_5	*T. harzianum*	60.00	30.00	66.66 (41.80)
T_6	*T. koningi*	50.00	40.00	55.55 (33.75)
T_7	*T. longibrachiatum*	45.67	44.33	50.73 (30.49)
T_8	*T. viride*	62.67	27.33	69.62 (44.14)
T_9	Control	0.00	90.00	00.00 (00.00)
	SE ±	1.21	1.21	0.91
	C. D . at 1%	3.61	3.61	2.71

Botanicals

Tr. No.	Treatment (Botanical Names)	Conc. v/v (%)	Colony Dia. of test pathogen * (mm)	Per cent inhibition
T_1	*Allium sativum*	10	12.67	85.64 (59.02)
T_2	*Azadiracta indica*	10	30.67	65.10 (40.66)
T_3	*Curcuma longa*	10	25.67	70.82 (45.15)
T_4	*Eucalyptus sp.*	10	68.33	22.34 (12.90)
T_5	*Lantana camera*	10	65.67	25.38 (14.70)
T_6	*Ocimum sanctum*	10	42.67	51.46 (30.99)
T_7	*Parthenium histeropous*	10	61.33	30.33 (17.65)
T_8	*Zingiber offcinalis*	10	14.67	83.34 (56.55)
T_9	Control (Untreated)		88.00	0.00 (0.00)
	SE ±	1.57	1.45	
	C.D .at 1%	4.66	4.30	

*Mean of three replications, Dia. Diameter, Figures in Parentheses are arc sin values.

CONCLUSION

All of the fungicides tested in vitro were proven to be antifungal and fungistatic against *S. rolfsii*. Nevertheless, Azoxystrobin, 23% SC, one of the tested fungicides Penconazole (10 EC), Hexaconazol (5 EC), and Propiconazole (25 EC) M45 Mancozeb 75% WP was the most efficient treatments were Captan 50% WP and Copper Oxychloride 50% WP. When bioagents were tested in vitro, *T. viride*, *T. harzianum*, *T. hamatum*, *T. koningi*, and *T. longibrachiatum* were the most effective potential antagonists against *S. rolfsii*. All 8 botanicals examined in vitro and their aqueous extracts were proven to be fungistatic or antifungal to *S. rolfsii*. *Allium sativum* (garlic), *Zingiber officinalis* (ginger), *Curcuma longa* (turmeric), *Azadiracta indica* (neem), and *Ocimum sactum* were the botanicals with the greatest promise (Tulsi).

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CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

REFERENCES

Alam, A., Islam, M. R., Sarkar, M. A., Alam, M. S., Han, K. D., Shim, J. O., Lee, T. S and Lee, M. W. 2004. *In vitro* inhibitory effect of plant extracts, urine, fertilizers and fungicides on stem rot caused by *Sclerotium rolfsii*. *Mycobiol*. **32**(3): 128.

Banyal, D. K., Mankotia, V and Sugha, S. K. 2008. Integrated management of tomato collar rot caused by *Sclerotium rolfsii*. *J. Mycol. Pl. Pathol.* **38**(2): 164-167.

Farooq, M. A., Iqbal, U., Iqbal, S. M., Afzal, R and Rasool, A. 2010. *In vitro* evaluation of plant extracts on mycelium growth of *Sclerotium rolfsii*, cause of root rot of Sugarbeet. *Mycopathol.* **8**(2): 81-84.

Gour, H. N and Sharma, P. 2010. Evaluation of fungicides *in vitro* and *in vivo* against *Sclerotium rolfsii* Sacc., causing root rot of groundnut. *Ind. Phytopathol.* **63** (3): 352-353.

Hameeda, B., Harini, G., Rubela, O. P., Rao J.V.D.K.K and Reddy, G. 2010. Biological control of chickpea collar rot by co-inoculation of antagonistic bacteria and compatible *rhizobia*. *Ind. J. Microbiol.* **50**(4): 419-424.

Islam, M. M., Raithan, M. G and Rafiq, Z. A. 2005. *In vitro* evaluation of *Trichoderma*, fungicides and

plant extracts against *Rhizoctinia solani* and *Sclerotium rolfsii* of peanut. *Int. J. Sustain. Agric. Tech*. **1**(1) 14-23.

Khanzada, S. A., Iqbal, S. M and Akram, A. 2006. *In vitro* efficacy of plant leaf extracts against *S. rolfsii* Sacc. *Mycopathology*. **4**(1): 51-53.

Kumar, R., Mishra P., Singh, G and Yadav, R. S. 2008. Integration of bio agents and fungicides for management of collar rot of chickpea. *J. of Bio. Control.* **22**(2): 487-489.

Mundhe, V. G., Diwakar, M. P., Kadam, J. J., Joshi, M. S and Sawant, U. K. 2009. *In vitro* evaluation of bio agents and botanicals against *Sclerotium rolfsii*, causing foot rot of Finger millet (Nagli). *J. Pl. Dis. Sci.* 4(2): 183-186.

Paredes-Escalante, J. E., Carrillo-Fasio, J. A., Garcia-Estrada, R. S., Allende-Molar, R., Sanudo-Barajas, J. A and Valdez-Torres, J. B. 2009. Antagonistic microorganisms for control of the fungal complex that cause wilt in chickpea (*Cicer arietinum* L.) in the state of Sinaloa, Mexico. *Revista Mexicana de Fitopatologia* **27**(1): 27-35.

Patil, S. K. H and Raut, S. P. 2008. Efficacy of fungicides, bioagents and botanicals against collar rot of Betelvine. *J. Pl. Dis. Sci.* **3**(1): 93-96.

Prasad, C., Gupta, S., Tyagi, V and Pathak, S. 2003. Biological control of *S. rolfsii* (Sacc.). The incitant of cauliflower collar rot. *Ann. Pl. Prot. Sci.* **11**(1): 61-63.

Prasad, R. D and Rangeshwaran, R. 2001. Biological control of root and collar rot of chickpea caused by *Sclerotium rolfsii*. **9**(2): 297-303.

Reddy, A. S. R., Madhavi, G. B., Reddy, K. G., Yellareddy, S. K and Reddy, M. S. 2011. Effect of seed bio priming with *Trichoderma viride* and *Pseudomona fluorescens* in chickpea (*Cicer arietinum*) in Andhra Pradesh, India. PGPR for sustainable agriculture. Proceedings of the 2nd Asian PGPR Conference, Beijing, China. pp.324-429.

Sahu, K. C., Narian, A and Swain, N. C. 1990. Bioassay of selected seed treating fungicides against *Sclerotium rolfsii* on groundnut. *Environ. Ecol*. **8**(1b): 485-487.

Seshakiran, K. 2002. Use of phytochemicals in the management of stem rot of groundnut caused by *S. rolfsii* (Sacc.) M.Sc (Agri), Thesis submitted to UAS, Dharwad. PP-86.

Sharma, K. D., Pannu, R. K., Tyagi, P. K., Chaudhary, B. D. and Singh, D. P. 2007. Effect of soil moisture stress on biomass partitioning and yield of chickpea genotypes. *J. Food legumes.* **20**(1):71-74.

Shivapuri, A., Sharma, O. P and Jhamaria, S. L. 1997. Fungitoxic properties of plant extracts against pathogenic fungi. *Ind. J. Myco. Pl. Pathol*. **27**(1): 29-31.

Singh, A., Sangeeta, M., Singh, H. B and Nautiyal, C. S. 2003. Bio control of collar rot disease of betel vine (*Piper betel* L.) caused by *S. rolfsii* by using rhizosphere competent *Pseudomonas fluorescences* NBRI-N6 and *P. fluorescences* NBRI. *N. Curr. Microbiol.* **47**(2): 153-158.

Sultana, J. N., Pervez, Z., Rahman, H and Islam, M. S. 2012. Integrated management for mitigating root rot of chilli caused by *Sclerotium rolfsii*. *Bangladesh Res. Publ. J*. **6**(3): 270-280.

Suryawanshi, A. P., Ladkat, G. M.. Dhoke, P. K., Somwanshi, S. D and Pensalwar, S. N. 2007. Evaluation of some plant extracts against *S. rolfsii* on pigeon pea. *J. Pl. Dis. Sci*. **2**(1): 32-33.

Tiwari, R. K. S. 1995. Comparative evaluation of three systemic fungicides against *Sclerotium rolfsii* causing root rot in gram and sunflower. *Ind. J. Mycol. Pl. Pathol*. **25**(3): 243-245.

Tripathi, B. P and Khare, N. 2005. Growth of *S. rolfsii* of chickpea as influenced by bio agents. *Ann. Pl. Prot. Sci*. **13**(2): 465-529.

Tripathi, B. P and Khare, N. 2006. Testing of fungicides against *Sclerotium Rolfsii. J. Myco. Pl. Pathol*. **36**(2): 347-348.

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