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Full Research Article

Integrated Management of Stem Rot of Sunflower (*Helianthus annuus* L.) Incited by *Sclerotium rolfsii* Sacc.

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Abstract

Sunflower is an important oil seed crop all over the world and is also an important oilseed crop in Nagaland. The present investigation was an attempt to explore the possibilities and develop effective management of stem rot disease caused by *Sclerotium rolfsii* in sunflower (*Helianthus annuus* L.) through the use of bioagents and botanicals. In in vitro studies, 3 (three) bioagents viz., T*richoderma harzianum, Trichoderma viride* and *Trichoderma virens* were tested following dual culture method where S. *rolfsii* was observed to be inhibited up to 61.8% , 58.5% and 56.4% respectively. Altogether 10 (ten) botanicals viz. *Mimosa pudica, Azadirachta indica, Allium cepa, Curcuma aromatica, Zingiber officinale Roscoe, Duranta sp., Acmella oleracea, Jatropha curcas, Eucalyptus* sp*.,* and *Allium sativum* were also tested in in vitro using poison food technique. Among which *Allium cepa bulb* extract and *Curcuma aromatica rhizome* extract at 5% conc. inhibited the growth of *S. rolfsii* completely (100%) followed by *Zingiber officinale Roscoe, rhizome* extract at 15% conc. with 82.6% mycelial growth reduction. Under field condition, seed treatment with *T. harzianum* @ 4 gm 10 ml^{-1} of water kg^{-1} of seed was found to be the best for management of stem rot of sunflower which resulted in lowest percentage plant mortality (5.62%), highest percentage disease control (94.69%), highest germination (77.65%) and plant height (118.57 cm) accompanied by highest yield (567.30 g $plot^{-1}$) respectively

1. Introduction

Sunflower (*Helianthus annuus* L.) is an important edible and fastest growing oilseed crop in India as well as in Nagaland. In Nagaland, it is grown in an area of 2060 ha with a production of 1130 tonnes (Statistical Handbook of Nagaland, 2012). The crop is seriously affected with stem rot caused by *Sclerotium rolfsii* and poses persistent problem to its production and cultivation in the state. *S. rolfsii* is a soil borne pathogen causing root rot, stem rot, collar rot, wilt and foot rot diseases in more than 500 plant species of agricultural and horticultural crops throughout the world (Agrios, 2005). The antagonistic effect of *T. harzianum, T. viride* and *T. virens* (Madhavi and Bhattiprolu, 2011) as a bioagent have been well known. *Trichoderma harzianum* and *Trichoderma virens* are the most commonly cited species in biological control (Papavizas, 1985).Besides bioagents some botanicals, cultural, chemicals has also been citied and worked against *S. rolfsii* by many researchers. However little has been done to explore the potentialities of different tools of integrated management specific to the environmental conditions of Nagaland for the management of stem rot in sunflower. Therefore the present study on effect of integrated management of stem rot of sunflower incited by *Sclerotium rolfsii* has been undertaken.

2. Materials and Methods

2.1. Source of the pathogen

The pure culture of the pathogen, *Sclerotium rolfsii* was isolated from naturally infected sunflower plants collected from the experimental farm of the School of Agricultural Sciences and Rural Development, Medziphema, Nagaland. The part of collar or stem region showing typical symptoms of the disease was cut into small pieces. These pieces were surface sterilized with 0.2% mercuric chloride solution for 30 sec. Such pieces were washed thoroughly in sterile distilled water thrice to remove traces of mercuric chloride and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. They were incubated at 28±1 °C for three days for the growth of the fungus. Later, the bit of fungal growth was transferred to

PDA slants. The pure culture of the fungus was obtained by further growing the culture and following hypal tip culture under aseptic conditions (Rangaswamy, 1972). The culture thus obtained was identified as *Sclerotium rolfsii* based on the morphological description given by Barnett (1960). Maize-Meal Sand Medium (MSM) was prepared for mass culture of the pathogen and stored at room temperature until needed.

2.2. Source of bioagents

The pure culture of bioagents used in the present investigation viz., *T. harzianum, T. viride* and *T. virens* were isolated from the same research field. The soil samples were made from 5 cm depth near the root zone of plants grown at the same research field. They were pooled and representative sample was drawn. The bioagents were isolated from the representative sample by following the serial dilution plate technique. After which pure cultures of antagonists were isolated. Sub culturing was done periodically to maintain the purity of the cultures. Mass cultures of the bioagents were prepared in MSM and kept at room temperature.

2.3. In vitro evaluation of bioagents

Three bioagents viz., *T. harzianum, T. viride* and *T. virens* were evaluated in vitro against *S. rolfsii*, the causal organism of stem rot of sunflower using dual culture method described by Rangeswaran and Prasad (2000). The experiment was laid out in a Completely Randomized Design (CRD) and each treatment was replicated three times. The% growth inhibition of the test pathogen as compared to that of control was also calculated using the following formula:

$$PI = \frac{A1\text{-}A2}{A1 \times 100}$$

Where, PI=% inhibiton; A1=Radial growth of *S. rolfsii* in control plates and A2=Radial growth of *S. rolfsii* in treated plates.

2.4. Preparation of botanicals

The botanicals that were selected for the present investigation were all locally available in Medziphema, Nagaland. They are leaves of *Azadirachta indica, Mimosa pudica, Duranta sp., Eucalyptus* sp*., Jatropha curcas, Acmella oleracea, rhizomes* of *Zingiber officinale Roscoe, Curcuma aromatica and corms* of *Allium sativum* and *Allium cepa. Acqueous* extracts of ten plants were evaluated against *S. rolfsii.* Fresh leaves of *A. indica, M. pudica, Duranta* sp. *Eucalyptus* sp*., J. curcas, A. oleracea,* rhizomes of *Z. officinale* Roscoe*, C. aromatica* and corms of *A. sativum* and *A. cepa* were collected and washed properly with distilled water. Hundred grams of fresh and washed plant materials (leaves, rhizomes and corms) were ground well with 100 ml (1:1 w v^{-1}) sterilized distilled water separately. The macerate was filtered through double layer muslin cloth (Banik and Pratibha, 2011). The extract thus obtained was considered as standard extract (100% stock).

2.5. In vitro evaluation of botanicals

Poisoned food technique was adopted for evaluating the plant extracts against *S. rolfsii* at 5, 10, and 15 percentage concentrations. Medium with plant extracts were autoclaved at 121±1 °C for 20 minutes. Mycelial discs (5 mm diam.) of 4 days old culture were incised out using a sterilized cork borer and transferred in the centre of Petri plates. Plates without plant extracts served as control. The experiment was laid out in a Completely Randomized Design (CRD) maintaining three replicates of each treatment. The plates were then incubated at 25±1 °C. Observation on radial growth of *S. rolfsii* was recorded at 24 hrs interval up to 72 hrs. The percent growth inhibition of the test pathogen as compared to that of control was also calculated following the same formula employed to evaluate the bioagents.

2.6. Field test

The field experiment was conducted at the experimental farm of the School of Agricultural Sciences and Rural development, Medziphema, Nagaland during the year 2012-2013. The experiment was laid in Randomized Block Design (RBD) with three replications comprising of the following treatments, T_0-Uninoculated control, T_1-Inoculated control, T_2-Mulching with simple polyethylene sheet, T_3-Burning of soil, T_4-Seed treatment with Allium sativum, T_5-Seed treatment with Zingiber officinale Roscoe, T_6-Seed treatment with Curcuma aromatic, T_7-Seed treatment with Trichoderma viride, T_8-Seed treatment with Trichoderma harzianum, T_9-Seed treatment with Trichoderma virens, T_{10}-Seed treatment with carbendazim, T_{11}-Soil treatment with Allium sativum, T_{12}-Soil treatment with Zingiber officinale Roscoe, T_{13}-Soil treatment with Curcuma aromatic, T_{14}-Soil treatment with Trichoderma viride, T_{15}-Soil treatment with Trichoderma harzianum, T_{16}-Soil treatment with Trichoderma virens, T_{17}-Soil treatment with carbendazim and T_{18}-Combined treatment respectively.

Mulching of the soil with polyethylene sheets (50 µm thick) was done 7 days before sowing. Burning the surface of the soil was done by using crop debris along with other plant debris available in the field 7 days before sowing. Prior to sowing, seeds were soaked in spore suspension of *T. harzianum, T. viride* and *T. virens* @ 1×108 spore ml^{-1} for 1 hour with Talc powder @ 2% w v^{-1} (2 g kg^{-1} seed) for 30 seconds and later dried in shade for about 12 hrs. Soil treatment was done by application of cultures of *T. harzianum, T. viride* and *T. virens* grown on MSM medium to the soil @10 g medium/m^2 at the time of sowing. Prior to sowing, seeds were soaked in 5% extract of *A. sativum,* and *C. aromatica* @ 15% extract of *Z. officinale Roscoe.* for 5 minutes. Soil treatment was done

by drenching the soil with *A.sativum* and *C. aromatica* @ 5% conc. plot^{-1} and *Z. officinale Roscoe*. for 15% conc. plot^{-1}. The botanicals which proved effective in in vitro studies were used for seed and soil treatment.

Seeds were also treated with carbendazim 50WP @ 0.2% for 30 minutes and then air dried at room temperature for 24 hrs before planting. Soil treatment was done by drenching the soil with carbendazim @ 0.2% plot^{-1} at the time of sowing. All the above treatments mentioned in the treatment methods viz., mulching with polyethylene sheets, burning, seed and soil treatments with bioagents, botanicals and chemicals were combined together in this treatment.

3. Results and Discussion

3.1. In vitro evaluation of bioagents against Sclerotium rolfsii

In dual culture, *T. harzianum, T. viride* and *T. virens* subdued the growth of *S. rolfsii* showing significant increase in their biocontrol influence with time (Table 1). Observations taken at 24 hrs interval showed that the bioagents wielded varied degree of stress and restricted the mycelial growth of *S. rolfsii* in culture. Considerable variations in the inhibitory properties of the bioagents were apparent when assessed in time. It was observed that with the passage of time the per cent inhibition of *S. rolfsii* in the presence of the bioagents accelerated. At 72 hrs incubation, the radial growth of *S. rolfsii* in control plates measured 90 mm but the treated plates were only 34.3, 37.3, 39.2 mm respectively. The growth of *S. rolfsii* was either overrun by the bioagents or their mycelia strands when came in contact with the bioagents gradually got lysed. Attempts to reisolate *S. rolfsii* from the culture plates yielded only the bioagents. Microscopic examination also showed clear constrictions of the mycelia of *S. rolfsii* as compared to the structures in control. Similar inhibitory ability of *T. harzianum* and *T. viride* to arrest growth by way of parasitism or antagonism or lysis of the mycelial strands of pathogenic fungi was observed by (Patil et al., 2004).

Table 1: Antagonistic effect of bioagents on radial growth (mm) of *S. rolfsii* in culture, bioassayed in PDA at 28±1 °C at different hours of incubation

Treatments	24 hrs*	PI	48 hrs*	PI	72 hrs*	PI
S. rolfsii+*T. harzianum*	17.26	41.09	24.26	52.89	34.3	61.8
S. rolfsii+*T. viride*	17.2	41.29	25.66	50.17	37.3	58.5
S. rolfsii+*T. virens*	17.73	39.48	27.73	46.15	39.2	56.4
Control	29.30	-	51.5	-	90	-
CD (p=0.05)	1.08		2.64		1.57	

*Average growth of five replications; PI: Percent inhibition

The inhibitory potentials of *Trichoderma* sp. is attributed to a number of merits including production of antibiotics, which diffused air filled pores and are detrimental to the growth of *S. rolfsii* and also synthesis of enzymes capable of degrading and digesting the cell wall (Elad et al., 1980; Elad et al., 1983); (Jones and Hancock, 1988; Karthikeyan, 1996; Bhagwat, 1997; Harman and Björkman, 1998). (Henis et al., 1983) has observed *T. harzianum* to secrete large amount of chitinase of β–(1, 3) -glucanase enzyme and an array of toxic metabolites, antifungal antibiotics, volatile gases and various cell wall degrading enzymes. *T. harzianum* has also been reported by (Claydon et al., 1987) to produce a pyrone compound, 6-n-pentyl-2H-pyrone-2-one which has antibiotic properties. In the present investigation, the highest percentage inhibition on the radial growth of *S. rolfsii* was exhibited by *T. harzianum* at 61.8% and hence more potent and superior to *T. viride* at 58.5% and *T. virens* at 56.4% which is probably due to the production of these toxic metabolites, antifungal antibiotics, volatile gases and various cell wall degrading enzymes.

3.2. In vitro evaluation of botanicals against S. rolfsii

The effect of all the botanicals significantly varied at different intervals of incubation from that of control (Table 2 and 3). Observations taken at 24, 48 and 72 hrs showed that the botanical extract restricted the growth of pathogen. Among the botanical extracts, *C. aromatica* and corms of *A. sativum* were observed to be most promising against *S. rolfsii*. The growth of *S. rolfsii* in the presence of extracts of *C. aromatica* and corms of *A. sativum* was recorded to be completely inhibited at 5%, 10% and 15% concentration at 24, 48, 72 hrs of incubation respectively, followed by application of 15% conc. of *Z. officinale* Roscoe at 72 hrs which resulted in the growth of *S. rolfsii* of 15.6 mm. The growth of *S. rolfsii* however, could not be effectively controlled with Leaf extracts of *Mimosa pudica* and *Eucalyptus* sp., in all the treatment concentrations applied at different incubation period (Table 2). The deterring qualities of the botanical extracts in the present investigation might be attributed to constituent of the plants that impede the growth of *S. rolfsii*.

Turmeric contains an essential oil, which consists of a variety of sesquiterpenes. Most important for the aroma are turmorene, ar-turmorene and zingeberene. Conjugated diarylheptanoids are responsible for the orange colour and probably for the pungent taste (Katzer, 1998). The antimicrobial property of turmeric has been attributed to the presence of essential oil, an alkaloid, curcumins and other curcuminoids, turmeric oil, turmerol and veleric acid (Negi et al., 1999; Chandrana et al., 2005;

Table 2: Effect of botanicals on radial growth (mm) of *S. rolfsii* in presence of botanicals in PDA at 28±1 °C at different concentration and different hours of incubation

Treatments	24 hrs			48 hrs			72 hrs		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
S. rolfsii+M. pudica	20.3	18.1	15.3	54.5	49.8	48.5	72.8	69.8	70
S. rolfsii+Duranta sp.	21.5	15.3	11.8	44	28	15.6	58.5	39.8	21.0
S. rolfsii+Eucalyptus sp.	23.6	16.6	13.3	61.3	46.8	35.2	72.8	65.6	69.6
S. rolfsii+J. curcas	11.5	13.3	15.6	14.6	24.1	24.3	34.8	36.2	39.2
S. rolfsii+A. indica	14.3	12.2	11.6	25	23.1	19.5	42.2	40.6	38.1
S. rolfsii+A. oleracea	13.3	12.3	15.1	16.8	14.8	21.8	23.5	27.6	36.1
S. rolfsii+Z. officinale	14.5	12.1	11.5	44	21	14.4	75.6	36.5	15.6
S. rolfsii+C. aromatica	0	0	0	0	0	0	0	0	0
S. rolfsii+A. sativum	0	0	0	0	0	0	0	0	0
S. rolfsii+A. cepa	12.2	12.3	12.3	17.3	18.2	17.9	24.6	23.6	23.5
Control	30.3	30.3	30.3	67.2	67.2	67.2	90	90	90
CD (p=0.05)	1.80	2.46	2.07	6.13	4.53	17.8	2.39	4.24	4.49

*Average of five replications

Table 3: % inhibition of *S. rolfsii* in presence of botanicals at different concentrations and different hours of incubation

Treatments	24 hrs			48 hrs			72 hrs		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
S. rolfsii+M. pudica.	33	40.26	49.5	18.89	28.89	27.82	19.11	22.44	22.22
S. rolfsii+Duranta sp.	29.04	49.5	61.05	34.52	58.33	76.78	35	38.03	76.6
S. rolfsii+Eucalyptus sp.	22.11	45.21	56.1	8.77	30.35	47.61	19.11	69.8	22.6
S. rolfsii+J. curcas	62.04	56.1	48.51	78.27	64.1	63.83	61.33	59.7	56.44
S. rolfsii+A. indica.	52.8	59.73	61.71	62.7	65.6	70.98	53.11	54.88	57.6
S. rolfsii+A. oleracea	56.1	59.4	50.16	75	77.9	67.5	73.88	69.33	59.8
S. rolfsii+Z. officinale	52.14	60	62.04	34.52	68.75	78.5	75.6	59.4	82.6
S. rolfsii+C. aromatica	100	100	100	100	100	100	100	100	100
S. rolfsii+A. sativum	100	100	100	100	100	100	100	100	100
S. rolfsii+A. cepa	59.73	59.4	59.4	74.25	72.91	73.36	72.6	73.77	73.88
Control	30.3	30.3	30.3	67.2	67.2	67.2	90	90	90

Cikrikci et al., 2008). Turmeric has also been shown to induct predominant antifungal activity (Arora, 1999). As for ginger it contains mostly sesquiterpenes, bisabolene, famsene and monoterpinoides (Katzer, 1998). Garlic contains 0.1-0.36% of a volatile oil these volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic. Garlic contains at least 33 sulfur compounds like aliin, allicin, ajoene, allylpropl, diallyl, trisulfide, sallylcysteine, vinyldithiines, S-allylmercaptocystein, and others. Besides sulfure compounds garlic contains 17 amino acids and their glycosides, arginine and others (Motteshard, 2008). The antimicrobial action of the botanical extracts observed in the present investigation could therefore be ascribed to the presence of such anionic components naturally occurring in the plant material. Observations similar to the present study with extracts of *C. aromatica* and corms of *A. sativum* and *Z. officinale* Roscoe on *S. rolfsii* has also been reported by earlier workers (Islam et al., 2005; Mandhare and Suryawanshi, 2009; Gupta, 2012; Kumar et al., 2012).

3.3. Field test

The results presented in Table 4 to 5 revealed that % plant mortality, disease control,% germination, plant height and yield were significantly influenced by all the treatments as compared to inoculated control. Amongst the treatments, seed treatment with *T. harzianum* was significantly superior in decreasing plant mortality (5.62%) with consequent increase in disease control

Table 4: Effect of different treatments on mortality, disease control at 120 DAS

Treatments	Mortality (%)	Disease control (%)
T_0	0 (0.71)*	100 (90)**
T_1	94.56 (9.75)	0.00 (0)
T_2	34.51 (5.92)	65.02 (53.72)
T_3	23.77 (4.93)	54.79 (47.73)
T_4	22.54 (4.80)	64.35 (53.32)
T_5	22.89 (4.84)	63.16 (52.61)
T_6	25.58 (5.11)	50.03 (45.00)
T_7	11.77 (3.50)	81.43 (64.45)
T_8	5.62 (2.47)	94.69 (76.65)
T_9	21.68 (4.71)	66.85 (54.83)
T_{10}	12.13 (3.55)	80.95 (64.10)
T_{11}	17.39 (4.23)	57.8 (49.47)
T_{12}	17.39 (4.23)	66.19 (54.43)
T_{13}	29.09 (5.44)	60.12 (50.82)
T_{14}	19.45 (4.47)	56.81 (48.90)
T_{15}	19.04 (4.42)	68.29 (55.71)
T_{16}	21.59 (4.70)	64.65 (53.50)
T_{17}	24.66 (5.02)	62.3 (52.10)
T_{18}	19.05 (4.45)	68.99 (56.14)
CD (p=0.05)	0.67	7.70

*Figures in parenthesis represent square root transformed values;**Figures in parenthesis are in Arc sine transformed values

Table 5: Effect of different treatments on germination, plant height and yield of sunflower in the field

Treatments	Germination (%)	Plant height (cm)	Yield (g plot^{-1})
T_0	83.33	112	389.56
T_1	22.32	55.85	121.40
T_2	61.12	115.00	432.23
T_3	65.50	86.75	434.73
T_4	66.66	84.50	431.40
T_5	63.34	85.50	406.73
T_6	62.20	84.43	427.80
T_7	68.95	113.37	522.47
T_8	77.65	118.57	567.30
T_9	66.65	98.33	402.23
T_{10}	68.50	107.53	478.26
T_{11}	65.00	95.23	399.37
T_{12}	65.45	93.27	395.10
T_{13}	61.11	95.40	405.30
T_{14}	63.40	96.20	415.10
T_{15}	64.50	97.17	434.63
T_{16}	63.50	96.50	430.20
T_{17}	61.50	96.93	435.80
T_{18}	65.50	92.35	416.87
CD (p=0.05)	1.13	2.18	43.18

*Average of three replications

(94.69%). It also recorded the highest germination (77.65%) followed by seed treatment with *T. viride* (68.95%). Seed treatment with *T. harzianum* also recorded maximum plant height (118.57 cm) at 120 DAS followed by mulching with simple polyethylene sheets (115.00 cm) and seed treatment with *T. viride* (13.37 cm) respectively. Analysis of the data however, reveals that mulching with simple polyethylene sheets and seed treatment with *T. viride* are statistically at par. The highest yield was recorded in plots seed treated with *T. harzianum* (567.30 g plot^{-1}) followed by plots seed treated with *T. viride* (522.47 g plot^{-1}). Increased yield was observed to be inversely proportional to the reduction in per cent plant mortality.

The efficacy of *Trichoderma* sp. as observed in the present study in repressing *S. rolfsii*. is in conformity with similar account made by earlier workers (Elad et al., 1983); (Harlapur, 1988); (Virupaksha et al., 1997; Anahosur et al., 1998, 2001) reported effective role of bioagents as tuber treatment on Sclerotium wilt of potato with *T. harzianum* and *T. viride* which gave good germination. Hanumanthe (1999) also reported that, seed treatment with *T. harzianum* recorded low incidence of groundnut stem rot caused by *S. rolfsii*. (Lahre et al., 2012) accounted similar findings that seed treatment with Trichoderma was found to be most effective with maximum germination (96.29%) and least total mortality (18.51%). Singh (2008) also concluded in his findings that higher yield was observed when seeds were treated with a combination of *T. viride* in sunflower crops. Furthermore, increase growth parameters and yield in the presence of *Trichoderma* sp. have been reported by many workers (Ao, 1999; Daiho and Upadhyay, 2004; Khatso and Ao, 2013). In the recent decades, research on Trichoderma has shown that different strains of Trichoderma can interact directly with roots and thereby increase plant growth potential, and resistance to disease and tolerance to abiotic stress (Hermosa et al., 2012). The reduction of the severity of stem rot disease by inhibiting *S. rolfsii* through highly potent antagonistic and mycoparasitic activity of *T. harzianum* in the present study is thus in conformity with the reports of the earlier workers.

4. Conclusion

Seeds of sunflower treated with the spore suspension of *T. harzianum* @ 1×108 spores ml^{-1} for one hour before sowing can effectively mitigate stem rot disease in sunflower. Notwithstanding the fact that micro-organisms as bioagents typically have a relatively narrow spectrum of activity compared with chemicals, their use poses serious consequences to the environment. The prospect of use of bioagents as inferred in the present study therefore offer as a suitable strategy for management of stem rot of sunflower under Nagaland conditions.

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