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RESEARCH ARTICLE

ECO-FRIENDLY APPROACHES FOR MANAGEMENT OF SCLEROTINIA ROT IN FENNEL CAUSED BY SCLEROTINIA SCLEROTIORUM (LIB.) DE BARY

***Richhapal Kumawat, S. Godika, S. K. Goyal and Jitendra Sharma**

Sri Karan Narendra Agriculture, University Jobner -303329

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ABSTRACT

Fennel (*Foeniculum vulgare* Mill.) is one of the major seed spice crop. It suffers from various diseases. Among these diseases, stem rot disease is becoming a considerable threat to thefennel. The present investigation aimed to evaluate eco-friendly approaches *viz*., Bio agent, plant extracts, and oil cakes to control Sclerotinia rot of fennel under *in vitro* and *in vivo* conditions. Maximum disease reduction was observed in the seed treatment + soil application of *T. harzianum*. All the tested plant extracts inhibit the mycelial growth of *S. sclerotiorum* under *in vitro* conditions. Garlic (*Allium sativum*) clove extract gave maximum inhibition (40.40%, 62.60%, and 76.30%) of mycelial growth at 5, 10, and 15 per cent concentration. Seed treatment (ST) and foliar spray (FS) of plant extracts were most effective in managing disease, followed by seed treatment and foliar spray alone. Among plant extracts, garlic (*Allium sativum*) clove extract was found best in reducing the disease incidence (38.50%) followed by datura (*Datura stramonium*). Extracts of six oil cakes were screened *in vitro* for fungitoxicity against *S. sclerotiorum*, castor, and neem cake were effective in inhibiting mycelial growth. While castor cake was most effective under *in vivo* conditions for reducing disease incidence, followed by neem cake.

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INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is one of the major seed spice crop cultivated in post rainy season. Fennel seeds contain about 5% protein, 10.0% fat, 42.30% carbohydrates, 18.5% crude fibre and 13.40% minerals. Indian fennel contains about 70 per cent anethole and 6 per cent fenchone. However, European fennel contains up to 90 per cent anethole and absence of fenchone responsible for its delicate sweet odour and flavour (Anonymous, 2015). The area under fennel in Rajasthan is 45200 ha. It is cultivated in Sirohi, Nagaur, Jodhpur, and Tonk districts. Fennel crop suffers from various diseases *viz.,* Ramularia blight (*Ramularia foeniculi*), root rot (*Rhizoctonia solani*), wilt (*Fusarium oxysporum* f. sp. *funiculi*), powdery mildew (*Leveillula taurica var. languinosa*), Alternaria blight (*Alternaria alternata*), and stem rot (*Sclerotinia sclerotiorum*). Among these diseases, the stem rot disease is becoming a measure threat to the fennel cultivation and growers under changing climatic conditions. *Sclerotinia sclerotiorum* (Lib.) de Bary is the most ubiquitous, omnivorous, soil-borne, and destructive plant pathogen, inciting more than 500 plant species (Purdy, 1979; Boland and Hall, 1994, Saharan and Mehta, 2008 and Sharma, 2014). The fungus belongs to class *Ascomycetes*, order *Helotiales* and family *Sclerotiniaceae*, characterized by the formation of hard blackish sclerotia, which on germination produce cup-shaped brown coloured apothecia. The least information is available until date about the incidence and yield losses due to stem rot disease of the fennel. Singh *et al*. (2016) reported 20.43-22.52 per cent disease intensity of stem rot of fennel in India. Sehgal and Agrawal (1971) first reported the stem rot disease of fennel caused by *Sclerotinia sclerotiorum* from Rajasthan as exhibiting drooping off around the infected portion.

****Corresponding author: Richhapal Kumawat,***
Sri Karan Narendra Agriculture, University Jobner -303329

The fungus grows in the vascular tissue and forms black hard resting structure sclerotia. The application of fungicides is often cost-prohibitive, impractical, and hazardous to the environment and human health. Therefore, the present investigation aimed to test some alternative eco-friendly approaches to control Sclerotinia rot of fennel under *in vitro* and *in vivo* conditions.

MATERIAL AND METHODS

Collection of disease sample and isolation of the pathogen: Sclerotinia rot infected plants of fennel (Plate 1) were collected from areas of KVK, Ajmer, and isolations were made on potato dextrose agar (PDA) medium from black sclerotia present inside the diseased stem as well as from individual stem rot lesion as per procedure and purified by hyphal tip method (Riker and Riker, 1936).

In vivo efficacy of Trichoderma harzianum: Field studies were conducted at the Department of Plant Pathology (Sri Karan Narendra College of Agriculture, Jobner) to manage the Sclerotinia rot through T. harzianum during post rainy season (Rabi) 2016-17 under artificial soil inoculation conditions. The field study consists of four different T. harzianum and control treatments, with five replications using fennel variety RF-178 in RBD design. The talc-based formulation of T. harzianum formulation ($2x10^8$ cfu) was used as a seed treatment (ST), soil application (SA), and seed + soil (SS) application. The pathogen cultured on sorghum grains at 25 ± 1 ˚C for one week was used as a soil inoculum. Sowing was done using T. harzianum as seed treatment @ 10 g/kg seed and soil application @2.5 kg/ha pre-incubated in 50 kg well-decomposed farmyard manure for fifteen days. The row spacing (30 cm) and plant spacing (10 cm) were maintained ten days after sowing (DAS). All recommended agronomic practices followed, and in case of aphid

infestation crop was sprayed with oxy-demeton methyl (0.1%) was sprayed. Per cent disease incidence (PDI) and per cent disease control (PDC) was calculated by using the following formula:

PDI = No. of infected plants/ Total No. of plants x 100
PDC = Disease in control – Disease in treatment / Disease in control x 100

In vitro fungitoxicity of plant extracts: A laboratory experiment was carried out to find out the fungitoxicity of six plant extracts *viz., Calotropis procera* (Aak); *Withania somnifera* (Ashwagandha); *Parthenium hysterophorus* (Carrot grass); *Datura stramonium* (Datura); *Allium sativum* (Garlic); *Zingiber officinalis* (Ginger) against *S. sclerotiorum*. One hundred gram leaves/cloves/rhizomes from each were collected and washed twice with water and allowed to dry at room temperature (25 ± 1 ˚C) for six hours. Before extraction, leaves/cloves/rhizomes of each plant (100 g) were crushed separately with 100 ml sterilized water. The extract was filtered through cheesecloth and centrifuged at 5000 rpm for 30 min (Sharma *et al*., 2016). The extract obtained of every species was diluted to achieve three concentrations viz., 5, 10, and 15 per cent. Petri plates containing PDA supplemented with different plant extracts, eachwith three concentrations and replicated three times, were inoculated with seven days old culture (5 mm dia. disc). A suitable check (without plant extract) was also maintained. The fungal colony was measured after seven days of inoculation at 25 ± 1 ˚C. The linear growth of test fungus was recorded, and per cent mycelial growth inhibition was calculated by using Bliss (1934) formula-

I = (C-T)/C x 100

Where,
I = Per cent mycelial inhibition
C = Growth of fungal plant pathogen in control (mm)
T = Growth of fungal plant pathogen in dual culture plate (mm)

Efficacy of plant extract (*In- vivo*): The experiment was carried out in earthen pots (30 cm dia.) with cultivar RF- 178. The pathogen multiplied on sorghum grains at 25±1 ˚C for one week was used as a soil inoculum. Before sowing, pots were filled with sterilized soil. The soil was sterilized at 1.045 kg/ cm^2 for one hour for three consecutive days. RF- 178 variety of fennel was sown in these pots as susceptible check with four replications. Five plant extracts [*Calotropis procera* (Aak); *Parthenium hysterophorus* (Carrot grass); *Datura stramonium* (Datura); *Allium sativum* (Garlic); *Zingiber officinalis* (Ginger)] were tested by applying as a seed treatment (for 15 min), foliar spray (30 DAS) and seed treatment and foliar spray. Seeds were dipped in freshly prepared, aqueous leaf and clove (garlic) extract (5 % v/v) for 15 min., the seeds were drained of water andthen air-dried before sowing. The pots were inoculated with fungal inoculum multiplied on sorghum grains before sowing. For inoculation, the upper 5 cm layer of soil of each pot was thoroughly mixed with inoculum @ 20 g/pot. Ten seeds were maintained per pot and kept in the cage house. At 30 DAS, each extract of the 5 per cent concentration was used as a single foliar spray. (Meena *et al.,* 2013). The disease incidence and per cent disease control were calculated.

In vitro efficacy of oil cakes: Six oil cakes *viz.*, neem, mustard, castor, groundnut and sesame were tested *in vitro* to evaluate their inhibitory effect on radial growth of the pathogen. One hundred gram oil cake was taken in 1000 ml water and preserved in an earthen pot. Polythene bags wrapped pots to conserve moisture. Oil cake extract was filtered with cheesecloth, mixed @ 3 per cent in PDA in a conical flask and autoclaved. Twenty ml PDA was poured in each sterilized Petri dish and allowed for solidifying. Each plate was inoculated with a 5 mm diameter bit of 7 days old culture of fungus. Inoculated plates were incubated at 25±1 ^{0}C for seven days. The linear growth of test fungus was recorded, and per cent mycelial growth inhibition was calculated using Bliss's following formula (1934).

Efficacy of oil cakes (In vivo): The experiment was carried out in plots (1.0 m^2) with cultivar RF-178. The pathogen multiplied on sorghum grains at 25±1 ˚C for one week was used as the soil inoculum. Six oil cakes *viz.*, neem, mustard, castor, groundnut and sesame were tested by applying as soil application @ 50 g/ plot in four replications. These plots were inoculated with fungal inoculum multiplied on sorghum grains before sowing. For inoculation, the upper 5 cm layer of soil of each plot was thoroughly mixed with inoculum @ 20 g/ plot. Thirty seeds were maintained per plot in control conditions. The disease incidence and per cent disease control were calculated.

RESULTS AND DISCUSSIONS

In vivo* efficacy of *Trichoderma harzianum: Field studies were conducted at the Department of Plant Pathology, SKNCOA, Jobner to manage the Sclerotinia rot through *T. harzianum* during Rabi 2016-17 under artificial soil inoculation conditions. Minimum disease incidence (23.0%) was recorded with seed treatment + soil application of *T. harzianum* @ 10g/kg seed and 2.5 kg/ha, respectively, followed by soil application of *T. harzianum* @ 2.5 kg/ha (36.26%) as compared to control (71.56%). Maximumreduction in disease incidence over control was observed with seed treatment + soil application of *T. harzianum* (67.85%) followed by soil application of *T. harzianum* (49.32%). Minimum per cent disease control (44.1%) was observed in seed treatment of *T. harzianum* alone (Table 1). *T. harzianum* was used as a seed treatment, soil application, and seed treatment + soil application to manage the disease. The least disease incidence was observed in the seed + soil application of *T. harzianum*. These results are in agreement with the results of Dutta *et al.* (2008), Yadav *et al.* (2012), and Yadav *et al.* (2015). They reported the efficacy of various *Trichoderma sp*. in disease control against *S. sclerotiorum* in French bean, Indian mustard, and oilseed Brassica.

In vitro fungitoxicity of plant extracts: The efficacy of six plant extracts (Table 2 and Plate 2) was tested *in vitro* at three concentrations *viz.*, 5, 10 and 15 per cent against *S. sclerotiorum* on PDA by poisoned food technique. Among six plant extracts, extract of *Allium sativum* clove was found most effective in inhibiting mycelial growth (40.4, 62.6, and 76.3 %) of *S. sclerotiorum* at 5, 10, and 15 per cent, respectively, followed by *Datura stramonium* leaf extract (8.9, 15.5 and 46.8 %) over control. Extract of *Parthenium hysterophorus* was not inhibiting mycelial growth of *S. sclerotiorum* over control (90 mm). All the concentrations (5, 10 and 15%) of *Allium sativum* clove extract were significantly superior to other treatments. At 5 per cent concentration, *Calotropis procera* and *Zingiber officinalis* were found at par with 5.9% and 6.9% mycelial growth inhibition, respectively. All the plant extracts inhibit the mycelial growth of *S. sclerotiorum* under *in vitro* conditions. *Allium sativum* clove extract gave maximum inhibition of mycelial growth at each concentration. Similar results have been observed by Yadav (2009), Tripathi and Tripathi (2009),and Sharma *et al.* (2016) while working with *S. sclerotiorum in vitro.*

Efficacy of plant extracts (*In vivo*)Seed treatment: A perusal of data (Table 3) revealed that minimum disease incidence was observed with Allium sativum (53.56%) followed by Datura stramonium (66.85%), as compared to control (78.56%). Maximum reduction in disease incidence over control was observed with Allium sativum (31.82%) followed by Datura stramonium (14.9%) over control. Per cent disease incidence of Calotropis procera (72.98%) was found at par with Zingiber officinalis (73.26%). A minimum reduction in disease incidence was observed in Withania somnifera (0.48 %).

Foliar spray: The highest reduction in disease incidence over control (82.96 %) was observed in Allium sativum (29.89 %), followed by Datura stramonium (11.81 %). Minimum reduction in disease incidence was observed in Parthenium hysterophorus (0.20 %) (Table 3).

Seed treatment and foliar spray: *A perusal of data (Table 3) revealed minimum disease incidence was in* Allium sativum (48.56%) followed by Datura stramonium (64.96%) over control (78.96%). Overall maximum reduction in disease incidence over control was observed in Allium sativum (38.50%) followed by Datura stramonium (18.21%). Per cent disease incidence with Datura stramonium (64.58%) was found at par with Zingiber officinalis (64.96%). Minimum reduction in disease incidence was observed in Parthenium hysterophorus (3.11%). Among these methods, seed treatment and foliar spray of plant extracts were found most effective to control the disease for minimizing disease incidence, followed by seed treatment and foliar spray alone. Allium sativum clove extract was found most effective in reducing the disease incidence, followed by Datura stramonium.

Table 1 Efficacy of *Trichoderma harzianum* against stem rot of fennel *(in vivo)*

Treatments	Disease incidence* (%)	Disease control (%)
Seed application with *Trichoderma harzianum* @ 10 g/kg seed	40.00 (41.56)	44.10
Soil application with *Trichoderma harzianum* @ 2.5 kg/ha	36.26 (37.18)	49.32
Seed treatment with *Trichoderma harzianum* @ 10 g/kg seed + soil application with *Trichodermaharzianum* @ 2.5 kg/ha	23.00 (28.16)	67.85
Control (untreated)	71.56 (58.16)	0.00
SEm±	0.71	-
CD (p=0.05)	2.36	-

*Average of five replications
Figures given in parentheses are angular transformed values

Table 2. Fungitoxicity of plant extracts against S. sclerotiorum by poisoned food technique after 7days of incubation at 25 ± 1 ^{0}C

Plant extracts	Part used	Per cent mycelial growth inhibition*at different concentrations (%)			
		5	10	15	Mean
Calotropis procera	Leaf	5.90	11.60	21.50	13.00
(Aak)		(14.11)	(19.94)	(27.40)	
Datura stramonium	Leaf	8.90	15.50	46.80	23.73
(Datura)		(17.40)	(23.43)	(44.14)	
Allium sativum	Clove	40.40	62.60	76.30	59.77
(Garlic)		(40.45)	(52.73)	(60.86)	
Zingiber officinalis	Rhizome	6.90	8.50	25.86	13.75
(Ginger)		(14.98)	(16.85)	(31.56)	
Withania somnifera	Leaf	2.75	10.69	31.68	15.04
(Ashwagandha)		(9.95)	(19.15)	(33.87)	
Parthenium	Leaf	0.00	0.00	0.00	0.0
hysterophorus		(0.00)	(0.00)	(0.00)	
(Carrot grass)					
Control	-	0.00	0.00	0.00	0.00
		(0.00)	(0.00)	(0.00)	
Mean		9.26	15.56	28.88	
		SEm±		CD (p=0.05)	
		P	0.39	1.08	
		Con.	0.34	0.97	
		P x Con.	0.75	2.16	

*Average of three replications
Figures given in parentheses are angular transformed values

Table 3. Efficacy of plant extracts against Sclerotinia rot of fennel (in vivo)

Plant extracts	Concentration (%)	Seed application*		Foliar application*		Seed-cum- foliar* application	
		Disease incidence(%)*	Per cent disease control	Disease incidence(%)*	Per cent disease control	Disease incidence(%)*	Per cent disease control
Calotropis procera (Aak)	5.0	72.98 (58.96)	7.10	78.95 (62.80)	4.83	71.96 (58.10)	8.86
Datura stramonium (Datura)	5.0	66.85 (54.80)	14.90	73.16 (58.76)	11.81	64.58 (53.78)	18.21
Allium sativum (Garlic)	5.0	53.56 (47.30)	31.82	58.16 (50.00)	29.89	48.56 (44.21)	38.50
Zingiber officinalis (Ginger)	5.0	73.26 (60.20)	6.74	71.56 (58.06)	13.74	64.96 (53.56)	17.73
Withania somnifera (Ashwagandha)	5.0	78.18 (62.16)	0.48	72.56 (58.58)	12.53	73.96 (58.96)	6.33
Parthenium hysterophorus (Carrot grass)	5.0	76.86 (63.58)	2.16	81.26 (64.08)	0.20	76.50 (60.26)	3.11
Control	-	78.56 (62.58)	0.00	82.96 (65.58)	0.00	78.96 (62.87)	0.00
SEm±		0.61	-	0.69	-	0.76	-
CD (p=0.05)		1.86	-	2.16	-	2.45	-

*Average of four replications
Figures given in parentheses are angular transformed values

Allium sativum has been known for its antifungal and antibacterial activities for decades. Chemical compounds from Allium sativum such as allicin and ajoene are well known to be effective against bacteria and fungi. These results are in agreement with the results of Prasad and Kumar (2007), Meena et al. (2013), Sharma et al. (2016), and Fagodia et al. (2017). They reported the effectiveness of Allium sativum clove extract in disease control against S. sclerotiorum.

observed in castor cake (39.49%) followed by neem cake (33.26%). Per cent disease incidence with mustard (72.98%) was found at par with sesame (71.96%). Minimum reduction in disease incidence was observed in groundnut cake treated plots (2.59%) (Table 4). Among these, castor cake was found most effective for reducing disease incidence, followed by neem. These results are in agreement with the results of Yadav *et al.* (2016).

Table 4. In vitro and in vivo efficacy of oil cakes against S. sclerotiorum

Oil cakes	In vitro			*In vivo*		
	Concentrationused (%)	Mycelial growth (mm)*	Percent mycelial growth inhibition	Dose (g/plot)	Disease incidence (%)*	Percent disease control
Mustard	3	83.86	6.82 (16.03)	50	72.98 (58.96)	9.07
Neem	3	54.00	40.00 (39.23)	50	53.56 (47.30)	33.26
Castor	3	50.00	44.44 (41.61)	50	48.56 (44.21)	39.49
Groundnut	3	86.00	4.44 (13.10)	50	78.18 (62.16)	2.59
Sesame	3	70.50	21.66 (28.12)	50	71.96 (58.10)	10.34
Cotton	3	82.50	8.33 (16.95)	50	76.50 (60.26)	4.68
Control	-	90.00	0.00 (0.00)	-	80.26 (64.26)	0.00
SEm±	-	-	0.34	-	0.56	-
CD (p=0.05)	-	-	1.43	-	1.87	-

*Average of three replications
Figures given in parentheses are angular transformed values

Plate 1. Symptoms of Sclerotinia rot of fennel

In vitro efficacy of oil cakes: The efficacy of six organic oil cakes (Table 4 and Plate 3) was tested *in vitro* against *Sclerotinia sclerotiorum.* The castor cake was found significantly superior over all the tested oil cakes with maximum (44.44%) inhibition of mycelial growth of *S. sclerotiorum* over control followed by neem cake (40.0%). Groundnut cake was found least effective (4.44%) in inhibiting mycelial growth of *S. sclerotiorum*. It was observed that castor and neem cake was found most effective in inhibiting mycelial growth. Earlier workers have also been reported oil cakes as a source for inhibition of fungal growth. Chand and Rai (2008) said castor cake and neem cake significantly superior in inhibiting mycelial growth of *S. sclerotiorum*. The inhibitory effect of six organic amendments on the mycelial growth of *S. sclerotiorum* has also been reported by Tripathi *et al.* (2011).

Efficacy of oil cakes (In vivo): Minimum disease incidence in castor cake (48.56%) followed by neem cake (53.56%) over control (80.26%). Maximum reduction in disease incidence over control was

They reported the effectiveness of oil cakes in disease control against *S. sclerotiorum*.

CONCLUSION

Stem rot caused by *Sclerotinia sclerotiorum* is a severe disease of Fennel (*Foeniculum vulgare* Mill.). Stem rot infected samples of fennel were collected from KVK, Ajmer. Pathogen isolated, purified by hyphal tip method. The present investigation evaluated some eco-friendly approaches *viz*., Bio agent, plant extracts, and oil cakes to control Sclerotinia rot of fennel under *in vitro* and *in vivo* conditions.

Among bioagents, *Trichoderma harzianum* was found most effective under *in vivo* conditions. Among plant extracts and oil cakes, *Allium sativum* clove extract and castor cake were found most effective under *in vitro* and *in vivo* conditions, respectively, for the management of Sclerotinia rot of fennel.

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