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Management of Ashwagandha root rot disease with fungicides, biocontrol agents and botanicals

R. P. S. Jetawat* and K. Mathur

Department of Plant Pathology, RCA, Maharana Pratap University of Agriculture and Technology, Udaipur, (Rajasthan), INDIA
*Corresponding author. E-mail: jaitawat.ravindra@gmail.com

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Abstract: The experiment was conducted to study fungicides, biocontrol agents and botanicals for management of ashwagandha root rot disease. Ashwagandha root rot disease caused by two pathogen *Fusarium solani* and *Rhizoctonia solani*. In field trial, seed treatments with integration of fungicides, neem cake manure, neem oil and *Trichoderma viride* agent evaluated as seed treatments individually as well as in different combination of seed treatment and soil application of neem cake was found effective integrated treatment (ST SAAF + neem cake manure + *T. viride*) and soil application of neem cake manure@500g/plot showed minimum per cent root rot and maximum per cent germination and maximum yield of Ashwagandha as compared to their individual applications over the untreated control.

Keywords: Ashwagandha, *Fusarium solani*, Neem cake manure, *Rhizoctonia solani* and *T. viride*

INTRODUCTION

Ashwagandha (*Withania somnifera*), also known as Indian ginseng, belonging to the family Solanaceae, is an important ancient medicinal plant, used in the Indian traditional systems of medicine, *Ayurveda* and *Unani*. Ashwagandha roots and their extracts are used in preparation of herbal tea, powders, tablets and syrups which help in reducing arthritis, disability, fatigue, high cholesterol and stress, increase healing processes, have positive effect against impotence and also normalize the sugar content of the blood.

It grows well in dry and sub-tropical regions of India, Sri Lanka and Bangladesh. Rajasthan, Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra and Madhya Pradesh are the major producing states of India. The estimated production of its roots in India is more than 1500 tonnes, while the annual requirement is about 7000 tonnes, necessitating increase in its cultivation and higher production. (Anonymous, 1976., Sharma, 2004 and Baghel *et al* 2010). The roots, leaves and fruits (berry) possess medicinal values due to alkaloids and steroidal lactones with anoides which is anti-Inflammatory (Anabalagan and Sadique, 1984), anti-arthritis (Begum and Sadique, 1988) and immuno-suppressive activities (Singh and Kumar, 1998), antioxidant (Dhuley, 1998), immunomodulatory (Davis and Kuttan, 2000), antidepressant (Bhattacharya *et al,* 2000).

The pharmaceutical industries are mainly dependent upon the wild population of Ashwagandha for the supply of tuberous roots for forskolin and withafarin extraction.The root rot of ashwagandha [*W. somnifera* (L.) Dunal] is an important disease causing significant economic and yield losses. *Fusarium solani* is a widely distributed soil-borne fungus pathogenic to at least 111 plant species spanning over 87 genera. It causes root rot diseases on a wide variety of crops. *Rhizoctonia solani* is also a widespread and destructive fungal pathogen of many plant species. Different types of disease symptoms like damping off, root, crown and stem rot, sheath blight etc. are caused by the pathogen. In view of increasing importance of root rot of ashwagandha, the present study was undertaken to investigate the efficacy of fungicides, biocontrol agents and botanicals against the disease.

MATERIALS AND METHODS

Diseased roots of Ashwagandha (*W. somanifera*) were collected from farmer's field of Udaipur and Rajasthan College of Agriculture, Udaipur.

Isolation, purification and identification of the pathogen: For isolation of the pathogen, small pieces of diseased roots were cut, washed in water and surface sterilized by dipping in 0.10 per cent mercuric chloride solution for 2 minutes followed by three washings in sterilized distilled water and plated on 2 per cent PDA plates aseptically. These were incubated at 25 ± 2°C in incubator for growth. Sub-cultures from peripheral growth were made on PDA slants.

The culture of *Rhizoctonia* was purified by single hyphal-tip method and that of *Fusarium* by single spore method using a dummy objective. The cultures were identified by comparing the morphological and

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culture characters described by Mordue (1988) for *Rhizoctonia* and Booth (1971) for *Fusarium* and were identified as *Rhizoctonia solani* and *Fusarium solani.*

Planting method and multiplication of *F. solani* and *R. solani* pathogens: The fungicide, botanicals and biocontrol agents found effective *in vitro* was evaluated in field for management of root rot ashwagandha. The plots size was 4 x 2 meter (8 sqm) in randomized block design (RBD) three replication of each treatment were maintained. Total 14 treatments were taken including control. For the field experiment cultures of *F. solani* and *R. solani* were reportedly on corn meal-sand (1:1) medium. This mixture was filled in 500 ml flasks to about 2/3rd of the capacity. The flasks were autoclaved for one hour at 1.056 kg/cm^2 pressure twice and inoculated with *R. solani* and *F. solani* incubated at 28 ± 2°C for 10 days. Inoculated flasks were shaking daily to avoid clumping. Mycelium growth and profuse sporulation of the pathogens occurred on the media after 10 days and then mixed in to the soil for multiplication of the pathogen. All the plots were lightly irrigated immediately after inoculation and to allow establishment of the pathogen before sowing. For comparison two controls were kept. In one control, plots had inoculated soil and another set of control plots had sterilized soil without inoculation. For each treatment and control three plots with three replications were maintained.

Treatment with cake, neem formulation, biocontrol agent and fungicide (integrated management): Fungicides and biocontrol agents found effective *in vitro* were evaluated individually and in combinations
Seed treatment with SAAF (Carbendazim 12% + Mancozeb 63% WP)
Seed treatment with Neem oil
Seed treatment with *T. viride* ICRISAT
Soil application of Neem cake
Seed treatment with SAAF + Neem oil
Seed treatment with SAAF + *T. viride* ICRISAT
Seed treatment with Neem oil + *T. viride* ICRISAT
Seed treatment with SAAF + Neem oil + *T. viride* ICRISAT
Soil application of Neem cake + Neem oil
Soil application of Neem cake + SAAF
Soil application of Neem cake + *T. viride* ICRISAT
Soil application of Neem cake + SAAF +*T. viride* ICRISAT
Inoculated untreated control.
Uninoculated untreated control

For seed treatment, cultures of the bio-control agents were individually grown on 2% malt extracts agar (MEA). The sporulatings colonies were harvested by suspending in 20 ml water in each Petri dish and mixed with sterilized fine clay (talc powder)10 gm to make a slurry. These formulations of the individual BCA's were used for seed treatments @ 10 g/kg seed. The coated seeds were kept overnight in moist chamber so as to enable the antagonists to establish on seeds and for chemical/botanical seed treatment, small quantity of each fungicide/botanical were used and seeds were soaked in fungicide SAAF 75 WP (0.20 %) and botanical Achook (3%) separately for 30 minutes, air dried in shade and sown.

$$\text{Per cent root rot} = \frac{\text{Total number of infected plants}}{\text{Total number of plants assessed}} \text{ X } 100$$

RESULTS AND DISCUSSION

Evalution of integrated disease management modules against root rot of Ashwagandha: Based on *in vitro* studies, the effective botanicals (Neem oil) and bicontrol agent (*Trichoderm viride,* take based formulation) and fungicide SAAF were soil application of neem cake manure evaluated individually as well as in various combination in a sick plot (infected with *F.solani* and *R. solani*) for management of ashwagandha root rot (Table 1).

In the field trial conducted in the sick plot, high plant mortality was observed in the untreated control plots .In the inoculated control, mortality was 88.9% while in the uninoculated control, it was70.4% the seed treatment with SAAF and *Trichoderma* formulation and Neem cake manure resulted in 32.2% 49.3% and 30.3% mortality, respectively, while plots having seed treatments with Neem oil showed 64.7 % mortality. Sinha and Padamini (2015) studied effect of volatile and non-volatile compounds produced by native *Trichoderma spp.* on *R. solani* causing sheath blight of rice in Manipur The results showed that all the isolates of *Trichoderma spp*. have potential to inhibit the mycelial growth of *R. solani*. The volatile compounds produced from the nine *Trichoderma* isolates showed inhibition of 35.33 to 79.53% of mycelial growth of *R. solani*. Combined treatment resulted in considerably reduced mortality, and with SAAF and Neem oil resulted in 36.0 % mortality, Seed treatment with Neem oil +*T. viride* also was at per showing 35.7 % mortality. Combination of three treatments further reduced the plant mortality due to *F. solani* and *R. solani.* The lowest mortality (11.8 %) was in combined treatment having ST SAAF +*T. viride* and soil application of Neem cake. This was closely followed by ST SAAF + soil application of neem cake which had 17.9% mortality. Next effective was the treatment having ST *T. viride* + soil application of Neem cake, where 23.7% mortality was recorded. Dhingani *et al* (2013) studied four organic extracts were tested against *M. phaseolina* by poisoned food technique in vitro. Significantly least growth of mycelium and maximum mycelium inhibition was recorded in extracts of neem cake (59.40 %) followed by farm yard manure (42.56 %). Next best in order of merit were castor cake and mustard cake. The difference in percent in mortality among these three treatments were statically significant. In the un-inoculated control plots

Table 1. Evaluation of integrated disease management modules against root rot of Ashwagandha.

S. No.	Treatment	Per cent mortality	Dry root yield		Seed yield		Root parameters		Alkaloid %
			g/plot (5sqm)	q/ha	g/plot (5sqm)	q/ha	Length (cm)	Diameter (mm)	
1.	Seed treatment (ST) SAAF 75 WP @ 0.2%	38.8 (38.55)	245	4.90	185	3.70	16.85	5.57	0.48
2.	ST Neem oil @ 1.0%	64.7 (53.54)	175	3.50	115	2.30	15.73	5.32	0.48
3.	ST *T.viride* 10 g/kg	49.3 (44.62)	210	4.20	152	3.03	16.13	5.55	0.57
4.	Soil application (SA) Neem cake manure 500 g/sqm	30.3 (33.41)	275	5.50	215	4.30	17.53	5.80	0.57
5.	Seed treatment SAAF + Neem oil (1.0%)	36.0 (36.87)	285	5.70	225	4.60	19.70	5.90	0.57
6.	SAAF 0.2% + *Trichoderma* formulation	28.8 (32.42)	283	5.67	223	4.47	18.87	6.00	0.66
7.	ST Neem oil 1.0% + *T. viride*	35.7 (36.70)	260	5.20	200	4.00	16.80	5.68	0.57
8.	ST SAAF 0.2% + Neem oil 1.0% + *T. viride*	26.8 (31.21)	300	6.00	243	4.87	20.50	6.17	0.66
9.	ST Neem oil 1.0% + Soil application of Neem cake	29.0 (32.58)	277	5.53	218	4.37	18.60	6.00	0.65
10.	ST SAAF 0.2% + Soil application of Neem cake	17.8 (25.00)	330	6.60	270	5.40	21.47	6.30	0.73
11.	ST *T. viride* + Soil application of Neem cake manure	23.7 (29.16)	310	6.20	250	5.00	21.30	6.27	0.66
12.	ST SAAF 0.2% + ST *T. viride* + Soil application of Neem cake	11.8 (20.13)	345	6.90	292	5.83	22.10	6.53	0.74
13.	Inoculated untreated control	88.9 (70.54)	75	1.50	20	0.40	10.38	2.40	0.32
14.	Uninoculated untreated control	70.3 (57.00)	125	2.50	65	1.30	12.50	2.93	0.40
	SEm±	1.05	3.842	0.077	3.895	0.078	0.349	0.099	
	C.D. at 5%	3.06	11.168	0.223	11.323	0.226	1.014	0.287	
	CD at 1%	4.14	15.098	0.302	15.306	0.306	1.371	0.388	
	CV%	4.72	2.666	2.666	3.533	3.533	3.404	3.136	

Figures in parenthesis are Arc sin √percent angular transformed; *Values of root parameters are mean of values of three replications (five observations in each replications)

the root yield was 125g/plot (2.5 q/ha) seed yield was 65g (1.30 q /ha), root length 12.5 cm, root diameter 2.93 mm and alkaloids 0.47%. In the inoculated control ashwagandha plots, yield was 75g (1.5g/ha), 20g (0.40q/ha), root length 10.38 cm, root diameter 2.40 mm and alkaloids 0.32%. All the treatment resulted in increased dry root and seed yield and the alkaloids contents highest root yield 345 g/plot enhanced root parameters and (6.90q/ha) was obtained with combined treatment of ST SAAF + *T. viride* and soil application of Neem cake the seed yield in this was 292g/plot (5.83 q/ha) mean root length was 22.1 cm, root diameter 6.53 and alkaloids content 0.74 %. In second best treatment ashwagandha plots (ST SAAF + soil application of neem cake. The dry rot root yield was 330 g/plot (6.6 q/ha), seed yield was 270 g/plot (5.40 q/ha), root length 21.47cm, root diameter 6.30 mm and alkaloid content 0.73%. In treatment having ST *T. viride* + soil application of Neem the root yield was 310 g/plot (6.20 q/ha), seed yield was 250g/plot (5.0q/ha), root length was 21.30 cm root diameter 6.27 mm and alkaloid 0.66%.

In brief, all the remaining treatment resulted in root yield ranging from(173-300) g/ plot (3.50-6.0 q/ha) seed yield ranging from 115-243g/plot(2.30-4.87q/ha), root length 15.73-21.47cm, root diameter 5.32-6.0 mm and alkaloid content ranging from (0.48-0.66%) in ashwagandha plots. In these parameter also, the combined treatments was better effective than the individual ones.

In the three best treatments, the alkaloid content was 1.8-2.3, times higher than control (Table 1). In the three best effective treatments the roots yield was four time higher seed yield was 30 times, root length was two times, root diameter. 2.6 time and alkaloid content was 2.3 times higher over the inoculated control, and 2.7, 4.5, 1.8, and 1.8 times high over the uninoculted control. The fungicides, oil cakes, neem formulation and bio-control agents which were found effective *in vitro*, were further evaluated as seed treatment individually as well as in different combination for suppression of Ashwangadha root-rot in field conditions. In this experiment, two types of controls were maintained inoculated and uninoculated. In uninoculated control also, considerable disease severity occurred, as it was a sick plot where Ashwagandha was being grown for two *kharif* season. But to ascertain adequate disease pressure moderate level of inoculum was added, hence disease and population density of both the pathogens was higher in the inoculated control over the uninoculted one. In the field trial, integrated treatment (ST SAAF + Neem cake + *T. viride*) showed minimum per cent root rot, maximum per cent germination and maximum yield of Ashwagandha. The different methods of application of fungicides and biocontrol agents, seed treatments have been most favoured and used, and there are several studies to show that the BCAs applied on seed can

establish in the rhizosphere and provide good suppression of the pathogens and diseases (De and Mukhopadhyay, 1990, Vyas, 1994 and Xue *et al* 2007). Gyanendra and Verma (2005) reported good compatibility of fungicides carbendazim, Neem products and biocontrol agents (*T. harzianum* and *T. viride*), for control of soybean root-rot. It was observed that integrated treatments were more effective over their individual applications as well as over the untreated control. Similar results on integration of fungicides with BCAs have been observed by Mousa (1996), who reported *T. viride* and *T. harzianum* with carbendazim were found effective for reduction of *F. solani* and *R. solani* and good compatibility of fungicides carbendazim, neem products and biocontrol agents (*T. harzianum* and *T. viride*) for the control of *F. solani* and *R. solani* causing root rot complex of soybean. Jatav and Mathur (2005) and Tetarwal (2011) studied that BCAs and two neem formulations with carbendazim and Tebuconazole were highly effective against *R. solani* and *F. solani (*root-rot complex in cluster bean and soybean).

Conclusion

The experiment was conducted to study fungicides, biocontrol agents and botanicals for management of ashwagandha root rot caused by two pathogen *F. solani* and *R. solani*. In the inoculated control, mortality was 88.9% while in the uninoculated control, it was70.4% the Seed treatment with SAAF and *Trichoderma* formulation and Neem cake manure resulted in 32.2% 49.3% 30.3% mortality, respectively, while plots having seed treatments with Neem oil showed 64.7 % mortality. Combined treatment resulted in considerably reduced mortality, and with SAAF and Neem oil resulted in 36.0 % mortality, Seed treatment with Neem oil +*T.viride* also was at per showing 35.7 % mortality. Combination of three treatment further reduced the plant mortality due to *F.solani* and *R.solani.* The lowest mortality (11.8 %) was in combined treatment having ST SAAF +*T. viride* and soil application of Neem cake. In the un-inoculated control plots the root yield was 125 g/ plot (2.5 g/ha) seed yield was 65 g (1.30 g/ha), root length 12.5 cm, root diameter 2.93 mm and alkaloids 0.47%. In the inoculated control, these value was 75g (1.5g/ha), 20g (0.40q/ha), root length 10.38 cm, root diameter 2.40 mm and alkaloids 0.32%. All the treatment resulted in increased dry root and seed yield and the alkaloids contents highest root yield 345 g/plot enhanced root parameters and (6.90q/ha) was obtained with combined treatment of ST SAAF +*T. viride* and soil application of Neem cake the seed yield in this was 292g/plot (5.83g/ha) mean root length was 22.1 cm, root diameter 6.53 and alkaloids content 0.74 %. In second best treatment (ST SAAF + soil application of neem cake. The dry rot root yield was 330 g/plot (6.6 q/ha), seed yield was 270 g/plot (5.40 q/ha), root length 21.47cm, root diameter 6.30mm and alkaloid content 0.73%. As such, the treatments found effective in sick plot conditions seem to be promising for practical disease management in farmer's field also. Consequently, integration management appeared not only economical but eco-friendly strategy for better control of root rot of Ashwagandha.

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