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Integrated Root Rot Management of Ashwagandha(*Withania somnifera* (L) Dunal)

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Abstract

Ashwagandha [*Withania somnifera* (L) Dunal] is an important medicinal herbs, mainly proned to the pathogens causing root rot disease resulted significant economic yield losses. An organic module consisting of fungicides, Neem-based formulations and biocontrol agent *Trichoderma viride* was evaluated for sustainable organic management of root rot (*Rhizoctonia solani* and *Fusarium solani*) of Ashwagandha under sick plot condition during *Kharif*- 2010 - 12. Integration of soil amended with Neem cake manure @ 500 g/m^2 + seed treatment with fungicide Mancozeb 63% + Carbendazim 12% (SAAF) @ 0.2% plus with *T. viride* talc based formulation @ 10g/kg resulted in the highest germination, minimum root rot and maximum quality root yield. Determination of population density by dilution plating revealed that disease suppression due to various treatments was mainly due to reduction in population density of *R. solani* and *F solani*. The integrated treatment also improved root quality parameter like, number of roots/plot, length (cm), and diameter (mm) as compared to their individual treatment as well as over the untreated control.

Keywords: *Fusarium solani, Rhizoctonia solani*

Introduction

The world health organization has estimated that more than 80% of the world population in developing countries depends primarily on herbal medicines for basic healthcare needs (Vines, 2004). One such popular medicinal herb is an Indian ginseng or winter cherry (*Withania somnifera* (L.) Dunal), family *Solanaceae* commonly known as “Ashwagandha” (smell of horses) is due to presence of steroidal lactones ‘Withanolides’ (Bhatnagar et al 1976), used as a quiet valuable herb in Ayurvedic and indigenous medical system for over 3000 years (Atal and Schwarting, 1961; Dash and Junius, 1983). It is hardy, drought tolerant and erect growing, branching shrub with a normal height of 1.50 m. 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 t, while the annual requirement is about 7000 t, 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 immunosuppressive activities (Singh and Kumar, 1998), antioxidant (Dhuley, 1998), immunomodulatory (Davis and Kuttan, 2000) antidepressant (Bhattacharya et al 2000), nootropic (Dhuley, 2001) , anti-parkinsonian (Ahmad et al 2005), antimicrobial (Owais et al 2005), anti-venom (Machiah et al 2006), anti-cancerous, anti-carcinogenic and potentiating apoptotic signalling in cancerous cell lines (Mathur et al 2006; Ichikawa, 2006). Although Indian states are cultivating Ashwagandha, but ever increasing demand for its dry matter produce and chemicals derived from them is steadily increasing has led to its commercial cultivation in many states. Its protract production is challenged by several foliar and root diseases that cause quantitative and qualitative losses in yield. While foliar pathogens can be managed by judicious use of fungicides, root pathogens like *Rhizoctonia solani* and *Fusarium solani* being intermittently dispersed in soil and surviving as resistant structures, are difficult to control by conventional methods.

Under favourable conditions its cause severe yield losses and may become destructive at all the growth stages of the crop. The pathogen is soil borne; hence use of chemical fungicides alone to control is an inadequate and uneconomical. Keeping this in view, sustainable and eco-friendly management was evaluated by the addition of organic amendments-oil cakes, botanical and biocontrol agents.

Materials and Methods

Causal Agents and Antagonists

Fungal pathogens were isolated, purified and pathogenicity of the culture was confirmed by growing healthy Ashwagandha in pathogens-inoculated soil. Pathogen was identified by comparing characters with the standard references description (Sneh et al 1991 and Mordue 1988) for *Rhizoctonia* and Booth (1971) for *Fusarium* and their identity was confirmed as *R. solani* and *F. solani.* Isolation of fungal biocontrol agents (*Trichoderma viride*) from rhizosphere soil of both healthy and diseased Ashwagandha plants was attempted by using selective media (Budge and Whipps 1991) by dilution plate method (Warcup 1955) , and the cultures available in the Department of Plant Pathology, RCA (Mathur et al 2006) were also used.

Evaluation of fungicides, botanical formulations, oil cakes, and bio agent's against *R. solani* and *F. solani in vitro*

Relative efficacy of Eleven systemic and non-systemic fungicides *viz.,* carbendazim-50 WP, tebuconazole 250 EC, propiconazole 25 EC, carbendazim 12% + mancozeb- 63% (SAAF), mancozeb-75 WP, captan 50 WP, trifloxystrobin 50WG, thiophanate methyl 70WP, chlorothalonil 75WP, Super-XL (Mancozeb 65% + Thiophanate methyl 10%), Thiophanate methyl and pencycuron at 0.05, 0.1 and 0.2 *a.i.* per cent concentrations; Five Neem-based formulations *viz.,* Neem oil, Neem formulation no.1, Neem formulation no. 2, Achook, Neem fungicide at 1, 2 and 3 per cent concentration and five oil cakes *viz.,* Neem, Jatropha, Mahua, Linseed and Mustard cake at 10, 20 and 40 per cent concentration were evaluated using poisoned food technique (Schmitzs 1930). Whereas, efficacy of biocontrol agents (*T. viride*) were tested by dual culture technique (Morsy et al 2009) and inverted plate technique (Dennis and Webster 1971) on PDA medium.

Evaluation of Organic Management Module in Field An integrated disease management package against root rot of Ashwagandha was evaluated under sick plot condition using fungicides, botanicals and biocontrol agents found effective *in vitro* individually and in combinations during the year 2010-2012.The plots size was 4 × 2 meter in randomized block design (RBD) and total 14 treatments were taken including control. Cultures of *R. solani* and *F. solani* were multiplied on corn meal-sand (1:1) medium in flasks at 28 $\pm$ 1 C for 10 days and then properly mixed @ 500 g/ plot in to the field for multiplication of the pathogen one week before sowing. All the plots were lightly irrigated immediately after inoculation to allow establishment of the pathogen. For comparison, two controls were kept. In one control, plots received inocula, while another set of control plots was kept without inoculation. For each treatment three plots as three replications were maintained. For seed treatment, culture of *T. viride* was grown on potato dextrose broth for 7 days at 28 C, mixed in talcum powder @ 1% W/V and carboxymethyl cellulose was added at 2%. The formulation of *T. viride* was 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. The variously treated seeds were sown in each plot@ 260/plot. The initial plant stand was recorded at 30 days after sowing. The numbers of root rot infected plants were recorded from germination till 40 - 45 days after sowing or at the time of harvesting and dry root and seed yield, root parameter *i.e.* number of roots/plot, length (cm), diameter (mm) were recorded for each treatment at the time of harvesting. Soil samples were collected from Ashwagandha rhizosphere and around from both diseased and healthy plants after 30 days of sowing at 30 days interval, i.e. 30, 60, 90 DAS by carefully uprooting the plants and lightly shaking these to remove the extra soil. The plants were air dried and the rhizosphere soil was collected by gentle scrapping with a hard brush. The population of *Trichoderma* spp. was determined using PDA-Triton X-Chloramphenicol medium (Mathur et al 2006) peptone PCNB and Rice agar medium for *F. solani* and *R. solani,* respectively (Mathur and Bohra 2004).

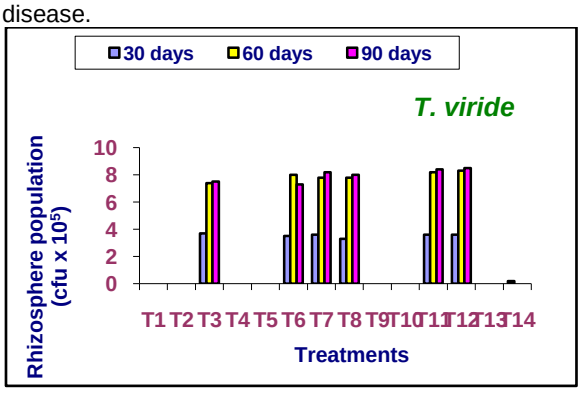
Results and Discussion

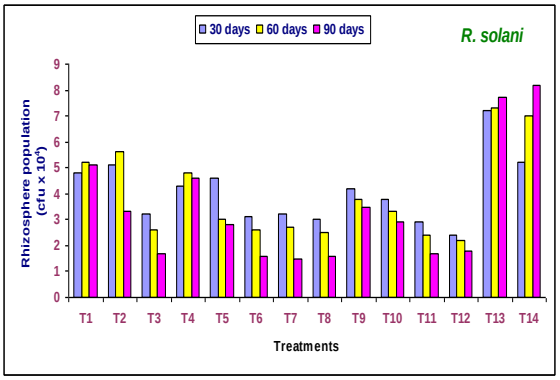
The twelve fungicides tested *in vitro* at three concentrations (0.05, 0.1 and 0.2 % *a.i.*). Mancozeb 63% + carbendazim 12% (SAAF) were highly effective against *R. solani* and *F. solani* at all the concentrations. Seed treatment with fungicides carbendazim, thiram and captan has been reported effective for control of soybean root-rot caused by *R. solani* (Wang et al 2004, Taya et al 1990). Out of five Neem-based formulations at 1, 2 and 3% concentrations and six oil cakes evaluated *in vitro* at 10, 20 and 40% concentrations, Neem oil as well as Achook and Neem cake were highly effective against *R. solani* and *F. solani* at all the concentrations. The efficacy of Neem-based formulation and Neem cake against *R. solani* in various crops is well documented. Singh et al (1980) reported that Neem oil at 0.1 % showed significant suppression in growth of *R. solani* and Kapoor *et al*. (2006) stated that botanicals, particularly Neem (*Azadirachta indica* A. Juss.) have shown good fungicidal potential against pathogenic fungi including *F. solani* and *R. solani* and seem promising as eco-friendly fungicide. Prasad et al (1998) reported the effect of oilcakes (Neem cake, groundnut cake, sesame cake) and 3 green leaf manures (*Calotropis* sp, *Crotalaria* sp and *Phaseolus* sp) on sclerotial survival of *R. solani* (causing rice sheath blight). Neem cake and the combined effect of *Calotropis* with groundnut cake was the most effective combination in reducing sclerotial viability in soil. Neem cake was also reported to be effective control of *F. solani* and *R. solani* (Jatav and Mathur, 2005; Tetarwal, 2011).Similarly, *T. viride* (ICRISAT) showed

high efficacy in suppressing the pathogen in dual culture method and production of volatile antibiotics by inverted plate technique maximum per cent inhibition of growth of *R. Solani*. The combined application of *T. viride* (ICRISAT) + *T. viride* strain Local 1 in the farmer`s field is highly effective against *R. solani*. Similar results have been observed by several workers, where biological control agents like *T. harzianum, T. viride, Gliocladium virens, Bacillus subtilis* and *Streptomyces* spp. have been reported to be effective for control of soybean root-rot pathogens (*R. solani, F. oxysporum* and *F. solani*) (Haque et al 1990; Mousa 1996;Hassanein et al 2000 ; Sen 2000), root rot of French bean (Hazarika and Das 1998), *F. solani* causing wilt of chilli (Rani *et al.,* 2009;), soil-borne diseases of cotton, egg plant, okra and sunflower (Mathivanan *et al.,* 2000). Among the fourteen treatments evaluated in the field, the minimum disease incidence with integration application of soil application with Neem cake manure @ 500 g/ m^2 as organic amendment + seed treatment with mancozeb 63% + carbendazim 12% (SAAF) @ 0.2% + seed treatment with *T. viride* talc-based formulation (10^8 cfu/g) @ 10g/kg resulted in maximum germination (89.2%) and plant stand (232/plot) and the lowest root rot incidence (13.0%). This treatment also resulted in the highest dry root yield 340 g/plot, maxium root length (21.96 cm), diameter (6.46 mm and alkaloid content (0.74%). This was found at par with integration of soil application with Neem cake manure + seed treatment with mancozeb 63% + carbendazim 12% (SAAF). The untreated inoculated control plots showed the lowest germination (37.1%), plant stand (97/plot) with maximum root rot incidence (89.9%) and lowest dry root yield (74 g/plot, and minimum root length (10.24 cm), diameter (2.33 mm) and the lowest alkaloid content (0.32%). It was observed that integrated treatments were more effective over their individual applications as well as over the untreated control (Table1).

The bio-control agent *T. viride* effectively established in Ashwagandha rhizosphere and reached high population densities during 30-90 days, while the population of *R. solani* was low in most of the treatments over the untreated control. The highest population counts (8.5 ×10^5 ; 8.4 c.f.u. / g soil) of *T. viride* were observed in the treatment comprising of soil application of Neem cake manure @ 500 g/m^2 + seed treatment with mancozeb 63% + carbendazim 12% (SAAF) @ 0.2% + seed treatment with *T. viride* talc based formulation @ 10g/Kg and seed treatment with *T. viride* and soil application of Neem cake manure, respectively. While seed treatment with SAAF + *T. viride* talc- based formulation exhibited lowest (7.3×10^5 c.f.u. / g soil) population counts as compared to other treatments (Table 2, Fig 1).

The disease suppression in these treatments seemed to be due to reduction of the inoculum density of *R. solani* and *F. solani* (c.f.u. /g soil) in the rhizosphere. At 90 DAS, the c.f.u. of *R. solani* and *F solani* in the untreated control was 8.2 ×10^4 / g and 8.0 x 10^4 c.f.u. /g soil, respectively, while in treatment having Neem cake manure + seed treatment with SAAF+ seed treatment with *T. viride*, it was 1.8×10^4 and 1.4×10^4 c.f.u. / g soil, respectively followed by 1.7 × 10^4 and 1.5×10^4 c.f.u. / g soil, respectively in soil application Neem cake manure + seed treatment with *T. viride* (Table 2, Fig 1). *Trichoderma* spp. are natural soil inhabitants and once established in the rhizosphere, these are able to suppress the pathogen by active antagonism, mainly antibiosis and competition (Chet, 1989 and Fravel et al 1985) and also enhance plant growth by production of volatiles (Harman et al 2006). Of 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*).* 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, and need to be further studied at pilot scale and popularized among the cultivators for mitigating the losses caused by this disease.





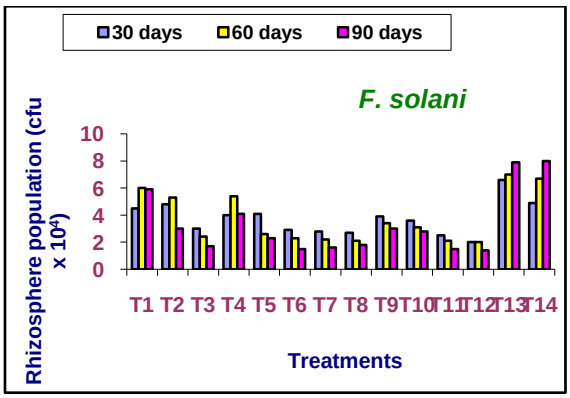


Fig.-1: Population dynamics of *Trichoderma viride, R. solani* and *F. solani* in rhizosphere of Ashwagandha under various integrated management module

1. Seed treatment (ST) SAAF 75 WP @ 0.2%
2. ST Neem oil @ 3.0%
3. ST *T. viride* (10^8 cfu/g) @10g/kg
4. Soil application (SA) Neem cake manure 500 g/sqm
5. Seed treatment SAAF 0.2% + Neem oil (3.0%)
6. ST SAAF 0.2% + *T.viride* @10g/kg
7. ST Neem oil 3.0% + *T.viride* @10g/kg
8. ST SAAF 0.2% + Neem oil 1.0% + *T. viride*
9. ST Neem oil 3.0% + SA Neem cake manure
10. ST SAAF 0.2% + SA Neem cake manure
11. ST *T.viride* @10g/kg + SA Neem cake manure
12. ST SAAF 0.2%+ ST *T.viride*+ SA Neem cake manure
13. Inoculated untreated control
14. Uninoculated untreated control

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soybean under *Rhizoctonia solani* inoculated field conditions in Ontario. Canadian Journal of Plant Science 87: 167-173.

Table 1
Evaluation of integrated disease management modules against root rot of Ashwagandha (Data pooled over 2010-2012)

S. No.	Treatment		Plant stand		Dry root yield		Seed yield		Root Quality parameters		Alkaloid %
		Germination (%)	/plot	Per cent mortality	g/plot	q/ha	g/plot	q/ha	Length (cm)	Diameter (mm)	
1.	Seed treatment (ST) SAAF 75 WP @ 0.2%	72.4 (58.3)	188	40.24 (39.37)	241	4.83	182	3.64	16.71	5.49	0.48
2.	ST Neem oil @ 3.0%	54.3 (48.1)	142	66.27 (54.49)	172	3.45	113	2.26	15.59	5.24	0.48
3.	ST T. *viride* (108 cfu/g) @10g/kg	62.2 (52.2)	162	50.92 (45.53)	207	4.14	149	2.99	15.99	5.48	0.57
4.	Soil application (SA) Neem cake manure 500 g/sqm	80.3 (64.2)	209	31.91 (34.40)	271	5.42	212	4.23	17.39	5.73	0.57
5.	Seed treatment SAAF 0.2% + Neem oil (3.0%)	72.4 (58.4)	188	37.49 (37.76)	281	5.61	222	4.43	19.56	5.83	0.57
6.	ST SAAF 0.2% + *T.viride* @10g/kg	80.3 (64.1)	209	28.33 (32.16)	279	5.58	220	4.40	18.72	5.93	0.66
7.	ST Neem oil 3.0% + *T.viride* @10g/kg	73.4 (58.4)	189	37.26 (37.62)	256	5.12	197	3.94	16.66	5.61	0.57
8.	ST SAAF 0.2% + Neem oil 1.0% + *T. viride*	82.2 (65.3)	214	30.27 (33.38)	296	5.91	240	4.79	20.36	6.09	0.66
9.	ST Neem oil 3.0% + SA Neem cake manure	82.1 (65.2)	213	30.50 (33.52)	273	5.45	215	4.30	18.46	5.93	0.65
10.	ST SAAF 0.2% + SA Neem cake manure	88.2 (70.2)	230	19.41 (26.14)	325	6.50	266	5.32	21.32	6.23	0.73
11.	ST *T.viride* @10g/kg + SA Neem cake manure	86.4 (68.1)	223	25.09 (30.06)	305	6.11	246	4.92	21.16	6.19	0.66
12.	ST SAAF 0.2% + ST *T.viride* + SA Neem cake manure	89.2 (71.1)	232	13.08 (21.20)	340	6.80	287	5.74	21.96	6.46	0.74
13.	Inoculated untreated control	37.1 (38.2)	97	89.95 (71.51)	74	1.48	20	0.39	10.24	2.33	0.32
14.	Uninoculated untreated control	49.3 (44.5)	128	71.34 (57.63)	123	2.46	64	1.28	12.36	2.86	0.40
	CD at 5%	5.20	19.13	2.84	11.10	0.22	11.15	0.23	1.02	0.29	
	CD at 1%	7.03	25.87	3.83	14.84	0.30	15.10	0.31	1.37	0.39	

*Average of three replications Figures in parentheses is Arc sin √percent angular transformed values.

Table 2
Population dynamics of *Trichoderma viride, R. solani* and *F. solani* in rhizosphere of Ashwagandha under various integrated management organic modules

DAS* / Treatment	Rhizosphere population of biocontrol agent and pathogens** c.f.u./ g soil								
	30 days			60 days			90 days		
	T. viride $\times 10^5$/ g	*R. solani* $\times 10^4$ /g	*F.solani* $\times 10^4$ /g	*T. viride* $\times 10^5$/ g	*R. solani* $\times 10^4$/ g	*F solani* $\times 10^4$/ g	*T. viride* $\times 10^5$/ g	*R. solani* $\times 10^4$/ g	*F solani* $\times 10^4$/ g
Seed treatment (ST) SAAF 75 WP @ 0.2%	0.0	4.8	4.5	0.0	5.2	6.0	0.0	5.1	5.9
ST Neem oil @ 3.0%	00	5.1	4.8	0.0	5.6	5.3	0.0	3.3	3.0
ST *T. viride* (10^8 cfu/g) @10g/kg	3.7	3.2	3.0	7.4	2.6	2.4	7.5	1.7	1.7
Soil application (SA) Neem cake manure 500 g/sqm	0.0	4.3	4.0	0.0	4.8	5.4	0.0	4.6	4.1
Seed treatment SAAF 0.2% + Neem oil (3.0%)	0.0	4.6	4.1	0.0	3.0	2.6	0.0	2.8	2.3
ST SAAF 0.2% + *T.viride* @10g/kg	3.5	3.1	2.9	8.0	2.6	2.3	7.3	1.6	1.5

ST Neem oil 3.0% + *T.viride* @10g/kg	3.6	3.2	2.8	7.8	2.7	2.2	8.2	1.5	1.6
ST SAAF 0.2% + Neem oil 1.0% + *T. viride*	3.3	3.0	2.7	7.8	2.5	2.1	8.0	1.6	1.8
ST Neem oil 3.0% + SA Neem cake manure	0.0	4.2	3.9	0.0	3.8	3.4	0.0	3.5	3.0
ST SAAF 0.2% + SA Neem cake manure	0.0	3.8	3.6	0.0	3.3	3.1	0.0	2.9	2.8
ST *T.viride* @10g/kg + SA Neem cake manure	3.6	2.9	2.5	8.2	2.4	2.1	8.4	1.7	1.5
ST SAAF 0.2%+ ST *T.viride*+ SA Neem cake manure	3.6	2.4	2.0	8.3	2.2	2.0	8.5	1.8	1.4
Inoculated untreated control	0.2	5.2	4.9	0.0	7.0	6.7	0.0	8.2	8.0
Uninoculated untreated control	0.0	7.2	6.6	0.0	7.3	7.0	0.0	7.7	7.9
CD 5%	0.23	0.43	0.48	0.35	0.40	0.47	0.51	0.36	0.48
CD 1%	0.32	0.58	0.61	0.48	0.54	0.51	0.69	0.49	0.58

***Days after sowing **Average of three replications ***Initial**cc **population of *F. Solani 1.9* × *10*^{4} c.f.u. / g soil and *R. solani* 1.4 ×10^4 c.f.u. / g soil**