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Effect of Bio-Agents and Organics Against *Fusarium* Wilt of Ashwagandha

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Ashwagandha (*Withania somnifera*) roots are the major source of alkaloids including tropine, pseudotropine and somniferine. Production of Ashwagandha root is less as compared to other medicinal plants because of wilt disease caused by *Fusarium solani*. The wilt infected plant has fully disturbed and finally whole plant is wilted. Therefore bio-agents and bio-fumigants (crusiferea plants) were evaluated. All treatments were significantly reduced the disease infection from 13.79 % - 90.8 %, 11.08 % - 72.44 %, 15.36 % - 68.48 % and 14.04 % - 61.44 % at 30, 60, 90 and 120 days after sowing. The per cent disease control was maximum 90.86 % in *Trichoderma harzianum* @ 2.0 %. The highest plant growth was observed in *Trichoderma harzianum* such as plant height (42.87 cm), stem length, (39.52 cm) and root length (12.07 cm). Highest seed yield per pot was recorded in T_1- *Trichoderma harzianum* (1.95 g) which was statistically at par with T_5 (1.87 g) and T_2 (1.65 g).

Keywords: Ashwagandha; wilt disease; Trichoderma spp.; Gliocladium spp.; Penicillium spp.; Aspergillus spp.

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1. INTRODUCTION

Ashwagandha (*Withania somnifera* L.) is also known as Indian ginseng, belonging to the family Solanaceae, native to the Indian subcontinent [1]. It is an important ancient medicinal plant, used in the Indian traditional systems of medicine, *Ayurveda* and *Unani* [2]. 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, stress and increase healing processes. The total alkaloid content of Ashwagandha roots varies between 0.13 to 0.31 per cent [3], along with starch, reducing sugars, glycosides, aspartic acids, glycine, tyrosine, and praline. Ashwagandha is grown in dry and sub-tropical regions of India, Sri Lanka and Bangladesh. It is mostly grown in dried region of India like Rajasthan, Punjab, Haryana, Uttar Pradesh, Gujarat, Madhya Pradesh and Maharashtra. Only two species of Ashwagandha are found in India, such as *Withania coagulans* and *Withania somnifera* [4]. Ashwagandha plants are hardy, drought tolerant, have erect branches and 1.5 M in height. It has produced about 300 to 500 kg of roots and 50-75 kg seeds from one hectare land. The estimated annual production of roots in India is more than 1500 tonnes, while the annual requirement is about 7000 tonnes; therefore is need hours for more cultivation to higher production [5, 6]. Production of Ashwagandha roots are less as compared to other medicinal plants because fungi like *Fusarium solani* causes wilt disease in Ashwagandha. The wilt infected plant has fully disturbed and finally whole plant is wilted. The demand for Ashwagandha is increasing day by day but farmers do not poduce more yield due to this pathological problems. Hence the gap between demand and supply of Ashwagandha to the pharmaceuticals companies are drastically increased. Its properties include anti-cancer , anti-Alzheimer, anti-Parkinson, anti-schizophrenic, anti-stress, and neuroprotective effects [22-24]. *Fusarium* spp. enters the host through fine roots and colonizes different plant parts [7]. Hence management of wilt disease through fungicides applied in the soil such as Thiram, Ferbum, Carbendazim and copper oxychloride have been reported by different workers. But indiscriminate use of these chemicals has led to development of fungicides resistance strain [8], and more importantly, environmental pollution posing a potential risk to animal and human health [9]. The biological control agents have been found safer for the environment and pesticide free agriculture. *Trichoderma* spp., *Gliocladium*spp., *penicillium* spp. and *Aspergillus* spp. have been found as effective bio control agents against soil borne plant pathogenic fungi such as *Fusarium*, *Sclerotium*, *Rhizoctonia* etc. In addition to biological control many bio-fumigants (botanicals) are known to have antifungal activity such as cabbage leaves, radish leaves, mustard leaves etc. The bio-fumigants are used for the suppression of soil borne pathogens incorporation into the soil. These bio-fumigants (crusiferea plants) have volatile compounds essentially isothyocyanates produce by the hydrolysis of glucosinolides [10]. Isothiocyanates have been shown highly suppressive to the soil borne pathogens as compared to synthetic fumigants metham sodium [11].

2. MATERIALS AND METHODS

A pot experiment was conducted in four replication with10 treatments viz; T_1 :*Trichoderma harzianum* 2% broth culture/500g FYM + soil.T_2 :*Gliocladium catenulatum* 2% broth culture/500g FYM + soil,T_3 :*Penicillium notatum* 2% broth culture/500g FYM + soil,T_4 :*Aspergillus niger*2% broth culture/500g FYM + soil, T_5 :*Trichoderma* local 2% broth culture/500g FYM + soil, T_6 : Cabbage leaf 10% per 500g FYM + soil, T_7 : Mustard leaf 10% per 500g FYM + soil, T_8 : Radish leaf 10% per 500g FYM + soil , T_9 : Cauliflower leaf 10% per 500g FYM + soil, T_{10} : Control in complete randomized design (CRD). 10 per cent formalin solution was used for sterilization of soil for 48hrs before adding the pathogen, bio agents and botanicals. Broth culture of *Fusarium solani* and bio agents were mixed in pots. The small pieces of botanicals were mixed in moisturized soil before 21 days of sowing. Inoculated pots were kept unsown for 4days for uniform growth of the inoculum. Inoculum was mixed in soil at 1:10 proportion (one part of inoculum and 10 parts of sterilized soil). The Fifteen seeds of Ashwagandha variety Nagori were sown in sick pots. Each treatments were maintained in four replication including control. The pots were watered with sterilized water periodically in order to provide sufficient moisture for seed germination and seedling growth. The development of disease symptoms was regularly observed up to four months and observed critically for appearance of disease symptoms. The plants which showed disease symptoms on roots were considered infected. The per cent disease incidence was calculated after first

disease appearance by adopting standard formula given below-

Percent disease incidence (PDI) = (Total no. of infected plants / Total number of plant) ×100

$$\text{Percent disease control(PDC)} = \frac{C-T}{C} \times 100$$

Where,

C = disease per cent in control
T = disease per cent in treatment

2.1 Growth and Yield

Plants height, stem length, root length, number of branches, number of leaves, number of berries, seed yield, fresh weight and dry weight of stem, root &leaves were recorded manually. Percent seed germination was calculated using formula given

Seed Germination Percent (GI) = (Germinated seeds / Total sown seeds) ×100

3. RESULTS AND DISCUSSION

Perusal of results from the Table 1 showed that the highest seed germination was recoded 91.73% in *Aspergillus niger* followed by 89.66 % in *Trichoderma* local and 88.86% in Cauliflower leaf extract. The lowest seed germination was observed 71.06% in control. Khan et al. [12] reported that the seed treatment with *Trichoderma* spp reduced the inhibitory effect of the pathogens and increased the seed germination 42% with *T*. *harzianum* and 41% *T. viride. S*eed treatments with *Trichoderma virens, Trichoderma harzianum* and *Aspergillus niger* have significantly increased the seed germination [13].

3.1 Percent Disease Incidence

Data presented in Table 2 revealed that the effect of all treatments was significant for reducing the percept disease incidence. The per cent disease incidence was 1.92 in *Trichoderma harzianum* followed by 2.08 in *Trichoderma* local, 6.62 in *Aspergillus niger*, 8.77 in *Penicillium notatum* 11.27 in *Gliocladium* catenulatum and 12.30 Cabbage leaf extract. The per cent disease incidence was maximumin in control 20.94 % at 30 days after sowing. Similar trends were found at 60 and 90 day after sowing. However, 120 days after sowing**,** the per cent disease incidence was minimum 30.53 % in *Trichoderma harzianum* and maximum in control (78.69 %). *Trichoderma viride* was more efficient than *Pseudomonas fluorescens* in arresting the growth of pathogen as compared to their individual applications over the control against the root rot of ashwagandha [14]. Antagonist fungi are producing a huge number of secondary metabolites during the metabolic activities which are responsible to inhibit the growth of pathogens example *Penicillium* spp produces a wide variety of beneficial secondary metabolites that enhance the plant growth [15] and defend their host from the pathogens [16]. Induction of mechanical resistance in the host and the attenuation of hormonal disruption produced by the pathogen are both the mechanisms enhanced by the *T. harziznum,* it might be the region for control of Fusarium wilt [17].

3.2 Per Cent Disease Control

Result of experiment (Table 3.) revealed that the all treatments were significantly reduced the disease infection from 13.79 % - 90.8 % at 30 days, 11.08 % - 72.44 % at 60 days, 15.36 % - 68.48 % in 90 days and 14.04 % - 61.44 % at 120 days. The disease control was maximum in *Trichoderma harzianum* (90.86 %), followed by *Trichoderma local* (90.00 %), *Aspergillus niger* (68.48 %), *Penicillium notatum* (58.25 %), *Gliocladium catenulatum* (46.36 %), Cabbage leaf extract (41.34 %), Mustard leaf extract (31.35 %), Radish leaf extract (21.89 %) and Cauliflower leaf extract (13.79 %). In between treatments maximum disease control was recorded in *Trichoderma harzianum* 90.86 %, 72.44 %, 68.27 % and 61.44 % at 30, 60, 90, and 120 days respectively. Minimum was found 13.79 %, 11.08 %, 15.36 % and 14.04 % in cauliflower leaf extract at 30, 60, 90, and 120 days respectively. Bio control agents were effectively established in ashwagandha root rhizosphere and reached high population densities during 30-90 days, while the population of *F. solani* was low in most of the treatments over the control. It is seemed due to the suppression of inoculum densities by the antagonistic fungi. The similar finding was reported by Joshi and Raut [18]. Tetarwal [19] studied that BCAs and two neem formulations with carbendazim and Tebuconazole were highly effective against *R. solani* and *F. solani* causing root rot complex in cluster bean. Disease reduction 77.5 percent was recorded in soil drenching of carbendazim (0.1%) followed by 73.6% in soil application of consortia (Seribed

waste +Pf1+BS4+Tv1+neem cake) @ 200g at 45 days after inoculation [20].

3.3 Growth and Yield

Data of the Table 4 revealed that all the treatments significantly increased the growth of ashwagandha in comparison to control. The maximum increase of plant height, stem length, root length were observed 42.87 cm, 39.52 cm and 12.07 cm, respectively in the treatment of *Trichoderma harzianum*. Whereas minimum effect was recorded in control 31.02 cm, 28.20 cm and8.85 cm of plant height, stem length, root

Table 1. Effect of bio-agents and botanicals on percent seed germination of Ashwagandha under pot experiment

Treatments		Dose (%)	Number of Seeds sown/pots	Number of seeds Germinated /pots	Germination (%)
T1	*Trichoderma harzianum*	2.0	15	13.25	88.33
T2	*Gliocladiumm catenulatum*	2.0	15	12.48	83.20
T3	*Penicillium notatum*	2.0	15	10.65	71.00
T4	*Aspergillus niger*	2.0	15	13.76	91.73
T5	*Trichoderma* local	2.0	15	13.45	89.66
T6	Cabbage leaf extract	10.0	15	11.56	77.06
T7	Mustard leaf extract	10.0	15	10.33	68.86
T8	Radish leaf extract	10.0	15	12.86	85.73
T9	Cauliflower leaf extract	10.0	15	13.33	88.86
T10	Control	-	15	10.66	71.06
	CD at 5%			2.85	3.45
	SE(m) ±			0.95	1.16

**Mean of four replications*

Table 2. Effect of bio-agents and organics products on percent disease incidence of fusarium wilt of Ashwagandha

Treatment	Dose (%)	Percent Disease Incidence (PDI)			
		30 DAS	60 DAS	90 DAS	120 DAS
Trichoderma harzianum	2.0	1.92 (7.93)	10.7 (19.02)	17.79 (24.86)	30.53 (33.42)
Gliocladium catenulatum	2.0	11.27 (19.53)	23.67 (28.99)	32.64 (34.70)	50.37 (45.01)
Penicillium notatum	2.0	8.77 (17.60)	23.30 (28.74)	28.85 (32.35)	45.60 (42.30)
Aspergillus niger	2.0	6.62 (14.85)	17.46 (24.60)	26.78 (31.04)	42.84 (40.72)
Trichoderma local	2.0	2.08 (8.29)	12.18 (20.41)	19.96 (26.52)	35.84 (36.76)
Cabbage leaf extract	10.0	12.30 (20.47)	29.49 (32.78)	34.16 (35.65)	54.04 (47.16)
Mustard leaf extract	10.0	14.31 (22.22)	31.93 (34.40)	37.58 (38.80)	55.24 (48.00)
Radish leaf extract	10.0	16.29 (23.79)	33.95 (35.62)	41.02 (39.81)	60.34 (50.95)
Cauliflower leaf extract	10.0	17.70 (24.91)	37.83 (37.89)	47.32 (43.55)	67.68 (55.35)
Control	-	20.94 (27.17)	42.49 (40.64)	56.10 (48.48)	78.69 (62.55)
CD (P=0.05)		0.41	0.50	0.86	1.08
SE(m)±		0.14	0.17	0.29	0.37

**Figures in parentheses are angular transformed values*

Table 3. Effect of bio-agents and organics products on percent disease control of fusarium wilt of Ashwagandha

Treatment	Dose (%)	Percent Disease Control (PDC)			
		30 DAS	60 DAS	90 DAS	120 DAS
Trichoderma harzianum	2.0	90.86 (72.38)	72.44 (58.37)	68.27 (55.70)	61.44 (51.59)
Gliocladiumn catenulatum	2.0	46.36 (24.89)	44.86 (42.03)	42.18 (40.48)	36.43 (37.09)
Penicillium notatum	2.0	58.25 (49.73)	45.46 (42.37)	50.68 (45.37)	42.43 (40.62)
Aspergillus niger	2.0	68.48 (55.82)	59.03 (50.18)	52.54 (46.44)	45.91 (42.63)
Trichoderma local	2.0	90.00 (71.73)	71.31 (57.59)	64.42 (53.36)	54.49 (47.56)
Cabbage leaf extract	10.0	41.34 (40.00)	30.87 (33.73)	39.38 (38.85)	31.69 (34.22)
Mustard leaf extract	10.0	31.35 (34.02)	24.75 (29.82)	32.97 (35.03)	29.84 (33.09)
Radish leaf extract	10.0	21.89 (27.86)	19.96 (26.52)	26.75 (31.13)	23.40 (28.91)
Cauliflower leaf extract	10.0	13.79 (21.58)	11.08 (19.42)	15.36 (23.01)	14.04 (21.99)
Control	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD (P=0.05)		2.64	2.89	3.02	2.18
SE(m)±		0.90	0.99	1.03	0.74

**Figures in parentheses are angular transformed values*

length, respectively. In between the treatments $T_1$and$T_5$was found at par in all growth characters. In case of number of branches, the effect was found highest in *Trichoderma harzianum* (5.25) which was statistically at par with *T*. local (5.0), *A. niger* (4.75), *P. notatum* (4.50), *G. catenulatum* (4.25) and Mustard leaf extract (3.75). Lowest number of branches was found in control (2.0) which was statistically at par with Radish leaf extract (3.50) and Cauliflower leaf extract (3.0). The maximum no. of leaves per pot was observed 53.25 in *T. harzianum* which was statistically at par with *T.* local (53.0), *A. niger* (51.75) andn *P. notatum* (49.75). The minimum was observed in control (29.25). Highest no. of berries was found 35.75 in *T. harzianum* which was statistically at par with T_5 (32.25), cabbage leaf extract (37.75), *A. niger* (30.50) and *P. notatum* (30.25). The lowest no. of berries was found in control (11.25) which was statistically at par with cauliflower leaf extract (15.0). Maximum fresh weight of stem, root & leaves were observed in *T. harzianum* which gave 56.02 g, 12.52 g and 22.50 g, respectively. The minimum fresh weight of stem, root and leaves were observed in control 26.80 g, 7.67 g and 10.02 g respectively. Maximum dry weight of stem, root and leaves was recorded in *T. harzianum* which gave 56.45 g, 2.32 g and 2.0 g respectively. The minimum dry weight of stem, root and leaves were recorded in control 3.00 g, 0.80 g and 1.05 g respectively. Highest seed yield per pot (g) was observed in *T. harzianum* (1.95 g) which was statistically at par with *T.* local (1.87 g) and *G. catenulatum* (1.65 g). Lowest seed yield was observed in control. Similar trends of results have reported by Hassan and Kareem [21] under the pot experiments. Asharaf and Zuhaib [13] have reported the increased growth parameters of ashwagandha i.e plant height, stem length, root length and thickness of root with treated *Trichoderma virens* and *Aspergillus niger* in comparison to control. Bioagents produce (*Trichoderma, Aspergillus* and *Penicillium*) a wide variety of beneficial secondary metabolites that enhance the plant growth [15]. Borade et al. [14] reported that *Trichoderma viride* was enhancing the growth and yield of ashwagandha *i.e.* fresh weight of stem, root, leaves and grain yield [22, 23, 24].

Table 4. Effect of bio-agent and botanicals on the growth and yield of Ashwagandha

Treatments		Dose (%)	Growth Characters					No. of Berries	Fresh Weight (g)			Dry Weight (g)			Seed yield/ Pot (g)
			Plant Height (cm)	Stem Length (cm)	Root Length (cm)	No. of Branch/ Plant	No. of Leaves/ Plant		Stem	Root	Leaves	Stem	Root	Leaves	
T_1	*Trichoderma harzianum*	2	42.87	39.52	12.07	5.25	53.25	33.75	56.02	12.32	22.50	6.45	2.32	2.00	1.95
T_2	*Gliocladium catenulatum*	2	35.10	32.35	10.50	4.25	46.50	26.75	48.92	10.72	19.00	4.84	1.80	1.22	1.65
T_3	*Penicillium notatum*	2	37.60	35.10	11.20	4.50	49.75	30.25	49.55	11.05	20.27	5.05	1.00	1.37	1.30
T_4	*Aspergillus niger*	2	39.25	36.77	11.42	4.75	51.75	30.50	51.27	11.55	22.05	5.92	1.95	1.00	1.40
T_5	*Trichoderma* local	2	42.07	39.37	11.77	5.00	53.00	32.25	54.95	12.05	22.37	6.32	2.10	1.82	1.87
T_6	Cabbage leaf extract	10	35.35	32.10	10.27	4.00	46.25	31.75	36.92	10.72	18.67	6.30	1.20	1.75	1.42
T_7	Mustard leaf extract	10	33.37	31.37	9.85	3.75	44.00	20.00	33.05	10.47	17.65	4.45	1.82	1.17	1.25
T_8	Radish leaf extract	10	32.40	30.15	9.55	3.50	40.75	18.25	32.05	9.77	15.62	3.95	1.35	1.00	1.32
T_9	Cauliflower leaf extract	10	31.02	28.20	8.85	3.00	38.25	15.00	29.50	8.75	14.87	3.97	1.25	1.07	1.20
T_{10}	Control	-	27.97	24.77	6.87	2.00	29.25	11.25	26.80	7.67	10.02	3.00	0.80	1.05	0.00
SE(m)±			0.65	0.68	0.49	0.58	1.25	1.31	2.37	0.84	0.57	0.22	0.15	0.13	0.12
CD (P=0.01)			1.89	1.99	1.44	1.69	3.63	3.80	6.87	2.45	1.65	0.65	0.45	0.40	0.35

**Mean of four replications*

4. CONCLUSION

Lowest disease intensity was recorded in *Trichoderma harzianum* and *Trichoderma* local, who was statistically at par, however, in case of bio-fumigant Cabbage leaf extract was more effective over the control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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