International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(1): 2050-2054

Received: 01-11-2017
Accepted: 02-12-2017

AS Borade
Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, M.S., India

RW Ingle
Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, M.S., India

VP Kendre
Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, M.S., India

Correspondence
VP Kendre
Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, M.S., India

Management of Ashwagandha root rot disease with bioagents and fungicides

AS Borade, RW Ingle and VP Kendre

Abstract
The experiment was conducted to study the effect of bioagents and fungicides for management of ashwagandha root rot disease. *Fusarium solani* causing root rot disease is one of the serious pathogen because it attacks on root and deteriorate the quality of alkaloid. Among various treatments, minimum disease attack was observed in fungicide seed treatment with Mancozeb 63% + Carbendazim 12%. This combined fungicidal seed treatment also increases the germination (plant stand), fresh and dry root weight, shoot and root length and alkaloid content. In case of bio agents, *Trichoderma viride* was more efficient than *Pseudomonas fluorescens* in arresting the growth of pathogen, as compared to their individual applications over the un-treated control.

Keywords: Ashwagandha, *Fusarium solani, T. viride* and *Pseudomonas fluorescens*

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, Madhya Pradesh and Maharashtra are the major producing states of India. Ashwagandha mostly grown on dried region of India. In Maharashtra, especially in Vidarbha region. Ashwagandha belongs to genus *Withania* and family *Solanaceae.* Only two species of Ashwagandha are found in India, such as *Withania coagulans* and *Withania somnifera.* 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) [4, 26, 5]. 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) [2], anti-arthritis (Begum and Sadique, 1988) [6] and immuno-suppressive activities (Singh and Kumar, 1998) [24], antioxidant (Dhuley, 1998) [13], immunomodulatory (Davis and Kuttan, 2000) [10], antidepressant (Bhattacharya *et al*, 2000) [7].

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.) Root rot caused by *Fusarium solani* has been considered among the most deleterious disease, which causes great losses in many parts of the world. Initial symptoms were withering and drooping of the plants while at later stages, plants showed severe wilting leading to death and decay of underground parts. The root of infected plant showed pulpiness with brownish colour. White cottony growth of the fungus was observed at the basal part of infected plants near ground level. The plant in the nurseries also showed symptoms of yellowing, drooping and decay at seedling stage leading to 30-40% mortality. Further investigations to characterize the infecting fungus led to identification of *Fusarium solani* as the causative organism (Gupta *et al*. 2004) [14].

Materials and methods
Diseased roots of Ashwagandha (*W. somanifera*) were collected from the field of Department of Plant Pathology, Dr. P. D. K. V., Akola.

Experimental site

The field experiment was conducted at the Field of Department of Plant Pathology, Dr. P. D. K. V., Akola during 2016-17 to find out the effect of different treatments for the management of root rot of Ashwagandha. For experimental layout the Randomized Block Design was used with three replications and seven treatments.

Sr. No.	Treatment No	Treatment Details
1.	T_1	Seed treatment with Carbendazim @(1.25g/kg)
2.	T_2	Seed treatment with Mancozeb @(2.5g/kg)
3.	T_3	Seed treatment with Mancozeb 63% + Carbendazim 12% (SAAF) 75WP @(2.5g/kg)
4.	T_4	Seed treatment with *Trichoderma viride* @(4g/kg)
5.	T_5	Seed treatment with *Pseudomonas fluorescens* @(10 g/kg) of seed
6.	T_6	Seed treatment with *Pseudomonas fluorescens* @(5g/kg) + *Trichoderma viride* @(2g/kg)
7.	T_7	Control

Symptomatology

The diseased root of Ashwangandha infected with *Fusarium solani* were collected from the field of department of plant pathology, Dr. PDKV, Akola. Initial symptoms were withering and drooping of the plants while at later stages, plants showed severe wilting leading to death and decay of underground parts. The root of infected plant showed pulpiness with brownish colour. White cottony growth of the fungus was observed at the basal part of infected plants near ground level. The plant in the nurseries also showed yellowing, drooping and decay symptoms at seedling stage leading to 30-40% mortality. Further investigations to characterize the infecting fungus led to identification of *Fusarium solani* as the causative organism. Similar type of symptoms also described by (Gupta *et al*. 2004) [14]. Leaf blight symptoms were noted in the field and observed microscopic characters in the laboratory with the help of simple microscope.

Isolation of leaf pathogen

The isolation of pathogen was done on Potato dextrose agar medium by tissue method. The plates were incubated in a B. O. D. incubator at 25±2 ^{0}C for 5 to 6 days and the growth of fungal colonies recorded every day. The root rot disease incidence was recorded at 30, 60, 90 and 120 DAS (Harvesting stage) by counting the number of diseased plants and total plants. Then Per cent disease incidence was calculated by using formula given by McKinney (1973).

$$\text{Per cent Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

And Percent alkaloid content in roots was estimated by following Formula**:**

Formula

$$\text{Total alkaloid content (\%w/w)} = \frac{\text{VT (blank)}}{\text{VT (blank) - VT (sample)}} \times \frac{\text{N of sample}}{\text{W}}$$

Results and discussion

Evalution of integrated disease management modules against root rot of Ashwagandha

The incidence of *Fusarium solani* causing root rot disease of Ashwagandha was observed in the field condition at 30, 60, 90, and 120 DAS. Data presented in Table 1. revealed that, the minimum root rot incidence was recorded in T_3 (Mancozeb 63% + Carbendazim 12%) is 6%. It was followed by T_1 Carbendazim), T_4 (*T. viride*) and T_2 (Mancozeb) is 15.01%, 19.01%, 21.02% respectively. Maximum root rot incidence was recorded in control treatment i.e. 31.63%. At 60 DAS, minimum root rot incidence was observed in fungicidal treated seed with (Mancozeb 63% + Carbendazim 12%) 8.9%. It was followed by T_1 (Carbendazim), T_4 (*T. viride*) and T_2 (Mancozeb) is 20.01%, 24.02%, 26.92% respectively. Maximum root rot incidence was recorded in control treatment i.e. 45.94%. At 90 DAS, fungicide treatment with (Mancozeb 63% + Carbendazim 12%) was again found effective with minimum (10.20%) root rot incidence followed by T_1 (Carbendazim), T_4 (*T. viride*) and T_2 (Mancozeb) is 21.02%, 25.02%, 28.22% respectively. Maximum root rot incidence was recorded in control treatment i.e. 47.24%. At harvesting stage (120 DAS), fungicide treatment with (Mancozeb 63% + Carbendazim 12%) was found effective with minimum (18.91%) root rot incidence followed by T_1 (Carbendazim) 28.53%, T_4 (*T. viride*) 33.13%, and T_2 (Mancozeb) 36.53%. Maximum root rot incidence was again recorded in control treatment i.e. 48.64%. Gupta and Mishra (2004) [14] reported that, *F. solani* causing root rot of Ashwagandha leads to 30-50% mortality of plants. Andrabi *et al.* (2011) [1] reported that seed treatment with Carbendazim + Mancozeb reduced disease incidence (8.56%) significantly. Similar results were recorded by Chavan *et al.* (2009) [9] in Patchouli.

Shoot and root length (cm) at different growth stages of Ashwagandha

Shoot length (cm)

Data presented in Table 3 revealed that at 60 DAS, maximum shoot length of plant was recorded in fungicidal seed treatment with Mancozeb 63% + Carbendazim 12% (37.39 cm), followed by seed treatment like Carbendazim (35.19 cm), followed by seed treatment with bioagent *T. viride* (36.13 cm). Minimum shoot length was recorded in control treatment (33.52 cm). At 90 DAS, seed treatment with fungicides like Mancozeb 63% + Carbendazim 12% again found effective with maximum shoot length (42.46 cm), followed by seed treatment with Carbendazim (42.12 cm) followed by antagonist seed treatment with *T. viride* (41.72 cm). Minimum shoot length was recorded in control treatment (39.93 cm). At the time of harvesting (120 DAS), maximum shoot length, was observed in fungicidal seed treatment with Mancozeb 63% + Carbendazim 12% (47.41cm) followed by seed treatment with Carbendazim(46.53 cm) followed by antagonist seed treatment with *T. viride* (46.32 cm). Minimum shoot length was recorded in control treatment (43.00 cm).

Root length (cm)

The data on root length of ashwagandha is presented in table 3 at harvesting stage (120 DAS), maximum root length was observed in fungicidal seed treatment with Mancozeb 63% +

Carbendazim 12% (26.33 cm), followed by seed treatment with Carbendazim (23.00 cm), followed by seed treatment with bioagent *T. viride* (21.93 cm). Minimum root length was recorded in control treatment (18.33 cm). Shanmugaiah (2009) [27] reported that, the seed treatment with biocontrol agent's *viz., Trichoderma viride* and *Pseudomonas fluorescens* was responsible for higher shoot and root length due to production of IAA, MG-6, UV-10, MNT-7 and other plant growth regulators. Maitlo *et al*. (2015) [19] reported that the maximum shoot length and root length was observed in plants treated with Bavistin D.F. while Mancozeb were observed least effective. Similar results were concprdant with Marnoranjitham *et al*. (2000) [18] in chilli seedling.

Fresh and dry weight (g) of roots after harvesting of Ashwagandha

Fresh weight (g)

At the time of harvesting, maximum fresh weight per plot was observed in seed treatment with fungicide *i.e.* Mancozeb 63% + Carbendazim 12% (468 g)is mentioned in table 4. It was followed by seed treatment with Carbendazim (406 g), followed by seed treatment with bio agent *T. viride* (369 g). Minimum fresh weight of roots was observed in control treatment (255 g). Also maximum fresh weight per root was observed in seed treatment with fungicide i.e. Mancozeb 63% + Carbendazim 12% (1.73 g). It was followed by seed treatment with Carbendazim (1.70 g), followed by seed treatment with bio agent *T. viride* (1.65 g). Minimum fresh weight per root was observed in control treatment (1.50 g).

Dry weight (g)

After harvesting, the fresh roots were collected and dry in clear sunlight condition for 7 days. Further the dried roots were weighted separatel in table 3. Among this dried root samples the maximum dry weight per plot was obtained in seed treatment with fungicide i.e. Mancozeb 63% + Carbendazim 12% (264.14 g). It was followed by seed treatment with Carbendazim (196 g), followed by seed treatment with antagonist *T. viride* (180 g). Minimum dry weight was observed in control treatment (109.81 g). fungicide i.e. Mancozeb 63% + Carbendazim 12% (0.91 g). It was followed by seed treatment with Carbendazim (0.82 g), followed by seed treatment with bio agent *T. viride* (0.80 g). Minimum fresh weight per root was observed in control treatment (0.64 g). Anju Tanwar *et al*. (2013) [3] recorded that, the application of *T. viride* alone increases fresh root weight (5.93 g) and dry root weight (2.51 g) in Brocoli. Similar results were recorded by Shanmugaiah *et al.* (2009) [27].

Per cent alkaloid content in roots of Ashwagandha

Alkaloids are special group of secondary compounds and are non toxic when stored but toxic when pathogens are attacked to the plants. Alkaloid was extracted from the root powder of Ashwagandha for comparing the difference in alkaloid percentage among various treatments.

The data in Table 4 revealed that, the maximum alkaloid per cent was recorded in seed treatment with fungicide, Mancozeb 63% + Carbendazim 12% (0.37%). It was followed by seed treatment with Carbendazim (0.36%), followed by seed treatment with bio agent *T. viride* (0.35%). Minimum alkaloid per cent was recorded from control plot (0.33%). Alkaloid per cent was estimated on the basis of dry weight of plant. Alkaloid percent per root and per plot were recorded maximum in fungicide treatment (T_3) and antagonist's treatment (T_4) because dry weight per root and per plot was maximum in chemical treatment (T_3) and antagonist treatment (T_4). Karthikeyan *et al*. (2009) [17] reported that, the seed priming and seedling treatments of native $PGPR_s$ can be used as a good tool in the enhancement of biomass yield and alkaloid contents in medicinal plant cultivation. Similar results were recorded by Nigam and Kandalkar (1995) [22] and Rawal *et al*. (2014) [23] in Ashwagandha.

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

Sr. No.	Treatments	Days after sowing							
		30 DAS	Per cent reduction over control	60 DAS	Per cent reduction over control	90 DAS	Per cent reduction over control	Harvesting Stage (120DAS)	Per cent reduction over control
T_1	Carbendazim	15.01 (22.78)*	52.54	20.01 (26.57)	56.44	21.02 (27.28)	55.50	28.53 (32.28)	47.71
T_2	Mancozeb	21.02 (27.28)	33.54	26.92 (31.25)	41.40	28.22 (32.09)	40.26	36.63 (37.24)	25.14
T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	06.00 (14.14)	81.03	8.9 (17.35)	80.62	10.20 (18.62)	78.40	18.91 (25.77)	61.34
T_4	*Trichoderma viride*	19.01 (25.85)	39.89	24.02 (29.33)	47.71	25.02 (30.00)	47.03	33.13 (35.14)	32.31
T_5	*Pseudomonas fluorescens*	28.02 (31.96)	11.41	40.43 (39.48)	11.99	41.54 (40.12)	12.06	44.74 (41.98)	08.60
T_6	*Pseudomonas fluorescens* + *Trichoderma viride*	23.02 (28.67)	27.22	33.43 (35.32)	27.23	34.73 (36.10)	26.48	39.03 (38.66)	20.24
T_7	Control	31.63 (34.22)	-	45.94 (42.66)	-	47.24 (43.41)	-	48.94 (44.39)	-
	'F' test	Sig.		Sig.		Sig.		Sig.	
	SE(m)±	0.43		0.67		0.66		0.69	
	CD at 5%	1.33		2.08		2.04		2.14	

Table 2: Shoot and root length (cm) at different growth stages of Ashwagandha

Sr. No.	Treatments	Shoot length (cm)			Root length(cm) Harvesting stage (120DAS)
		60 DAS	90 DAS	Harvesting stage (120 DAS)	
T_1	Carbendazim	35.19	42.12	46.53	23.00
T_2	Mancozeb	35.72	41.06	46.06	20.80

T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	37.39	42.46	47.41	26.33
T_4	*Trichoderma viride*	36.13	41.72	46.32	21.93
T_5	*Pseudomonas fluorescens*	34.72	39.99	44.73	19.55
T_6	*Pseudomonas fluorescens + Trichoderma viride*	35.39	40.92	45.46	19.86
T_7	Control	33.52	39.93	43.00	18.33
	'F' test	Sig.	Sig.	Sig.	Sig.
	SE(m)±	0.61	0.55	0.28	0.95
	CD at 5 %	1.88	1.71	0.86	2.99

Table 3: Fresh and dry weight (g) of roots after harvesting of Ashwagandha

Sr. No.	Treatments	Plant count (Harvesting stage)	Fresh weight of roots per plot	Fresh weight per root	Dry weight of roots per plot	Dry weight per root
T_1	Carbendazim	238	406	1.70	196	0.82
T_2	Mancozeb	211	345	1.63	161.67	0.76
T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	270	468	1.73	246.14	0.91
T_4	*Trichoderma viride*	222.66	369	1.65	180	0.80
T_5	*Pseudomonas fluorescens*	184	296	1.60	130	0.70
T_6	*Pseudomonas fluorescens + Trichoderma viride*	203	330	1.62	147	0.72
T_7	Control	170	255	1.50	109.81	0.64
	'F' test	Sig.	Sig.		Sig.	
	SE(m)±	3.11	8.54		5.58	
	CD at 5%	9.59	26.33		17.21	

Table 4: Per cent alkaloid content in roots of Ashwagandha

Sr. No.	Treatments	Per cent alkaloid content	Per cent Increase over control	Per cent alkaloid per root	Per cent Increase over control	Per cent alkaloid per plot	Per cent Increase over control
T_1	Carbendazim	0.36 (0.60)*	16.12	0.59	47.50	141.12	107.27
T_2	Mancozeb	0.34 (0.58)	09.67	0.52	30.00	109.93	61.47
T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	0.37 (0.61)	18.81	0.67	67.50	182.14	167.53
T_4	*Trichoderma viride*	0.35 (0.59)	13.44	0.56	40.00	126.00	85.07
T_5	*Pseudomonas fluorescens*	0.32 (0.56)	01.61	0.45	12.50	83.20	22.20
T_6	*Pseudomonas fluorescens + Trichoderma viride*	0.33 (0.57)	05.67	0.48	20.00	97.02	42.50
T_7	Control	0.31 (0.55)		0.40		68.08	
	'F' test	Sig.					
	SE(m)±	0.004					
	CD at 5%	0.013					

*Figures in parenthesis are square root transformed values

References

1. Andrabi M, Vaid A, Razdan VK. Evaluation of different measures to control wilt causing pathogens in chickpea. J. pl. protect. res. 2011; 51(1).
2. Anabalagan K, Sadique J. Role of prostaglandins in acute phase proteins in inflammation. Biochem. Med. 1984; 31:236-245.
3. Anju Tanwar, Aggarwal A, Sunita Kaushish, Sonika Chauhan. Interactive effect of AM fungi with *Trichoderma viride* and *Pseudomonas fluorescens* on growth and yield of Broccoli. Pl. Protect. Sci. 2013; 49(3):137-145.
4. Anonymous. The Wealth of India, (Raw Materials); CSIR: New Delhi, India. 1976; 10:580-585. England. 16.
5. Baghel G, Jahan T, Afreen B, Naqvi QA, Snehi SK, Raj SK. Detection of a begomovirus associated with yellow mosaic disease of Ashwagandha (*Withania somnifera* L.) and its impact on biomass yield. Medicinal Plants. 2010; 2(3):219-223.
6. Begum VH, Sadique J. Long term effect of herbal drug *Withania somnifera* on adjuvant induced arthritis in rats. Indian J. Exp. Biol. 1988; 26(11):877-882.
7. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Anxiolytic- antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. Phytomedicine, 2000; 7(6):463-469.
8. Booth C. The genus *Fusarium. Commonwealyh Mycological Institute*, Kew, 1971.
9. Chavan SC, Hegde YR, Prashanthi SK. Management of wilt of patchouli caused by *Fusarium solani*. J. Mycol. Pl. Pathol. 2009; 39:32-34.
10. Davis L, Kuttan G. Immunomodulatory activity of *Withania somnifera*. *J. Ethnopharmaol.* 2000; 71(1-2):193-200.
11. De RK, Mukhopadhyay A. Control of damping-off in tomato by seed coating with *Gliocladium virens*. *Proc. Int. Conf. Seed Sci. Technol. New Delhi*. 1990, 120.

12. Dhingani JC, Solanky KU, Kansara SS. Management of root rot disease [*Macrophomina phaseolina* (tassi.) Goid] of chickpea through botanicals and oil cakes. The Bioscan, 2013; 8(3):739-742.
13. Dhuley JN. Effect of Ashwagandha on lipid peroxidation in stress-induced animals. *J. Ethnopharmacol*, 1998; 60(2):173-178.
14. Gupta ML, Misra HO, Kalra A, Khanuja S. Root rot and wilt: A new disease of ashwagandha (*Withania somnifera*) caused by *Fusarium solani*. J. Medicinal and Aromatic PI. Sci. 2004; 26(3):285-287.
15. Gyanendra S, Verma RK. Compatibility of fungicides and Neem Products against *Fusarium solani* f.sp. *glycines* causing root-rot of soybean and *Trichoderma spp*. Journal of Mycological Research. 2005; 43:211-214.
16. Jatav RS, Mathur K. Bio-agents and neem based seed treatment for management of root-rot complex in cluster bean. Indian Phytopathology, 2005; 58:235-236.
17. Karthikeyan B, Jaleel CA, Azooz MM. Individual and combined effects of *Azospirillum brasilense* and *Pseudomonas fluorescens* on biomass yield and ajmalicine production in *Catharanthus roseus*. Academic J. Pl. Sci. 2009; 2(2):69-73.
18. Marnoranjitham SK, Prakasam V, Rajappan K. Biological control of chilli damping-off using talc based formulations of antagonists. Annals Pl. Protec. Sci. (India), 2000.
19. Maitlo WA, Markhand GS, Abul-Soad AA, Lodhi AM, Jatoi MA. Evaluation of various fungicides against *Fusarium solani* (mart.) sacc causing sudden decline disease of date palm (*Phoenix dactylifera*) In Sindh (Pakistan). Pak. J. Phytopathol. 2015; 27(1):07-12.
20. Mordue JEM. International course on the identifi-cation of fungi and bacteria of agriculture importance. Commonwealth Mycological Institute, 1988; 69:102-116.
21. Mousa ESM. Biological management of soil-borne pathogens and root knot nematode complexes on soybean (edited by Mousa, E.S.M.). *ShebinEiKom Egypt*, Menoufiya University, 1996, 60-69.
22. Nigam KB, Kandalkar VS. Ashwagandha. Advances in Horticulture- Medicinal and Aromatic Plants. 1995; 11:337-344.
23. Rawal P, Singh RP, Lekha. Integrated Root Rot Management of Ashwagandha [*Withania somnifera* (L.) Dunal)], 2014; III:III.
24. Singh S, Kumar S. *Withania somnifera*: The Indian ginseng Ashwagandha, Central Institute of Medicinal and Aromatic Plants, Lucknow, India, 1998.
25. Sinha B, Padamini RK. *In vitro* study of *Trichoderma spp.* Against sheath blight of rice caused by *Rhizoctonia solani* in Manipur. *The Ecoscan.* 2015; 9:295-298.
26. Sharma R. Agro-Techniques of Medicinal Plants. Daya Publishing House: New Delhi, India, 2004, 31-33.
27. Shanmugaiah V, Balasubramanian N, Gomathinayagam S, Manoharan PT, Rajendra A. Effect of single application of *Trichoderma viride* and *Pseudomonas fluorescens* on growth promotion in cotton plant. African J. Agril. Res. 2009; 4(11):1220-1225.
28. Tetarwal JP. Mix mycelia of *F. solani* and *R. so-lani* causing root rot of soybean M.Sc. Thesis, MPUAT, Udaipur (Rajasthan), 2012.
29. Vyas SC. Integrated biological and chemical con-trol of dry root-rot on soybean. Indian J Mycol Pl. Pathol. 1994; 24:132-134.
30. Xue AG, Cozber E, Morrison MJ, Voldeng HD, Ma BL. Effect of seed treatments on emergence, yield and root-rot severity of soybean under *Rhizoctonia solani* inoculated field conditions in Ontario. Canadian Journal of Plant Science. 2007; 87:167-173.