The Pharma Innovation Journal 2021; 10(9): 1714-1716

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(9): 1714-1716

www.thepharmajournal.com
Received: 10-06-2021
Accepted: 19-08-2021

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Effects of fungicides and organics on bio-molecules of Ashwagandha roots in *Alternaria* leaf spot infected plants

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Abstract
Ashwagandha (*Withania somnifera*) roots are major source of alkaloids including tropine, pseudotropine and somniferine. Deterioration of the biochemical and secondary metabolites of Ashwagandha by the *Alternaria* leaf spot disease are very scanty. Therefore, study has been done for management of leaf spot disease with fungicides and organics and their effects on bio-molecules of Ashwagandha roots. The total phenol and flavonoids content increased with decreasing disease intensity. The maximum increase of total phenol was recorded 17.12mg/g in ridomil MZ, and flavonoids in Kovach 16.90 mg/g with disease intensity 19.20%. Withanolides content was recorded maximum in ridomil (3.73%) followed by panchgavya (3.66%) and cow urine (3.39%).

Keywords: Ashwagandha, secondary metabolites, *Alternaria*, Leaf spot, Disease

Introduction
The pharmacological effects of Ashwagandha (*Withania somnifera*) are attributed to its active ingredients present in roots, which has a wide range of therapeutic application (Uma Devi, 1996). It have rich antioxidents properties with vitamin E, vitamin C, polyphenols including phenolic acids, phenolic diterpens, flavonoides, and catechins. Global interest are increasing in this plants due to high demands for its roots which proved ample scope to cultivate the Ashwagandha on commercial scale because it gives economically remunerative returns in comparison to other traditional crops. Ashwagandha crops are encountered many diseases in the field condition especially leaf spot disease caused by *Alternaria alternata* which is significantly affect the secondary metabolites production (Pati *et al*, 2008) [7]. Spray of Fungicides is common management practices but repeated use of fungicides lead to development of resistance in pathogen against fungicides. Therefore bioagents and organics were evaluated for management of *Alternaria* leaf spot and its effects on bio-molecules.

Materials and Methods
Pathogen
The pathogen *Alternaria alternata* was collected from the infected leaves of *Withania somnifera* and maintained on natural host.

Field Experiment
Field experiment was conducted in sandy loam soil, the plot size 3.6 X 3.0 m^2 adopting a standard spacing 30 X 10 cm for Ashwagandha sowing. Farm yard manures (FYM) 10 tones / ha was mixed in the soil before sowing. The variety Nagori was sown (@5 g seed/plot with 13 treatments *viz*; T_1- Three foliar spray of ridomil MZ @ 0.25% concentration at 45, 60 and 90 days after sowing, T_2- Three foliar spray of Streptocycline sulphate @ 0.30% concentration at 45, 60 and 90 days after sowing, T_3- Three foliar spray of Antracol (Propineb) @ 0.20% concentration at 45, 60 and 90 days after sowing, T_4- Three foliar spray of Kovach (Chlorothalonil) @ 0.20% concentration at 45, 60 and 90 days after sowing, T_5- Three foliar spray of Bayleton (Triodemefon) @ 0.05% concentration at 45, 60 and 90 days after sowing, T_6- Three foliar spray of Metalaxyl @ 0.25% concentration at 45, 60 and 90 days after sowing, T_7- Three foliar spray of *P. fluorescens* @ 0.20% concentration at 45, 60 and 90 days after sowing, T_8- Three foliar spray of *Trichoderma harzianum* @ 0.20% concentration at 45, 60 and 90 days after sowing, T_9- Three foliar spray of Cow urine @ 10% concentration at 45, 60 and 90 days after sowing, T_{10}- Three foliar spray of Cow dung slurry @ 10% concentration at

45, 60 and 90 days after sowing, T_{11}- Three foliar spray of Panchgavya @ 10% concentration at 45, 60 and 90 days after sowing, T_{12}- Three foliar spray of Vanaspativash @ 10% concentration at 45, 60 and 90 days after sowing, T_{13}- Control. Three replication in randomized block design were maintained for each treatments. Percent disease intensity (PDI) of *Alternaria* leaf spot disease was scored in the field at 12th standard week in randomly selected three plants of each Ashwagandha plots. The percentage of leaves affected by disease was assessed visually in 0- 4 point scale as described by Meena *et al*; (2019) [8]. Total phenol, flavonoids, withanolids and alkaloids contents were estimated in dry roots after 180 days of sowing. The total phenol content was estimated by the method of Bray and Thorpe (1954) [9]. Total flavonoids contents were determined according to methods described by Nabavi *et al.*, (2008) [10]. Alkaloids and withanolides content of Ashwagandha roots were analyzed by the method of Pati, *et al*., (2008) [7].

Results and Discussion

Phenolics are substances that are involved in plant pathogen interaction. Therefore, the total contents of phenol, flavonoids, withanolides and alkaloids were determined in the dry root of Ashwagandha. The total phenol content increased with decreasing disease intensity. The maximum content was recorded 17.12mg/g in ridomil MZ followed by Kovach 16.80mg/g and cow urine 14.43mg/g with disease intensity 17.70%, 19.20% and 34.53% respectively. The increased of flavonoids content was highest in Kovach 16.90 mg/g with disease intensity 19.20%. Total flavonoids content in panchgavya was 16.03 mg/g at 31.17% disease intensity. Alkaloids have been described as components of plant defense response, The total alkaloids were found higher amount in fungicides treated plants such as kovach (2.94%), bayleton (2.93%) and ridomil MZ (2.78%).Whereas in organics, alkaloids was found lesser amount, it is might be due to high disease intensity. Withanolides content was recorded maximum in ridomil (3.73%) followed by panchgavya (3.66%) and cow urine (3.39%). Percent disease intensity was significantly minimized by all the treatments. The minimum disease intensity was recorded in Ridomil MZ (17.70%) followed by Kovach (19.20%) and Antracol (20.07%). Bioagents and cow products were also significantly minimized percent disease intensity. The effect of fungicides and organics were seen to increase the accumulation of phenolics around the infection site, which mitigate the effects of pathogens. The similar finding was reported by Grayer (1989) [6] and Buchanan, *et al.* (2001) [5]. Pati *et al.* (2008) [7] reported that total withanolides and withaferin content were declined (76.3%) due to infection of *Alternaria alternata*. Neeraj and Verma (2010) [1] observed that total sugar and total phenols contents were reduced with plant age and percent disease infection of *Alternaria alternate* in *Raphanus sativus*. Jayapal and Mahadevan, (1968) [3], Kushwaha and Narain (2005) [4] and Bhardwaj *et al.* (1985) [2] also reported similar results.

Table 1: Effects of fungicides and organics on bio-molecules of Ashwagandha roots in *Alternaria* leaf spot infected plants

Treatments	Dose (%)	Percent Disease Intensity	Total Flavonoids in dry roots (mg/g)	Total Phenol contents in dry roots (mg/g)	Total Withanolides in dry roots (%)	Alkaloids in dry roots (%)
Ridomil MZ	0.25	17.70 (24.88)	16.73	17.12	3.73	2.78
Streptocycline sulphate	0.30	24.07 (29.38)	15.37	13.27	3.19	2.20
Antracol (propineb)	0.20	20.07 (26.58)	16.57	12.83	3.12	2.60
Kovach (Chlorothalonil)	0.20	19.20 (25.99)	16.90	16.80	3.52	2.94
Bayleton (Triodimefon)	0.05	27.90(31.88)	15.77	14.77	3.15	2.93
Metalaxyl	0.25	31.20 (33.96)	14.83	13.00	3.24	2.77
Pseudomonas fluorescence	0.50	33.20 (35.18)	15.83	13.13	2.97	2.63
Trichoderma harzianum	0.50	32.90 (35.00)	14.97	13.20	2.96	2.70
Cow urine	10.0	34.53 (35.99)	15.23	14.43	3.39	2.45
Cow dung slurry	10.0	35.97 (36.84)	16.00	14.30	3.20	2.21
Panchgavya	10.0	31.17 (34.55)	16.03	13.53	3.66	2.63
Vanaspativash	10.0	36.00 (36.87)	15.77	13.33	3.04	2.43
Control	Water	42.20 (40.51)	14.70	11.33	2.83	2.20
S.Em∓		0.470	0.373	0.401	0.153	0.113
CD at 5%		1.372	1.090	1.171	0.445	0.330
CV%		2.476	4.126	4.985	8.176	7.380

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