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Effect of fungicides on the mycelial growth of *Alternaria alternata* causing leaf spot disease in ashwagandha

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ABSTRACT

A Leaf spot disease was caused by *Alternaria alternata* ten isolates were collected from the ashwagandha growing different regions of Tamil Nadu. The virulence of the isolates were tested based on the Per cent Disease Index (PDI) and symptom expressed on the foliar surface. The maximum disease intensity of 65.13 PDI was recorded in Chempatti (I_6) isolate. The minimum disease intensity was recorded in I_{10} from Cumbum (36.83 PDI). Seven fungicides *viz.*, carbendazim(0.05%), mancozeb (0.2%) copper oxy chloride (1%), chlorothalanil (0.2%), fosetyl (0.1%), Ridomil MZ (0.05%) and dithane M 45(0.05%) were tested against *A. alternata*. Among the fungicides, the minimum diameter of mycelial growth of *A. alternata* (0.75cm) and maximum percentage of inhibition (91.34%) were recorded in mancozeb (0.2%).

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INTRODUCTION

Ashwagandha (*Withania somnifera* L. Dunal) is an important medicinal plant that is used in the Indian traditional system of medicine ayurveda and unani. The sanskrit name of ashwagandha which means smelling like a horse. In Tamil it is called as Amukkrang kilangu. Ashwagandha roots are compared with ginseng roots for their restorative properties and have been popularly known as Indian ginseng. It is belongs to the family Solanaceae. Ashwagandha is an errect, evergreen, annual, drought resistant medicinal shrub growing to height of 30-150 cm.

The plant roots, leaves, fruits and seeds contain a number of alkaloids that is withanin and somniferine. (Asthana and Raina, 1989). The crop is affected by various diseases like leaf spot, seedling blight and wilt. Among these diseases, leaf spot caused by *Alternaria alternata*. The initial symptoms are leaves having brown to black spot of two to nine mm in diameter surrounded by a yellow halo appear on both dorsal and ventral surfaces of the infected leaves (Inoue and Nasu, 2000). Both poisoned food technique and spore germination assay, mancozeb (0.2%) was the most effective fungicide against *A. alternata* (Kamalalakshmi, 1996).

MATERIAL AND METHODS

Collection and isolation of the pathogen :

Leaves of ashwagand has howing typical symptoms of dark coloured spots with concentric rings caused by *Alternaria alternata* were collected from ashwagandha growing areas of Tamil Nadu. The pathogen was isolated from the diseased tissues of ashwagandha by tissue segment method (Rangaswami, 1958). The infected tissues were first washed with tap water to remove the soil particles; cleaned tissues were swabbed with 80 per cent ethanol to eradicate the external microbial contaminants. The infected portions of diseased leaves were cut into small pieces using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in sterile distilled water thrice and then placed on previously poured and solidified Petri dish containing Potato dextrose agar (PDA) medium. These plates were incubated at room temperature (28 ± 2^0C) for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogen was identified based on their cultural and morphological characters.

Virulence (Pathogenicity) of the isolates :

The pathogenicity of purified isolates of *A. alternata* was tested and it was proved by Koch's Postulates conducted on the local varieties.Ashwagandha seedlings were planted in each pot and replicated three times. The conidial suspension (5×10^5 spores ml^{-1}) was prepared in sterile distilled water from 9-day-old PDA cultures of the different isolates of *A. alternata*. These spore suspensions were sprayed on the pinpricked ashwagandha plants of 25-day-old plants. The plants sprayed with sterile distilled water served as control. The severe symptoms were observed on seven days after inoculation and the disease intensity was recorded. The symptoms were observed and compared with the original symptoms. The fungus was reisolated from artificially inoculated ashwagandha leaves and compared with original culture isolate (Table A).

The Per cent Disease Index (PDI) was worked out by using McKinney (1923) formula *viz.*,

$$\text{PDI} = \frac{\text{Over all of numerical rating}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

Table A : Per cent Disease Index (PDI) grades

Grades	Leaf area spotted (%)
0	Healthy
1	1-5
3	5-10
5	10-25
7	25-50
9	More than 50

Efficacy of fungicides against *A. alternatain vitro* (Poisoned food technique):

Seven fungicides *viz.*, carbendazim(0.05%), mancozeb (0.2%) copper oxy chloride (1%), chlorothalanil (0.2%),fosetyl (0.1%), Ridomil MZ (0.05%) and dithane M 45(0.05%) were tested against *A. alternata*. Twenty ml of the PDA medium was poured in sterilized Petri plates and allowed to solidify. Before adding the medium the required concentration of fungicides was added. A nine mm of actively growing virulent isolate culture disc of *A. alternata* was placed on the centre of the Petri plate. PDA medium without adding the fungicides served as control. The plates were incubated at room temperature (28 ± 2°C). When the control plate showed the full growth of the pathogen, the diameter of the mycelial growth was measured in all the treatments. The results were expressed in per cent inhibition over control.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Survey and collection of isolates :

Ashwagandha leaves showing typical leaf spot symptoms were collected from ten ashwagandhagrowing areas of Tamil Nadu *viz*., Ottanchatram, Coimbatore, Periyakulam, Theni, Nilakottai, Chempatti, Palur, Vadipatti, Virudhunagar and Cumbum with a view to assess disease intensity and to identify the variability of the pathogen. The maximum disease intensity of 65.13 PDI was recorded in Chempatti of Madurai district, followed by 60.07 PDI in Vadipatti. The remaining areas recorded the disease incidence of 57.13 to 30.14 PDI. The lowest disease incidence (29.31 PDI) was recorded in Coimbatore (Table 1; Plate 1). The maximum disease intensity was recorded in I_6 from Chempatti (81.03 PDI)

Table 1 : Sources of collection of isolates of *Alternaria alternata* causing leaf spot of ashwagandha

Sr. No.	Isolate	Places	PDI *
1	I_1	Ottanchatram	37.01 (38.09)**
2	I_2	Coimbatore	29.31 (30.63)
3	I_3	Periyakulam	44.07 (41.84)
4	I_4	Theni	47.18 (41.27)
5	I_5	Nilakottai	57.13 (48.79)
6	I_6	Chempatti	65.13 (61.37)
7	I_7	Palur	48.05 (41.33)
8	I_8	Vadipatti	60.07 (52.12)
9	I_9	Virudhunagar	30.14 (34.01)
10	I_{10}	Cumbum	32.18 (35.91)
	C.D. (P=0.05)		1.45

* Mean of three values
** Values in the parentheses are arcsine transformed values

followed by I_4 from Theni (63.15 PDI), I_3 from Periyakulam (60.08 PDI) and I_8 from Vadipatti (58.69 PDI). The minimum disease intensity was recorded in I_{10} from Cumbum (36.83 PDI) (Table 2).

Severity of the leaf spot disease and its effect on the yield was already cautioned by Pati *et al.* (2008). Sharma *et al.*(2005) reported that pathogenicity of *A. alternata* of apple proved under semi controlled condition. Typical brown spot symptoms were observed after five to seven days of inoculation on all the inoculated apple plants.Such variations in the virulence of isolates have been reported by Berry (1960) in *A. sesame*, Awasthi and Kolte (1989) in *A. brassicae*, Babu (1994) in *A. solani*, Karthikeyan (1999) in *A. palandui*. Virulence of the best isolate may be due to more toxin production as well as its adaptability to favourable environment condition.

Table 2 : Virulence of different *A. alternata* isolates

Sr. No	Isolates	PDI *
1.	I_1	49.17 (32.49)**
2.	I_2	51.01 (37.9)
3.	I_3	60.08 (38.55)
4.	I_4	63.15 (42.86)
5.	I_5	48.17 (36.31)
6.	I_6	81.03 (56.74)
7.	I_7	52.74 (42.93)
8.	I_8	58.69 (54.02)
9.	I_9	40.69 (34.15)
10.	I_{10}	36.83 (30.12)
CD (P=0.05)		1.32

* Mean of three values
** Values in the parentheses are arcsine transformed values.

Efficacy of fungicides against *A. alternata :*

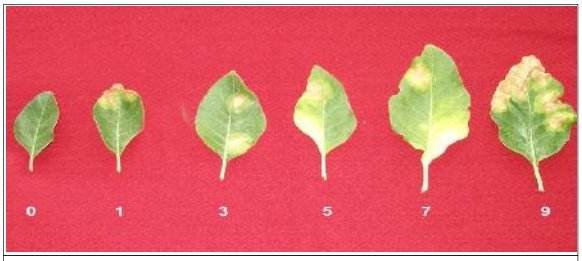
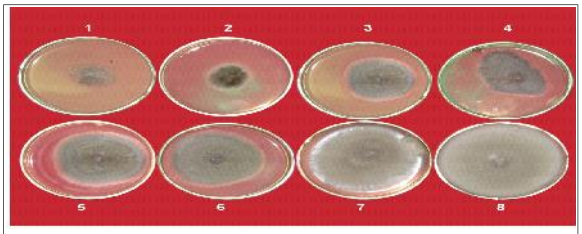


Plate 1 : Leaf spot disease – Grade chart



1. Mancozeb (0.2%)
2. Fosetyl AI (0.1%)
3. Copper Oxy Chloride (1%)
4. Metalaxyl) (.05%)
5. Chlorothalanil (0.2%)
6. Carbendazim (0.05%)
7. Metalxyl + Mancozeb (0.05%)
8. Control

Plate 2 : Effect of fungicides on the mycelial growth of *A. alternata*

Table 3 : Effect of different fungicides on the mycelial growth of *A. alternata* (isolate)

Sr. No.	Treatments	Mycelial growth (cm)*	Per cent reduction over control
1.	Fosetyl Al (0.1%)	3.55	59.01
2.	Mancozeb (0.2%)	0.75	91.34
3.	Copper Oxy Chloride (1%)	1.83	78.87
4.	Mancoceb+mealaxyl (0.05%)	7.20	16.86
5.	Carbendazim (0.05%)	6.85	20.90
6.	Chlorothalanil (0.2%)	6.42	25.87
7.	Metalaxyl (0.05%)	4.67	46.07
8.	Control	8.66	0.00
	C.D. (P = 0.05)	0.72	

* Mean of three replications

Among the fungicides *viz.*, fosetyl Al (0.1%), copper oxy chloride (1%), metalaxyl + mancozeb MZ (0.05%), carbendazim (0.05%), chlorothalanil (0.2%), metalaxyl (0.05%) and mancozeb (0.2%) tested, the minimum diameter of mycelial growth (0.75cm) and maximum percentage of inhibition (91.34%) were recorded in mancozeb (0.2%) followed by copper oxy chloride (1.83 cm and 78.87%) and fosetyl Al (3.55cm and 59.01%). The maximum diameter of mycelial growth (7.20cm) and minimum percentage of inhibition (16.86%) was recorded in metalaxyl + mancozeb MZ (0.05%) (Table 3; Plate 2).The evaluation of fungicides against *A. alternata*revealed that mancozeb (0.2 %) showed maximum per cent inhibition of pathogen in poisoned food technique. Previous authors have also reported the similar findings. Gupta *et al*. (1986) stated that Dithane M-45 at 1000 ppm concentration was effective in inhibiting *A. porri.* The efficacy of mancozeb against *Alternaria* spp. was reported by several workers, (Maheswari and Singh, 1998; Kannan and Subbaraja, 1999; Muthulakshmi, 1990; Babu, 1994; Mohan, 1996 and Sumathi, 1997). The reason may be the fungicidal compound may affect the sterol biosynthesis in fungal metabolism (Vidyasekaran, 1998). Dar *et al*. (2013) evaluated nine fungicides namely: carbendazim, hexaconazole, thiophonate methyl, triadimefan, metalaxyl, mancozeb, captan, copper oxychloride and chlorothalonil. A mongthe systemic fungicides maximum inhibition in mycelial growth and spore germination was observed in the carbendazim followed by other fungicides. Among the non -systemic fungicides maximummycelial growth inhibition and spore germination was observed in mancozeb followed by other fungicides.

Conclusion :

The isolate I_6 collected from Chempatti was found highly virulent followed by I_8 which was collected from Periyakulam. Under *in vitro* condition, among the fungicides were tested, mancozeb (0.2%) registered the maximum mycelial growth reduction followed by copper oxy chloride.

REFERENCES

Asthana, R. and Raina, M.K. (1989). Pharmacology of *Withania somnifera* (L.) Dunal.A Review. *Indian Drugs,* **26** : 199-205.

Awasthi, R.P. and Kolte, S.J. (1989). Variability on *Alternariabrassicae* affecting rape seed and mustard. *Indian Phytopath.*, **42** : 863.

Babu, S. (1994). Studies on leaf blight of tomato (*Lycopersicon esculentum* Mill.) caused by *Alternaria solani* (EII. and Mart.) Jones and Grout. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.193 p.

Berry, S.N. (1960). Pathogenicity and Morphology studies of *Alternaria* isolates from *Sesamum indicum. Phytopathol.*, **50** : 552-556.

Dar, W.A., Davis, R.D., Moore, N.Y. and Kochman, J.K. (2013). Characterization of a population of Fusarium oxysporum f. sp. vasinfectum causing vascular wilt of cotton in Australia. *Aust. J. Agric. Res.*, **47**: 1143-1156.

Gupta, R.P., Srivastava, V.K. and Pandey, U.B. (1986). Control of purple blotch disease of onion. *Seed Crop,* **39** : 303-304.

Inoue, K. and Nasu, H. (2000). Black spot of Peach caused by *Alternaria alternata* (Fr.) Keissler. *J. Gen Plant Pathol.*, **66** : 18-22.

Kamalalakshmi, C. (1996). Studies on leaf blight disease of jathimalli *Jasminum grandiflorum* L. incited by *A. alternata* (Fr.) Keissler.agg. M.Sc. (Ag.). Thesis, Tamil Nadu Agricultural University, Coimbatore, India.199 p.

Kannan, R. and Subbaraja, K.T. (1999). Comparative evaluation of selected plant extracts and fungicides on the incidence of leaf blight of onion caused by *Alternaria alternata* (Fr.) Keissler. *Pestol.*, **23** (5): 31-33.

Karthikeyan, M. (1999). Studies on onion (*Allium cepa* var *aggregatum* L.) leaf blight caused by *Alternaria palandui* Ayyangar. M.Sc., (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.120 p.

Maheswari, S.K. and Singh, D.V. (1998). Control of *Alternaria* leaf spot of Dolichos bean by foliar application of fungicides. *Ann. Pl. Protec. Sci*., **6** (2): 191-193.

Mckinney, H.H. (1923). A new system of grading plant diseases. *J. agric. Res.*, **26** : 195-218.

Muthulakhsmi, P. (1990). Studies on fruit rot of chillies (*Capsicum annum* L.) caused by *Alternaria tenuis* Nees. M.Sc., (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.139 p.

Pati, P.K., Sharma, M., Salar, R.K., Sharma, A., Gupta, A.P. and Singh, B. (2008). Studies on leaf spot disease of *Withania somnifera* and its impact on secondary metabolites. *Indian J. Microbiol*., **48** : 432-437.

Rangaswami, G. (1958). An agar blocks technique for isolating soil micro organisms with special reference to pythiaceous

fungi. *Sci. & Cul*., **24** : 85.

Sharma, J.N., Gupta, D., Bhardwaj, L.N. and Kumar, R. (2005). Occurance of *Alternaria* leaf spot (*Alternaria alternata)* on apple and its management. *Integrated Plant Disease Management*, pp.25-31.

Sumathi, P. (1997). Biological management of sesame (*Sesamum indicum* L.) leaf blight caused by *Alternaria sesame* (Kawam) Mohanty and Behera, M. Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India. 159 p.

Vidyasekaran, P. (1998). Physiology of disease resistance in plants.Vol. I. CRC Press, Florida, 149 p.