Biological Forum – An International Journal **14(2): 540-545(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Management of Alternaria Leaf Spot of Cauliflower by using Plant Extracts

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(Received 03 February 2022, Accepted 16 April, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Alternaria leaf spot caused by *Alternaria brassicicola* (Schwein) Wiltshire is one of the most destructive disease of the cauliflower (*Brassica oleracea* var. *botrytis*), causing high losses. The present investigations were taken for management of Alternaria leaf spot of cauliflower with botanicals. Result revealed that all the treatments significantly reduced the leaf spot disease incidence over control. Experiment was taken for the evaluation of efficacy of botanicals against *A. brassicicola* in *in vitro* as well as for the management of Alternaria leaf spot disease in field conditions. Six plant extracts at three concentrations *viz.*, 5%, 10%, 20% were evaluated in *In vitro* conditions against *A. brassicicola* using poisoned food technique. Leaf extract of neem (*Azadirachta indica*) exhibited maximum inhibition (85.39 %) in mycelial growth followed by parthenium (79.22%). Plant extracts (10%) were applied as foliar application against the disease incidence under field conditions. Neem (*Azadirachta indica*) extract proved most effective in reducing the disease incidence (38.60%) followed by parthenium (*Parthenium hysterophorous)* (29.91%). Plant extract can play a major role in coming days for the management of plant diseases at large scale and protect the farmers from indebtedness by decreasing the cost of production.

Keywords: Alternaria, Cauliflower, Disease, Botanical.

INTRODUCTION

Cauliflower is one of the most popular *Brassica* vegetables after cabbage in area and production across the world. Major cauliflower growing countries in the world are China, India, United States of America, Spain, Mexico, Italy, France, Poland, Pakistan and Egypt. India is the one of leading cauliflower and broccoli producing country having 32.5% share in the world after China. India's contribution in global cauliflower production is 8.66 million metric tonnes with an area of 4.52 lakh hectares (Anonymous, 2018).

In vegetable Brassica seeds, especially white cabbage and cauliflower [*Brassica oleracea* (L.) var. *botrytis*], *Alternaria brassicicola* (Schwein) Wiltshire is the dominant pathogen (Maude and Humpherson-Jones1980, Humpherson-Jones 1985; Maude *et al.* 1984; Deep and Sharma 2012; Sharma *et al.,* 2013). It can affect cauliflower crop at all stages of growth on leaves, curd and seeds. Alternaria leaf spots usually appear on the oldest leaves first and later spread to the newer leaves towards the tips (Chattopadhyay, 1999; Meena *et al.* 2010; Deep and Sharma, 2012). Alternaria leaf spot disease decrease nutritive value of vegetables (Azevdo *et al*. 2000). Alternaria blight may cause about 47.8 per cent reduction in yield of cauliflower seed (Hossain and Hossain 2010). The yield loss due to *A. brassicae* is 5-30% in entire cauliflower and cabbage growing area of India (Pandey *et al*., 2002). Disease severities of 52.25 were reported from Sikar district in Rajasthan due to Alternaria leaf spot disease of cauliflower (Sharma and Dhandapani 2006).

Due to the limitation in use of fungicides as it causes the environmental pollution and health hazards, use of plant extracts may be safer and least expensive. It is now widely recognized as a biorational approach to control Alternaria leaf spot disease. The present investigations were carried out to generate information on potentiality of some promising plant extracts under *in vitro* as well as *in vivo* conditions against Alternaria leaf spot of cauliflower and its causal agent is *Alternaria brassicicola* (Schwein) Wiltshire.

MATERIAL AND METHODS

Evaluation of plant extracts against *Alternaria brassicicola* (*in vitro*)

Collection and extraction of botanical extracts Fresh samples of medicinal plants with antifungal constituents (listed in Table 1) were collected. 100 g of leaves from each botanical was washed thoroughly under running

tap water, dried with blotting paper, cut into smaller pieces and ground using a sterile mortar and pestle by adding 100 ml of sterile distilled water. Finally, it was filtered through two layers of cheesecloth and the extract was then centrifuged at 10,000 rpm for 15 min and the supernatant alone was transferred to a fresh tube. The extract was then sterilized using 0.2 µm disposable syringe filters. The filtrate (100%) was further diluted to required concentrations for further use (Tiwari and Singh, 2005). The antifungal activity of following plant extracts were tested *in vitro* against *Alternaria brassicicola* by Poisoned Food Technique.

Table 1: Efficacy of plant extracts against *A. brassicicola.*

Name of Plant	Botanical name	Part used	Concentration (%)	
			In vitro	*In vivo*
Neem	*Azadirachta indica*	Leaves	5,10,20	10
Parthenium	*Parthenium hysterophorous*	Leaves	5,10,20	10
Sadabahar	*Catharanthus roseus*	Leaves	5,10,20	10
Giloy	*Tinospora cordifolia*	Leaves	5,10,20	10
Wild Amaranthus	*Amaranthas viridis*	Leaves	5,10,20	10
Wild Sunflower	*Helianthus annus*	Leaves	5,10,20	10

The effect of each plant extracts was tested at three different concentrations i.e. 5, 10 and 20 per cent. The effect of plant extracts against mycelial growth of *Alternaria brassicicola* were tested by poison food technique. Required quantity of each plant extracts were mixed thoroughly in melted PDA media, just before pouring in sterilized Petriplates and was allowed to solidify. Each plate was inoculated with 5 mm disc of 7 days old culture of *Alternaria brassicicola* with the help of sterilized cork borer. The Inoculated Petriplates were incubated at 25±1°C for 7 days. Control was also maintained in which medium was not supplemented with any of the plant extracts. The experiment was conducted in completely randomized design with three replications. Colony diameter (two diagonals) was measured after 7 days of incubation. The per cent growth inhibition was calculated by using Vincent's formula (1947).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,
C= Diameter of the colony in control (Average of both diagonals), T= Diameter of the colony in treatments (Average of both diagonals)

Bioefficacy of different plant extracts against Alternaria leaf spot of cauliflower under field conditions

The field experiment was conducted during 2020-21 in the field located behind the Department of Plant Pathology, SKNCOA, Jobner, Jaipur in RBD design with three replications and seven treatments. Cauliflower's seedlings were transplanted in the field with all the recommended agronomic practices. The Plant extracts *viz.*, neem leaf (*Azadirachta indica*), Parthenium (*Parthenium hysterophorous),* Sadabahar (*Catharanthus roseus),* Giloy (*Tinospora cordifolia),* Wild Amaranthus (*Amaranthas viridis),* Wild Sunflower (*Helianthus annus*)were tested by applying as foliar spray on 60 days old plants after 48 hrs of inoculation of the pathogen. For comparison inoculated control was maintained without plant extracts application. Observations of the disease severity were recorded after 10 days of inoculation on a standard disease rating scale (0-5 score).

Assessment of Disease severity. In order to record severity of Alternaria leaf spot disease, randomly 25 leaves from ten plants were selected from different treatments to record the data on disease severity. The data on the severity of the disease were recorded by using the 0-5 scale with slight modification (Mc Kinney, 1923)

Table 2: Scale for assessing disease severity of Alternaria leaf spot of cauliflower.

Rating	Infected leaf area (%)	Disease reaction
0	No visible infection	Immune (I)
1	1-5.0	Resistance (R)
2	5.1-15.0	Moderately resistant (MR)
3	15.1-30.0	Moderately susceptible (MS)
4	30.1-50.0	Susceptible (S)
5	>50.0	Highly susceptible (HS)

Where,
I= Immune, R = Resistant. MR = Moderately resistant, MS = moderately susceptible, S = Susceptible and HS = Highly susceptible

Per cent disease intensity (PDI) and per cent disease control (PDC) were calculated using following formula suggested by Conn *et al.* (1993).

$$\text{PDI} = \frac{\text{Sum of rating of the leaves infected}}{\text{Number of leaves} \times \text{maximum disease rating}} \times 100$$

$$\text{PDC} = \frac{\text{PDI in check - PDI in treatment}}{\text{PDI in check}} \times 100$$

RESULTS AND DISCUSSION

In vitro effect of plant extracts on mycelial growth of *A. brassocicola*

In several researches, extract of various higher plants has been reported to exhibit anifungal properties under *in vitro* laboratory trials. In particular wild plants seem to be a promising source of antifungal metabolites thus the efficacy of six plant extracts namely, giloy, neem, parthenium, wild sunflower, sadabahar and wild amaranthas (Table 3, Fig. 1 and Plate 1) was tested under *in vitro* conditions against *A. brassicicola* at three concentrations *viz*., 5, 10 and 20 per cent. The results revealed that at 5 per cent concentration, the maximum growth inhibition (83.34%) obtained in neem followed by parthenium which showed 77.11 per cent inhibition. Wild amaranthas was found least effective against *A. brassicicola* which showed only 51.25 per cent growth inhibition. More growth inhibition was observed at 10 per cent concentration as compared to 5 per cent. Neem exhibited maximum inhibition (85.11%) which is significant to other plant extracts. Parthenium was next to the neem which showed 79.51 per cent mycelial growth inhibition. Wild amaranthas was least effective which showed just 55.45 per cent growth inhibition.Likewise at 20 per cent concentration the extracts were highly toxic to *A. brassicicola* growth. Neem was found most effective, exhibiting 87.73 per cent growth inhibition followed by parthenium (81.03%). Least growth inhibition was exhibited by wild amaranthas (57.23%).

Mean of treatments showed that neem was proved superior to rest of the extracts tested in respect of growth inhibition percentage being 85.39 followed by parthenium (79.22). Wild amaranthas was found least effective with only 54.64 per cent inhibition. Results also depicted that as the concentration of extracts increased in medium, the inhibition percentage also increased. Highest mycelial growth inhibition was proved at 20 per cent concentration at par with 10 per cent and 5 per cent. Inhibition of mycelial growth of the *A. brassicicola* shown in the present study was consistent with that reported earlier by workers Anamika and Simon (2011); Sasode *et al*. (2012).

Table 3: *In vitro* evaluation of plant extracts against *A. brassicicola.*

Sr. No.	Plant extracts	Part used	Percent mycelia growth inhibition at different concentration*			Mean
			5%	10%	20%	
1.	Neem	Leaves	83.34	85.11	87.73	85.39
			(65.91)	(67.30)	(69.50)	(67.57)
2.	Parthenium	Leaves	77.11	79.51	81.03	79.22
			(61.42)	(63.09)	(64.18)	(62.89)
3.	Sadabahar	Leaves	62.13	64.20	66.30	64.21
			(52.02)	(53.25)	(54.51)	(53.26)
4.	Giloy	Leaves	67.06	69.10	71.43	69.20
			(54.98)	(56.23)	(57.69)	(56.30)
5.	Wild amaranthus	Leaves	51.25	55.45	57.23	54.64
			(45.72)	(48.13)	(49.16)	(47.67)
6.	Wild sunflower	Leaves	72.23	74.60	77.50	74.78
			(58.20)	(59.74)	(61.68)	(59.87)
7.	Control		0.00	0.00	0.00	0.00
			0.00	0.00	0.00	0.00
	Mean		59.02	61.14	63.03	
			(50.19)	(51.44)	(52.55)	
			SEm+	CD (p=0.05)		CV (%)
Plant extract (P)			0.54	1.51		5.01
Concentration (C)			0.83	2.30		
P × C			1.44	3.99		

* Average of three replications

Figures given in parentheses are angular transformed value

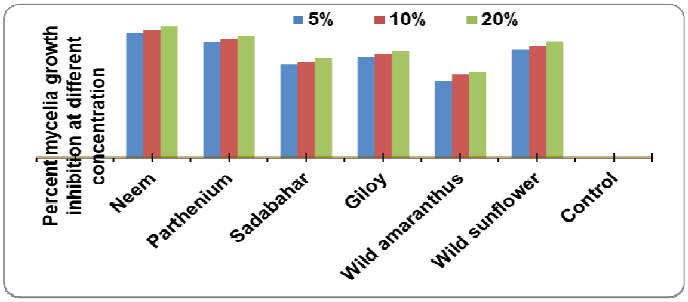

Fig. 1. *In vitro* evaluation of plant extracts against *A. brassicicola* at 25±1°C.

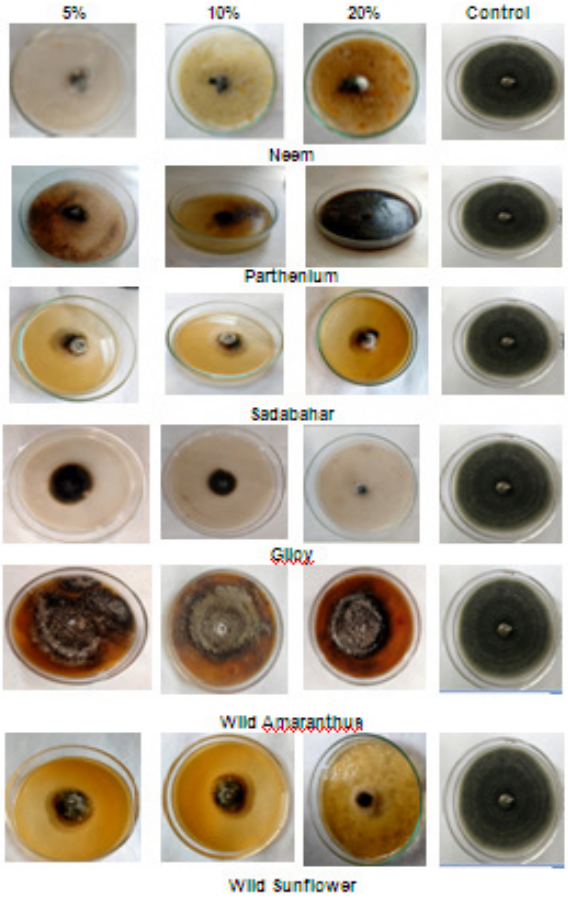

Plate 1: *In vitro* evaluation of plant extracts against *A. brassicicola.*

Six plant extracts were used to manage Alternaria leaf spot of cauliflower by foliar application. Results clearly indicated that among all plant extracts, minimum disease incidence was exhibited by neem (32.44%) followed by parthenium (38.63%) and followed by wild sunflower (43.50%), giloy (45.13%), sadabahar (46.59%) and wild amaranthas (49.60%) as compared to control (54.40%). Maximum reduction in disease incidence over control was exhibited by neem (38.60%) significantly followed by parthenium (29.91%). Wild amaranthas proved least effective, rendered minimum protection (8.82%) from disease incidence (Table 4, Fig. 2 and Plate 2). These findings are similar to those earlier findings, Mahapatra and Das (2013); Singh *et al.* (2013); Bhanage *et al.* (2019).

Table 4: Bioefficacy of different plant extracts against Alternaria leaf spot of cauliflower under field conditions.

Sr. No.	Plant extracts	Concentration	PDI	PDC
1.	Neem	10	32.44	38.60
			(34.76)	
2.	Parthenium	10	38.63	29.91
			(38.43)	
3.	Sadabahar	10	46.59	14.36
			(43.04)	
4.	Giloy	10	45.13	17.04
			(42.21)	
5.	Wild amaranthus	10	49.60	8.82
			(44.77)	
6	Wild sunflower	10	43.50	20.04
			(41.27)	
7	Control	-	54.40	0.00
			(47.52)	(0.00)
SEm±			1.09	
CD (p=0.05)			3.37	
CV (%)			4.54	

* Average of three replications
Figures given in parentheses are angular transformed values

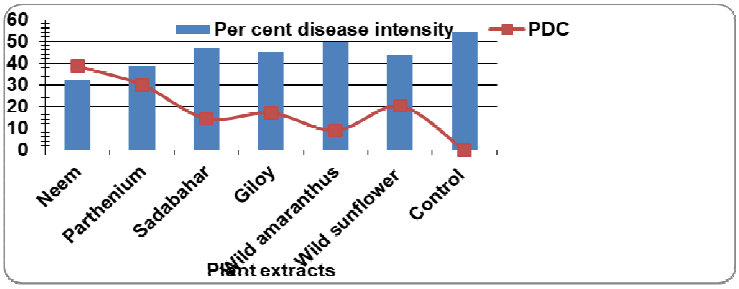

Fig. 2. Bioefficacy of different plant extracts (10%) against Alternaria leaf spot of cauliflower under field conditions.

Plate 2: Overview of the field experiment.

CONCLUSION

Among the tested plant extracts, Neem (*Azadirachta indica*) leaves extract was found most effective against *A. brassicicola* in *in vitro* conditions by inhibiting 85.39 per cent mycelial growth. *Azadirachta indica* leaf extract (10%) also found most effective in the management of the Alternaria leaf spot disease in field conditions by showing 38.60 per cent disease inhibition.

FUTURE SCOPE

In future, use of plant's extract based management technique may become more popular due to its eco-friendly nature and low cost input as now a days government of India is focusing on zero budget natural farming. Plant extract can play a major role in coming days for the management of plant diseases at large scale and protect the farmers from indebtedness by decreasing the cost of production.

Acknowledgments. The authors are grateful to Head, Department of Plant Pathology and Dean SKN Agriculture University, Jobner, Jaipur, Rajasthan 303329, India for valuable guidance, support and facilities provided to conduct this research experiment successfully.
Conflict of Interest. None.

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How to cite this article: Kiran, Shailesh Godika, A.K. Meena, Shivam Maurya, Astha Sharma and Anil Kumar (2022). Management of Alternaria Leaf Spot of Cauliflower by using Plant Extracts. *Biological Forum – An International Journal*, *14*(2): 540-545.