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Akhilesh Patel

Ex-M.Sc. Student, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj (Allahabad), Uttar Pradesh, India

Mohd. Danish

Ph.D. Scholar, Department of Entomology, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj (Allahabad), Uttar Pradesh, India

Mukesh Kumar Prajapati

Ph.D. Scholar, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj (Allahabad), Uttar Pradesh, India

Anoop Kumar

Ph.D. Scholar, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj (Allahabad), Uttar Pradesh, India

Abhilasha A Lal

Assistant Professor, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj (Allahabad), Uttar Pradesh, India

Corresponding Author:

Akhilesh Patel

Ex-M.Sc. Student, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj (Allahabad), Uttar Pradesh, India

In vitro efficacy of plant extracts against *Alternaria lini* (leaf blight of linseed)

Akhilesh Patel, Mohd. Danish, Mukesh Kumar Prajapati, Anoop Kumar and Abhilasha A Lal

Abstract

This research work deals with the study of “*In vitro* efficacy of plant extracts against *Alternaria lini* (leaf blight of linseed)”. It is the second most important *rabi* oilseed crop and stands next to rapeseed-mustard in area of cultivation and seed production in India. *Alternaria lini* is a highly destructive pathogen. The disease caused by this fungus is characterized by leaf spot appearance on the plant leaves. Small circular dark brown lesion with paler margin and yellow halo, usually circular in shape, first small and then enlarged to 2 to 3 cms in diameter. Leaf blight is one of the most economically important diseases worldwide. It affects every part of infected plant. Regular use of fungicide will be hazardous for the soil and humans as well. The lab experiment was analysed using C.R.D. (Complete Randomized Design) with three replications and eight treatments including six botanicals. Treatments like control (Untreated), Jatropha (5%, 8% and 12%), Tulsi (5%, 8% and 12%), Neem (5%, 8% and 12%), Neem (5%, 8% and 12%), Bougainvillea (5%, 8% and 12%), Gulmohar (5%, 8% and 12%) and Parthenium (5%, 8% and 12%) Observations were recorded at different time intervals at 72 and 96hrs for the mycelium growth of the pathogen and inhibition per cent. Jatropha leaf extract @12% recorded lowest radial growth and highest radial inhibition (%) against *Alternaria lini* causing leaf blight of linseed (*Linum usitatissimum* L.). The results of present experiment are under Allahabad conditions as such more trials should be carried out in future to validate the findings.

Keywords: *Alternaria lini*, botanicals, management

Introduction

Linseed or flax (*Linum usitatissimum* L., 2n= 30, X = 15) belongs to the order Malpighiales, the family Linaceae, and the tribe Lineae. Linseed is an important *rabi* oil seed crop and a major source of oil and fibre. The average productivity of this crop is very low (about 3.0 q/ha), for which diseases are one of the major reasons. Among fungal diseases, *Alternaria* leaf /bud blight caused by *A. lini* Dey is a serious threat in Northern high humidity regions of the country causing 58.44% yield losses (Singh *et al.*, 2014) [16]. Linseed is one of the most important crops of the world cultivated in over 2.6 million ha. The important linseed growing countries are India, Canada, China, USA and Ethiopia. India ranks first in the world in respect of acreage accounting for 23.8% of the world total and third in production contributing of 10.2% of the world total. Canada and China are other main linseed producers in the world. The productivity is the highest in Romania (1751.4 kg/ha). It is cultivated in the world over an area of 22.70 lakh ha with a production of 22.39 lakh t and productivity of 986 kg/ha. In India, it occupies an area of 3.38 lakh ha with a production of 1.47 lakh t and a productivity of 435 kg/ha (FAO, 2013) [5]. The major part of linseed growing area lies in the states of Madhya Pradesh, Himachal Pradesh, Chhattisgarh, Uttar Pradesh, Maharashtra, Bihar, Odisha, Jharkhand, Karnataka and Assam accounting for more than 97 per cent of the total area. (Anonymous, 2014) [2]. India area of linseed in India 0.31 lakh hectare and production of linseed 1.55 metric ton and its productivity rate is 1285 q/hac in 2016-2017 and the major linseed producing states in India are: West Bengal, Bihar, Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Orissa, and Uttar Pradesh. The area, production and productivity of linseed in Uttar Pradesh were 0.23 mha, 60.48 MT, 0.12 Mt/ha and 4.78 q/ha, respectively (National Horticulture Board, 2016-2017) [12]. Disease was first reported by Dey (1933) from Kanpur in Uttar Pradesh. The disease was found to cause heavy damage, especially in low lying, ill-drained fields with the yield losses ranging from 28-60 per cent. (Chauhan and Srivastava 1975) [4].

Materials and Methods

Keeping in view the present study entitled “*In vitro* efficacy of plant extracts against *Alternaria lini* (leaf blight of linseed)” under *in vitro* was conducted in the Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad during *rabi* season in NOV2016-March2017. A field trial was also conducted with 7 treatments. Linseed was sown during 2015 in CRBD with 3 replications. For the cultural studies fifteen days old culture of *Alternaria* associated with linseed which was grown on PDA medium was used for inoculating the solid media in petri plates. The size of the inoculum was standardized by cork borer having an internal diameter of 7 mm. The inoculum from agar plate culture was cut with sterilized Cork borer and was placed at the centre of the petriplate in the inverted position with the help of inoculating loop, so that the inoculums come in direct contact with the surface of the medium, then the inoculated petri plates were kept in an Incubator at 25±1°C for 12 days. Radial growth of the fungus was recorded and the diameter of the fungal colony grown on PDA media in petri plates was measured in two directions at right angle to each other in mm. For preparation of leaf extract were leaves *Jatropha* *Gulmohar* *Parthenium* *Neem* *Tulsi* and *Bougainvillea* washed in running tap water followed by washing in distilled water, air dried. The tissues were homogenized in distilled water (1:1 w/v) using a blender. The mixture was filtered through a four layer of moistened muslin cloth and washed the filter and centrifuged. The supernatant thus obtained was designated as concentrated leaf extract. Leaf extract was used as *in vitro* condition @ 5%, 8% and 12%. The lab experiment was analysed by using C.R.D. (Complete Randomized Design) with three replications and eight treatments under *in vitro* condition with six botanicals. Treatments like control (Untreated), *Jatropha* (5%, 8% and 12%), *Tulsi* (5%, 8% and 12%), *Neem* (5%, 8% and 12%), *Neem* (5%, 8% and 12%), *Bougainvillea* (5%, 8% and 12%), *Gulmohar* (5%, 8% and 12%) and *Parthenium* (5%, 8% and 12%) Observations were recorded at different time intervals at 72 and 96 hrs for the mycelium growth of the pathogen and inhibition per cent.

Results and Discussion

The results of study entitled, “*In vitro* efficacy of plant extracts against *Alternaria lini* (leaf blight of linseed)” under lab condition were conducted at the Department of Plant Pathology, Sam Higginbottom University of Agricultural, Technology and Sciences (SHUATS), Allahabad. Observations were recorded at successive stages of radial growth (mm) and Radial growth inhibition (%) T_0 . The data reported in table 4.1 revealed that after 72 hours of inoculation, minimum average radial growth (mm) of *A. lini* was observed in T_1 -*Jatropha* (15.22 mm) followed by T_2 -*Tulsi* (16.66 mm), T_3 -*Neem* (16.96 mm), T_4 -*Parthenium* (16.97 mm), T_5 -*Bougainvillea* (17.05 mm) and T_6 -*Gulmohar* (17.97 mm) as compared to the untreated-control (25.32 mm). Whereas 96 hours of inoculation, minimum average radial growth (mm) of *A. lini* was observed in T_1 -*Jatropha* (23.58 mm) followed by T_2 -*Tulsi* (24.30 mm), T_3 -*Neem* (24.87 mm), T_4 -*Parthenium* (24.93 mm), T_5 -*Bougainvillea* (24.94 mm) and T_6 -*Gulmohar* (25.22 mm) as compared to the untreated T_0 -control (33.18 mm). All the treatments were found statistically significant over T_0 - Control and among the treatments (T_3 , T_4 and T_5) were found non-significant to each other CD (0.05%) 1.150.

The data reported in table 4.2 revealed that after 72 hours of inoculation, minimum average radial growth (mm) of *A. lini* was observed in T_1 -*Jatropha* (13.26mm) followed by T_2 -*Tulsi* (16.26 mm), T_3 -*Neem* (16.48 mm), T_4 -*Parthenium* (16.49 mm), T_5 -*Bougainvillea* (16.82 mm) and T_6 -*Gulmohar* (16.96 mm) as compared to the untreated T_0 -control (25.33 mm). Whereas after 96 hours of inoculation, minimum average Radial growth (mm) of *A. lini* was observed in T_1 -*Jatropha* (21.07 mm) followed by T_2 -*Tulsi* (23.26 mm), T_3 -*Neem* (23.55 mm), T_4 -*Parthenium* (23.57 mm), T_5 -*Bougainvillea* (23.64 mm) and T_6 -*Gulmohar* (23.71 mm) as compared to the untreated T_0 -control (33.18 mm). All the treatments were found statistically significant over T_0 - Control and among the treatments (T_2 and T_3) and (T_3 , T_4 , T_5 and T_6) were found non-significant to each other. CD (0.05%) 0.065.

The data reported in table 4.3 revealed that after 72 hours of inoculation, minimum average radial growth (mm) of *A. lini* was observed in T_1 -*Jatropha* (11.27 mm) followed by T_2 -*Tulsi* (12.31 mm), T_3 -*Neem* (12.33 mm), T_4 -*Parthenium* (12.78 mm), T_5 -*Bougainvillea* (12.79 mm) and T_6 -*Gulmohar* (12.95 mm) as compared to the untreated T_0 -control (25.33 mm). Whereas, after 96 hours of inoculation, minimum average radial growth (mm) of *A. lini* was observed in T_1 -*Jatropha* (17.15 mm) followed by T_2 -*Tulsi* (18.07 mm), T_3 -*Neem* (18.18mm), T_4 -*Parthenium* (18.43 mm), T_5 -*Bougainvillea* (18.64 mm) and T_6 -*Gulmohar* (18.85 mm) as compared to the untreated T_0 -control (33.18 mm). All the treatments were found statistically significant over T_0 - Control and among the treatments (T_3 , T_4 and T_5) were found non-significant to each other. (CD (0.05%) 0.279).

The data reported in table 4.4 showed the response of plant leaf extracts on radial mycelia growth inhibition (%). Leaf extract of *Jatropha* (5%), *Tulsi* (5%), *Neem* (5%), *Parthenium* (5%), *Bougainvillea* (5%) and *Gulmohar* (5%) were tested against *A. lini*. All the botanicals tested were significantly effective in inhibitor growth (%) of pathogen over control (Untreated). Among different plant extracts tested *Jatropha* (39.91%) @ 5% showed maximum inhibition of *A. lini* followed by *Tulsi* (34.23%), *Neem* (33.04%), *Parthenium* (33.00%) and *Bougainvillea* (32.69%) least effectiveness was found in *Gulmohar* (29.06%). Leaf extract of *Jatropha* (8%), *Tulsi* (8%), *Neem* (8%), *Parthenium* (8%), *Bougainvillea* (8%) and *Gulmohar* (8%) were tested against *A. lini*. All the botanicals tested were significantly effective in inhibition growth (%) of pathogen over control (Untreated). Among different plant extracts tested *Jatropha* (47.65%) @ 8% showed maximum inhibition of *A. lini* followed by *Tulsi* (35.81%), *Neem* (34.94%), *Parthenium* (34.90%) and *Bougainvillea* (33.60%) least effectiveness was found in *Gulmohar* (33.04%). Leaf extract of *Jatropha* (12%), *Tulsi* (12%), *Neem* (12%), *Parthenium* (12%), *Bougainvillea* (12%) and *Gulmohar* (12%) were tested against *A. lini*. All the botanicals tested were significantly effective in inhibition growth (%) of pathogen over control (Untreated). Among different plant extracts tested *Jatropha* (55.51%) @ 12% showed maximum inhibition of *A. lini* followed by *Tulsi* (51.40%), *Neem* (51.32%), *Parthenium* (49.55%) and *Bougainvillea* (49.51%) least effectiveness was found in *Gulmohar* (48.87%).

The data reported in table 4.5 showed the response of plant leaf extracts on radial mycelia growth inhibition (%). Leaf extract of *Jatropha* (5%), *Tulsi* (5%), *Neem* (5%), *Parthenium* (5%), *Bougainvillea* (5%) and *Gulmohar* (5%) were tested against *A. lini*. All the botanicals tested were significantly

effective in inhibition growth (%) of pathogen over control (Untreated). Among different plant extracts tested *Jatropha* (28.93%) @ 5% showed maximum inhibition of *A. lini* followed by Tulsi (26.76 %), Neem (25.05%), Parthenium (24.86%) and Bougainvillea (24.83%) least effectiveness was found in Gulmohar (23.99%). Leaf extract of *Jatropha* (8%), Tulsi (8%), Neem (8%), Parthenium (8%), Bougainvillea (8%) and Gulmohar (8%) were tested against *A. lini*. All the botanicals tested were significantly effective in inhibition of growth (%) of pathogen over control (Untreated). Among different plant extracts tested *Jatropha* (36.50%) @ 8% showed maximum inhibition of *A. lini* followed by Tulsi (29.90%), Neem (29.02%), Parthenium (28.96%) and Bougainvillea (28.75%) least effectiveness was found in Gulmohar (28.54%). Leaf extract of *Jatropha* (12%), Tulsi (12%), Neem (12%), Parthenium (12%), Bougainvillea (12%) and Gulmohar (12%) were tested against *A. lini*. All the botanicals tested were significantly effective in inhibition of growth (%) of pathogen over control (Untreated). Among different plant extracts tested *Jatropha* (48.31%) @ 12% showed maximum inhibition of *A. lini* followed by Tulsi (45.54%), Neem (45.21%), Parthenium (44.45%) and Bougainvillea (43.82%) least effectiveness was found in Gulmohar (43.19%). The probable reason for such findings about *Jatropha* leaf extract may be due to cytotoxicity, metabolite profile and anti-microbial activity. The presence of alkaloids, flavonoids, leucoanthocyanins, saponins, tannins and phenolics may have inhibited the growth of pathogen. Similar findings have been reported by Singh *et al.* (2017)^[18]. The pathogenic fungus (*Alternaria lini*) was isolated from the affected tissues on potato dextrose agar medium. The isolated pathogen produced a similar cultural character which was similar to the description given by Simmons, (1967)^[15]. In

order to understand the fungal properties of leaf extracts of different species of *Jatropha*, the extracts of four species of *Jatropha* at 20% concentration were tested against growth plant pathogenic fungi. All the four species of *Jatropha* showed inhibitory nature for mycelia growth of all the fungi tested, however *Jatropha curcas* exhibited maximum inhibitory action. Similar results on the efficacy of plant extracts against *Alternaria* sp. have been reported by Shivpuri *et al.* (1998)^[14], Fawzi *et al.* (2009)^[6], Taskeen *et al.* (2010)^[19], Baraka *et al.* (2011)^[3]. This reduction was gradually increased by increasing the concentration of extracts in the growth medium. Similar effect of various other plant products effective against *Alternaria spp.* have been reported by several workers (Latha *et al.*, 2009, Goussous *et al.*, 2010)^[10, 8]. The inhibitory effect of the tested plant extracts may be due to their direct toxic effect to the pathogen as reported by Vijayan (1989)^[20]. Investigations on mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly (Amadioha, 2000)^[1], or induce systemic resistance in host plants resulting in reduction of disease development (Kagale *et al.*, 2004)^[9]. All the botanicals plant extracts evaluated *in vitro* were found fungistatic and significantly inhibited mycelia growth of *Alternaria lini*. The fungistatic effect of the botanicals might be due to the presence of specific antifungal chemical compounds like phenols, tannins, alkaloids, resinous and non-volatile substances. Similar results of antifungal/ fungistatic effect of botanicals plant extracts against *Alternaria lini* and *Alternaria spp.* were reported earlier by several workers (Singh and Mujumdar, 2001; Ghosh *et al.*, 2002; Mesta *et al.*, 2009; Ranaware *et al.*, 2010)^[17, 7, 11, 13].

Table 4.1: Radial growth (mm) of *Alternaria lini* as affected by different treatments

Treatment@ 5%		Radial growth (mm) of <i>Alternaria lini</i> and radial inhibition per cent		
		72 hrs	96 hrs	Radial inhibition (%)*
T ₀	Control (Untreated)	25.33	33.18	00.00
T ₁	<i>Jatropha</i> leaf extract	15.22	23.58	76.00
T ₂	Tulsi leaf extract	16.66	24.30	75.16
T ₃	Neem leaf extract	16.96	24.87	75.13
T ₄	Parthenium leaf extract	16.97	24.93	74.95
T ₅	Bougainvillea leaf extract	17.05	24.94	73.23
T ₆	Gulmohar leaf extract	17.97	25.22	71.06
Mean		18.02	25.86	73.55
F- test		S	S	S
S. Ed. (±)		0.002	0.206	0.094
C. D. (P = 0.05)		0.166	1.150	0.285
C.V.		0.688	3.824	2.629

Table 4.2: Radial growth (mm) of *Alternaria lini* as affected by different treatments

Treatment@ 8%		Radial growth (mm) of <i>Alternaria lini</i> and radial growth inhibition per cent		
		72hrs	96hrs	Radial growth inhibition (%)*
T ₀	Control (Untreated)	25.33	33.18	00.00
T ₁	<i>Jatropha</i> leaf extract	13.26	21.07	71.45
T ₂	Tulsi leaf extract	16.26	23.26	71.24
T ₃	Neem leaf extract	16.48	23.55	70.98
T ₄	Parthenium leaf extract	16.49	23.57	70.85
T ₅	Bougainvillea leaf extract	16.82	23.64	70.10
T ₆	Gulmohar leaf extract	16.96	23.71	63.50
Mean		17.37	24.56	69.52
F- test		S	S	S
S. Ed. (±)		0.041	0.001	0.0.98
C. D. (P = 0.05)		0.515	0.065	0.296
C.V.		2.465	0.244	0.688

Table 4.3: Radial growth (mm) of *Alternaria lini* as affected by different treatments

Treatment@ 12%		Radial growth (mm) of <i>Alternaria lini</i> and radial growth inhibition per cent		
		72 hrs	96 hrs	Radial growth inhibition (%)*
T ₀	Control (Untreated)	25.33	33.18	00.00
T ₁	Jatropha leaf extract	11.27	17.15	56.81
T ₂	Tulsi leaf extract	12.31	18.07	56.17
T ₃	Neem leaf extract	12.33	18.18	55.54
T ₄	Parthenium leaf extract	12.78	18.43	54.79
T ₅	Bougainvillea leaf extract	12.79	18.64	54.46
T ₆	Gulmohar leaf extract	12.95	18.85	51.69
Mean		14.25	20.36	53.25
F- test		S	S	S
S. Ed. (±)		0.002	0.011	0.075
C. D. (P = 0.05)		0.042	0.279	0.228
C.V.		0.305	1.300	0.640

Table 4.4: Radial growth (mm) of *Alternaria lini* as affected by different treatments at 72 hours

Treatment	Concentration					
	5%		8%		12%	
	Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition
Control (Untreated)	25.33	0.00	25.33	0.00	25.33	0.00
Jatropha leaf extract	15.22	39.91	13.26	47.65	11.27	55.51
Tulsi leaf extract	16.66	34.23	16.26	35.81	12.31	51.40
Neem leaf extract	16.96	33.04	16.48	34.94	12.33	51.32
Parthenium leaf extract	16.97	33.00	16.49	34.90	12.78	49.55
Bougainvillea leaf extract	17.05	32.69	16.82	33.60	12.79	49.51
Gulmohar leaf extract	17.97	29.06	16.96	33.04	12.95	48.87
	Result	S. Ed. (±)	C.D. at 5%			
Due to Concentration	S	0.524	1.111			
Due to Treatment	S	0.800	1.697			

Table 4.5: Radial growth (mm) of *Alternaria lini* as affected by different treatments at 96 hours

Treatment	Concentration					
	5%		8%		12%	
	Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition
Control (Untreated)	33.18	0.00	33.18	0.00	33.18	0.00
Jatropha leaf extract	23.58	28.93	21.07	36.50	17.15	48.31
Tulsi leaf extract	24.3	26.76	23.26	29.90	18.07	45.54
Neem leaf extract	24.87	25.05	23.55	29.02	18.18	45.21
Parthenium leaf extract	24.93	24.86	23.57	28.96	18.43	44.45
Bougainvillea leaf extract	24.94	24.83	23.64	28.75	18.64	43.82
Gulmohar leaf extract	25.22	23.99	23.71	28.54	18.85	43.19
	Result	S. Ed. (±)	C.D. at 5%			
Due to Concentration	S	0.694	1.472			
Due to Treatment	S	1.061	2.249			

Conclusion

Jatropha leaf extract @12% recorded lowest radial growth and highest radial inhibition (%) against *Alternaria lini* causing leaf blight of linseed (*Linum usitatissimum* L.). The results of present experiment are under Allahabad conditions as such more trials should be carried out in future to validate the findings.

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