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Evaluation of non-conventional chemicals for the management of Alternaria blight disease of mustard

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Abstract
Alternaria blight of mustard incited by *Alternaria brassicae* (Berk.) Sacc.) Is one of the most devastating fungal disease which causing significant yield losses. The aim of this study was to evaluate the fungicidal effect of non-conventional chemicals *viz.*, salicylic acid (SA), acetyl salicylic acid (ASA), indole acetic acid (IAA), indole butyric acid (IBA), zinc sulfate ($ZnSo_4$) and magnesium sulfate ($MgSo_4$) against alternaria blight disease of mustard. The experiment was carried out through poison food technique under *in vitro* and through foliar spray under screen house conditions. Salicylic acid inhibited mycelial growth up to 49.2 per cent at 150µg/ml concentration followed by indole acetic acid which inhibited the fungal growth up to 41.7 per cent at 150µg/ml concentration. Zinc sulphate was found least effective among all six non-conventional chemicals, as this chemical inhibited 1.1 per cent of mycelial growth even at 100µg/ml concentration. Screen house experiment was conducted to test the efficacy of these non-conventional chemicals. A maximum control of 24.5 per cent was recorded by salicylic acid at 100µg/ml concentration after six weeks of challenge inoculation by the pathogen followed by indole acetic acid, as it controlled the disease 22.9 per cent at 100µg/ml concentration in cv. RH 30.

Keywords: Alternaria blight, *A. brassicae*, mycelia growth, non-conventional chemicals

Introduction
Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is one of the major oilseed crops cultivated in India and around the world. Among the various oilseed crops cultivated in India, the rapeseed mustard is accounted for 25 per cent total area and 1/3 of total oil production in the country after groundnut. India ranked third after Canada and China in area (19.3%), and production *i.e.* 11.1 per cent (Shekhawat *et al.*, 2012; Saharan *et al.*, 2016) [15, 14]. In India, rapeseed mustard crops are cultivated on an area of 66.52 lack hectare with a production of 71.09 lack tonnes and with an average yield of 1069 kg/ha (Anonymous, 2016) [1]. Despite considerable increase in productivity and production in recent years, still a wide gap exists between yield potential and yield realized at farmer's field, which is largely due to biotic and abiotic stresses. Among biotic stresses, Alternaria blight disease incited by the necrotrophic pathogen *Alternaria brassicae* (Berk.) Sacc. has been reported to be most widespread and destructive fungal disease of rapeseed-mustard throughout the world. In Haryana state the Alternaria disease appears every year in severe form at pod initiation stage (Rathi and Singh, 2009) [13]. It affects seed germination as well as quality and quantity of oil (Meena *et al.*, 2010) [5]. Various options are accessible to the farmers to protect their crop from disease including development of resistant cultivars, biological control, and use of fungicides. Fungicides check the disease spread but have poor compliances including human health hazards (Thakur *et al.*, 2014) [16]. The pathogen continuously changes its nature and rapidly the resistant cultivars become susceptible (Chaudhary *et al.*, 2001) [2]. Therefore, there is need to alleviate infection by activating plant's own defense system with the help of low molecular weight non-conventional chemicals called elicitors (Cohen *et al.*, 1999) [3]. The use of non-conventional chemicals lacks environmental and toxicological side-effects and is effective against a variety of plant pathogens. Kumar *et al* (2015) [6] reported that out of five eco-friendly chemicals, K_2So_4 1000 ppm (64.28%) followed by $ZnSo_4$ 1000 ppm (63.88%) showed maximum inhibition of mycelial growth under *in vitro* conditions. The spray of elicitors has been reported to effectively manage Alternaria blight of *B. juncea* (Meena *et al.*, 2004; Patni and Kolte, 2006) [11, 12]. The eco-friendly approach to defeat biotic stress helped to optimize the economic yields and enable farmers to grow healthy Indian mustard crops (Meena *et al.*, 2011; Meena *et al.*, 2013; Kumar *et al.*, 2014) [9, 10, 5]. Kumar *et al* (2014) [5] reported that foliar

application of $CaSo_4$ at 0.5 per cent concentration showed maximum fungicidal effect against Alternaria blight of mustard, while zinc sulphate was found least effective in controlling the disease. Foliar spray of the treatment containing BTH (3 ppm) + SA (33 ppm) showed maximum reduction in disease severity (33.9%) as compared to control (Thakur and Sohal, 2014) [16]. The present investigations were carried out to find out the fungicidal effect of non-conventional chemicals for management of Alternaria blight disease of mustard.

Materials and methods

In vitro screening of non-conventional chemicals for fungitoxic effect against *Alternaria brassicae*

The efficacy of six non-conventional chemicals *viz.*, salicylic acid (SA), acetyl salicylic acid (ASA), indole acetic acid (IAA), indole butyric acid (IBA), zinc sulfate ($ZnSo_4$) and magnesium sulfate ($MgSo_4$) on the growth of *A. brassicae* were tested *in vitro* using the standard method of poison food technique as given by Mayer (1962). Stock solution of each non-conventional chemical was prepared in double strength *i.e.* 10, 50 and 100 µg/ml by dissolving weighed or measured quantity of non-conventional chemical in a measured volume of sterilized water. The double strength PDA medium was also prepared and sterilized at 15 lbs pressure for 20 minutes. An equal volume of chemical solution and PDA was mixed in a sterilized conical flask and poured aseptically in the Petri plates. After solidification of medium, each Petri plate was centrally inoculated with 5 mm disc of fungus taken from 10 days old culture of *A. brassicae* with the help of sterilized cork borer and incubated at 26±1°C. Suitable controls were maintained for each chemical. Four replications of each fungicide were maintained and completely randomized design was followed. Colony diameter of the *A. brassicae* of each treatment along with control was recorded with metric scale (mm) till the fungus of controlled treatment occupied the full area of Petri plate within which it was growing. Radial growth of fungus was measured with metric scale (mm). The per cent inhibition of mycelial growth over control was calculated by following formula given by Vincent (1947).

$$\text{Growth inhibition (\%)} = \frac{(C-T)}{C} \times 100$$

Where,
C= Radial growth of *A. brassicae* mycelium in control.
T= Radial growth of *A. brassicae* mycelium in treatment.

Efficacy of different non-conventional chemicals for management of Alternaria blight under screen house conditions

Forty-five to fifty days old plants of two mustard varieties RH 30 and RH 0749 were raised in the pots in screen house conditions and sprayed by selected non-conventional chemicals (SA, ASA, IAA, IBA, $ZnSo_4$ and $MgSo_4$) in different concentrations *viz.*, 10, 50 and 100µg/ml. The spore suspension was made from 7-8 days old culture of *A. brassicae* in sterile distilled water. After 24 h of chemical treatment both the varieties was inoculated by spray spore suspension (10^5spores/ml) method. A hand atomizer was used for spraying the suspension and atomizer was pre sterilized with 90 per cent ethanol before the spraying. The experiment was carried out with four replications (Five plants/ replication) and completely randomized design was followed. Suitable controls were also maintained for each chemical by plants were sprayed with sterile water before challenge inoculation by the pathogen. Observation on disease severity was calculated by using 0-6 disease rating scale (Conn *et al.*, 1990).

Per cent disease severity was calculated by using following formula:

$$\text{Per cent disease severity} = \frac{\text{Sum of all numerical ratings}}{\text{No. of leaves examined} \times \text{maximum grade}} \times 100$$

Results and discussion

In vitro screening for fungitoxic effect against *Alternaria brassicae*

Efficacy of six non-conventional chemicals was tested *in vitro* for the per cent mycelia growth inhibition of *Alternaria brassicae* through standard procedure of poison food technique. The results presented in Table 1 revealed that salicylic acid inhibited mycelial growth up to 49.2 per cent at 150µg/ml concentration followed by indole acetic acid which inhibited the fungal growth up to 41.7 per cent at 150µg/ml concentration. Indole butyric acid provided inhibition up to 38.6 per cent at 150µg/ml concentration as compare to 6.7 per cent inhibition at 10µg/ml concentration. Acetyle salicylic acid inhibited fungal growth up to 29.4 per cent at 150µg/ml concentration, while it found ineffective in inhibition of fungal growth at 10µg/ml concentration. Zinc sulphate was found least effective among all six non-conventional chemicals, as this chemical inhibited 1.1 per cent of mycelial growth even at 100µg/ml concentration.

Efficacy of different non-conventional chemicals for management of Alternaria blight under screen house conditions

Efficacy of six non-conventional chemicals was tested under screen house conditions for control of Alternaria blight on two mustard varieties RH 30 and RH 0749. The results of experiment presented in Table 2 & 3 revealed that as the concentration of different non-conventional chemicals increased from 10µg/ml to 100µg/ml there was increase in per cent disease control under screen house conditions. A maximum control of 24.5 per cent was recorded by salicylic acid at 100µg/ml concentration after six weeks of challenge inoculation by the pathogen followed by indole acetic acid, as it controlled the disease 22.9 per cent at 100µg/ml concentration in cv. RH 30 (Table 2). Indole butyric acid controlled the disease 20.9 per cent at 100µg/ml concentration after six weeks of challenge inoculation, while it controlled 10.0 per cent at 100µg/ml after two weeks of challenge inoculation. However, zinc sulphate was found least effective in reducing the disease severity, as it controlled the disease up to 8.1 per cent after six weeks of challenge inoculation (Table 2). Similar trends of results were observed for above mentioned treatments under screen house conditions on cultivar RH 0749 (Table 3). A maximum control of 25.6 per cent was observed by salicylic acid at 100µg/ml concentration after six weeks of challenge inoculation followed by indole acetic acid, as it controlled the disease up to 23.5 per cent. Indole butyric acid controlled the disease 21.6 per cent at 100µg/ml concentration after six weeks of challenge inoculation, while it controlled 10.5 per cent at 100µg/ml after two weeks of challenge inoculation. A minimum control of 8.6 per cent was recorded by zinc sulphate even at 100µg/ml concentration after six

week of challenge inoculation. Results are in agreement with that of Kumar *et al* (2014) [5] where they reported that foliar application of $CaSo_4$ at 0.5 per cent concentration showed maximum fungicidal effect against Alternaria blight of mustard, while zinc sulphate was found least effective in controlling the disease. Foliar spray of the treatment containing BTH (3 ppm) + SA (33 ppm) showed maximum reduction in disease severity (33.9%) as compared to control (Thakur and Sohal, 2014) [17].

Table 1: *In vitro* evaluation of different non-conventional chemicals against *Alternaria brassicae.*

Treatments	Growth inhibition (%) at different concentration (µg)				Mean
	10*	50*	100*	150*	
Salicylic acid	21.4 (27.5)	29.4 (32.8)	38.6 (38.4)	49.2 (44.5)	34.7 (35.8)
Acetyle salicylic acid	0.0 (2.9)	4.4 (12.1)	15.8 (23.4)	29.4 (32.8)	12.4 (17.8)
Indole acetic acid	13.6 (21.6)	23.9 (29.2)	30.3 (33.3)	41.7 (40.1)	27.4 (31.0)
Indole butyric acid	6.7 (14.8)	13.3 (21.3)	23.1 (28.7)	38.6 (38.4)	20.4 (25.8)
Zinc sulfate	0.0 (2.9)	0.0 (2.9)	1.1 (5.9)	23.3 (28.9)	6.1 (10.1)
Magnesium sulfate	5.8 (13.6)	10.6 (18.9)	20.0 (26.5)	35.6 (36.6)	18.0 (23.9)
Mean	7.9 (13.9)	13.6 (19.6)	21.5 (26.0)	36.3 (36.9)	
	Treatment	Concentration	Treatment ×Concentration		
SEm±	0.4	0.3	0.8		
C.D. (p=0.05)	1.1	0.9	2.3		

*Mean of four replications
Figures in parenthesis indicate angular transformed values

Table 2: Effect of non-conventional chemicals on Alternaria blight severity on mustard cv. RH 30 under screen house conditions.

Treatments	After two weeks						After four weeks						After six weeks					
	10 µg/ml		50 µg/ml		100 µg/ml		10 µg/ml		50 µg/ml		100 µg/ml		10 µg/ml		50 µg/ml		100 µg/ml	
	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C
SA	21.9 (27.9)	10.6	19.7 (26.3)	11.2	18.2 (25.2)	15.0	23.8 (29.1)	14.9	22.2 (28.0)	15.8	21.5 (27.6)	16.6	28.4 (32.1)	17.5	27.4 (31.5)	20.9	27.0 (31.3)	24.5
ASA	23.7 (29.1)	3.1	21.4 (27.5)	3.9	20.4 (26.8)	4.4	26.0 (30.6)	6.8	24.2 (29.4)	8.2	23.4 (28.9)	9.3	32.0 (34.4)	7.1	30.7 (33.6)	11.3	30.1 (33.2)	15.8
IAA	22.1 (28.0)	9.5	20.1 (26.6)	9.6	18.7 (25.6)	12.5	24.1 (29.4)	13.8	22.6 (28.3)	14.3	21.9 (27.9)	15.1	28.9 (32.5)	15.9	28.0 (31.9)	19.2	27.6 (31.6)	22.9
IBA	22.9 (28.5)	6.5	20.4 (26.8)	8.4	19.2 (25.9)	10.0	24.5 (29.6)	12.4	23.0 (28.6)	12.8	22.3 (28.1)	13.5	29.4 (32.8)	14.6	28.4 (32.1)	18.2	28.3 (32.1)	20.9
$ZnSo_4$	24.3 (29.5)	0.8	22.0 (27.9)	1.0	21.0 (27.2)	1.6	27.4 (31.5)	2.0	25.7 (30.5)	2.4	24.7 (29.8)	4.0	33.5 (35.3)	2.5	33.3 (35.2)	3.9	32.9 (34.9)	8.1
$MgSo_4$	23.3 (28.8)	4.9	21.0 (27.2)	5.5	19.9 (26.5)	6.9	25.4 (30.3)	8.9	23.8 (29.2)	9.6	22.9 (28.6)	11.1	30.2 (33.3)	12.3	29.4 (32.8)	15.3	29.1 (32.6)	18.6
Control	24.5 (29.6)	-	22.2 (28.1)	-	21.4 (27.5)	-	27.9 (31.9)	-	26.4 (30.9)	-	25.8 (30.5)	-	34.4 (35.9)	-	34.7 (36.0)	-	35.8 (36.7)	-
C. D. (p=0.05)	0.71	-	0.55	-	0.58	-	0.63	-	0.48	-	1.35	-	0.78	-	1.06	-	0.74	-
C. V.	1.40	-	1.14	-	1.24	-	1.17	-	0.94	-	2.67	-	1.30	-	1.79	-	1.25	-

Note: Values in parentheses are angular transformed
PDS- Percent disease severity, % C- Per cent control

Table 3: Effect of non-conventional chemicals on Alternaria blight severity on mustard cv. RH 749 under screen house conditions.

Treatments	After two weeks						After four weeks						After six weeks					
	10 µg/ml		50 µg/ml		100 µg/ml		10 µg/ml		50 µg/ml		100 µg/ml		10 µg/ml		50 µg/ml		100 µg/ml	
	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C	PDS	% C
SA	20.6 (26.9)	11.5	18.3 (25.3)	12.9	17.5 (24.7)	16.3	22.9 (28.5)	15.2	21.6 (27.6)	16.3	19.2 (25.9)	17.8	27.1 (31.3)	18.6	26.7 (31.0)	21.9	26.0 (30.6)	25.6
ASA	22.4 (28.2)	3.7	20.0 (26.5)	4.8	19.7 (26.4)	5.7	25.0 (29.9)	7.4	23.3 (28.8)	9.7	20.8 (27.1)	10.7	30.8 (33.7)	7.5	29.5 (32.9)	13.5	29.3 (32.8)	16.2
IAA	21.0 (27.3)	9.7	18.9 (25.8)	10.0	18.0 (25.0)	13.9	23.0 (28.6)	14.8	21.9 (27.9)	15.1	19.4 (26.1)	16.8	27.6 (31.7)	17.1	27.0 (31.2)	21.0	26.8 (31.1)	23.5
IBA	21.7 (27.8)	6.7	19.1 (25.9)	8.9	18.7 (25.6)	10.5	23.3 (28.8)	13.7	22.1 (28.0)	14.2	19.9 (26.5)	14.6	28.0 (31.9)	15.9	27.5 (31.6)	19.5	27.4 (31.5)	21.6
$ZnSo_4$	23.0 (28.7)	1.0	20.6 (26.9)	1.9	20.4 (26.9)	2.2	26.4 (30.9)	2.3	25.0 (29.9)	3.1	21.9 (27.9)	6.0	32.2 (34.5)	3.4	32.0 (34.4)	6.3	32.0 (34.4)	8.6
$MgSo_4$	22.0 (27.9)	5.4	19.7 (26.4)	6.0	19.2 (25.9)	8.2	24.1 (29.4)	10.7	22.8 (28.5)	11.5	20.2 (26.7)	13.5	28.8 (32.4)	13.6	28.0 (31.9)	18.0	28.3 (32.1)	19.1
Control	23.3 (28.8)	-	21.0 (27.2)	-	20.9 (27.1)	-	27.0 (31.3)	-	25.8 (30.5)	-	23.3 (28.8)	-	33.3 (35.2)	-	34.2 (35.7)	-	35.0 (36.2)	-
C. D. (p=0.05)	0.29	-	0.19	-	0.73	-	0.54	-	0.67	-	0.61	-	0.73	-	0.48	-	0.99	-
C.V.	0.58	-	0.42	-	1.59	-	1.03	-	1.32	-	1.28	-	1.26	-	0.82	-	1.71	-

Note: Values in parentheses are angular transformed
PDS- Percent disease severity, % C- Per cent control

Conclusion

In conclusion, salicylic acid was found to be the most effective among all non-conventional chemicals in controlling the *A. brassicae* pathogen under *in vitro* and screen house conditions. The findings of this study may lead to effective field strategies for the management of alternaria blight disease of mustard.

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