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Salicylic Acid as Inducer of Resistance against Chilli Fruit Rot Pathogen *Colletotrichum capsici*

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Studies were conducted to test the efficacy of different non-conventional chemicals against *Colletotrichum capsici* under -*in vitro* and screen house conditions at Department of Plant Pathology, CCS Haryana Agricultural University, Hisar. Inhibition of spore germination inhibition method was followed for *in vitro* assay while foliar spray on red fruits of chilli followed by pathogen inoculation by pin prick method was adopted for assessing efficacy under screen house conditions. The pathogen, *C. capsici* was found most sensitive to salicylic acid among different non-conventional chemicals tested under *in vitro* conditions while under screen house conditions, salicylic acid at the concentration of 5mM induced resistance in plants and gave 67.86 and 78.63 per cent disease control in susceptible (Pusa jwala) and resistant (Pusa Sadabahar) variety, respectively over check.

Key words: Chilli, *Colletotrichum capsici*, non-conventional chemicals, salicylic acid.

Chilli (*Capsicum annuum* L.) belonging to the family solanaceae is mainly cultivated for its green fruit and for dry chilli as the spice. It is cultivated in an area of about 7.94 lakhs hectares in India with annual production of 13.04 lakh tones (NHB, 2013). The major chilli growing states are Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu and Rajasthan. Chilli crop suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also by abiotic stresses. Among the major diseases of chilli, fruit rot caused by *Colletotrichum capsici* (Sydow) Butler and Bisby is one of the major constraints in the production of chilli. The disease causes severe damage on red chilli fruits. Many authors reported that only red ripe stages of chilli fruits to be vulnerable for attack by the pathogen rather than green fruits (Chowdhury, 1957). It has been reported to cause 8-27 per cent yield losses in Maharashtra, 20-60 per cent in Punjab and Haryana and 30-76 per cent in Tamil Nadu (Datar, 1995; Bansal and Grover, 1969 and Sujathabai, 1992). Host plant resistance is considered as most practical, feasible and an economical method of plant disease management. Non-conventional chemicals along with host plant resistance can be effective management strategy for this disease.

The non-conventional chemicals are an important signal molecules that plays a critical role in plant defense against pathogen invasion. These chemicals reduce the fungus growth or inhibit spore germination *in vitro*. Rohilla *et al*. (2001) while studying mustard *Albugo candida* system reported that salicylic acid exhibited curative effect. Plants have evolved complex, integrated defense mechanisms against diseases that include

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preformed physical, chemical barriers, as well as inducible defenses such as the production of antimicrobial compounds, enhanced strengthening of cell walls and production of various antifungal proteins. However, survey of literature reveals that there seems to be no report on the effect of salicylic acid in controlling *Colletotrichum capsici* causing anthracnose disease in *C.annuum*. Malamy and Klessig (1992) suggested that salicylic acid plays a key role in the induction and maintenance of plant resistance against wide range of pathogens. Salicylic acid is one of the favoured signal substances. Gaffney *et al*. (1993) studied the requirement of salicylic acid for the induction of systemic acquired resistance in plants. They reported, in plants transformed with the Nah G gene (naphthalene hydroxylase G), the salicylic acid levels were low and systemic acquired resistance was blocked which indicated that salicylic acid is required for induction of systemic acquired resistance. Kauss *et al*. (1992) found salicylic acid and its derivatives as a potential activator for induction of resistance in the plants. Datar (1996) found that dipping chilli fruits in Indole-3-butyric acid or Naphthallic acetic acid at 200 ug/L concentration for 30 minutes delayed the fruit rot caused by *Colletotrichum capsici*, *Alternaria alteranate*, *Aspergillus niger* and *Fusarium solani* for six days.

Meena *et al.* (2001) investigated the effect of salicylic acid in inducing resistance in groundnut against leaf spot caused by *Cercosporidium personatum*. Foliar application of salicylic acid at the concentration of 1mM significantly reduced late leaf spot disease intensity and increased the pod yield under green house conditions.

MATERIALS AND METHODS

Evaluation of non-conventional chemicals *in vitro*

The effect of five non-conventional chemicals *viz.*, salicylic acid, zinc sulphate, magnesium sulphate, indole-acetic acid, indole-butyric acid and a fungicide carbendazim was evaluated *in vitro* by inhibition of spore germination method against *C. capsici.*

Five ml sterilized water was added to culture tubes under aseptic conditions. Spores were harvested by gently scraping the surface growth of the culture. The double strength spore suspension (30 spores/microscopic field) was maintained and double strength of non-conventional chemicals were (50, 100, 250, 500 and 1000 µg/ml) also prepared. Moist chambers containing two cavity slides in a Petridish were prepared. An equal volume of non-conventional chemicals and conidial suspension was mixed and thereafter two drops of this suspension were put on each slide. Petridishes were incubated at 28±1°C for 24 and 48 hours. The spores that had a germ tube to a length exceeding its width were considered as germinated (Singh, 1970). Suitable control with carbendazim (50, 100, 250, 500 and 1000 µg/ml) was also maintained. Four replications of each treatment were maintained and conidial germination was observed after 24 and 48 hours. per cent spore germination inhibition was calculated by the formula suggested by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where

I = per cent inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

Effect of non-conventional chemicals on disease development under screen house conditions

An attempt was made to study the effect of following chemicals against fruit rot of chilli caused by *C. capsici.*

The chemicals used were salicylic acid, zinc sulphate, magnesium sulphate, indole acetic acid, indole butyric acid and fungicide used was Carbendazim. These resistance inducing chemicals and fungicide were used at three conc. i.e. 0.2mM, 1mM and 5mM for determining the effect of non-conventional chemicals and fungicide on disease development under screen house conditions. Stock solution of non-conventional chemicals and fungicide were prepared by dissolving calculated amount of these in 20 ml of acetone and final volume made up to 2000 ml with sterilized distilled water. Subsequent dilution of each non-conventional chemical and fungicide with different conc. (0.2mM, 1mM and 5mM) were sprayed on red fruits of chilli on susceptible variety 'Pusa jwala' and resistant variety 'Pusa sadabahar' in the evening using pin prick method . The red fruits were inoculated with standard spore suspension (3×10^4 spores/ml)

prepared from 8 days old culture after one day of chemical spray. To maintain the higher relative humidity the plants having red fruits were covered with polythene sheet and sprayed with water thrice (morning, noon and evening) in a day. Four replications of each chemical and fungicide with different conc. were maintained. Control plants having red fruits were sprayed with water and pathogen only. Completely randomized design was followed. The disease severity in both varieties was recorded. The length and breadth of the lesions were also measured in case of fruit sprayed with non-conventional chemicals, fungicide as well as in control plants in both the varieties. The fruits were observed after 15 days of pathogen inoculation. The disease severity was measured using 0-5 scale (Jayalakshmi, 1998). (Table 1)

Per cent disease control was computed with following formula:

$$\text{Disease control (\%)} = \frac{\text{Infection index in control - Infection index in treatment}}{\text{Infection index in control}} \times 100$$

RESULTS

Effect of non-conventional chemicals on inhibition of spore germination of *C. capsici* under *in vitro* conditions

It is evident from Table 2 that salicylic acid was effective by inhibiting 61.65 per cent spore germination at 1000 µg/ml concentration followed by indole butyric acid (32.56%) and indole acetic acid (30.17%). Carbendazim was most effective by giving 100 per cent inhibition at 1000 µg/ml conc.

Effect of non-conventional chemicals on disease development under screen house conditions

It is clear from the Table 3 that salicylic acid was most effective in reducing the disease severity as compared to other non-conventional chemicals. At all the conc. Salicylic acid controls more per cent disease as compare to other non-

Table 1. For calculation of disease severity the following disease rating scale (Jayalakshmi, 1998) was used

Fruit area affected	Disease grade
Healthy	0
1.0-5.0%	1
5.1-10.0%	2
10.1-25.0%	3
25.1-50.0%	4
>50.1%	5

Table 2. Effect of non-conventional chemicals on inhibition of spore germination of *Colletotrichum capsici* under *in vitro* conditions

Non –conventional Chemicals	Concentration (µg/ml)																			
	50				100				250				500				1000			
	24h		48h		24h		48h		24h		48h		24h		48h		24h		48h	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Salicylic acid	65.03	31.55	63.25	34.11	60.65	36.82	57.97	40.24	57.33	39.66	56.03	42.24	55.06	42.64	53.1	45.26	52.69	44.54	37.2	61.65
Indole butyric acid	78.96	16.89	74.52	22.38	74.35	22.56	73.57	24.16	72.15	24.05	69.94	27.9	68.96	28.16	68.78	29.1	68.23	28.18	65.41	32.56
Indole acetic acid	77.56	18.36	75.16	21.71	74.93	21.95	74.36	23.34	73.53	22.61	72.08	25.7	70.92	26.12	70.63	27.19	69.52	26.82	67.74	30.17
Zinc sulphate	78.86	16.99	78.55	18.18	77.68	19.09	77.66	19.94	76.58	19.39	75.39	22.28	75.1	21.77	74.91	22.77	73.9	22.21	74.13	23.58
Magnesium sulphate	79.83	15.97	78.83	17.89	78.69	18.03	77.9	19.69	77.53	18.39	77.08	20.54	76.6	20.21	76.18	21.47	75.76	20.25	75	22.68
Carbendazim	23.8	74.9	22.2	76.8	18.3	80.9	16.3	83.1	12.08	87.2	10.7	89.01	5.5	94.3	0.8	99.2	0	100	0	100
Control	95	0	96	0	96	0	97	0	95	0	97	0	96	0	97	0	95	0	97	0
C.D. (p=0.05)	TimeChemicalsConcentrationInteraction																			
	(A)				(B)				(C)				(A×B×C)							
	0.295				0.511				0.466				1.615							

1= % germination 2= % inhibition over control

Table 3. Effect of non-conventional chemicals on chilli fruit rot development in resistant and susceptible variety under screen house conditions during 2013-14 crop season

Chemical	Concentration‘	Pusa jwala’ (Susceptible)		‘Sadabahar’ (Resistant)	
	(mM)	Disease severity (%)	Disease control (%)	Disease severity (%)	Disease control (%)
Salicylic acid	0.2	44.05	45.46	8.25	75.87
	1	39.09	51.60	8.10	76.31
	5	25.96	67.86	7.30	78.63
Indole butyric acid	0.2	59.70	26.07	9.85	71.17
	1	56.13	30.50	9.79	71.36
	5	48.05	40.50	8.20	76.00
Indole acetic acid	0.2	64.21	20.50	9.90	71.04
	1	61.58	23.75	9.80	71.32
	5	51.92	35.71	8.30	75.71
Zinc sulphate	0.2	66.83	17.25	9.99	70.76
	1	61.94	23.31	9.89	71.05
	5	53.12	34.22	8.50	75.13
Magnesium sulphate	0.2	68.3	15.43	9.95	70.87
	1	62.52	22.59	9.90	71.04
	5	54.18	32.91	8.55	74.98
Carbendazim	0.2	60.45	25.15	25.45	25.53
	1	57.49	28.81	18.65	45.42
	5	49.39	38.85	14.62	57.23
Water spray	%	%	%	%	%
Pathogen sprays	%	80.76	00.00	34.17	00.00
C.D. (p=0.05)	Time (A)	Chemicals (B)	Concentration (C)	Interaction (A×B×C)	
	0.174	0.301	0.213	0.737	

conventional chemicals. At the conc. 5mM it controls 67.86 and 78.63 per cent disease in susceptible (Pusa jwala) and resistant (Sadabahar) variety, respectively over check. Indole acetic acid at 5mM controls 40.50 per cent disease in Pusa jwala and 76.0 per cent in Sadabahar. Least reduction in disease control was observed when red fruits were sprayed with zinc sulphate and magnesium sulphate.

DISCUSSION

In the present study, screening of non-conventional chemicals along with fungicide (carbendazim) was done with a view to find out the curative effect of these compounds for inhibition of growth of pathogen *in vitro*. In the present study salicylic acid showed 61.65 per cent spore germination inhibition at 1000 µg/ml conc. The inhibition of spore germination may probably be due to toxic effect at higher conc. The remaining non-conventional chemicals did not show much inhibition of spore germination under *in vitro* conditions. Maximum spore germination inhibition was obtained at 1000 ppm with carbendazim. However, Rohilla *et al*. (2001) when sprayed mustard plants with different concentrations of salicylic acid 1 hour before inoculation with *Albugo candida* observed that it had curative effect. Salicylic acid was phytotoxic at higher conc.

Kauss *et al*. (1992) found that salicylic acid and its derivatives act as a potential activator for induction of resistance in plants. The present studies indicated that salicylic acid was effective in reducing the disease severity significantly under screen house as compared to other non-conventional chemicals. Several workers reported that the salicylic acid was potential activator for inducing resistance when applied before pathogen inoculation (Kauss *et al*., 1992; Malamy and Klessig, 1992; White, 1998 and Meena *et al*., 2001). Rohilla *et al*. (2001) during their study found that

salicylic acid mediated resistance to the treated tissue indicating that salicylic acid does not translocate efficiently when applied exogenously conjugated with â-glucoside and there may be the lack of phloem mobility. When salicylic acid was sprayed on *Brassica juncea* plant one hour before the spray of spores of *Albugo candida*, it exhibited curative effect. It had also phytotoxic effect to .*B. juncea* above 5mM conc.

In the present study indole butyric acid when sprayed at 5mM conc. on red fruits of chilli 24 hours before inoculation showed significant disease control, 40.50 and 76.00 per cent in susceptible and resistant variety, respectively over check. However, zinc sulphate and magnesium sulphate were least effective in controlling the disease in susceptible as well as resistant variety.

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