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Efficacy of chemical inducers against wilt of chickpea incited by *Fusarium oxysporum* f.sp. *ciceri*

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Abstract
The aim of present study was evaluation of different inducers against *Fusarium oxysporum* f.sp. *ciceri* in *in-vitro* in seven treatments with three replications. Efficacy of different chemical inducers *viz*. Salicylic acid, Oxalic acid and Ascorbic acid in controlling the growth of *Fusarium oxysporum* f.sp. *ciceri*, were used in different concentrations i.e. 100ppm and 200ppm using poisoned food technique. The pathogen was isolated from the disease affected roots of chickpea from the field of Uttaranchal University. The fungal discs of seven days old culture of the pathogen were inoculated in poisoned PDA media. The chemical inducers showed decent effective control in the growth of the pathogen at every observation taken. Salicylic acid at 200ppm showed the highest control on the growth of the pathogen while the lowest was shown by Oxalic acid at 100ppm after 7 days of inoculation. The maximum percent inhibition was done by Salicylic acid at 200ppm whereas, the minimum inhibition was done by Oxalic acid at 100ppm.

Keywords: *Fusarium* wilt, chemical inducers, salicylic acid, oxalic acid, ascorbic acid, poisoned food technique

Introduction
The chickpea (*Cicer arietinum*) also called Bengal gram or garbanzo bean is an annual vegetable of the family Fabaceae, subfamily Faboideae. It is significant in Indian, Mediterranean and Middle Eastern cooking eaten both whole fried or boiled and also as split pulse. Chickpea provides great amount of energy, protein, minerals, vitamins, fiber, and also contains potentially health-beneficial phytochemicals (Wood & Grusak, 2007) [14]. Chickpea in India accounts for about 45% of total pulses production. (Shakya *et al.*, 2016) [8] similar to the case of other pulses, India is the major chickpea producing country and contributing for over 75% of total world chickpea production. In India, Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra, Gujarat, Andhra Pradesh and Karnataka are the major chickpea producing states sharing over 95 per cent area. Madhya Pradesh is the biggest chickpea producing state in India, adding to 39% of the nation's production.

A significant reduction in cropping area and production has been recorded during the most recent twenty years. A few biotic and abiotic constraints underlie this decline. Regardless of the efforts conveyed in breeding and selection of a few chickpea varieties with high yield potential that are tolerant to diseases, the circumstance has continued as before for the last decade. *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend Fr. *f. sp. ciceri* (Padwick) Matuo & K. Sato is the major soil-borne fungus affecting chickpeas around the world. *Fusarium* wilt epidemics can destroy harvests and cause up to 100% loss in severely infested fields and under favourable conditions. At national level the yield losses suffered due to wilt may vary between five to ten per cent (Singh and Dahiya, 1973) [9]. The pathogen is both seed and soil borne; facultative saprophyte and can survive in soil up to six years in the absence of susceptible host (Haware *et al*., 1986) [2]. The symptoms appear on leaves start yellowing and eventually dry up and early stages of flowering, 6-8 weeks after sowing and can also appear upto podding stage (late wilt). Although lacking an immune system comparable to animals, plants have developed a stunning array of structural, chemical, and protein-based defenses designed to detect invading organisms and stop them before they are able to cause extensive damage. For the controlling of pathogen use of plant defense inducers is one such technique that has been in use recently. Components such as salicylic acid, ascorbic acid, oxalic acid have shown induction of systemic resistance against several pathogens. The higher levels of polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), β-1, 3-glucanase (PR-2) and phenolics were observed in roots and shoots of resistant cultivar than that of susceptible cultivar on treatment with salicylic acid and *Fusarium oxysporum f. sp. ciceri.*

The pathogen invasion was also more in susceptible cultivar compared with resistant cultivar (Raju *et al.*, 2008) [4]. Oxalic acid has been able to control the growth of *Fusarium oxysporum* when treated at a concentration of 20mM and higher and the resistant is totally OA dependent. They have also suggested that the use of OA signifies that a rather simple organic substance can be used for induction of resistance and that this compound does not need to be pathogen inhibiting itself (Attitalla and Brishammar, 2003) [1]. *In vitro* experiment by application of different SA & chitosan concentrations and observed that they had a direct effect on mycelium growth of both (*F. oxysporum* f. sp. *ciceri*) and (*F. solani*) on PDA. Chitinase and b-1, 4 glucanase enzymes activity and total phenol content in chickpea leaf tissues were evaluated as induced resistance indicators (among others) at 0, 48, 96, and 168 h after inoculation. Increased levels of enzymes activities were observed in 200 ppm of SA however, no significant effects on root rot disease control were obtained in this treatment (Veladi *et al.*, 2013) [12]. The present study was done to understand the efficacy of chemical inducers against the pathogen in lab conditions using suitable nutrient medium.

Materials and methods

Isolation of pathogen

The diseased plant parts showing characteristic symptoms of chickpea wilt were taken from the fields of School of Agriculture, Uttaranchal University for isolation of the causal organism. These diseased plants were washed thoroughly in distilled water to remove the dust particles and surface contaminants.

Maintenance of pure culture

The culture of *Fusarium oxysporum* f.sp. *ciceri* was isolated and maintained on Potato Dextrose Agar medium by regular sub-culturing. The cultures of Foc was grown in sterilized petri plates on Potato Dextrose Agar (PDA) medium for 8 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10 x) of compound microscope and transferred to PDA slants for maintenance. These culture tubes were incubated at 24±1^{0}C for about a week and again sub-cultured on PDA medium and then stored in a refrigerator at 05^0±1C for further use.

Preparation of chemical inducers of different concentration

All the chemicals i.e. Salicylic acid, Oxalic acid and Ascorbic acid were obtained from the plant pathology and soil science labs of School of Agriculture, Uttaranchal University. The chemicals were then made up to 100ppm and 200ppm using distilled water and stored in air tight glass wares in refrigerator.

Table 1: List of the treatments done

Treatment	Chemical inducers	Chemical formula
T1	Salicylic acid	$C7H_6O_3$
T2	Oxalic acid	$C_2H_2O_4$
T3	Ascorbic acid	$C_6H_8O_6$

In-vitro evaluation of treatments

Poisoned food technique (Nene and Thapliyal, 1993) [3] was used to check the efficacy of treatments. Purified plate of 7-10 days old culture was taken and 8mm pieces of culture was cut out with the help of Cork borer. Media was poisoned with treatments at different concentrations of Plant chemical inducers (100ppm and 200ppm) and placed inside incubator at 25±2ºC. Completely Randomized Design was followed (CRD) with three replications of each treatment and plate having no treatments served as control. Growth was checked after 3 days, 6 days and 7 days intervals. Zone of Inhibition was calculated by using formula (Vincent, 1947) [13];

I=[C-T)/C] X 100

Where,
I = % of growth inhibition
C=control plates growth
T=Treated plates growth

Statistical analysis

Data was analysed by using complete randomized design (CRD) with the help of analysis of variance table (ANOVA) wherever required. The F value will be calculated and critical difference (CD) was tested at five per cent level of significance for comparing treatment means (Steel *et al.*, 1997) [11].

Result and Discussion

Efficacy of chemical inducers on radial mycelia growth and percent mycelia inhibition of *Fusarium oxysporum* f. sp. *ciceri*

Radial mycelia growth

All the chemical inducers at both concentrations showed effective control against the mycelia growth of the pathogen (Table 2). The lowest radial growth at after 3 DAI was shown by Salicylic acid @ 200 ppm (21.67mm) followed by Ascorbic acid @ 200 ppm (25.67), Salicylic acid @ 100ppm (27.00mm), Oxalic acid @ 200 ppm (27.67mm), Ascorbic acid @ 100 ppm (32.33mm) followed by the highest radial growth with treatment of Oxalic acid @ 100ppm (35.67mm) which is same as the untreated control. At 5 DAI, however the highest control was shown by Ascorbic acid at 200ppm (40.33mm), followed by Salicylic acid at 200ppm (43.67mm), Oxalic acid at 200ppm (44.00mm), Ascorbic acid at 100ppm (50.00mm), Salicylic acid at 100ppm (51.67mm) and the lowest control was shown by Oxalic acid at 100ppm (53.00mm) over the untreated control growth of 65.00mm.
At 7 DAI, the highest control against the radial growth was shown by Salicylic acid at 200ppm (64.00mm), followed by Ascorbic acid at 200ppm (66.00mm), Oxalic acid at 200ppm (67.00mm), Ascorbic acid at 100ppm (74.00mm), Salicylic acid at 100ppm (74.67mm) and the least control was shown by Oxalic acid at 100ppm (75.00mm). The untreated control showed growth of 82.67mm.

Mycelial inhibition

The results obtained from the experiment suggest that the chemical inducers have been effective in inhibiting the mycelia growth of the pathogen. The percent mycelia inhibition was calculated using the formula,

I = [C-T)/C] X 100

Where,
I = % of growth inhibition
C=control plates growth
T=Treated plates growth.

At 3 DAI, Oxalic acid at 100ppm had the poorest inhibition percentage (0%). However, the highest inhibition percentage

was shown by Salicylic acid at 200ppm (39.33%) followed by Ascorbic acid at 200ppm (28.03%), Oxalic acid at 200 ppm (22.43%). Ascorbic acid at 100ppm showed low mycelia growth inhibition of 9.36%.

At 5 DAI, the highest inhibition percentage was shown by Ascorbic acid at 200ppm (37.95%) followed by Salicylic acid at 200 ppm (32.82%), Oxalic acid at 200 ppm (32.31%), Ascorbic acid at 100ppm (23.08%), Salicylic acid at 100ppm (20.51%) and the least inhibition percentage was shown by Oxalic acid at 100ppm (20.51%).

The lowest inhibition percentage at 7 DAI was shown by Oxalic acid at 100ppm (9.28%). The highest was shown by Salicylic acid at 200ppm (22.58%) followed by Ascorbic acid at 200ppm (20.16%), Oxalic acid at 200ppm (18.95%), Ascorbic acid at 100ppm (10.49%) and Salicylic acid at 100ppm (9.68%). The study has shown that the chemical inducers have effectively controlled the growth of *Fusarium oxysporum* f.sp. *ciceri* in both the concentration.

Previous studies have shown that use of Bion, SA and Riboflavin in chickpea against *Fusarium* wilt has controlled the growth of the pathogen (Sarwar *et al.*, 2003; Saikia *et al*., 2006) [6, 5]. Similar results were seen when Salicylic acid was used to control Ascochyta blight in chickpea, which showed the maximum control (Sarwar *et al.*, 2011) [7]. Treatment with Oxalic acid has also shown that induction of systemic resistance in tomato against *Fusarium* wilt increase with increase in the concentration of the inducers (Attitalla and Brishammar, 2003) [1]. Our study has also shown that in comparison to 100ppm, 200ppm has shown better results in controlling the pathogen growth. Studies have also shown that seeds when bioprimed with ascorbic acid and antagonistic microbes have controlled *Fusarium* wilt (Singh *et al*., 2020) [10]. The findings in our experiment are in accordance with the above findings. Therefore, we can say that the use of chemical inducers along with other methods can be beneficial in controlling the disease intensity.

Table 2: Effect of different chemical inducers against *Fusarium oxysporu* f.sp. *ciceri* (Mycelia Growth Colony Diameter in mm)*

Treatment	Radial growth (mm)		
	3DAI	5DAI	7DAI
Salicylic acid @ 100ppm	27.00	51.67	74.67
Salicylic acid @ 200ppm	21.67	43.67	64.00
Oxalic acid @ 100ppm	35.67	53.00	75.00
Oxalic acid @ 200ppm	27.67	44.00	67.00
Ascorbic acid @ 100ppm	32.33	50.00	74.00
Ascorbic acid @ 200ppm	25.67	40.33	66.00
Control	35.67	65.00	82.67
C.D.	2.410	4.010	2.809
SE(m)	0.787	1.309	0.917

*= Mean of Three Replications.
C.D. = Colony Diameter Mean
SE (m) = Standard Error Mean

Table 3: Mycelial percent inhibition of *Fusarium oxysporum* f.sp. *ciceri* by chemical inducers

Treatment	Mycelial percent inhibition (%)		
	3DAI	5DAI	7DAI
Salicylic acid @ 100ppm	24.31	20.51	9.68
Salicylic acid @ 200ppm	39.33	32.82	22.58
Oxalic acid @ 100ppm	0.00	18.46	9.28
Oxalic acid @ 200ppm	22.43	32.31	18.95
Ascorbic acid @ 100ppm	9.36	23.08	10.49
Ascorbic acid @ 200ppm	28.03	37.95	20.16
Control	0.00	0.00	0.00

Conclusion

The results obtained from the experiment conducted to study the effect of different chemical inducers against the growth of *Fusarium oxysporum* f.sp. *ciceri* are as follows: All the chemical inducers have effectively controlled the growth of the pathogen when compared to the growth in the untreated control. The most effective chemical inducer that showed the maximum control on the mycelia growth of the pathogen was Salicylic acid, followed by Ascorbic acid. Oxalic acid showed the least effect in controlling the mycelia growth of the pathogen. Therefore, the use of chemical inducers in controlling the growth of the pathogen can be done as a means to ensure reduction in the pathogenicity of the pathogen and also reduce the seriousness of the disease.

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