Emerging Growth Inducers And Bioagent During The Seedling Of Chickpea Against *Fusarium Oxysporum* f. sp. *ciceri*

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Abstract
In this present study, studies on chickpea wilt using of different chemical inducers and bio-control agents the *Fusarium oxysprum* f. sp. *ciceri*. The use of chemical inducers in PD broth media and pro-tray amended (SA, OA and AA) with concentrations (100 and 200 ppm). Use of plant defense inducers is a newer approach and can prove to be a beneficial alternative and therefore this study was conducted to know the efficacy of chemical inducers and bio control agents. The experiments were conducted in both *in vitro* as well as in portray method. The result revealed that maximum growth of bio-agent was recorded (30.60 mm) in T1 while the pathogen growth (40.73 mm) and mycelial percent inhibition (38.89%) in T1. In *T. viride* maximum wet weight of fungal mat was recorded in T_{10} (9.25 g) in case of *T. harzianum* was shown in T8 (6.16 g). The maximum dry weight of *T. viride* fungal mat was found in T2 (2.05 g) and in case of *T. harzianum* (1.79 g). Tested chemical inducers and bioagents on poo-tray method after ten days of sowing, the maximum average number of healthy seedlings were recorded in T2 (27.00).

Keywords: Chickpea, wilt, chemical inducers, bioagents, broth media, pro-tray and inhibition.

1. Introduction

Fungal wilt, which exhibits itself by drooping or root rots, is one among the foremost devastating and exigent diseases, which may damage the crop at any stage. Fusarium wilt epidemics can destroy harvests and can even lead to around 100% yield loss in fields heavily infested with the disease, given under favourable conditions. The symptoms appear on leaves as they start yellowing and eventually dry up during the beginning stages of flowering, 6-8 weeks after sowing and can also appear upto pod formation stage (late wilt). Plants are not only the major autotrophs on this planet but are also a great energy source for several organisms for their feed and survival. Although lacking a defense system like animals, plants have known to develop an interesting range of structural, chemical, and protein-based defenses intended to sense invading organisms and stop them before they become capable to cause broad injury. Use of plant defense inducers is one such technique that has been in use recently. Components such as Salicylic Acid (SA), Ascorbic Acid (AA), Oxalic Acid (OA) have shown initiation of systemic resistance against several pathogens. SA application induces an amassing of PR proteins. (Pieterse and Loon, 1999) SA affects both resistance to biotic stress (pathogens), tolerance to abiotic stresses and several aspects of plant growth and maturity. There are two main pathways to synthesize SA: isochorismate and phenylalanine ammonia-lyase pathways (Dempsey *et al*., 1999). AA communicates with vital elements of the complex networkof plants, eventually affecting the outcome of plant–pathogen interaction (Boubakri, 2017). OA, the simplest dicarboxylic acid is a natural product that is present in many plants and animals. It is available as an antioxidant in several plants. Majorly, it is present as a calcium salt but also sometimes exist as a free acid (Pieterse and Loon 1999).

Several studies have shown that the acid is capable of controlling the disease and mediating disease resistance response against various invading/affecting pathogens. Several of the major pathogens that have been controlled by the acid are *Fusarium, Sclerotium, Alternaria, Alternaria, Botrytis* and post harvest pathogens as well. Some biocontrol agents are used against the several pathogens for the controlling. *Trichoderma* spp. usually grows in its natural habit i.e., on plant root surface can control root diseases specifically. (Poddar *et al*., 2004) reported that *Trichoderma* spp. are effective for the control of wilt pathogen and have shown better potential in domineering the chickpea wilt under field condition.

2. MATERIALS AND METHODS

2.1. Isolation of Pathogen:

The infected plant parts showcasing characteristic symptoms of chickpea wilt were taken for isolation of Foc. These diseased plants were rinsed thoroughly using distilled water to eliminate the dust particles and surface contaminants.

2.2. Maintenance of pure culture

The culture of FOC was isolated and maintained on PDA medium by regular sub culturing. The cultures of FOC were grown in germ-free petri plates on PDA medium for 8 days. Single branched hyphae from the edge of the rising 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 around a week and yet again sub-cultured on PDA medium and finally stored in a refrigerator at 05 ±1 ^{0}C for further use.

2.3. 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 chemical inducers

Sr. No.	Chemical inducers	Chemical formula
1.	Salicylic acid	$C_7H_6O_3$
2.	Oxalic acid	$C_2H_2O_4$
3.	Ascorbic acid	$C_6H_8O_6$

2.4. Maintenance of bio-control agent

The BCAs namely *Trichoderma viride* and *Trichoderma harzianum* were procured from the School of Agriculture, Uttaranchal University for this study.

2.5. Preparation of inoculum

T. viride and *T. harzianum* strains were grown in conical flasks (250 ml) containing 100 ml of PD broth amended with salicylic acid, ascorbic acid and oxalic acid (50ppm and 100ppm). Then the growth of the mycelium was observed. Fungal antagonists *viz., T. viride* and *T. harzianum* strains were cultured in PD broth by transferring a disc of vigorously multiplying mycelium and incubated for 10 days.

2.6. Dual culture technique

The inhibitory activity of the BCAs against Foc was studied by dual culture technique Rabindran and Vidhyasekaran, (1996).

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

Where, I = Per cent growth inhibition C= pathogen colony diameter in control (mm) T= pathogen colony diameter in treatment (mm).

2.7. Protray method

Treated seeds were sown in the pro trays maintaining three replications for each treatment. Fungal discs of FOC of seven days old culture were inoculated in each well of the pro trays using forceps. Seeds were soaked in the chemical solution overnight and were sown on the following day. Compost had only seeds soaked in water. Vermi compost and coco peat were thoroughly mixed in the ratio of 1:1 using hand, unwanted pebbles and dirt were removed during the treatment Khaliq *et al*., (2006).

2.8. Effectiveness of chemical inducers against Foc

The effect of CIs against the growth of pathogen (*Fusarium* spp.) was evaluated by food poisoning technique. The use of 20 ml of PDA media in each plates containing the chemical inducers and later inoculated Fusarium spp. was done Nene and Thapliyal, (1993).

2.9. Effect of combination between bio-control agent/chemical inducers

250 mL conical flasks were taken and filled with 200 mL PD broth and amended with chemical inducers individually. After that fungal disc was cut and placed in each flask. For control treatment, only the fungal disc was placed inside the flask. Observations were taken after 10 days. The wet and dry mycelia were weighed.

2.10. 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 was calculated and critical difference (CD) was tested at five per cent level of significance for comparing treatment means Steel *et al*., (1997)

3. RESULT AND DISCUSSION

In the present study was conducted using of chemical inducers and biocontrol agents to determine their efficacy for the control of *F. oxysporum* f. sp. *ciceri*.

3.1. The antagonistic effect against *F. oxysporum* f. sp. *ciceri* in-vitro.

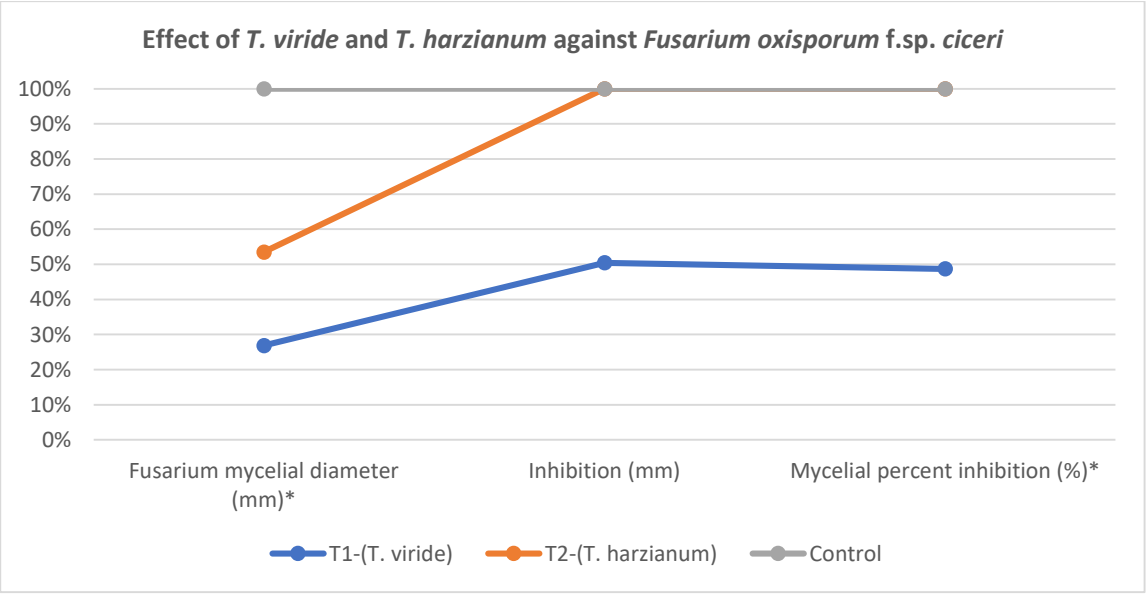
Mycelial inhibition:

The result revealed the growth of bioagents and *F. oxysporum* f.sp. *ciceri*. The maximum growth of bio-agent was recorded (30.60 mm) in T1 while the pathogen growth (40.73 mm) followed by treatment T2 (30.07 mm) growth of bioagent was recorded while pathogen growth (44.40 mm). The mycelial percent inhibition in T1 was recorded as 36.89% while the mycelial percent inhibition in T2 was 38.89%. Control showed mycelial diameter of 70.50 mm whereas there was 0% mycelial inhibition as no bio control disc was added in the plate. (Table 2)

T. viride and *T. harzianum* are fast growing fungi and are well known to inhibit the growth of various plant pathogens. They are been recommended in various crop disease management. The pathogen showed low colony growth of 40.73 mm and 40.40 mm in comparison of control which had growth of 70.50 mm. This result is in line with the studies done by (Dhar *et al*., 2005) which showed that *Trichoderma* spp. and *Gliocladium virens* could efficiently restrict the growth of *F. udum*. The mycelia percent inhibition of *T. viride* and *T. harzianum* was observed as 36.89% and 38.89% respectively depicting good control over the growth of the pathogen. Related observations were made by (Sundaramoorthy and Balabaskar, 2013)

Table-2: Effect of *T. viride* and *T. harzianum* against *Fusarium oxisporum* f.sp. *ciceri*

Treatment	*Fusarium* mycelial diameter (mm)*	Inhibition (mm)	Mycelial percent inhibition (%)*
T1-(*T. viride*)	40.73	30.60	36.89
T2-(*T. harzianum*)	40.40	30.07	38.89
Control	70.50	0.00	0.00
CD	0.748	0.724	-----
SE(m)	0.186	0.180	-----



3.2. Effect of chemical inducers in combination with the bio control agents.

In this study, fungal discs of bio control agents *Trichoderma viride* and *Trichoderma harzianum* discs were inoculated in PD broth cultures amended with chemical inducers (SA, OA and AA) of concentrations (100 and 200 ppm). Their growth was observed for ten days after and following are the results: Both fungal mats of *T. viride* and *T. harzianum* showed fast growth in all the broth cultures. Initial mat colour was found to be dull white with irregular margins of colony. As the growth progressed of fungal mats started changing colour from dull white to green. The surface of the fungal mats remained uneven. The fungal mats on gaining maturity became harder, thicker and produced numerous spores on their upper surfaces. Eventually, all the treatments showed increase in radial growth of the bio control agents. When the fungal mats were dried in the hot air oven, the weight reduced drastically depicting that the mats absorbed much amount of the broth liquid during their growth in the flasks.

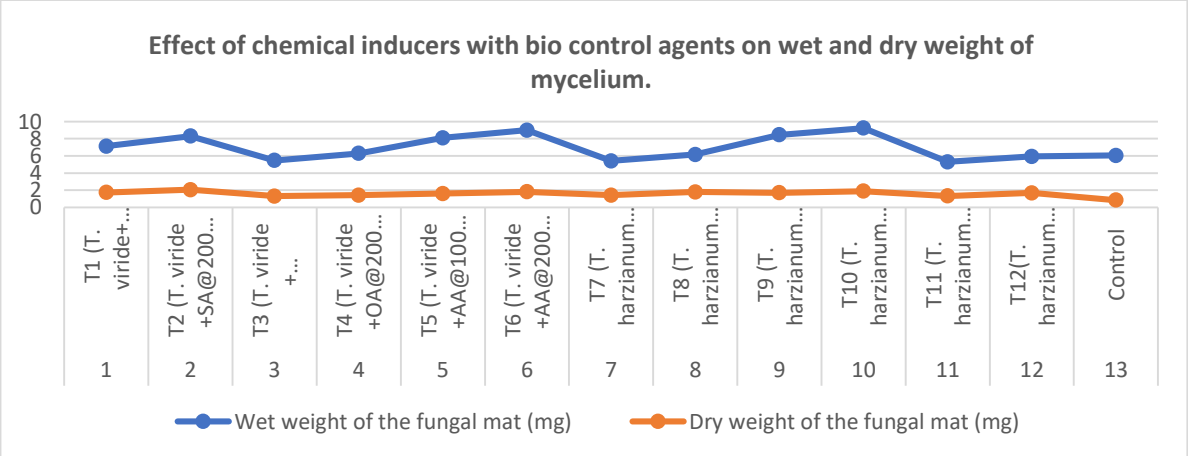
Both the bio agents i.e. *Trichoderma viride* and *Trichoderma harzianum* showed positive growth when placed in chemical inducer amended potato dextrose broth (as seen in Table 3). All the broth cultures had higher growth when treated with the inducers at both concentrations (100 and 200ppm) in comparison to the untreated control.
Previous studies of (Zehra *et al*., 2017) have shown that a combination of salicylic acid and methyl jasmonate with *Trichoderma harzianum* have proven to be effectual in controlling *F. o. f. sp. lycopersici* and have shown synergism effect with each other. Fungal discs of both *T. viride* and *T. harzianum* showed more growth in PDA broth cultures which were amended with chemical inducers 9.25 mg followed by 8.99 mg and 8.46 mg while the control consisting of only bio agent culture with no amended chemical inducer had weight of 6.04g. This is also in line with the observation made by (Saikia *et al*., 2003) suggesting that a combination of salicylic acid, acetylsalicylic acid, indole-3- carbinol, DL-norvaline and lichenan in combination with Pseudomonas fluorescens provided better protection in chickpea against Fusarium wilt.

Fungal mat weight of *Trichoderma viride* and *T. harzianum*.
Wet weight
The control (only *T. viride*) had wet weight of 6.04g while the dry weight was 0.83g. The maximum wet weight of *T. viride* fungal mat was seen in T10 (9.25 g) followed by T6 (8.99 g) and T9 (8.46 g) while the minimum wet weight was shown in control I (6.04 g) followed by T1 (7.14 g). On *T. harzianum* had wet weight of 4.99g and dry weight of 0.79g. The maximum wet weight of the fungal mat of *T. harzianum* was shown in T8 (6.16 g) followed by T4 (6.30 g) and T12 (5.93 g) while the minimum growth was shown in T11 (5.30 g) followed by T7 (5.41 g) and T11 (5.30 g).

Dry weight
The maximum dry weight of *T. viride* fungal mat was seen in T2 (2.05 g) followed by T10 (1.89 g) and T6 (1.80 g) while the minimum fungal mat was seen in T5 (1.61 g) followed by T9 (1.70 g) and T1 (1.74 g). In this experiment the maximum dry weight of fungal mat T8-*T. harzianum* (1.79 g) was observed followed by T12 (1.67 g) and T4 (1.42 g) while the minimum dry weight was observed in T3 (1.30 g) followed by T11 (1.33 g) and T7 (1.41 g).

Table-3: Effect of chemical inducers in combination with the bio control agents on wet and dry weight of mycelium.

Sr. No.	Treatment	Wet weight of the fungal mat (mg)	Dry weight of the fungal mat (mg)
1.	T1 (*T. viride*+ SA@100ppm)	7.14	1.74
2.	T2 (*T. viride* +SA@200ppm)	8.30	2.05
3.	T3 (*T. viride* + OA@100ppm)	5.46	1.30
4.	T4 (*T. viride* +OA@200ppm)	6.30	1.42
5.	T5 (*T. viride* +AA@100ppm)	8.10	1.61
6.	T6 (*T. viride* +AA@200ppm)	8.99	1.80
7.	T7 (*T. harzianum*+SA@100ppm)	5.41	1.41
8.	T8 (*T. harzianum* +SA@200ppm)	6.16	1.79
9.	T9 (*T. harzianum*+OA@100ppm)	8.46	1.70
10.	T10 (*T. harzianum*+OA@200ppm)	9.25	1.89
11.	T11 (*T. harzianum*+AA@100ppm)	5.30	1.33
12.	T12(*T. harzianum* +AA@200ppm)	5.93	1.67
13.	Control	6.04	0.83
	CD	0.216	0.218
	SE(m)	0.074	0.075

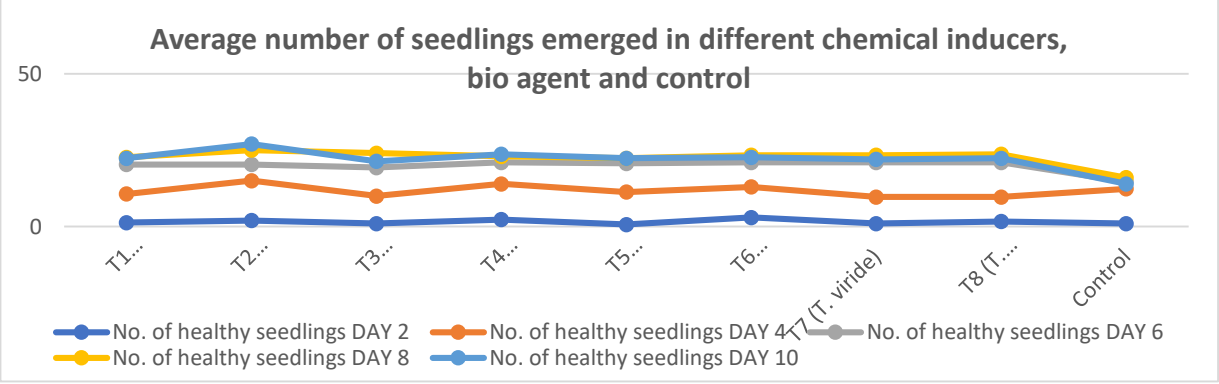


3.3. Efficiency of chemical inducers and bio agents on growth of healthy seedlings of chick pea by pro tray method.
In this study, the treated seeds (both with chemical inducers and bio control agents at both concentrations) were sown in pro trays that were already inoculated with *Fusarium oxysporum f. sp. ciceri* discs a day before and observation was recorded daily for continuously 10 days.

Healthy seedlings growth
Two days after sowing (2 DAS), the maximum average number of healthy seedlings was in T6 (3.00), followed by T4 (2.33) and T2 (2.00) while the minimum number of average healthy seedlings was shown by T5 (0.67).
Four days after sowing (4 DAS), the maximum number of average healthy seedlings was shown by T2 (15) followed by T4 (14) and T6 (13) while the minimum average number of healthy seedlings was shown by both T7 and T8 (9.67). Six days after sowing (6 DAS), the maximum average number of healthy seedlings (21) was shown by four treatments *viz*., T4, T6, T7 and T8 while the minimum number of average healthy seedlings was shown by control (14.67). Eight days after sowing (8 DAS), the highest average number of seedlings was shown by T2 (25), followed by T3 (24) and T8 (23.67) while the minimum average number of seedlings was again shown by control (16). The last day of observation i.e. ten days after sowing (10 DAS), the maximum average number of healthy seedlings was seen in T2 (27), followed by T4 (23.67) and T6 (22.67). The minimum average number of healthy seedlings was again shown by control (14) (Table-4).
Affected plants were weak, showed wilting symptoms with poor growth. The results show that the bio agents and the chemical inducers have been effective in decreasing the disease incidence of Fusarium wilt and were able to produce better number of healthy seedlings than when compared to the untreated control.
The results also showed that at initial days i.e. till day 4 DAS, the untreated control was able to produce good number of healthy seedlings when kept in comparison with the other treatments. However, the number started declining and eventually became the least from day DAS. Fusarium wilt had affected the growth of the seedlings resulting in the increase of the wilt disease and further death of the seedlings. The other treatments maintained a good number of healthy seedlings from 2 DAS till the last day of observation i.e. 10 DAS, showing that they were capable of reducing the pathogen inoculums in the soil and hence producing better results.
This observation lies in accordance with (El-Mougy *et al*., 2004; Karlidag *et al*., 2009; Zahra *et al*., 2010) where it was reported that application of chemical inducers showed an increase in the growth parameters, yield components and quality of fruits and vegetables. Similar results were obtained by (El-Mohamedy *et al*., 2014) when the tomato plants were treated with salicylic acid and sorbic acid producing good reduction in root rot incidence (72 and 60.2%, respectively) and disease severity (55.8 and 50.0%, respectively). Treatment consisting of bioagents had better growth, (22) and (22.33) when *T. viride* and *T. harzianum* was used respectively. Previous studies have also shown that seeds when bio-primed with antagonistic microbes have controlled Fusarium wilt (Singh *et al*., 2020). Similar results were found by (Larkin and Fravel, 1998) that when bio-agents were used as seed dressing, they effectively controlled Fusarium wilt diseases of crops like watermelon and muskmelon.

Table-4: Average number of seedlings emerged in different chemical inducers, bio agent and control

Treatment	No. of healthy seedlings				
	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10
T1 (SA@100ppm)	1.33	10.67	20.33	22.67	22.33
T2 (SA@200ppm)	2.00	15.00	20.33	25.00	27.00
T3 (OA@100ppm)	1.00	10.00	19.33	24.00	21.33
T4 (OA@200ppm)	2.33	14.00	21.00	23.00	23.67
T5 (AA@100ppm)	0.67	11.33	20.67	22.33	22.33
T6 (AA@200ppm)	3.00	13.00	21.00	23.33	22.67
T7 (*T. viride*)	1.00	9.67	21.00	23.33	22.00
T8 (*T. harzianum*)	1.67	9.67	21.00	23.67	22.33
Control	1.00	12.33	14.67	16.00	14.00
C.D.	N/A	3.13	3.34	3.67	2.93
SE(m)	0.729	1.04	1.11	1.22	0.98



CONCLUSION

We can conclude from the observation made and results obtained that inducers can effectively control the growth of the pathogen and induce systemic resistance in the affected as well as the healthy plants. The chemical and bi-control agents are quite compatible with each other and can be recommended in a combination for disease management. They can be used in chickpea and also other various crops that are affected by *Fusarium* spp. or soil inhabiting pathogen. A combination of both (chemical inducers and bioagents) can also be used as both are synergistic in nature to each other therefore enhancing the inhibitory effect and reducing the disease incidence to a greater extent.

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