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Effectiveness of isolates of *Trichoderma viride* containing carbon and nitrogen sources against major fungal pathogens of chickpea

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Abstract
Isolate of *Trichoderma viride* supplemented with various carbon and nitrogen sources was evaluated for their potential in inhibiting major fungal pathogens using chickpea as test crop. Peptone, mannitol and glucose were used as carbon sources; ammonium sulphate, urea and potassium nitrate were taken as nitrogen sources. Among the carbon source, isolate obtained from treatment with peptone recorded the least disease incidence as compared to control whereas, isolate of *T. viride* containing Ammonium sulphate as nitrogen source obtained the lowest disease incidence for all the three pathogens. Combined application of both carbon and nitrogen with *Trichoderma* isolate also enhance the potential in reducing disease severity. The severity of wilt, collar rot and root rot was controlled by 67.2%, 59.0% and 55.9%, respectively as compared to control.

Keywords: Biocontrol, carbon, disease, isolate, nitrogen, pathogens

Introduction
Chickpea (*Cicer arietinum* L.) is one of the major pulses of India cultivated on an area of about 26.28 million hectare and producing around 75 per cent of the world's consumption (FAO, 2008; Tomar *et al*., 2010) [2, 19]. Chickpea is reported to be infected by a number of pathogens, of nearly 172 pathogens (Nene *et al*., 1996) [10]. Of these, some are minor in infection and lesser known; whereas the others occur worldwide and have caused a devastating reduction in the yield and quality of chickpea. Dry root rot (*Rhizoctonia bataticola*) is one such example, where the disease incidence is becoming severe in semi-arid regions because of changes in climatic conditions during the flowering to pod filling stage (Sharma *et al*., 2010) [16]. Soil borne diseases viz., *Fusarium oxysporum* f. sp. *ciceri*, *Sclerotium rolfsii* and *Rhizoctonia solani* have be considered as the most devastating diseases of chickpea (Singh *et al*., 1986; Khan *et al*., 2002) [17, 4].
Biological control has been in the front run lately because they are environmentally safe, low cost and sustainable over chemical fertilizers. The fungus belonging to the genus *Trichoderma* species have been effectively used as biocontrol agents for years. They are known to produce antibiotics, mycoparasitize the host pathogen and also produce cell wall degrading enzymes, the key role in reducing disease incidence. A number of experiments conducted reported showed that *Trichoderma* application as seed or soil treatment are effective in controlling root rot, collar rot and wilt of chickpea and other pathogens belonging to different crops (Kumar *et al*., 2006; Rajput *et al*., 2010; Dubey *et al*., 2012; Kumar *et al*., 2012; Khan *et al*., 2014; Sahu *et al*., 2015; Nirmalkar *et al*., 2017) [6, 13, 1, 7, 5, 15, 11]. It is also well known fact that addition of carbon and nitrogen sources enhances the enzyme activity and thereby boosts the biocontrol activity of *Trichoderma* species. With that in mind the present investigation was carried out to assess the field performance of *Trichoderma viride* with carbon and nitrogen sources in managing root rot, collar rot and wilt of chickpea.

Materials and methods
Preparation of inoculum
The fungus *Trichoderma viride* was cultured on potato dextrose medium in a conical flask. After preparation of media, the broth was distributed into 16 different conical flasks. The media was supplemented with all the treatment combinations viz., peptone, glucose and mannitol as carbon source @ 0.2% (w/v) and ammonium sulphate, potassium nitrate and urea as nitrogen source @ 0.1% (w/v) concentration, as per the treatment combination given below (Table 1).

Table 1: Treatment combinations

No.	Treatment combination	No.	Treatment combination
T_1	Peptone	T_{10}	Glucose + ammonium sulphate
T_2	Glucose	T_{11}	Glucose + potassium nitrate
T_3	Mannitol	T_{12}	Glucose+ urea
T_4	Ammonium sulphate	T_{13}	Mannitol+ ammonium sulphate
T_5	Urea	T_{14}	Mannitol+ potassium nitrate
T_6	Potassium nitrate	T_{15}	Mannitol+ urea
T_7	Peptone + ammonium sulphate	T_{16}	Control
T_8	Peptone+ potassium nitrate	T_{17}	FUI (Fertilized Uninoculated)
T_9	Peptone+ urea		

The flasks were then autoclaved at 15 psi for 20 minutes after which strain of *T. viride* was inoculated into each flask and kept at room temperature. The isolates obtained from the treatment combinations were then used as seed treatment in the field.

Field preparation and sowing

The experiment was conducted at research field of department of Soil Science and Agricultural Chemistry, JNKVV Jabalpur. The experiment in field was carried out following Randomized Block Design (RBD) experimental design. A total of 16 treatments as mentioned under component-I along with FUI (fertilized uninoculated) were taken with three (03) number of replications. Chickpea var. JG-16 was used as the test crop for carrying out the experiment. Plot size of 2 m x 3 m = 6 m^2 with 40 cm row to row spacing was maintained and recommended dose of fertilizers for chickpea 2020:80:20 ($N:P_2O_5:K_2O$ kg ha^{-1}) was applied through Urea, SSP and MOP. Before sowing the crop, seeds were treated with the *Trichoderma* strain obtained from the laboratory @ 10 ml/plot.

Disease incidence percentage

The number of diseased plants in each plot was counted at a periodic interval of 30, 60 and 90 DAS. Then the disease incidence percentage was calculated using the following formula:

$$\% \text{ disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Results

Wilt of chickpea (*Fusarium oxysporum* f. sp. *ciceri*)

Fusarium wilt is a very common disease and is the chief cause in yearly yield losses of chickpea. The number of wilted plants was taken at intervals of 30, 60 and 90 DAS and the disease incidence percentage is presented in Table 2. The disease was observed to affect the entire plots and incidence increased with time up to 60 DAS and thereafter declined.

Amongst all the treatments, the incidence of wilt was recorded minimum with the isolate received the treatment of Peptone+Ammonium sulphate with 1.51% corresponding to 67.2% control of disease severity over that of FUI (4.61% disease incidence), followed by the isolates from Mannitol+Ammonium sulphate, Glucose+Potassium nitrate and Ammonium sulphate with 1.61, 1.64 and 1.64% disease incidence. The isolate from the treatment of Glucose+Urea exhibited the highest wilt incidence of 2.41%.

Collar rot (*Sclerotium rolfsii*)

Collar rot is also a prevalent disease of chickpea and is favoured with high moisture particularly at the time of sowing. Incidence of the disease was not observed at 30 DAS. The incidence of the disease increased at 60 DAS and then decreased again at 90 DAS. The data on disease incidence percentage at 60 DAS ranged from 13.21 to 33.10%, with the overall mean 19.89%. Isolates from all the treatment combinations significantly decreased the disease incidence as compared to control FUI (33.10%).

The isolate from the treatment of Peptone+Ammonium sulphate produced the least incidence of the disease of 13.21% which corresponded to 60.1% of control over that of control FUI. This was followed by the treatment combinations of Glucose+Ammonium sulphate and Mannitol+Ammonium Sulphate for 13.56 and 14.04% incidence of disease with response by 59.0 and 57.6% control respectively, when compared to FUI. The treatment which exhibited the highest disease incidence was Urea with 23.13%, followed by Mannitol and Mannitol+Urea for the disease incidence of 23.10% each.

Dry root rot (*Rhizoctonia solani*)

Dry root rot is another major root rot disease of all chickpea growing areas. The disease is mostly observed during flowering or post flowering and even up to podding stage. As per the observations recorded there was no sign of the disease incidence at 30 DAS. However, the incidence of root rot disease was exhibited at 60 DAS and further increased with growth at 90 DAS.

The least disease incidence was noted with the isolate from Peptone+Ammonium sulphate for 8.62% which corresponded to 55.9% control over that of control FUI (19.54% disease incidence), followed by isolates from the treatment combinations of Glucose + Ammonium sulphate, Mannitol + Ammonium Sulphate, Glucose + Potassium nitrate, Mannitol + Potassium nitrate and Ammonium sulphate with 52.3, 51.5, 50.4, 49.5 and 49.0% control respectively, when compared to untreated FUI.

Table 2: Effect of inoculation of different isolates of *T. viride* derived from treatment of different C and N on disease incidence percentage (%)

Treatments	Fusarium Wilt			Sclerotium Collar rot			Rhizoctonia root rot		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T1	1.81	9.22	4.78	0.00	17.27	8.86	0.00	10.04	13.61
T2	1.76	10.13	5.35	0.00	20.47	9.32	0.00	10.12	14.22
T3	2.02	10.02	5.18	0.00	23.10	8.94	0.00	10.24	14.21

T4	1.64	9.42	4.80	0.00	17.50	8.91	0.00	9.96	13.48
T5	2.34	13.86	7.18	0.00	23.13	10.19	0.00	11.09	17.61
T6	1.77	10.39	5.33	0.00	19.73	9.14	0.00	10.14	14.17
T7	1.51	8.76	4.23	0.00	13.21	8.59	0.00	8.62	11.41
T8	2.06	11.26	6.59	0.00	21.40	9.74	0.00	10.54	14.41
T9	1.70	11.06	5.25	0.00	16.63	9.10	0.00	10.23	13.23
T10	1.66	8.94	4.35	0.00	13.56	9.26	0.00	9.32	12.56
T11	2.41	14.44	7.02	0.00	22.07	11.45	0.00	11.45	16.81
T12	1.64	9.09	4.51	0.00	15.33	10.12	0.00	9.69	12.94
T13	1.61	8.94	4.42	0.00	14.04	8.68	0.00	9.48	12.86
T14	2.11	15.64	6.87	0.00	23.10	12.03	0.00	10.63	18.85
T15	1.74	9.94	4.62	0.00	15.40	8.74	0.00	9.87	14.24
Control	3.47	17.78	9.98	0.00	29.04	14.70	0.00	16.10	24.40
FUI	4.61	20.81	11.20	0.00	33.10	15.54	0.00	19.54	29.32
Mean	2.11	11.80	6.00	0.00	19.89	10.19	0.00	11.00	15.79
SEm	0.155	0.839	0.459	0.000	1.380	0.722	0.000	0.902	1.121
CD 5%	0.46	2.47	1.35	0.00	4.07	2.13	0.00	2.66	3.30

Discussion

The findings recorded showed that *Trichoderma* application led to a decline in disease severity of Fusarium wilt, collar rot and root rot of chickpea as compared to untreated control. Similar finding was reported by Rudresh *et al*. (2005) [14] wherein the incidence of diseases viz., wilt, root rot and collar rot were found to be lesser in *Trichoderma* isolates inoculated treatment compared to pathogen control and fungicide treatments. Patole *et al*. (2017) [12] also drew the same conclusion that *T. viride* and *T. harzianum* as single or in combined treatment significantly inhibited the growth of pathogen, thereby reduced the incidence of Fusarium wilt in comparison with fungicides. This gives all the more reason to choose biocontrol agents over chemical fungicides, having known its potential. Several other studies have reported the inhibition of pathogenic fungi by *Trichoderma* isolates, like (Khan *et al*., 2014) [5], whose studies stated that soil application of *Trichoderma* is as effective as carbendazim in checking the severity of wilt and root rot disease. The structure of fungi cell wall is considered to be made of chitin, β-(1,3)- glucanase, β-(1,4)- linked N-acetylglucosamine polymer (Latge, 2007) [8]. *Trichoderma* species are capable of producing cell wall degrading enzymes such as cellulase, xylanase, pectinase, glucanase, lipase, amylase, arabinase, and protease (Strakowska *et al*., 2014) [18]. Cell wall degrading enzymes produced by *Trichoderma* species leads to attack of fungal cell wall by making holes in the pathogenic fungi cell wall and consume nutrients for their own development. This notably explains the reason for superiority of *Trichoderma* application in reducing disease incidence. Also, the inhibition of pathogens could also be possibly due to production of secondary metabolites (such as glioviridin, viridin and gliotoxin) by *Trichoderma* species which inhibits the host pathogen (Inbar *et al*., 1994) [3]. Control of root rot and wilt diseases by *Trichoderma* spp. might be attributed to the pronounced colonization of rhizosphere by antagonist to the pathogens (Mathew and Gupta, 1998) [9].

Conclusion

The addition of carbon and nitrogen sources to *Trichoderma* isolate possibly led to an increase potential of *T viride* in reducing disease incidence since cell wall degrading enzyme activities increases with media supplemented with carbon and nitrogen sources. *Trichoderma* can therefore use as an effective bioagent in checking the severity of disease incidence. The control response of the isolates of *T. viride* against disease causing phytopathogenic fungus might be due to their cell wall was constituted with chitin and β-glucan both embedded into the protein matrix which were readily degradable by the hydrolytic enzymes. *T. viride* made it more advantageous over the pathogenic fungi while competing for space and nutrients. The difference in control response could be because of different environmental factors affecting the host and culture, resulting in differential secretion of antifungal enzymes.

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Conflict of Interest: None declared

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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