



Effect of mycorrhizal fungi in combination with botanicals, fungicides and bio-agents in ameliorating Fusarium wilt management in chickpea (*Cicer arietinum*)

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ABSTRACT

Fusarium wilt incited by *Fusarium oxysporum* f. sp. *ciceris* causes substantial economic losses in chickpea. To study the effect of mycorrhizal inoculation in combination with different bio-agents, fungicides and botanicals on the Fusarium wilt incidence and yield of chickpea, field trials were conducted during 2019–20 at the research field of CCS Haryana Agricultural University, Hisar. Mycorrhizal fungi were found to be beneficial in alleviating chickpea wilt incidence and upsurging the yield components of chickpea. The mycorrhizal fungus *Glomus mosseae* performed better than *Glomus hoi*, evincing the importance of mycorrhizal species. Seed soaking provided more disease control in comparison to soil drenching of plant extracts, bio-agents and fungicides except azoxystrobin 23% SC in variety JG 62 in which mode of application of fungicide had no significant effect on disease incidence. Amongst mycorrhiza × treatment interaction, carbendazim 50% WP provided utmost disease control (>80%) in the soil pre-inoculated with *G. mosseae* and *Glomus hoi*, followed by azoxystrobin 23% SC and *Trichoderma viride*, while aqueous extract of datura leaves was the least effective in both chickpea varieties JG 62 and C 235.

Keywords: *Fusarium oxysporum*, *Glomus mosseae*, *Glomus hoi*, Plant extracts

Wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is one of the most devastating diseases of chickpea (*Cicer arietinum* L.) in different parts of the world including India. Average annual losses due to wilt have been estimated to be 10–30% in different chickpea growing regions, reaching up to 90–100% under favourable climatic conditions (Jimenez-Diaz *et al.* 2015). Many crop protection tactics used to manage plant pathogens relies on the utilization of resistant varieties and application of fabricated antimicrobial compounds. However, in recent times, a paradigm shift towards eco-friendly, ecologically sound microbe derived antimicrobials is burgeoning. Arbuscular mycorrhizal fungi (AMF) help the plants by enhancing nutrient uptake, exacerbating abiotic stress resistance and upsurged resistance to disease development by plant pathogens (Schaefer *et al.* 2021). The symbionts (mycorrhizal fungi) are known to impart considerable benefits to the plant development under greenhouse conditions in sterile soil. In earlier studies, seed treatment with extracts of neem oil (Singh *et al.* 1980) and soil drenching with carbendazim (Mahmood *et al.* 2015)

were effective in curtailing chickpea wilt. In addition, *Trichoderma* species provided 89–94% protection against chickpea wilt under field conditions coupled with enhanced seed yield (Maitlo *et al.* 2019).

For sustainable crop production, identification and use of effective plant extracts and bio-agents along with need-based application of fungicides is promoted throughout the world. The response of mycorrhizal fungi is much diversified under field conditions. The compatibility of AMF with plant extracts, fungicides and bio-control agents, and their combined effect against chickpea wilt has not been fully explored. In the present study, an attempt was made to develop an integrated disease management module comprising of mycorrhizal application along with different plant extracts, fungicides and bio-agents under field conditions.

MATERIALS AND METHODS

Multiplication of pathogen, bio-agents and mycorrhizal fungi: The fungal bio-agents, *Trichoderma viride* and *T. harzianum* obtained from the local habitat of CCS HAU, Hisar, were mass cultured on sterilized sorghum grains. The inoculated flasks were incubated at 27±1°C for 10 days. The mycorrhizal fungi, *Glomus mosseae* and *Glomus hoi* were maintained on pearl millet plants grown in earthen pots filled with 5 kg sterilized soil. The pots were irrigated regularly and Hoagland's nutrient solution was applied at 10 ml/pot

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after every 30 days of sowing to support the multiplication of *Glomus* spp. The pot soil along with small pieces of rootlets, collected after growing pearl millet for three months was used as mycorrhizal inoculum. The artificial inoculation of field soil was done with the 10 days old culture of *F. oxysporum* f. sp. *ciceris* multiplied on sorghum grains.

Evaluation of plant extracts, fungicides and bio-agents against Fusarium wilt under field conditions: Based on field trials conducted during spring 2018–19, each of the two bio-agents, *T. viride* and *T. harzianum*, plant extracts, viz. aqueous leaf extract of neem and datura and fungicides, carbendazim 50% WP and azoxystrobin 23% SC were found to be promising in chickpea against Fusarium wilt in our earlier study (Khanna *et al.* 2021). The extracts of neem (*Azadirachta indica*) and datura (*Datura stramonium*) leaves were prepared by following the procedure described by Khanna *et al.* (2021).

The experiment was conducted in a split plot design with three replications at the research farm of CCS Haryana Agricultural University, Hisar during spring 2019–20. The main plots were inoculated with mycorrhiza, sub-plots were based upon mode of application of treatments and sub-sub plots corresponded to different treatments comprising of bio-agents, fungicides and aqueous plant extracts. The field soil (upper 5 cm layer) was first inoculated with mycorrhizal fungi at 100 g of mycorrhizal soil mixed with pearl millet rootlets having 450–500 sporocarp/kg soil followed by inoculation with *F. oxysporum* f. sp. *ciceris*. The plant extracts, bio-agents and fungicides were applied as seed soaking and soil drenching. The chickpea seeds were soaked in suspension of bio-agents having a concentration of 2×10^6 spores/cfu per ml. The fungicides, viz. carbendazim 50% WP and azoxystrobin 23% SC were applied at 2 g and 1 ml/kg seed while bio-agents and plant extracts were applied at 0.4 and 10% concentration, respectively. The seeds of wilt susceptible and moderately susceptible chickpea varieties JG 62 and C 235, respectively, were treated separately with

fungicides, plant extracts and bio-agents before sowing, dried under shade and sown in soil pre-inoculated with *G. mosseae* and *G. hoi* along with untreated control in 3×2 m² plots with a row to row and plant to plant spacing of 30 cm and 10 cm, respectively. The crop was raised using recommended agronomic practices.

Disease assessment: Observations on disease incidence (DI) were recorded by counting the infected and healthy plants along with seed yield and yield parameters such as number of pods/plant, number of seeds/pod and seed weight/plant in different treatments. The seed yield was recorded on plot basis and converted to kg/ha.

Statistical analysis: The analysis of variance (ANOVA) was performed following least significant difference (LSD) test at $P < 0.05$ using agricolae package of R studio.

RESULTS AND DISCUSSION

DI for variety JG 62 and C 235 was influenced by mycorrhizal inoculation, mode of application, different treatments, interaction between mode of application \times treatment (Table 1). DI was suppressed more in the presence of *G. mosseae* than *G. hoi* (Table 2) and application of different treatments through seed soaking had significantly lesser DI compared to its soil application (Table 2). In the present study, soil amendment with mycorrhiza substantially reduced DI. The results were in line with Shukla *et al.* (2015), where early inoculation of plants with mycorrhizal fungi facilitated reduction in wilt severity index. Among mycorrhiza \times treatment, carbendazim 50% WP performed better with soil amended with mycorrhiza in curtailing wilt incidence. Ipsilantis *et al.* (2012) observed transient effect of carbendazim on colonization and community structure of indigenous mycorrhizal fungi in comparison to pots where it totally impeded mycorrhizal colonization. This could be attributed to microbial degradation of pesticide in the field soil compared to the sterile soil substrate used in pot experiment (Pedersen and Sylvia 1997). The effects

Table 1 Summary of Analysis of variance of Fusarium wilt incidence in chickpea variety JG 62 and C235

Variety	JG 62					C 235				
	Disease incidence	Number of pods/plant	Seeds/pod	Seed weight	Seed yield	Disease incidence	Number of pods/plant	Seeds/pod	Seed weight	Seed yield
Mycorrhiza	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
Mode of application	<0.01	0.01	<0.01	0.40	<0.01	<0.01	0.38	0.01	0.22	0.03
Treatment	< 0.01	<0.01	<0.01	<0.01	<0.01	< 0.01	<0.01	<0.01	<0.01	<0.01
Mycorrhiza \times Mode of application	0.81	0.24	0.26	0.26	0.11	0.68	0.08	0.38	0.42	0.09
Mycorrhiza \times Treatment	0.04	0.67	<0.01	0.62	0.03	0.70	0.87	0.26	<0.01	0.37
Mode of application \times Treatment	<0.01	0.71	0.48	0.15	0.76	0.04	0.98	0.18	<0.01	0.06
Mycorrhiza \times Mode of application \times Treatment	0.94	0.73	0.98	0.48	0.71	0.96	0.9	0.28	<0.01	0.77

Table 2 Mean values of the disease incidence, number of pods/plant, seeds/pod, seed weight and seed yield for an experiment with three level of mycorrhiza, seven level of treatments and two levels of mode of application on two chickpea varieties

Variety	JG 62					C 235				
	Disease incidence	Number of pods/plant	Seeds/pod	Seed weight	Yield (kg/ha)	Disease incidence	Number of pods/plant	Seeds/pod	Seed weight	Yield (kg/ha)
<i>Mycorrhiza</i>										
<i>G. mosseae</i>	26.07 (29.20) c	42.40 a	1.40 a	12.44 a	1699.32 a	17.98 (24.07) b	58.47 a	1.36 a	11.91 a	2176.00 a
<i>G. hoi</i>	34.29 (35.01) b	39.12 b	1.23 b	11.72 b	1534.48 b	27.80 (31.01) a	55.19 b	1.23 b	11.59 b	2083.66 a
Without mycorrhiza	38.04 (37.31) a	37.00 c	1.01 c	11.32 b	1568.72 b	28.93 (31.79) a	45.23 c	1.06 c	10.88 c	1969.93 b
LSD	1.25	1.64	0.03	0.71	46.65	1.12	3.07	0.06	0.11	106.69
<i>Treatment</i>										
Neem leaves extract	41.53 (39.93) c	39.71 bc	1.26 a	11.69 c	1564.91 b	32.64 (34.60) b	48.82 de	1.25 a	11.27 d	2047.11 c
Datura leaves extract	46.67 (43.07) b	37.39 cd	1.16 b	11.30 cd	1578.47 b	35.97 (36.73) c	49.96 cde	1.12 b	11.48 cd	2036.08 c
Carbendazim 50WP	9.86 (17.69) g	43.40 a	1.29 a	12.99 a	1717.52 a	8.75 (16.73) g	61.66 a	1.29 a	12.63 a	2219.73 a
Azoxystrobin 23SC	14.03 (21.51) f	41.86 ab	1.29 a	12.39 ab	1669.75 a	12.22 (19.93) f	55.23 b	1.32 a	11.60 bc	2132.33 b
<i>T. viride</i>	23.33 (28.40) e	39.62 bc	1.28 a	11.82 bc	1594.91 b	18.75 (25.30) e	54.16 bc	1.24 a	11.38 cd	2032.14 c
<i>T. harzianum</i>	30.69 (33.36) d	38.33 cd	1.24 a	11.77 c	1589.37 b	24.58 (29.45) d	53.47 bcd	1.28 a	11.81 b	2144.02 ab
Control	63.47 (52.90) a	36.22 d	0.94 c	10.85 d	1490.94 c	41.39 (39.94) a	47.45 e	1.01 c	10.05 e	1924.28 d
LSD	2.17	2.41	0.07	0.61	49.35	2.10	4.81	0.09	0.29	82.42
<i>Mode</i>										
Seed	28.29 (31.17) b	40.66 a	1.27 a	11.95 a	1634.71 a	22.26 (26.92) b	55.29 a	1.28 a	11.59 a	2102.74a
Soil	36.71 (36.50) a	38.35 b	1.15 b	11.71 a	1566.97 b	27.54 (30.99) a	50.64 b	1.16 b	11.33 a	2050.32b
LSD*	1.85	1.64	0.06	0.67	20.61	1.18	3.37	0.09	-	46.50

*Least significant differences at $P < 0.05$. Means followed by different alphabets within the same treatment are statistically different. Figures in parentheses are angular transformed values.

of pesticides on mycorrhizal fungi in field depend on the physico-chemical behaviour of the active substances, the soil type and other soil microorganisms (Hage-Ahmed *et al.* 2019).

Amongst various treatments, carbendazim 50% WP provided maximum disease control i.e. 84.47% in variety JG 62 and 78.86% in variety C 235 (Table 2). In variety JG 62 and C 235, minimum wilt incidence was observed in seed priming with suspension of carbendazim 50% WP (Fig 1). Effective management of Fusarium wilt with carbendazim 50% WP has been documented by earlier researchers (Mahmood *et al.* 2015). Soil application of *T. viride* and *T. harzianum* has been found effective in managing wilt diseases (Dubey *et al.* 2012). *Trichoderma* species have been highlighted as potential bio-agent in controlling wilt. In our

study, bio-priming seeds of chickpea variety JG 62 with *T. viride* and *T. harzianum* provided 46.31 and 36.94% disease control, respectively under field conditions irrespective of mode of application of treatments in presence of mycorrhizal fungi. Moradi *et al.* (2012) reported that seed and liquid inoculation of *B. subtilis* and *T. harzianum*, singly or in combination reduced the disease severity up to 40%.

Jamwal *et al.* (2017) reported that aqueous extract of neem was effective against *F. oxysporum* f. sp. *ciceris*. In the present experiment, the disease control provided by aqueous extract of neem (24.51%) and datura (18.58%) leaves in presence of both the *Glomus* species was significantly less in comparison to fungicides and bio-agents. The results were in accordance with Flor-Peregrin *et al.* (2017) who witnessed negative interaction with respect to

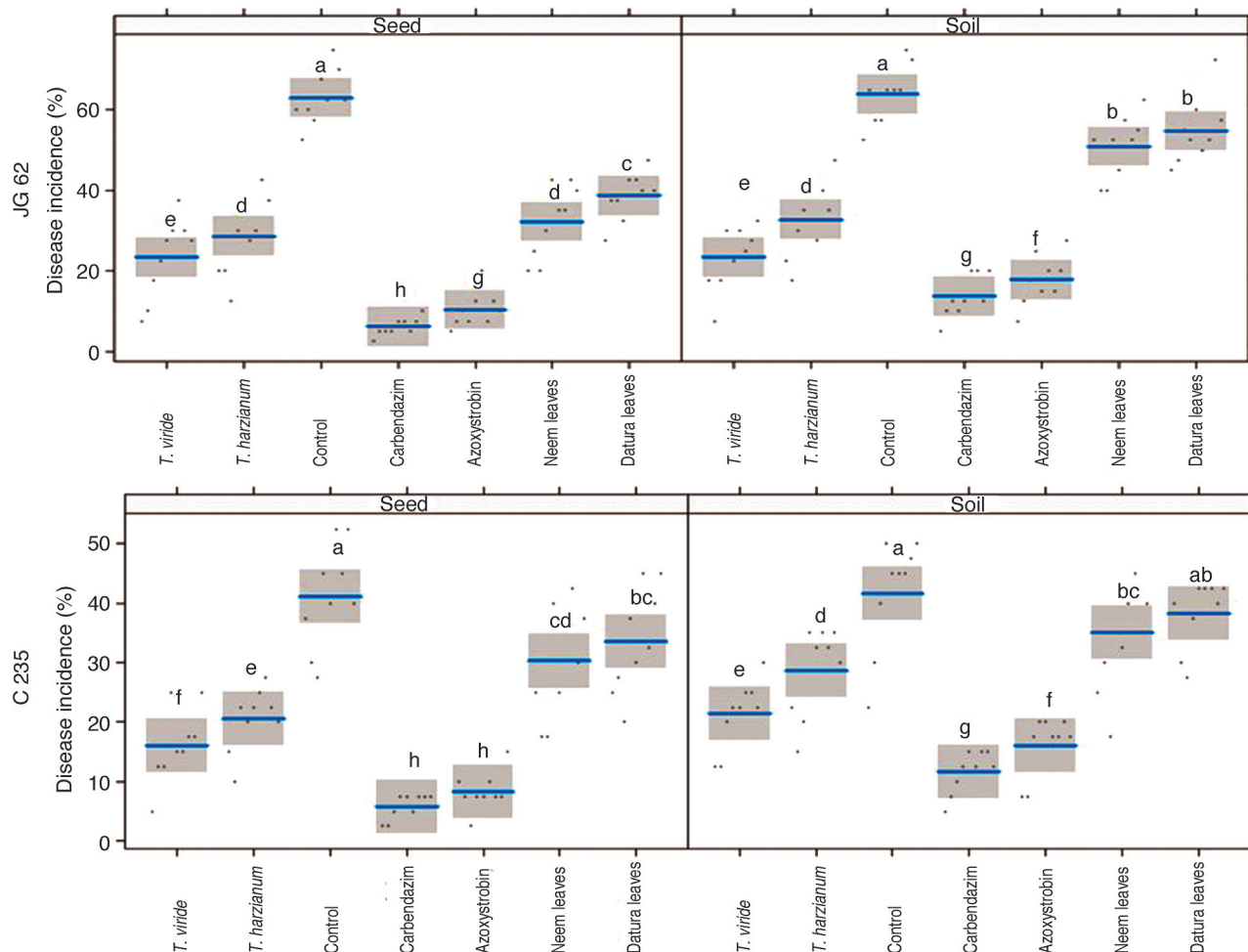


Fig 1 Effect of mode of application and different treatments on fusarium wilt incidence in chickpea variety a. JG 62 and b. C 235.

mycorrhization by *Funneliformis mosseae* in presence of neem and thyme extracts used to reduce nematode damage in tomato. In variety JG 62, a significant interaction between mycorrhizal fungi and other treatments revealed that *G. mosseae* inoculated soil along with carbendazim 50% WP and azoxystrobin 23% SC, and azoxystrobin 23% SC and *T. viride* were found to be statistically at par with each other (Fig 2).

Seed yield and yield attributes: The difference in DI in different treatments was reflected in terms of yield attributes and seed yield of chickpea. On mean basis, soil amendment with *G. mosseae* and *G. hoi* resulted in significant increase in number of pods/plant (42.40% and 39.12% in chickpea variety JG 62 and 58.47% and 55.19% in variety C 235). Treatment with carbendazim 50% WP resulted in maximum number of pods/plant (43.40% in JG 62 and 61.66% in C 235) coupled with enhanced number of seeds/pod and increase in seed weight/plant by 19.7 and 25.6% in variety JG 62 and C 235, respectively (Table 2).

Mycorrhiza, mode of application and treatments effectuated number of seeds/pod in both the varieties JG 62 and C 235, however in variety JG 62, the interaction between mycorrhiza \times treatments was also significant (Supplementary Fig 1). Pre inoculation of soil with *G.*

mosseae and *G. hoi* resulted in 38.61% and 21.78% and 28.30% and 16.04% increase in number of seeds/pod (1.40 and 1.23 vs 1.01 in JG 62 and 1.36 and 1.23 vs 1.06 in C 235) over the plots without mycorrhiza. Amongst various treatments, azoxystrobin 23% SC and carbendazim 50% WP (statistically at par) resulted in maximum number of seeds/pod while in datura leaves extract, seeds/pod was minimum. In chickpea variety JG 62 seed weight was influenced by mycorrhiza, treatment, interaction between mode of application \times treatment and mycorrhiza \times mode of application \times treatment while in variety C 235, interaction between mycorrhiza \times treatments was found to be significant. Among combined effect of mode of application \times treatment, 24.33% and 14.99% (13.39 vs 10.77 and 12.58 vs 10.94 g/plant) increased in seed weight/plant was observed in seed and soil treatment with carbendazim 50% WP in variety JG 62 while maximum increase (32.68%) was recorded in seed treatment with carbendazim 50% WP in variety C 235. A significant three way interaction indicated that in variety JG 62 the highest seed weight of 14.08 and 13.78 g/plant (statistically at par) was recorded in seed treatment with carbendazim 50% WP and azoxystrobin 23% SC along with *G. mosseae*. In *G. hoi* amended soil, maximum seed weight was observed in seed and soil application with carbendazim

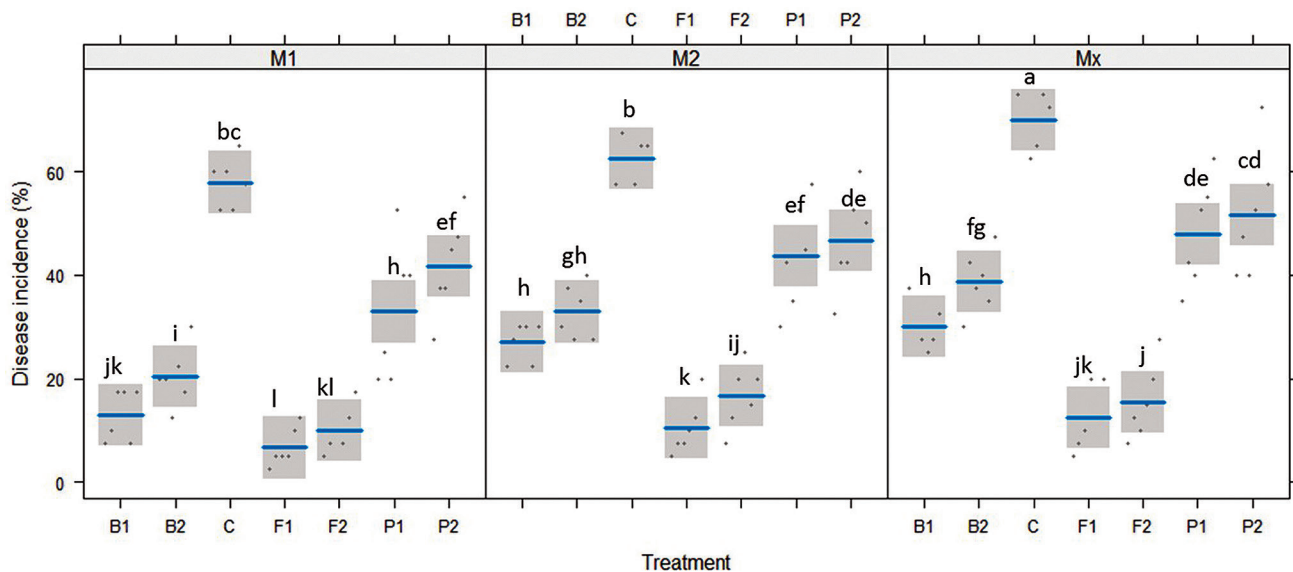


Fig 2 Effect of mycorrhiza application and different treatments on Fusarium wilt incidence in chickpea variety JG 62.

Treatments B1, *T. viride*; B2, *T. harzianum*; F1, Carbendazim 50WP; F2, Azoxystrobin 23 SC; P1, Neem leaves extract; P2, Datura leaves extract; C, Control; Mycorrhiza M1, *G. mosseae*; M2, *G. hoi*; Mx, Without mycorrhiza.

50% WP (13.46 and 13.28 g/plant) in variety JG 62.

Application of *G. mosseae* resulted in 10.74% increase in seed yield in comparison to plot amended with *G. hoi*. On mean basis, all the treatments significantly enhanced the seed yield of chickpea variety JG 62 over control. However, the highest seed yield was obtained from carbendazim 50% WP and azoxystrobin 23% SC treated plots in JG 62 while in C 235, carbendazim 50% WP and *T. harzianum* treated plots were significantly superior over other treatments with respect to seed yield of chickpea. Seed soaking with different treatments gave significantly higher seed yield of 1634.71 kg/ha and 2102.73 kg/ha in variety JG 62 and C 235, respectively than their soil application (Table 2). The effect of interaction between mycorrhiza and different treatments revealed that maximum seed yield of JG 62 was recorded with carbendazim 50% WP (1865.65 kg/ha) and azoxystrobin 23% SC (1835.11 kg/ha) along with *G. mosseae*. However, the minimum seed yield of 1494.02 kg/ha was obtained from plots treated with *T. harzianum* along with *G. hoi* (Supplementary Fig 2), the outcome of interaction on the seed yield for the variety C 235 was non-significant.

The results of the present investigation were in line with the findings of Arshadi *et al.* (2017) and Javanmard *et al.* (2020) who found an increase in chickpea yield on inoculation with *G. mosseae*. Sohrabi *et al.* (2015) found that *G. mosseae* inoculation resulted in maximum enhancement in chickpea plant growth parameters. Solaiman *et al.* (2005) reported that dual inoculation of arbuscular mycorrhiza and *Rhizobium* along with poultry litter enhanced number of pods/plant, number of seeds/pod and seed yield (3.96 g/plant) of chickpea. The results regarding enhanced seed yield of chickpea following inoculation with *G. mosseae* were in agreement with Karagiannidis *et al.* (2002), indicating

that the mycorrhiza are a key factor for optimizing crop productivity in the sustainable agriculture system.

The findings of our study would help in devising the integrated disease management strategy against chickpea wilt and in enhancing the crop productivity by avoiding the losses inflicted by such a devastating disease. In both the chickpea varieties JG 62 and C 235, seed treatment with carbendazim 50 WP provided maximum disease control. In variety JG 62, soil pre inoculated with *G. mosseae* along with carbendazim 50 WP was the most suitable combination in mitigating the Fusarium wilt incidence and enhancing the chickpea seed yield.

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