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Effect of Planting Techniques and Organic Amendments on the Management of Fusarium Wilt of Common Bean

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ABSTRACT: Fusarium wilt is the most serious disease of common bean causing 10 to15 per cent yield losses each year. It is a soil-borne fungal disease where water conducting (xylem) vessels become blocked. Based on morpho-cultural characters fungal pathogen isolated from infected roots of bean plant was identified as *Fusarium oxysporum* f.sp. *phaseoli* (Kendrick and Synder). Microscopic examination revealed mycelium as septate producing micro-macro conidia. The isolated fungus was put to pathogenicity test. The pathogenicity of the isolated fungus was proving by Koch's postulates. Organic amendments like vermicompost @ 12.5 q ha^{-1}+ seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), Vermicompost @ 12.5 q ha^{-1} and FYM @ 20t ha^{-1} along with planting techniques *viz*., ridges, raised bed and flat bed was tested against *Fusarium oxysporum* to manage the disease in field conditions. Vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^{9}cfu ml^{-1}) along with raised bed combination was used. The most effective treatment combination i.e. vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) exhibiting minimum disease incidence (16.42 %) was obtained when compared to control having 49.63 per cent disease incidence.

Keywords: Fusarium wilt, common bean, incidence, *Trichoderma harzianum*, organic amendment, planting technique.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) belong to family Fabaceae and is a native to South Mexico and Central America. It is a major grain legume consumed worldwide for its edible seeds and pods and is an important source of human dietary protein and calories (Pachico, 1993).

It is traditionally a basic food crop in many developing countries and serves as a major plant protein source for rural and urban areas. The crop is consumed principally for its dry beans and green pods. It provides 15 per cent protein with high contents of lysine and methionine and 30 per cent caloric requirement to the world's population and represents 50 per cent of the grain legume consumed worldwide (McConnell *et al*., 2010). The dry pulse bean and green snap bean possess 22 per cent and 6.1 per cent protein, respectively and are increasingly being consumed as an alternative to animal protein by low income families in developing countries (Bhat *et al*., 2017). Dry beans contain high levels of chemically diverse components like phenols, starch, vitamins and fructooligosaccharides giving protection against conditions like oxidative stress, cardiovascular diseases, diabetes, metabolic syndrome and many types of cancers (Camara *et al*., 2013). Moreover, beans are consumed as boiled, baked, fried or ground into flour. Crop residues such as dried pods, stems and processed byproducts are used as fodder. Although common bean is less efficient in fixing N than other legumes, yet it is able to fix up 125 kg N ha^{-1} and nodulates with several rhizobia (Wortmann, 2006).

Globally the production of common bean is 26.83 million tonnes in an area of 29.39 million ha with a productivity of 0.91 t ha^{-1}. In India it is grown in an area of 9.47 million ha with a production of 3.90 million tonnes and productivity 0.41t ha^{-1} (Anonymous, 2017). It is the premier green legume crop of Jammu and Kashmir where its cultivation is mainly confined to rainfed and karewa areas covering an area of about 2000 ha with an annual production of about 1600 tonnes and yield of about 0.8 t ha^{-1} (Choudhary *et al*., 2017).

A number of biotic and abiotic stresses like diseases, insect-pests, soil and environmental factors are responsible in reduction of crop yield. Among diseases, Ascochyta blight (*Ascochyta phaseolorum*), bean rust (*Uromyces appendiculatus*), angular leaf spot (*Phaeoisariopsis griseola*), powdery mildew (Erysiphe polygony), Bacterial blight (*Xanthomonas phaseoli*), anthracnose (*Colletotrichum lindemuthianum*), Charcoal rot (*Macrophomina phaseolina*), white mold (*Sclerotinia sclerotiorum*) and Fusarium wilt of bean (*Fusarium oxysporum*) are mostly prevalent (Junior *et al*., 2001). Fusarium wilt is one of the most important economic diseases of common bean worldwide and has caused significant economic losses (Saremi, 2000; Okungbowa and Shittu 2014; Xue *et al*., 2015). The Fusarium wilt, was first reported and identified on common bean in USA (Harter, 1929). It is ubiquitous fungal pathogen causing wilt diseases in a broad range of important crop spp. such as cotton, sugar beet, banana, tomato, flowers and legumes (Webb *et al*., 2013). The disease was subsequently reported from other common bean growing areas of the world as well (Brayford, 1997). In India, Gupta *et al*. (1993) for the first time reported the association of *Fusarium* spp. with common bean inciting rot symptoms on roots, resulting in severe wilting. The pathogen perpetuates in soil for pretty long periods of time and monoculture of bean crop result in build-up of pathogen inoculum in soil (Cross *et al*., 2000).

The mode of infection of pathogen takes place through penetration of plant root tissue, subsequent colonization of the vascular bundles, causing xylem blockage, internal stem discoloration and total plant wilt. The conspicuous symptoms of bean crop infected with *Fusarium* spp. are severe alterations and physiological abberrations like stunting and complete wilting alongwith extensive chlorosis and necrosis leading to loss of turgidity and/or plant death (Ramos *et al*., 2007). The disease show lifeless yellow-green color on the lower or primary

leaves as an indication of initial symptoms, which then progress quickly upward to younger leaves. The yellowing is uniform, but in some cases unilateral yellowing affects one side of the plant first. Due to loss of turgidity the leaf margins may curl inwards and the leaflets droop. At advanced stages of the disease, leaves develop chlorosis, becoming progressively more discernible as they become brightly yellow (El-Mougy *et al*., 2007). The vascular bundles develop a dark reddish-brown discolouration extending from the roots up the stem to the petioles and into the pods. After the plant is dead, the fungus sporulates, forming slimy masses of microconidia and macroconidia at the host surface and form chlamydospores in colonized tissues (Brayford, 1997; Dhingra and Coelho Netto 2001). Fusarium wilt is a problem in intensively cropped areas which witness high or frequent rainfall with various variability's like high moisture, excessive irrigation or poorly drained field, soil texture, plant residues/debris and a lack of crop rotation (Horsley *et al*., 2000; Ogg *et al*., 2000). The pathogen is soil-borne and is disseminated through introduced infected seeds or contaminated farm implements, irrigation or flood water, animals and wind move infested soil and plant parts from one area to other (Toledo-Souza *et al*., 2012). The disease usually develops at an optimum temperature of 20°C. Moreover, the roots are partially or totally reddish-brown in color.

The need of research, ipso facto, arises in this soil-borne disease with adoption of different planting methods and organic amendments for management.

MATERIALS AND METHODS

Field trial on effect of planting techniques and organic amendments on the management of Fusarium wilt of common bean was conducted during kharif 2017 at Faculty of Agriculture, SKUAST-K Wadura. The experiment was laid in Split Plot Design (SPD) with 54 plots having 1.5×1m plot size, each with 50 plants and replicated thrice, as per Package of Practices of SKUAST-K (Anonymous, 2011). In this design, there are two factors combination *i.e.* planting methods and organic amendments. The details of treatments given are as under:

Observations on disease incidence were recorded using formula:

Disease incidence (%) =

$$\frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

The data on wilted plants at reproductive stage were recorded at the initiation of physiological maturity, when cent percent killing of the susceptible check had occurred.

The disease incidence which was recorded at the initiation of physiological maturity, that data was analyzed using standard statistical procedure.

Symptomatological studies. The symptoms of the disease were studied in situ and also on uprooting the plants. The leaf colour and turgidity, and the overall growth of the diseased plant was compared with the healthy ones. The main stem of the plant was vertically cut open to study the vascular discolouration, if any. Plants of susceptible common bean cv. Shalimar French Bean-1 (SFB-1) were raised in a sick plot and keenly observed for development of various symptoms of the wilt disease at different phenological stages of crop.

Details of treatments

Treatment No.	Treatment Code	Treatment combination	
		Planting methods	Organic amendments
T1	P1A1	Planting on	Vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml-1)
T2	P1A2		FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (109cfu ml^{-1})
T3	P1A3		Vermicompost @ 12.5 q ha^{-1}
T4	P1A4		FYM @ 20 t ha-1
T5	P1A5		Seed treatment with carbendazim @ 0.1% (standard check)
T6	P1A0		Control (No treatment)
T7	P2A1	Planting on raised bed	Vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1})
T8	P2A2		FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (109cfu ml^{-1})
T9	P2A3		Vermicompost @ 12.5 q ha^{-1}
T10	P2A4		FYM @ 20 t ha^{-1}
T11	P2A5		Seed treatment with carbendazim @ 0.1% (standard check)
T12	P2A0		Control (No treatment)
T13	P0A1	Planting on flat bed	Vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1})
T14	P0A2		FYM @ 20 t ha-1 + seed priming with *Trichoderma harzianum*(109 cfu ml^{-1})
T15	P0A3		Vermicompost @ 12.5 q ha^{-1}
T16	P0A4		FYM @ 20 t ha^{-1}
T17	P0A5		Seed treatment with carbendazim @ 0.1% (standard check)
T18	P0A0	Overall control	Control (No treatment)

Isolation, purification and maintenance of pathogen

Isolation of casual pathogen. The entire work of isolation and purification was done under aseptic laboratory conditions. The pathogen was isolated in Potato Dextrose Agar medium (PDA) by following standard tissue isolation method. Plants showing typical wilt symptoms were selected for isolation of pathogen. The isolations were made from the stem portion, about 5 cm above the ground-level and also from the roots of diseased plants. After uprooting the diseased plants, the infected roots were first washed gently under running tap water. The infected roots of each infected common bean plant samples were split opened longitudinally with the help of sterilized scalpel. The plant parts showing brown discoloration of vascular tissues were cut into small bits along with healthy portion. These pieces were surface sterilized by dipping in 0.1 per cent hypochlorite solution for 1minute after three consecutive washing with sterilized distilled water, the pieces were transferred to PDA medium borne petriplates/slants and incubated at 25±1ºC in BOD incubator for seven days. The fungal colonies emanating from bits were examined after seven days of incubation. The mycelium emanating from these bits were transferred on fresh medium in petri plates and slants and incubated again at 25±1ºC for seven days and use for purification.

Purification of the pathogen. Dilute spore suspension of the isolated pathogen was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates and the excess of which was aseptically drained. Such plates were incubated at 25±1˚C and periodically observed for germination of spores under the microscope. Hyphae coming from each end cell of the single spore was traced and marked with the ink on the reverse side of the Petri plates. Then tip of hypha was cut and transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at temperature of 25±1˚C for 10 days. Later, the mycelial bits of the fungus were placed in the centre of Petri plates containing PDA medium and incubated at room temperature for 10 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of the fungus. Such cultures were used for further studies.

Maintenance of the pathogen. The slants and petriplates containing pathogenic fungal isolate were sub cultured on PDA slants and petriplates and allowed to grow at room temperature (25±1°C) for ten days and such slants and petriplates were preserved in a refrigerator at 4°C and reviewed once in 30 days.

Pathogenicity test. To ascertain ability of the organism to cause Fusarium wilt in common bean plant.

The isolated and purified fungus from diseased roots was tested for its pathogenicity.

Method employed by Rava *et al*. (1996) was adopted to prepare the inoculum for performing the pathogenicity test. The 16-20 days old seedlings were carefully uprooted and roots were washed gently under tap running water to remove excess sand. Then the roots of 16 days old seedlings of bean were immersed into 10^6conidia ml^{-1} suspension. One plant was inoculated with spore suspension while other plant was kept as it is which served as check. Both the inoculated and uninoculated plants in the tubes were kept under polyhouse and after 20-25 days inoculated plant developed yellowing and wilting symptoms and after 30 days symptoms were also appeared on the collar region of stem therefore confirmed the pathogenicity of the fungus. The plant showing the wilting symptoms was compared with the uninoculated plant to confirm the pathogenicity. This method has been successfully adopted by France and Abwai (1994b) to reproduce the same disease.

Identification of the pathogen. The isolated fungus was identified on the basis of various cultural and morphological studies, *viz*., colony characteristics, mycelia growth, colour, size and sporulation of the fungus under compound microscope and also on the basis of the descriptions given in various monograph and literature (Booth, 1971). The culture was sent to Indian Type Culture Collection (ITCC) Division of plant pathology, IARI, New Delhi for further confirmation and identification of fungus.

Morphological characteristics of the pathogen. The morphological characters of the pathogen were studied on culture in laboratory. Semi-permanent slides were prepared from 7 days old culture stained with cotton blue or lacto phenol. The slides were examined under microscope (400X) with respect to following characters of the casual organism.

Colony: Colour, shape and size

Mycelium: Colour, breadth, septation and branching

Chlamydospore: Colour, shape and size

Conidia: Colour, shape, size and septation

RESULTS

Symptomatology. To study the symptoms of Fusarium wilt of common bean, under field conditions plants of susceptible cultivar 'Shalimar French Bean-1' were sown on 20th of April, 2017. The plants used for studying the symptoms of disease were not inoculated with pathogen. These plants were examined for appearance of characteristic symptoms of disease which were initiated three weeks after sowing. The symptoms were observed at timely interval, the typical among those were drooping of leaves followed by discoloration and finally death of plants. On splitting the collar region of wilted plants reddish brown discoloration was observed in xylem and pith tissue. The symptoms of wilt were studied at two different stages *i.e.* seedling and adult stages.

Symptoms at seedling stage. Initially yellowing of lower leaves was observed followed by yellowing of upper leaves with the progression of disease. On uprooting the affected seedlings, uneven shrinking of the stem above and below the collar region was observed.

Symptoms at adult stage. Wilt symptoms in adult plants were quite common at flowering and pod filling stages. The affected plants showed characteristic wilting *viz*., drooping of the petioles, rachis and leaflets. Gradually, all the leaves became yellow which later turned light brown or straw coloured. Two types of wilt symptoms were observed *viz*., complete wilt and partial wilt. In case of partial wilt, only a few branches were affected at one side of the plant while in case of complete wilt all branches were affected on both sides of the plant. Later, the dried leaflets were shed at maturity. When the wilted stem was split open longitudinally, internal discoloration was seen.

Isolation, purification and maintenance of pathogen

Isolation of casual pathogen. Wilt infected plants of common bean were collected from the field and brought to the laboratory of Plant Pathology for further study. The fungus associated with disease was isolated on PDA under aseptic conditions. The fungal culture was continuously examined for the mycelial growth. The white aerial growth of fungus was observed within 7 days of inoculation. By 10th day, old mycelium turned to grayish white then light pinkish-whitish in colour.

Purification and maintenance of the pathogen. The pure culture of the fungus was maintained on potato dextrose agar medium using single spore technique. Further sub-culturing was done at monthly intervals and the culture was stored in a refrigerator at 4°C for further studies (Plate 1).

Pathogenicity test. The pathogenicity of the isolated fungus was established by confirming Koch's postulates using root dip inoculation technique (Plate 2). Observations regarding the pathogenicity of the test fungus revealed the initiation of typical symptoms of the disease after 16-20 days of inoculation. However, no symptom development was observed in control. The fungus on re-isolation from artificially inoculated plants resembled initially isolated and inoculated pathogen and hence pathogenicity of the isolate was proved.

Identification of the pathogen. The pathogen identified on the basis of morpho-cultural characters was *Fusarium oxysporum* f.sp. *phaseoli*. Further, the culture was sent to ITCC, Division of Plant Pathology IARI, New Delhi for confirmation. The pathogen was confirmed as *Fusarium oxysporum* f.sp. *phaseoli* under Acc. No. 12, 770;18.

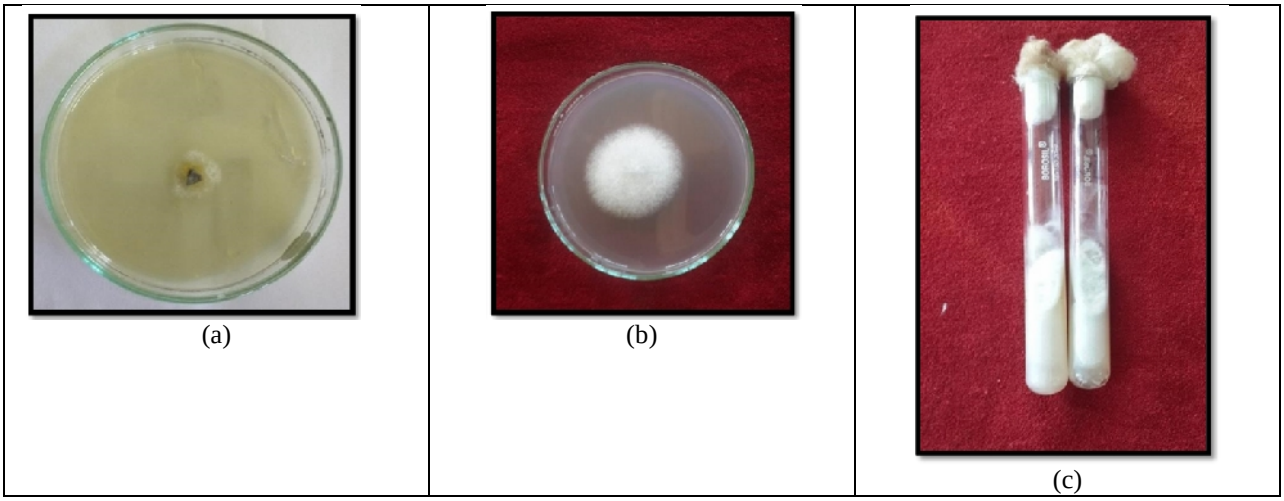


Plate 1: (a) Isolation (b) Purification and (c) Maintenance of *Fusarium oxysporum* f. sp. *Phaseoli.*

Plate 2: Pathogenicity test of Fusarium wilt on common bean plant.

Morphological characteristics of the pathogen

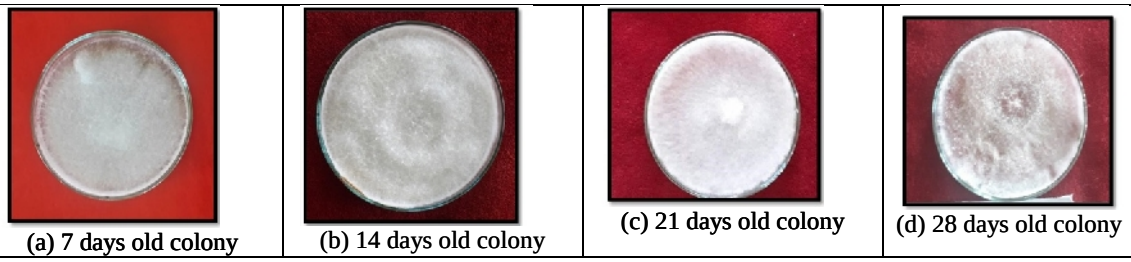
Morphological characteristics in cultur. The morphological characters of different structures *viz.* mycelium, micro and macro conidia and chlamydospores produced by the pathogen were studied using 5-10 day old culture and the results are presented in Table 1.

The pure culture of the fungus, was critically observed for colony characters. The fungal colony was observed to be white, cottony with profuse, fluffy aerial mycelium after 7 days of incubation which gradually turned light pinkish, grayish white or creamish in colour. Microscopic observations of the isolated fungus revealed that the mycelium was septate and hyaline with hyphal width of 1.5-2 µm. The fungus produced cottony and fluffy growth on PDA which was initially white and later turned light pinkish after 25-30 days of incubation (Plate 3). The pathogen produced three types of spores within the culture i.e. microconidia, macroconidia and chlamydospores. Microconidia were hyaline, aspetate, cylindrical to falcate with ovoid shape. The length × breadth of the microconidia ranged from 2.5-(3.2)-3.5× 7.0-(9.5)-13.0 µm. Macroconidia ranging from 3.0-(3.5)-4.0 × 28-(33.5)-40 µm were found to be septate (4-5 septa).

Table 1: Morphological characteristics of *Fusarium oxysporum* f.sp. *phaseoli.*

Morphological stage	Colour	Type	Shape	Size (µm)*	Septation
Mycelium	Hyaline	…..	……	Hyphal width 1.5-2.0	Septate
Conidia	Hyaline	Micro	Ovoid	2.5-(3.2)**-3.5 × 7.0-(9.5)-13.0	Aseptate
		Macro	Sickle shaped	3.0-(3.5)-4.0 × 28-(33.5)-40	Septate 4-5 septa
Chlamydospores	Hyaline	……	Globose	8.2-10.5 × 6.60-9.90	Aseptate

*Values are means of 15 replications ; **Values in parenthesis are mean values



(a) 7 days old colony (b) 14 days old colony (c) 21 days old colony (d) 28 days old colony

Plate 3: Colony characteristics of *Fusarium oxysporum* f.sp. *phaseoli.*

(a) Mycelium (b) Microconidia (c) Macroconidia (d) Macroconidia with septation (e) Chlamydospores

Plate 4: Morphological characteristics of *Fusarium oxysporum* f.sp. *phaseoli*

They were sickle-shaped with slightly foot shaped basal cell. Chlamydospores were produced 12-15 days after incubation in cultures. They were globose, single celled, aseptate, produced terminally or intercalary with 8.210.5 × 6.60-9.90 µm size (Plate 4).

Effect of organic amendments and planting techniques on the management of Fusarium wilt of common bean. The treatments comprised of six organic amendments and three planting techniques *viz*., raised bed, ridges and flat bed. Among six treatments there were two control treatments *viz*., negative control which was not given any treatment and positive control which was treated with 0.1% carbendazim. To manage the disease in vivo the field was equally divided into raised bed, ridges and flat bed. Analysis of the data (Table 2 and Plate 5) revealed that all the treatments had significant effect on per cent disease incidence over negative control. Among the treatments, the most effective treatment combination found was vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) which showed the wilt incidence of 16. 42 per cent as compared to negative control exhibiting wilt incidence of 44.32 per cent when applied on raised bed followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} exhibiting wilt incidence of 22.21 per cent, 30.35 per cent and 32.96 per cent respectively. The positive control showed least disease incidence of 9.72 per cent as compared to other treatments when applied on raised beds.

Table 2: Effect of organic amendments and planting techniques on the per cent incidence of Fusarium wilt of common bean.

Organic amendments	Planting techniques			Mean
	Raised bed	Ridges	Flat bed	
Vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1})	**16.42** (23.89)***	20.40 (26.84)	22.11 (28.04)	**19.64 (26.25)**
FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1})	22.21 (28.10)	24.01 (29.32)	31.57 (34.17)	**25.93 (30.53)**
Vermicompost @ 12.5 q ha^{-1}	30.35 (33.41)	33.26 (35.20)	36.34 (37.05)	**33.32 (35.22)**
FYM @ 20 t ha^{-1}	32.96 (35.02)	34.51 (35.96)	36.54 (37.17)	**34.67 (36.05)**
Seed treatment with carbendazim @ 0.1% (standard check)	**9.72** (18.14)	12.52 (20.71)	13.62 (21.64)	**11.95 (20.16)**
Control (No treatment)	**44.32** (41.72)	46.36 (42.89)	49.63 (44.77)	**46.77 (43.13)**
Mean	**26.00 (30.05)**	**28.51 (31.82)**	**31.69 (33.80)**	

C.D (p≤0.05)
P = 0.97; O= 1.6; P×O =2.61 where,
P = Planting techniques; O = Organic amendments
*Figures in parenthesis are arc sine transformed values
**Average of three replications

(a) Field trial

(b) Crop without disease symptoms

(c) Crop with disease symptoms

(d) Symptoms on uprooted plants

Plate 5: Management of Fusarium wilt through organic amendments and planting techniques.

The data from Table 2 further revealed that when the same treatments were applied on ridges, the most effective treatment combination found was vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) which showed the wilt incidence of 20. 40 per cent as compared to negative control exhibiting wilt incidence of 46.36 per cent followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} exhibiting wilt incidence of 24.01 per cent, 33.26 per cent and 34.51 per cent respectively. The positive control showed least disease incidence of 12.52 per cent as compared to other treatments when applied on ridges. The data from Table 2 further revealed that when the same treatments were applied on flat beds, the most effective treatment combination found was vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) which showed the wilt incidence of 22.11 per cent as compared to negative control exhibiting wilt incidence of 49.63 per cent followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} exhibiting wilt incidence of 31.57, 36.34 and 36.54 per cent respectively. The positive control showed lowest disease incidence of 13.62 per cent as compared to other treatments when applied on flat beds.

A significant combination existed between organic amendments and planting techniques. The range of disease incidence in treatments varied from 9.72 to 49.63 per cent. Minimum disease incidence of 16.42 per cent was recorded in treatment combination T7 i.e. vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}).

Effect of organic amendments and planting techniques on average number of pods $plant^{-1}$. The effect of treatment combinations on average number of pods plant-1 was recorded commencing from 5 days after completion of flowering stage till harvest. The data in Table-3 revealed that there was a significant increase in pod number per plant when combination of seed priming with *Trichoderma harzianum* + organic amendments in the soil with different planting techniques *viz*., raised bed, ridges and flat bed was used. The data further revealed that maximum number of pods per plant was obtained with treatment combination combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (20.41) indicating an increase of 5.58 compared to negative control (14.83) followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1}, with number of pods per plant i.e. 19.25, 17.91 and 16.58 respectively while planted on raised bed. It was further revealed that the treatment combinationvermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) showed maximum number of pods per plant when compared with positive control.

When the same treatments were applied on ridges, maximum number of pods per plant was obtained with treatment combination vermicompost @ 12.50 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (19. 66) indicating an increase of 5.83 compared to negative control (13.83) followed by FYM @ 20 t ha^{-1} + seed priming *with Trichoderma harzianum* (10^9 cfu ml^{-1}), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} with number of pods per plant i.e. 19.08, 17.66, 17.33 and 15.66 respectively. It was further revealed that the treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) showed maximum number of pods per plant when compared with positive control.

The data further revealed that on flat bed, maximum number of pods per plant was obtained with treatment combination maximum vermicompost @ 12.50 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (18.50) indicating an increase of 4.75 compared to negative control (13.75) followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml-1), vermicompost @ 12.5 q ha^{-1}, FYM @ 20 t ha^{-1}, exhibiting number of pods per plant i.e. 18, 17.00 and 14.58 respectively. It was further revealed that the treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) showed maximum number of pods per plant when compared with positive control.

Table 3: Effect of organic amendments and planting techniques on average number of pods $plant^{-1}$.

Organic amendments	Raised bed	Ridges	Flat bed	Mean
Vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9cfuml^{-1})	20.41*	19.66	18.50	**19.52**
FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1})	19.25	19.08	14.41	**17.58**
Vermicompost @ 12.5 q ha^{-1}	17.91	17.33	17.00	**17.41**
FYM @ 20 t ha^{-1}	16.58	15.66	14.58	**15.61**
Seed treatment with carbendazim @ 0.1% (standard check)	18.33	17.66	17.08	**17.96**
Control (No treatment)	14.83	13.83	13.75	**14.13**
Mean	**17.73**	**16.97**	**16.27**	

C.D (p≤0.05)
P = 1.14; O= 1.33; P×O =1.66; where,
P= Planting techniques; O= organic amendments
*Average of three replications

Effect of organic amendments and planting methods on average number of seeds pod^{-1}. The effect of various treatments on average number of seeds per pod is presented in Table-4. A perusal of the data revealed a significant increase in seed number per pod by the treatment combination and their interaction with planting techniques. It was observed that maximum number of seeds per pod was obtained with treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (7.16) compared to negative control which exhibiting least number of seeds per pod (4.58) followed by FYM @ 20 t ha^{-1}+ seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), Seed treatment with carbendazim @ 0.1% (positive control), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} with number of seeds per pod i.e. 6.50, 5, 83, 5.08 and 4.83 respectively while planted on raised bed.

A significant combination existed between the planting techniques and organic amendments. Maximum numbers of pods per plant was observed in treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) with 20.41 while planting on raised bed followed by same treatment combination with 19.66 while planting on ridges.

When all the treatments were applied on ridges, It was observed that maximum number of seeds per pod was obtained with treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (6.91) compared to negative control which exhibiting least number of seeds per pod (3.91) followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), Seed treatment with carbendazim @ 0.1% (positive control), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} with number of seeds per pod i.e. 6.33, 5.75, 4.91 and 4.66 respectively.

When all the treatments were applied on flat beds, maximum number of seeds per pod was recorded in treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (5.33) compared to negative control which exhibiting least number of seeds per pod (3.75) followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), seed treatment with carbendazim @ 0.1% (positive control), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} with number of seeds per pod *i.e.* 4.58, 4.25, 3.83 and 3.75 respectively.

A significant combination existed between the planting techniques and organic amendments. Among all the treatment combinations maximum number of seeds per pod *viz.*, 7.16 and 6.91 seeds per pod was observed in treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) when planted on raised beds and ridges respectively.

Table 4: Effect of organic amendments and planting methods on average number of seeds pod^{-1}.

Organic amendments	Planting techniques			Mean
	Raised bed	Ridges	Flat bed	
Vermicompost @ 12.5q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9cfu ml^{-1})	7.16*	6.91	5.33	**6.47**
FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1})	6.50	6.33	4.58	**5.80**
Vermicompost @ 12.5 q ha^{-1}	5.08	4.91	4.16	**4.72**
FYM @ 20 t ha^{-1}	4.83	4.66	4.00	**4.50**
Seed treatment with carbendazim @ 0.1% (standard check)	5.83	5.75	4.25	**5.72**
Control (No treatment)	4.58	3.91	3.75	**4.08**
Mean	**5.56**	**5.41**	**4.34**	

C.D (p≤0.05)
P = 0.44; O= 0.36; P×O =0.72 where,
P= Planting techniques and O= organic amendments
*Average of three replications

Effect of organic amendments and planting methods on average seed yield (q ha^{-1}) of common bean. Table 5 presented the data of various treatments on average number of seeds per pod. A perusal of the data revealed a significant increase in seed yield by the treatment combinations and their interaction with planting techniques. It was found that among all the treatments combinations, the maximum seed yield was recorded in treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (13.1 q ha^{-1}) while the minimum seed yield was obtained in case of negative control (8.6 q ha^{-1}) followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), seed treatment with carbendazim @ 0.1% (positive control) vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} with seed yield *i.e.* 11.6, 10.6, 10 and 9.6 q ha-1 while planted on raised bed technique.

When all the treatments were applied on ridges, the maximum seed yield was recorded in treatment combination vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (12.4 q ha^{-1}) while the minimum seed yield was obtained in case of negative control (8.7 q ha^{-1}) followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), seed treatment with carbendazim @^{9} 0.1% (positive control), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} with seed yield i.e. 11.1, 9.8, 9.2 and 8.1 q ha^{-1} respectively.

When all the treatments were applied on flat bed, the maximum seed yield was recorded in treatment combination vermicompost @ 12.5q ha^{-1}+ seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) (11 q ha^{-1}) while the minimum seed yield was obtained in case of negative control (5.4 q ha^{-1}) followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}), seed treatment with carbendazim @ 0.1% (positive control), vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} with seed yield *i.e.* 9.4, 8.4, 8.3 and 6.8 q ha^{-1} respectively.

A significant combination existed between the planting techniques and organic amendments. The most effective treatment of all the tested ones was combination of vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) in which the yield recorded was 13.1 q ha^{-1} and 12.4 q ha^{-1} when planted in raised beds and ridges respectively.

Table 5: Effect of organic amendments and planting methods on average seed yield (q ha^{-1}) of common bean.

Organic amendments	Planting techniques			Mean
	Raised bed	Ridges	Flat bed	
Vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9cfu ml^{-1})	13.1*	12.4	11.0	**12.1**
FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1})	11.6	11.1	9.4	**10.7**
Vermicompost @ 12.5 q ha^{-1}	10.0	9.2	8.3	**9.16**
FYM @ 20 t ha^{-1}	9.6	8.1	6.8	**8.1**
Seed treatment with carbendazim @ 0.1% (standard check)	10.6	9.8	8.4	**9.6**
Control (No treatment)	8.7	8.6	5.4	**6.9**
Mean	**10.6**	**9.8**	**8.1**	

C.D (p≤0.05)
P = 0.38; O=0.59; P×O =1.16 where,
P= Planting techniques; O = organic amendments
*Average of three replications

DISCUSSION

The present study was undertaken to evaluate the effect of planting techniques and organic amendments on the management of Fusarium wilt of common bean and screening of germplasm against the disease. Using organic amendments, for control of soil borne plant pathogens is a vital area of plant pathological research all over the world. It is regarded as highly important, as use of chemicals against plant pathogens is becoming very expensive and ecologically dangerous leading to serious health problems in human beings and animals besides polluting soil and water by chemical residues. In present study, three planting techniques *viz*., raised, ridges and flat bed were used along with organic treatments. The most effective treatment combination observed was vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) which recorded the lowest disease incidence of 16.42, 20.40 and 22.11 per cent when the seeds were sown on raised, ridges and flat beds respectively. When compared with the negative control, all the treatments were significantly showing the less disease incidence whereas, when the same treatments were compared with positive control the disease incidence was more in treatments than that recorded in positive control. Our results are in agreement with the results of Merkuz and Getachew, 2012 who worked on influence of chickpea Fusarium wilt (*Fusarium oxysporum* f.sp. *ciceri*) on desi and kabuli-type of chickpea in integrated disease management option at wilt sick plot in North Western Ethiopia and found that on raised bed disease incidence was lesser as compared to ridges and flat bed. Our results are supported by Ahamad and Ahmed (1999) ; Prasad *et al*. (2012) who studied management of pigeonpea wilt caused by *Fusarium oxysporum* f.sp. *udum* Butler through integrated approaches and found that seed treatment with bioagents and application of organic amendments in the soil reduced the disease incidence to 20 per cent. Similar results were also revealed by Kumar *et al*. (2013) who worked on management of vascular wilt on lentil through biocontrol agents and organic amendments in Tarai area of Uttrakhand state and observed that seed treatment with *Trichoderma harzianum* and application of organic manure reduced the disease incidence from 7.5 to 9.5 per cent. Our results are in conformity with the results

of Hossain *et al.* (2013) who studied integrated management of Fusarum wilt of chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* with microbial antagonist, botanical extract and fungicide and reported that seed treatment with *Trichoderma harzianum* and application of FYM reduced the disease incidence to 13 per cent. The results of present investigation are also in agreement with the results of Karcho *et al*. (2015) who worked on influence of organic amendments and fungicides on population dynamics of fungi in chickpea ecosystem and found that application of FYM and vermicompost alongwith seed treatment with bioagents reduced the disease incidence to 20 per cent. Our results are in conformity with the results of Najar *et al*. (2016) who studied an ecofriendly approach for the management of Fusarial wilt of chilli and observed that combined application of FYM + *Trichoderma* recorded significantly least wilt incidence of 20.39 to 30.75 per cent. Our results are also supported by the results of Kala *et al*. (2016) who found that the disease incidence was least when Trichoderma treated seeds were sown in soils amended with vermicompost in case of chickpea wilt. Our results are also in conformity with results of Patidar (2017) who reported that combined application of FYM and seed treatment with *Trichoderma harzianum* reduce the disease incidence to 24 percent in case of chickpea wilt.

Besides significantly reducing the wilt incidence, organic amendments and their interaction with planting techniques also exhibited significant effects on other yield contributing factors such as number of pods per plant, number of seeds per pod and yield per plot. The results of present investigation on number of pods per plant revealed that the organic amendments + *Trichoderma harzianum* have significantly higher pod number than negative control. In general, the average number of pods per plant ranged from 13.75 to 20.41. Maximum number of pods per plant (10.41) was recorded when seeds were sown in raised bed treated with vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) which was found to be at par when compared with ridges and flatbed techniques with same organic treatments. When compared with the negative control, all the treatments were significantly showing higher number of pods per plant whereas, when the same treatments were compared with positive control the number of pods per plant is more in treatment *i.e*. FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) but less in treatments, vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} than that recorded in positive control. Our results are in conformity with results of Sreeja (2014); Pandey (2016) who studied that seed treatment with bioagents and organic amendments increased number of pods per plant in case of cowpea wilt and chickpea wilt. Our results are in agreement with result of Kailas, 2017 who found that seed treatment with bioagents increased the pod number in case of chickpea wilt.

The results of present investigation on number of seeds per pod revealed that the organic amendments + *Trichoderma harzianum* has significantly higher seed number than negative control. In general, the average number of seeds per pod ranged from 3.75 to 7.16. Maximum number of seeds per pod (7.16) was recorded when seeds were sown in raised bed treated with vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) which was found to be at par when compared with ridges and flatbed techniques with same organic treatments. When compared with the negative control, all the treatments were significantly showing higher number of pods per plant whereas, when the same treatments were compared with positive control the number of pods per plant is more in treatment *i.e*. FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) but less in treatments, vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} than that recorded in positive control. Our results are in conformity with results of Sreeja (2014) who reported that seed treatment with bioagents and organic amendments increased seed number per plant in case of cowpea wilt. The results are in agreement with results of Najar *et al*. (2016) who found that seed treatment with bioagents and organic amendments increased the fruit weight per plant in case of Fusarium wilt of chilli.

The results of present study on seed yield revealed that the organic amendments + *Trichoderma harzianum* has significantly higher seed yield than negative control. In general, the average number of pods per plant ranged from 8.2 to 12.4. Maximum seed yield (13.1 q ha^{-1}) was recorded when seeds were sown in raised bed treated with vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) which was found to be at par when compared with ridges and flatbed techniques with same organic treatments. When compared with the negative control, all the treatments were significantly showing higher number of pods per plant whereas, when the same treatments were compared with positive control the number of pods per plant is more in treatment *i.e*. FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* (10^9 cfu ml^{-1}) but less in treatments, vermicompost @ 12.5 q ha^{-1} and FYM @ 20 t ha^{-1} than that recorded in positive control. Our results are in conformity with the results of Hossain *et al*. (2013); Kumar *et al*. (2013); Sreeja, 2014 and Pandey, 2016 who reported that seed treatment with bioagents and organic amendments increased the seed yield in case of fusarium wilt of chickpea. The results are in agreement with results of Najar *et al*. (2016) who found that seed treatment with bioagents and organic amendments increased the fruit weight in case of Fusarium wilt of chilli. Our results are in conformity with result of Kailas, 2017 who found that seed treatment with bioagents increased the seed yield in case of chickpea wilt.

CONCLUSION

Under natural conditions, the disease symptoms first appeared on lower leaves in first week of August as yellowing of lower leaves followed by drying of the leaves, drooping of petioles and when stem was split open, reddish brown discolouration of the xylem vessels was noticed.

Fungus isolated from infected roots of wilted common bean plants was tested for pathogenicity test by root dip inoculation techniques. The pathogenicity of the isolated fungus was established by proving Koch's postulates. The culture of the pathogen produced characteristic disease symptoms after 16-20 days of inoculation. Therefore, pathogen was reisolated on potato dextrose agar medium and incubated at 25±1oC. The pathogen was found pathogenic on common bean and was identified as *Fusarium oxysporum* f.sp. phaseoli.

The fungus produced colonies that were observed to be circular having white colour, cottony with profuse, fluffy aerial mycelium which gradually turned light pinkish, grayish white or creamish white. The mycelium is septate and hyaline with hyphal width 1.5-2 µm. The microconidia ranged from 2.5-(3.2)-3.5 × 7.0-(9.5)-13.0 µm. Macroconidia varied between 3.0-(3.5)-4.0 × 28-(33.5)-40 µm. Microconidia were aseptate. Macroconidia were septate with 4-5 septa. Chlamydospores were produced 12-15 days after incubation in cultures. They were globose, single celled, aseptae, produced terminally or intercalary with 8.210.5 × 6.60-9.90 µm size. On the basis of morphological characteristics of conidia, the pathogen was identified as *Fusarium oxysporum* f.sp. phaseoli. The culture was sent to the ITCC at IARI in New Delhi, and the identity of the isolate was confirmed as *Fusarium oxysporum* f.sp. phaseoli (Kendrick and Synder) under accession No. 12, 770;18.

Studies on the effect of planting techniques and organic amendments on the management of Fusarium wilt of common bean revealed that vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* was found to be most effective treatment exhibiting minimum disease incidence of l6.42 per cent followed by FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* showed incidence of 22.21 per cent as compared to negative control plot having 49.63 per cent incidence. This was followed by vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* and FYM @ 20 t ha^{-1} + seed priming with *Trichoderma harzianum* sown in ridges exhibiting 20.40 per cent and 24.01 per cent disease incidence respectively.

Minimum per cent disease incidence was recorded in *Trichoderma harzianum* + vermicompost, which was at par with *Trichoderma harzianum* + FYM and was significantly superior over rest of the treatments including negative control.

Among the treatments used, vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}). Among the interaction vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}) along with raised beds exhibited highest per cent disease control over negative control. This was followed by vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}) along with ridges.

The highest numbers of pods per plant was recorded in the treatment vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}) when seeds were sown on raised bed (20.41) and vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}) when seeds were sown on ridges (19.66) compared with other treatments.

The highest numbers of seeds per pod was recorded in the treatment vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}) when seeds were sown on raised bed (7.16) and vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}) when seeds were sown on ridges (6.91) compared with other treatments.

The highest seed yield was recorded in the treatment vermicompost @ 12.5 q ha^{-1} + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}) when seeds were sown on raised bed (13.1 q ha^{-1}) and vermicompost @ 12.5 q ha-1 + seed priming with *Trichoderma harzianum* (109 cfu ml^{-1}) when seeds were sown on ridges (12.4 q ha^{-1}) compared with other treatments.

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