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ORGANIC MANAGEMENT OF CHICK PEA WILT (*CICER ARIENTINIUM*) CAUSED BY *FUSARIUM OXYSPORUM* F.SPP. *CICERIS*

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ABSTRACT

Antagonistic potentiality of *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *Fusarium oxysporum* f. spp. *ciceris* under *in vitro* conditions. The effect of organic amendments, plant extract and essential oils *viz*; seaweed powder, mustard cake, Neem oil, Garlic bulb extract and Karanj oil on disease control potentiality of test antagonists against chickpea wilt pathogen in soil. In present study all the tested bioagents, organic amendment, plant extract and essential oils were found the effective against the *F. oxysporum* f. spp. *ciceris.* Among all the bioagents minimum 11.33 and 16.33 per cent disease incidence were recorded in T1 (Soil application of *Trichoderma* spp. @ g/m^2) followed by 14.00 and 18.33 in T2 (Soil application of *Pseudomonas* @ g/m^2). In case of control (T9) 26.33 and 33.33 percent disease incidence was recorded after 45 and 60 DAS. Whereas maximum 28.25 and 38.25 plant height were observed in T6 (Soil application of mustard cake @ 100 g/m^2) followed by 27. 00 and 37.00 cm in T1 (Soil application of *Trichoderma* spp. @ g/m^2). In case of control minimum 22.25 and 32.25cm plant height was recorded. On the basis of present investigation, it may be concluded that the used of *Trichoderma* spp. @ 10 gm/$m^{2,}$ and *Pseudomonas*@ 10 gm/m^2 as a soil application may be recommended for the management *Fusarium oxysporum* f. spp. *ciceris* of chickpea.

Keyword **:** *F. oxysporum* f. spp. *Ciceris*, *Trichoderma* spp., *Pseudomonas,* disease incidence

Introduction

Chickpea (*Cicer arietinum* L.) belongs to the family Leguminosae. It is an annual grain legume (pulse crop) that is extensively cultivated for human consumption throughout the world, including the Mediterranean basin, the Near East, Central and South Asia, East Africa, South and North America, and Australia**.** It is the second-most important pulse crop in the world (after dry bean), covering 15% (10.2 million ha) of the area dedicated to pulse cultivation and accounting for 14% (7.9 million tons) of pulse production worldwide

In India, chickpea contributes 42- 47% of total pulse production, with major production concentrated in Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka, and Andhra Pradesh. Chickpea is rich in protein (17.1%), fat (5.3%), and minerals (3.0%), and it is known for its high content of polyunsaturated fatty acids, including linoleic acid, as well as bioactive compounds like folic acid, tocopherols, and β-carotene (Aharon *et al*., 2012). It has health benefits such as reducing coronary heart disease risk, improving glucose tolerance, and controlling blood pressure. Despite its benefits, chickpea yield is low, averaging 15-20 quintals per hectare, due in part to diseases caused by a range of pathogens, including fungi, viruses, bacteria, nematodes, and mycoplasma. Fusarium wilt, caused by *Fusarium oxysporum* f. spp. *ciceris*, is a major threat, reducing yields significantly. This soil-borne pathogen can persist in the soil for up to six years and causes symptoms ranging from early wilting to late wilting, leading to substantial yield losses. Management of Fusarium wilt primarily involves using resistant varieties, although resistance can break down over time due to pathogen variability. Integrated management strategies, including cultural practices, resistant varieties, and reduced chemical use, along with

promoting beneficial microbes, are essential for effective disease control.

Therefore, integrated management strategies are the possible solution to maintain plant health mainly for soil borne plant pathogen. These strategies include modification of cultural practices, growing of resistant varieties with minimum application of chemical and encouragement of beneficial microbial population to reduce pathogen inocula.

Keeping this in view, the present investigations have been undertaken to develop Organic management of chick pea wilt (*Cicer arietinum*) caused by *Fusarium oxysporum* F. spp. *ciceris* pathogens of chickpea and their management with the following objectives: -

- To study about the Symptoms and pathogenicity of *Fusarium oxysporum* F. spp. *ciceris*
- To study about the organic management of chick pea wilt caused by *Fusarium oxysporum* F. spp. *ciceris*

Material and Methods

Experimental site and location

In the present study, field experiments were conducted at Karguaji Organic Research Farm of Institute of Agricultural Science, Department of Plant Pathology, situated in the main campus of the Bundelkhand University, Jhansi (U.P.) during 2023. A virulent isolate of *Fusarium oxysporum* f. spp. *ciceris* (Padwick) Synd. & Hans. isolate from wilt infested chickpea plants was used in the present studies.

To study the management of *Fusarium oxysporum* F. spp. *ciceris*

Biocontrol agents viz., *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* obtained from the Culture Collection Section, the University. were used for the management study conducted under field conditions.

Culture medium and their preparation

During case study, for growing fungi, bacteria etc. different culture media were used.

PDA preparation procedure

The required quantity of peeled potatoes was cut into small pieces and boiled in 500 ml of distilled water till the pieces became soft. The potato extract was filtered through muslin cloth and the filtrate was collected in the beaker. Rest of the 500 ml water was made warm and 20 gm agar and 20-gram dextrose was added properly by shaking through glass rod. Two hundred ml of this solution was dispensed in each conical flask of 250 ml capacity. Flasks were plugged with nonabsorbent cotton plugs. Flasks containing medium were sterilized at 121^{0}C at 15lbs pressure/inch^2 for 15 minutes in an autoclave. Transferred medium was allowed to cool up to 40-42 ºC before pouring into Petri plates.

Procedure for preparation of NAM medium

All the ingredients were mixed properly in 500 ml distilled water with the help of glass rod and volume was made up 1000 ml by adding required amount of distilled water. Two hundred ml of this solution was poured in each 250 ml capacity of conical flasks. Flasks were tightly plugged with nonabsorbent cotton plug and wrapped with silver foil and autoclaved at 121ºC temperature at 15lbs pressure/inch^2 for 15 minutes. Sterilized medium was allowed to cool up to 40-42 ^{0}C before pouring into Petri plates.

Nutrient broth preparation procedure:

Similar procedure was followed for the preparation of Nutrient Agar Medium except addition of Agar.

Preparation of mass culture of bioagents:

Wheat grains were used for mass culture of fungal bio agents *Trichoderma* spp. Wheat grains were soaked overnight in water for 12 hours and then spreaded on towel paper to remove the extra water. Dextrose was added in wheat seeds @ 2% of seed and then 250 g of wheat grain were taken in each 500 ml conical flasks. Flasks with wheat grains were plugged with nonabsorbent cotton and wrapped with aluminum foil and sterilized in autoclave at 121^{0}C temperature at 15 lbs. pressure/inch2 for 15 minutes. Flask was taken out from autoclave allow to cool and kept on the bench of laminar flow. The conical flask containing wheat seed were inoculated with 5 mm diameter PDA discs punched from the periphery of actively growing 7 days old culture of *Trichoderma* spp. All inoculated conical flasks were incubated in a BOD incubator at 25±2 °C temperature. Bioagents were allowed to grow with periodic shaking of the flasks, after 15 days of *Trichoderma* colonized the surface of all wheat seeds. Whole grains were taken out from flask, and spreaded in neat and clean plastic trays, clumps of grains were broken, then after fungal growth covered grain were shade air dried, after proper drying grains were converted in fine powder with the help of mixer grinder. This fine powder was used for conducting research trials.

Preparation of plant extract

Extracts of plant were prepared by crushing leaves of garlic bulb extract with sterilized distilled water. The material was dried at room temperature (23 ^{0}C) for 6

hours before extraction to remove the excess water. 100g cloves were crushed separately with 100 ml sterilized water. The extract was filtered through a muslin cloth and centrifuged for 5000 rpm at 30 min. The extracts were sterilized by passing them through a Whatman filter paper (0.22-micron pore size).

Isolation of wilt causing pathogens *Fusarium oxysporum* f. spp. *ciceris* of chickpea:

Isolation of (*Fusarium oxysporum f.* spp. *ciceris),* the infected roots of chickpea plant showing characteristic symptoms of wilt disease were collected from field. The roots of infected chickpea plants were washed under tap water to remove soil particles. The roots were cut into small pieces of 5 - 8 cm long and surface sterilized by dipping in 0.1% $HgCl_2$ solution for 30 seconds followed by three-time wash with sterilized distilled water. These bits were transferred in the Petri plates containing PDA media under aseptic condition. These Petri plates were incubated at 27 ± 1^0C temperature in BOD incubator. After 3 days of inoculation, fungal mycelium growth was observed around the inoculated root. Identification of Pathogen was confirmed by observing the morphological features of colony, spore characteristics referred in the literature by Booth's key (1977). Pure culture was maintained in slants on PDA media for further study.

Pathogenicity Test

The pathogenicity of *Fusarium oxysporum* f. spp. *ciceris* were tested according to method given by (Dashgupta, 1988). The susceptible chickpea variety (BGM-10216 variety) was used to assess the pathogenicity of pathogens. Pot soil was collected from the organic research farm, Institute of Agricultural Science, Bundelkhand University, Jhansi (U.P.). The collected soil was thoroughly mixed and filled in polythene begs sterilized at 121^0C at 15 lbs. pressure/inch^2 for 15 minutes in an autoclave. Sterilize soil was filled in pots for conducting experiments and 20 gm mass culture of pathogens was mixed thoroughly in sterilize pots soil. Then apparently healthy15 chickpea seeds were sown in each pot. Pot without inoculums were maintain as control. Soil moisture was maintained by adding water on throughout the period. Re-isolation of *Fusarium oxysporum* f. spp. *ciceris,* was made from such infected plants and culture were compared with original culture.

Study the effect of bio agent, Plant extract, essential oil and organic amendment against wilt of chickpea in field condition

In order to evaluate the efficacy of bio agent, organic amendment, plant extract and essential oils on management of wilt associated fungal pathogen of chickpea in field condition. The crop was sown during third week of November at a spacing of 30 x 10 cm. The experiment was conducted in Randomized Block Design with three replications for each treatment. Plot size was maintained 3 X 3 meter for each replication. Bio agents were used with vermicompost to enriched the antagonist's activity of soil. And they were used at the time of sowing of the crop. Control plot was maintained without treatment.

Observation Recorded

Plant Disease incidence was recorded using following formula

$$\text{PDI} = \frac{\text{Number of diseased leaves/ treatment}}{\text{Total number of leaves/ treatment}} \times 100$$

Results and Discussion

Symptomatology

Symptoms of wilt disease causing pathogens *Fusarium oxysporum* f. spp. c*iceris* are drooping, yellowing, drying of the leaves and discoloration of vascular system. Susceptible plants showed the symptoms at 25 days after sowing. Wilted plants lie down on the ground and finely died. The fungus attacked the root system, made its way through the epidermis, cortex and eventually into xylem vessel of the tap root from where it spread. As a result, the lateral roots might wither off. Pods from the wilted plants look healthy but seeds were smaller in size, wrinkled and discolor.

Collection, isolation and purification of *Fusarium oxysporum* f. spp. *ciceris*:

Infected plants of Chickpea were collected from Organic Research Farm, Karguaji, Department of Plant Pathology, Institute of Agricultural Science, Jhansi where disease was prevalent and collected. Samples were brought to the laboratory for isolation and further studies. The fungus was isolated on PDA from infected roots of chickpea plants under aseptic conditions. The fungus emerging form root bits placed on PDA was observed to have profuse white aerial mycelium later turn brown to black on PDA. The culture was purified by hyphal tip technique.

Identification of the pathogen

Identification of the isolated fungus was done on the basis of cultural characteristics and morphological characters of the conidia. The young hyphae of the fungus were observed to be hyaline, thin-walled light brown to dark brown in colour and having septa.

Pathogenicity

The *Fusarium oxysporum* f. spp. *ciceris* isolated from infected roots of chickpea plant found pathogenic when seed and soil was inoculated artificially to chickpea plant under pot conditions. The characteristic symptoms of root appeared after 15 days. The most recognizable symptoms were sudden death of chickpea seedlings. First leaves start withering and drying the root followed gradually killing whole plant. Roots of the disease plant showed brown to blackish lesions. Re-isolation of *Fusarium oxysporum* f. spp. *ciceris,* was made from such infected plants and culture were compared with original culture for the confirmation of the pathogen.

Nikam *et al.* (2011) reported similar symptoms of yellowing, dropping and wilting of leaves of diseased plants caused by *Fusarium oxysporum* f. spp. *ciceris,* after 25 days. From the disease plants re-isolation of the pathogens was made which yielded the fungi, *Fusarium oxysporum* f. spp. *ciceris,* identical with the original one that was inoculated. Similar results have been reported by Kaur *et al*., (2007), Khalil (2007) and Hossain *et al*., (2013),

Management of *Fusarium oxysporum* f. spp. *ciceris*

To test the effectiveness of *Trichoderma spp.* @ 10 g/m^{2,}, *Pseudomonas spp.* @ 10 g/m^2, *Bacillus subtilis* g/m^2, seaweed powder @ 10 g/m^2, mustard cake @ 100 g/m^2 were taken as the soil application and Neem oil @ 10 %, Garlic bulb extract @10 % and Karanj oil @10 % as a foliar application. Control plot was maintained without any application.

Effect of different treatment on disease incidence and plant height at 45 DAS:

All the tested bioagents and organic amendment significantly reduced the disease incidence, increase the crop yield and plant height as compare the control. Data given in Table 1 and fig. 1 that, treatment T1 (Soil application of *Trichoderma* spp. @ 10 g/m^2), recorded the minimum 11.33 per cent disease incidence after 45 DAS of sowing followed by 14.00 in T2 (Soil application of *Pseudomonas* @ 10 g/m^2), 16.33 in T3 (Soil application of *Bacillus subtilis* @ 10 g/m^2) and 17.67 in T7 (Foliar application of garlic bulb extract @ 10%) after 45 days of sowing. Whereas maximum 19.33 per cent disease incidence was recorded in T6 (Soil application of mustard cake @ 100 g/m^2) followed by 18.33 in T5 (Foliar application of neem oil @ 10%). While in case of T8 (Foliar application of Karanj oil @ 10% conc.) 21.00 per cent disease incidence was recorded. In case of control (T9) 26.33 per cent disease incidence was recorded after 45 DAS.

Maximum 28.25 plant height was observed in T6 (Soil application of mustard cake @ 100 g/m^2) followed by 27. 00 cm in T1 (Soil of *Trichoderma* spp. @ 10 g/m^2) 26.33 cm in (Soil application of *Pseudomonas* @ g/m^2) and 25 cm in T3 (Soil application of *Bacillus subtilis* @ 10 g/m^2). Whereas 24.25 cm plant height in recorded in T8 (Foliar application of Karanj oil @ 10%) after 45 DAS. In case of control minimum 22.25 cm plant height was recorded. Similar result was observed by Meena *et al.* (2013) reveled that seed treatment with *T. harzianum* + Neem oil was also effective in suppression of the disease. This treatment useful for suppression of soil borne pathogens in organic farming of chickpea as well as for root rot complex in other crops.

Effect of different treatment on plant height and crop yield at 60 DAS:

Maximum 38.25 plant height was observed in T6 (Soil application of mustard cake @ 100 g/m^2) followed by 37 cm in T1 (Soil of *Trichoderma spp.*. @ g/m^2) 35.33 cm in T2 (Soil application of *Pseudomonas* @ g/m^2) and 35 cm in T3 (Soil application of *Bacillus subtilis* @ 10 g/m^2). Whereas 34.25 cm plant height in recorded in T8 (Foliar application of Karanj oil @ 10%) after 60 DAS. In case of control minimum 32.25cm plant height was recorded.

All the tested bioagents and organic amendment significantly increase the crop yield as compare the control. Data given in Table no. 2 and fig. 2 that, treatment T1 (Soil application of *Trichoderma spp.* @ g/m^2), recorded the maximum 15.75 q/ha. crop yield followed by 15.25 in T2 (Soil application of Pseudomonas @ g/m^2), 15.00 q/ha. in T3 (Soil application of Bacillus subtilis @ 10 g/m^2) and 13.50 in T7 (Foliar application of garlic bulb extract @ 10%) after 45 days of sowing. Whereas minimum 12.75 q/ha was recorded in T8 (Foliar application of Karanj oil @ 10% conc.). In case if control (T9) 10.89 q/ha. crop yield was recorded after 60 DAS. Wav are *et al.* (2017) Khan *et al.* (2014) reported that seed treatment with carbendazim 50 WP @ 2 g/ kg +soil treatment with *Trichoderma* spp. (1X 10^8 CFU) @ 5 Kg with vermicompost @ 100 kg /ha significantly enhanced seed germination, reduced disease incidence and promoted plant growth and yield of chickpea. Similar result had been observed by Kamdi *et al.* (2012) and Kaur *et al.* (2007) reported that under field conditions the isolates of *Trichoderma viride* and *T. harzianum,* significantly enhanced seed germination, reduced disease incidence and promoted plant growth and yield of chickpea as compared to control plot.

Table 1: Effect of different treatment on wilt of chick pea disease incidence after 45 DAS:

Treatments	Treatments details	No. of wilted plant/ plot		
		PDI	% disease reduction	Plant height
T1	Soil of *Trichoderma* spp.p. @ g/m^2,	11.33	56.96	27
T2	Soil application of *Pseudomonas* @ g/m^2,	14.00	46.82	26.33
T3	Soil application of *Bacillus subtilis* @ 10 g/m^2,	16.33	37.97	25
T4	Soil application of Sea weed powder @ 10 g/m^2,	19.00	27.83	22.75
T5	Foliar application of neem oil @ 10%	18.33	30.38	24
T6	Soil application of mustard cake @ 100 g/m^2,	19.33	26.58	28.25
T7	Foliar application of garlic bulb extract @ 10%	17.67	32.89	23.33
T8	Foliar application of Karanj oil @ 10%	21.00	20.24	24.25
T9	Control	26.33		22.25
CD @ 5 % level		1.96		2.43
SEm		0.65		0.81

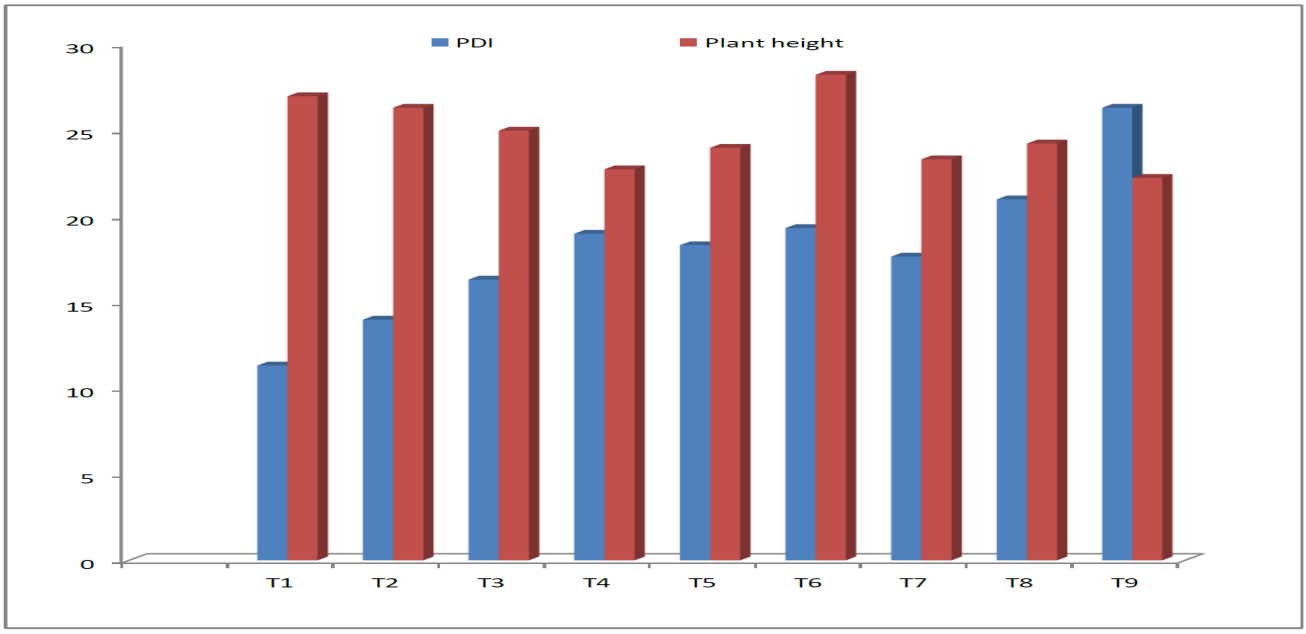

Fig. 1: Effect of different treatment on disease incidence and Plant height after 45 DAS

Table 2: Effect of different treatment on wilt of chick pea disease incidence after 60 DAS

Treatments	Treatments details	No. of wilted plant/ plot			Yield q/ha.	% yield increasing
		PDI	% disease reduction	Plant height		
T1	Soil of *Trichoderma* spp.p. @ g/m^2,	16.33	51.00	37	15.75	44.62
T2	Soil application of *Pseudomonas* @ g/m^2,	18.33	45.00	35.33	15.25	40.00
T3	Soil application of *Bacillus subtilis* @ 10 g/m^2,	23.33	30.00	35	15	37.74
T4	Soil application of Sea weed powder @ 10 g/m^2,	25.00	24.99	32.25	12.25	12.48
T5	Foliar application of neem oil @ 10%	25.00	24.99	34.75	13.15	20.75
T6	Soil application of mustard cake @ 100 g/m^2,	27.67	16.98	38.25	13.00	19.37
T7	Foliar application of garlic bulb extract @ 10%	24.67	25.98	33.50	13.50	23.96
T8	Foliar application of Karanj oil @ 10%	26.00	21.99	34.25	12.75	17.07
T9	Control	33.33		32.25	10.89	
CD @ 5 % level		**2.58**		**3.41**	**1.32**	
SEm		**0.86**		**1.14**	**0.44**	

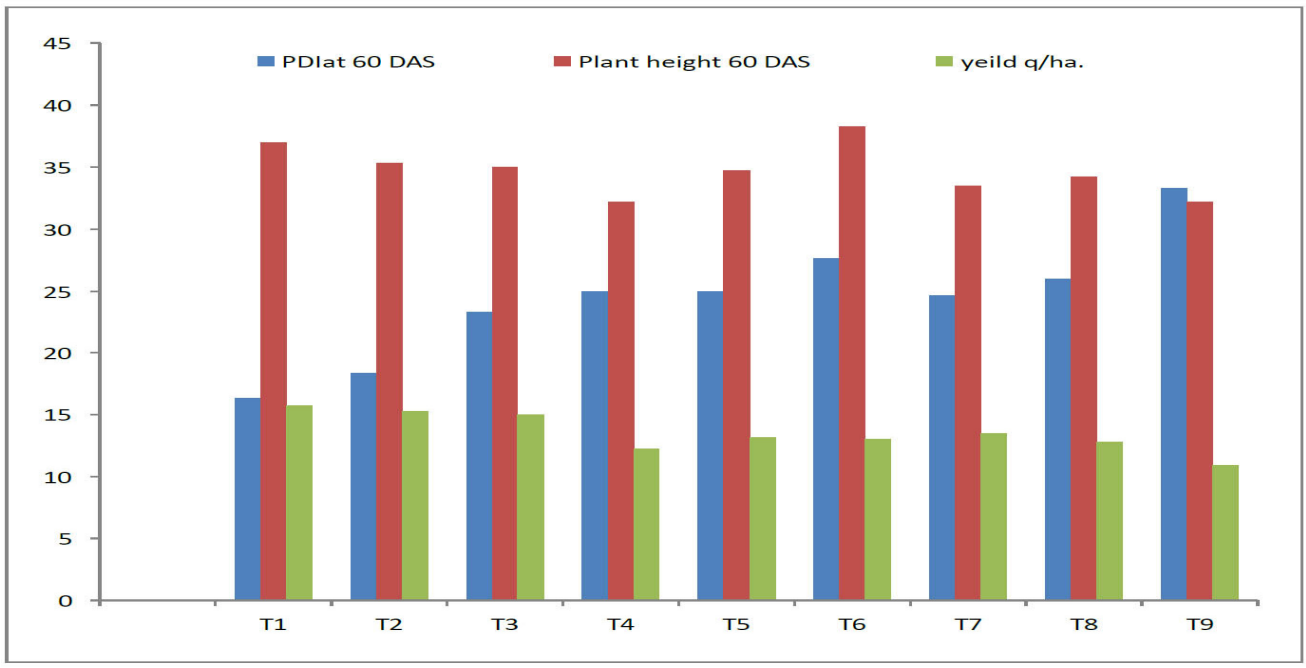

Fig. 2: Effect of different treatment on PDI, plant height and yield at 60 DAS

Conclusion

- Among all the various tested treatments against the management of the disease, minimum disease incidence was found in soil application of *Trichoderma* spp. @ gm/m^2 and *Pseudomonas*@ gm/m^2 found most effective against wilt causing pathogen
- In case of plant extract, that the foliar application of garlic bulb extract @ 10% concentration found most effective for this pathogen.
- On the basis of present investigation, it may be concluded that the treatments which was used as a soil application viz. *Trichoderma* spp. @10 g/m^2 and *Pseudomonas*@ 10 g/m^2 and foliar application of garlic bulb extract @ 10% concentration may be recommended for the management *Fusarium oxysporum* f. spp. *ciceris*

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