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Management of Fusarium Wilt of chickpea (*Cicer arietinum* L.) under the undulating red and lateritic belt of West Bengal

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Chickpea (*Cicer arietinum* L.) is a vital source of plant derived edible protein in many countries. Indian subcontinent accounts for 90% of the total world chickpea production. The major limiting factor in chickpea production is Fusarium wilt which is caused by *F. oxysporum* Schlechtend.Fr. f.sp. *ciceris* causing 10-15% yield losses annually. Experiments were conducted with a view to minimize the Fusarium wilt with different approaches under field condition at the agricultural farm, Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan,West Bengal, during the *rabi* season of 2012-14. The incidence of Fusarium wilt of chickpea was found to be influenced greatly by changing the date of sowing. The incidence of disease was found to decreased with the early sowing. While, it increased considerably in late sown crop, which sown after the schedule time of sowing i.e. second week of November (11.11.2014). Maximum disease incidence of wilt 21.20 % was recorded from 30 days late shown crop. The disease was reduced greatly in early shown crop and reached the lower level of 6.8% at 30 days early sowing. Of all the bicontrol agents evaluated against *F. oxysporum* f. sp. *ciceris, Trichoderma harzianum* showed maximum growth inhibition (80%) followed by *Pseudomonas fluorescens* 75.55 % and *T. viride* 64.44 % which differed significantly from each other. Systemic fungicide Carbendazim exhibited maximum fungal growth inhibition (100%) at 0.1, 0.15 and 0.2% concentrations followed by Combi product (Carbendazim 12% + Mancozeb 63%), which gave 88.7 % , 100 % and 100% growth inhibition at 0.1, 0.15 and 0.2 % concentrations respectively. Among the different organic composts, spent mushroom substrate + cow dung+ earth worm compost (EWC) 1:1:1 was found to be most effective in minimizing the disease incidence in field (67.8 %) and gave maximum plant height (49.53 cm) at 75 DAS, 1000 seed weight (181g) and 1780 kg/ha yield. Among the oil seed cakes, Til (sesame) cake @ 500 kg/ha performed better in terms of reduction in disease incidence (50.55%) and yield (1680 kg/ha). The present findings highlighting the various approaches of wilt disease management of chickpea which will help the farmers for decision making and maximization of their profit.

Key words : Chickpea, disease incidence, *Fusarium oxysporum* f. sp. *ciceris,* management, *Pseudomonas fluorescens*

INTRODUCTION

India is the largest chickpea producer as well as consumer in the world. Its cultivation is mainly concentrated in semi-arid environments. It is the most important pulse crop of India and accounts for approximately 75% of world's chickpea production. In West Bengal chickpea is cultivated over an area of 38.0 hectare with a production of 38.9 tons and productivity of 1024 kg per ha. which is low compared to the world average of 1348 kg per hectare. The major limiting factor in chickpea production is Fusarium wilt which is caused by

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F.oxysporum Schlechtend.Fr. f.sp. *ciceris*. This disease has been reported from 33 countries of the world causing 10-15% yield losses annually depending upon the environment condition. Chemical pesticides have its own limitations such as high cost, no availability, toxicity, development of resistant strains, environmental pollution and adverse effect on beneficial soil microflora and fauna has compounded the problem in India and all other countries producing chickpea. Biological control is a potential alternative to chemical fungicides The best control method found for *F. oxysporum* is planting resistant varieties, although not all have been bred for every formae specialis of the pathogen. Nagamani *et al.* (2015) reported the biocontrol efficacy of antagonistic organism in managing the chickpea major root rot pathogens *viz., F. o*. f. sp. *ciceris, R. bataticola* and *S. rolfsii* under *in vitro* condition and observed 81.1% efficiency of *Trichoderma isolates,* KNN 4 against *R. bataticola*, KNPG 3 showed against *S. rolfsii* (80.7%) and ATPU 1 inhibited the mycelia growth of *F. o* f. *sp. ciceris* (84.1%). Organic amendments of soil enhance the activity of *Trichoderma*. Soil amendment of *Trichoderma* with various organic substrates have showed its effectiveness against few soil borne diseases of various crops (Bhaskar *et al.* 2007; Nikam *et al.* 2007; Singh *et al,* 2007).However, very little works has been done so far in West Bengal with particular reference to lateritic belt where Bengal gram considered being a good choice for the farmers. Therefore, present study was undertaken with a view to manage this disease by slightly changing its time of sowing, by changing the soil status through organic amendments and also to exploit the higher efficacy of bio-agents, and chemicals for better utilization to productivity of chickpea.

MATERIALS AND METHODS

Evaluation of biological agent and fungicides against Fusarium wilt of chickpea

In vitro evaluation of bio-control agents

Five biocontrol agents *viz*., *Trichoderma viride, Trichoderma harzianum, T. hamatum, Gliocladium virens* and *Pseudomonas fluorescens* were evaluated against *F. oxysporum* f. sp*. ciceris* through dual culture technique. For evaluation of fungal biocontrol agents, mycelial discs of test fungus were inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at ends of the Petri plates and mycelial discs of the fungus was placed at the centre. Four replications were maintained for each treatment.The plates were incubated at 27±1^{0}C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula.

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

where, X is the mycelial growth of the pathogen (*F. oxysporum* f. sp. *ciceris*) in the absence of antagonist and Y is the mycelial growth of the pathogen (*F. oxysporum* f. sp. *ciceris*) in the presence of the antagonist.

In vitro evaluation of selected fungicides

Two systemic fungicides, two non-systemic fungicides and two Combi fungicidal products at the concentrations of 500, 1000, 1500 and 2000 ppm were screened against *F. oxysporum* f. sp*. ciceris.* Fungicides were mixed at different concentration in autoclaved PDA medium by poisoned food techniques. 5 mm diameter agar disc of test fungi was cut from 4-day old culture and placed in the center of Petri plates containing different concentration of fungicides. The plates without fungicides served as control. The radial growth was recorded after 5 to 7 days of incubation when the fungus covered the plates completely in control. The per cent inhibition of the fungus over control was calculated by using the per cent inhibition of the fungus over control was calculated. The fungicides used in this study are as given below in Table 1

Effect of date of sowing on the incidence of chickpea wilt

To study the effect of date of sowing on the incidence of wilt disease of chick pea in field, common variety Mahamaya-1 was selected. The date of sowing were fixed on the basis of schedule time of sowing date i.e. second week of November, generally being followed by the farmers in lateritic belt of West Bengal and in present study it was taken as 11.11.2014 and served as control. Accordingly, different dates were fixed with

Table 1 : Details of fungicides used in *in vitro* evaluation

Common name	Chemical name	Trade name
	Systemic fungicides	
Carbendazim 50% WP	2-(Methoxy-Carbaryl)–benzimidazole	Bavistin
Thiophanate methyl 70%WP	(Dimethyl 4,4'-(o-phenylene)bis (3-thioallophanate)	Roko
	Non systemic fungicides	
Chlorothalonil 75% WP	Tetrachloroisophthalonitrile	Kavach
Mancozeb 75%WP	Manganese ethylene bis dithiocarbonate + Zinc	Indofil M45
	Combi product	
(Carbendazim 12%+ Mancozeb 63% WP	Manganese zinc ethylene bisdithiocarbamate +2-(Methoxy carbaryl) – benzimidazole	Saaf
Captan 70 % + Hexaconazole 5%WP	N (trichloromethylthio) Cyclohix-4 ere-1, 2 dicarbozimide + (RS)-2-C2, 4-dichlorophe myl)-1-(14-1, 2, 4-triazole-1-41) hexan-2-Ol	Kick

15 days intervals from the scheduled date of sowing viz. 15 days early, 30 days early, 15 days late and 30 days late and 45 days late. Incidence of the wilt disease was recorded at an interval of 10 days after appearance of first disease symptoms. Accordingly, data on the interval between the date of sowing and the appearance of first symptoms and total interval were recorded .Apparent infection rate of spread of the disease was calculated.

$$R = \frac{2.3}{t_2 - t_1} \left\{ \text{Log}\left(\frac{X_2}{1-X_2}\right) - \text{Log}\left(\frac{X_1}{1-X_1}\right) \right\}$$

where,

r = Apparent infection rate at exponential growth stage

t_1 = First day of observationt_2= Last date of observation

X1 = Production of the disease on first day of observation

X_2= Production of the disease on last day of observation

Effect of organic amendments on Fusarium wilt of chickpea

Various oilseed cakes viz., Groundnut cake: 500 kg/ha, Neem seed cake, mustard seed cake 500 kg/ha, Til (sesame) cake 500 kg/ha, Earth worm compost (EWC)+ FYM, Spent mushroom substrate (SMS), Spent mushroom substrate + FYM (1:1) and Spent mushroom substrate + cow dung + Earthworm compost (EWC) 1:1:1 were evaluated to find out their effect on wilt disease of chickpea. Organic composts were mixed with soil after first ploughing of the soil, after mixing the organic composts the field was kept undisturbed for 7 days for building up of the soil microflora. Soil without any amendment served as control. Chickpea variety Mahamaya-2 was selected for the study. Observations were recorded on per cent disease incidence, plant height and 1000 seed weight. Per cent disease incidence was calculated by using the following formula,

$$\text{Per cent Disease Incidence} = \frac{\text{No. of plants showing wilting symptom}}{\text{Total No. of plants}} \times 100$$

RESULTS AND DISCUSSION

In vitro evaluation of bio control agents against F. oxysporum f. sp. ciceris

Five biocontrol agents *viz*., *Trichoderma viride, Trichoderma harzianum, T. hamatum, Gliocladium virens* and *Pseudomonas fluorescens* were evaluated against *F. oxysporum* f. sp. *ciceris* and the data are presented in Table 2. The results revealed that the antagonists significantly reduced the growth of *F. oxysporum* f. sp. *ciceris* either by over grow-

Table 2 : *In vitro* evaluation of bio control agents against *Fusarium oxysporum* f.sp. *ciceris*

Bioagent	Diameter of the mycelium growth after 7 days of inoculation		% inhibition in mycelium growth of *F. oxysporum* f. sp. *ciceris*
	Bioagents (mm)	*F.oxysporum* f. sp. *ciceris* (mm)	
T. viride	58	32	64.44 (8.089)
T. harzianum	72	18	80 (8.999)
T. hamatum	50	40	55.5 (7.515)
Gliocladium virens	61	29	67.77 (8.291)
P.fluorescens	68	22	75.55 (8.748)
Control	0	90	0 (1.000)
S. Em. +		1.745002	0.60961
CD at 5%		7.533670	0.18781

* Data in parenthesis are square root transformed value

Table 3 : *In vitro* evaluation of fungicides against *F. oxysporum* f. sp. *ciceris*

Common name	Trade Name	Percentage inhibition of mycelium growth Concentration			
		500 ppm	1000 ppm	1500 ppm	2000 ppm
Carbendazim 50%WP	Bavistin	98.00	100.00	100.00	100.00
Thiophanate methyl 70% WP	Roko	48.80	85.50	100.00	100.00
Mancozeb 75% WP	Indofil M45	60.50	86.40	98.30	100.00
Chlorothalonil 75% WP	Kavach	45.30	72.75	87.20	98.50
Carbendazim 12% + Mancozeb 63%	Saaf	49.50	88.70	96.45	100.00
Captan 70 % + Hexaconazole 5%WP	Kick	47.00	68.50	86.50	98.20
S. Em. +		1.0313	1.0657	1.3896	2.1098
CD at 5%		4.1972	4.3378	5.6552	6.2683

ing or by exhibiting inhibition zones (Table 2). Of all the biocontrol agents evaluated against *F. oxysporum* f. sp. *ciceris* the *T. harzianum* showed maximum growth inhibition (80%) and found most effective than all other treatments. This was followed by *P. fluorescens* 75.55 % and *T. viride* 64.44 %. Other antagonists showed fair response against the pathogen (Fig.1, 7,8).

The results of dual culture technique against *F. oxysporum* f. sp. *ciceris* revealed that all the five bioagents significantly reduced the growth of *F. oxysporum* f. sp. *cerceris* either by over growing or by exhibiting inhibition zones, except bacterial bioagent. Maximum reduction in mycelium growth was observed with *T. harzianum* (80%) and found most effective than all other treatments. This was followed by *P. fluorescens* 75.55 % and *T. viride* 64.44 %. which were significantly superior than other bioagents tested. Results are in accordance with Rani and Mane (2014).

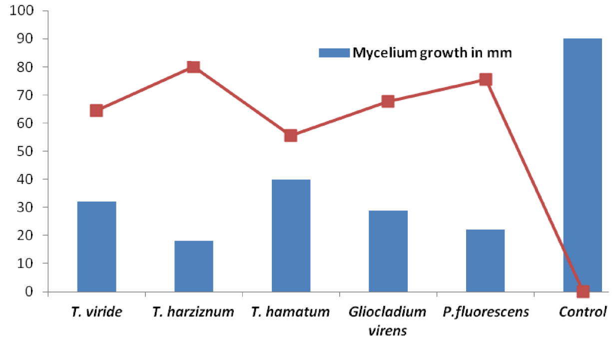

Fig. 1 : Effect of different bioagentson the mycelial growth of *F.oxysporum* f.sp. *ciceris*

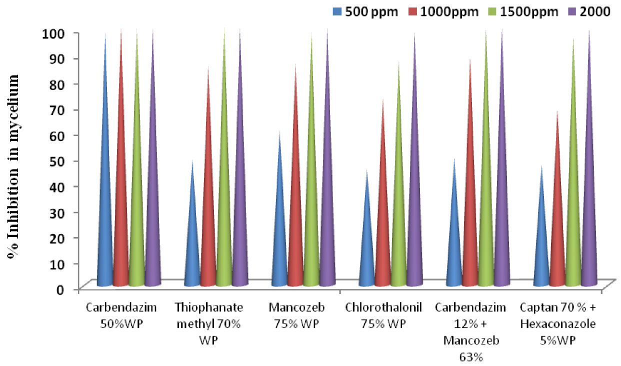

Fig. 2 : *In vitro* evaluation of fungicides ahgainst *F.oxysporum* f. sp.*ciceris*

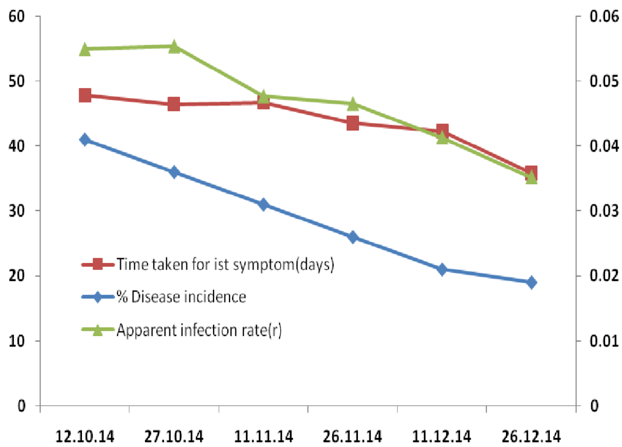

Fig. 3 : Relationship between disease incidence, time talken for first symptoms and rate of infection in field of chickpea

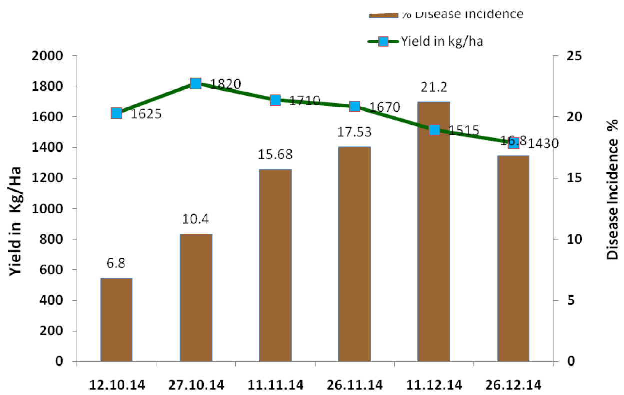

Fig. 4 : Effect of date of sowing on the incidence of Fusarium wilt and yield of chickpea

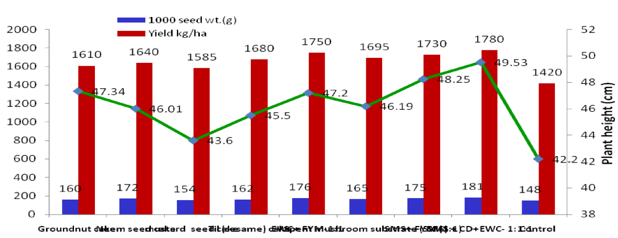

Fig. 5 : Effect of organic ammendment on plant growth, seed weight and yield of chickpea

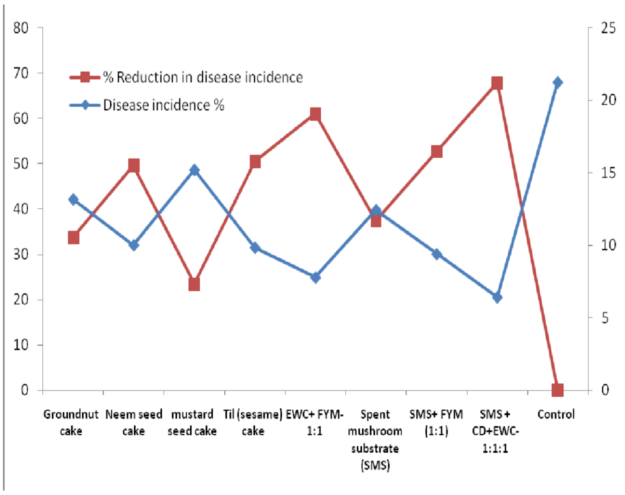

Fig. 6 : Effect of organic amendments on Fusarium wilt of chickpea

In vitro evaluation of fungicides against F. oxysporum f. sp. ciceris

The efficacy of two systemic fungicides, two non-systemic fungicides and two combi fungicidal products at the concentrations of 500,1000,1500 and 2000 ppm were screened against *F. oxysporum* f. sp. *ciceris.* The results indicated the positive response of all fungicides in inhibiting the growth of *F. oxysporum* f. sp. *ciceris* (Table.3). Among the two systemic two non-systemic and two combi fungicidal products evaluated, Carbendazim exhibited maximum fungal growth inhibition (100%) at 0.1, 0.15 and 0.2% concentrations followed by combi product of Carbendazim 12% + Mancozeb 63% which gave 88.7 % , 100 % and 100% growth inhibition at 0.1, 0.15 and 0.2 % concentrations respectively. Lower dose of fungicides (500 ppm) was found to be less effective against F. *oxysporum* f. sp. *ciceris* (Fig.2).

Among systemic fungicides, Carbendazim at 0.1, 0.15 and 0.2 % inhibited the growth of *F. oxysporum* f. sp*. ciceris* completely under *in vitro* condition. Carbendazim being a benzimidazole group of fungicides, they interfere with energy production and cell wall synthesis of fungi Carbendazim induced nuclear instability by disturbing the mitosis and meiosis. Results of two combi products, *viz.,* carbendazim 12% + mancozeb 63% and Captan 70% + Hexaconazole 5% gave 96.54% and 86.5% inhibition with 0.15% concentration respectively.

Table 4 : Effect of different date of sowing on the incidence of chickpea wilt

Date of sowing	Time taken for developing 1st disease symptoms in field (Days)	Interval between initial to final incidence (Days)	Initial Disease Incidence (%)	Final Disease Incidence (%)	Apparent infection rate(r)	Yield (Kg/ha)
12.10.14	41	35	0.85	6.8	0.05491	1625
27.10.14	36	38	1.02	10.4	0.05537	1820
11.11.14	31	38	1.96	15.68	0.04767	1710
26.11.14	26	34	2.75	17.53	0.04653	1670
11.12.14	21	36	3.56	21.20	0.04134	1515
26.12.14	19	33	4.21	16.8	0.03520	1430
S.Em+-				1.091441	0.582309	38.29940
CD at 5%				3.438183	0.183435	120.6482

Table 5 : Effect of organic amendments against of *Fusarium* wilt of chick pea in field

Treatment	Dose kg/ha	Plant height in (cm) (75 DAS)	1000 seed weight (g)	Percent disease incidence	% reduction in disease incidence	Yield kg/ha
Groundnut cake	500	47.34	160	13.16 21.265	33.7	1610
Neem seed cake	500	46.01	172	10 18.431	49.6	1640
mustard seed cake	500	43.6	154	15.2 22.938	23.4	1585
Til (sesame) cake	500	45.5	162	9.83 18.263	50.5	1680
Earth worm compost (EWC)+ FYM (1:1)	500	47.2	176	7.76 16.171	60.9	1750
Spent mushroom substrate (SMS)	500	46.19	165	12.42 20.631	37.4	1695
Spent mushroom substrate + FYM (1:1)	500	48.25	175	9.38 17.827	52.7	1730
Spent mushroom substrate + cow dung+ Earth worm compost (EWC) 1:1:1	500	49.53	181	6.4 16.625	67.8	1780
Control		42.2	148	21.25 27.441	0.0	1420
S. Em. +		5.1208	2.9689	.444315		121.950
CD at 5%		15.214	8.8209	1.32008		353.867

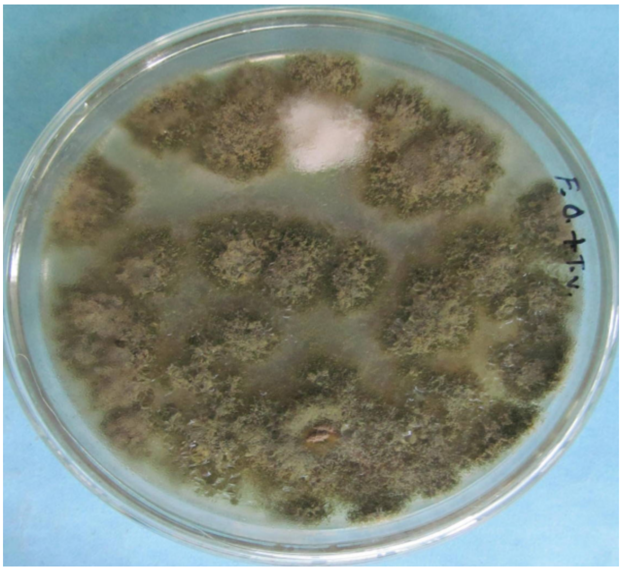

Fig. 7 : *In vitro* evaluation of *T. viride* against *F. oxysporum* f. sp. *ciceris*

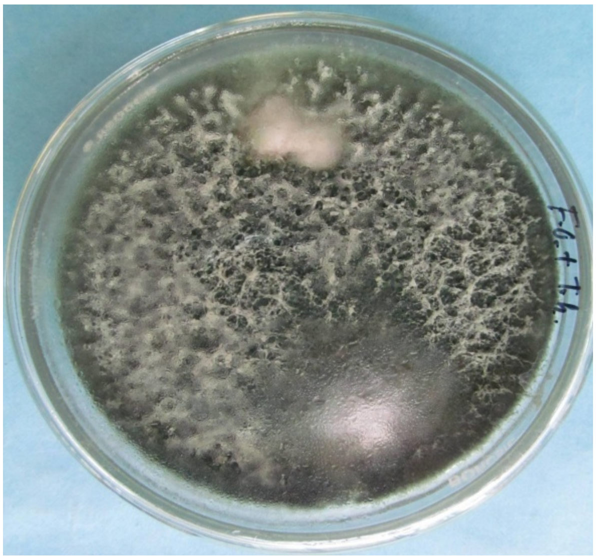

Fig. 8 : *In vitro* evaluation of *T. viride* against *F. oxysporum* f. sp. *ciceris*

Effect of different date of sowing on the incidence of chickpea wilt and yield

The incidence of *Fusarium* wilt of chickpea was found to be influenced greatly by changing the date of sowing. The incidence of disease was found to be decreased with the early sowing. While, it was increased considerably in late sown crop, which was sown after the schedule time of sowing i.e. second week of November (11.11.2014). Maximum disease incidence of wilt 21.20 % was recorded from 30 days late shown crop followed by 17.53% from 15 days later than scheduled time. The disease was reduced greatly in early shown crop and reached the lower level of 6.8% at 30 days early sowing. There was a great correlation exist between time taken for developing first symptoms in field and level of incidence. The infection rate was found to be more in early sowing of crop i.e. 0.05537, and 0.05537 respectively with 30 days and 15 days early sown crop. Maximum yield of chickpea (1820 kg/ha) was obtained from the field sown on 27.10.14 i.e. 15 days earlier than scheduled time of sowing (11.11.2014) followed by (1710 kg/ha) from the field sown at scheduled time (11.11.2014) (Fig 3 and 4). Higher incidence of *Fusarium* wilt in late sown crop was probably due to appropriate soil temperature and moisture level during the month of February and March which helped the pathogen for faster growth and multiplication. However decreased yield in late sown chickpea was probably due to the interaction between inherent genetic potential of crop and environment and also due to the congenial environment for the development of *Fusarium oxysporum* f. sp. *ciceris* (25-30°C) and the optimum soil temperature for root infection is 30°C or above.

Effect of organic amendments and nitrogen fixing bacteria against Fusarium wilt disease of chickpea

Results indicated the positive response of different treatments towards disease management and yield (Table 4). Spent mushroom substrate + Cow dung+ Earthworm compost (EWC) 1:1:1 was found to be most effective in minimizing the disease incidence in field (67.8 %) and gave maximum plant height (49.53 cm) at 75 DAS, 1000 seed weight (181g) and 1780 kg/ha yield followed by Earth worm compost (EWC)+ FYM (1:1) which exhibited 60.90 % reduction in disease, 47.2 cm plant height, 176 g weight of 1000 seeds and 1750 kg yield per hectare. Both of the treatments differed significantly over untreated control. The performance of various organic composts and their combinations were found better than the oil cakes tested. Among the oil seed cakes, Til (Sesame) cake @ 500 kg/ha performed better in terms of reduction in disease incidence (50.55) and yield (1680 kg/ha) followed by Neem seed cake which reduce 49.6 % disease incidence and gave 1640 kg/ha yield. A range of 23.4 to 37.4 % reduction in disease incidence and fair quantity of yield i.e. 1585 to 1695 kg/ha was obtained from other treatments which differ significantly over control (Fig.3,4)

Present findings of spent mushroom substrate (SMS) + vermicompost over the growth and yield of chickpea were corroborated with the reports of Hernandez *et al*. (2010), obtained from strawberry and lettuce crops respectively.

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