

“Studies on interaction of *Meloidogyne incognita* and *Fusarium oxysporum* in blackgram (*Vigna mungo* (L) Hepper).”

THESIS

Submitted to the

Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur

**In partial fulfillment of the requirement for
the Degree of**

MASTER OF SCIENCE

In

**AGRICULTURE
(PLANT PATHOLOGY)**

By

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2015

CERTIFICATE - I

*This is to certify that the thesis entitled, “**Studies on interaction of Meloidogyne incognita and Fusarium oxysporum in blackgram.**” submitted in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE in Agriculture (Plant Pathology)** of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur is a record of the bonafide research work carried out by Mr. DILIP KUMAR, I.D. No. AP/JB-543/2013 under my guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee and the Director of Instruction.*

All the assistance and help received during the course of the investigation has been acknowledged by him.

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List of Symbols

| Symbol | Abbreviation | Stand for |
|----------------|---------------|---------------------|
| @ | | At the rate of |
| % | | Per cent |
| ± | | Plus or minus |
| % | | Percentage |
| ^o C | | Degree centigrade |
| μ | | micron |
| | μm | micrometer |
| | Avg | Average |
| | W / W | Wet by wet |
| | Sp | Species |
| | i.e. | that is |
| | viz., | Namely |
| | <i>et al.</i> | co- workers |
| | Fig | Figure |
| | CD | Critical difference |
| | Cm | Centimeter |
| | Ha | Hectare |
| | Hrs | Hours |
| | Mt | million tonnes |
| | MI | Milliliter |
| | Mm | Millimeter |
| | G | Gram |
| | Mg | Milligram |
| | NS | Non significant |

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(Dilip Kumar)

INTRODUCTION

In Indian agriculture pulses, play an important role in maintaining soil fertility and supplying protein to the large vegetarian population of the country. Nearly 11,000 species of legumes are known and many are of importance as industrial, medicinal or food plants.

Black gram (*Vigna mungo L*), one of the pulses, is mostly produced in Asian countries as their tropical climate and soil type suits its cultivation. India occupies 31 lakh hectares of land with 14 lakh tones production and 451.61 kg/ha productivity. Madhya Pradesh alone contributes 4.72 lakh hectares of land with 1.66 tones production and 351.69 kg/ha productivity (Anon.2012).

Urdbean contains about 26 percent proteins. In addition, being an important source of human food and animal feed, it also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen.

Several fungi have been reported in black gram viz. *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Colletotrichum* sp. *Fusarium solani*, *F. oxysporum*, and *Macrophomina phaseolina*. Some of these are seed borne in nature and seed transmissible (Raut and Ahire, 1988, Rahman *et al.*1999) causing considerable losses in yield.

Meloidogyne incognita is considered to be one of the most severe pests of blackgram. The nematodes adversely affect nodulation Nitrogen fixation and yield (Hussaini and Seshadri, 1975). *Meloidogyne* infection, which primarily impairs water and nutrient uptake, and upward translocation by the root system (Karssen and Moens, 2006).

Disease complexes involving nematodes and fungal pathogens may cause significantly more crop losses than individually (Hussey and McGuire, 1987). The interaction between the root infecting fungus and the nematode results in the reduction of seed emergence and increase in both galling and nematode fecundity (Kassab & Ali 1996). Simultaneously the disease development caused by soil-borne fungal pathogens is also stimulated (Shawadfy & Mousa, 1997).

Meloidogyne incognita has evolved a specialized adoptive mechanism with the vascular wilt fungus *F. oxysporum* to cause a disease complex etiology in a variety of crop plants (Powell, 1971; Mai and Abawi, 1987). Such a disease complex, involving both the organisms has also been reported in black gram inflicting an appreciable loss in yield to the tune of 49 percent (Mahapatra and Swain, 1999).

Application of biological control agents (BCA) is a promising and ecofriendly tool in improving current levels of agricultural production. It assists in reducing use of chemical pesticides thereby controlling release of their residues into environment. One of the most efficient ways to achieve this objective is to develop BCAs for disease control alone, or to integrate it with reduced doses of chemicals in the control of phytopathogens resulting in minimal impact of chemicals on the environment (Harman and Kubicek, 1998). If complexities exist between the two organisms, application of either a fungicide or a nematicide alone will reduce the respective pathogen only. More over extensive use of chemicals have adverse effect on the environment (Latha et al, 2000).

Information is lacking on the complex pathogenic behaviour of the nematode and the pathogen parasitizing black gram simultaneously. Similarly there is megere information on the management of such a disease complex using bio control agents. Hence, it was thought desirable to undertake studies on interaction of *M. incognita* and *F. oxysporum* in blackgram with following objectives.

1. To establish relationship between *M. incognita* and *F. oxysporum* in disease complex.
2. To manage the disease complex using bio control agents.

REVIEW LITERATURE

The root knot nematode (*Meloidogyne incognita*) is one of the limiting factors affecting blackgram yield adversely and has evolved a specialized adaptive mechanism with the vascular wilt fungus *Fusarium oxysporum* to cause a disease of complex etiology (Mahapatra and Swain 2006).

2.1 Effect of different levels of inoculum of *Meloidogyne incognita* on growth of black gram

Nassar and Mustafa (1981) inoculated 22 days old tomato plants with *Meloidogyne hapla* and *M. arenaria* at the rate of 100 to 1600/ 1500 cm³ soil and found that 100 and 200 J₂/500 cm³ produced galling. Stephan (1983) recorded reduced growth parameters at 4000 and 8000 eggs of *M. hapla* /pot.

Mani and Sethi (1984) reported that all the growth parameters of chickpea were reduced when inoculated with *M. incognita* at different inoculum levels (0, 1, 2, 4, and 8 larvae/g soil). The root galls were increased with increasing inoculum density. Up to 1000 J₂/pod and there after declined. The root galls were also increased with increasing level of inocula. Das and Sukul (1984) in a pots experiment on tomato noted that number of root galls increased with increasing inoculums density.

Bhatt *et al.* (1994) studied the pathogenicity of *M. incognita* on soybean. There was significant reduction in growth with respect to root length and fresh and dry weight of shoot and root recorded at 2500 second stage larvae (L₂)/500 cm³ soil.

Mahapatra *et al.* (1999) recorded significant growth reduction of blackgram (*Vigna mungo*) at an initial inoculation level of 1000 J₂ of *Meloidogyne incognita*.

Sanchez and Rodriquez (2000) reported that inoculum level of 600, 1500, 2000, 4500 and 6000 eggs of *M. incognita* per plant reduced plant growth of pumpkin and root galling decreased with increase in inoculum levels.

Singh and Goswami (2000) studied pathogenicity of *Meloidogyne incognita* on cowpea with freshly hatched nematode larvae at 10, 100, 1000 and 10000 second stage juveniles (J₂) per 500g soil. Increase in the level of root knot resulted in proportional decrease in plant vigour and rhizobium nodulation. Significant reduction in plant growth was observed at 1000 nematode /500g soil.

Haider *et al.* (2003) reported initial inoculum level of 100 juveniles of *Meloidogyne incognita* per plant caused significant reduction in growth characters of pulse crops. viz; blackgram and redgram.

Hasan *et al.* (2003) reported that number of adult females and juvenile of *M. javanica* were higher with lower plant growth, nodulation and yield per plant at lower to higher inocula ranging from 4- 10 egg masses of *M. javanica* infecting groundnut.

Khan *et al.* (2004) studied pathogenic effect of *M. javanica* on bitter gourd (*Momordica charantia*), bottle gourd (*Lagenaria siceraria*), red gourd (*Cucurbita maxima*) and sponge gourd (*Luffa cylindrica*) by inoculating at 0, 250, 500, 1000, 2000, 4000 and 8000 J₂/kg a soil and reported that there was significant reduction in growth of bottle gourd and red gourd at initial inoculum level. Damaging threshold of *M. javanica* was 500 and 2000 J₂/kg of soil respectively.

Agwu *et al.* (2005) observed that when seedlings of okra were inoculated with different numbers of egg masses (0, 4, 8 and 12) of *M. incognita*. Root galls increased progressively and significantly with increased level of inoculum.

Olabiya (2008) studied the impact of graded inocula of 5,000; 10,000; 15,000; 20,000 and 25,000 eggs of *M. incognita* on tomato. At inoculum levels of 15,000; 20,000 and 25,000, all the growth parameters and fruit yield were significantly reduced while root galls were the maximum.

Hussain, *et al.* (2011) found that all of inocula levels reduced the shoot and root lengths, and fresh and dry weights. Increasing the nematode inoculum level increased the number of galls; egg masses and nematode population build up. The reductions in growth parameters and nematode infestation were found to be directly proportional to the inoculum level (okra).

Azhagumurugan and Rajan (2014) while working with *Vigna mungo* and *M. incognita* found that all the growth parameters were decreased with increasing concentration of inocula.

Abbasi and Hisamuddin (2014) reported that there was a significant reduction in plant length, fresh and dry weight, leaf area, chlorophyll, seed protein, nitrogenase and leghaemoglobin contents in the root nodules in *V. radiata* at 400 J₂ while at higher levels i.e.; 800 J₂ and 1,600 J₂ per kg. Soil the reduction was more pronounced and significant. Azhagumurugan *et al.* (2015) noted that growth characteristics of blackgram decreased with increasing inoculum levels of egg-masses of *M. incognita*.

2.2 Effect of Plant Age on multiplication of *Meloidogyne incognita*

Nayga and Salaries (1990) observed that population of root-knot nematode decreased significantly when *Momordica charantia* plants were inoculated at six to eight weeks after planting. Per cent yield loss was lower in eight weeks old plants regardless of inoculum density used.

Jadav and Kurundkar (1991) observed that plant age significantly influenced root-knot nematode multiplication in okra. Maximum females and egg masses/gm root were found in 20 weeks old inoculated plants followed by four, five and three weeks old inoculated plants. Number of eggs/plant was significantly higher in four weeks old inoculated plants than other plant ages.

Eapen (1992) inoculated two 12 and 24 months old seedlings and mature vegetative suckers of cardamom plants with 500 second staged juveniles of *M. incognita*. The highest multiplication rate, gall and egg mass indices were observed in 24 months old seedling while the per gram root population was highest in 12 months old seedlings. The galls on the root system of mature plants were comparatively small and supported fewer nematodes. He also observed that young cardamom seedlings were more susceptible to root knot nematodes than mature plants.

Khan *et al.* (2000) studied the influence of three initial inoculum densities (2000, 4000, and 6000) of root-knot nematode (*M. incognita*) and plant age on root-knot disease of tomato. Significant reduction in the plant height, number of leaves, fresh and dry weight of shoot and root was observed as initial inoculum

level was increased. Little effect was observed on five weeks old nursery transplanted plants as compared to those 3 and 4 weeks old.

Mukhtar *et al.* (2013) inoculated okra plants at 2nd 3rd 4th and 5th week and recorded that plants inoculated after second week of emergence were heavily damaged. However, with the increase in plant age at the time of inoculation, the damaging effects lowered significantly. With an increase in the initial inoculum density and plant age there was a corresponding decrease in the reproduction factor being inversely proportional to inoculum densities and plant ages.

2.3 Pot trials for interrelationship of *Meloidogyne incognita* and *Fusarium oxysporum*

Khan *et al.* (1991) reported that *M. incognita* alone caused greater suppression in plant growth in papaya than *F. solani*, but greatest plant growth suppression was caused by their simultaneous inoculation. The symptoms were more pronounced when both were present concomitantly than any one of them separately.

Fazal, *et al.* (1994) studied the interaction between *M. incognita* and *F. oxysporum* f. sp. *lentis* on lentil (*Lens culinaris*) using various combinations of each pathogen. Individually, *F. oxysporum* f. sp. *lentis* was the most aggressive pathogen. Reduction in growth parameters in concomitant inoculation was greater than the pathogens acting independently, thus showing a synergistic relationship. Rate of nematode multiplication and galling in the presence of fungus were significantly reduced.

Rao *et al.* (1996) conducted a pot experiment to investigate the interaction of *F. oxysporum* f.sp. *ciceri* with *M. incognita* on chickpea. The results indicated that inoculation of fungus either along with nematode or seven days before or after the nematode inoculation resulted significant reduction in fresh and dry weight of shoot and fresh weight of root as well as increase in the wilt incidence compared to plant inoculated with the fungus alone.

Mahapatra and Swain (2001) studied interaction between *M. incognita* and *F. oxysporum* on black gram and recorded that Individually, *M. incognita* was relatively more pathogenic than *F. oxysporum*. However, with the combined inoculation, plant growth along with rhizobial nodulation were adversely affected.

Simultaneous inoculation of both pathogens and nematode inoculation prior of fungus synergistically reduced plant growth and nodulation. These effects were less significant when the fungus preceded the nematode inoculation. The multiplication of nematodes and galling on roots decreased with pre inoculation of fungus.

Naji *et al.* (2004) observed that plants to completely lost their resistance when infected with *M. javanica*. Cent percent wilting was noted in muskmelon when inoculated with *M. javanica* and *F. oxysporum* f sp. melois.

Akram *et al.* (2006) observed that *Fusarium* wilt become considerably severe in the presence of root-knot nematode. The plants inoculated with nematode before the fungus (pre-inoculation) exhibited decline in yield, followed by concomitant inoculation. Gall formation and egg mass production was adversely affected in the presence of *F. oxysporum* f.sp *lycopersici*. There number was further declined in pre and concomitant inoculations.

Ravishankar and Singh (2008) reported that inoculation of *M. incognita* 15 day prior to *R. solani* significantly reduced all the plant growth parameters as compared to inoculation of *R. solani* 15 day prior to *M. incognita*. The effect of root-knot nematode with fungus enhanced the suppression of plant growth of tomato than alone treatment (Samuthiravalli & Sivakumar, 2008).

Kumar and Haseeb (2009) noted high root rot incidence in tomato crop the presence of fungus a predisposed the roots by *M. incognita* for subsequent damage by *R. solani*.

Kumar *et al.* (2009) observed that the presence of root knot nematode (*M. incognita*) irrespective of the presence of other organism *F. oxysporum* f. sp. pisi showed reduction in shoot length to a significant manner. However, maximum reduction in shoot length was observed in nematode inoculated one week prior to fungus.

Malhotra *et al.* (2011) carried out studies under pot conditions to determine the effect of *M. incognita* and *R. solani* on disease development and growth parameters in chilli. He also reported that when nematode inoculation was done 15 days prior to fungal inoculation, it showed maximum synergistic effect followed by treatment where both the pathogens were inoculated

simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression.

Askary *et al.* (2012) observed reduced plant growth characters such as fresh and dry shoot-root weight and formation of rhizobial nodules on roots of pigeonpea when *M. javanica* was associated with *F. udum*. Greatest reduction in growth parameters was observed when plants were inoculated with *M. javanica* and *F. udum* simultaneously and it was followed by *M. javanica* prior to *F. udum* and *F. udum* prior to *M. javanica*, respectively.

Ahmed *et al.* (2014) noted that simultaneous and sequential inoculation of *M. incognita* 15 days prior to *F. solani* showed greater reduction in plant growth parameters as compare to damage caused by either of the pathogen alone. Nematode multiplication and number of galls/root system were reduced significantly as compared to individual inoculations. Intensity of root rot was increased in presence of *M. incognita*.

Hesamedin (2014) reported that *M. incognita* and *F. oxysporum* suppressed the plant growth parameters of chickpea when inoculated simultaneously.

2.4 Efficacy of different biocontrol agents against *M. incognita* and *F. oxysporum*

Jatala (1985) noted that since many of the most commonly used pesticides are expensive or are being withdrawn from the market due to their harmful effect on humans, their persistence in the soil or their contamination of ground water, investigators are concentrating their efforts on integrating biological control agents in plant disease management strategies.

Siddiqui *et al.* (1999) observed that *Paecilomyces lilacinus* not only reduced nematode infection but also effectively suppressed root colonization by soil borne fungi. *P. lilacinus* has been reported to control *Macrophomina phaseolina*, *R. solani* and *F. solani* infection of sunflower, chickpea, mungbean and mashbean.

Latha, *et al.* (2000) reported that among the biocontrol agents tested combination of *P. lilacinus* with *T. viride* and *P. fluorescens* as seed treatment

resulted in lesser root rot incidence and nematode population with more pod yield.

Haseeb *et al.* (2005) conducted studies on the effect of *Trichoderma harzianum*, *Pseudomonas fluorescens*, *T. harzianum* + *P. fluorescens* on *M. incognita* and *F. oxysporum* disease complex on *Vigna radiata* and reported that all the bioagents were highly effective against nematode + fungus disease complex. Highest increase in the fresh and dry weight of root due to severe galling was observed in plants treated with *P. fluorescens*.

Haseeb *et al.* (2005) conducted studies to determine the effect of *T. harzianum*, *P. fluorescens*, *T. harzianum* + *P. fluorescens* against *M. incognita* and *F. oxysporum* disease complex on *Vigna mungo*. The treatments were highly effective against nematode + fungus disease complex. Highest increase in the fresh and dry weight of root due to severe galling was observed in plants treated with *P. fluorescens* followed by *T. harzianum*, and *T. harzianum* + *P. fluorescens*, respectively.

Nagesh *et al.* (2006) observed that the combination of *T. harzianum* + *F. oxysporum*, resulted in 42 per cent *inhibition* of *F. oxysporum*. Inoculation of *M. incognita* juveniles prior to *Fusarium* inoculation advanced the wilt of tomato by a week, while nematode inoculation after *Fusarium* inoculation did not increase wilting. *Trichoderma harzianum* controlled wilt in the presence of *M. incognita* for six weeks after transplanting.

Sharma and Pandey (2009) reported that when bio-control agents are applied in the soil, they improve plant health and thus contributing to overall productivity. These bio agents are also self propagating under favorable condition and therefore, may remain in the soil for long period.

Ambreen *et al.* (2012) observed that maximum inhibition of root knots was observed in urdbean plants inoculated with *M. incognita* and simultaneously treated with 20 ml of *Pseudomonas fluorescens* and *Bacillus subtilis*.

Yaqub *et al.* (2012) observed treatment with *Paecilomyces lilacinus* resulted in significantly lesser damage to plant growth of blackgram inoculated with *M. incognita*.

MATERIALS AND METHOD

The following materials and methods were employed during the course of present investigation

3.1. GENERAL

3.1.1 Preparation of Soil composite

Sandy loam soil was collected from experimental area of Jawaharlal Nehru Krishi Vishwa Vidhyalaya, Jabalpur. Soil was thoroughly washed with 3-4 changes of water so as to remove the soluble leachates and air dried. Soil was then mixed with well decomposed FMY (3:1), to prepare soil composite and was used throughout the course of investigation.

3.1.2 Sterilization of soil composite

The composite soil was disinfested with four percent commercial formaldehyde (E. Merck) by sealing the heap corners with polyethylene sheet for 15 days and later exposed to direct sunlight after spreading in a thin layer to allow complete evaporation of remnants of formaldehyde. Sterilized soil composite was stored in a clean aluminum tray, well covered with polyethylene sheet and was utilized as and when required for the experimental purpose (Plate 1–A).

3.1.3 Sterilization of Pots

Ten-centimeter diameter earthen pots containing 500 cm³ soil were used during experimentations. Pots were sterilized by exposing them to the fumes of four per cent commercial formaldehyde solution in a closed container and stored.

3.1.4 Irrigation of Pots

Optimum moisture level was regulated in each pot by providing 50 ml of purified water, obtained through Aqua quard (Eurekaphobes Pvt. Ltd. Mumbai)

so as to remain the soil free of all biological entities, physical and chemical impurities.



Plate - 1 (A) Sterilized soil Composite.



Plate - 1 (B) Black gram Roots Showing Galls Formed by *Meloidogyne incognita*.

3.1.5 Cleaning of Glassware

Glasswares of Corning, Borosil and Yera make ware used throughout the investigations. These were first cleaned by detergent, washed with tap water and then dipped over night in cleaning solution (Sulfuric acid 300ml, Potassium dichromate 80g and distilled water 400 ml) followed by rinsing with distilled water four times.

3.1.6 Medium used

Potato Dextrose Agar (PDA) medium was used during the course of study for isolation and maintenance of culture.

The ingredients of the medium were as follows:

| | | |
|--------------------------|---|--------|
| Peeled and sliced Potato | : | 200g |
| Dextrose | : | 20g |
| Agar | : | 20g |
| Distilled water | : | 1000ml |

Two hundred grams of peeled and sliced potatoes were washed and boiled in 500 ml. of distilled water for 30 minutes. The extract was filtered through cheese cloth. Twenty gram Agar-agar strips, washed in distilled water, and 20g Dextrose were added to the extract and total volume was made up to 1000ml by adding distilled water. The medium so prepared was kept in water bath so as to obtain complete dissolution of the ingredients. Fifty ml medium was poured into 250ml conical flasks and plugged. The flasks were sterilized at 121.6⁰ c temperature and 1.05 kg/cm² pressure for 30 minutes in an autoclave before use.

3.2 Collection and purification of nematode culture

Root knot infested blackgram plants (Plate 1-B) were collected from the fields of nearby villages. The roots were washed in a gental stream of water and egg masses were collected in Syracuse watch glass and surface disinfested with 1000 ppm dihydrogen streptomycin sulphate before inoculation. Seven days old seedlings of blackgram grown in sterilized soil in ten centimeter diameter pots were inoculated by placing a single egg mass near the root and then covered with a thin layer of sterilized moist soil (Jacob, 1979). Pots were examined forty

five days after inoculation. The egg masses developed on the galls were collected using 400 mesh sieve and thus subsequently multiplied on tomato plants to meet the requirement of larval population for experimentation.

3.3 Extraction of Nematode population

Cobb's sieving and decanting and modified Baermann funnel techniques (Christies and Perry, 1957) were employed. Egg masses were collected over 400 mesh British Standard (BS) Sieve and were further subjected to extraction of juveniles at room temperature ($25^{\circ}\text{C} \pm 2$). An extraction assembly had PVC ring of 110 millimeter diameter holding double layered wet tissue paper supported by a gauze cloth and tightly stretched with a rubber band.

Extraction dish was placed over a piece of sponge measuring 15 cm x 15cm x 15cm for providing firm support from the bottom to the tissue paper and allowing a fast passage of water. Suspension containing freshly washed egg masses on 400 mesh B.S. Sieve was poured with the help of a gentle stream of water. Assembly was later kept on a glass bowl (Yera make) holding 60 ml. of aqua-guard water. Extraction assembly was so placed, that the upper layer of water in glass bowl touches the stretched base of extraction dish to ensure a thin film leaving no air bubble. The extraction was carried out at room temperature ($25^{\circ}\pm\text{C}$) and the second stage juveniles (J_2) were collected 24 and 48 hr after. The extraction was further continued till 72 to 96 hr and juveniles emerged within 96 hr were used for inoculation after calibrating the population.

3.4 Method of Inoculation

Inoculum containing surface sterilized (1000 ppm, Dihydrogen streptomycin sulphate) second stage juveniles (J_2) were sprayed on the sterilized soil composite with an atomizer, mixed thoroughly and homogenized nematode soil mixture was prepared. Ten samples from this lot were washed to work out the initial population of juveniles/100 gm soil. The soil inoculated in this way was further used to raise the seedling and treatments were applied accordingly.

3.5 Collection of diseased plants

Diseased plants of blackgram showing characteristic symptoms of wilt (*Fusarium oxysporum*) were collected from the fields of Urdua village of Tahsil

Panagar. The infected plants were carefully uprooted from the soil to get whole root system intact. The roots were washed with tap water and dried with the help of blotter paper to remove traces of water, for isolation of casual organism.

3.6 Isolation, purification and pathogenicity of *Fusarium oxysporum*

Isolations were made from the roots of plants showing characteristic symptoms of wilt (Plate -3 B). Small pieces of about one cm, from affected plant roots were cut by sterilized knife. These were then surface sterilized with 1:1000 mercuric chloride solution for 30 seconds followed by three changes of sterilized distilled water and dried on sterilized blotter paper before keeping them on Petri plates containing PDA. The inoculated plates were kept for incubation at $25\pm 1^{\circ}\text{C}$ and examined for the growth of fungus after seven days.

The casual organism was identified and transferred on fresh Petri plate containing PDA. On the growth of fungus it was again identified under research binocular microscope and pure culture was developed following hyphal tip method. The pure culture so obtained was maintained on PDA slants and used as and when required (Plate 2-A,B).

3.6.1 Pathogenicity test:

Following method was employed to test the pathogenicity of *F. oxysporum*

Sorghum Seed Medium:

The soil Sorghum Seed Medium (SSM) was prepared to confirm pathogenicity of *F. oxysporum*. This consisted of soil and finely pulverized Sorghum seed and dextrose as:

| | | |
|--------------|---|-------|
| Soil | : | 930 g |
| Sorghum seed | : | 50 g |
| Dextrose | : | 20 g |

All the contents were thoroughly mixed and enough water was added to moistened this soil mixture. This medium was then dispensed @ 50g/250 ml. Erlenmeyer flask and plugged with non-absorbent cotton and autoclaved at 1.05 kg/cm^2 for 30 minutes. The flasks were later inoculated with seven mm disc of *F. oxysporum* and incubated at $25^{\circ}\pm 1^{\circ}\text{C}$ for 10 days.



Plate -2 (A) Mass culture of *Fusarium oxysporum* on Sorghum Seeds.

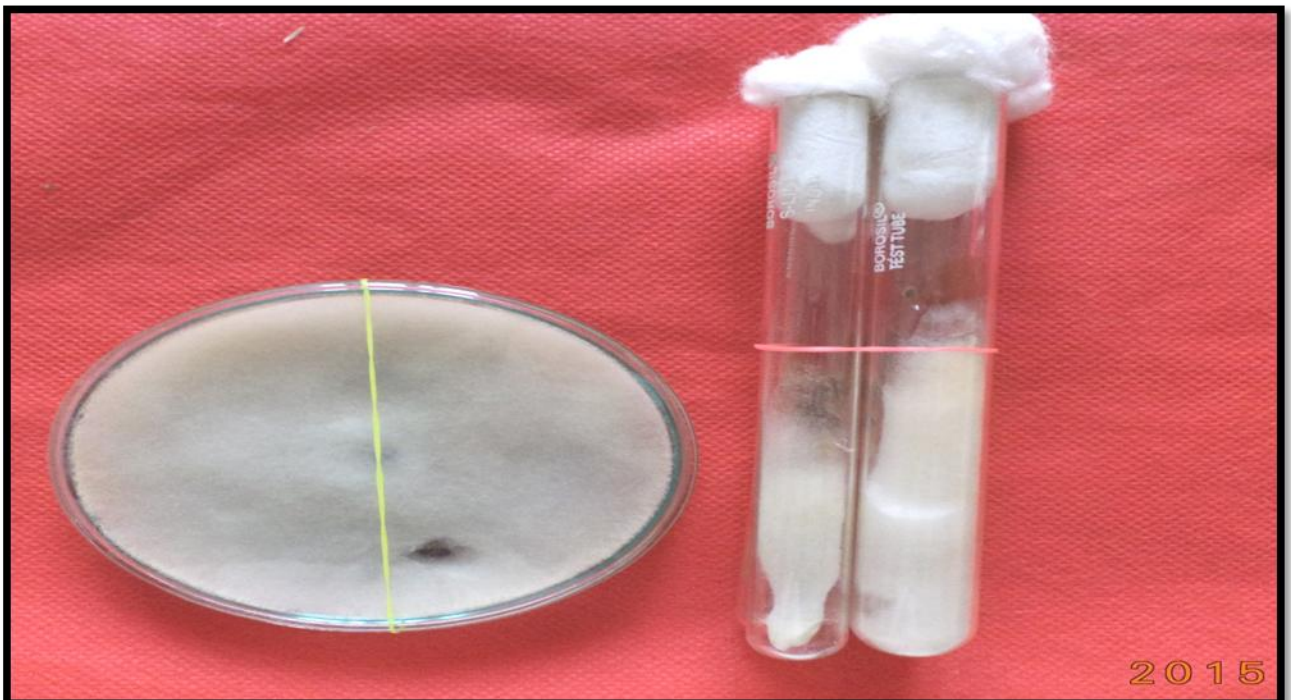


Plate -2 (B) Isolated and Purified culture of *Fusarium oxysporum*.



Plate -2 (C) Macro conidia with Mycelium of *Fusarium oxysporum*.

Ten days after, the contents of flask were well mixed with 450 cm³ of sterile soil and were transferred in sterilized 10 cm pots, irrigated with 100ml sterilized distilled water and kept covered with clean aluminium trays for five days before sowing seeds.

The good, bold and surface sterilized seeds of black gram (var. Tu 98-14) were dibbled at depth of two cm, and covered with fresh sterile soil. The pots were watered immediately.

Seven days after, the seedlings were removed by washing them in running tap water. This technique resulted in discolored lesions on hypocotyls and roots of blackgram. The identity of the fungus was confirmed by re-isolation of the fungus from necrotic lesions.

This isolate was, then, purified and maintained in PDA slants and kept in a Frigidaire at 4-8⁰C by sub culturing the same (Plate-2 C).

3.7 Effect of different levels of inoculation of *Meloidogyne incognita* on growth of black gram

Seven days old black gram seedlings (var.TU 98-14) were inoculated with different level of inocula. The treatments consisted of an uninoculated control and four levels of inocula as 10, 100, 1000 and 10,000 second stage juveniles of *M. incognita*. The extraction of these nematodes and their disinfestations were carried out as per the technique described earlier and inoculation with appropriate treatments/ level of inocula was carried out seven days after germination.

The soil around the seedling in a circumference area of four cm was removed and then dispersing nematodes over this area. After appropriate inoculation, the roots were covered by fresh and sterilized soil. Each treatment was replicated four times and randomized on the glass house bench following complete randomized design (CRD). The glass house temperature during this period ranged from 21 to 25⁰C.

The pots were irrigated with 100ml fresh water every day or if and when needed and thereafter with equal quantity of water per day as and when required. The experiment was terminated 45 days after inoculation.

The observation on plant height, fresh and dry shoot and root weights, root length, number of galls, nematode population in soil and root and number of galls were recorded. The entire root system along with the soil was taped out of the pot and washed in a container with a gentle stream of water. For obtaining fresh weight, the roots were pressed gently between two pads of blotting paper

and air dried then the was weight recorded. The roots were then kept in an oven maintained at $60 \pm 1^{\circ}\text{C}$ for 72 hr. to record oven dry weights.

The data so obtained were statistical analyzed using ANOVA.

3.8 Effect of plant age on growth and multiplication of *Meloidogyne incognita*

The experiment was conducted in ten cm earthen pots containing 500 cm^3 sterilized soil. The blackgram seedling of four different ages vis., 7, 14, 21, 28 days were chosen for the experiment. Each pot had one seedling. Freshly hatched 1000 second stage juveniles of *M. incognita* were inoculated in each pot at 7, 14, 21 and 28 days old seedlings. An uninoculated control was also maintained. Each treatment was replicated five times and randomized on glass house bench following CRD. The pots were irrigated with 100ml fresh tap water as and when required. The glass house temperature during this period of investigation ranged from 21 to 26°C .

The experiment was terminated 45 days after inoculation. Observations on plant height, root length, fresh and dry shoot and root weights, number of galls/plant and nematode populations in soil and in roots were recorded at the time of termination of the experiment.

The data so obtained were statistical analyzed using ANOVA.

3.9 Pot trials for interrelationship of *Meloidoyne incognita* and *Fusarium oxysporum* :

Seed of Blackgram (var.TU 98-14) ware used for the present studies. A mixture of soil and SSM was specifically prepared as per the method described earlier in the ration of 100 kg soil and 1kg Sorghum. This soil mixture was subsequently autoclaved at 1.05 kg/cm^2 for two hours before use. Ten cm earthen pots holding 500 cm^3 of soil were used in the present studies.

The treatments consisted of control without fungus and nematode, nematode alone (N), Fungus alone (F), simultaneous inoculation of nematode and fungus (NF), nematode at the time of sowing and fungus one week after (N1F7) fungus at the time of sowing and nematode after one week (F1N7).



Plate -3 (A) symptoms of *Fusarium oxysporum* on foliage.



Plate -3 (B) symptoms of *Fusarium oxysporum* on roots.

A constant level of 1000 second stage juveniles was inoculated per pot as per treatment. The technique for extraction and disinfection of nematodes was the same as described in earlier. The nematodes were pipette around the pregerminated seeds growing in sterile moist chambers prepared out of Petri

dishes. The radical length at the time of sowing ranged between 0.3 to 0.5 mm. Pregerminated seeds were dibbled 2cm deep. Due precautions were observed to avoid contamination from one pot to another.

Wherever, the nematodes were to be inoculated, one week after, three glass rods were fixed two cm deep in a circle of two cm in diameter. At the time of inoculation, the glass rods were removed and the nematode suspension was evenly distributed in the holes and these were then plugged with sterile soil. For inoculating the soil with fungus, *Fusarium oxysporum*, and 50g content of each flask containing SSM inoculated with the fungus was mixed in each pot for fungus, fungus + nematode one week after, and simultaneous inoculation of fungus and nematode.

The other pots which were to be inoculated by nematode, nematode + fungus, fungus one week after, and control were mixed with un inoculated SSM. When the fungus was to be inoculated on week after nematode inoculation, the technique suggested by Grewal and Pall (1974) was adopted with slight modification of placing three glass rods equidistant in a circle of two cm diameter and plugging the holes with sterile soil after introducing the actively growing *Fusarium oxysporum* plug derived from the culture.

A total of 30 pots were thus randomized over the glass house bench following CRD and watered daily with an equal quantity of sterilized distilled water if and when required. The experiment was concluded after 45 days after inoculation. The glass house temperature during the course of experiment was ranged from 27 to 34⁰C.

The observation on plant height, fresh and dry shoot and root weights, root length, number of galls, nematode population in soil and root and number of galls were recorded. The entire root system along with the soil was taped out of the pot and washed in a container with a gentle stream of water. For obtaining fresh weight, the method described earlier was followed. The roots were then kept in an oven maintained at 60 ± 1⁰C for 72 hr. to record oven dry weights.

The data so obtained were statistical analyzed using ANOVA.

3.10 Efficacy of different biocontrol agents against *Meloidoyne incognita* and *Fusarium oxysporum* complex.

The experiment was conducted in ten cm earthen pots containing 500 cm sterilized soil employing five bio-control agents viz, *Trichoderma viride*, *Paecilomyces lilacinus*, *Psuedomonas fluorescens*, *Bacillus subtilis* and *Pochonia chlamydosporia*.

The fungus *P. lilacinus* and *T. viride* were isolated from the soils of J.N.K.V.V Jabalpur from by sprinkling it on Petri plates containing sterilized Potato Dextrose Agar (PDA) medium. Both the organisms were purified and maintained on PDA plants.

The fungus *T. viride* was multiplied on wheat seeds. The seeds were boiled in water for half an hour and excess moisture was drained out. The boiled seeds were filled in polypropylene bags @ 500g seeds per bag and autoclaved at 1.05 kg/ cm² for 20 minutes. After cooling the bags were inoculated with pure culture of *T. viride* and inoculated at 24⁰C for 10 days. When sufficient growth was achieved the seeds along with fungus were mixed with the pot soil @ 2g/pot. Before mixing the fungus spore load was calculated by haemocytometer.

Paecilomyces lilacinus was mass multiplied on wheat seeds following the technique described earlier and at maximum growth and spore load mixed with the pot soil @ 2g per pot.

Commercial formulations of *Pochonia chlamydosporia*, *Psuedomonas fluorescence* and *Bacillus subtilis* were obtained from Department of Nematology, Tamil Nadu Agricultural University with a spore load 2×10⁷ colony forming units (cfu). These formulations were mixed with the pot soil @ 2g/pot.

After mixing the test bioagents in to the soil infested with *F. oxysporum* the pot soil was sprayed by freshly hatched and surface sterilized second stage juveniles of *M. incognita*. The population of root-knot nematode (2 j₂/g soil) was determined by the method described earlier.

The soil with test bioagents and *M. incognita* was filled in ten cm earthen pots and sown with blackgram seeds. Each treatment was replicated five times and randomized on glass house earlier.

The experiment was terminated 45 days after inoculation and observations on plant height, root length, shoot weight (fresh and dry), root weight (fresh and dry), number of galls, and nematode population in soil and roots were recorded.

The data were analyzed statically.

RESULT

4.1 Effect of different inoculum levels of *Meloidogyne incognita* on growth of blackgram.

The experiment on the effect of different levels of inocula of *Meloidogyne incognita* on various growth parameters of blackgram is conducted in pots under glass house conditions and the data is presented in the Table1 and Plate 4 (A-B) and Fig -1(A-E).

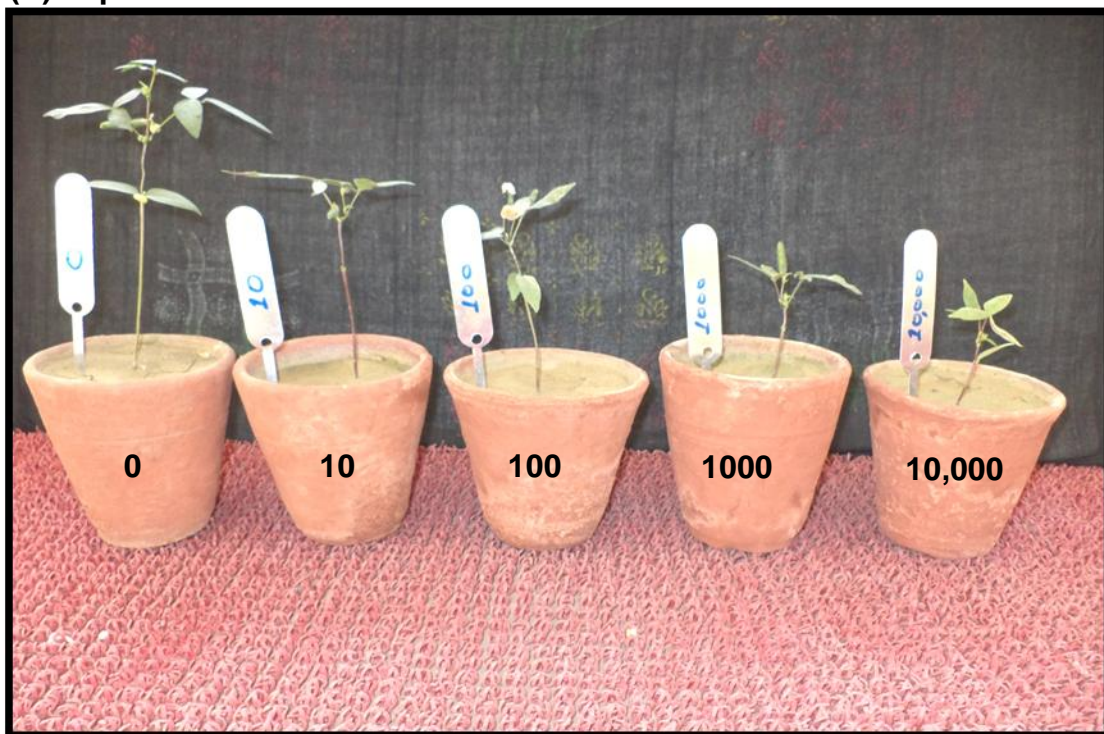
It is evident from the data that the plant height of black gram declined as the levels of inocula increased. Maximum (18.94 cm) plant height was noted in control followed by 10 (18.00 cm), 100 (16.06 cm) and 1000 J₂/plant (14.02cm). Minimum (12.22 cm) plant height was recorded with 10,000 J₂/plant level of inocula. At this level the plants were severely stunted looked sick and devitalized.

Similar trend was noted with the root length of crop. There was a progressive decrease in the length of the roots of black gram with increase in inoculums levels. Maximum (18.48cm) root length was recorded in uninoculated control and minimum (9.06 cm) in highest level (10,000 N/plant) of inoculum. Inoculation with 10 J₂/plant exhibited 17.00 cm root length followed by 100 (14.74 cm), and 1000 J₂/plant (10.34 cm).

On fresh weight basis maximum shoot weight was recorded with control (0.85g) and minimum (0.64g) with 10,000 J₂ inoculations. Ten J₂/plant exhibited (0.74g). Shoot weight 100 and 10, 00 J₂ recorded 0.72g and 0. 65g shoot weight respectively. Significant and progressive decline in the fresh weight of shoot was also noticed with increase in the levels of inocula against maximum in control. (Table 1)

Minimum (0. 58g) fresh root weight was recorded with 10,000 J₂/plant against maximum (0.87g) in uninoculated control, 10, 100 and 1000 second stage juveniles inoculation showed 0.72g, 0.70g and 0.62g root weight respectively.

(A) In pots



(B) Infected roots



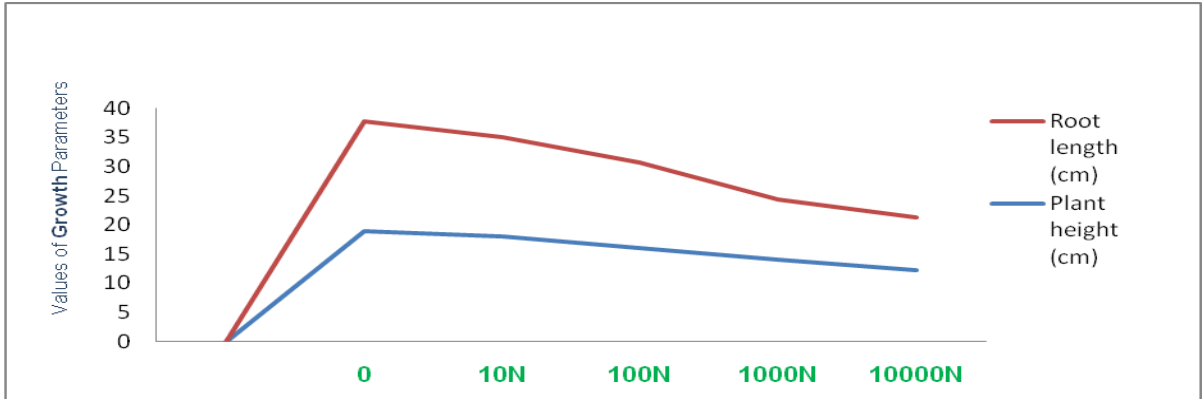
Plate 4:- Effect of different levels of inocula of *Meloidogyne incognita* on growth of blackgram.

Table: 1 Effect of different levels of inocula of *Meloidogyne incognita* on growth of blackgram.

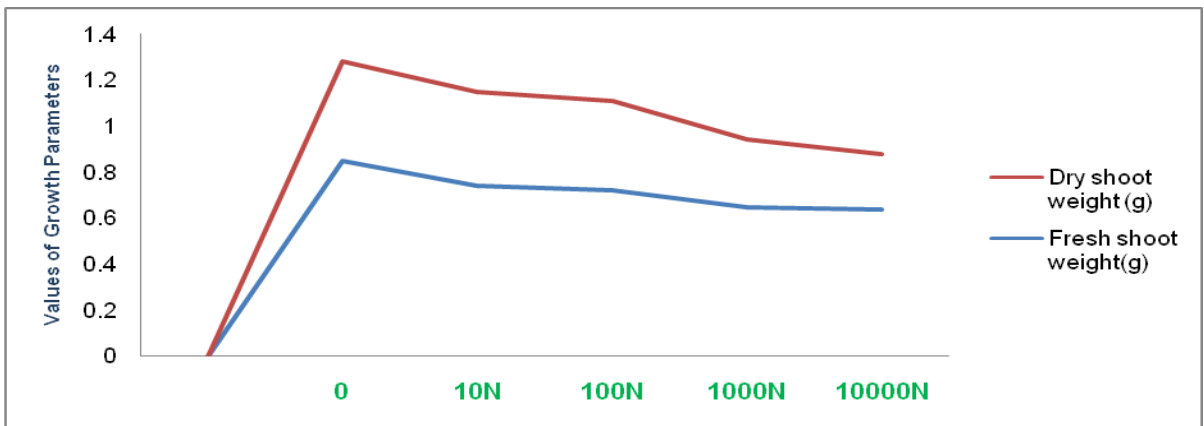
| S.no | Treatment | Plant height (cm) | Root length (cm) | Fresh weight (g.) | | Dry weight (g.) | | No. of galls/plant | Total No. of nematode |
|--|-----------|-------------------|------------------|-------------------|-------|-----------------|-------|--------------------|-----------------------|
| | | | | Shoot | Root | Shoot | Root | | |
| 1 | 0 | 18.94 | 18.48 | 0.85 | 0.87 | 0.43 | 0.42 | 0.00 (0.70) | 0.00 (0.70) |
| 2 | 10 | 18.00 | 17.00 | 0.74 | 0.72 | 0.41 | 0.37 | 5.20 (2.28) | 142.00 (11.91) |
| 3 | 100 | 16.06 | 14.74 | 0.72 | 0.70 | 0.39 | 0.33 | 10.80 (3.28) | 1134.00 (33.67) |
| 4 | 1000 | 14.02 | 10.34 | 0.65 | 0.62 | 0.29 | 0.21 | 18.20 (4.26) | 1960.00 (44.27) |
| 5 | 10,000 | 12.22 | 9.06 | 0.64 | 0.58 | 0.24 | 0.19 | 22.40 (4.73) | 14500.00 (120.41) |
| S.E.(M) ± | | 0.727 | 0.575 | 0.028 | 0.026 | 0.008 | 0.006 | 1.654 | 82.50 |
| CD (P=0.05) | | 2.161 | 1.709 | 0.084 | 0.079 | 0.023 | 0.019 | 4.914 | 245.11 |
| *Mean of five replication | | | | | | | | | |
| ** Values in parentheses are $\sqrt{n+1}$ transformation | | | | | | | | | |

Fig: 1 Effect of different levels of inocula of *Meloidogyne incognita* on growth of blackgram

A



B



C

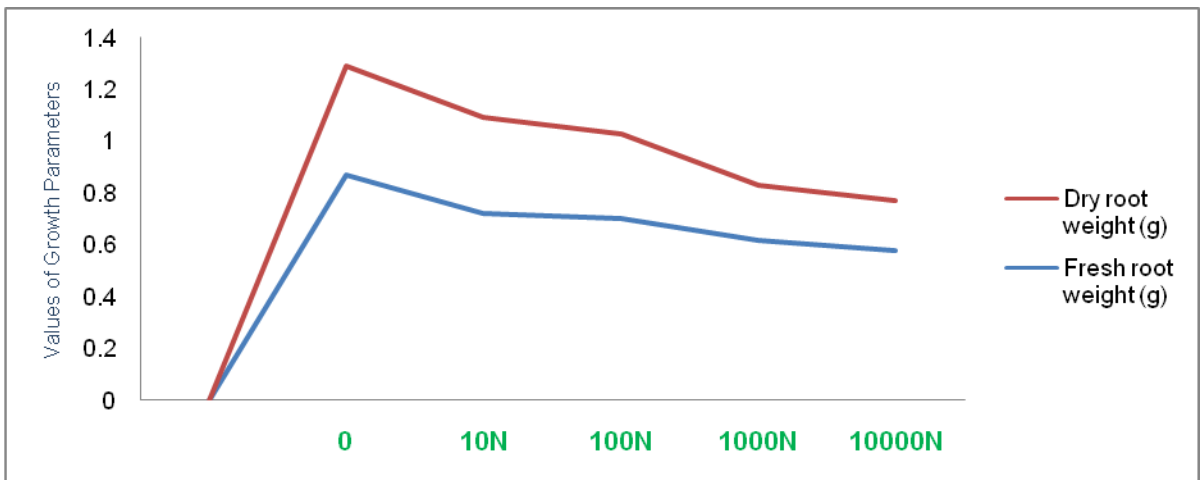
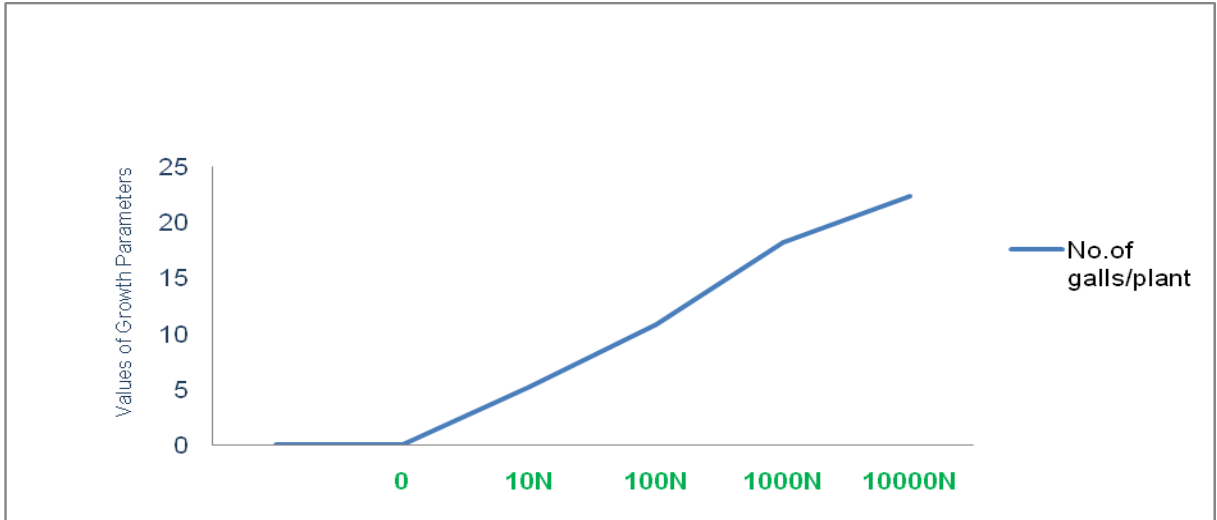
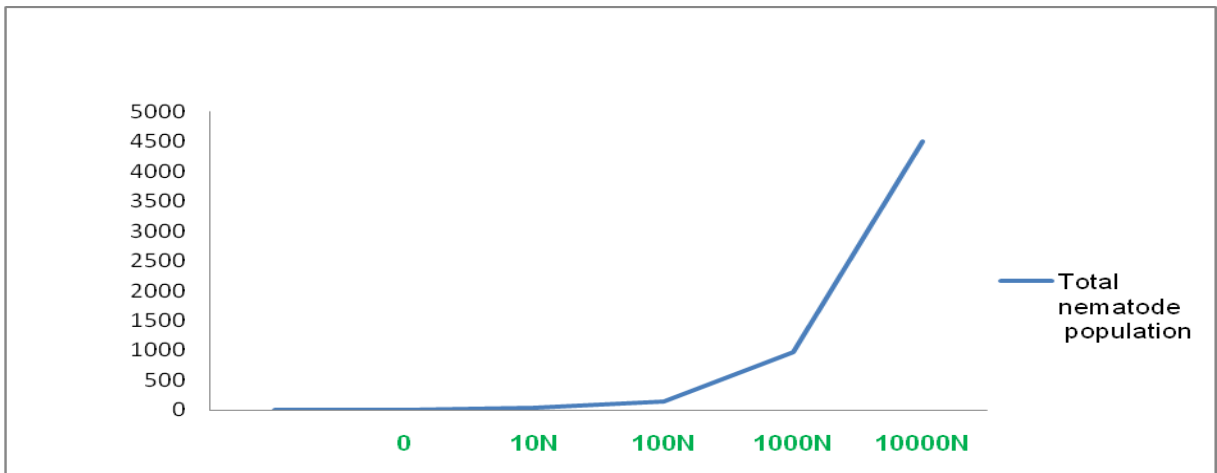


Fig: 1 (Contd.) Effect of different levels of inocula of *Meloidogyne incognita* on growth of blackgram

D



E



Similar trend was also recorded with dry weights of shoot and root when inoculated with progressive levels of inocula. Maximum (0.43g) dry weight of shoot was recorded in uninoculated control which declined sharply (0.24g) at 10,000 J_2 /plant. Inoculations with 10, 100 and 1000 juveniles/plant recorded 0.41, 0.39 and 0.29g shoot weight respectively. The root weight, on dry weights

basis was also affected severely as the levels of inocula increased. All the treatment affected the dry root weight significantly. Minimum (0.19g) root weight was recorded with highest level of inocula i.e. 10,000 J₂/plant. Inoculations with 10 J₂/plant exhibited (0.37g) root weight which declined at 100 J₂/plant (0.33g). A sharp decline (0.21g) was recorded at 1000 J₂/plant against maximum (0.42g) in uninoculated control.

Maximum (22.40) galling caused by root knot nematode was noted with highest level of inoculum and minimum (5.20) with 10 N/plant which increased in 100 (10.80) and 1000(18.20) N/plant against no galling in control.

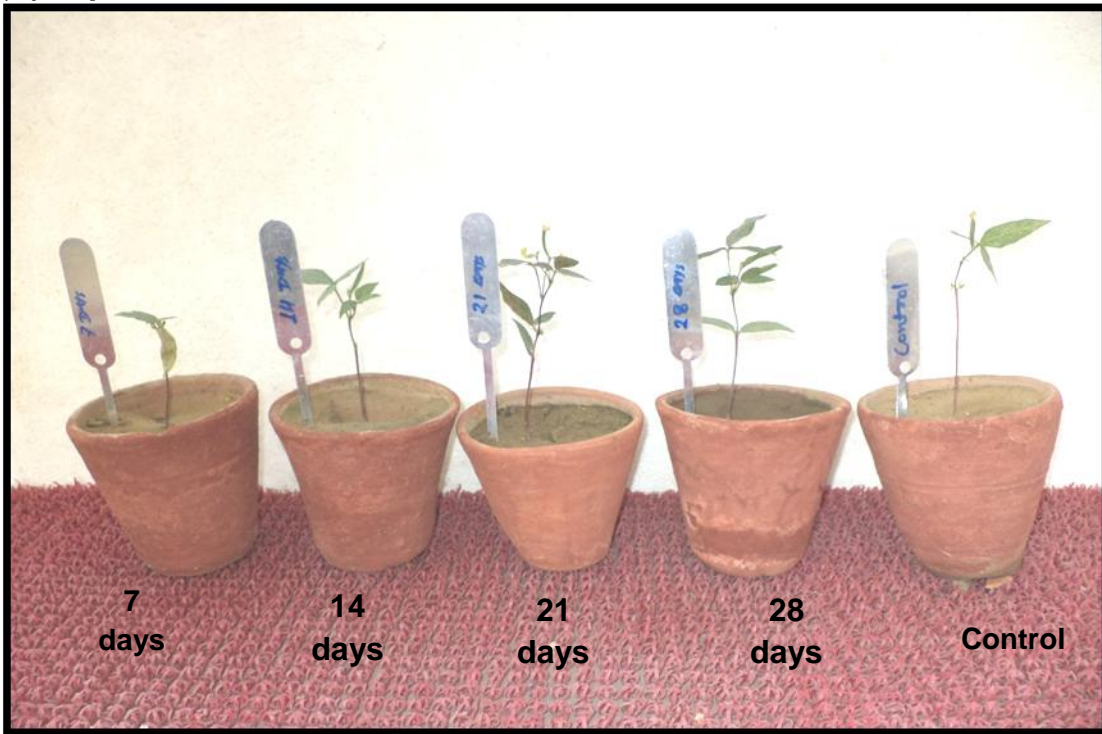
Maximum (14500 N) nematode recovery was recorded with 10,000 N/plant and minimum (142N) with ten nematode /plant. Other inoculum levels i.e. 100 and 1000 N/plant recovered 1134 and 1960 nematode (root + soil) respectively against no recovery in uninoculated control.

4.2 Effect of plant age on multiplication of *Meloidogyne incognita*.

The data on effect of root knot nematode (*M. incognita*) is presented in Table 2, Plate 5 (A-B) and Fig 2 (A-E). The data obtained by inoculating 1000 J₂/plant at 7, 14, 21 and 28 days old black gram plants respectively indicated that minimum (12.90) plant height was recorded in seven days old plants against maximum (19.38 cm) in control and 28 days old plant (18.42 cm). 7, 14, 21 days old plant recorded 12.90, 14.94 and 17.10 cm plant heights.

Similar trend was also recorded with lengths of roots at various plant growth stages. Maximum (18.22) root length was recorded in control and minimum (9.70 cm) in seven days old plants. Increasing trend in the lengths of root was in 14, 21 days old plants which were 12.52 and 15.06 cm respectively. The root length in 28 days old plant (16.02 cm) was at par with that of control.

(A) In pots



(B) Infected roots

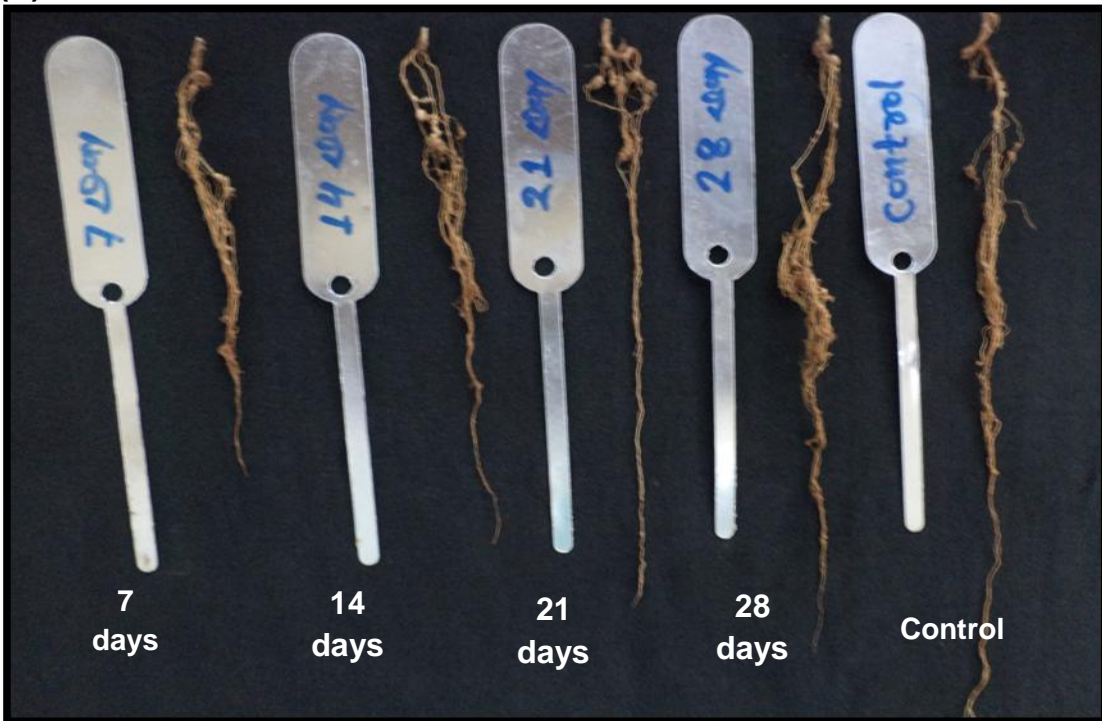


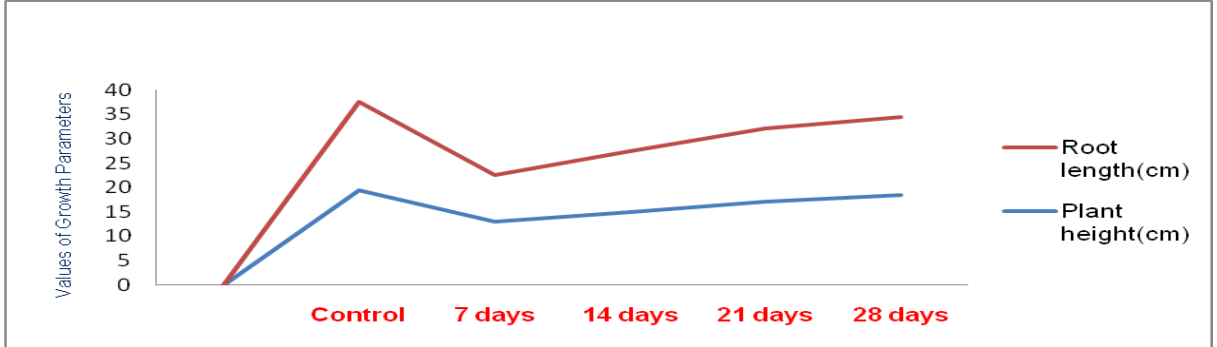
Plate 5:- Effect of *Meloidogyne incognita* on various ages of blackgram

Table: 2 Effect of plant age on plant growth and multiplication of *Meloidogyne incognita*.

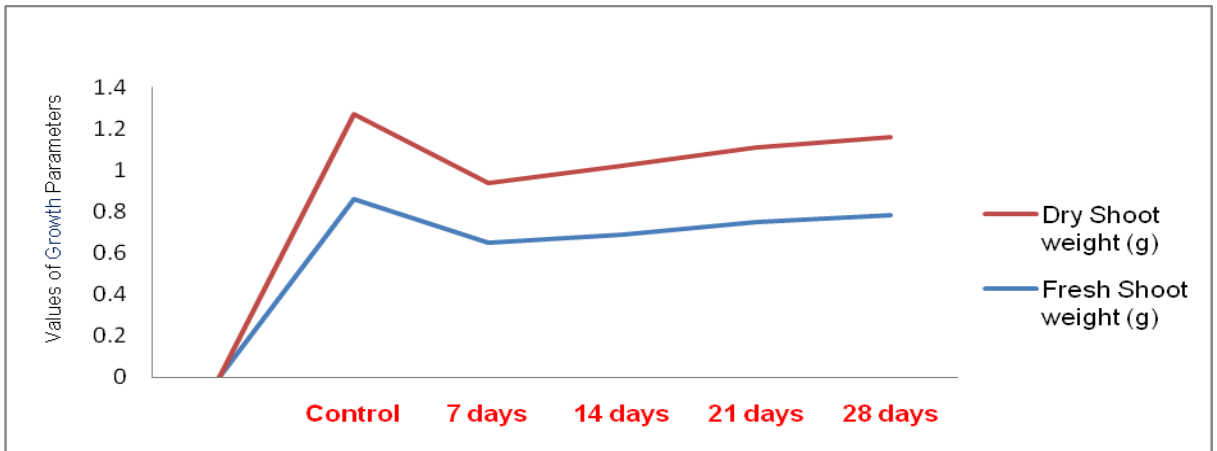
| S.no | Treatment | Plant height (cm) | Root length (cm) | Fresh weight (g.) | | Dry weight (g.) | | No of galls/plant | Total No. of nematode |
|--|-----------|-------------------|------------------|-------------------|-------|-----------------|-------|-------------------|-----------------------|
| | | | | Shoot | Root | Shoot | Root | | |
| 1 | Control | 19.38 | 18.22 | 0.86 | 0.85 | 0.41 | 0.40 | 0.00 (0.70) | 0.00 (0.70) |
| 2 | 7 days | 12.90 | 9.70 | 0.65 | 0.62 | 0.29 | 0.27 | 15.80 (4.03) | 1986.00 (44.56) |
| 3 | 14 days | 14.94 | 12.52 | 0.69 | 0.64 | 0.33 | 0.32 | 12.60 (3.54) | 1725 (41.53) |
| 4 | 21 days | 17.10 | 15.06 | 0.75 | 0.70 | 0.36 | 0.33 | 15.00 (3.87) | 1494.20 (38.65) |
| 5 | 28 days | 18.42 | 16.02 | 0.78 | 0.75 | 0.38 | 0.36 | 11.80 (3.43) | 1407.00 (37.50) |
| S.E.(M) ± | | 0.746 | 0.516 | 0.026 | 0.024 | 0.007 | 0.007 | 1.899 | 20.929 |
| CD (P=0.05) | | 2.217 | 1.534 | 0.077 | 0.072 | 0.021 | 0.019 | 5.643 | 62.174 |
| *Mean of five replication | | | | | | | | | |
| ** Values in parentheses are $\sqrt{n+1}$ transformation | | | | | | | | | |

Fig: 2 Effect of plant age on multiplication of *Meloidogyne incognita*

A



B



C

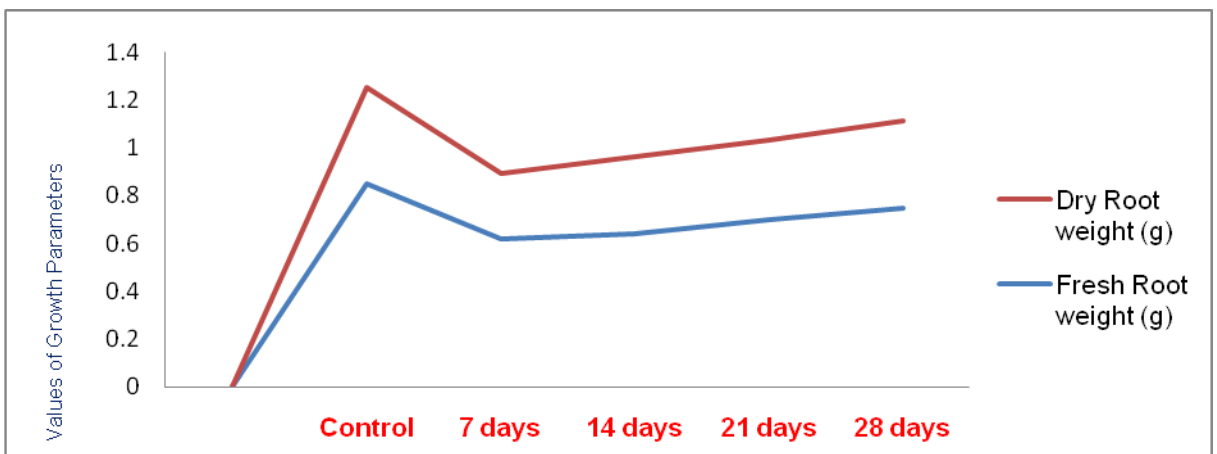
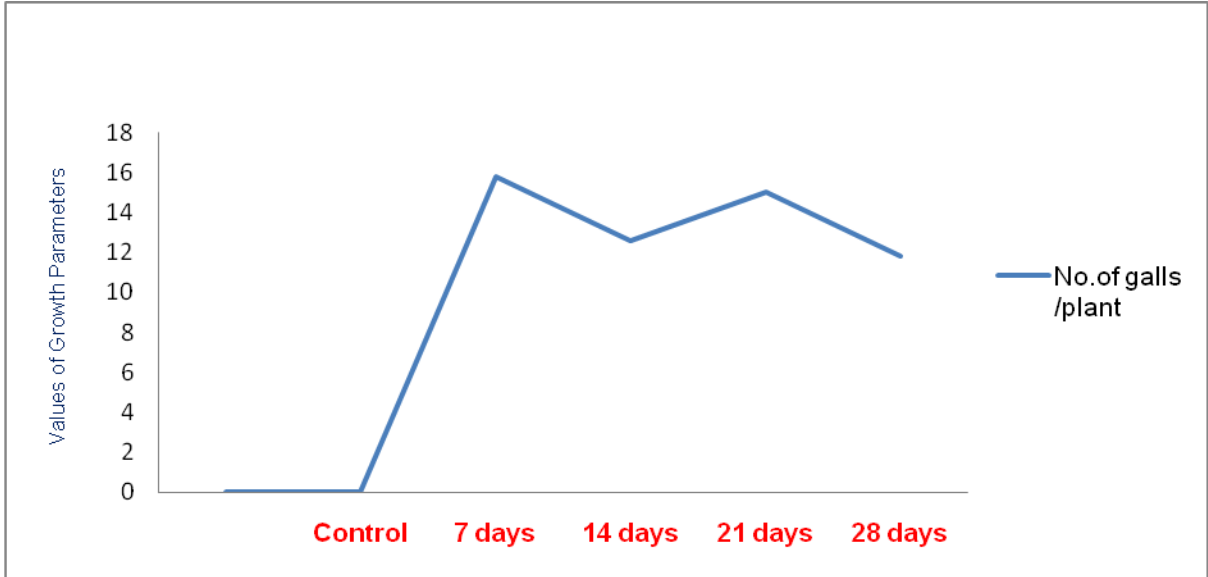
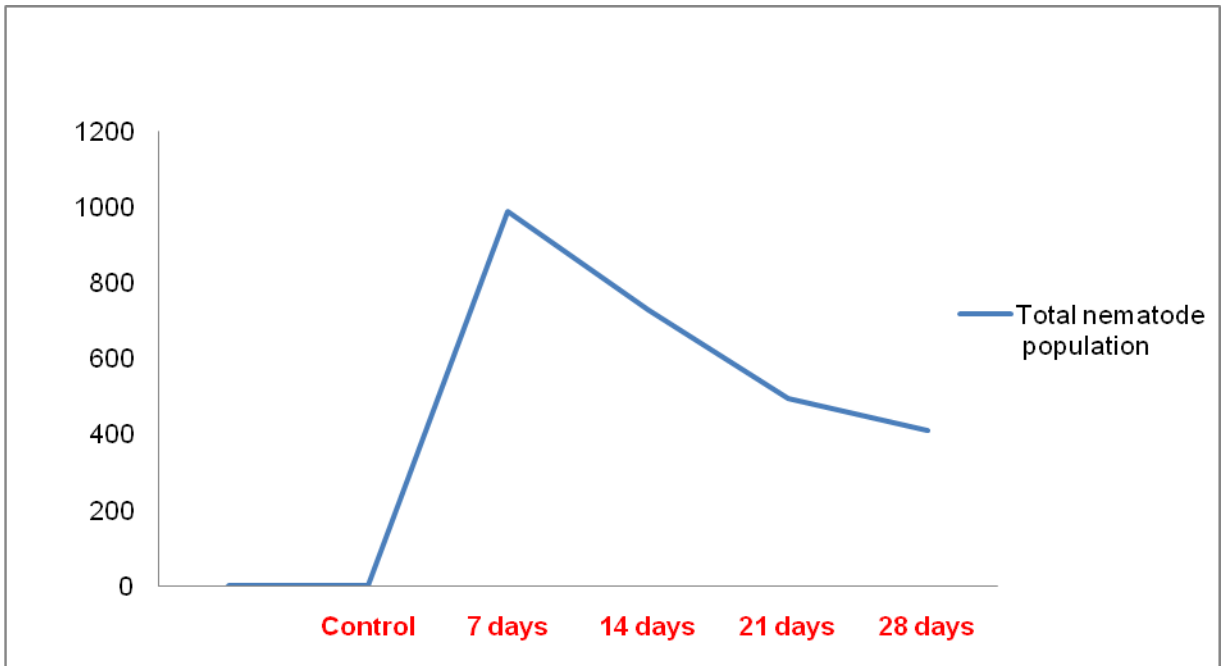


Fig: 2 (Contd.) Effect of plant age on multiplication of *Meloidogyne incognita*

D



E



On fresh weight basis maximum (0.86g) shoot weight was recorded in uninoculated control. Which was significantly higher than in 28 days old plant and rest of the ages. Seven days old plants recorded 0.65g fresh weight which was significantly lower than 14 days (0.69g) and 21 days (0.75g) old plants. Root weights on fresh weight basis have also shown the similar trend. Uninoculated control recorded maximum (0.85g) weight of fresh root followed by rest of the plant ages. Seven, 14 and 21 days old plants recorded 0.62, 0.64, and 0.70g root weights respectively.

However, on dry weight basis minimum (0.29g) shoot weight was recorded in seven days old plants. That was increased gradually in 14 (0.33g), 21 (0.36g) and 28 (0.38g) days old plant. Maximum (0.41g) shoot weight was recorded in control. The root weights have also shown the similar trend on dry weight basis. Minimum (0.27g) root weight was recorded with 7 days old plant which increased gradually as the age of the plant increased from 14 to 28 days. Fourteen days old plant recorded 0.32g root weight and 21 and 28 days old plants recorded 0.33 and 0.36 root weights respectively. Maximum (0.40g) root weight was recorded in control.

Maximum (15.80) galls were noted on roots of seven days old plants which were at par with 21 days old plants (15.00). Fourteen days old plants recorded 12.60 galls and 28 days old plants recorded 11.80 galls/plants which were observed to be non significant against no galling in control.

Nematode recovery was maximum (1986 N) in seven days old plants which gradually declined as the age of the plant increased. Fourteen, 21 and 28 days old plants recovered 1725, 1494 and 1407 nematodes respectively against no recovery in uninoculated control.

4.3 Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* :

The experiment on interaction between *M. incognita* and *F. oxysporum* was conducted under pot conditions and the data is presented in the Table 3, Plate 6 (A-B) and Fig 3 (A-E).

The data revealed that significantly reduced (13.40) plant height was recorded in the treatment where nematode inoculated first and fungus seven days after followed by the inoculation by fungus first and nematode seven days after. Simultaneous inoculation by both the organisms recorded 16.06 cm plant height which was at par with nematode inoculation only (15.36) cm). Fungus inoculated plants attained 16.56 cm plant height against 18.62 cm plant height in control.

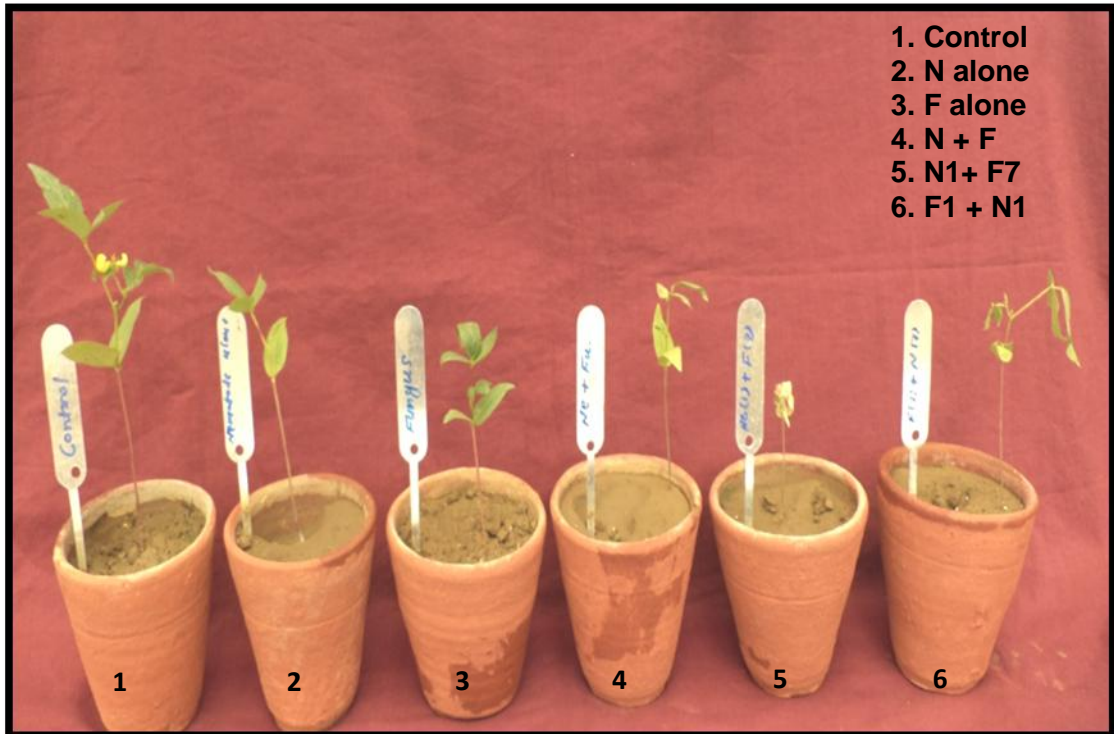
Minimum (11.52 cm) length of root was noticed in the treatment were nematode preceded fungus followed by the treatment where fungus inoculated first and nematode seven days after (12.98 cm). Reduced root length was also noted in nematode (14.28 cm) and fungal inoculation (13.06 cm) which were at par with each other. Significant increase in root length was also recorded in simultaneous inoculation by both the organisms. Uninoculated control recorded maximum (19.02 cm) root length.

The fresh shoot weight significantly declined (0.60g) in the treatment where nematode preceded fungus seven days before followed by fungus inoculated first and nematode seven days after (0.61g) simultaneous inoculations of nematode and fungus (0.64g) and nematode alone (0.63g). Fungus alone exhibited 0.68g root weight against maximum (0.79g) in control.

Similarly minimum (0.58g) root weight was recorded in the same treatment were nematodes inoculated first and fungus after words followed by fungus inoculated first and nematode seven days after (0.61g) followed by nematode alone (0.63g) and simultaneous inoculations by both the organisms (0.64g). Fungus alone exhibited 0.68g fresh root weight against maximum (0.79g) in control.

On dry weight basis minimum (0.29g) shoot weight was recorded in nematode inoculation followed by fungal inoculations and fungus inocufirst and nematode seven days after (0.32g) simultaneous inoculation by both the organism and nematode inoculation remained at par. Dry weight of root also exhibited the same trend.

(A) In pots



(B) Infected roots



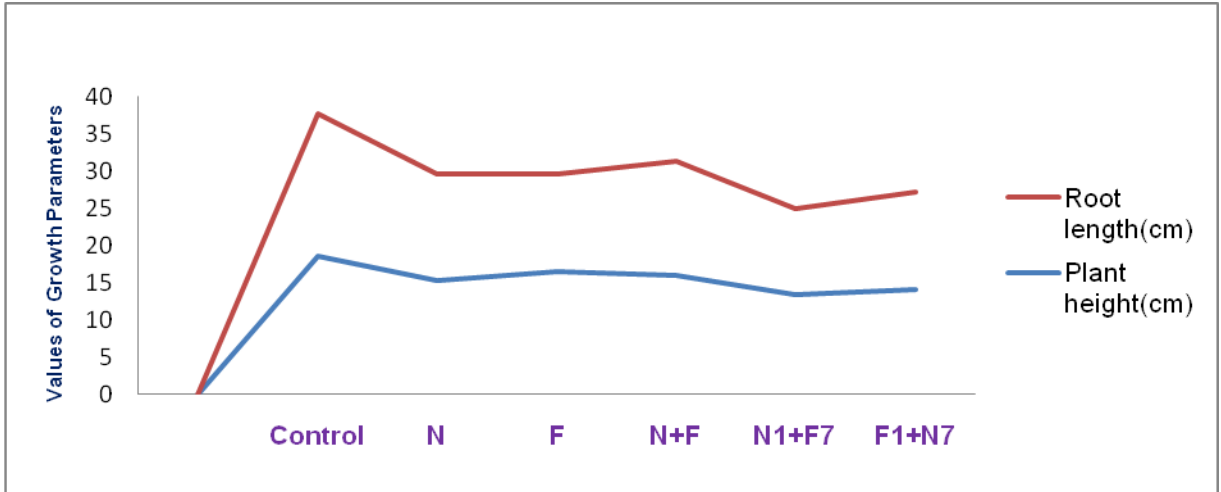
Plate 6:- Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* in blackgram.

Table: 3 Influence of *Meloidogyne incognita* and *Fusarium oxysporum* on plant growth parameters.

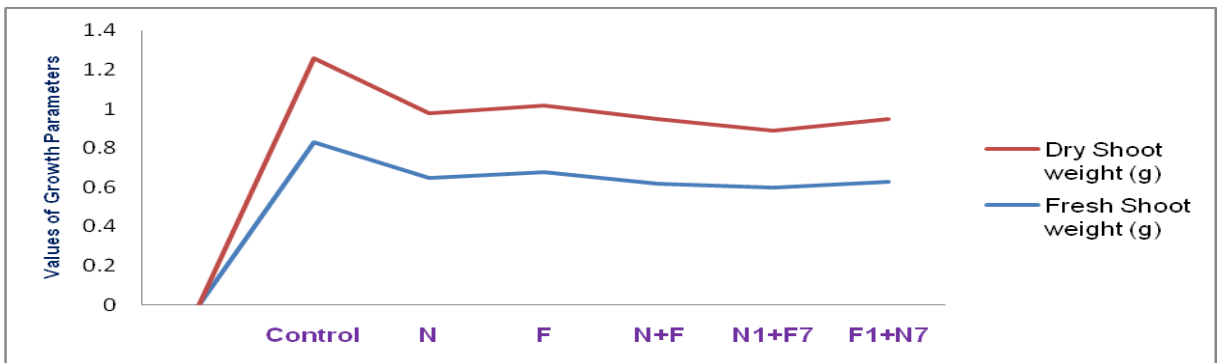
| S.no | Treatment | Plant height (cm) | Root length (cm) | Fresh weight(g) | | Dry weight(g) | | No of galls/plant | Total No. of nematode |
|--|-----------|-------------------|------------------|-----------------|-------|---------------|-------|-------------------|-----------------------|
| | | | | Shoot | Root | Shoot | Root | | |
| 1 | Control | 18.62 | 19.02 | 0.83 | 0.79 | 0.43 | 0.41 | 0.00 (0.70) | 0.00 (0.70) |
| 2 | N | 15.36 | 14.28 | 0.65 | 0.63 | 0.33 | 0.34 | 14.40 (3.79) | 1849.00 (43.00) |
| 3 | F | 16.56 | 13.06 | 0.68 | 0.68 | 0.34 | 0.31 | 0.00 (0.70) | 0.00 (0.70) |
| 4 | N+F | 16.06 | 15.24 | 0.62 | 0.64 | 0.33 | 0.33 | 10.20 (3.19) | 1549.00 (39.35) |
| 5 | N 1 + F7 | 13.40 | 11.52 | 0.60 | 0.58 | 0.29 | 0.26 | 14.00 (3.74) | 1655.00 (40.68) |
| 6 | F1 + N7 | 14.10 | 12.98 | 0.63 | 0.61 | 0.32 | 0.27 | 11.40 (3.37) | 1545.00 (39.30) |
| S.E.(M) ± | | 0.810 | 0.737 | 0.033 | 0.030 | 0.011 | 0.010 | 1.282 | 66.711 |
| CD (P=0.05) | | 2.378 | 2.163 | 0.098 | 0.089 | 0.032 | 0.029 | 3.764 | 195.875 |
| *Mean of four replication | | | | | | | | | |
| ** Values in parentheses are $\sqrt{n+1}$ transformation | | | | | | | | | |

Fig: 3 Pot trials for interaction of *Meloidogyne incognita* and *Fusarium oxysporum*

A



B



C

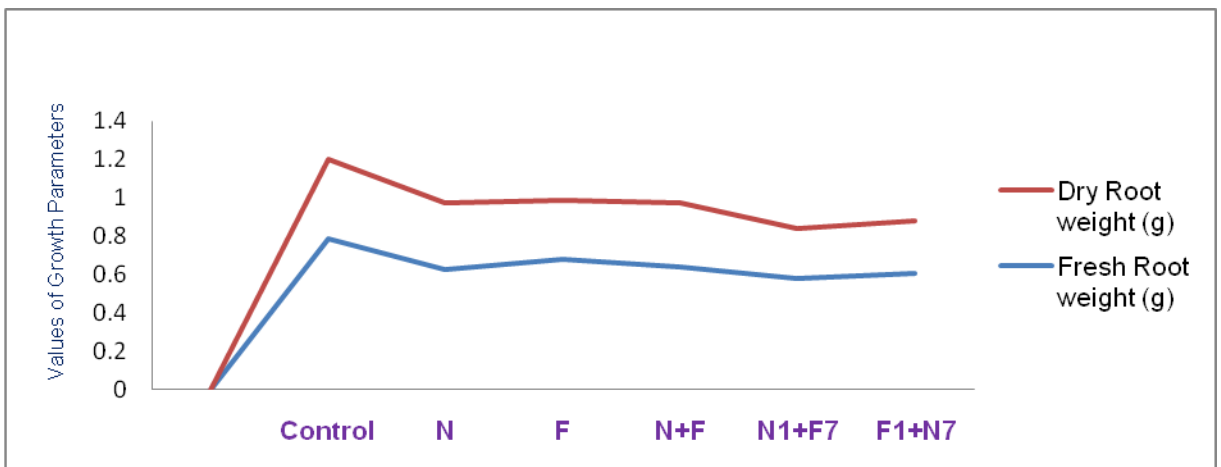
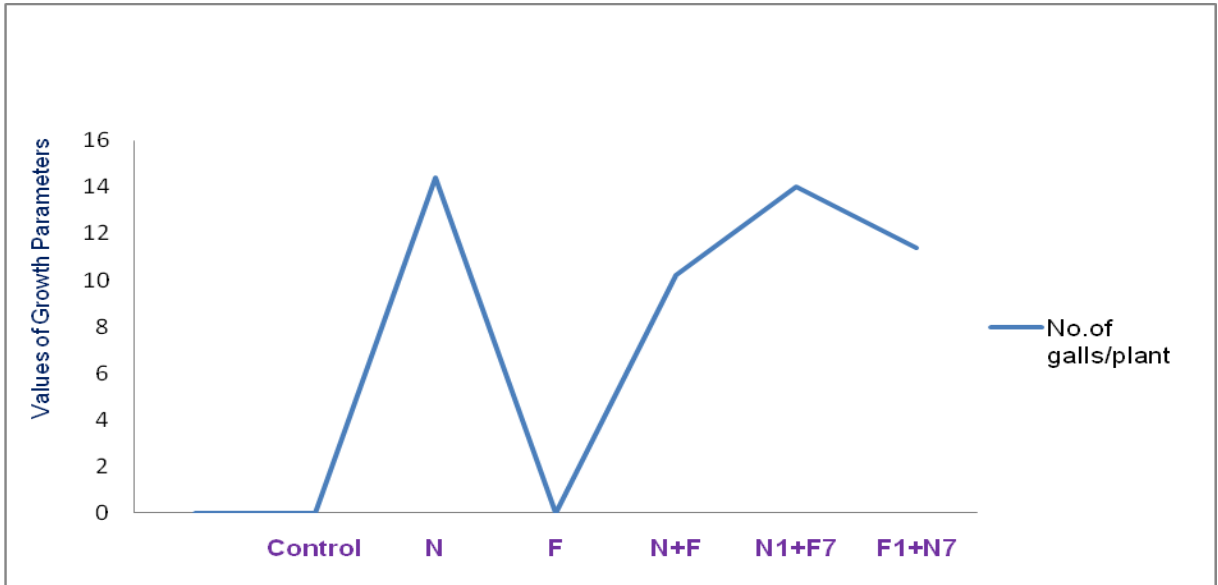
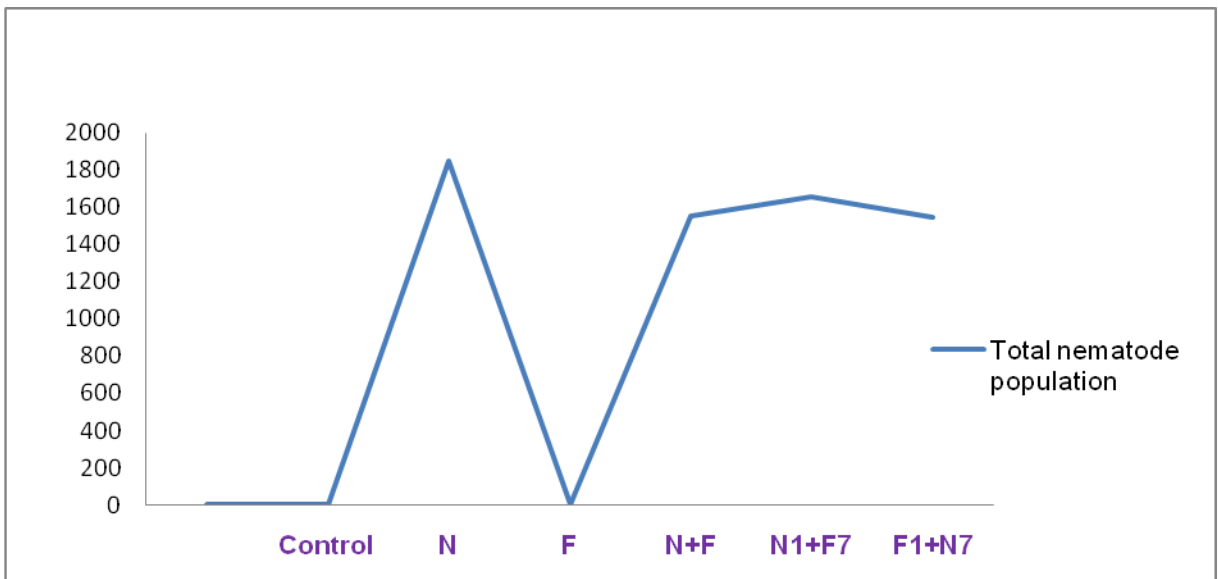


Fig: 3 (Contd.) Pot trials for interaction of *Meloidogyne incognita* and *Fusarium oxysporum*

D



E



Maximum (14.00) number of galls was observed in nematode inoculated first followed by fungus inoculated first and nematode seven days after and nematode alone. Simultaneous inoculation by both the organisms exhibited

minimum (10.20) number of gall followed by fungus inoculated first and nematode after words.

Maximum number (1849) of nematodes were recovered with inoculation of nematode alone followed by nematode inoculated first and fungus seven days after (1655). Fungus inoculated first and simultaneous inoculation of both the organism remained at par.

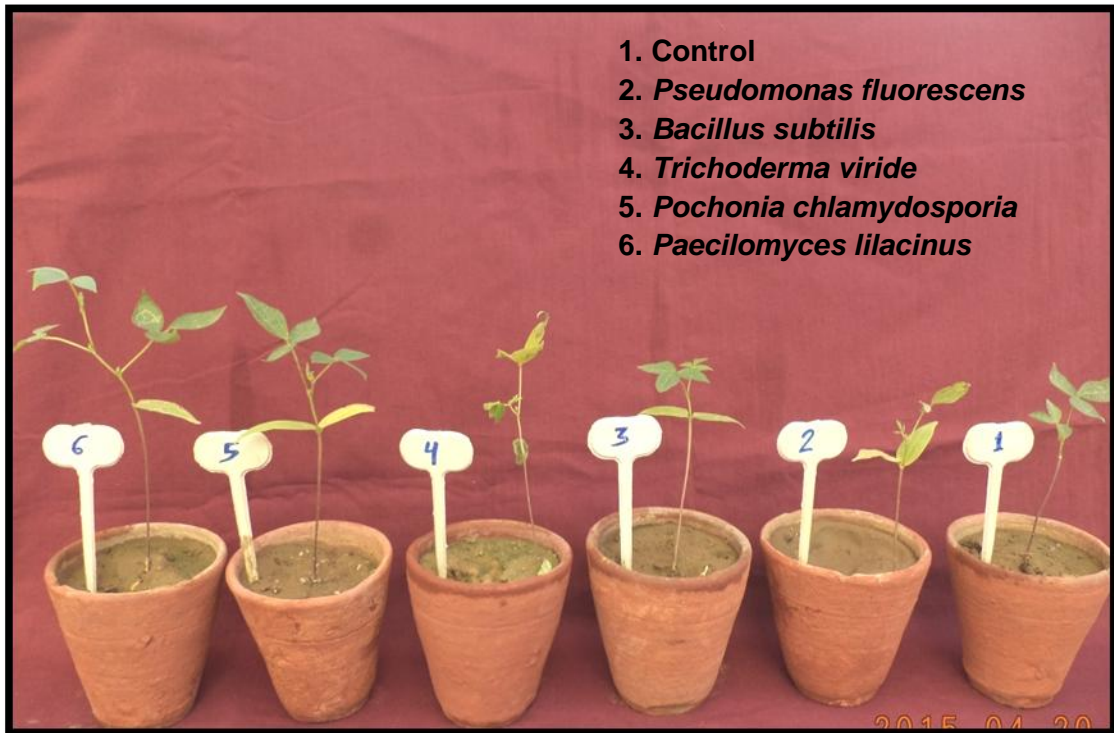
4.4 Efficacy of different bio control agents against *M. incognita* and *F. oxysporum*

The five biocontrol agents viz, *Trichoderma viride*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Bacillus subtilis* and *Pseudomonas fluorescense* were tested against disease complex produced by *M. incognita* and *F. oxysporum* in black gram and the data is presented in the Table 4 and Plate 7 (A-B) Fig -4(A-E).

All the treatments reduced incidence of nematode and fungus on growth of black gram and multiplication of nematode. The data presented in the Table 4 revealed that maximum plant height (19.02 cm) was recorded with *Paecilomyces lilacinus* followed by *Pochonia chlamydosporia* (17.70 cm), *Trichoderma viride* (16.46 cm), *Bacillus subtilis* (15.68 cm) and *Pseudomonas fluorescense* (13.96 cm). Inoculated control recorded minimum (11.10 cm) plant height.

Similarly maximum (18.38 cm) root length was noted with *P. lilacinus* and minimum (11.24 cm) with control rest of the treatments were significantly superior over control but inferior over *P. lilacinus*, *P. chlamydosporia*, *T. viride*, *B. subtilis* and *P. fluorescense* which recorded 16.66, 15.44, 11.78 and 15.12 cm lengths respectively the effect of *P. fluorescense* and *T. viride* remained at par with each other.

(A) In pots



(B) Infected roots

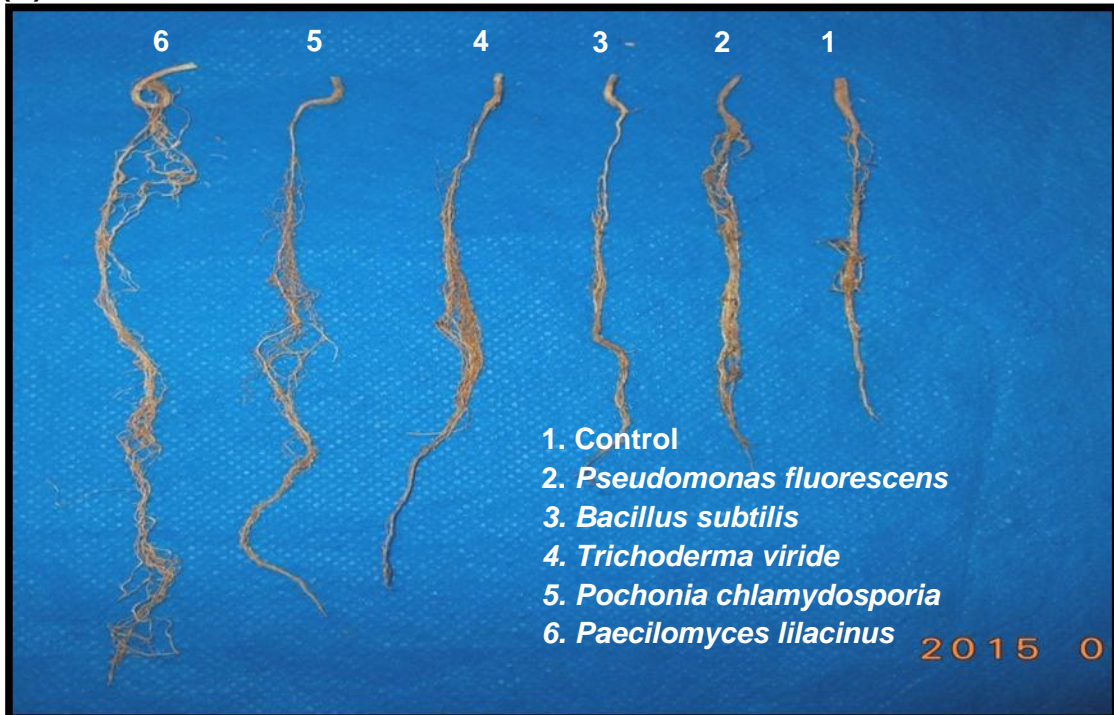


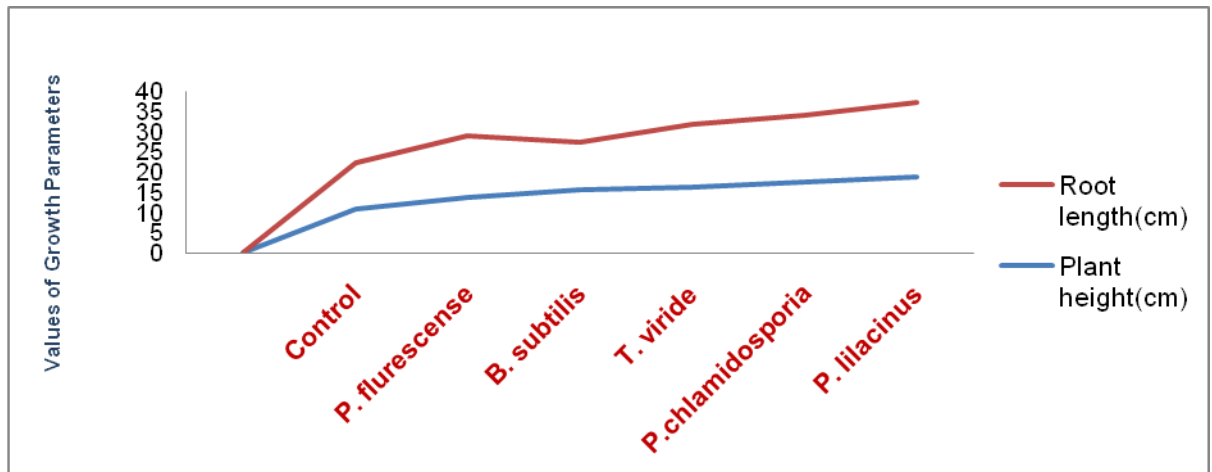
Plate 7:- Efficacy of different bio control agents against *Meloidogyne incognita* and *Fusarium oxysporum* disease complex.

Table: 4. Efficacy of different biocontrol agents *Meloidogyne incognita* and *Fusarium oxysporum* on plant growth parameters.

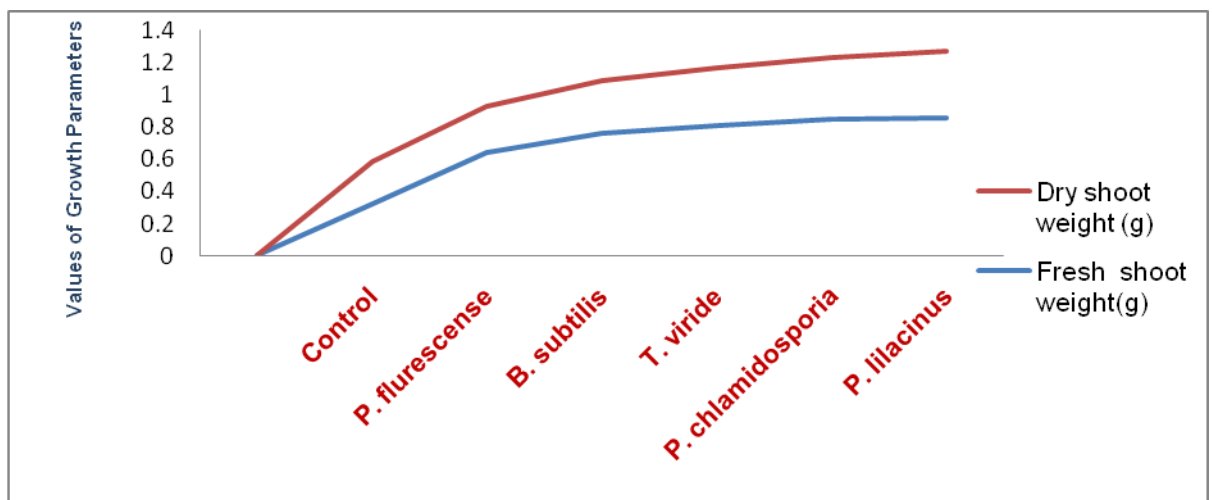
| S. no | Treatment | Plant height (cm) | Root length (cm) | Fresh weight(g) | | Dry weight(g) | | No of galls/plant | Total No. of nematode |
|--|---------------------------------|-------------------|------------------|-----------------|-------|---------------|-------|-------------------|-----------------------|
| | | | | Shoot | Root | Shoot | Root | | |
| 1 | Control | 11.10 | 11.24 | 0.62 | 0.32 | 0.26 | 0.25 | 13.80 (3.71) | 1890.20 (43.47) |
| 2 | <i>Psuedomonas fluorescense</i> | 13.96 | 15.12 | 0.69 | 0.64 | 0.29 | 0.28 | 11.00 (3.31) | 1342.40 (36.63) |
| 3 | <i>Bacillus subtilus</i> | 15.68 | 11.78 | 0.78 | 0.76 | 0.33 | 0.32 | 12.20 (3.49) | 1230.40 (35.07) |
| 4 | <i>Trichoderma viride</i> | 16.46 | 15.44 | 0.85 | 0.81 | 0.36 | 0.34 | 10.40 (3.22) | 1178.60 (34.33) |
| 5 | <i>Pochonia chlamyosporium</i> | 17.70 | 16.66 | 0.87 | 0.85 | 0.38 | 0.36 | 9.80 (3.13) | 1156.40 (34.00) |
| 6 | <i>Paecilomyces lilacinus</i> | 19.02 | 18.38 | 0.89 | 0.86 | 0.41 | 0.39 | 8.20 (2.86) | 1115.40 (33.39) |
| S.E.(M) ± | | 0.957 | 0.844 | 0.043 | 0.035 | 0.013 | 0.012 | 0.503 | 16.160 |
| CD (P=0.05) | | 2.809 | 2.480 | 0.126 | 0.103 | 0.037 | 0.034 | 1.478 | 47.450 |
| *Mean of four replication | | | | | | | | | |
| ** Values in parentheses are $\sqrt{n+1}$ transformation | | | | | | | | | |

Fig: 4 Efficacy of different biocontrol agents against *Meloidogyne incognita* and *Fusarium oxysporum* complex

A



B



C

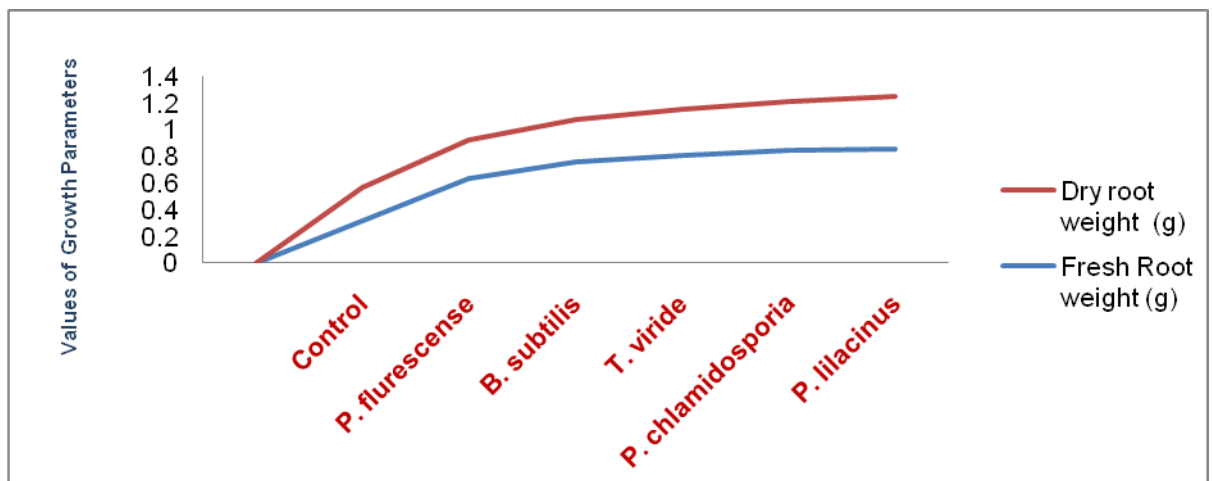
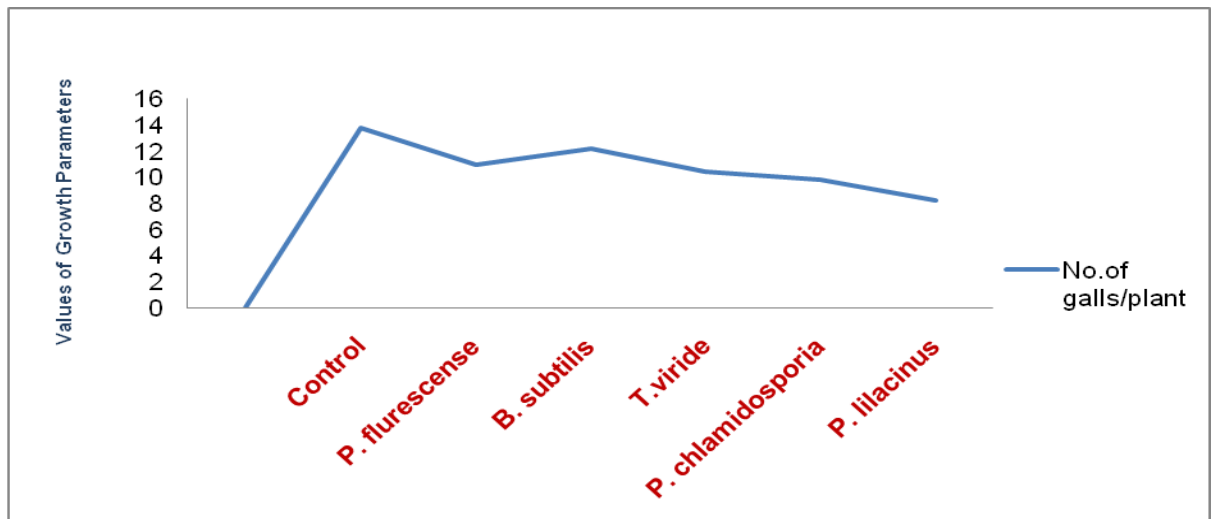
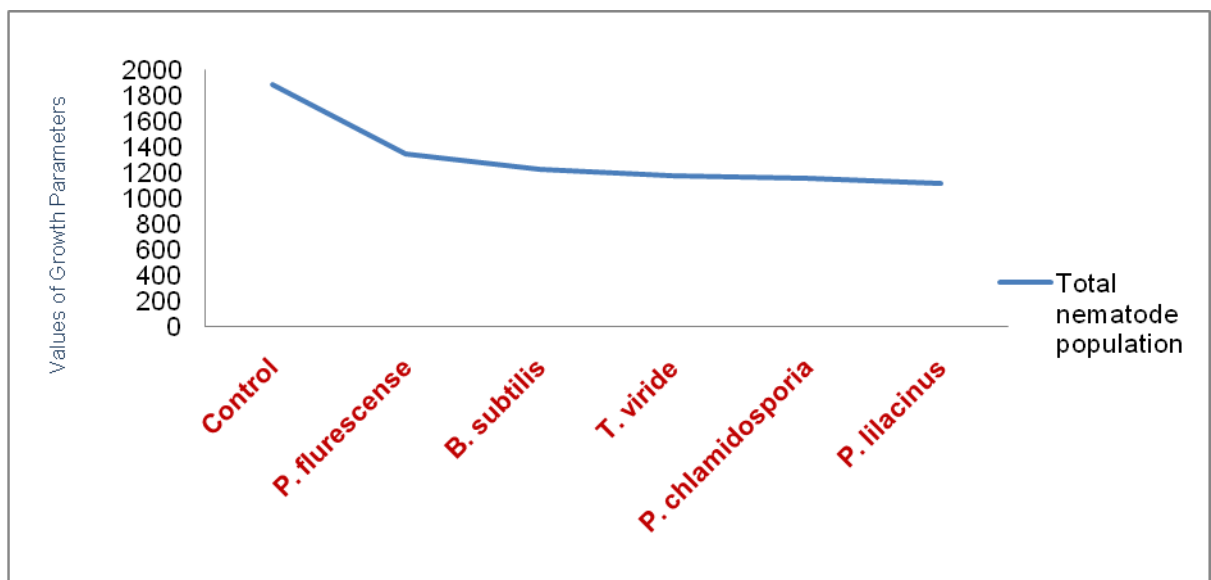


Fig: 4 (Contd.) Efficacy of different biocontrol agents against *Meloidogyne incognita* and *Fusarium oxysporum* complex

D



E



Significantly increased fresh weight of shoot (0.89g) was recorded with *P. lilacinus* which remained almost at par with *P. chlamidosporia* (0.87g) and *T. viride* (0.85), *B. subtilis* and *P. fluorescense* recorded 0.78 and 0.69g shoot weight respectively against minimum (0.62g) in control. Maximum (0.86g) root weight on fresh weight basis was noted with *P. lilacinus* and *P. chlamidosporia* (0.85g) which remained at par with each other. This treatment was followed by *T.*

viride (0.81g), *B. subtilis* (0.76g) and *P. fluorescence* (0.64g) Minimum root weight was recorded with inoculated control (0.32g).

Dry weight of shoot was significantly increased (0.41g) in *P. lilacinus* against minimum (0.26g) in control. The effect of *P. chlamydosporia* and *T. viride* remained at par with each other. These treatments recorded 0.38 and 0.36g root weights respectively. Significantly lower shoot weights were recorded in *B. subtilis* (0.33g) and *P. fluorescence* (0.29g) but superior over inoculated control.

All the treatments have significantly increased dry root weight of black gram. Maximum (0.39g) dry root weight was recorded with *P. lilacinus* followed by *P. chlamydosporia* (0.36g) and *T. viride* (0.34g), *B. subtilis* and *P. fluorescence* recorded 0.32 and 0.28g dry root weight respectively. Minimum (0.25g) dry root weight was recorded in inoculated control.

Minimum number (8.20) of galls were recorded in *P. lilacinus* followed by *P. chlamydosporia* (9.80) and *T. viride* (10.40), *B. subtilis* recorded 12.20 and *P. fluorescence* recorded 11.00 galls/plant respectively with maximum (13.80) galling in inoculated control.

Maximum (1890.20) nematode recovery was noted in inoculated control. Which was significantly reduced in *T. viride* (1178.60) followed by *P. chlamydosporia* (1156.40) and *P. lilacinus* (1115.4). *B. subtilis* and *P. fluorescence* recorded 1230.40 and 1342.40 nematode (Root+ Soil).

DISCUSSION

The destructive plant parasitic nematodes are one of the major limiting factors in the production of pulses throughout the country. Roots damaged by the nematodes are not efficient in the utilization of available moisture and nutrients in the soil resulting in reduced functional metabolism. The visible symptoms of nematode attack often include reduced growth of individual plants. The deleterious effects on plant growth result in reduced yield and poor quality of crop (Anon, 1988).

The random surveys conducted in pulses growing areas in Madhya Pradesh have revealed the cent percent frequency of root knot nematode *Meloidogyne incognita* (Anon, 1997). Total 27 per cent loss in yield due to root knot nematode was reported by Bhatti and Jain, (1977). The damage to global agricultural crops due to root knot nematode is estimated around US\$ 157 billion annually (Abad *et al.*, 2008).

The influence of different inoculum levels of *M. incognita* on the growth of blackgram (*Vigna mungo* L.) revealed that the nematode mitigated the plant height drastically at higher levels i.e. 1000 and 10,000 nematodes per pot. Similarly significant reduction in root length, fresh and dry root and shoot weights was also noticed at these levels. Highest level of nematode inoculum indicated inhibitory and damaging potential on plant growth parameters of blackgram. Similar findings were also brought forward by Birat (1968) in okra with *M. incognita* and Reddy *et al.* (1975) in chickpea plants with *M. javanica* where 1000 and 10,000 juveniles of *Meloidogyne* sp. per pot were applied. Reduction in plant length and weights due to root knot nematode has also been reported by Dhawan and Sethi (1976), Gaur and Prasad (1980), Mani and Sethi (1984), Hisamuddin *et al.* (2005); Azam and Hisamuddin (2008), and Azam *et al.* (2008) that inoculum level of two larvae per g of soil was the damaging threshold level of *M. incognita* on chickpea in accordance with the above findings in the present study also 1000 juveniles per 500g of soil were found to be the damaging threshold level for root knot nematode on black gram (Cv TU 98-14). Damage caused by nematodes to plants is directly proportional to their population densities in soil, and their reproduction potentials on the plant (Barker and Olthof,

1976). The minimal density that causes a measurable reduction on plant growth and yield is regarded as the threshold density (Barker and Nusbaum, 1971).

The effects of *M. inconita* on different plant stages of blackgram were most pronounced when inoculations were carried out on 7, 14 and, 21 days old seedlings of blackgram. Maximum damage appeared 28 days after inoculation. All the growth parameters were significantly reduced at Seven and 14 days of plant growth by 1000N/plant. The plant growth parameters were at seven, 14 days old seedlings were tend to be completely suppressed and the plants remained stunted and devitalized. Sharp decline in growth parameters was noticed at seven days old seedling inoculations.

There was a gradual increase in the number of galls/plant with decrease the plant age stages. Maximum number of galls was recorded in seven days old seedling stage followed by 14 days, 21 days and 28 days old age. The number of galls significantly reduced at 28 days old seedling inoculation. Which may be due to the deterioration in the root system and colonization of nematode population in the pericycle and subsequently continuous feeding by the nematodes. Similarly maximum number of egg mass/gall was noted at 7, 14, and 21 days old seedlings. 28 days old seedling recovered correspondingly less number of eggs when compared to other stages of crop growth. These results are in according with the findings of Mishra and Gaur (1981) on mothbean; Kalita and Phukan (1993) on blackgram; Gupta and Verma (1990) on mungbean; Sharma and Bhatti (1992) on peas, okra, tomato and bottle gourd.

It is evident from the findings that root knot nematode continues to penetrate the roots at all the stages of plant growth in blackgram. These results confirm the findings of Olthof, (1983) on sugar beet Pandey (1984) on mungbean; Jadhav and Kurundkar (1991) on okra. They also reported that young seedlings were more susceptible. Similar results have also been reported by Eapen (1992) in cardimum.

The low number of galls on the roots of 28 days old plants may be due to low penetration or because of low multiplication rate of nematode (Eapen, 1992) Weiser (1985) reported that actively elongating roots are more attractive to nematodes juveniles than roots in which extension had stowed.

Studies were carried out under pot conditions to determine the effects of cohabitation of *Meloidogyne incognita* and *Fusarium oxysporum* on disease development and growth parameters in blackgram. The results indicated that

plant growth was adversely affected in all the cases where the plant was inoculated with *M. incognita* and *F. oxysporum* when compared with uninoculated control. Generally, the treatments receiving the nematode inoculation prior to fungus resulted in higher reduction of plant growth than the other treatments.

When nematode inoculation was done seven days prior to fungal inoculation, showed maximum synergistic effect followed by treatment where both the pathogens were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression. (Fazal *et al.* 1994, Malhotra *et al.* 2011).

In a sequential etiology, one pathogen of the disease complex infects host before the invasion by the other pathogen and brings about certain histophysiological and biochemical alterations within the host, rendering it more suitable substratum for establishment and growth (Anwar and Khan, 2002).

Although, each pathogen was able to reduce the plant growth, the combined infection of nematode and fungus resulted in synergistic effect. Haseeb *et al.* (2005) and Ravishankar and Singh (2008) observed that inoculation of *M. incognita* 15 days prior to *R. solani* significantly reduced all the plant growth parameters as compared to inoculation of *R. solani* 15 days prior to *M. incognita* in *Vigna mungo*. Similar effect on suppression of plant growth of tomato than alone treatment was also recorded by (Samuthiravalli & Sivakumar 2008).

The host infestation by *M. incognita* as represented by root knot index was maximum in plants inoculated nematode seven days prior to fungus followed by nematode and fungus simultaneously, and fungus seven days prior to nematode, nematode alone inoculations showed maximum galling, number of galls and egg masses. This was Followed by plants inoculated with fungus first and nematode a week after.

The reduction might be due to reduced root system, thus nematode faced competition for food. In addition to fungal disruption of nematode feeding sites, plants affected by disease complexes may be more prone to early senescence and death which in turn might prevent nematode from completing its life cycle leading to reduced reproduction. Similar type of reduced galling and population density in presence of *R. solani* was also reported by Roy and Mukhopadhyaya (2004).

Visually highest root infection by *F. oxysporum* was observed in nematode-fungus simultaneously inoculated plants followed by nematode seven days prior to fungus; seven days prior to nematode and fungus alone inoculated plants, respectively. There was a significant increase in percent disease incidence when *M. incognita* inoculation preceded the fungal inoculation. The results corroborate with the research findings of Bhagwati *et al.* (2007).

Minimum root infection in plants inoculated with *F. oxysporum* alone than the ones where nematode was present together with fungus suggested that delay in entry of fungus was due to absence of predisposing agent (*M. incognita*) or due to absence of nutrient rich cells, which were responsible for attracting the fungus to galled roots. Similar types of results were reported by Haseeb *et al.* (2007) on *Pisum sativum*.

These observations on nematode-fungal interaction suggested that they were due to nematode providing a readymade means of entry into the host for the fungus. This occurred when root knot nematode caused superficial root injury and so enhances fungal access. The results of the present investigation suggested that *M. incognita* and *F. oxysporum* together caused greater damage to black gram than either of them alone. Anwar and Khan. (2002) observed slight to moderate galling in nematode inoculated prior to fungus in soybean. This difference might be due to the variety used during the study. The reduction in galling and nematode population could be possibly attributed to deleterious effects of metabolites of *F. oxysporum* on the juveniles of root-knot nematode. This is further supported by greater reductions when fungi and nematodes were inoculated simultaneously. These results are in conformity with the results recorded by Haseeb *et al.* (2007) on *V. mungo*.

Minimum nematode population was recorded when fungus was inoculated first and nematode a week after. Similar results in reduction of population of nematode in presence of *R. solani* was also reported by Goswami *et al.* (1975), Choo *et al.* (1990), Kumar and Haseeb (2009) and Vidyasagar (2012). This is possibly attributed to deleterious effects of metabolites of *fungus* on the juveniles of root knot nematode in terms of their development and multiplication. Decrease in the rate of nematode multiplication and galling in the presence of fungus showed antagonistic effect of the fungus on the development and reproduction of nematodes. This could be ascribed to the possible toxic effect of the fungus

metabolites on nematodes (Haseeb *et al.* 2007). This was followed by simultaneous inoculations with nematode and fungus in terms of total nematode population (Root +Soil). These findings are in accord with of Anwar and Khan (2002) who observed lowest reproduction of *M. incognita* during the simultaneous inoculation by fungus and nematode.

The increase in nematode population in the treatment where nematode inoculated first and fungus a week after might be due to the fact that nematode has colonized the feeding site first and modifies the host substrate unfavourable for fungus similar results has been recorded by Bhatt (1986).

The data presented in the table 4 indicated that *Paecilomyces lilacinus* significantly increased the growth parameters of the blackgram viz., plant height, root length, fresh and dry weight of shoot and roots with significant decrease in the number of galls and final nematode population. These result are in accord with the finding of Latha *et al.*, (2000) who observed increase in growth parameter and decrease in nematode multiplication of *Hetrodera cajani* in presence of *Macrophomina phaseolina* on *Vigna mungo*. Similar result have also been observed by Verma *et al.*; (2005). On *P. lilacinus* has been reported to produce peptidal antibiotic viz; lilacin and paecilotoxin that are toxic to root knot nematode (Verma *et al.*; (2005).

The biocontrol agent *Pochionia chlamydosporia* was also noted to be superior and took second rank in improving the plant growth and reduced nematode population .similar finding have also been reported by Siddiqui and Mahmood on pigeon pea in the presence of *Hetrodera cajani* and *Fusarium udum*.

Bacillus subtilis, and *Psuedomonas fluorescens* showed its efficacy in reducing the nematode population and enhancing the growth characters of the blackgram. These findings have also been supported by the result shown by Siddiqui and Mohmood (1995) on chickpea with *M. incoginita* and *M. phaseolina*, Poornima *et al.*; (2007) on banana with *Fusarium oxysporum* and *Helicotylenchus multicinctus*, Latha *et al.* (2000) on blackgram Haseeb *et al.* (2005) on green gram and Akhtar *et al.* (2012) on blackgram.

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

The root knot nematode (*Meloidogyne incognita*) caused significant reduction in plant height, root length fresh and dry weights of shoots and roots. Root galls and final nematode population increased with the increasing levels of nematode inoculum. Highest level of nematode inoculum indicated inhibitory and damaging potential on plant growth parameters on blackgram. Two second stage larvae of *M. incognita* were found to the damaging level on blackgram (Cv TU 98-14).

The root knot nematode continues to penetrate at all the stages of plant growth. However, seven days old plant were observed to be most susceptible to root knot. Significant reduction in plant growth parameters at all stages of plant growth was observed seven days old seedling stage.

The treatments receiving the nematode inoculation prior to fungus resulted in higher reduction and plant growth than the other treatments. When nematode inoculation was done seven days prior to fungal inoculation, it showed maximum synergistic effect followed by treatment where both the pathogens were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression.

Paecilomyces lilacinus significantly increased the growth parameters of the black gram viz., plant height, root length, fresh and dry weight of shoot and roots with significant decrease in the number of galls and final nematode population. *Pochionia chlamydosporia* was also noted to be superior and took second rank in improving the plant growth and reduced nematode population.

CONCLUSION:

- Two second stage/500g of the soil were the damaging threshold on black gram.
- The nematode continues to penetrate the roots at all stages of plant growth but young seedlings were more susceptible to root knot than mature roots.
- The root knot nematode, *M. incognita* not only predisposed the host but also shortened the incubation period for diseases expression.
- The egg parasitic *Paecilomyces lilacinus* and *Pochionia chlamydosporia* were found to have a significant effect in reducing galls and enhancing plant growth.

SUGGESTIONS FOR FURTHER WORK:

Systemic and comprehensive surveys of blackgram growing areas are needed to work out the economic importance of nematode fungus wilt complex involving *M. incognita* and *Fusarium oxysporum*. Agro ecological factors have to be studied to know the distribution and prevalence of this complex disease. Genetic basis of mechanism of interaction, physiologically and histological changes in diseases complex have to be workout .Characterization of translocatable metabolites in nematode fungal interactions and determination of their biochemical nature and mode of action and specificity are the areas of further research.

Exploration and exploitation of other biocontrol agents are required to manage this complex disease.

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*Original not seen.

APPENDICES

Analysis Variance Table 1- Effect of different levels of inocula of *Meloidogyne incognita* on growth of blackgram

Analysis of Variance Table 1- Plant height

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 153.702 | 38.426 | 14.522 | 0.00001 |
| Error | 20 | 52.920 | 2.646 | | |
| Total | 24 | 206.622 | | | |

Analysis of Variance Table 1- Root length

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 336.941 | 84.235 | 50.922 | 0.00000 |
| Error | 20 | 33.084 | 1.654 | | |
| Total | 24 | 370.025 | | | |

Analysis of Variance Table 1- Fresh shoot weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 0.152 | 0.038 | 9.492 | 0.00018 |
| Error | 20 | 0.080 | 0.004 | | |
| Total | 24 | 0.231 | | | |

Analysis of Variance Table 1- Fresh root weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 0.245 | 0.061 | 17.504 | 0.00000 |
| Error | 20 | 0.070 | 0.003 | | |
| Total | 24 | 0.315 | | | |

Analysis of Variance Table 1- Dry shoot weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 0.134 | 0.034 | 108.948 | 0.00000 |
| Error | 20 | 0.006 | 0.000 | | |
| Total | 24 | 0.140 | | | |

Analysis of Variance Table 1- Dry root weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 0.205 | 0.051 | 253.770 | 0.00000 |
| Error | 20 | 0.004 | 0.000 | | |
| Total | 24 | 0.209 | | | |

Analysis of Variance Table 1- No. of galls/plant

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 1,679.840 | 419.960 | 30.699 | 0.00000 |
| Error | 20 | 273.600 | 13.680 | | |
| Total | 24 | 1,953.440 | | | |

Analysis of Variance Table 1- Total nematode population

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|-----------------|-----------------|--------------|--------------|
| Treatment | 4 | 762,422,904.000 | 190,605,726.000 | 5,599.874 | 0.00000 |
| Error | 20 | 680,750.000 | 34,037.500 | | |
| Total | 24 | 763,103,654.000 | | | |

Analysis of Variance Table – 2 Effect of plant age on growth and multiplication of *Meloidogyne incognita*

ANALYSIS OF VARIANCE TABLE 2- PLANT HEIGHT

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 138.612 | 34.653 | 12.441 | 0.00003 |
| Error | 20 | 55.709 | 2.785 | | |
| Total | 24 | 194.321 | | | |

Analysis of Variance Table 2- Root length

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 216.156 | 54.039 | 40.516 | 0.00000 |
| Error | 20 | 26.675 | 1.334 | | |
| Total | 24 | 242.831 | | | |

Analysis of Variance Table 2- Fresh shoot weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 0.128 | 0.032 | 9.572 | 0.00017 |
| Error | 20 | 0.067 | 0.003 | | |
| Total | 24 | 0.195 | | | |

Analysis of Variance Table 2- Fresh root weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 0.173 | 0.043 | 14.879 | 0.00001 |
| Error | 20 | 0.058 | 0.003 | | |
| Total | 24 | 0.232 | | | |

Analysis of Variance Table 2- Dry shoot weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 0.044 | 0.011 | 45.082 | 0.00000 |
| Error | 20 | 0.005 | 0.000 | | |
| Total | 24 | 0.049 | | | |

Analysis of Variance Table 2- Dry root weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 0.050 | 0.013 | 59.373 | 0.00000 |
| Error | 20 | 0.004 | 0.000 | | |
| Total | 24 | 0.054 | | | |

Analysis of Variance Table 2- No. of galls/plant

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 4 | 816.160 | 204.040 | 11.310 | 0.00006 |
| Error | 20 | 360.800 | 18.040 | | |
| Total | 24 | 1,176.960 | | | |

Analysis of Variance Table 2- Total nematode population

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|---------------|--------------|--------------|
| Treatment | 4 | 11,939,329.360 | 2,984,832.340 | 1,362.912 | 0.00000 |
| Error | 20 | 43,800.800 | 2,190.040 | | |
| Total | 24 | 11,983,130.160 | | | |

Analysis of Variance Table 3- Influence of *Meloidogyne incognita* and *Fusarium oxysporum*.

Analysis of Variance Table 3 - Plant height

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 86.800 | 17.360 | 5.292 | 0.00204 |
| Error | 24 | 78.724 | 3.280 | | |
| Total | 29 | 165.524 | | | |

Analysis of Variance Table 3- Root length

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 170.778 | 34.156 | 12.593 | 0.00000 |
| Error | 24 | 65.096 | 2.712 | | |
| Total | 29 | 235.874 | | | |

Analysis of Variance Table 3- Fresh shoot weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 0.179 | 0.036 | 6.508 | 0.00059 |
| Error | 24 | 0.132 | 0.006 | | |
| Total | 29 | 0.312 | | | |

Analysis of Variance Table 3- Fresh root weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 0.142 | 0.028 | 6.131 | 0.00086 |
| Error | 24 | 0.111 | 0.005 | | |
| Total | 29 | 0.253 | | | |

ANALYSIS OF VARIANCE TABLE 3- DRY SHOOT WEIGHT

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 0.057 | 0.011 | 19.525 | 0.00000 |
| Error | 24 | 0.014 | 0.001 | | |
| Total | 29 | 0.072 | | | |

Analysis of Variance Table 3- Dry root weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 0.071 | 0.014 | 28.784 | 0.00000 |
| Error | 24 | 0.012 | 0.000 | | |
| Total | 29 | 0.083 | | | |

Analysis of Variance Table 3- No. of galls/plant

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 1,103.467 | 220.693 | 26.859 | 0.00000 |
| Error | 24 | 197.200 | 8.217 | | |
| Total | 29 | 1,300.667 | | | |

Analysis of Variance Table 3- Total nematode population

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|---------------|--------------|--------------|
| Treatment | 5 | 18,443,256.667 | 3,688,651.333 | 165.770 | 0.00000 |
| Error | 24 | 534,040.000 | 22,251.667 | | |
| Total | 29 | 18,977,296.667 | | | |

Analysis of Variance Table 4- Efficacy of different bio control agents against *Meloidogyne incognita* and *Fusarium oxysporum*.

Analysis of Variance Table 4- Plant height

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 198.874 | 39.775 | 8.694 | 0.00008 |
| Error | 24 | 109.800 | 4.575 | | |
| Total | 29 | 308.674 | | | |

Analysis of Variance Table 4- Root length

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 192.883 | 38.577 | 10.818 | 0.00002 |
| Error | 24 | 85.580 | 3.566 | | |
| Total | 29 | 278.463 | | | |

Analysis of Variance Table 4- Fresh shoot weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 0.296 | 0.059 | 6.417 | 0.00064 |
| Error | 24 | 0.222 | 0.009 | | |
| Total | 29 | 0.518 | | | |

Analysis of Variance Table 4- Fresh root weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 1.054 | 0.211 | 34.427 | 0.00000 |
| Error | 24 | 0.147 | 0.006 | | |
| Total | 29 | 1.201 | | | |

Analysis of Variance Table 4- Dry shoot weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---------------------|----|----------------|--------------|--------------|--------------|
| Treatment | 5 | 0.080 | 0.016 | 20.248 | 0.00000 |
| Error | 24 | 0.019 | 0.001 | | |
| Total | 29 | 0.099 | | | |

Analysis of Variance Table 4- Dry root weight

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|----------------------------|-----------|-----------------------|---------------------|---------------------|---------------------|
| Treatment | 5 | 0.069 | 0.014 | 20.114 | 0.00000 |
| Error | 24 | 0.017 | 0.001 | | |
| Total | 29 | 0.086 | | | |

Analysis of Variance Table 4- No. of galls/plant

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|----------------------------|-----------|-----------------------|---------------------|---------------------|---------------------|
| Treatment | 5 | 94.300 | 18.860 | 14.889 | 0.00000 |
| Error | 24 | 30.400 | 1.267 | | |
| Total | 29 | 124.700 | | | |

Analysis of Variance Table 4- Total nematode population

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|----------------------------|-----------|-----------------------|---------------------|---------------------|---------------------|
| Treatment | 5 | 2,111,353.900 | 422,270.780 | 323.385 | 0.00000 |
| Error | 24 | 31,338.800 | 1,305.783 | | |
| Total | 29 | 2,142,692.700 | | | |

Table: 1 Effect of different levels of inocula of *Meloidogyne incognita* on growth of blackgram

| Source of variation | D.F. | Mean squares | | | | | | | |
|---------------------|------|------------------|-------------|-------|-------|----------------|-------|------------------|---------------------------|
| | | Fresh weight (g) | | | | Dry weight (g) | | | |
| | | Plant height | Root length | Shoot | Root | Shoot | Root | No of gall/plant | Total nematode population |
| Treatment | 4 | 38.426 | 84.235 | 0.038 | 0.061 | 0.034 | 0.051 | 419.960 | 190,605,726.000 |
| Error | 20 | 2.646 | 1.654 | 0.004 | 0.003 | 0.000 | 0.000 | 13.680 | 34,037.500 |

Table: 2 Effect of plant age on growth and multiplication of *Meloidogyne incognita*

| Source of variation | D.F. | Mean squares | | | | | | | |
|---------------------|------|------------------|-------------|-------|-------|----------------|-------|------------------|---------------------------|
| | | Fresh weight (g) | | | | Dry weight (g) | | | |
| | | Plant height | Root length | Shoot | Root | Shoot | Root | No of gall/plant | Total nematode population |
| Treatment | 4 | 34.653 | 54.039 | 0.032 | 0.043 | 0.011 | 0.013 | 204.040 | 2,984,832.340 |
| Error | 20 | 2.785 | 1.334 | 0.000 | 0.003 | 0.000 | 0.000 | 18.040 | 2,190.040 |

Table: 3 Influence of *Meloidogyne incognita* and *Fusarium oxysporum*.

| Source of variation | D.F. | Mean squares | | | | | | | |
|---------------------|------|------------------|-------------|-------|-------|----------------|-------|------------------|---------------------------|
| | | Fresh weight (g) | | | | Dry weight (g) | | | |
| | | Plant height | Root length | Shoot | Root | Shoot | Root | No of gall/plant | Total nematode population |
| Treatment | 5 | 17.360 | 34.156 | 0.036 | 0.028 | 0.011 | 0.014 | 220.693 | 3,688,651.333 |
| Error | 24 | 3.280 | 2.712 | 0.006 | 0.005 | 0.001 | 0.000 | 8.217 | 22,251.667 |

Table: 4 Efficacy of different bio control agents against *Meloidogyne incognita* and *Fusarium oxysporum*.

| Source of variation | D.F | Mean squares | | | | | | | |
|---------------------|-----|------------------|-------------|-------|-------|----------------|-------|------------------|---------------------------|
| | | Fresh weight (g) | | | | Dry weight (g) | | | |
| | | Plant height | Root length | Shoot | Root | Shoot | Root | No of gall/plant | Total nematode population |
| Treatment | 5 | 39.775 | 38.577 | 0.059 | 0.211 | 0.016 | 0.014 | 18.860 | 422,270.780 |
| Error | 24 | 4.575 | 3.566 | 0.009 | 0.006 | 0.001 | 0.001 | 1.267 | 1,305.783 |

CURRICULUM VITAE

The author of this thesis **Dilip Kumar S/o Shri Bhawati Prasad** was born on 04th Oct. 1988 at Village-Belsara, Tehsil- Takhatpur, Distt- Bilaspur (C.G.).



He took admission for B.Sc. (Honours) Agriculture in the College of Agriculture, Dantewada affiliated to I.G.K.V.V. Raipur Chhattisgarh in the year 2008-09. He has successfully completed his graduation with 6.52 OGPA in the year 2012. He passed his High School (10th) with first division (61.33%) in the year 2006 and Higher Secondary School (12th) with first division (71.2%) in the year 2008 from B.R.SAO. H. Sec. School Mungeli Chhattisgarh.

After graduation for further study, he got admission in M.Sc.(Ag.) for specialization in Plant Pathology at the college of Agriculture, JNKVV, Jabalpur (M.P) where successfully completed all the course requirements for master's degree with OGPA 6.68 out of 10 point scale in the year 2015-16.

For the partial fulfilment of the Master's degree research work on "Studies on interaction *Meloidogyne incognita* and *Fusarium oxysporum* in black gram" under Jabalpur condition, which was successfully conducted by him and being submitted in the form of this thesis.