

**Eco friendly management of Root knot nematode,  
*Meloidogyne incognita* infecting Bitter gourd  
(*Momordica charantia*)**

**A  
Thesis submitted to the  
Odisha University of Agriculture and Technology Bhubaneswar  
in partial fulfillment of the requirement  
for the degree of  
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(Nematology)**

**BY**

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**CERTIFICATE- I**

This is to certify that the thesis entitled “**Eco friendly management of Root knot nematode, *Meloidogyne incognita* infecting bitter gourd (*Momordica charantia*)**” submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (NEMATOLOGY)** to the Odisha University of Agriculture and Technology, Bhubaneswar is a faithful record of *bonafide* research work carried out by **SARMISTHA PANDA** under my guidance and supervision. No part of the thesis has been submitted for the award of any other degree or diploma.

It is further certified that the assistance and help availed by her from various sources during the course of investigation has been duly acknowledged.

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## CERTIFICATE-II

This is to certify that the thesis entitled “**Eco friendly management of Root knot nematode, *Meloidogyne incognita* infecting bitter gourd (*Momordica charantia*)**” submitted by **SARMISTHA PANDA** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE IN AGRICULTURE (NEMATOLOGY)** has been approved/ not approved by the Students’ Advisory Committee and the External Examiner.

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## **CONTENTS**

| <b>CHAPTER</b> | <b>TITLE</b>                      | <b>PAGE NO.</b> |
|----------------|-----------------------------------|-----------------|
| <b>1.</b>      | <b>INTRODUCTION</b>               | <b>1-3</b>      |
| <b>2.</b>      | <b>REVIEW OF LITERATURES</b>      | <b>4-13</b>     |
| <b>3.</b>      | <b>MATERIALS AND METHODS</b>      | <b>14-20</b>    |
| <b>4.</b>      | <b>RESULTS</b>                    | <b>21-28</b>    |
| <b>5.</b>      | <b>DISCUSSION</b>                 | <b>29-31</b>    |
| <b>6.</b>      | <b>SUMMARY AND<br/>CONCLUSION</b> | <b>32-34</b>    |
|                | <b>REFERENCES</b>                 | <b>i-vii</b>    |

## LIST OF FIGURES

| <b>FIGURE</b> | <b>PARTICULARS</b>  | <b>PAGE NO.</b> |
|---------------|---|-----------------|
| 1.            | Effect of treatments on plant growth parameters                           | 22              |
| 2.            | A view of bittergourd plants grown in replicated pots                     | 24              |
| 3.            | Photograph showcasing roots of bittergourd plants in different Treatments | 27              |

## LIST OF TABLES

| TABLE | PARTICULARS  | PAGE NO. |
|-------|--|----------|
| 1     | Root Knot Index Scale  | 18       |
| 2     | Effect of neem cake, bioagents and carbofuran on growth of bitter gourd infected with root-knot nematode, <i>Meloidogyne incognita</i> . | 23       |
| 3     | Effect of neem cake, bioagents and carbofuran on infection parameter of <i>Meloidogyne incognita</i> in bitter gourd.                    | 25       |
| 4     | Effect of neem cake, bioagents and carbofuran on growth and Multiplication of <i>Meloidogyne incognita</i> in bitter gourd.              | 28       |

## **ABBREVIATIONS**

|       |                                 |
|-------|---------------------------------|
| %     | Percentage                      |
| @     | At the rate of                  |
| cm    | Centimeter                      |
| G     | Gram                            |
| kg    | Kilogram                        |
| SE(m) | Standard error mean             |
| CRD   | Completely Randomised<br>Design |
| Fig.  | Figure                          |
| Viz.  | Namely                          |
| i.e.  | That is                         |
| ha    | Hectare                         |
| CD    | Critical difference             |
| RKN   | Root knot nematode              |
| Max   | Maximum                         |
| Min   | Minimum                         |



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**CERTIFICATE OF ANTI-PLAGIARISM**

This is to certify that the M.Sc.(Ag.) thesis of **Sarmistha Panda** Adm.No. **191222002**, Department of Nematology, College of Agriculture, OUAT, Bhubaneswar has been checked for antiplagiarism by using Turnitin web portal and similarity index was found within 15% level (from Abstract to Summary & Conclusion) as prescribed by OUAT.

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**Chairman**

**Head of the Department**

## ABSTRACT

A pot culture experiment was conducted in the net house of the Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar with seven treatments and four replications to study the efficacy of Eco-friendly management modules of Root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) infecting bittergourd (*Momordica charantia* L). Well pulverized sandy loam soil collected from the experimental plots of Central Farm, Odisha University of Agriculture and Technology, Bhubaneswar was mixed thoroughly with sand and FYM in the ratio of 2:1:1 and sterilized in autoclave. Earthen pots of 15 cm diameter were cleaned with tap water, drained, surface sterilized and filled with 1 kg aerated sterilized soil. Neem cakes were applied 15 days prior to sowing according to treatments. Pre-incubated bioagent *P.fluorescens* and *P. lilacinus*, as well as Carbofuran were applied at the time of sowing to the pot soil as per treatments. Overnight soaked seeds of bittergourd cultivar green long, were sown in soil and watered regularly to keep the soil moist. After germination, when they were at 3-4 leaved stage, one plant per pot was maintained. Freshly hatched J2 of *Meloidogyne incognita* isolated from pure culture were inoculated to the 10 days old bittergourd plants @ one J2/g soil following standard procedure. Watering and weeding were continued as per requirement. Stackings were provided in each pot to support the growing vines. The effect of treatments on growth parameters of bittergourd plant as well as population build-up of root knot nematode was noted down. The treatment combination i.e. soil application of neem cake @1ton/ha 15 days prior to sowing + soil application of pre incubated *Psuedomonas fluorescens* @2.5kg/ha at the time of sowing of seeds enhanced the vine length, vine weight, root length and root weight of bitter gourd plants to the tune of 73%, 52.6%, 66.1%, and 81% respectively over untreated control. It also reduced the number of galls, number of egg masses and nematode population of pot soils by 91.4%, 93.4% and 22.4% respectively with RKI (2.0) and reproduction factor (1.05) and thus proved to be the best among all other treatments. On the other hand, the treatment combination comprising of soil application of neem cake @1ton/ha 15 days prior to sowing + soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing was found to be second best treatment with 62.6%, 44.8%, 49.1% and 61.5% enhancement of vine length, vine weight root length and root weight of the bittergourd plant respectively. This treatment also recorded 73.5% less gall formation, 75.8% less egg mass production and 18.4 % less final nematode population as compared to untreated control pot.

# INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is an indigenous vegetable of tropical regions of Asia. Bitter gourd is a member of the Cucurbitaceae family. It is also known as bitter melon in England, balsam-pear & margose in France, *Balsambirne peria* in Malaysia and karella in India. Bitter gourd is acknowledged as a vegetable all over the world (Taylor, 2002). In terms of area and production, bitter gourd is cultivated in 95,000 ha area with annual production of 10,30,000 MT in India (Horticulture at a glance, 2017). Among the different states, Chattisgarh ranks first followed by Telangana, Andhra Pradesh, Orissa and Madhya Pradesh.

Bittergourd plants include active phytochemicals that are beneficial to humans such as triterpenes, proteins and steroid hormones. Minerals such as Cu, Fe, Mg, Zn, and Ca are abundant in *M. charantia*, according to researchers. Fatty acids such as lauric, myriatic, palmitic, stearic, and linolaic have also been discovered in *M. charantia* ( Yuwai *et al.*,1991).According to researchers, charantin chemical is having anti-diabetic effects that might be used to treat diabetes (Krawinkel *et al.*,2006). Momordicin, a functional component of bitter gourd, may be found in the fruits, leaves, and roots of the plant. The other components of charantin have been shown to be extremely effective in regulating blood glucose levels in those with insulin resistance. In addition, research is undertaken to improve functionality in areas including obesity and colon cancer prevention (Manaku, 1980). Diabetes, hypertension, heart disease and cancer are all on the rise, despite advancements in medical understanding. Bitter gourd is known for its hypoglycemic effects. It has also been found to prevent the formation of gastric and duodenal ulcers. Bitter gourd fruits, leaves, and seeds have been utilised as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antibacterial, anti-diabetic and anti-tumor remedies for centuries.

Bitter gourd is regarded as a nutritious vegetable and has become a valuable commodity. There are, however, pest concerns associated with bitter gourd production, which are strongly influenced by biotic factors such as fungus, bacteria, insect pests, and nematodes. *Meloidogyne* spp., *Rotylenchulus reniformis*, *Heterodera* spp., *Pratylenchus* spp., *Helicotylenchus* spp. and *Hoplolaimus* spp. are plant parasitic nematodes linked with vegetables in India. Root-knot nematodes (*Meloidogyne* spp.) are the most extensively spread and damaging of the plant parasitic nematodes.

Damage produced by plant parasitic nematodes, particularly the root-knot group, *Meloidogyne spp.*, is one of the greatest barriers to the production of adequate food supply in many poor countries (Sasser, 1979). It is quite widespread and causes significant damage to crops, particularly vegetables. There are around 97 recognised species of root-knot nematode worldwide, but only 14 *Meloidogyne species* have been identified in India. *Meloidogyne indica*, *M. lucknowica*, *M. triticooryzae*, and *M. piperi* are four species of root-knot nematodes that have been reported from India only. According to Khan *et al.* (2014). *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria*, and *Meloidogyne hapla* are the most common root-knot nematode species. However *Meloidogyne incognita* and *Meloidogyne javanica* are regarded as one of the primary limiting factors in the development of vegetable crops due to their worldwide spread, vast host range, and destructive character. Infected plants have shown stunted development and dried leaves, which ultimately affects the plant's growth and production. Nematode losses can reach 100% resulting in the crop's total collapse (Gupta, 1986). Root-knot nematodes are recognised to be a problem for cucumbers (Singh and Sharma, 1998). Root-knot nematode has been found to cause yield losses of 36.72 percent in bitter melon, 46.56 percent in bottle melon (Darekar and Mhase, 1998), 41.30 to 45.50 percent in okra (Manaku, 1980), and 69.2 percent in cucumber (Krishnaveni and Subramanian, 2002).

Root-knot nematodes generate a distinct symptom known as a "root gall" or "knotted root," which may be seen with the naked eye. The root-knot nematodes damage almost every vegetable crop. As a result, it is commonly regarded as a limiting element in vegetable growing. Due to their soil-borne origin and vast host range, these nematodes are difficult to manage. The primary causes of yield losses are nematode inoculum accumulation and repetitive cultivation of the same cultivars in the same area every year. Because use of most chemical nematicides have been prohibited due to their negative effects on humans and the environment, scientists have shifted to soil amendments using organic amendments, botanicals, and biological control agents to find a safer way to manage these nematodes. However, because biocontrol agents may be easily integrated with other management methods, they are becoming more significant as an alternative strategy for controlling root knot nematodes. Currently, a variety of commercial bio-formulations comprising various bio-agents are available on the market. The discovery of oviparasitic fungus, *Paecilomyces lilacinus* (*Purpureocillium lilacinum*) have a huge potential for sustainable nematode management which can be seen as an important milestones in this direction. Also

bacterial antagonists like *Pseudomonas fluorescens* have shown effective control of phytonematodes affecting different crops in varied agro-ecological conditions. *Paecilomyces liliacinus* is a soil born fungi which parasitise on nematode eggs and/or adult females. It produces toxin that prevents hatching of nematode eggs or kills the nematode. *Paecilomyces* has been one of the principal genera in biological control of nematodes. This fungus penetrates the nematode eggs directly by individual hyphae. *Pseudomonas fluorescens* is a nematode antagonists and endospore forming bacteria popularly known as Plant Growth Promoting Rhizobacteria (PGPR). It can grow in the plant rhizosphere and enable frontline defence to plant roots against pathogens and plant parasitic nematodes. It is having the ability to colonise plant roots and helps in synthesis of various enzymes and growth hormones contributing to a number of positive effects on plant growth. Rhizobacteria reduce nematode population mainly by regulating nematode behaviour, interfering with plant-nematode recognition and competition for essential nutrients in an ecological niche or a substratum. Production of inhibitory allelochemicals and induction of systemic resistance (ISR) by fortifying the physical and mechanical strength of the cell wall by means of cell wall thickening is another important mechanism. The production of iron chelated siderophore, which further limits the availability of iron necessary for the growth and development of nematode is another important characteristics of rhizobacteria which contributes towards the limitation of nematode population in soil. Plant rhizosphere, being the site of intensive interaction between plants, microorganisms and majority of phytonematodes, possesses huge opportunity for plant health management. Soil types, variation in pH and other abiotic factors are also having tremendous impact on the efficiency of these biocontrol agents present in the rhizosphere in nematode control thereby reduce soil and root populations of *Meloidogyne incognita* (Kalele et al., 2010).

Keeping in view the research gap in *Meloidogyne* management and adverse effect of associated chemical nematicides on human and environment, an experiment entitled “Eco-friendly management of root knot nematode *Meloidogyne incognita* (Kofoid and White, 1919) infecting bittergourd (*Momordica charantia* L.)” is met with the following objectives –

1. To study the effect of eco-friendly management practices on plant growth character of bittergourd infected by *Meloidogyne incognita*.
2. To study the effect of eco-friendly management practices on growth and multiplication of *Meloidogyne incognita* infecting bittergourd.

## REVIEW OF LITERATURES

Eco-friendly management is now been seen one of the most emerging field to control the phytonematodes, among which bioagents have shown excellent properties to reduce the population of plant parasitic nematodes in different crops in varied agro ecological situations. It is environmentally safe and also cost effective. A good number of literatures are available in this context. Some of them are listed here under.

Ahl *et al.* (1986) suggested the mechanism of reduction of nematode population which was due to premature egg hatching and reduction in viability and mortality of juveniles caused by secondary metabolites such as 2,4-Diacetylphloroglucinol and lytic enzymes, antibiotics and hydrogen cyanide produced by *Pseudomonas fluorescens* and non-cellular extracts and toxic metabolites like bacillopeptidase, subtilin and lactamase from *Bacillus* spp.

Bhattacharya and Goswami (1987) observed that combination of bioagents like *Pseudomonas fluorescens*, *Purpureocillium lilacinum*, *Bacillus pumilus* etc., reduced the population of *Meloidogyne incognita* in tomato and also increase the plant vigour.

Cabanillas and Barker (1989) carried out a micro plot experiment to evaluate the effects of inoculum level and time of application of *Paecilomyces lilacinus* on the protection of tomato against *Meloidogyne incognita*. The best protection against *M. incognita* was found with 10 and 20 g of fungus-infested wheat kernels per micro plot which resulted in a 3 fold and 4 fold increase in yield of tomato, respectively, compared with tomato plants treated with the nematode alone. But the greatest protection against this pathogen was observed when *P. lilacinus* was introduced into soil 10 days prior to planting and again at planting. Yield was increased two-fold as compared to the yield of nematode-alone plots and plots with *M. incognita* in combination with the fungus.

De Leij and Kerry (1991) suggested that *Verticillium chlamydosporium* as biocontrol agent against *Meloidogyne incognita* on tomato plants under greenhouse conditions helped in reducing the population of nematodes of less than 80% after the first nematode generation.

Wetscott and Kluepfel (1993) reported that the production of metabolites by rhizosphere bacteria (*Pseudomonas fluorescens*, *Bacillus subtilis*) which causes lysis of

nematode eggs, affects vitality of second stage juveniles and deteriorates release of specific root exudates resulting in reduced attraction and penetration of *Meloidogyne incognita* in bittergourd .

El-sherif *et al.* (1994) revealed that the culture of bacteria like *Pseudomonas fluorescens*, *Bacillus subtilis* etc. lowered the hatching of *Meloidogyne incognita* in cucumber and was highly lethal to juveniles. The bacterium also advanced the plant growth there by improving the plant vigour.

Rao *et al.* (1997) observed that seed treatment with *Purpureocillium lilacinum* have shown lowest root knot index and final population of *Meloidogyne incognita* with increased fruit yield in cucumber.

Regina *et al.* (1998) assessed that mortality of *Meloidogyne incognita* increases with increase in exposure time as well as concentration of rhizo-bacterial toxins which caused the reduction of nematode reproduction, egg hatching, and survival of juveniles and direct killing of nematodes through paralysis and conclusive movements in tomato.

Holland *et al.* (1999) conducted a study on the in vitro interaction of *Paecilomyces lilacinus* strain 251 with eggs, 3<sup>rd</sup> and 4<sup>th</sup> stage juveniles and adult females of *Meloidogyne javanica*. Eggs of all stages, with those containing unhatched juveniles, were infected by *P. lilacinus*. Infection of eggs occurred following flattening of hyphae to the egg surface and formation of appressoria. Hyphae grew out of the egg to continue growing or to form conidiophores. 3<sup>rd</sup> and 4<sup>th</sup> stage juveniles and adult females were infected with hyphae and conidiophores penetrating into the body wall.

Jonathan *et al.* (2000) studied the efficacy of plant growth promoting rhizobacteria, *Bacillus pumilus*, *Bacillus subtilis*, *Agrobacterium radiobacter*, *Pseudomonas fluorescens* against *Meloidogyne incognita* on tomato and banana. All bacteria and actinomycetes have shown increased growth of both crops, and reduced the root-gall development as compared to control plants.

Rao *et al.* (2000) observed that *Trichoderma* spp. individually or in combination with castor or neem cake was able to parasitize the egg mass and ultimately suppressed the galling and population of *Meloidogyne incognita* nematode in tomato. Application of *Trichoderma* spp. starts penetrating the larval cuticle through some enzymatic

activity and dissolves the chitin layer, which is the protective layer of the nematodes against many pathogenic organisms.

Ameer-Zareen (2001) applied fungi *Purpureocillium lilacinum*, *Trichoderma harzianum*, *Trichoderma flavus* as seed treatment and soil drench in a greenhouse cultivation of okra. Seed treatment with *Purpureocillium lilacinum* suppressed the gall formation, egg mass production, population of *Meloidogyne incognita* in soil and roots as compared to control while, *Trichoderma harzianum*, *Trichoderma flavus* were on par.

Ali *et al.* (2002) reported that the seed treatment or soil drench with *Pseudomonas aeruginosa* and *Pseudomonas* spp., significantly lowered the population of *Meloidogyne javanica* and subsequently reduced root knot disease severity with increased protein content and yield of mungbean.

Mittal and Prasad (2003) suggested that application of bioagents enhanced the plant growth parameters of soyabean and reduced the multiplication of *Meloidogyne incognita*.

Siddiqui and Shaukat (2004) observed that the application of *Pseudomonas fluorescens* and *Trichoderma harzianum* to sterilized sandy loam soil lowered the population densities of *Meloidogyne incognita* in tomato roots.

El-Hamshary *et al.* (2004) reported that *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* had an impact on *Meloidogyne incognita* juvenile survival in vitro in sunflower. The mortality percentage of the nematodes were dependent on the bacterial concentration and exposure period.

Khan *et al.* (2004) applied *Purpureocillium lilacinum* at 0.5, 1.0, 2.0, 4.0, 5.0, 6.0, 7.0 and 8.0 gm per pot against *Meloidogyne incognita* in okra and observed an increase in plant growth parameters with a remarkable reduction of nematode population. No significant differences were found above 6.0, 7.0, 8.0gm of dosage.

Krishnaveni and Subramanian (2004) studied the efficacy of *Pseudomonas fluorescens* and *Trichoderma viride* as soil and seed treatments in control of *Meloidogyne incognita* attacking cucumber cv. Green long in pot culture experiments.

Pathan *et al.* (2005) evaluated the activity of *Paecilomyces lilacinus*, a soil borne hyphomycete biological control agent, against *Meloidogyne incognita*, a root-knot nematode, on tomato. *P. lilacinus* significantly enhanced the growth of tomato plants infested with *M. incognita*. They found that *P. lilacinus* was more effective when applied prior nematode inoculation or together with *M. incognita* but was not so effective to reduce nematode reproduction when applied after nematode infestation.

Khan *et al.* (2006) revealed that *Pseudomonas fluorescens*, an egg parasitic fungus which is infected by direct hyphal penetration, where the hyphal branches develops across the egg shell is the most widely used biocontrol agent against *Meloidogyne incognita* in cucurbits.

Kiewnick and Sikora (2006) studied the efficacy of *Paecilomyces lilacinus* strain 251 for the biological control of the northern root-knot nematode *Meloidogyne hapla*. It was revealed that under different temperature regimes for biocontrol activity against the northern root-knot nematode *Meloidogyne hapla*. When the temperature was favourable for the fungal parasite (25°C), efficacy was 90%. In glasshouse experiments with unfavourable temperatures for *M. hapla* development (25°C-33°C), tomato plants repeatedly treated with PL251 exhibited remarkable higher fruit yield (23-102%).

Kiewnick and Sikora (2006) reported that *Pseudomonas fluorescens* and *Purpureocillium lilacinum* were deadly to *Meloidogyne incognita* juveniles through mechanism of direct infection of secondary stages infesting bitter gourd.

Haseeb and Kumar (2006) studied the effects of bio control agents against *Meloidogyne incognita* and *Fusarium solani* disease complex of brinjal (cv. Pusa kranthi). Significantly reduced root knot index and root infection by *Fusarium solani* were reported with the application of bioagents like *Trichoderma viride* and *Pseudomonas fluorescens*.

Sharma *et al.* (2007) conducted a greenhouse study to control *Meloidogyne incognita* in okra with *Purpureocillium lilacinum* alone or in combined application with carbofuran, phorate, neem cake. The *Purpureocillium lilacinum* alone lowered the number of galls, eggs per egg mass by 32% and soil nematode population by 77%.

Kannan and Veeravel (2008) conducted a glass study experiment to assess the effects of *Paecilomyces lilacinus*, an egg parasitoid against the root knot nematode *Meloidogyne incognita* in tomato. Out of different inoculums of fungal culture multiplied on pounded sorghum grains at 2, 4, 6, 8 and 10 g and 100 M. *incognita* females showed maximum enhancement in yield, shoot length, shoot weight and root length in the treatment that was inoculated with 10 g fungal inoculum/kg soil and remarkable reduction in soil nematode population, galls/plant and egg mass/g root was also the findings of this treatment.

Seenivasan and Devrajan (2008) evaluated the effects of commercial formulations of the plant growth promoting rhizobacterium *Pseudomonas fluorescens*, an antagonistic fungus *Trichoderma viride*, the egg parasitic fungi *Paecilomyces lilacinus* and *Verticillium lecanii*, and arbuscular mycorrhizal fungus *Glomus mosseae*, on root-knot nematode, *Meloidogyne incognita*, affecting medicinal coleus. The root colonization of *P. fluorescens* was more and the next best treatment was soil application of *P. lilacinus* and application of *V. lecanii* as dip treatment of softwood cuttings was not much effective treatment.

Ahmad and Siddiqui (2009) conducted a study to manage the root knot nematode in tomato by comparing the effects of six organic additives and nematicides viz., chopped neem leaves, chopped *ficus* leaves, fresh cow dung, NPK fertilizers, phorate (Thimet 10G) and carbofuran (Furadan 3G) on *Meloidogyne incognita* and tomato (*Lycopersicon esculentum* Mill. cv. „K-25“) and its growth parameters were examined in a greenhouse trial. They ascertained that the application of organic additives and nematicides lowered the pathogenic effect and resulted in significant reduction in gall index and population density of *Meloidogyne incognita* in roots and soil but the highest reduction in root knot index was observed in plants treated with carbofuran.

Arevalo *et al.* (2009) conducted an experiment on the egg parasitic fungi, *Pochonia chlamydosporia* var. *chlamydosporia*, *P. chlamydosporia* var. *catenulata* and *Lecanicillium psalliotae*, that were isolated from *M. mayaguensis* eggs and assessed based on their cultural and morphological characteristics. The best growth was at 30°C and in the range of 28-30 °C. In solid state formulation, *P. chlamydosporia* isolates exhibited between 3.5 and 5.2 x 10<sup>6</sup> chlamydospores per g of

colonized substrate, whereas *L. psalliotae* isolate exhibited  $8.5 \times 10^8$  conidia per g of colonized substrate.

Brand *et al.* (2010) worked on the production of fungal biological control agents through solid state fermentation for the eco-friendly management of root-knot nematodes with *Paecilomyces lilacinus* as solid state fermentation was a suitable biological process for the mass production of biological control agents and the conidiospores, so produced were also cost-effective and exhibited good stability and viability for field applications on a commercial scale.

Kalele *et al.* (2010) evaluated *Purpureocillium lilacinum* strain (PL251) and *Arthrobotrys conoides* for their efficacy against *Meloidogyne* spp. in tomato and cucumber under greenhouse conditions. Both pre-planting and planting application of *Purpureocillium lilacinum* was found to suppress the nematode population and root galling in both tomato and cucumber.

Vinod kumar and Jain (2010) conducted a screen house study for assessment of some fungal and bacterial antagonist as seed dressing treatment against root knot nematode *Meloidogyne incognita* infesting okra. Okra seeds were treated with *Pseudomonas fluorescens*, *Trichoderma viride* and *Trichoderma harzianum*. Growth parameters of okra plant were impressive and population of root knot nematode *Meloidogyne incognita* was low in all the treatments.

Wang *et al.* (2010) examined the effects of *Paecilomyces lilacinus* against *Meloidogyne incognita* eggs by over-production of a serine protease at the transcriptional level and the bioassay results revealed that the relative parasitizing rates of the transformants against *M. incognita* eggs increased by about 20% in both conidial suspension and mycelium treatment groups.

Hashem and Abo-elyousr (2011) reported that the application of different biocontrol agents (*Pseudomonas fluorescens* & *Purpureocillium lilacinum*) in combination or alone not only had a deadly effect on nematode and increases the plant growth by supplying many nutritional elements and introduction of the systemic resistance in tomato plants.

Luangsa-Ard *et al.* (2011) conducted a study on the fungus, *Paecilomyces lilacinus*. They compared the clinical isolates with strains isolated from soil, insects and

nematodes using 18S rRNA gene, internal transcribed spacer (ITS) and partial translation elongation factor 1- $\alpha$  (TEF) sequences. The new genus name *Purpureocillium* is suggested for *P. lilacinus* and the new combination *Purpureocillium lilacinum* is developed here.

Kumar *et al.* (2012) have tested the seeds of okra treated with *Trichoderma harzianum*, *Trichoderma viride*, *Pochonia chlamydosporia*, *Purpureocillium lilacinum* and *Pseudomonas fluorescens* at 20g/kg of seed. All treatments significantly lowered the population density of the nematode in soil and enhanced the plant growth.

Majzoub *et al.* (2012) evaluated the effects of rhizosphere bacteria on root knot nematode *Meloidogyne javanica* on cucumber in green house conditions. All showed enhanced growth of plants and reduced root knot indices, but *Pseudomonas fluorescens* CHAO was the most suitable isolate which brought about 55.31 and 55.72% reduction in gall numbers besides 91.13% and 82.84% reduction in reproduction factor of *Meloidogyne javanica*.

Mensin *et al.* (2012) conducted a study on the selection of efficient nematophagous fungi against root-knot nematodes in the highland area. A number of highly virulent nematophagous fungi were recovered by soil sprinkling technique from both infested nematode plantation sand areas rich in organic matter in four provinces of Thailand. Four isolates of genus *Arthrobotrys* and two isolates of genus *Monacrosporium* chosen from forty-five soil samplings destroyed 90-100% second stage juveniles (J2) of root knot nematodes (*Meloidogyne incognita*) using adhesive net structures while two isolates, genus *Paecilomyces* and genus *Pochonia* damaged 70-75% of eggs by means of appressoria. Results of morphological and molecular identification were generally concordant and were in agreement with the four fungal isolates.

Pavithra (2014) suggested that the soil application of *Pseudomonas fluorescens* and *Trichoderma viride* per plant was effective to control root knot nematode infestation and to increase the growth of brinjal with enhanced fruit yield and decreased nematode population.

Hano and Khan (2016) conducted a trial in both pot and field conditions for two consecutive seasons which resulted that *Paecilomyces lilacinus* (PL)-formulation (25% SC/25% WP) was effective at different doses to lower the population of *Meloidogyne*

*incognita* affecting tomato. However, the efficacy of PL-formulations was not as effective as carbofuran at 2.0 kg a.i. /ha for managing root knot nematode in tomato.

Song *et al.* (2016) suggested that the production of microsclerotia by *Purpureocillium lilacinum* in liquid culture to use this as a biocontrol agent for managing root-knot nematode, *Meloidogyne incognita* by culturing in liquid media against various concentrations of ferrous sulphate. Under these conditions mycelia began to form microsclerotia which showed excellent nematophagous ability and greater thermo tolerance and UV-B radiation tolerance as compared to conidia.

Nama and Sharma (2017) found that *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* could enhance the plant growth by decreasing the reproduction of *Meloidogyne incognita* in cowpea. Maximum shoot length, root length, shoot weight, root weight and minimum number of galls per plant, egg masses per plant, eggs per egg mass, nematode population per soil and total nematode population were assessed with *Trichoderma harzianum* @ 10g/kg seed.

Xiang *et al.* (2017) reported that the biological control of *Meloidogyne incognita* by spore forming plant growth promoting rhizobacteria in cotton.

Das and Borgohain (2018) evaluated the efficacy of biocontrol agent viz., AM fungi, *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Bacillus subtilis* and *Heterorhabditis indica* to manage the plant parasitic nematodes around banana rhizosphere alone and in combination. Results obtained from the experiment were that all the treatments significantly superior to the untreated check, increasing the plant growth parameters and yield of crops, and decreasing the population of *Meloidogyne incognita* and *Pratylenchus* spp. Among the biocontrol agents, combined application of *Pseudomonas fluorescens* and *Paecilomyces lilacinus* (*Purpureocillium lilacinum*) were found to be the most effective in reducing the nematode population of *M. incognita* and *Pratylenchus thornei*, root knot index; and enhancing the plant growth parameters and yield of tomato.

Das *et al.* (2018) observed that the bio-control of plant parasitic nematodes by using fungal bioagents is an effective sustainable management practice in rice. The application of carbofuran 3G @ 0.3g a.i./m<sup>2</sup> in the nursery bed and application of fungal bioagent *Trichoderma viride* @2.5 kg/ha mixed with 25 kg FYM at 45 days

after transplanting was reported to be most effective for management of root knot nematode in rice.

Prusty *et al.* (2018) studied the effects of liquid formulations of biological control agents to manage root knot nematode (*Meloidogyne incognita*) in brinjal. They found that nursery treatment with *Trichoderma viride* @ 50g per m<sup>2</sup> followed by application of vermicompost @ 1t per ha enriched with *Purpureocillium lilacinum* @10ml/kg revealed highest increase of 98.0, 73.2, 88.5, 93.6, 93.8, and 93.4 per cent in plant height, fresh shoot weight, fresh root weight, shoot dry weight, root dry weight, and total bio mass over untreated set, respectively with minimal production of root galls per plant (92.2%), number of egg masses per plant (94.9%) as well as the final nematode population (83.9%) in soil with less root knot index (2.667).

Ragavi *et al.* (2019) suggested that the use of biocontrol agents and resistant sources were found to be better when compared to chemical means, both in terms of cost and its side effects. Hence in vitro biocontrol studies were conducted using four efficient biocontrol agents such as three fungi (*Pochonia chlamydosporia*, *Purpureocillium lilacinum*, *Trichoderma harzianum*) and one bacteria (*Bacillus subtilis*). Among these bio control agents, *P. lilacinum* and *P. chlamydosporia* were almost equally effective as other biocontrol agents in controlling the root knot nematode in mango. *P. lilacinum* was very effective in reducing the egg hatching of *M. incognita* (78%) whereas *P. chlamydosporia* was significant among the four organisms in juvenile mortality test (90.75%). In case of screening, the rootstock of polyembryonic variety H- 13- 1 was moderately resistant (Root knot index- 3) as compared to the other four variety rootstocks.

Dahlin *et al.* (2019) conducted a study to manage *Meloidogyne incognita* in tomatoes using fluopyram and *Purpureocillium lilacinum* strain 251. They suggested that the combined effect of chemical and biological nematicides can successfully reduce the root-knot nematode *M. incognita* population and enhance the yields. Fluopyram regulated the nematode population at-planting and reinforced the biological efficacy of *P. lilacinum* throughout the growing season.

Sarven *et al.* (2019) suggested that the efficacy of various doses of *Purpureocillium lilacinum* PLSAU-1 (*P. lilacinum* PLSAU-1). It was examined against brinjal root-knot pathogen, *Meloidogyne incognita* at different inoculum levels. Among

the doses, the rate of *P. lilacinum* PLSAU-1 of  $1 \times 10^6$  CFU/g soil increased plant growth parameters. Inoculation of *M. incognita* decreased the plant growth significantly, and the reduction increased with the increase of inoculum density of *M. incognita*. The application of *P. lilacinum* PLSAU-1 at  $1 \times 10^6$  CFU/g soil lowered a maximum of 72% gall index and 84% egg masses when the crop was challenged by 800 and 400 eggs/100 cm<sup>3</sup> soil, respectively.

Mahalik *et al.* (2020) conducted a field experiment to study the effects of oil cakes i.e. neem and jatropha cakes & biocontrol agents like *P. lilacinum*, *Trichoderma viridae* and *P.fluorescens* on the root knot nematode infesting okra. The authors found that soil application of jatropha oil cake @ 1 ton/ha 15 days prior to sowing, seed treatment with *Trichoderma viridae* and *Pseudomonas fluorescens* each @ 5 g per kg seed and soil application of *Purpureocillium lilacinum* revealed maximum increase in plant growth characters but reduction in nematode population. They also found that the significant increase in plant height (68.1 per cent), dry weight of shoot (90.9 per cent), dry weight of root(77.5 per cent ), no. of eggs/ egg mass(48.3%), nematode population(81.1%) and root knot index(34 per cent ) over untreated control check.

Padmanavan *et al.* (2021) carried out an experiment to study the effect of certain biocontrol agents and neem cake against *Meloidogyne incognita* affection banana. They concluded that neem cake increased the plant growth and root characters and reduced the root gall development in comparison with untreated control treatments. Application of *Pseudomonas fluorescens* @ 10g/ plant + *Trichoderma viridae* @ 10g/ plant + neem cake @ 100g/ plant caused maximum advancement in plant height (38.8 cm), no. of leaves (7), girth of pseudostem (15 cm), root length (25.6 cm), healthy roots (17), less no. of infected roots (3.3) and suppressed the nematode population in soil and root.

## MATERIALS AND METHOD

To study the eco-friendly management of root knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) infecting bitter melon (*Momordica charantia* L.) a pot culture experiment was done in the net house of the Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar during the year 2020-2021. The whole experiment was conducted with bacterial (*Pseudomonas fluorescens*) and fungal (*Paecilomyces lilacinus*) bioagents, neem cake along with a chemical check by application of carbofuran. The experiment was carried out in 15 cm earthen pots following Complete Randomised Design (CRD) with seven treatments, each replicated four times. In this chapter, the materials and methods used for the experimentation are discussed below.

### 3.0 Materials:

#### 3.0.1 Laboratory materials:

Different laboratory equipments and materials used to perform the experiment are as follows.

#### Glass wares:

Glass beakers, Measuring cylinder, Petri dish, Pipettes, 100ml beakers, Counting dish, Glass slides.

#### Laboratory chemicals :

Sodium hypochlorite (NaOCl), Distilled water, Glycerol, Formalin (40% Formaldehyde)

#### Other materials:

Aluminium wire gauge, Aluminium pans, Bamboo stick, Needle, Meter scale, Gunny bags, Envelops, Threads, Earthen pots, Bucket, Sieve sets, Tissue paper, Electric heater, Polythene bags, Meter scale, Envelops, Spades and Picks

#### 3.0.2 Equipments :

Electric balance, Autoclave, Binocular research microscope, Binocular stereoscopic microscopic and Hot air oven.

### **3.0.3 Bitter gourd Seeds:**

Bitter gourd seeds of variety Green long was collected from local market, Bhubaneswar.

### **3.0.4 Bio-agents and Nematicides:**

Talc formulation of *Paecilomyces lilacinus* (cfu  $2 \times 10^6$ /g) and *Pseudomonas fluorescens* (cfu  $2 \times 10^6$ /g) ( collected from Officer in charge, AICRP on Nematodes, Bhubaneswar centre), neem cake and Nematicide Carbofuran 3G

## **3.1 Experimentation:**

### **3.1.1. Culturing of nematodes:**

From the tomato root, a single egg mass of *Meloidogyne incognita* was collected and the populations was multiplied on a susceptible tomato variety (Bt-10) that are grown in pots containing sterilized soil. This was done two months before the start of the experiment.

### **3.1.2. Incubation of bioagent:**

The fungal (*Paecilomyces lilacinus*) and bacterial (*Pseudomonas fluorescens*) bioagents which are received from AICRP was incubated in well decomposed FYM @ 1:1000 ratio. For this 1g of talc formulation of bioagent was mixed with 1kg moist FYM. After mixing thoroughly it was kept in one gunny bag and stored as such in shade away from direct sunlight. After 7 days, again a little water was sprinkled, mixed thoroughly and kept as such for 15 days for its multiplication.

### **3.1.3. Collection and sterilization of soil:**

For experimental purpose well pulverized sandy loam soil free from plant debris and gravels was collected from the experimental plots of Central Farm, Odisha University of Agriculture and Technology, Bhubaneswar. The soil was mixed thoroughly with sand and FYM in the ratio of 2:1:1 which was filled in gunny bag and autoclaved at 1.1kg/cm<sup>2</sup> pressure for one hour daily for two consecutive days. The soil was then spread on a polythene sheet and mixed thoroughly.

#### **3.1.4. Preparation of pots:**

15 cm diameter earthen pots of 15 cm were cleaned with tap water, drained and surface sterilized with 1% formaldehyde solution and were air dried. Then the pots were filled with 1 kg aerated sterilized soil, were kept in net house and as per treatments and replication they were labelled properly. The preincubated bioagent (*P. lilacinus* and *P. fluorescens*), neem cake and Carbofuran were thoroughly mixed and they were added to pot soil as per the treatment details given below.

#### **Treatment details:**

T1-Soil application of neem cake @1ton/ha 15 days prior to sowing.

T2- Soil application of pre incubated *Psuedomonas fluorescens* @2.5kg/ha at the time of sowing.

T3- Soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing.

T4- Soil application of neem cake @1ton/ha 15 days prior to sowing +soil application of pre incubated *Psuedomonas fluorescens* @2.5kg/ha at the time of sowing.

T5- Soil application of neem cake @1ton/ha 15 days prior to sowing + soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing.

T6- Application of Carbofuran @ 1kg a.i. / ha at the time of sowing.

T7-Untreated control

#### **3.1.5. Application of neem cake:**

Neem cake as per requirement of T1, T4, and T5 was mixed to the pot soil 15 days prior to the sowing of seeds but the bioagents and the carbofuran were applied as per the requirement during sowing.

#### **3.1.6. Sowing of bittergourd seeds:**

In luke warm water the bittergourd seeds of Var. Green long were soaked for 24 hrs and were surface sterilized in 2.5% sodium hypochlorite solution for two minutes followed by washing in clean distilled water and then are dried in shade. Two to three seeds were sown in each pot, covered with soil and watered to keep the soil moist to facilitate germination. Seeds germinated within a 7-10 days. When they was at 3-4 leaved stage thinning was done and per pot one plant was maintained. Watering and weeding were continued as per requirement.

### **3.1.7. Inoculation of nematode**

Egg masses were collected from roots of cultured plants and were allowed to hatch in distilled water to give rise to second stage juveniles for inoculation. The J2 isolated from pure culture were surface sterilized by treating with aqueous solution of 0.1% Mercurochrome solution for a period of 30 mins in order to get rid of any bacterial or fungal contamination. In sterilized distilled water the juveniles were washed three times. By counting replicated aliquots of the suspension the juvenile population was estimated. A major volume of hatched juvenile suspension containing 1000 second stage juveniles of *M. incognita* was pipetted out and put around the root zone of ten days old bittergourd seedlings grown in pots by making small holes around the base of the plant. Holes were then filled with soil followed by light watering. Stacking was provided for crawling of vines with bamboo sticks in each pot .

### **3.1.8. Recording of observation of plant growth parameters:**

The pot soil was saturated with water after 45 days of sowing and made so soft, as to facilitate easy uprooting of plants. The plants were uprooted from the pot soil carefully. Roots were washed properly so that the soil are and adhering particles are removed under slow stream of water. Observations of effect of different treatments on different plant growth parameters (vine length, root length, vine weight and root weight) were recorded as follows.

#### **Vine length (cm):**

The length of the vine from the base of the shoot to the top-most point of the plant was measured with a meter scale.

#### **Vine weight (g):**

Fresh weight of vine of individual plant was recorded on an electronic weighing balance in the laboratory after cutting them from the base of the plant, separating the roots and weighing the entire vines and leaves.

#### **Root length (cm):**

After separating the root portion from the shoot portion with blade, the root length was measured with the help of a meter scale from the base of the plant up to the end of the main root.

**Root weight (g):**

The whole root system was washed properly to remove the soil and adhering inert material. After washing, the excess water from the root surface was removed by placing them between the blotting papers carefully, air dried and measured in balance to record the fresh root weight.

**3.1.9. Recording of observation of nematode infection parameters:**

Nematode infection parameters like as number of galls per plant and number of egg masses was recorded. Further, nematode population in a pot soil as well as plant root was estimated as follows.

**Root gall index**

After uprooting the plants from the pot soil, the roots were washed properly with water to remove the soil and galls formed by the nematode as well as the egg masses were counted with the help of hand tally counter and rated as per (1-5) scale as follows (as suggested by Johnson and Campbell, 1980).

**Table 1. Root knot index**

| No of galls   | Rating |
|---------------|--------|
| No galls      | 1      |
| 1-10          | 2      |
| 11-30         | 3      |
| 31-100        | 4      |
| More than 100 | 5      |

**Number of egg masses per root system**

After uprooting the plants, roots were separated from plants and egg masses were counted per root system with the help of a lens.

**Estimation of Root knot nematode population in pot soil:**

Soil samples of 200cc from each pot were collected after drying and mixing the entire soil mass thoroughly over a polythene sheet and processed by Cobb's sieving, decanting and modified Baermann's funnel technique (Cobb 1918, Schnider 1961). After screening the nematode suspension collected from different mesh sieves was

compounded and poured over a double layered tissue paper supported by aluminium wire guage which is mounted on a petridish (10 cm in diameter) having sufficient water to completely touch the tissue paper placed over it. The whole system was covered with a lid and was kept undisturbed for 24 hrs, so that the nematodes move from tissue paper into the water on the petridish and it was collected in another beaker. After 24 hr, the nematode suspension was examined under a binocular stereoscopic microscope and nematodes were counted.

#### **Estimation of Root knot nematode population in root:**

For estimating the root knot nematode population in root, 2g root sample from each treatment and replication were separated out. Then the samples were stained by lactophenol-acid fuchsin and cleared in pure lactophenol following the standard procedure. After staining the root samples are arranged on glass slides and tightly pressed by other glass slides. The glass slides were examined under stereoscopic microscope to record the population of root knot nematode in 2g roots. From that the entire population of root knot nematode for each pot was worked out.

#### **Estimation of reproductive rate (Rf)**

Reproductive factor (Rf) was determined from the ratio of final nematode population level to initial nematode population level by using the formula:

$$Rf. = Pf/Pi$$

Where

Rf= Rate of nematode multiplication

Pf=final nematode total population

Pi = Initial inoculated nematode population

### **3.2. Statistical analysis and interpretation of data :**

Statistical analysis of different plant growth parameters, nematode were carried out following Fisher's (1970) "F" test and analysis of variance in a complete randomized design. Further the comparison of the treatment was done by taking S.E (m) and L.S.D for different factors which were calculated to test the significance of means in the following manner.

S.E (m) for treatment =  $\sqrt{2EMS/R}$

L.S.D at 0.05= S.E (m)  $\pm$   $\times$  t (0.05) at error d.f., where,

d.f. =degree of freedom

R = replication

EMS= Error means sum of square

S.E (m) = Standard error mean

L.S.D (0.05) = Least significant difference at 5% level.

The difference between two treatment means if greater than the LSD value indicated the significant difference between the treatments. In this way, comparison between the treatments was made.

## RESULTS

The pot experiment was conducted to test the efficacy of Eco-friendly management of root knot nematode infecting bittergourd in net house of Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar. The effect of treatments on plant growth parameters i.e., vine length, vine weight, root length, root weight, overall nematode population in soil as well as root and change in population growth of root knot nematode were recorded. The observation and salient results of the experiment are as follows.

### 4.1. Effect of treatments on plant growth parameters of bittergourd :

Application of bioagent *P. lilacinus*, *P. fluorescens*, neem cake and carbofuran to the pot soil influenced the growth parameters of bittergourd plant differently as per the treatments. Their effect on different plant growth parameters are discussed below.

#### 4.1.1. Vine length (Table 2):

The vine length of bittergourd plant sown in earthen pots with/ without application of bioagent *P. lilacinus*, *P. fluorescens*, neem cake and carbofuran varied between 54.25 to 93.87 cm. Highest vine length 93.87 cm was recorded in T<sub>4</sub> (*Pseudomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) and the lowest vine length was noticed in T<sub>7</sub> (untreated control pot). The variation of vine length in treated pots over untreated control were in the range of 9.8% (T<sub>3</sub>) to 73% (T<sub>4</sub>). Among the treatments, T<sub>4</sub> (*Pseudomonas fluorescens* @ 2.5Kg/ha+ Neem cake @ 1ton/ha) exhibited highest change of shoot length (73%) followed T<sub>5</sub> (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) that is 62.6% which are statistically superior over other treatments.

#### 4.1.2. Vine weight (Table 2):

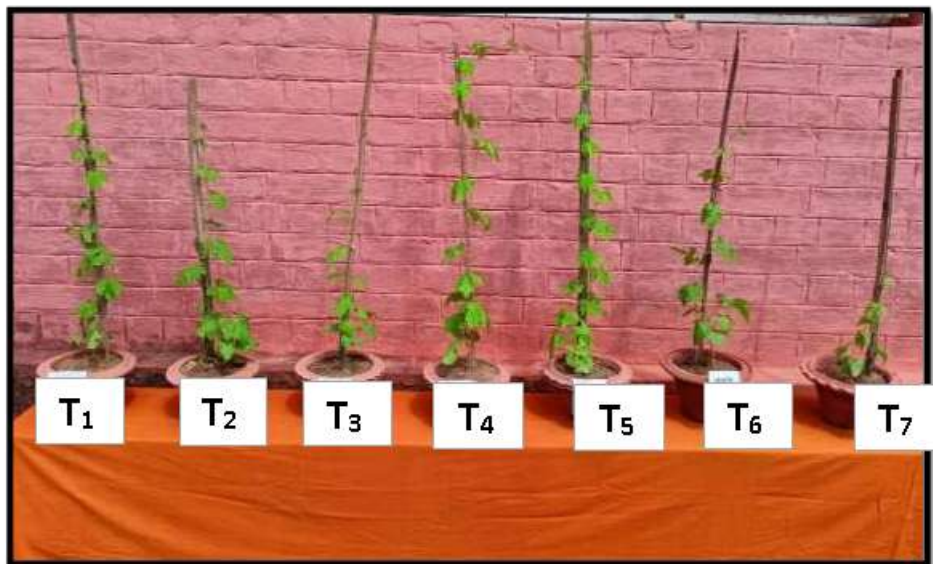
The vine weight of bittergourd plants in different treatments were in the range of 92.67g to 60.7g. Highest vine weight (92.67g) was in T<sub>4</sub> followed by (87.9g) in T<sub>5</sub> which are superior to other treatments. Among the treatments, (*Pseudomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) in T<sub>4</sub> (92.67g) exhibited maximum vine weight followed by (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) in T<sub>5</sub> (87.9g). They are statistically significant. The variation in different treatments over untreated control were between 4.9% to 52.6% highest being in T<sub>4</sub> (52.6%). The lowest variation in fresh vine weight (4.9%) was recorded in T<sub>3</sub> (*P.lilacinus*) only.

#### 4.1.3. Root length (Table 2):

The root length of bittergourd plant varies between 12.82cm to 21.30 cm. Highest root length 21.3cm was noticed in T<sub>4</sub> (*Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) and untreated control (T<sub>7</sub>) exhibited the lowest root length of 12.8cm. The treated pots exhibited the variation in root length in the range of 12% (T<sub>3</sub>) to 66.1% (T<sub>6</sub>). Among the treatments maximum root length of 21.3cm was observed in T<sub>4</sub> (*Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) followed by T<sub>5</sub> (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) i.e., 19.12cm. Therefore T<sub>4</sub> and T<sub>5</sub> are statistically superior and significantly different than other treatments.

#### 4.1.4. Root weight (Table 2):

The bittergourd plants in all the treated pots exhibited enhanced fresh root weight over untreated control. The root weight varied between 8.02g to 14.52g. Highest root weight was observed in T<sub>4</sub> (14.52g) followed by T<sub>5</sub> (13.2g) which are statistically significant. The highest change in root weight over control was observed in T<sub>4</sub> (81%) (*Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) followed by T<sub>5</sub> (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) that is 61.5%.



**Fig 1. Effect of treatments on plant growth parametres.**

**Table 2. Effect of neem cake, bioagents and carbofuran on growth of bitter gourd infected with root- knot nematode, *Meloidogyne incognita*.**

| Treatments     | Vine length (cm) | Increase over check (%) | Vine weight (g) | Increase over check (%) | Root length (cm) | Increase over check (%) | Root weight (g) | Increase over check (%) |
|----------------|------------------|-------------------------|-----------------|-------------------------|------------------|-------------------------|-----------------|-------------------------|
| T <sub>1</sub> | 80.32            | 48.0                    | 80.22           | 32.1                    | 17.40            | 35.7                    | 12.40           | 54.6                    |
| T <sub>2</sub> | 64.65            | 19.1                    | 66.37           | 9.3                     | 15.32            | 19.5                    | 10.50           | 30.9                    |
| T <sub>3</sub> | 59.60            | 9.8                     | 63.71           | 4.9                     | 14.37            | 12.0                    | 8.62            | 7.4                     |
| T <sub>4</sub> | 93.87            | 73.0                    | 92.67           | 52.6                    | 21.30            | 66.1                    | 14.52           | 81.0                    |
| T <sub>5</sub> | 88.22            | 62.6                    | 87.90           | 44.8                    | 19.12            | 49.1                    | 13.20           | 61.5                    |
| T <sub>6</sub> | 70.97            | 30.8                    | 70.85           | 16.7                    | 16.50            | 28.7                    | 10.32           | 28.6                    |
| T <sub>7</sub> | 54.25            |                         | 60.70           |                         | 12.82            |                         | 8.02            | 54.6                    |
| SE(m) ±        | 1.17             |                         | 2.11            |                         | 0.26             |                         | 0.55            |                         |
| C.D. (0.05%)   | 3.48             |                         | 6.26            |                         | 0.77             |                         | 1.64            |                         |

T1-Soil application of neem cake @1ton/ha 15 days prior to sowing.

T2- Soil application of pre incubated *Pseudomonas fluorescens* @ 2.5kg/ha at the time of sowing.

T3- Soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing.

T4- Soil application of neem cake @1ton/ha 15 days prior to sowing +soil application of pre incubated *Pseudomonas fluorescens* @2.5kg/ha at the time of sowing.

T5- Soil application of neem cake @1ton/ha 15 days prior to sowing + soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing.

T6- Application of Carbofuran @ 1kg a.i./ha at the time of sowing.

T7-Untreated control



**Fig 2. A view of bittergourd plants grown in replicated pots.**

#### **4.2. Effect of treatments on root knot nematode infection parameters:**

The effect of the bioagent *P. lilacinus*, *P.fluorescens*, neem cake alone and combined along with a chemical check with carboguran was recorded on infection parameters of root knot nematode in terms of number of galls produced in the root system of bittergourd plants, number of egg masses per root system, nematode population (J2) in pot soil, nematode population in plant roots and the reproduction factor for multiplication of root knot nematode which has been given below.

##### **4.2.1. Number of galls per plant (Table 3):**

The gall formation by the root knot nematode was observed to be different in different treatments. The range of number of galls formed varies between 9.00 to 105.00. The highest number of galls was observed in T7 i.e. untreated control plot without any bioagent or chemical treatment (105.00 ) and the lowest number of galls(9.00) observed in T4 (*Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil). The second best treatment was observed to be T5 (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) with number of galls being 27.75.

**Table 3. Effect of neem cake, bioagents and carbofuran on infection parameter of *Meloidogyne incognita* in bitter gourd**

| Treatments         | No of galls formed | % decrease over control | No of egg mass/ plant | % decrease over control | Root knot Index |
|--------------------|--------------------|-------------------------|-----------------------|-------------------------|-----------------|
| <b>T1</b>          | 34                 | 67.6                    | 31                    | 69.7                    | 4               |
| <b>T2</b>          | 58.50              | 44.2                    | 56                    | 45.3                    | 4               |
| <b>T3</b>          | 74.25              | 29.2                    | 71.75                 | 30                      | 4               |
| <b>T4</b>          | 9.00               | 91.4                    | 6.75                  | 93.4                    | 2               |
| <b>T5</b>          | 27.75              | 73.5                    | 24.75                 | 75.8                    | 3               |
| <b>T6</b>          | 42.50              | 59.5                    | 40.25                 | 60.7                    | 4               |
| <b>T7</b>          | 105                | 0                       | 102.50                | 0                       | 5               |
| <b>S.E.(m)±</b>    | 0.96               |                         | 0.98                  |                         |                 |
| <b>C.D.(0.05%)</b> | 2.84               |                         | 2.92                  |                         |                 |

T1-Soil application of neem cake @ 1ton/ha 15 days prior to sowing.

T2- Soil application of pre incubated *Psuedomonas fluorescens* @ 2.5kg/ha at the time of sowing.

T3- Soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing.

T4- Soil application of neem cake @ 1ton/ha 15 days prior to sowing +soil application of pre incubated *Psuedomonas fluorescens* @2.5kg/ha at the time of sowing.

T5- Soil application of neem cake @ 1ton/ha 15 days prior to sowing + soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing.

T6- Application of Carbofuran @ 1kg a.i./ha at the time of sowing.

T7-Untreated control

#### **4.2.2. Number of egg mass/ plant (Table 3):**

The number of egg mass per plant observed in the range of 6.75 to 102.50. The lowest number of egg mass per plant were observed in T4 with application of *Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil i.e. 6.75. T5 (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) produced 24.75 number of egg masses per plant which is also low in number. The number of egg masses in control plot (T7) found to be 102.50.

#### **4.2.3. Root knot index (Table 3)**

The root knot index was measured and it was found that T4 (application of *Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) recorded the lowest RKI (2.0) followed by T5 (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) with RKI of 3.0. All other treatments recorded RKI of 4.0 in the scale 1-5 as suggested by Johnson and Campbell, 1980 except T7 (untreated control) which was found to be having RKI of 5.0.

#### **4.2.4. Root knot nematode population in pot soil (Table 4):**

The population growth of *Meloidogyne incognita* was found to be influenced by different treatments including untreated control. Root knot nematode population was in the range of 185.5 to 239.25 J2/200cc soil. Lowest population in soil (185.5 J2/200cc soil) was recorded in T4 with application of *Psuedomonas fluorescens* @ 2.5Kg/ha of soil+ Neem cake @ 1ton/ha of soil. T5 (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) exhibited moderate control of nematode population recording soil population of 195.0 J2 / 200 cc of soil. Highest population was observed in untreated control pot (239.25 J2/200cc soil) at 30 days after inoculation. The nematode population of rest other treatments varied from 206.25 to 222.75 J2/200cc of soil.

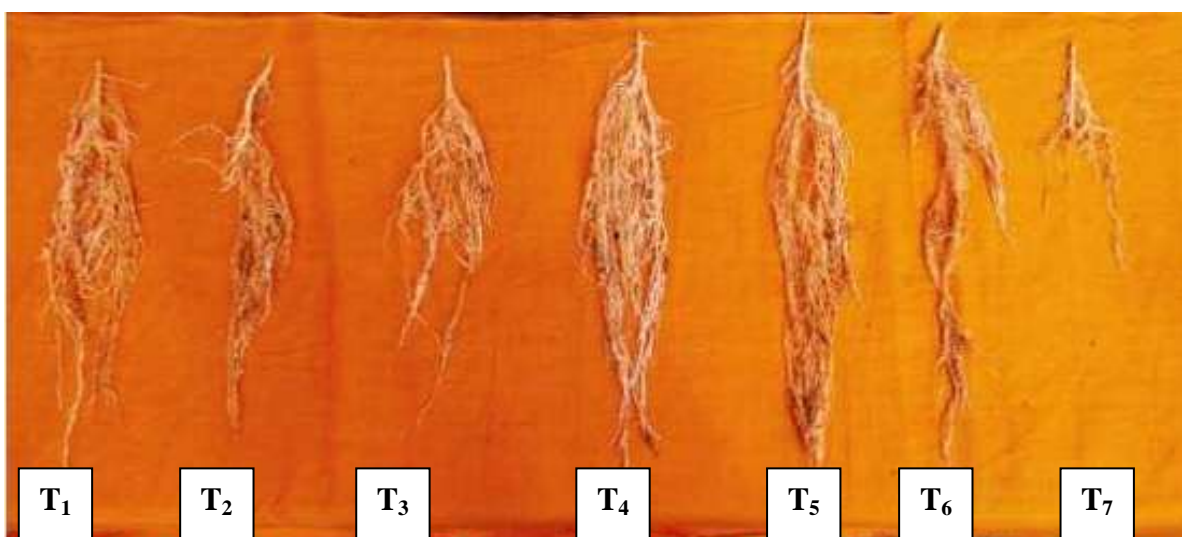
#### **4.2.5. Nematode population in plant root (Table 4):**

The population of root knot nematode *Meloidogyne incognita* in roots was also found to be influenced by different treatments. Lowest root population was recorded in T4 i.e. 18.25 per 2 gm root followed by T5 (22.50) and T1 (24.50). The roots of untreated control pot exhibited the highest population (47.75) of root knot nematode of

different stages. Maximum per cent decrease over control was noticed in T<sub>4</sub> (*Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil ) i.e. 61.7 followed by T<sub>5</sub> (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) with 52.8. Overall total population of root knot nematode both in pot soil and plant root was maximum (1387.72) in control pot (T<sub>7</sub>). Minimum population was recorded in T<sub>4</sub> (1059.99) with application of *Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil. Maximum decrease in total nematode population over control was seen in T<sub>4</sub> (*Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) i.e. 23.6.

#### 4.2.6. Reproduction factor of root knot nematode (Rf) (Table 4):

The reproduction factor was found to be least in T<sub>4</sub> (*Psuedomonas fluorescens* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) i.e. 1.05 followed by T<sub>5</sub> (*Paecilomyces lilacinus* @ 2.5Kg/ha of soil + Neem cake @ 1ton/ha of soil) i.e. 1.12 . The reproduction factor for untreated control was recorded to be highest i.e. 1.38.



**Fig 3. Photograph showcasing roots of bitter gourd plants in different treatments.**

**Table 4. Effect of neem cake, bioagents and carbofuran on growth and multiplication of *Meloidogyne incognita* in bitter gourd.**

| Treatments   | Final nematode(J2)/200 cc of soil | % decrease over control | Nematode population/2gm root | % decrease over control | Total nem. Population/pot | % decrease over control | Rf (Pf/Pi) |
|--------------|-----------------------------------|-------------------------|------------------------------|-------------------------|---------------------------|-------------------------|------------|
| T1           | 206.25                            | 13.7                    | 24.50                        | 48.6                    | 1183.15                   | 14.7                    | 1.18       |
| T2           | 218.00                            | 8.8                     | 33.75                        | 29.3                    | 1267.18                   | 8.6                     | 1.26       |
| T3           | 222.75                            | 6.8                     | 42.25                        | 11.5                    | 1295.84                   | 6.6                     | 1.29       |
| T4           | 185.50                            | 22.4                    | 18.25                        | 61.7                    | 1059.99                   | 23.6                    | 1.05       |
| T5           | 195.00                            | 18.4                    | 22.50                        | 52.8                    | 1123.50                   | 19.0                    | 1.12       |
| T6           | 212.50                            | 11.1                    | 35.50                        | 25.6                    | 1243.90                   | 10.3                    | 1.24       |
| T7           | 239.25                            |                         | 47.75                        |                         | 1387.72                   |                         | 1.38       |
| S.E.(m)±     | 1.77                              |                         | 0.77                         |                         |                           |                         |            |
| C.D. (0.05%) | 5.25                              |                         | 2.28                         |                         |                           |                         |            |

**\*Initial nematode population (J2)/pot is 1000**

T1-Soil application of neem cake @1ton/ha 15 days prior to sowing.

T2- Soil application of pre incubated *Psuedomonas fluorescens* @ 2.5kg/ha at the time of sowing.

T3- Soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing.

T4- Soil application of neem cake @1ton/ha 15 days prior to sowing +soil application of pre incubated *Psuedomonas fluorescens* @2.5kg/ha at the time of sowing.

T5- Soil application of neem cake @1ton/ha 15 days prior to sowing + soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing.

T6- Application of Carbofuran @ 1kg a.i./ha at the time of sowing.

T7-Untreated control

## DISCUSSION

Bittergourd is considered to be one of the most important vegetable crop that is cultivated throughout the country. Its yield seems to be affected by several pests and diseases including phytonematodes. Root knot nematode *Meloidogyne incognita* is seen to be a major plant parasitic nematode affecting almost all cucurbitaceous crops including bittergourd. Different scientists and research scholars have suggested various management modules to keep this hidden enemy under control. But majority of them are based on the application of synthetic nematicides which are not only costly, but also having harmful effect on human health and environment. Besides, most of them are either banned for application in vegetables or they are phased out of local market day by day. So the development and popularization of alternative ecofriendly approach to protect the crops from nematode infestation is considered to be of utmost need of the hour. The application of bioagents like *P.fluorescens* and *P. lilacinus*, organic amendments with different oil cakes are considered as ecofriendly and also low cost sustainable approach of nematode management. With this backdrop, the present study was undertaken to assess the efficacy of eco-friendly management of modules comprising of above inputs for management of root knot nematode, *Meloidogyne incognita* infecting bittergourd with the following objectives:

1. To study the effect of eco-friendly management practices on plant growth character of bittergourd infected by *Meloidogyne incognita*
2. To study the effect of eco-friendly management practices on growth and multiplication of *Meloidogyne incognita* infecting bittergourd

The experiment was conducted in 15cm diameter earthen pots in the year 2020-21 in the net house of Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar. Seven treatments were replicated four times with bioagents i.e. *P. lilacinus*, *P.fluorescens*, neem cake either alone and in combination along with carbofuran as chemical check and untreated control under Complete Randomised Design (CRD). The effect of the treatments on different plant growth parameters of bittergourd, nematode infection parameters and root knot

nematode population both in soil and plant root was noticed 30 days after inoculation of second stage juveniles of root knot nematode.

Application of neem cake 15 days prior to sowing, pre-incubated bioagent *P.lilacinus* and *P.fluorescens*, as well as chemical nematicide carbofuran into pot soil during the time of sowing of seeds enhanced the plant growth parameters of bittergourd substantially over untreated control. Maximum vine length (93.87cm), vine weight (92.67g), Root length (21.3cm) and root weight (14.52g) were recorded with combined soil application of neem cake @1ton/ha 15 days prior to sowing and pre incubated *Psuedomonas fluorescens* @2.5Kg/ha at the time of sowing . The combined soil application of neem cake @1ton/ha 15 days prior to sowing and bioagent, *Paecilomyces lilacinus* @2.5kg/ ha at the of sowing also indicated better plant growth in terms enhanced of vine length (88.2cm), vine weight (87.9g), root weight (13.2g), and Root length (19.12cm). The vine length and vine weight of plants in pots treated with bioagent and neem cake are in the increasing order than that from bioagents and neem cake treated alone. The result revealed that the plant growth parameters are mostly positively influenced with application of neem cake, bioagents like *P. lilacinum*, *P.fluorescens* , either alone (T1, T2 and T3) or in combination with neem cake (T4, T5) and carbofuran (T6). Khan *et al.* in 2004 observed that there was significant increase in plant growth parameters when *Purpureocillium lilacinum* was applied at 0.5, 1.0, 2.0, 4.0, 5.0, 6.0, 7.0 and 8.0 g per pot. Similarly Mittal and Prasad in 2003 suggested that application of bioagents enhanced the plant growth parameters like shoot growth, root growth etc in soyabean. Nama and Sharma in 2007 found that *Trichoderma hazianum*, *T. viridae* and *Pseudomonas fluorescens* when applied at 10g / kg seed resulted maximum shoot length, root length, shoot weight and root weight in cowpea. Another experiment was conducted by Das and Borgohain revealed that *P.fluorescens* and *P.lilacinum* were most effective in enhancing the plant growth parameters in tomato.

Nematode populations in all treatments were found to be influenced 40 days after sowing of bittergourd seeds. All treatments except untreated control reduced the population of *Meloidogyne incognita*. The percentage of reduction of root knot nematode population in 200g/cc of soil was within the range of 6.8% to 22.4% in. Maximum reduction (22.4%) was observed in T4 followed by T5 (18.4%). *P.fluorescens* produces secondary metabolite 2,4-diacetylphloroglucinol which have antiphytopathogenic and biocontrol proprieties that helps to protect the roots of some plant species.(Bangera andThomashow, 1999) Similarly different enzymes like serine

proteases, collagenases, and chitinases secreted by egg parasitic fungal bioagent *Purpureocillium lilacinum* must have break down the physical and physiological integrity of nematode cuticle and egg cells facilitating penetration and colonization of both fungal antagonists leading to better control of nematodes as reported by Li *et al.*, 2015 and Kerry., 2000. The root knot index is found to be 2 in case of T4 followed by 3 in case of T5 (soil application of neem cake @1ton/ha 15 days prior to sowing and bioagent *Paecilomyces lilacinus* @ 2.5kg/ ha at the of sowing) All other treatment has been reported to be 3 in the scale except control which shows an index of 4. So T4 has been seen as significant treatment in terms of root knot index. The reproduction factor was found to be least in T4 (combined soil application of neem cake @1ton/ha 15 days prior to sowing and pre incubated *Psuedomonas fluorescens* @2.5Kg/ha at the time of sowing) i.e 1.05 followed by T5 (combined soil application of neem cake @1ton/ha 15 days prior to sowing and bioagent *Paecilomyces lilacinus* @ 2.5kg/ ha at the of sowing) with 1.12 Rf. It is 1.29 in case of T3 (Neem cake alone) followed by T6 (Carbofuran) i.e. 1.24. The reproduction factor for untreated control was observed to be 1.29. Bhattacharya and Goswami in 1987 observed that combination of bioagents like *P. fluorescens*, *P. lilacinum* and *Basilus pumilis* reduced the *M. incognita* in tomato. Ali *et al.* 2002 reported that soil drenching with *P. fluorescens* significantly lowered the population of *Meloidogyne* spp. subsequently reduced disease severity in mungbean. Similarly Sharma *et al.* In 2007 suggested that *P. lilacinum* reduced the nematode population by 77%. Another experiment was conducted by Kumar *et al.* in 2012, recommended that *P. fluorescens* at 20g /kg of seeds effectively lowered the population density of nematode in soil.

Further the number of galls formed ranged between 9.00 to 105.00. Maximum galls were formed in untreated control i.e. 105.00 and minimum galls were formed in T4 i.e. 9.00. Similarly number of egg mass per plant were found to be minimum in T4 (6.75) followed by T5(24.75) which are 93.4% and 75.8% less as compared to untreated control plots Majzoob *et al.* in 2012 observed that *P. fluorescens* brought about 55.72% reduction in gall number and 82.84% reduction in reproduction factor in *Meloidogyne* spp. Rao *et al.* in 2000 suggested that *Trichoderma* spp. in combination with neem cake was able to completely parasitized the egg mass and ultimately suppressed the galling. Ameer-Zareen in 2001 suggested that *P. lilacinum* suppressed the gall formation, egg mass production in soil and roots as compared to control. Similarly Sharma *et al.* In 2007 observed that *P. lilacinum* in combination with neem cake lowered the number of galls and number of eggs per egg mass by 32%. Hence the present findings derive ample support from the findings of previous authors.

## SUMMARY AND CONCLUSION

Bittergourd is a famous vegetable crop which have high market value as well as have disease preventing and health promoting phyto chemical compounds. Pest and diseases including phyto parasitic nematodes, creates hinderance in production of it. *Meloidogyne incognita* is the most devastating among them. A number of management practices are being adopted by farmers to control this dreaded pest. Different oil cakes like neem cake and various bioagents like *P. fluorescens*, *P. lilacinum* are widely used for management of plant parasitic nematode in various crops as sustainable and ecofriendly approach. In the present study a pot culture experiment was conducted to assess the efficacy of eco-friendly management modules of root knot nematode, *Meloidogyne incognita* infecting bittergourd with the following objectives :

1. To study the effect of eco-friendly management practices on plant growth character of bittergourd infected by *Meloidogyne incognita*
2. To study the effect of eco-friendly management practices on growth and multiplication of *Meloidogyne incognita* infecting bittergourd.

The experiment was conducted with seven treatments and four replications each following Complete Randomized Design. The treatments were- T1-Soil application of neem cake @1ton/ha 15 days prior to sowing, T2- Soil application of pre incubated *Psuedomonas fluorescens* @2.5kg/ha at the time of sowing, T3- Soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing, T4- Soil application of neem cake @1ton/ha 15 days prior to sowing +soil application of pre incubated *Psuedomonas fluorescens* @2.5kg/ha at the time of sowing, T5- Soil application of neem cake @1ton/ha 15 days prior to sowing + soil application of pre incubated *Paecilomyces lilacinus* @ 2.5 kg/ha at the time of sowing., T6- Application of Carbofuran @ 1kg a.i./ha at the time of sowing, T7-Untreated control. Well pulverized sandy loam soil free from plant debris and gravels was collected from the experimental plots of Central Farm, Odisha University of Agriculture and Technology, Bhubaneswar. Firstly, from the tomato root, a single egg mass of *Meloidogyne incognita* was collected and the populations was multiplied on a susceptible tomato variety (Bt-10) grown in pots containing sterilized soil.

This was done two months before the start of the experiment. Then the soil was mixed thoroughly with sand and FYM in the ratio of 2:1:1 then was filled in gunny bag and autoclaved at 1.1kg/cm<sup>2</sup> pressure for one hour daily for two consecutive days. The soil was then spread on a polythene sheet and mixed thoroughly. Earthen pots of 15 cm diameter were cleaned with tap water, drained and surface sterilized in 1% formaldehyde solution and made air dry. Pots were then filled with 1 kg aerated sterilized soil, kept in net house and labelled as per treatments and replication wise properly. Neem cakes were applied 15 days prior to sowing according to treatments in T1, T4, and T5. The pre-incubated bioagent *P. fluorescens* and *P. lilacinus* were applied to the pot soil as per treatments during sowing. One chemical check with nematicide Carbofuran and untreated control pot were also maintained for comparison. Seeds of bittergourd cultivar green long were soaked overnight. Two to three seeds were sown per pot, covered with soil and watered regularly to keep the soil moist to facilitate germination. After germination and at 3-4 leaved stage, one plant per pot was maintained by thinning of plants. Freshly hatched J2 of *Meloidogyne incognita* isolated from pure culture were inoculated to the 10 days old bittergourd plants @ one J2/g soil following standard procedure. Watering and weeding were continued as per requirement. Stackings were given in each pot to support the vines.

The effect of treatments on growth parameters of bittergourd plant as well as on population buildup of *Meloidogyne incognita* was noted down. It was revealed that combined application of bacterial bioagent *Pseudomonas fluorescens* @ 2.5Kg / ha at the time of sowing along with neem cake application @ 1ton /ha 15days prior to sowing increased the shoot length, root length, shoot weight and root weight of bittergourd plants to the tune of 73%, 66.1%, 52.6%, and 81% respectively over untreated control. It also reduced the number of galls, number of egg masses, nematode population of pot soils, nematode population in root and total nematode population by 91.4%, 93.4%, 22.4%, 61.7% and 23.6%, respectively over untreated control. Simultaneously, with it's application the population growth of root knot nematode both in soil and plant root was considerably reduced to minimum with least scope for further nematode multiplication. Likewise, the treatment combination of fungal bioagent, *Paecilomyces lilacinus* @ 2.5Kg / ha of soil at the time of sowing + Neem cake @ 1 ton/ ha of soil 15 days prior to sowing increased the shoot length, root length, shoot weight and root weight of bittergourd plants to the tune of 62.6%, 49.1%, 44.8%, and

61.5% respectively over untreated control. It also reduced the number of galls, number of egg masses, nematode population of pot soils, nematode population in root and total nematode population by 73.5%, 75.8%, 18.4%, 52.8% and 19.0%, respectively over untreated control. Therefore the above two treatment combinations exhibiting better result in terms of nematode management with lower multiplication rate (1.05 and 1.12) along with enhanced plant growth parameters and are considered to be better treatments than the rest others in management of root knot nematode i.e. *Meloidogyne incognita*.

Thus it could be concluded from the observation of this experiment that the application of bacterial bioagent *Pseudomonas fluorescens* or fungal bioagent *Paecilomyces lilacinus* @2.5kg/ h during sowing of seeds along with neem cake @1 ton/h two weeks before sowing can effectively manage the root knot nematode, *Meloidogyne incognita* in bittergourd which ultimately will be resulting in higher yield along with sustainability and environment safety. Such a small scale research information could be a base for field trails for development of more tangible result in this direction.

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