MANAGEMENT OF ROOT-KNOT NEMATODE, *Meloidogyne incognita* (KOFOID AND WHITE) CHITWOOD IN VEGETABLE COWPEA

by

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**MANAGEMENT OF ROOT-KNOT NEMATODE, *Meloidogyne incognita* (KOFOID AND WHITE) CHITWOOD IN VEGETABLE COWPEA**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Vellayani

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CERTIFICATE

Certified that this thesis entitled "**MANAGEMENT OF ROOT-KNOT NEMATODE, *Meloidogyne incognita* (KOFOID AND WHITE) CHITWOOD IN VEGETABLE COWPEA**" is a record of research work done independently by Ms. Divya, T. S. (2018-11-102) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS USED

a.i	Active ingredient
et al.,	And co-workers
@	At the rate
Cm	Centimeter
CD	Critical difference
Cc	Cubic centimetre
Cv	Cultivar
°C	Degree Celsius
Fig.	Figure
G	Gram
Ha	Hectare
H	Hours
Kg	Kilogram
L	Litter
M	Meter
µg	Microgram
Mg	Milligram
mL	Millilitter
Mm	Millimeter
Min	Minutes
Viz	Namely
Sl	Serial
sp. or spp.	Species (singular and plural)
T	Treatment
w w^{-1}	Weight by weight

Introduction

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is an annual legume, originated in Africa and adapted to a wide range of soils. It is one of the most important protein rich food legumes in human diet. Cowpea is rich in amino acids (lysine and tryptophan) and valued as a nutritional supplement to cereals. Cowpea is grown over sixty countries and it is cultivated in an estimated area of 14.5 million hectare and has an annual production of 6.5 million metric tonnes worldwide (Fatokun *et al.*, 2012). Cowpea can be also used green or as dry fodder. In Kerala, cowpea can be grown throughout the year as pure crop or intercrop. It is one of the major vegetable in homestead garden. The rhizobacteria present in the cowpea root help to fix nitrogen there by improves soil fertility. Hence it is used in crop rotation with cereals. Cowpea can also be grown as a cover crop as it reduces soil erosion.

Various pests and diseases challenge cowpea cultivation to cause immense yield loss world wide. Plant parasitic nematodes are found as a cosmopolitan pest in cowpea cultivation. Caveness and Ogunfowra (1985) reported 55 species of plant parasitic nematodes from cowpea growing area. Among the several plant parasitic nematodes that infest cowpea, *Meloidogyne incognita* (Kofoid and White) Chitwood was the most damaging (Sarmah and Sinha, 1995; Sikora *et al.*, 2005).

Root knot nematode, *Meloidogyne* spp. has a very wide distribution and causes serious damage to crops particularly in vegetables. Root knot nematodes are obligate parasites which causes severe root damage. The primary symptom of root knot nematode infestation is root galling. It affects nutrient uptake ability of plants and finally yield loss. *M. incognita* race 2 could cause 28.62 per cent losses in cowpea (Reddy and Singh, 1981). Besides the direct damage caused by root knot nematode, they interact with other organisms such as fungi and bacteria and leads to disease complexes. The nematode *M. incognita* enhances the infection of *Fusarium oxysporum* in cowpea and causes wilt complex (Singh and Goswami, 2001). Root kont nematode – *Fusarium* wilt complex is common in cowpea growing areas of Kerala.

Management of pests and diseases through resistant varieties is the cheapest and most convenient method. Identifying nematode resistance/tolerance in cowpea varieties will be a greater step towards nematode management.

Use of organic amendments like neem cake reduces the nematode infestation and improves plant nutrition. Decomposition of organic amendments release toxic compounds which is antagonistic to nematodes. Use of biocontrol agents is a specific pest management strategy which is environmentally safe. Bacterial and fungal bioagents are available for nematode management and these provide long term protection.

Chemical pesticides are used for the quick reduction of pest population. Commonly available chemical nemticides *viz*, carbofuran and phorate (red labeled) have been banned in Kerala. Now no registered nematicides are available in Kerala. Fluopyram is a new green labeled fungicide with nematicidal property. Integration of organic amendments, bioagents and green labeled nematicide with a resistant or tolerant variety will provide a promising management practice for plant parasitic nematodes in cowpea.

In this context, the study entitled 'Management of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in vegetable cowpea' was undertaken with following objectives.

- To screen vegetable cowpea varieties for resistance towards *M. incognita*
- To evaluate the new nematicide fluopyram 400 SC for management of *M. incognita* in cowpea
- To evaluate efficacy of biocontrol agents, organic amendment and new nematicide fluopyram for the management of root-knot nematode in vegetable cowpea
- To determine the residue of fluopyram in cowpea pods at harvesting time

Review of Literature

2. REVIEW OF LITERATURE

The literature related to the present study 'Management of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in vegetable cowpea' are reviewed in this chapter.

2.1. PLANT PARASITIC NEMATODES AND CROP LOSS

Plant parasitic nematodes are ubiquitous and one of the major agriculture pathogens. The estimated annual yield loss in major crops was 12.3 per cent amount to $157 billion (Abad *et al.*, 2008). In India, plant parasitic nematodes causes 21.30 per cent crop losses annually in which the loss was higher in horticulture crops (20.03 per cent) than field crops (18.23 per cent). The estimated loss in spices, fruits, pulses, fiber crops, vegetables, cereals and oil seeds was 29.50, 25.5 23.0 19.75 19.6 18.8 11.8 per cent, respectively (Kumar *et al.,* 2020).

Nematodes feed all parts of the plant *viz.,* root, stem, leaves, flowers and seeds. Majority of them infect root system, there by plant's ability to absorb water and nutrients were reduced. Around 4,100 species of plant-parasitic nematodes were described all over the world (Decraemer and Hunt, 2006). The most important plant parasitic nematodes are root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Heterodera* and *Globodera* spp.), root lesion nematodes (*Pratylenchus* spp.), burrowing nematode (*Radopholus similis* (Cobb) Thorne), stem and bulb nematode (*Ditylenchus dipsaci* (Khun)), pine wilt nematode (*Bursaphelenchus xylophilus* (Steiner and Buher) Nickle), reniform nematode (*Rotylenchulus reniformis* Linford and Olivera), dagger nematode (*Xiphinema index* Thorne and Allen), false root-knot nematodes (*Nacobbus aberrans* (Thorne) Thorne and Allen) and rice white tip nematode (*Aphelenchoides besseyi* Christie) (Jones *et al.*, 2013). Among these different plant parasitic nematodes, *Meloidogyne* spp. caused major loss in global food production and it is estimated as 78 billion dollars annually (Lima *et al.,* 2017).

2.2. ROOT KNOT NEMATODES

Meloidogyne spp. are polyphagous and around 3000 plant species including monocotyledons, dicotyledons, herbaceous and woody plants (Hussey and Janssen, 2002). The genus, *Meloidogyne* consists of 98 species and the most important species are *M. incognita, M. javaniaca* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood and *M. hapla* Chitwood (Jones *et al.*, 2013). Out of 14 species reported from India, *M. incognita* and *M. javanica* found to be widely distributed and damaging wide range of crops (Khan *et al.*, 2014). Yield losses caused by root knot nematodes on solanaceous vegetables, cucurbitaceous vegetables and root crops were 8-42, 6-23 and 18.20 per cent respectively (Gowda *et al.*, 2017). Kumar *et al.* (2020) reported that in India, 75.38 per cent of total yield loss by plant parasitic nematodes was caused by root knot nematodes.

Infective juveniles (J_2) of root knot nematode penetrate into the plant roots and induce formation of giant cells in stelar region by hypertrophy and hyperplasia. These giant cells act as nutrient sinks for nematode development (Berg *et al.*, 2009). This infection in the stelar region reduce water absorption and plants show yellowing, stunting, wilting and reduced yield (Perry *et al.*, 2009). Besides the direct damage, many bacterial and fungal pathogens are predisposed by root knot nematodes into plants that lead to disease complexes. In India, disease complexes by root knot nematodes caused estimated yield loss of 40-70 per cent (Gowda *et al.*, 2017).

Pulses grown worldwide are attacked by a wide range of plant parasitic nematodes, majorly root knot and reniform nematodes (Haider *et al.*, 2003). Bridge *et al.* (2005) found high reproduction rate of *M. incognita* on cowpea growing areas of Ghana. In the humid south-western part of Burkina Faso, 90 per cent cowpea fields were infested with root knot nematodes (Sawadogo *et al.*, 2009). In India, the estimated yield loss of 23 per cent in pulses was caused by root knot nematodes (Kumar *et al.*, 2020).

Integrated nematode management practices include use of resistant varieties, organic amendments (cultural methods), fungal and bacterial biocontrol agents (biological control), chemical nematicides (chemical methods) etc.

2.3. VARIETAL RESISTANCE

Cultivation of resistant variety is economically as well as ecologically viable means for controlling nematode infestation.

2.3.1. Pulses

2.3.1.1. Cowpea

Choudhury *et al.* (2005) screened 149 varieties of cowpea for their response against *M. incognita*. They recorded 19 resistant, 42 moderately resistant, 61 susceptible and 27 highly susceptible varieties to *M. incognita*. The resistant varieties were EC-955-B, EC-390213, EC-241049, EC-45771-A, IC-253181, IC-249591, IC253268, IC-257424, IC-253271, IC-259063, IC-259588, IC-259095, V-38 (547-2), NIC-15305, NIC-15304, NIC-15322, NIC-15321, NIC-15318 and L/B-46 with root knot index 1.1 – 2.0. Adegbite *et al.* (2006) conducted a field experiment to study the reaction of 15 cowpea cultivars against *M. incognita*. The cultivar IT84S-2246-4 reported as most resistant with root knot index 1.5 and reproduction factor 0.45. Five cultivars were tolerant and nine cultivars were susceptible to *M. incognita*.

Olowe (2007) screened 70 cowpea genotypes for their reaction to *M. incognta* and found five genotypes *viz.*, Vita 3, 82D4532CIT'85, Acc 64298 (cv. New Era), TVX2724-01F and IT89KD-288 as resistant. The reproduction factor in these genotypes ranged from 0.5 – 0.8 and gall index was 2. Three cultivars of cowpea were evaluated for tolerance to *M. incognita* by Claudius-Cole *et al.* (2010) who reported that the cultivar IT-97K-497-2 had the highest yield with more tolerance. The cultivar IT-85D-2865 was most susceptible with the lowest yield. Ononuju and Nzenwa (2011) screened six cowpea cultivars for their resistance to *M. incognita*. They reported that cultivars IT89KD485, IT89KD391 and Sokoto local were resistant with gall index 1.33, 1.66 and 1.33, respectively. Reproduction factor of nematode in these cultivars was less than 0.8.

Kumar *et al.* (2012) evaluated 100 genotypes of cowpea against *M. incognita* race-1. The genotypes GAU-1, VKP605 and EC-244372 showed resistance and 26 genotypes *viz.*, C-791, C-731/01, EC-109793/03-25, V-585, DCP-7, DCS-6, CALC-

21, V-240, GC-9040, DCP-5, CPD-45, C-1163, C-1161, DCP-11, EC-24431, HC03-4, C-1259, C-1255, C-1352, C-1291, EC-244137, IC-20683/P3, EC-244241, EC-240992, EC-244385 and IC-20504-2 showed moderate resistance to *M. incognita*.

Ten cowpea varieties were evaluated for their reaction towards *M. incognita* by Adomako *et al.* (2013). The varieties Asontem and Asetenapa recorded as resistant with gall index 2.7 and 2.0 respectively. The varieties Adom and Vidza were found tolerant to *M. incognita* infection. Kankam *et al.* (2019) screened ten genotypes of cowpea for resistance to *M. incognita*. They recorded the cowpea genotypes SARI 1-4-90, Padi tuya, Songotra, IT99K-1122, Sanzi and Apagbaala as moderately resistant and IT86D-610, Zaayura, SARI 5-5-5 and IT07K-299-6 were slightly resistant.

2.3.1.2. Chickpea

Bhagwat and Sharma (2000) screened twenty one genotypes for their resistance to *M. incognita*. ICCL 86102 showed tolerance to *M. incognita* infection. Charu and Trivedi (2000) screened forty seven chickpea accessions for their reaction to *M. incognita* under pot culture condition. The variety RSG 617 was found least susceptible to *M. incognita* with lowest number of galls, number of eggs and nematode population. The most susceptible variety was RSG 564.

Hussain *et al.* (2001) screened ten chickpea lines *viz.*, 90122, 93127, 91A001, 91A039, Nes 950193, Nes 950174, Nes 950012, Nes 95004, Nes 96003 and Nes 96002 and reported that all these varieties were moderately resistant to *M. incognita* with reproduction factor 1.2-1.88. Chakrabarti and Mishra (2002) screened ten cultivars of chickpea against *M. incognita* and reported that cultivar BG 1067 as tolerant cultivar with 56 galls plant^{-1}. Ansari *et al.* (2004) evaluated seven chickpea genotype for their resistance against *M. javanica*. They found ICC 11152, ICC 8932, ICCC 42 and ICCV 90043 as tolerant genotypes to *M. javanica*.

2.3.1.3. Soybean

Adegbite (2007) screened 34 varieties of soybean for their resistance against *M. incognita* infestation. GM 344, TGM 1784 and TGX 1448-2E were found resistant

with gall index 1.9, 1.6 and 1.6 respectively. The reproduction factors of *M. incognita* in these varieties were recorded as 0.54, 0.56 and 0.54 respectively. Ten soybean varieties showed tolerance to *M. incognita.*

2.3.1.4. Pigeon Pea

Sharma *et al.* (1994) screened 34 pigeon pea cultivars and 227 germplasm accessions for their response to *M. javanica.* Pant A3, BDN 2, and ANM 504 were found highly resistant with gall index 1. ICP 99 and ICP 24 were moderately resistant. Ten pigeon pea accessions were screened for their resistance to *M. incognita* under field conditions. None of the pigeon pea accessions was resistant. Two accessions *viz.* Cc 10B and Cc 12 showed tolerance to *M. incognita* infestation with gall index 1.7 and 1.9 respectively (Adegbite *et al.*, 2011). Khan *et al.* (2011) evaluated 47 cultivars of pigeon pea for their reaction against *M. incognita.* The cultivars SKNP 0217, JKE 114, PT 05-36 and WBP 216 showed resistance to *M. incognita* with reproduction factor 0.93, 0.98, 0.55 and 1.00 respectively. All varieties recorded gall index 1.

2.3.1.5. Blackgram

Bhagawati *et al.* (2018) screened 125 blackgram germplasms against *M. incognita* and reported that PDU 3, IPU 99-18, PLU 557, KU-1106 and UH-07-06 were moderately resistant to *M. incognita.*

2.3.1.6. Greengram

Chandraguru and Rajarajan (1990) screened 20 greengram cultivars against *M. incognita* and found NPRC-3, Pusa-102, Pusa-104 and Pusa-103 as resistant.

Pandey and Nayak (2016) screened 38 greengram cultivars for their resistance to *M. incognita* and found 34 cultivars as resistant. The resistant cultivars were 15 IPM 2K 15-4, 1 AKM 12-02, 9 GM 11-02, 3 AKM 8802, 14 IPM 2-14, 14 IPM 2-14, 6 DGG 6, 11 HUM-27, 30 PUSA 1371, 35 SGC 20, 28 PM 10-12, 22 MH-934, 33 RMG-1078, 10 HUM-1, 21 MH-810, 32 PUSA 1472, 27 PM 09-11, 19 KM-2342, 12 IGKM 05-26-3, 24 ML-233, 34 RMG-1030, 13 IPM-2-3, 16 IPM 410-3, 7 GGG 10-

14, 2 AKM-4, 17 IPM 9901-6, 8GM 04-02, 26 NVL-641, 23 ML-2056, 37 TMB-45, 31 PUSA-1471, 4 DGG-3, 37 TMB-45, 20 MH-175, 36 TARM-1and 18 IPM 9901-8.

2.3.1.7. Field Pea

Sharma *et al.* (2006) screened 23 selections of field pea for their reaction towards *M. incognita* under pot culture condition. They reported HFP-0129, HFP-990713 and Pant P-25 as resistant to *M. incognita* with root knot index 1.00, 0.25 and 0.25. The selections NDP-2 Pant, P-42 and P-2005 showed tolerance to *M. incognita.*

Simon and Dass (2010) screened 141 chickpea, 55 field pea, 141 lentil and 70 pigeon pea varieties for their resistance against *M. incognita.* Out of 141 chickpea varieties, 8 showed moderate resistance to *M. incognita.* One among 55 filed pea varieties and 9 among 141 lentil varieties showed resistance towards *M. incognita* with gall index 2. Out of 70 varieties of pigeon pea, 44 varieties showed high resistance with gall index 1 and 11 varities showed resistance with root knot index 2 to *M. incognita.*

Chakraborty *et al.* (2016) evaluated the response of 60 germplasms of chickpea, 22 germplasms of lentil, 23 germplasms of field pea, 14 germplasms of mungbean, 26 germplasms of pigeon pea and 12 germplasms of urdbean towards natural infestation of *M. incognita* race 2 in field. Out of 26 germplasms of pigeon pea screened, RVKT.298 showed high resistance to *M. incognita.* They recorded 9 chickpea germplams, 8 lentil germplasms, 7 field pea germplasms, 19 germplasms of pigeon pea as resistant to *M. incognita.* Three mungbean and two urdbean germplasms showed moderate resitance to *M. incognta.*

2.4. MANAGEMENT OF ROOT KNOT NEMATODE USING FLUOPYRAM

The green labeled broad spectrum fungicide fluopyram found effective on plant parasitic nematodes and it killed the nematodes by selectively inhibiting complex II of the mitochondrial respiratory chain, there by depletes the nematode's cellular energy (Broeksma *et al.*, 2014). Faske and Hurd (2015) reported that fluopyram affected the mobitlity of *M. incognita* and *R. reniformis.*

Kim *et al.* (2016) evaluated effect of nematicidal compounds *viz.*, fluopyram 40% SC, fosthiazate 30% SC, imicyafos 30% SC, abamectine 1.68% SC, terthiophene, and *Eclipta prostrata* extract in hatching of *Heterodera schachtii* Schmidt, cysts. No hatching was observed in fluopyram 400 SC (100 ppm) treated cysts. Jones *et al.* (2017) evaluated different nematicides *viz.*, fluopyram, spirotetramat, ethoprophos and oxamyl for the management of *M. incognita* in lima bean under greenhouse condition. Application of Luna Privilege SC (Fluopyram) 0.22 L a.i ha^{-1} as pre plant treatment to the micro plots controlled 94.00 per cent juveniles. The pod weight of lima bean increased to 342 from 256 g.

A study was conducted to evaluate the effect of fluopyram, fluensulfone, abamectin and furfural on nemtodes associated with turf grass. Fluopyram 500 g a.i ha^{-1} @1.25 L ha^{-1} reduced both plant parasitic and beneficifial nematode populations in turfgrass. Bacterivores and omnivores were significantly controlled by fluopyram but not fungal feeders. Fluopyram controlled all feeding groups of nematodes quickly after application and throughout the season (Waldo *et al.*, 2019).

Ji *et al.* (2019) evaluated the effect of fluopyram against *M. incognita* under both laboratory and field conditions. Fluopyram at 0.25, 0.5, 1.0, 5.0, 10, 25 and 50 mg L^{-1} were tested against *M. incognita* juveniles and egg masses under laboratory condition. They found that fluopyarm was highly toxic to *M. incognita* juveniles and egg masses with LC_{50} 2.78 and 1.70 mg L^{-1} respectively. In field experiment, fluopyram @ 320, 480 and 640 g ha^{-1} applied as soil drench before transplanting of tomato seedlings. Fluopyram @ 480 and 640 g ha^{-1} reduced *M. incognita* population and improved plant growth and yield (21.4 to 58.5 per cent).

Yue *et al.* (2020) evaluated four nematicides *viz.*, fluensulfone, avermectin B1a, fluopyram and fosthiazate against *M. incognita* on tomato. Soil application of fluopyram at 1 and 10 mg L^{-1} reduced galls in root by 96.20 and 99.20 per cent respectively. Fluopyram also reduced nematode population in root (90.60 - 96.10 per cent) and soil (65.70 - 63.70 per cent).

2.5. MANAGEMENT OF ROOT KNOT NEMATODES USING BIOAGENTS

2.5.1. Fungal Bioagents

2.5.1.1. Purureocillium lilacinum

Purpureocillium lilacinum (Thom.) is a fungal bio agent, which parasitize nematode eggs and there by control root knot nematodes (Jatala *et al.*, 1980). Anita and Vadivelu (1997) reported that application of *P. lilacinus* (10g plant^{-1}) to scented geranium (*Pelargonium graveolens* L) suppressed *M. hapla* population and improved plant biometric characters. Number of galls (1g root) and egg masses (1g root) reduced by 87.90 and 93.53 per cent respectively. Plant height increased from 26.60 to 42.00 cm and leaf yield increased from 111.50 to 194.50 g.

Devarajan and Rajendran (2001) reported that basal application of *P. lilacinum* @ 30g kg soil^{-1} significantly reduced number of galls, gall index, egg masses and population of *M. incognita* in banana. Verma *et al.* (2004) reported that application of *P. lilacinum* (10 g pot^{-1}) suppressed *M. incognita* population in brinjal and tomato and increased yield. Kiewnick and Sikora (2006) reported that *P. lilacinum* (1×10^6) strain 251 as basal application reduced root galling (66.00 per cent), number of egg masses in root system (74.00 per cent) and population of *M. incognita* (71.00 per cent) in tomato.

Priya and Kumar (2006) reported that soil application of *P. lilacinum* (8x 10^6 spores g^{-1}) @ 4 g 3 kg soil^{-1} significantly reduced number of galls (62.30 per cent), egg masses (29.00 per cent), females (57.3 per cent) and *M. incognita* population in soil (57.30 per cent) over untreated in tomato and improved plant growth. Plant growth parameters *viz.* shoot length, shoot weight, root length, root weight and yield were increased by 65.50, 127.50, 84.30, 65.10 and 195.20 per cent respectively. Kannan and Veeravel (2008) reported that application of *P. lilacinum* (1 x 10^8 cfu g^{-1}) @ 10 g kg^{-1} soil significantly reduced number of galls and improved plant growth in tomato.

Faria (2013) evaluated effectiveness of different concentrations of *P. lilacinum* for managing *M. incognita* in tomato plant in pot culture experiment under shade

house. The optimum concentration of *P. lilacinum* for nematode management was found as 5×10^4 cfu g^{-1}. The root galling and egg masses in root system were reduced by 76.53 and 91.13 per cent respectively. Highest rate of colonization of *P. lilacinum* in nematode eggs was observed at 5×10^5 cfu g^{-1} concentration. Kepenekci *et al.* (2017) reported that application of Bio Nematon (*P. lilacinum* strain PL1) @ 1×10^8 conidia mL^{-1} to the tomato plants significantly reduced the *M. incognita* population and improved yield. Number of galls reduced significantly and total yield of tomato increased from 11.18 to 18.80 kg.

P. lilacinum (1×10^7) @ 30g $plant^{-1}$ is effective for management of *M. incoginta* in cardamom plants. *P. lilacinum* significantly reduced the nematode population in both soil and root and improved yield (Narayana *et al.*, 2017). Nisha *et al.* (2017) reported that paring + treatment of sucker with *P. lilacinum* (5g $sucker^{-1}$) + application of *P. lilacinum* (20g pit^{-1}) 45 days after planting significantly reduced the nematode population in banana and increased yield.

Hore *et al.* (2018) reported that Bio-Nematon 1.15%WP @ 69 g ha^{-1} as soil drench reduced population of *M. incognita* (37.00 per cent over control) and increased yield (11.60 per cent over control) in tomato. Khan *et al.* (2019) stated that usage of *P. lilacinum* as biocontrol agent for management of *M. incognita* is a better alternative for chemical nematicides. Application of *P. lilacinum* at 15g pot^{-1} on 15 days before *M. incognita* inoculation to mung bean was found effective in improving growth parameters of the plant *viz.*, plant length (68.10 cm from 32.90 cm), fresh weight of plant (81.64 g from 19.62 g), dry weight of plant (16.31 g from 4.03 g), number of pods in single plant (51.00 from 19.00), number of nodules $plant^{-1}$(48.00 from 31.00) and chlorophyll content (2.84 mg $g^{-1}$from 1.21) as compared to untreated. The root knot index was reduced from 5.00 to 2.10 and egg masses $plant^{-1}$ reduced to 8.00 from 169.00 (untreated).

Metwally *et al.* (2019) reported that BioNematon 1.15% WP (*P. lilacinum* @ 1×10^8 cfu g^{-1}) at 10 mL pot^{-1} significantly suppressed nematode population (78.60 per cent over untreated) and enhanced plant growth characters. Total length and fresh weight of the plant increased to 34.4 cm from 18.2 cm and 10.00 g to 4.40 g

respectively. Reproductive factor of nematode, number of galls in root system and root gall index was reduced to 4.20 from 19.60, 32.20 from 82.20 and 3.80 from 4.00 respectively.

2.5.1.2. Other Fungal Bioagents

Goswami and Singh (2002) studied the effect of *Aspergillus niger* Tiegh and *Cladosporium oxysporum* Berk and M.A. Curtis on *M. incognita* multiplication in eggplant. They reported that application of *A. niger* Solan isolate ($5x10^6$ g^{-1}) and *C. oxysporum* Hissar isolate ($5x10^6$ g^{-1}) significantly reduced *M. incognita* multiplication in eggplant. The total number of galls in root, egg masses $plant^{-1}$, eggs egg $mass^{-1}$ and nematode population in soil reduced from 84.00 to 16.60, 71.30 to 13.60, 160.60 to 42.00 and 2577.00 to 157.30 respectively.

Arbuscular mycorrhizal fungus was effective in reducing population of *M. incognita* and improving plant growth in two species of *Acacia viz., Acacia farnesiana* (L.) Wight et Arn and *Acacia saligna* (Labill.) H.L. Wendl. Application of AMF (500 spores g^{-1}) @ 100 g $seedling^{-1}$ before nematode inoculation reduced the number of juveniles in pot (60 from 1100), egg masses (11 from 56), reproduction factor of nematode (0.63 from 6.2) and increased plant length (74.33 cm from 38.0 cm) in the variety *A. farnesiana* compared to untreated control. In the variety *A. saligna,* AMF (500 spores g^{-1}) @ 100 g $seedling^{-1}$ reduced the number of juveniles in pot (100.00 from 2600.00), egg masses (20.00 from 122.00), reproduction factor (2.30 from 19.70) and increased plant length (59.00 cm from 27.33 cm) (Soliman *et al.*, 2011).

Soil application of *Syncephalastrum racemosum* filtrate (10 mL m^{-2}) at two days before planting in the field reduced number of galls (62.80 per cent) and *M. incognita* population in soil (51.70 per cent) and root (54.40 per cent) on cucumber plant (Huang *et al.*, 2014). Karajeh (2013) reported that *Saccharomyces cerevisiae* Meyen ex E.C. Hansen @10 g $plant^{-1}$ significantly reduced *M. jvanica* and increased yield in cucumber.

Tian *et al.* (2014) isolated endophytic fungus *Acremonium implicatum* (J.C. Gilman and E.V. Abbott) W. Gams from tomato root galls infected with *M. incognita*. *A. implicatum* recorded 96.00 per cent juvenile mortality and 36.30 per cent egg hatching under *in vitro* condition.

Verma *et al.* (2009) reported that treatment of cowpea seeds with *Trichoderma viride* Pers. 1794 and *Gliocladium virens* (J.H. Mill., Giddens and A.A. Foster) @ 10 g kg^{-1} seed significantly reduced *M. incognita* population, number of galls, egg masses and number of females. This also increased plant growth parameters *viz.* root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight.

Priya (2015) reported that application of *T. viride* at 2.5 kg ha^{-1} one week before planting of rice suppressed *M. graminicola* population in root (79.82 per cent) and soil (64.39 per cent) and increased the yield (78.04 per cent). Number of galls, females and egg masses in the root system was reduced by 89.25, 81.25 and 79.92 per cent respectively. Reproduction factor of nematode was 0.46 in treated plants while it was 1.29 in control. Soil application of *P. lilacinum* (25 g m^{-2}) + *Pochonia chlamydosporia* (Goddard) Zare & W. Gams (25 g m^{-2}) significantly reduced *M. incognita* galls and population in tomato. The yield was increased by 53.00 per cent (Senapati *et al.*, 2016). Viggiano *et al.* (2015) reported that application of *P. chlamydosporia* isolate Pc-10 @18 g L^{-1} significantly reduced the number galls (21.0 per cent) and egg masses (43.50 per cent) of *M. javanica* in lettuce. Nama and Sharma (2017) reported that application of *T. harzianum* @ 10 g kg^{-1} seed improved plant biometric characters and decreased *M. incognita* population in cowpea plants.

2.5.2. Bacterial Bioagents

2.5.2.1. Stenotrophomonas maltophilia Palleroni and Bradbury

Huang *et al.* (2009) reported the nematicidal property of *S. maltophilia* strain G2 against *Panagrellus redivivus* (Linne) and *B. xylophilus* under *in vitro* condition. The crude extracellular proteins of *S. maltophilia* have nematicidal property and within 36 hours of treatment, it killed 90.00 per cent of *P. redivivus* and 65 per cent of *B. xylophilus*.

Jankiewicz *et al.* (2016) studied the nematicidal property of *S. maltophilia* against *Caenorhabditis elegans* (Maupas) and *P. redivivus*. They reported that culture supernatant and protease PN4 of *S. maltophilia* showed mortality of 50.00 and 35.00 per cent respectively against *C. elegans* after 30 h of incubation and 85.00 and 80.00 per cent respectively against *P. redivivus* after 10 h of incubation.

Vishnu (2018) found that soil drenching of *S. maltophilia* (1×10^7 cfu mL^{-1}) @ 50 mL pot^{-1} in tomato significantly reduced *M. incognita* population in soil (72.69 per cent reduction over untreated) and root (82.33 per cent reduction over untreated).

2.5.2.2. Other Bacterial Bioagents

Sheela and Nisha (2004) reported that soil drenching of *Bacillus macerans* Schardinger at 25 g m^{-2} in nursery and seven days after sowing in field significantly suppressed *M. incognita* population and increased yield in brinjal. Abo-Elyousr *et al.* (2010) reported that application of *Pseudomonas fluorescens* (Flugge) Migula (10^8 cfu mL^{-1}) 20 mL suspension in tomato plant under field condition significantly reduced *M. incognita* population. The tomato yield, fresh weight of shoot and root was increased by 67.00, 115.90 and 332.10 per cent, respectively over control. Number of galls, females, egg masses and total nematode population was reduced by 48.80, 57.20, 65.80 and 51.00 per cent, respectively over control.

El- Hadad *et al.* (2011) evaluated *Paenibacillus polymyxa*, *Bacillus circulans* Jordan and *Bacillus megaterium* de Bary against *M. incognita* in tomato plants under pot culture condition. *P. polymyxa* NFB7 significantly increased number of leaves in plant (30.80 per cent), shoots dry weight (70.30 per cent), root dry weight (14.20 per cent) and root length (32.60). *P. polymyxa* NFB7, *B. circulans* KSB2 and *B. megaterium* PSB2 resulted significant reduction in number of females in root (63.57 per cent), nematode population in root (95.80 per cent) and soil (57.80 per cent).

Anita and Samiyappan (2012) observed the accumulation of defense enzymes such as peroxidase (PO), phenol, polyphenol oxidase (PPO), super oxide dismutase (SOD), phenylalannine ammonia lyase (PAL) and chitinase in the rice roots by the

application of *P. fluorescens* isolate Pf1. These defence enzymes reduced *M. graminicola* infection in rice plants. Norabadi *et al.* (2014) reported that application of 20 mL suspension of *P. fluorescens* (10^9 cfu mL^{-1}) into tomato seedlings under pot culture condition reduced *M. javanica* infection. Number of galls, diameter of nematode gall, number of egg masses and eggs in single egg mass reduced to 112.00, 1.60 mm, 100.00 and 215.00 respectively. Chormule *et al.* (2017) reported that application of *P. fluorescens* @20 kg ha^{-1} against *M. incognita* significantly reduced nematode population (38.60 per cent), number of galls (26.10 per cent) and egg masses (28.80 per cent) and increased yield (23.90 per cent) over untreated in grape.

Nisha and Sheela (2012) reported that rhizome treatment of kacholam with *P. fluorescens* @ 3%w/w increased the plant growth parameters and suppressed the root knot and burrowing nematode population. Ann (2013) reported that application of *Bacillus* strain MPB93 and MPB04 in pepper reduced *M. incognita* population in root by 35.28 and 60.95 per cent respectively under pot culture condition. Chinheya *et al.* (2017) reported that seed treatment of soybean with *Bacillus* spp. isolates *viz.*, BC27 and BC29 caused significant reduction in root knot galling and number of egg masses. Among these two isolates BC29 resulted highest reduction in number of galls (83.71 per cent) and egg masses (86.48 per cent).

Safni *et al.* (2018) reported that four rhizobacteria *viz., Aremonas* sp., *Vibrio* sp., *Serratia* sp. and *Serratia marcescens* Bizio isolated from potato field have nematicidal property against *M. incognita* under *in vitro* condition. These bacteria showed juvenile mortality of 99.87, 99.40, 98.80 and 98.20 per cent, respectively.

Sohrabi *et al.* (2018) reported that application of 1 mL bacterial suspension (10^8 cfu mL^{-1}) of *P. fluorescens* and *B. subtilis* in tomato plant separately under pot culture, significantly reduced *M. javanica* population. The reproductive factor of nematode reduced from 112.15 to 24.94 and 24.96 respectively. Abd-El-Khair *et al.* (2019) observed that *B. subtilis*, *B. pumilus* and *P. fluorescens* reduced *M. incognita* population in cowpea.

2.6. MANAGEMENT OF ROOT KNOT NEMATODES USING ORGANIC AMENDMENTS

2.6.1. Neem Cake

Akhtar and Mahmood (1994) reported that extracts of both decomposed and undecomposed neem cake and leaves as bare-root dip treatment caused significant reduction of *M. incognita* population. Decomposed oil cake extract was more effective than others. Abulusoro and Oyedunmade (2005) used different concentrations of neem fruit powder for management of *M. incognita* in tomato var Roma UF. They observed higher yield (13.18 t ha^{-1}) and reduced gall index (2.40) when neem fruit powder @ 2 t ha^{-1} was incorporated into soil as compared to control plants which recorded a yield of 2.77 t ha^{-1} and gall index of 4.85. Claudius-Cole *et al.* (2010) reported that soil drenching with *Azadirachata indica* (A. Juss) extract in cowpea cultivar IT-85D-2865 reduced root knot index (9.8) compared to control (22.2) under pot culture condition. Seenivasan (2010) evaluated neem cake, pungam cake, castor cake and vermicompost for the management of *M. incognita* in medicinal coleus under pot condition. Neem cake (500 kg ha^{-1}) reduced nematode population in soil (30.80 per cent) and increased tuber yield (42.40 per cent) as compared to untreated control. Lal and Rana (2012) conducted a study to find the effect of neem products such as achook, econeem, nimbicidine, neem seed kernel powder (NSKP) and neem seed powder (NSP) on plant growth and *M. incognita* population in okra. All the neem products reduced root galling on okra. Among the commercial neem formulations, achook was found most effective followed by nimbicidine.

Chimbekujwo and Bukar (2013) evaluated *A. indica*, *Eucalyptus gigantean* (Dehnh) and *Cassia siamea* (Lamk.) for the management of *M. incognita* in cowpea and reported that application of neem leaf powder @ 75g in 4 Kg soil gave the highest reduction in *M. incognita* population. This treatment reduced nematode population in soil (80.00 per cent), root (88.73 per cent), reproduction factor (80.50 per cent) and increased yield (428.32 per cent) as compared to control.

Neem cake @1 t ha^{-1} as basal application significantly reduced number of galls (76.96 per cent), egg masses (64.20 per cent), eggs egg $mass^{-1}$ (18.87 per

nematode population (78.6 per cent) and improved growth of cucumber compared to control. Shoot length, fresh shoot weight, fresh root weight, root length, dry shoot weight and dry root weight increased by 26.90, 28.66, 52.63, 44.47, 40.87 and 78.90 per cent respectively (Devi and Das, 2016). According to KAU (2016) application of neem cake (1.0 t ha^{-1}) twice during planting and 45 days after planting in endemic areas was effective in managing nematode population in ginger.

2.6.2. Other Organic Amendments

Soil incorporation of *Calotropis* sp., *Eupatorium* sp., cashew and mango leaves at 5000 kg ha^{-1} three weeks before sowing of okra seeds reduced nematode population and number of galls in root and increased plant biometric characters (Kumar and Nair, 1976).

Application of soil amendments such as cocoa pods and poultry droppings significantly reduced the root knot nematodes and improved growth and yield of soybean. The basal application of cocoa pods and poultry droppings @ 200 g pot^{-1} reduced nematode multiplication rate to 36.20 and 29.00 respectively from 309.90. The gall index was reduced to 0.90 and 0.80 in cocoa pods and poultry droppings applied treatments while in control it was 4.20 (Oyedunmade *et al.*, 2001).

Incorporation of chopped *Tagetes* leaves @ 80 g kg $soil^{-1}$ significantly increased tomato plant growth and reduced *M. javanica* population. Number of nematode galls, egg masses and nematode population 100 cc $soil^{-1}$ was reduced from 40.00 to 18.00, 51.00 to 7.50 and 1185.00 to 465.00 respectively (Walia and Gupta, 1997). Rather *et al.* (2008) reported that application of chopped marigold leaves @100g pot^{-1} recorded the lowest gall index of 0.64.

Soil incorporation of *Gliricidia maculata* (H. B. K.) Steud. as green leaf manure (25 t ha^{-1}) significantly reduced the root knot nematode population and increased yield in tomato. Treated plants recorded lowest gall index (0.327), extent of galling (35.78), reproduction factor of nematode (0.411) and high yield (17.87 Mt ha^{-1}) (Pakeerathan *et al.*, 2009).

Hassan *et al.* (2010) reported that incorporation of refuse dump @ 45 t ha^{-1} into soil significantly reduced root knot nematode population (88.00 per cent) and increased yield (17.00 per cent) of tomato plant. Number of galls 5g $root^{-1}$ in treated plants was reduced to 1.0 from 39.70 in untreated. Rashad and Kesba (2011) reported that incorporation of rice straw compost at 5 and 7.5 % into rhizosphere of eggplant resulted 79.00 and 84.00 per cent reduction in *M. incognita* population respectively. Kankam *et al.* (2014) reported that application of Indian almond cake at 15 g pot^{-1} significantly reduced root galling of *M. incognita* on cowpea. Number of pods in single plant increased to 3.67 from 2.67 and number of galls in root were recorded as zero while it was recorded as 5.25 in control.

Saeed and Shawkat (2014) reported that application of *Peganum harmala* L. leaf powder @15 g $plant^{-1}$ significantly reduced *M. incognita* population in tomato plant under pot culture experiment. Number of galls, egg masses, nematode population in soil and root reduced by 94.00, 94.00, 99.00 and 91.00 per cent respectively. Dry leaf powder of *Tithonia diversifolia* (Hemsl.) at 50 and 100 g $plant^{-1}$ suppressed the nematode population (85.00 per cent reduction over untreated) associated with cabbage (Varghese, 2015). Frederick *et al.* (2015) reported that application of sunhemp leaf residues @ 6 kg ha^{-1} reduced *M. incognita* population (94.00 per cent) and increased yield (168.70 kg ha^{-1}) in tomato.

Nisha and Nimisha (2017) reported that mulching of green leaves of *G. maculata* @ 5 kg pit^{-1} significantly reduced *M. incognita* population in soil and increased yield in banana. Sowley *et al.* (2018) reported that incorporation of moringa leaf powder @ 60 g $plot^{-1}$one week after planting of cowpea significantly reduced *M. incognita* population in soil and improved plant growth parameters. Youssef *et al.* (2018) reported that soil amendment with mashed storage roots of sugar beets @ 20 g pot^{-1} suppressed nematode population (85.10 per cent) and improved yield (70.20 per cent) of cowpea under pot culture.

2.6.3. Combination of Fungal Bioagents and Organic Amendment

2.6.3.1. P. lilacinum + Neem Cake

Rao *et al.* (1997) reported that seed treatment of okra by neem cake suspension 5% with *P. lilacinum* spores significantly reduced *M. incognita* population. Soil solarisation in the nursery and field application of *P. lilacinum* (15 g m^{-2}) + neem cake (100 g m^{-2}) was effective in reducing *M. incognita* population in soil and increasing yield in coleus (Nisha and Sheela, 2006). Sharma *et al.* (2007) reported that combined application of 15 mL suspension of *P. lilacinum* (10^6 cfu mL^{-1}) and neem cake @ 10 g pot^{-1} significantly suppressed number of galls (81.00 from 225.00), egg masses (31 from 100) and nematode population (350 from 900) in okra.

Sundararaju and Kiruthika (2009) reported that application of *P. lilacinum* (10 g $plant^{-1}$) + neem cake (100 g $plant^{-1}$) against *M. incognita* in banana cv. Robusta resulted in significant increase of height of plant (5.30 cm), pseudostem girth (11.70 cm), number of leaves (5.30), length of root(36.00 cm) and weight of root (42.00 g). Lowest gall index (1.00), nematode population in 250 cc soil (30.00) and 5 g root (110.00) were recorded.

Ashraf and Khan (2010) reported that combined application of *P. lilacinum* @ 1 g and neem cake 10 g pot^{-1} significantly reduced *M. javanica* in eggplant. The number of galls in root system and reproduction factor of nematode in treated plants reduced to 42.00 and 3.50 respectively against 158.00 and 5.60 in untreated. Total plant length and plant dry weight increased to 54.10 cm and 15.9 g, respectively against 34.2 cm and 10.2 g in untreated. Thammaiah *et al.* (2012) reported that combined application of *P. lilacinum* (10 g $plant^{-1}$) + neem cake (250 g $plant^{-1}$) significantly reduced *R. similis* in banana and increased yield.

2.6.3.2. Other Fungal Bioagents and Organic Amendments

Verma *et al.* (2005) reported that combined application of *P. lilacinum* (50g) + *T. harzianum* (100g) + neem cake (250g) + marigold (3 plant) in each pit of pointed gourd field significantly reduced *M. incognita* infection and improved plant growth in

consecutive two years. Odeyemi *et al.* (2010) reported that combined application of organic fertilizer (10 t ha^{-1}) and 50 g mycorrhiza inoculum at planting time significantly suppressed *M. incognita* population on cowpea and enhanced plant growth. Number of pods $plant^{-1}$ and pod weight increased to 47.00 from 34.00 and 391.67 kg ha^{-1} from 316.66 kg ha^{-1}, respectively. Number of galls in root system and nematode reproduction rate were reduced to 7.45 from 45.66 and 0.66 from 4.55, respectively.

Shamalie *et al.* (2011) reported that *Trichoderma* incorporated organic manures improved the growth of Gotukola and also suppressed root knot nematodes significantly. Field application of *Trichoderma* ($1x10^{11}$ cfu mL^{-1}) + compost @ 2 kg m^{-2} recorded significant reduction in root gall per cent compared to control. Somasekhara *et al.* (2012) reported that application of *P. lilacinum* @ 50 g $plant^{-1}$ + castor cake @ 1 kg $plant^{-1}$ significantly reduced number of galls and nematode population of *M. incognita* in pomegranate. Singh *et al.* (2014) reported that seed treatment of *P. lilacinum* @ 10 g kg $seed^{-1}$ and soil application of *P. lilacinum* @ 10 kg ha^{-1} + FYM @ 1.5 t ha^{-1} significantly reduced *M. incognita* in okra and increased yield.

Ravindra *et al.* (2014) reported that combined treatment of *P. lilacinum* with acacia compost recorded maximum growth parameters and least nematode population of root knot nematode on brinjal. Application of *P. lilacinum* cfu 2×10^{6} (250 g m^{-2}) + acacia compost (1 kg m^{-2}) reduced root knot index from 3.67 to 1.47. Application of *P. lilacinum* (50 g m^{-2}) in nursery and main field application of *P. lilacinum* enriched FYM (2.5 t ha^{-1}) suppressed nematode population and increased yield in brinjal (Nisha and Sheela, 2015).

Kumar *et al.* (2017) reported that combined application of neem cake (600g $plot^{-1}$) + vermicompost (6 kg $plot^{-1}$) + *Trichoderma* (0.02 g $plot^{-1}$) significantly reduced *M. graminicola* population and increased yield (78.81 per cent) of rice. Number of galls in treated plants was 1.33 while it was 17.66 in control at 60 days after transplanting.

Patel *et al.* (2019) reported that combined application of *P. lilacinum* cfu 10^6 g^{-1} and poultry manure @ 5 t ha^{-1} suppressed *M. incognita* in potato. The treated plants recorded the highest plant height (38.33 cm) and tuber weight (129.71 g) against 23.17 cm and 69.21 g respectively in control.

2.6.3.2. Combination of Bacterial Bioagents and Organic Amendments

Akram *et al.* (2016) reported that compost of *Calotropis procera* (Aiton) + *Azotobacter chroococcum* Beijerinck + *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe significantly suppressed root knot nematode population in chickpea. Number of pods per plant, fruit weight and chlorophyll content were increased to 38.00 from 15.21, 72.41 g to 30.01 g and 3.21 mg g^{-1} from 1.36 mg g^{-1} respectively. Number of galls in root was reduced to 37.19 from 158.50 and reproduction factor was recorded as 0.63 against 7.33 in untreated.

Kar *et al.* (2018) reported that combined application of *P. fluorescens* 20 g m^{-2} and neem cake @100 g m^{-2} significantly reduced the *M. incognita* population and improved yield of cowpea plants under field condition. The treatment combination increased yield from 2.10 to 3.64 t ha^{-1} and reduced nematode population in 200 cc soil to 133.50 from 333.70.

Gogoi and Boruah (2019) reported that FYM enriched *P. fluorescens* @ 50 g $plant^{-1}$ was effective in suppressing root knot nematode population and improving plant growth parameters in long piper.

2.7. MANAGEMENT OF ROOT KNOT NEMATODES USING CHEMICALS

2.7.1. Carbosulfan

Seed treatment of okra using carbosulfan significantly reduced root knot nematodes in both soil and root (Jain and Gupta, 1990). Mishra and Prasad (1991) reported that cowpea seed treated with carbosulfan 25 STD significantly reduced *M. incognita* population thereby improved plant growth. Soaking and dressing of seed by carbosulfan significantly suppressed root not nematode population in okra (Meena and Mishra, 1993). Barman and Das (1994) reported that seed dressing with

carbosulfan 25 EC (3% w/w) and double spraying of carbosulfan 25 EC (0.1%) at 40 and 70 days after sowing in field significantly reduced *M. incognita* population, galls and egg masses in mugbean.

Jain and Dabur (2000) reported that soil solarization followed by seed treatment using carbosulfan (25 ST) at 3 % w/w and soil incorporation of neem cake (200 kg ha^{-1}) was effective in lowering nematode population (71.80 per cent). Mohanty *et al.* (2000) evaluated certain chemical nematicides *viz.* carbosulfan, monocrotophos and phosalone as seed treatment for management of *M. graminicola* in rice. They reported that carbosulfan 25 EC @ 0.1 % was more effective in managing *M. graminicola* and resulted in highest yield (0.374 kg $plot^{-1}$) and lowest nematode population in 250 cc soil (135.80) and 5g root (16.00) while in control it was 0.296 kg $plot^{-1}$, 267.60 and 30.20 respectively. Vadhera *et al.* (2000) used carbosulfan (25 ST) and triazophos (40 EC) for the management of *M. incognita* in cowpea and reported that carbosulfan was more effective than triazophos in increasing yield and reducing nematode population. They also reported that seedling bare root dip in carbosulfan (25 ST) at 0.1 % for 6 h increased the yield by 43.00 per cent with low gall index (3.5).

Sharma and Majumdar (2003) reported that carbosulfan 25 EC @ 500 ppm as seed soaking was effective in increasing root nodulation, grain yield as well as reducing root knot disease index of *M. incognita* in chickpea. Root knot index in plant reduced from 50.60 to 29.60 and yield was increased to 13.30 q ha^{-1} from 6.60 q ha^{-1}. Chawla *et al.* (2006) reported that soaking of tuberose (*Polianthes tuberosa* Linn) in carbosulfan 2000 ppm for one hour significantly reduced *M. incognita* multiplication. Number of galls, egg masses, eggs and the soil nematode population were reduced at harvesting time more than 70.00 per cent. Gowda *et al.* (2014) reported that soaking of tuberose bulbs in 2000 µg concentration of carbosulfan reduced total nematode population (81.04 per cent).

Mukhopadhyay *et al.* (2006) reported that dipping of pointed gourd vine in carbosulfan 25EC at 500 ppm for 6 h reduced *M. incognita* population in 200cc soil to 66.00 from 160.00 and increased yield from 10.70 to 12.00 kg $plot^{-1}$. Rajvanshi *et al.*

(2008) reported that seed soaking with carbosulfan 25EC @ 1000 ppm significantly reduced number of galls (49.66 per cent) and *M. incognita* population (63.35 per cent) and increased yield (72.72 per cent) in round melon.

Mohanty and Mahapatra (2009) reported that seed soaking with carbosulfan 25 EC @ 0.1% for 12 h significantly reduced the *M. graminicola* infestation in paddy. The multiplication of root-knot nematodes in soil and formation of egg masses were reduced by 60.07 and 63.54 per cent respectively. The paddy yield was increased by 29.80 percent.

Patel and Patel (2009) reported that soil application of carbosulfan (Marshal 25 EC) @ 2.5 L ha^{-1} one day prior to seeding + 25 days after seeding significantly reduced root knot nematodes associated with bidi tobacco in nursery. Carbosulfan reduced total nematode population in soil (100 cc) to 367.00 from 783.00 (untreated control) and root knot index was reduced to 0.50. Soil application of carbosulfan 6G @ 2 kg a.i ha^{-1} significantly lowered root knot nematode population and yield in okra. Carbosulfan reduced number of galls, gall index and nematode population by 69.57, 39.33 and 59.49 per cent respectively. Shoot length, root length, fresh weight of shoot, fresh weight of root and yield were increased by 71.37, 64.28, 83.67, 93.41 and 32.94 per cent respectively (Shendge *et al.*, 2010). Dwivedi *et al.* (2013) stated that soaking of okra seeds in carbosulfan 25 EC @ 0.1% effective in reducing gall index and increasing yield.

2.8. PERSISTENCE/DEGRADATION OF RESIDUES OF PESTICIDES

2.8.1. Fluopyram

Guan *et al.* (2011) studied the residues of fluopyram (500 g L^{-1} SC) at 225 and 300 mL ha^{-1} in cucumber and reported that the residues in cucumber and soil at both the dosages were below 0.5 mg kg^{-1} and was below the maximum residue limits (1 mg kg^{-1}). They also found that half lives of fluopyram 500g L^{-1} SC were less than 7 days in cucumber and soil. Dong and Hu (2014) reported that residue of fluopyram 300 g a.i ha^{-1} in watermelon was below maximum residue limit.

Chawla *et al.* (2018) applied fluopyram 400 SC at 250 and 500 g a.i ha^{-1} as soil drench in cucumber field with 15 days interval and reported that residue of fluopyram were below determination level (0.05 mg kg^{-1}) at both the dosages on 40^{th} day after second application.

Matadha (2019) reported that soil drenching of Luna Experience 400 SC (fluopyram 17.7% + tebuconazole 17.7%) at the time of tomato fruit setting resulted the residue of fluopyram in tomato fruit as 0.060 mg kg^{-1} which was below the maximum residue level. He also stated that fluopyram had less chance to enter into the food chain through the tomato fruits.

2.6.2. Carbosulfan

Kabir *et al.* (2008) reported that the residue of carbosulfan (1.5 mL L^{-1}) in yard long bean remained till at detected quantity up to seven days after application. The residue was above maximum residue level up to three days after application.

Bhattacherjee (2013) sprayed imidacloprid and carbosulfan (2.0 mL L^{-1}) into mango during fruit development stage to find out the persistence of these pesticides. The residue of carbosulfan in the mango peel was 0.05 mg kg^{-1} and in the pulp was below detectable level at 45 days after spraying (at harvesting time).

Zhang *et al.* (2016) reported that seed treatment of rice by carbosulfan at 840 g a.i per 100 kg seed resulted residue of carbosulfan in brown rice as 0.05 mg kg^{-1} which as lower than maximum residue level.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled 'Management of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in vegetable cowpea' was conducted at Department of Nematology, College of Agriculture, Vellayani during 2018-2020. Details of the materials used and methodologies followed for this study given in this chapter.

3.1. SCREENING OF VEGETABLE COWPEA VARIETIES AGAINST *M. incognita*

Seven (5 KAU released and 2 local) varieties of vegetable cowpea were screened for their resistance against *M. incognita* infection. This experiment was carried out in glass house of Department of Nematology.

3.1.1. Identification of *M. incognita*

M. incognita was identified by cutting perineal pattern of female nematode and observing under stereo microscope as given by Taylor and Netscher (1974) later modified and described by Hartman and Sasser (1985). *M. incognita* infected plants were collected from Department of Nematology, College of Agriculture, Vellayani and roots were gently washed with distilled water. Galls in the nematode infected roots were placed on a microscopic slide and dissected under stereo microscope. Mature females were collected from these galls using a sterilized forceps and kept in 45% lactic acid. Twenty females were collected from each sample randomly. The anterior part of nematode body was cut off by using scalpel. The nematode body was gently pressed to remove the inner tissues. These inner tissues were cleaned out using a brush. The posterior part of nematode body was cut using scalpel and the cut portion of the posterior region was kept in 45% lactic acid. This posterior cuticular part was trimmed into a square shape with the perineal pattern in the centre. The perineal pattern was transferred to a microscope slide in a small drop of glycerine and it aligned as anus oriented towards down. A glass cover slip was placed on it. Perineal pattern was observed and identified the species of root knot nematode with the help of

identification keys which contain dorsal arch, lateral field, striae and tail terminus as given by Eisenback (1985).

3.1.2. Preparation of Denematized Potting Mixture

Potting mixture was prepared by mixing sieved field soil, sand and farm yard manure in the ratio 1:1:1. This potting mixture was denematized with four per cent formaldehyde. Potting mixture was heaped on the floor and holes were made on it to pour formaldehyde solution. Formaldehyde solution (4%) was poured into the potting mixture and covered tightly with polythene sheets. Polythene sheets were removed after two weeks and the potting mixture was spread on the floor for evaporation of residues of formaldehyde solution. This denematized potting mixture was used for pot culture study (Radwan and Hassan, 2018).

3.1.3. Maintenance of Pure Culture of *M. incognita*

The pure culture of *M. incognita* was obtained from infested tomato plants maintained in the net house of Department of Nematology. Egg masses adhering on the root surface were carefully transferred to a beaker containing distilled water. Hatched juveniles were collected after 3-5 days and counted under stereo microscope in a counting dish. The number of juveniles (1mL water) was adjusted by adding sterile water. Inoculation of juveniles into root zone was done as per the method given by Venkitesan and Sethi (1977). Juveniles were inoculated @ two juvenile g soil^{-1} into the rhizosphere of fifteen days old cowpea seedlings raised in pots filled with denematized potting mixture (Jayakumar *et al.*, 2005). Sub culturing of nematode was done periodically for maintaining the pure culture. This pure culture of *M. incognita* was used for various experiments.

3.1.4. Staining of Plant Roots

Acid fuschin lactophenol staining solution was used for staining of roots to observe nematodes present inside the roots (Franklin and Goodey, 1949). Acid fuschin lactophenol is a differential stain which stains only nematodes to red colour. The nematode infected plants were collected and roots washed in tap water to remove

adhering soil particles. The roots were again washed in distilled water and cut into small bits of 1-2 cm size. Acid fuschin stock solution was prepared by dissolving acid fuschin (250 mL), acetic acid (250 mL) and distilled water (750 mL). Lactophenol solution was prepared by mixing liquid phenol (500 mL), glycerine (100 mL), lactic acid (500 mL) and distilled water (500 mL). Acid fuschin lactophenol stain was prepared by dissolving acid fuschin stock solution (1 mL) into lactophenol (100 mL). The root bits were boiled in acid fuschin lactophenol stain for 1 min. The stained root bits were rinsed with distilled water for removing excess stain and kept in lactophenol solution in 24-48 h for destaining. The stained nematodes appeared red in colour under microscope.

3.1.5. Sowing of Seeds

Five KAU released and two local varieties of vegetable cowpea (Table 1) were used for screening. Seeds collected and sowed into the pots filled with denematized potting mixture prepared as mentioned in para 3.1.2. The trial was laid out in completely randomized design with three replications. Second stage juveniles of *M. incognita* were inoculated into rhizosphere of cowpea plants fourteen days after sowing. Plants were watered regularly and maintained as per Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016).

Table 1: List of vegetable cowpea varieties for screening

Sl. No.	Varieties	Source
1	Geethika	KAU
2	Lola	KAU
3	VS 50	KAU
4	Vyjayanthi	KAU
5	Vellayani Jyothika	KAU
6	Kadakkal Local	Kadakkal, Kollam
7	Vellayani Local	Vellayani, Thiruvananthapuram

3.1.6. Recording Observations

Cowpea plants were carefully uprooted 45 days after nematode inoculation and observations on nematode population characteristics *viz.* number of galls, number of females, number of egg masses, number of eggs eggmass^{-1}, nematode population in soil and root were taken.

3.1.6.1. Number of Galls

Cowpea plants uprooted and gently washed under tap water to remove adhering soil particles. Five g root was taken and number of galls counted.

3.1.6.2. Gall Index (0-5 scale)

Based on the number of galls in root system, root knot indexing was done by the method of Heald *et al.* (1989).

No. of galls	Root Knot Index	Reaction
0	0	Highly resistant (HR)
1-25	1	Resistant (R)
26-50	2	Moderately resistant (MR)
51-75	3	Moderately susceptible (MS)
76-100	4	Susceptible (S)
>100	5	Highly susceptible (HS)

3.1.6.3. Number of Egg Masses

The method given by Southey (1986) was adopted to estimate the number of egg masses present in root. Five g root was placed in Phloxine B solution for 15 minutes to stain the egg masses. Phloxine B solution was prepared by dissolving

Phloxine B (0.15g) into water (1 L). The number of stained egg masses was counted under stereo microscope.

3.1.6.4. Number of Eggs Egg Mass^{-1}

The stained Egg masses from root bits were handpicked carefully by using a sterilized forceps and kept in a microscopic slide. These egg masses were crushed under stereo microscope and eggs in egg masses were counted.

3.1.6.5. No. of Females

The uprooted roots were washed under tap water to remove adhering soil particles. Five g root was cut into small bits of 2-3 cm length and stained by differential staining technique described in 3.1.4. The stained root bits were placed on a microscopic slide and dissected with a sterile needle. The females were observed under a stereoscopic microscope and counted.

3.1.6.6. Number of Nodules

Number of rhizobium nodules present in 5 g root was counted.

3.1.6.7. Nematode Population in Soil

Soil samples (200cc) were collected from rhizosphere of cowpea plants. Nematodes were extracted from soil samples by Cobb's sieving and decanting method (Cobb, 1918) and modified Baermann's funnel method (Schindler, 1961). The extracted nematodes were counted under stereo microscope in counting dish.

3.1.6.8. Nematode Juvenile Population in Root

Nematode population in root was estimated by direct examination method. Root sample (5 g) was washed thoroughly and chopped into small pieces and nematodes were collected by modified Baermann's funnel method (Schindler, 1961). Nematode suspension was collected in a beaker and nematodes were counted under stereoscopic microscope in a counting dish.

3.1.6.9. Nematode Reproduction Factor

Reproduction factor of nematode was calculated by the formula

Rf = Pf/Pi

Pf – Final nematode population

Pi – Initial nematode population

3.2. EVALUATION OF FLUOPYRAM AGAINST *M. incognita* IN COWPEA

Different doses of fluopyram 400 SC were tested against *M. incognita* in vegetable cowpea under pot culture condition in glass house of Department of Nematology.

3.2.1. Preparation of Different Concentrations of Fluopyram

Two doses of fuopyram were used in this experiment. Fluopyram 400 SC @ 250 g a.i ha^{-1} was prepared by dissolving 0.625 mL of fluopyram in 1 L water and fluopyram 400 SC @ 500 g a.i ha^{-1} prepared by dissolving 1.25 mL of fluopyram in 1 L water.

3.2.2. Testing of Doses of Fluopyram Against *M. incognita* in Cowpea

Nematode infested soil was collected from pots containing pure culture of *M. incognita* maintained in net house of Department of Nematology as described in para 3.1.3 and filled in 5 kg capacity pots. Different concentrations of fluopyram prepared as mentioned in 3.2.1 was drenched with nematode infested soil @ 200 mL pot^{-1}. Seeds of most susceptible variety obtained from 3.1 were sowed @ three seeds pot^{-1} one day after basal application of fluopyarm. Cowpea plants were watered regularly and maintained as per Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016)

The experiment was laid out in completely randomized block design.

Treatments – 5 Replications – 4

T1 - Fluopyram 400 SC @ 500 g a.i ha^{-1} as basal application

T2 - Fluopyram 400 SC @ 500 g a.i ha^{-1} as basal application, 500 g a.i ha^{-1} 25 days after first treatment.

T3 - Fluopyram 400 SC @ 250 g a.i ha^{-1} as basal application, 250 g a.i ha^{-1} 25 days after first treatment.

T4 - Fluopyram 400 SC @ 250g a.i ha^{-1} as basal application

T5 – U ntreated control

3.2.3. Recording Observations

Observations on nematode penetration and development of different stages of *M. incognita* were recorded 3, 11, 19 and 26 days after planting. Roots of plants uprooted from each replication were stained as described in 3.1.4. Stained roots were observed under stereo microscope and number J2, J3, J4 and females in the roots were recorded. Observations on nematode population $soil^{-1}$ (200 cc), nematode juvenile population $root^{-1}$ (5g), number of galls $root^{-1}$ (5g), number of females $root^{-1}$ (5g), number of egg masses $root^{-1}$ (5g), number of eggs egg $mass^{-1}$ and number of nodules $root^{-1}$ (5g) were taken 60 days after sowing. Phytotoxicity symptoms on cowpea plant after the application of fluopyram was also recorded.

3.3. MANAGEMENT OF *M. incognita* IN COWPEA

3.3.1. Preparation of Biocontrol Agents

3.3.1.1. Stenotrophomonas maltophilia

Talc based formulation of *S. maltophilia* containing 2×10^6 cfu g^{-1} was prepared in Department of Nematology, College of Agriculture, Vellayani and was applied to the soil @ 20 gm^{-2}. The dose was reduced to 10 gm^{-2} in combination treatment with neem cake.

3.3.1.2. Purpureocillium lilacinum

Talc formulation of *P. lilacinum* (cfu 2×10^6 g^{-1}) available in the trade name Bio-nematon 1.15% W.P obtained from T. Stanes & Company Ltd, Coimbatore was used for soil application @20 gm^{-2}. In combination treatment with neem cake the dose was reduced to 10 gm^{-2}.

3.3.2. Neem Cake

Neem cake was applied @ 100g m^{-2} before sowing. In combination treatments the rate was reduced to 50 g m^{-2}.

3.3.3. Fluopyram

Fluopyram 400SC @ 250g a.i ha^{-1} (effective dose obtained from 3.2) was soil drenched before sowing as basal application.

3.3.4. Carbosulfan

Carbosulfan 6G was applied to soil @ 5g m^{-2} before sowing seeds.

3.3.5. Field Experiment

Field experiment was conducted in nematode infected field located in Instructional Farm, Vellayani using the effective dose of fluopyram selected from 3.2 in comparison with bioagents (*P. lilacinum* and *S. maltophilia*) and neem cake for the management of *M. incognita* in cowpea (Plate 1). Seeds of most susceptible variety obtained from 3.1 were sowed in field @ 3 seeds per pit after basal application of treatments. The plants were maintained as per the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016).

The experimental details are as follows

Design: RBD

Plot size - 6×2 m

Treatments – 8

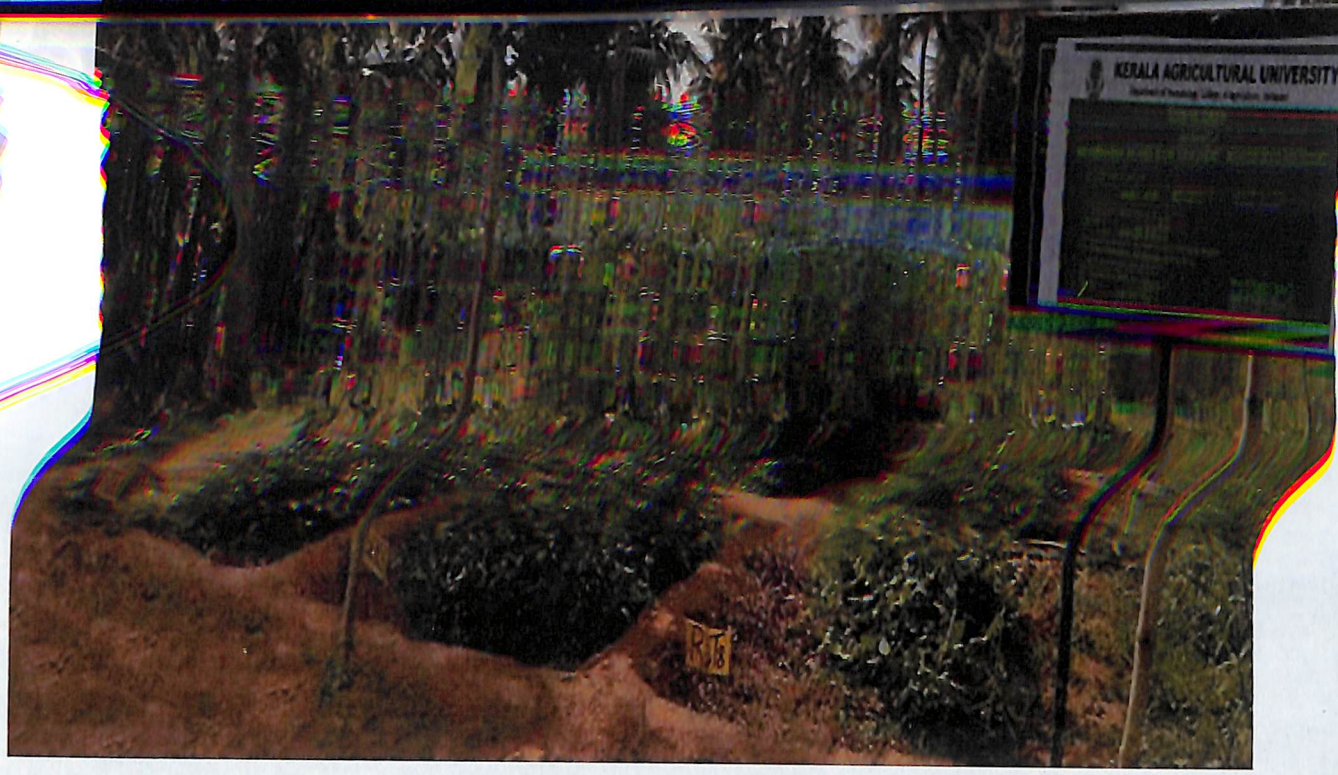


Plate 1. Field view

Plate 1. Field view

Replications – 3

Spacing - 2×2 m

T2 - Neem cake @100 g m^{-2}

T3 – *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}

T4 – *S. maltophilia* (cfu $2x10^6$ g^{-1}) @ 20 g m^{-2}

T5 – *S. maltophilia* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}

T6 - Fluopyram 400 SC @ 250g a.i ha^{-1} as basal application

T7 - Carbosulfan 6G @ 5 g m^{-2}

T8 - Untreated check

3.3.7. Recording of Observations

Root weight (g) and yield (kg $plot^{-1}$) was recorded at the time of harvest. Number of nodules and nematode population characteristics *viz,* number of galls $root^{-1}$ (5 g), number of egg masses $root^{-1}$ (5 g), number of eggs egg $mass^{-1}$, nematode population in soil (200 cc) and root (5 g) were recorded as mentioned in 3.1.8.

3.3.8. Re-isolation of Biogents

3.3.8.1. Estimation of Population of P. lilacinum

Population of *P. lilacinum* in cowpea rhizosphere was done by serial dilution and plating method as given by Ranganayaki *et al.* (2006). Rhizosphere soil (10 g) was collected from cowpea roots and added to a 250 mL Erlenmeyer flask containing 90 mL sterile water and mixed well. One mL aliquot from this stock solution was added to a test tube containing 9 mL sterile water and mixed well to get 10^{-2} dilution. One mL from 10^{-2} dilution mixed to 9 mL sterile water in a test tube. Pipetted out 0.1 mL from this solution (10^{-3} dilutions) and poured on Potato dextrose agar medium in a Petri plate and spreaded uniformly using L shaped glass rod. The Petri plate sealed

well with parafilm and kept for incubation for three days. Number of colony forming units (cfu) of *P. lilacinum* was counted and recorded in 10^3 dilutions.

Composition of the potato dextrose agar media is as follows

Potatoes (sliced, washed and unpeeled) – 4 g

Dextrose- 20 g

Agar powder – 20 g

pH adjusted to 5.6±0.2 at 25 °C

3.3.5.2. Estimation of Population of S. maltophilia

Population of *S. mlaltophilia* was estimated by serial dilution method as explained in 3.3.5.1. Dilution was done upto 10^{-5} and medium used was nutrient agar. The number of cfu of *S. maltophilia* was counted one day after incubation and recorded in 10^5 dilutions.

The composition of nutrient agar media is as follows

Peptone – 5 g

Beef extract/Yeast extract – 3 g

Agar – 15 g

Sodium Chloride – 5 g

Distilled water – 1000 mL

pH adjusted to neutral (6.8) at 25 °C

3.4. HARVEST TIME RESIDUES OF CHEMICALS

Residue of nematicides fluopyram and carbosulfan were evaluated in cowpea pods at harvesting time. Residue analysis was done at the Pesticide Residue Research

and Analytical Laboratory, Department of Agricultural Entomology, College of Agriculture, Vellayani.

3.4.1. Chemicals and Reagents

Certified reference material (CRM) of fluopyram and carbosulfan were purchased from Sigma- Aldrich Pvt. Ltd. Acetonitrile, water, anhydrous sodium sulphate, Sodium chloride and methanol (HPLC grade) were purchased from Merck, Germany. Primary secondary amine was supplied from Agilent technologies, USA. Magnesium sulphate, anhydrous sodium sulphate and sodium chloride were activated in a muffle furnace for 4 h at 350°C and kept in dessicators. The commercial formations of these chemicals were available in local market and purchased.

3.4.2. Preparation of Standards

Standard stock solution of fluopyram and carbosulfan were prepared in methanol. Different concentrations (1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01 $\mu g\ mL^{-1}$) of standard solutions were prepared from stock solution by serial dilution method and by injecting these standards calibration curve was made. All the standard solutions were stored at a temperature of -20°C.

3.4.3. Estimation of Residues of Nematicides

Cowpea pods (2 kg) were collected from nematicide applied plots at harvesting time. The pod samples were chopped, crushed, sub-sampled and extracted following the QuEChERS (Quick, easy, cheap, effective, rugged, safe) method as given by Sharma (2013). Estimation of the residue of fluopyram and carbosulfan were done in LC-MS/MS (Liquid chromatography- Mass spectrometer.

3.4.3.1. Extraction and Clean up

Twenty five gram of ground cowpea pods were taken in a 250 mL centrifuge bottle. Acetonitrile (50 mL) added to HPLC grade and the analyte was extracted. Activated sodium chloride (10 g) was added and centrifuged at 8000 rpm for 8 min. Supernatant (16 mL) was pipetted out and poured to a 50 mL centrifuge tube, 6 g

activated sodium sulphate added to it and vortexed. Twelve mL was pipetted from this and poured to a 15 mL centrifuge tube containing 0.2 g PSA and 1.2 g magnesium sulphate. After mixing well, it was centrifuged @ 2500 rpm for 5 minutes. Three mL was pipetted out from this for LC-MS/MS analysis. The residue was reconstituted in of methanol (1.5 mL). Prior to estimation in LC-MS/MS the residue was filtered through 0.2 micron PVDF filter.

3.4.3.2. Instrumentation

LC-MS/MS

Waters Acquity UPLC system equipped with a reverse phase Atlantis d c-18 (100×2.1 mm, 5µm particle size) column was used for chromatographic separation. The mobile phase for the separation of residues as a gradient system involved two components: such as (A) 10% methanol in water + 0.1% formic acid + 5mM ammonium acetate; (B) 10% water in methanol + 0.1% formic acid + 5mM ammonium acetate. The injection volume was 10µL and the flow rate remained constant at 0.8 mL min^{-1}.

3.4.3.3. Residue Quantification

The quantity of residue was determined based on the peak area of the chromatogram obtained for various insecticides, and it is given below.

Pesticide residue (mg kg^{-1}) = concentration obtained from chromatogram by using calibration curve × Dilution factor

$$\text{Dilution factor} = \frac{\text{Volume of the solvent added (mL)} \times \text{Final volume of extract (mL)}}{\text{Weight of the sample (g)} \times \text{Volume of extract taken for Concentration (mL)}}$$

3.5. STATISTICAL ANALYSIS

Data collected in 3.1 and 3.2 were analysed in completely randomized design and 3.3 in randomized block design. The variables which did not satisfy the basic assumption of ANOVA were square root transformed and analysis was done by using statistical software WASP 2.0. Significant difference among various treatments was assessed at 5% level.

Results

4. RESULT

The studies on screening of vegetable cowpea varieties for resistance to *M. incognita,* evaluation of new nematicide fluopyram against *M. incognita* and management of *M. incognita* in vegetable cowpea using organic amendment, biocontrol agents and chemical nematicides were carried out and the results are presented in this chapter.

4.1 SCREENING OF VEGETABLE COWPEA VARIETIES AGAINST *M. incognita*

Five KAU released varieties and two local collections of vegetable cowpea were screened to study resistance against *M. incognita* under pot culture condition. The initial nematode population in the experiment was 400 juveniles of *M. incognita* 200 cc soil^{-1}. The data on reaction of varieties on nematode population characteristics and number of nodules in roots are presented in Table 2 and 3.

4.1.1. Nematode Population Characteristics

4.1.1.1. Nematode Population in Soil

Data on population of *M. incognita* (Table 2) showed significant variation among different cowpea varieties. Lowest mean nematode population in soil (200cc) was recorded in the rhizosphere of Kadakkal local (7.33) which was found superior to all other varieties screened. The next lowest mean nematode population was recorded in Vellayani Jyothika (709 *M. incognita* juveniles 200cc soil) and it was on par with Vyjayanthi and Vellayani local which recorded mean nematode population of 712.33 and 720.67 in 200cc soil respectively. The variety Geethika was on par with Vellayani local and was inferior to Vellayani Jyothika and Vyjayanthi. Geethika and Lola were statistically on par with mean population of 749.33 and 771.67 *M. incognita* juveniles 200cc soil^{-1} respectively. Geethika showed significant superiority over VS 50 which recoded highest nematode population 200cc soil^{-1} (794.33) but Lola found to be statistically on par with VS 50.

Table 2. Response of cowpea varieties to population of root knot nematode *Meloidogyne incognita*

Treatments	Initial nematode population (200 cc soil)	Final nematode population			Total nematode population (soil+root+females)	Reproduction factor (Rf=Pf/Pi)
		Soil (200 cc)	**Number of juveniles (5g root)**	**Number of females (5g root)**		
Geethika	400.00	749.33 (27.39) ^{bc}	193.33 (13.90) ^{bc}	153.33 (12.38) ^{c}	1096.00 (33.11) ^{c}	2.74 (1.66) ^{bc}
Lola	400.00	771.67 (27.80) ^{ab}	205.33 (14.33) ^{b}	180.67 (13.44) ^{b}	1157.67 (34.02) ^{b}	2.81 (1.68) ^{b}
VS 50	400.00	794.33 (28.20) ^{a}	246.67 (15.70) ^{a}	246.33 (15.69) ^{a}	1287.33 (35.88) ^{a}	3.22 (1.79) ^{a}
Vyjayanthi	400.00	712.33 (26.71) ^{d}	160.33 (12.65) ^{d}	135.00 (11.62) ^{c}	1007.67 (31.74) ^{d}	2.52 (1.59) ^{d}
Vellayani Jyothika	400.00	709 .00 (26.64) ^{d}	141.33 (11.88) ^{e}	103.67 (10.17) ^{d}	954.00 (30.89) ^{e}	2.38 (1.54) ^{e}
Kadakkal Local	400.00	7.33 (2.85) ^{e}	6.33 (2.51) ^{f}	3.67 (1.86) ^{e}	16.33 (4.01) ^{f}	0.04 (0.20) ^{f}
Vellayani Local	400.00	720.67 (26.86) ^{cd}	175.67 (13.25) ^{cd}	148.00 (12.18) ^{c}	1044.67 (3.32) ^{d}	2.61 (1.62) ^{cd}
C D (0.05)	-	(0.568)	(0.699)	(0.785)	(0.619)	(0.041)

Pf – Final nematode population Pi – Initial nematode population

Figures in the parentheses are square root transformed values

4.1.1.2. Nematode Juvenile Population in Root

Nematode population in root (5g) showed significant variation among different varieties screened (Table 2). The mean population of *M. incognita* juveniles in root (5g) was significantly lower in Kadakkal local (7.33). Vellayani Jyothika recorded 141.33 *M. incognita* juveniles 5g root^{-1} and it was inferior to Kadakkal local but showed statistically significant superiority over other varieties. KAU released variety Vyjayanthi recorded 160.33 *M. incognita* juveniles 5g root^{-1} and was on par with Vellayani local (175.67). The performance of Vellayani local was on par with Geethika but was superior to Lola which recoded mean population of 193.33 and 205.33 *M. incognita* juveniles 5g root^{-1} respectively. Lola was statistically on par with Geethika and both these varieties found to be inferior to VS 50 which recorded mean population of 246.67 *M. incognita* juveniles 5g root^{-1}.

4.1.1.3. Number of Females

Analysis of the data (Table 2) showed significant difference in mean number of females in 5g root. Lowest mean number of females in root (5g) was recorded in variety Kadakkal local (3.67) and it was significantly different from all other varieties screened. Vellayani Jyothika recorded 103.67 females 5g root^{-1} and it showed significant superiority to other varieties screened. The KAU released variety Vyjanthi recorded mean number 135.00 females 5g root^{-1}and it was statistically on par with Vellayani local (148.00) and Geethika (153.33). The variety Lola recorded 180.67 females in 5g root and was superior to VS 50 (246.33).

4.1.1.4. Nematode Reproduction Factor

Among the five KAU varieties and two local collections screened, Kadakkal local showed lowest reproduction factor (0.04). Regarding total nematode population also, Kadakkal local recorded least number of nematodes (16.33) compared to VS 50 (1287.33). The second lowest reproduction factor (2.38) and total nematode population (954.00) was recorded in Vellayani Jyothika and it showed statistically significant superiority over other varieties. The variety Vyjayanthi recorded mean reproduction factor of 2.52 and it was on par with Vellayani local (2.61). Similar

trend was observed in total nematode population also which ranged from 1007.67 to 1044. 67. Regarding the mean reproduction factor Vellayani local was on par with Geethika (2.74) and was superior to Lola (2.81). Highest reproduction factor (3.22) and total nematode population (1287.33) was recorded by VS 50 and it was inferior to all varieties (Table 2).

4.1.1.5. Number of Galls in Root

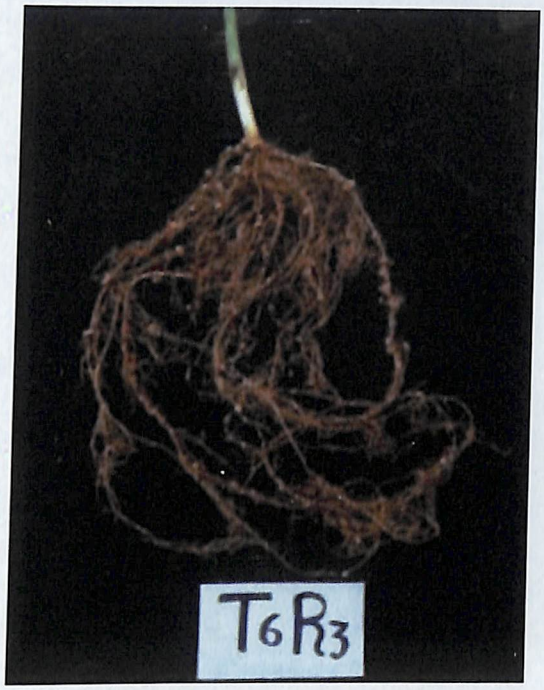
The data (Table 3) showed statistically significant variation in number of galls 5g root^{-1} among different varieties of cowpea. The lowest mean gall number 5g root^{-1} (5.67) was recorded by Kadakkal local and it was significantly different from all other varieties screened. KAU released varieties Vyjayanthi and Vellayani Jyothika recorded mean gall number of 215.30 and 214.67 in 5 g roots, respectively and these two varieties were statistically on par with Vellayani local (230.67 galls 5g root^{-1}). These varieties were inferior to Kadakkal local and showed statistically significant superiority over other three KAU varieties Geethika, Lola and VS 50 which recorded mean gall number of 239.33, 254.33 and 300.67 in 5 g roots, respectively. Geethika was statistically on par with Lola and Vellayani local but was significantly superior to VS 50 (Plate 2).

4.1.1.6. Gall Index (0-5 scale)

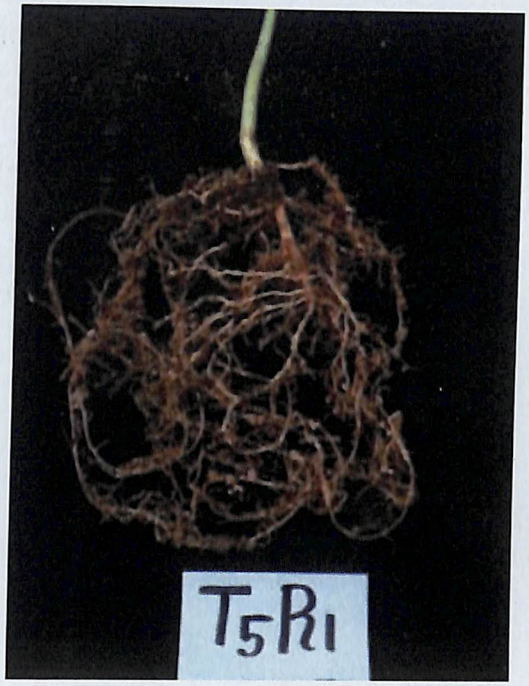
Gall indexing was done based on number of galls present in the roots (5g). Out of seven varieties of vegetable cowpea screened, the variety Kadakkal local was resistant against *M. incognita* infection with root knot index 1. All other six varieties were highly susceptible to *M. incognita* infection with root knot index 5.

4.1.1.7. Number of Egg Masses in Root

Statistical analysis of the data (Table 3) showed significant difference in number of egg masses 5g root^{-1} of different cowpea varieties. The mean number of egg masses 5g root^{-1} ranged from 2.33 to 224.33. Lowest number of egg masses in 5g root (2.33) was recorded in Kadakkal local and it showed statistically significant



Kadakkal local



Vellayani Jyothika

Vyjayanthi

VS 50

Plate 2. Response of cowpea varieties to root knot / galls caused by *Meloidogyne incognita*

Table 3. Response of cowpea varieties to number of galls and eggs of root knot nematode, *Meloidogyne incognita*

Treatments	Number of galls (5g root)	Gall index	Reaction	Number of egg masses (5g root)	Number of eggs (per egg mass)	Number of nodules (5g root)
Geethika	239.33 (15.50) ^{bc}	5.00	HS	180.67 (13.47) ^{b}	110.33 ^{bc} (10.54)	14.67 (3.96) ^{bc}
Lola	254.33 (15.98) ^{b}	5.00	HS	196.67 (14.06) ^{b}	112.33 (10.64) ^{b}	12.33 (3.64) ^{cd}
VS 50	300.67 (17.36) ^{a}	5.00	HS	224.33 (15.01) ^{a}	147 .00 (12.16) ^{a}	9.33 (3.20) ^{d}
Vyjayanthi	215.3 (14.71) ^{d}	5.00	HS	148.33 (12.22) ^{c}	96.67 (9.88) ^{c}	16.67 (4.20) ^{b}
Vellayani Jyothika	214.67 (14.68) ^{d}	5.00	HS	143 .00 (12.00) ^{c}	96.33 (9.86) ^{c}	15.33 (4.04) ^{bc}
Kadakkal Local	5.67 (2.56) ^{e}	1.00	R	2.33 (1.79) ^{d}	63.33 (8.02) ^{d}	22.67 (4.86) ^{a}
Vellayani Local	230.67 (15.22) ^{cd}	5.00	HS	156.33 (12.54) ^{c}	98.67 (9.98) ^{bc}	14.67 (3.94) ^{bc}
C D (0.05)	(0.686)	-	-	(0.739)	(0.759)	(0.528)

HS – Highly susceptible, R - Resistant

Figures in the parentheses are square root transformed values

superiority over other varieties. Vellayani Jyothika recorded 143.00 egg masses 5g root^{-1} and it was statistically on par with Vyjayanthi and Vellayani local which recorded 148.33 and 156.33 egg masses 5g root^{-1} respectively. These four varieties showed statistically significant superiority over Geethika and Lola which recorded mean number of egg masses of 180.67 and 196.67 in 5g roots respectively. Highest number of egg masses 5g root^{-1}(224.33) was recorded by VS50 and it was inferior to all varieties screened.

4.1.1.8. Number of Eggs in Egg Mass

Cowpea varieties showed statistically significant difference in mean number of eggs egg mass^{-1}. The variety Kadakkal local recorded 63.33 eggs egg mass^{-1} and it was significantly superior to all other varieties screened. The variety Vellayani local found to be statistically on par with KAU released varieties Vellayani Jyothika, Vyjayanthi and Geethika in which number of eggs egg mass^{-1} ranged from 98.67 to 110.33. The variety Lola recorded 112.33 eggs egg mass^{-1} and it was statistically on par with the variety Geethika. Maximum number of eggs eggmass^{-1} (147.00) was recorded by VS 50 and it was inferior to all other varieties screened (Table 3).

4.1.2. Number of Nodules in Root

Analysis of the data recorded on number of nodules present in root (5g) showed significant difference in performance of varieties (Table 3). Highest mean number of nodules 5g root^{-1} (22.67) was recorded in Kadakkal local and it was significantly superior to all other varieties. KAU released variety Vyjayanthi recorded 16.67 nodules 5g root^{-1} and it was statistically on par with Vellayani Jyothika (15.33), Geethika (14.67) and Vellayani local (14.67). The varieties Vellayani Jyothika, Vellayani local, Geethika and Lola (12.33) were statistically on par with mean nodule number ranging from 12.33 to 15.33. Lowest mean number of nodules 5g root^{-1} (9.33) was recorded by VS 50.

4.2. EVALUATION OF FLUOPYRAM AGAINST *M. incognita* IN COWPEA

Different doses of fluopyram were tested to find out the effective dosage for management of *M. incognita* in cowpea under pot culture condition. The variety used in this study was VS 50 (highly susceptible variety obtained from 4.1). The data recorded on effect of different dosages of fluopyram on nematode population characteristics of *M. incognita* in cowpea are presented in Table 4.

The mean initial nematode population soil^{-1} (200 cc) ranged from 510.00 to 618.00. No phytotoxicity symptoms were observed in fluopyram treated plants. Nematode penetration and completion of life cycle was observed only in untreated plants. Different stages of *M. incognita* juveniles (J_2, J_3, J_4), adult females and males were not observed in roots of fluopyram treated cowpea plants. Galls (257.75 in 5g root), females (218.75 in 5g root) and egg masses (227.75 in 5g root) were observed in uprooted cowpea plant roots in untreated whereas in fluopyram applied treatments it was zero. In the case of nematode population in soil and root also, no nematodes were observed fluopyram treated plants while in untreated control plants it was 761.50 (200cc soil) and 222.75 (5g root^{-1}). Regarding the number of eggs eggmasses^{-1} also, in untreated plants it was 130.25 while in untreated zero. Number of rhizobium nodules was significantly lower in untreated plants (17.75) while in fluopyram treated plants it ranged from 24.25 to 27.5 in 5g roots of cowpea plants. As all doses of fluopyram 400 SC was effective in reducing the nematode population in soil and root, number of galls (Plate 3), number of females, number of egg masses and number of eggs egg mass^{-1} the lowest dose (250g a.i ha^{-1}) was selected as effective dosage for managing *M. incognita* in cow pea.

4.3. MANAGEMENT OF *M. incognita* IN COWPEA

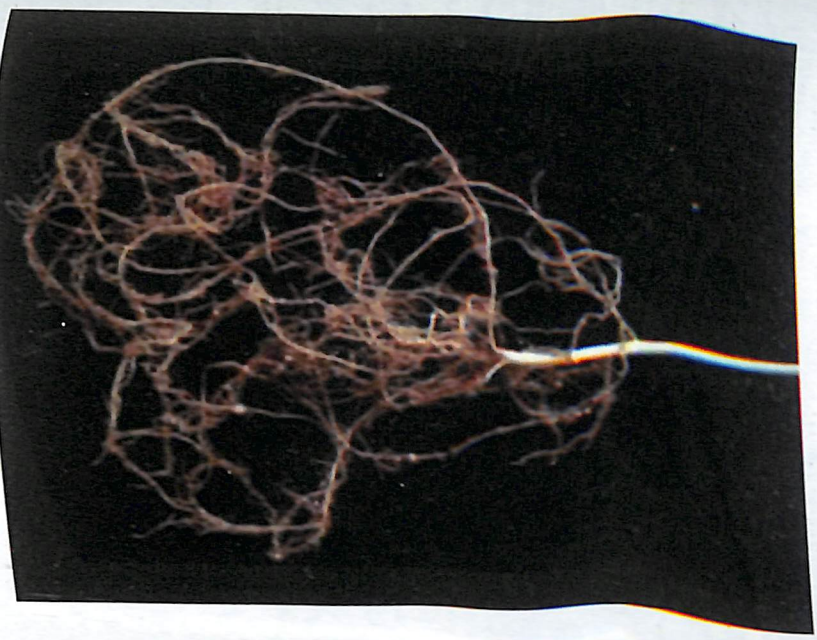
Field experiment was conducted by using the variety VS 50 (highly susceptible variety obtained from 4.1) to study the comparative effect of bio agents (*Purpureocillium lilacinum* and *Stenotrophomonas maltophilia*) and organic amendment (neem cake) in comparison with chemicals fluopyram and carbosulfan. Reisolation of bioagents was done at the time of harvest. Effect of different treatments

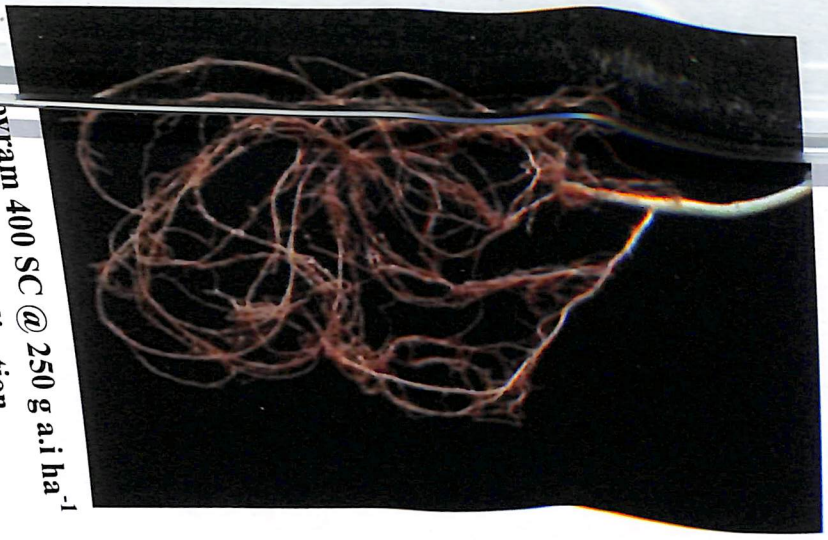
Table 4. Effect of different dosages of fluopyram on nematode population characteristics of *Meloidogyne incognita* in cowpea

Treatments	Initial nematode population (200 cc soil)	Final nematode population			Number of galls (5g root)	Number of egg masses (5g root)	Numbr of eggs (per egg mass)	Number of nodules (5g root)
		Soil (200 cc)	**Root (5g)**	**Number of females (5g root)**				
Fluopyram 400 SC @ 500g a.i ha^{-1} as basal application	543.50 (23.33)	0	0	0	0	0	0	27.75 (5.26) [a]
Fluopyram 400 SC @ 500g a.i ha^{-1} as basal application, 500g a.i ha^{-1} 25 days after first treatment	571.25 (23.91)	0	0	0	0	0	0	25.25 (5.01) [a]
Fluopyram 400 SC @ 250g a.i ha^{-1} as basal application, 250g a.i ha^{-1} 25 days after first treatment	560.50 (23.69)	0	0	0	0	0	0	26.00 (5.07) [a]
Fluopyram 400SC @ 250g a.i ha^{-1} as basal application	566.25 (23.81)	0	0	0	0	0	0	24.25 (4.91) [a]
Untreated control	555.75 (23.60)	761.5	222.75	218.75	257.75	227.75	130.25	17.75 (4.21) [b]
CD(0.05)	NS	-	-	-	-	-	-	(0.629)

Figures in the parentheses are square root transformed values

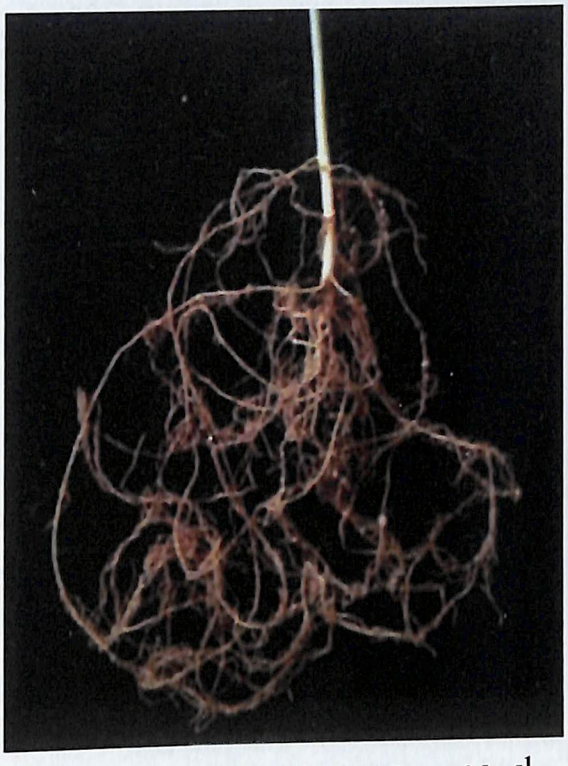
0	0	0	0	27.75 (5.26) [a]
0	0	0	0	25.25 (5.01) [a]
0	0	0	0	26.00 (5.07) [a]
0	0	0	0	24.25 (4.91) [a]
218.75	257.75	227.75	130.25	17.75 (4.21) [b]
-	-	-	-	(0.629)



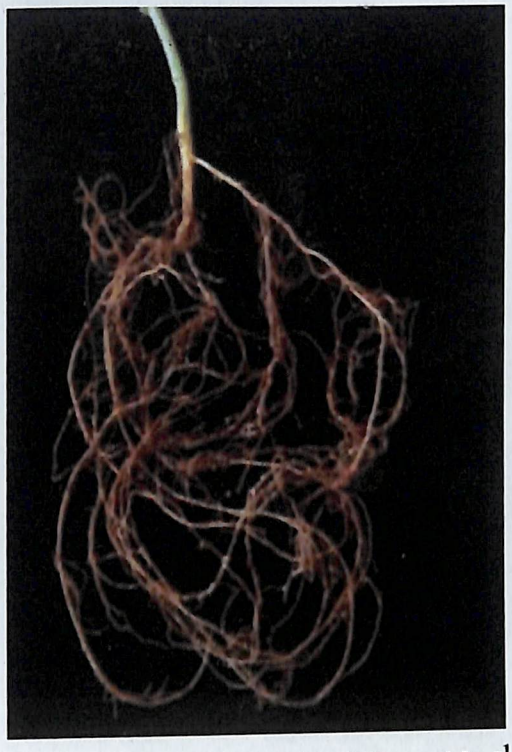


Fluo pyram 400 SC @ 250 g a.i ha^{-1}

l application



Fluopyram 400 SC @ 500 g a.i ha^{-1} as basal application



Fluopyram 400 SC @ 250 g a.i ha^{-1} as basal application

Untreated

Plate 3. Effect of different doses of fluopyram on root knots / galls caused by *Meloidogyne incognita* in cowpea

on nematode population characteristics, yield and root weight of cowpea and are presented in Tables 5 to 7. The initial nematode population in soil (200cc) ranged from 356.00 to 405.00.

4.3.1. Nematode population characteristics

4.3.1.1. Nematode Population in Soil

All the treatments were effective in reducing the nematode population in soil compared to the untreated (675.33 *M. incognita* juveniles 200 cc $soil^{-1}$). The treatment combination, *P. lilacinum* @ 10 g m^{-2} + neem cake @ 50 g m^{-2} significantly reduced nematode population in soil (200 cc) and found statistically on par with chemical treatment fluopyram 400SC @ 250 g a.i ha^{-1} (38.87 *M. incognita* juveniles 200cc $soil^{-1}$). It was followed by carbosulfan @ 5 g m^{-2} which recorded mean nematode population of 80.67 in 200 cc soil and was significantly different from other treatments. Treatment with *S. maltophilia* @10 g m^{-2} + neem cake@ 50 g m^{-2} and *P. lilacinum* @ 20 g m^{-2} were statistically on par with mean nematode population of 104.33 and 116.00 *M. incognita* juveniles 200 cc $soil^{-1}$, respectively. Other treatments in the order of effectiveness were neem cake @100 g m^{-2} and *S. maltophilia* @ 20 g m^{-2} which recorded mean population of 150.33 and 142.33 *M. incognita* juveniles 200 cc $soil^{-1}$ and effect of these two treatments was statistically on par (Table 5).

4.3.1.2. Nematode Population in Root

Nematode population in root (5g) estimated at the time of harvest varied significantly among treatments (Table 5). All the treatments were effective in reducing the nematode population in root. The mean nematode population in 5g root ranged from 22.67 to 87.67 in different treatments against 194.00 in untreated. Effect of *P. lilacinum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} was statistically on par with fluopyram 400 SC @ 250 g a.i ha^{-1}giving mean number of 25.33 and 22.67 *M. incognita* juveniles 200cc $soil^{-1}$ respectively. The mean nematode population in root (5g) was significantly lower in carbosulfan treatment (37.67) but was inferior to fluopyram and *P. lilacinum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2}. Treatment with *S. maltophilia* @ 10 g m^{-2} + neem cake @ 50 g m^{-2} was inferior to carbosulfan which

Table 5. Effect of different treatments on population of *Meloidogyne incognita* in cowpea at the time of harvest

Treatments	Initial nematode population (200 cc soil)	Final nematode population			Total nematode population (soil+root+females)	Reproduction factor (Rf=Pf/Pi)
		Soil (200cc)	**Root (5g)**	**Number of females (5g root)**		
Purpureocillium lilacinum @ 20 g m^{-2}	360.67 (18.98)	116.00 (10.76) ^{c}	60.33 (7.76) ^{d}	41.00 (6.40) ^{d}	217.33 (14.74)^{d}	0.61 (0.781) ^{c}
Neem cake @ 100 g m^{-2}	369.00 (19.23)	150.33 (12.25) ^{b}	87.67 (9.36) ^{b}	72.33 (8.50) ^{b}	310.33 (17.61)^{b}	0.84 (0.98) ^{b}
P. lilacinum @ 10 g m^{-2} + neem cake @ 50 g m^{-2}	356.00 (18.89)	46.67 (6.83) ^{e}	25.33 (5.03) ^{g}	16.33 (4.02) ^{f}	88.33 (9.40)^{g}	0.25 (0.50)^{f}
Stenotrophomonas maltophilia @ 20 g m^{-2}	357.33 (18.93)	142.33 (11.92) ^{b}	76.67 (8.75) ^{c}	56.67 (7.52) ^{c}	275.67 (16.60)^{c}	0.77 (0.88) ^{b}
S. maltophilia @ 10 g m^{-2} + neem cake @ 50 g m^{-2}	379.67 (19.51)	104.33 (10.21) ^{c}	48.33 (6.95) ^{e}	33.67 (5.79) ^{d}	186.33 (13.64)^{e}	0.49 (0.70)^{d}
Fluopyram 400 SC @ 250g a.i ha^{-1}	405.00 (20.14)	38.67 (6.20) ^{e}	22.67 (4.75) ^{g}	12.67 (3.55) ^{f}	74.00 (8.60)^{h}	0.18 (0.43) ^{f}
Carbosulfan 6G @ 5 g m^{-2}	371.33 (19.27)	80.67 (8.98) ^{d}	37.67 (6.14) ^{f}	25.00 (4.99) ^{e}	143.33 (11.97)^{f}	0.39 (0.62) ^{e}
Untreated control	359.00 (18.96)	675.33 (25.98) ^{a}	194.00 (13.93) ^{a}	191.67 (13.84) ^{a}	1061.00 (32.57)^{a}	2.97 (1.72) ^{a}
CD(0.05)	NS	(0.657)	(0.441)	(0.640)	(0.605)	(0.077)

Pf – Final nematode population Pi – Initial nematode populationFigures in the parentheses are square root transformed values

recorded 48.33 *M. incognita* juveniles 5g root^{-1}. The mean number of *M. incognita* juveniles was significantly lower in treatment with *P. lilacinum* @ 20 g m^{-2} and *S. maltophilia* @ 20 g m^{-2} in which the mean nematode population root^{-1} (5g) being 60.33 and 76.67 respectively and these two treatments showed significant superiority over neem cake @ 100 g m^{-2} (87.67).

4.3.1.3. Nematode Reproduction Factor

Reproduction factor of nematodes varied from 0.18 to 0.84 in different treatments against 2.97 in untreated. Mean reproduction factor of *M. incognita* in combination treatment *P. lilacinum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} was 0.21 and it was on par with fluopyram 400 SC @ 250g a.i ha^{-1} in which mean reproduction factor was 0.18. Total nematode population in these two treatments ranged from 74.00 to 88.33. In carbosulfan application @ 5 g m^{-2} mean reproduction factor of *M. incognita* was 0.39 and mean total nematode population was 14.33 and it was inferior to *P. lilacinum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} and fluopyram. Reproduction factor of *M. incognita* in *S. maltophilia* @10 g m^{-2} + neem cake @ 50 g m^{-2} was 0.49 and the total nematode population in this treatment was 186.33. It showed significant superiority over *P. lilacinum* @ 20 g m^{-2}, *S. maltophilia* @ 20 g m^{-2} and neem cake @ 100 g m^{-2} which recorded reproduction factor of 0.61, 0.77 and 0.84 respectively. Total nematode population in these treatments ranged from 217.33 to 310.33 against 1061.00 in untreated (Table 5).

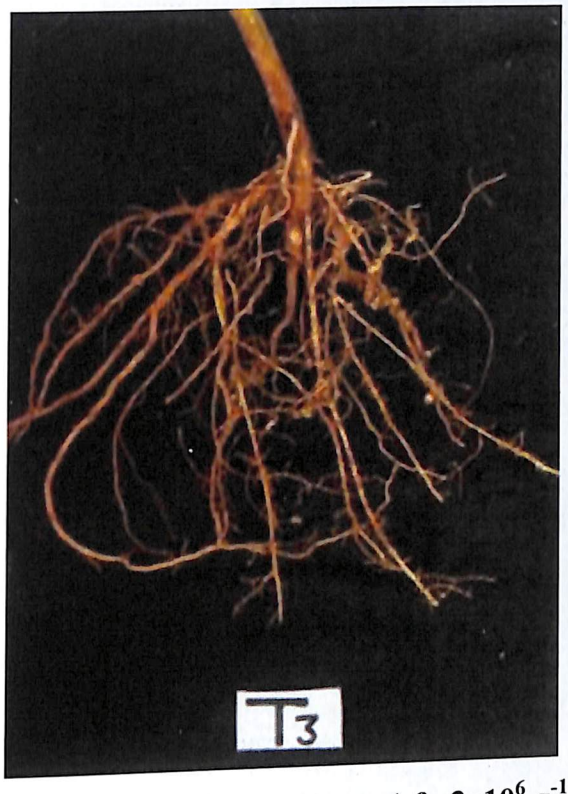
4.3.1.4. Number of Galls

The data (Table 6) regarding number of galls root^{-1} (5g) showed the effectiveness of all treatments in lowering gall formation in roots of cow pea. Plants treated with *P. lilacinum* @10 g m^{-2} + neem cake @ 50 g m^{-2} recorded 27.33 galls root^{-1} (5g) with gall index of 1.66. It was statistically on par with fluopyram 400 SC @ 250g a.iha^{-1} which recorded 22.33 galls 5 g root^{-1} with gall index of 1.33. Mean number of galls in plants treated with carbosulfan 6G @ 5g m^{-2} was 38.33 and it was statistically on par with *S. maltophilia* @10 g m^{-2} + neem cake @ 50 g m^{-2} (38.67 galls 5g root^{-1}). Gall index was 2.00 and 2.33. These two treatments showed

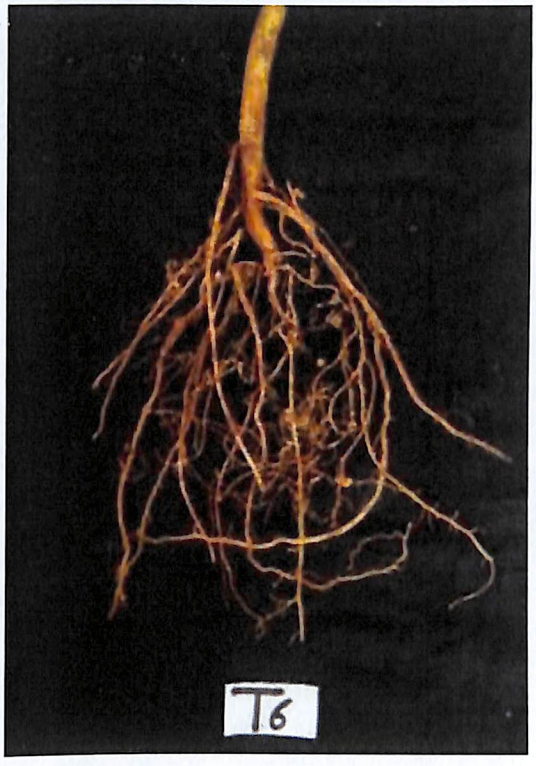
Table 6. Effect of different treatments on number of galls and eggs of *Meloidogyne incognita* in cowpea at the time of harvest

Treatments	Number of galls (5g root)	Gall index	Number of egg masses (5g root)	Number of eggs (one egg mass)	Number of nodules (5g root)
Purpureocillium lilacinum @ 20 g m^{-2}	58.67 (7.65) ^{c}	3.00	57.67 (7.59) ^{d}	75.67 (8.70) ^{ef}	16.33 ^{cd}
Neem cake @ 100 g m^{-2}	88.67 (9.41) ^{b}	4.00	82.67 (9.09) ^{b}	98.67 (9.93) ^{b}	14.33 ^{d}
P. lilacinum @ 10 g m^{-2} + neem cake @ 50 g m^{-2}	27.33 (5.22) ^{e}	1.67	22.33 (4.72) ^{f}	65.67 (8.10) ^{g}	29.33 ^{a}
Stenotrophomonas maltophilia (@ 20 g m^{-2}	77.67 (8.81) ^{b}	3.67	71.33 (8.44) ^{c}	91.67 (9.57) ^{bc}	13.33 ^{d}
S. maltophilia @ 10 gm^{-2} + neem cake @ 50 g m^{-2}	38.67 (6.21) ^{d}	2.33	36.67 (6.05) ^{e}	87.33 (9.34) ^{cd}	16.67 ^{cd}
Fluopyram 400 SC @ 250g a.i ha^{-1}	22.33 (4.71) ^{e}	1.33	17.67 (4.19) ^{f}	71.67 (8.64) ^{fg}	21.33 ^{b}
Carbosulfan 6G @ 5 g m^{-2}	38.33 (6.18) ^{d}	2.00	33.67 (5.79) ^{e}	81.00 (9.00) ^{de}	19.33 ^{bc}
Untreated check	212.33 (14.75) ^{a}	5.00	193.33 (13.90) ^{a}	135.33 (11.63) ^{a}	9.33 ^{e}
CD(0.05)	(0.724)	-	(0.575)	(0.456)	3.956

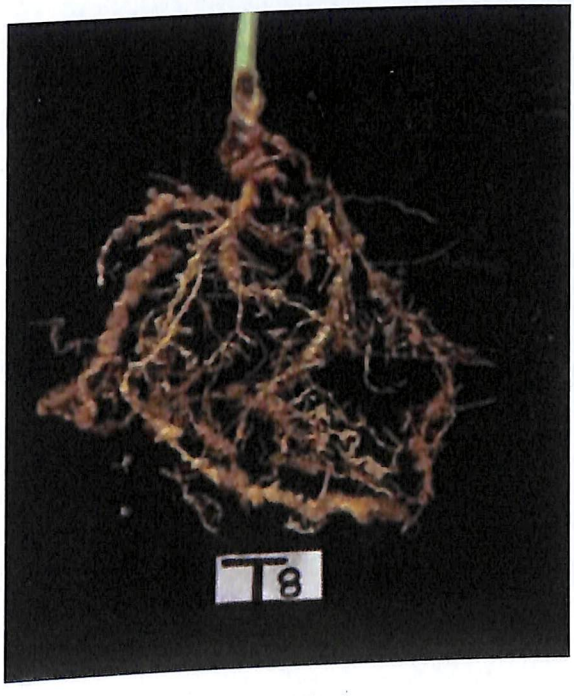
Figures in the parentheses are square root transformed values



Purpureocillium lilacinum (cfu $2x10^6$ g^{-1}) @ 10 gm^{-2} + neem cake @ 50 g m^{-2}



Fluopyram 400 SC @ 250 g a.i ha^{-1}



Untreated

Plate 4. Effect of different treatments on galls caused by *Meloidogyne incognita*

significant superiority over *P. lilacinum* @ 20 g m^{-2}, *S. maltophilia* @ 20 g m^{-2} and neem cake @ 100 g m^{-2} giving 58.67 to 88.67 galls 5 g $root^{-1}$. Among these treatments effect of *P. lilacinum* @ 20 $gm^{-2}$was significantly to superior to *S. maltophilia* @ 20 g m^{-2} and neem cake @ 100 g m^{-2} with gall index of 3.00. The gall index of neem cake @ 100 g m^{-2} treated plants was 4.00 and it was statistically on par with *S. maltophilia* @ 20 g m^{-2} with gall index of 3.67. Both the treatments showed significant superiority to untreated which recorded 212.33 galls 5g $root^{-1}$ and gall index of 5 (Plate 4).

4.3.1.5. Number of Females

Analysis of the data (Table 6) revealed that all the treatments significantly reduced the number of females in 5g root. The mean number of females $root^{-1}$ (5g) ranged from 12.67 to 72.33 in different treatments against 191.67 in untreated. *P. lilacinum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} recorded 16.33 females $root^{-1}$. It was statistically on par with fluopyram 400 SC @ 250 g a.i ha^{-1} in which mean number of females 5g $root^{-1}$ was 12.67. Both the treatments were superior to other treatments in reducing number of females. Carbosulfan applied treatment recorded 25.00 females in 5g cowpea root and it was inferior to *P. lilacinum* @10 g m^{-2}+ neem cake @ 50 g m^{-2} and fluopyram treatments. Plants treated with *S. maltophilia* @10 g m^{-2}+ neem cake @ 50 g m^{-2} recorded 33.67 females $root^{-1}$ (5g) and it was on par with *P. lilacinum* @ 20 g m^{-2}. Plants treated with *P. lilacinum* @ 20 g m^{-2} recorded 41.00 females 5g $root^{-1}$ and was significantly superior to *S. maltophilia* treated plants which recorded 56.67 females 5g $root^{-1}$. Neem cake @ 100 g m^{-2} (72.33 females 5g $root^{-1}$) was significantly superior to untreated (191.67 females 5g $root^{-1}$) but was inferior *S. maltophilia* @ 20 g m^{-2}.

4.3.1.6. Number of Egg masses

There was statistically significant difference in number of egg masses recorded in different treatments (Table 6). All the treatments significantly reduced number of egg masses in 5 g cowpea root compared to untreated (193.33 egg masses 5g $root^{-1}$). The mean number of eggmasses 5g $root^{-1}$ recorded in *P. lilacinum* @ 10 g

m^{-2}+ neem cake @ 50 g m^{-2} was 22.33 and it was statistically on par with fluopyram 400 SC @ 250 g a.i ha^{-1} in which mean number of egg masses $root^{-1}$ (5g) was recorded 17.6. It was followed by carbosulfan 6G @ 5 g m^{-2} which recorded 33.67 egg masses $root^{-1}$ (5g) which was inferior to above two treatments. Effect of *S. maltophilia* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} found as effective as carbosulfan treatment which recorded 36.67 egg masses $root^{-1}$ (5g). Mean number of eggmasses in *P. lilacinum* @ 20 g m^{-2} treatment was 57.67 and it was significantly superior to *S. maltophilia* @ 20 g m^{-2} (71.33) and neem cake @ 100 g m^{-2} (82.67).

4.3.1.7. Number of Eggs Egg Mass^{-1}

Analysis of the data regarding number of eggs egg $mass^{-1}$ showed that all the treatments were effective in reducing number of eggs in egg mass compared to untreated (135.33). Lowest number of eggs egg $mass^{-1}$ (65.67) was recorded in *P. lilacinum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} and it was statistically on par with fluopyram 400 SC @ 250 g a.i ha^{-1}(71.67). *P. lilacinum* @ 20 g m^{-2} was found as effective as chemical treatments (fluopyram and carbosulfan) in reducing the number of eggs egg $mass^{-1}$. Mean number of eggs egg $mass^{-1}$ in these treatments ranged from 75.67 to 81.00. Next best treatment, *S. maltophilia* @ 10 g m^{-2}+ neem cake @ 50 gm^{-2} (87.33 eggs egg $mass^{-1}$) was found statistically on par with carbosulfan 6G @ 5 g m^{-2} and *S. maltophilia* @ 20 g m^{-2} (91.67 eggs egg $mass^{-1}$). Treatment with neem cake @ 100 g m^{-2} recorded 98.67 eggs egg $mass^{-1}$ and it was equally effective to *S. maltophilia* @ 20 g m^{-2} (Table 6).

4.3.1.8. Number of Nodules in Root

All the treatments significantly increased the rhizobium nodules in cowpea root (Table 6). Highest mean number of nodules (29.33 in 5 g roots) was recorded in *P. lilacinum* @10 g m^{-2}+ neem cake @ 50 g m^{-2} followed by fluopyram 400 SC @ 250 g a.i ha^{-1} (21.33 nodules 5g $root^{-1}$). Effect of these two treatments was significantly different. Mean number of nodules $root^{-1}$ (5g) in carbosulfan 6G @ 5 g m^{-2} was 19.33 and it was statistically on par with fluopyram treatment. *S. maltophilia* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} was equally effective to *P. lilacinum* @ 20 g m^{-2}

and carbosulfan treatments giving 16.33 to 19.33 nodules 5g root^{-1}. Treatment with *S. maltophilia* @ 20 g m^{-2} was statistically on par with neem cake @ 100 g m^{-2}, *P. lilacinum* @ 20 g m^{-2} and *S.maltophilia* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} and mean number of nodules 5g root^{-1} in these treatments ranged from 13.33 to 16.67. Lowest number of nodules 5g root^{-1} (9.33) was observed in untreated.

4.3.4. Root Weight

Analysis of the data (Table 7) showed that all the treatments significantly increased the root weight of cowpea. Highest mean root weight (13.20 g) was recorded by *P. lilacínum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} treated plants and it showed statistically significant superiority over all other treatments. Next best treatments in the order of effectiveness were fluopyram 400 SC @ 250 g a.i ha^{-1}, carbosulfan 6G @ 5 g m^{-2}, *S.maltophilia* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2}, *P. lilacinum* @ 20 g m^{-2}, *S. maltophilia* @ 20 g m^{-2} and neem cake @ 100 g m^{-2} with mean root weight ranging from 8.29 to 12.32 g. Effect of *S. maltophilia* @ 20 g m^{-2} found to be statistically on par with neem cake and it showed significant superiority over untreated (7.14 g).

4.3.3. Yield

There was statistically significant variation in pod weight recorded in different treatments (Table 7). Highest yield (20.04 kg plot^{-1}) was recorded in *P. lilacinum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} and it was statistically on par with fluopyram 400SC @ 250 g a.i ha^{-1}(19.89 kg plot^{-1}). Carboulfan treated plants recorded mean yield of 18.02 kg plot^{-1} followed by *S. maltophilia* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} (17.07 kg plot^{-1}) and effect of these two treatments was significantly different. Yield recorded by *P. lilacinum* @ 20 g m^{-2} was 16.08 kg plot^{-1} and it was significantly superior to *S. maltophilia* @ 20 g m^{-2} and neem cake @ 100 g m^{-2}. Effect of *S. maltophilia* and neem cake was statistically on par with mean yield of 15.45 and 15.27 kg plot^{-1}respectively and these two treatments showed significant superiority over untreated (12.96 kg plot^{-1}). Per ha yield in treated plants ranged from 12.73 to 16.70 tones while in untreated 10.80 tones.

Table 7. Effect of different treatments on root weight and yield of cowpea

Treatments	Root weight (g)	Pod weight (kg plot^{-1})	Yield (t ha^{-1})
Purpureocillium lilacinum (cfu 2x10^6 g^{-1}) @ 20 g m^{-2}	9.07 ^{e}	16.08 ^{d}	13.40 ^{d}
Neem cake @ 100 g m^{-2}	8.29 ^{f}	15.27 ^{e}	12.73 ^{e}
P. lilacinum (cfu 2x10^6 g^{-1}) @ 10 gm^{-2} + neem cake @ 50 g m^{-2}	13.20 ^{a}	20.04 ^{a}	16.70 ^{a}
Stenotrophomonas maltophilia (cfu 2x10^6 g^{-1}) @ 20 g m^{-2}	8.55 ^{f}	15.45 ^{de}	12.87 ^{de}
S. maltophilia (cfu 2x10^6 g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}	9.71 ^{d}	17.07 ^{c}	14.22 ^{c}
Fluopyram 400 SC @ 250g a.i ha^{-1}	12.32 ^{b}	19.89 ^{a}	16.58 ^{a}
Carbosulfan 6G @ 5 g m^{-2}	11.27 ^{c}	18.02 ^{b}	15.02 ^{b}
Untreated check	7.14 ^{g}	12.96 ^{f}	10.80 ^{f}
CD(0.05)	0.462	0.708	0.589

4.3.4. Re-isolation of Bioagents

Data presented in Table 8 on population of *P. lilacinum* and *S. maltophila* estimated from soil at the time of harvest revealed that the number of cfu g soil^{-1} was higher in combination treatment, *P. lilacinum* @ 10 g m^{-2}+ neem cake @ 50 g m^{-2} and *S. maltophilia* @ 10 g m^{-2} + neem cake @ 50 g m^{-2} which recorded 8.33×10^{3} and 5.13×10^{5} respectively. Number of cfu g soil^{-1} with *P. lilacinum* @ 20 g m^{-2} and *S. maltophila* was 2.17×10^{3} and 1.23×10^{5} respectively.

4.4. CHEMICAL RESIDUE LEVELS AT HARVEST TIME

The residue of fluopyram and carbosulfan in cow pea pods were estimated at the time of harvest. Mean residues of fluopyram and carbosufan in cowpea pods were below the limit of quantification (LOQ-0.05 mg kg^{-1}) (Table 9).

Table 8. Population of bioagents in the rhizosphere of cowpea at the time of harvest

Treatments	cfu g soil^{-1}
Purpureocillium lilacinum @ 20 g m^{-2}	2.17×10^{3}
P.lilacinum @ 10 g m^{-2}+ neem cake @ 50 g m^{-2}	8.33×10^{3}
Stenotrophomonas maltophilia @ 20 g m^{-2}	1.23×10^{5}
S. maltophilia @ 10 g m^{-2}+ neem cake @ 50 g m^{-2}	5.13×10^{5}

Table 9. Residue of fluopyram and carbosulfan in cowpea pods

Harvest time residue	Mean residue (mg kg^{-1})	
	Fluopyram 400 SC	Carbosulfan 6G
	<LOQ	<LOQ

LOQ - Limit of quantification LOQ-0.05 mg kg^{-1}

Discussion

5. DISCUSSION

Cowpea (*Vigna unguiculata* (L.) Walp.) is mainly grown as a vegetable crop in Kerala. It is a protein rich nutritious component in human diet. Cowpea can be used as vegetable, forage, fodder and green manure. Root knot nematode, *Meloidogyne incognita* is one of the major pests of cowpea. In India, it causes 31 to 71 per cent yield loss in cowpea (Ali, 1997). Besides direct damage, the nematode also serves as predisposing agent in development of disease complexes with fungi and bacteria. Indiscriminate use of chemicals for the management of nematode may result in health issues due to the presence of pesticide residues in harvested produce.

Resistant varieties offer the cheapest and most convenient method of pest management. Resistant varieties that deter the penetration and development of nematodes will aid in the production of safe and quality produce without pesticide residues. Identification of *M. incognita* resistant variety is very important as the nematode act a predisposing factor of *Fusarium* wilt disease in cowpea which is common in Kerala. Hence present study was conducted to assess the reaction of varieties against *M. incognita* which can be used as a cost effective component in eco-friendly nematode management strategy.

Plant parasitic nematodes can be effectively controlled by using chemical nematicides. The primary advantage of chemical control over other method is the quick reduction of nematode population within days after application of chemical. Commonly used red labeled nematicides *viz,* phorate and carbofuran have been banned in Kerala. Fluopyram is a new fungicide having nematicidal property, which is grouped under green labeled chemical. Being new chemical, present investigation was carried out to standardize the dosage of fluopyram for the management of *M. incognita* in cowpea.

Field experiment was conducted by using the susceptible variety from screening experiment to study the comparative effect of bioagents (*Purpureocillium lilacinum* and *Stenotrophomonas maltophilia*) and organic amendment (neem cake) in comparison with chemicals fluopyram and carbosulfan. The results were assessed in

terms of reduction in nematode population (number of galls, number of egg masses, nematode population in soil and root) and increase in root weight, yield and number of nodules. Persistence of biocontrol agents in soil was studied by re-isolating the bioagents at the time of harvest. Residues of nematicides in cowpea pods were determined at the time of harvest. The results of the study are discussed in this chapter.

5.1. SCREENING OF VARITIES FOR RESISTANCE

The results presented in 4.1.1 on response of five KAU released varieties (Geethika, Lola, VS 50, Vyjayanthi, Vellayani Jyothika) and two local collections (Kadakkal local and Vellayani local) of vegetable cowpea revealed that population build up of nematodes was lowest in rhizosphere of Kadakkal local (7.33 *M. incognita* juveniles 200cc soil^{-1}). Highest nematode population (794.33 *M. incognita* 200cc soil^{-1}) was observed in KAU released variety VS 50. Kadakkal local recorded 99.08 per cent reduction in nematode population over variety VS 50 (Fig 1). Regarding the nematode population in root also, Kadakkal local differed significantly from other varieties screened recording the least number of juveniles (6.33 *M. incognita* juveniles 5g root^{-1}). Minimum number of females (3.67 in 5 g roots) was recorded in Kadakkal local while in other varieties it ranged from 103.67 to 180.67. Highest nematode population in root and number of females was recorded in VS 50. Kadakkal local showed 97.43 and 98.51 per cent reduction in nematode population in root and number of females over VS 50 respectively (Fig 1). Data on total nematode population revealed that nematode multiplication in Kadakkal local was significantly lower (16.33) compared to other varieties screened. Kadakkal local recoded the lowest reproduction factor (0.04). Reproduction factor in other varieties ranged from 2.52 to 3.22. This finding is in line with Adegbite *et al.* (2006) who screened 15 varieties of cowpea against *M. incognita* and reported lowest reproduction factor (0.45) in the resistant variety, IT84S2246-4.

Nematode population in soil, root and number of females was significantly reduced in Kadakkal local compared to other varieties screened. VS 50 was found to be highly susceptible to *M. incognita* infection as the recovery of nematode

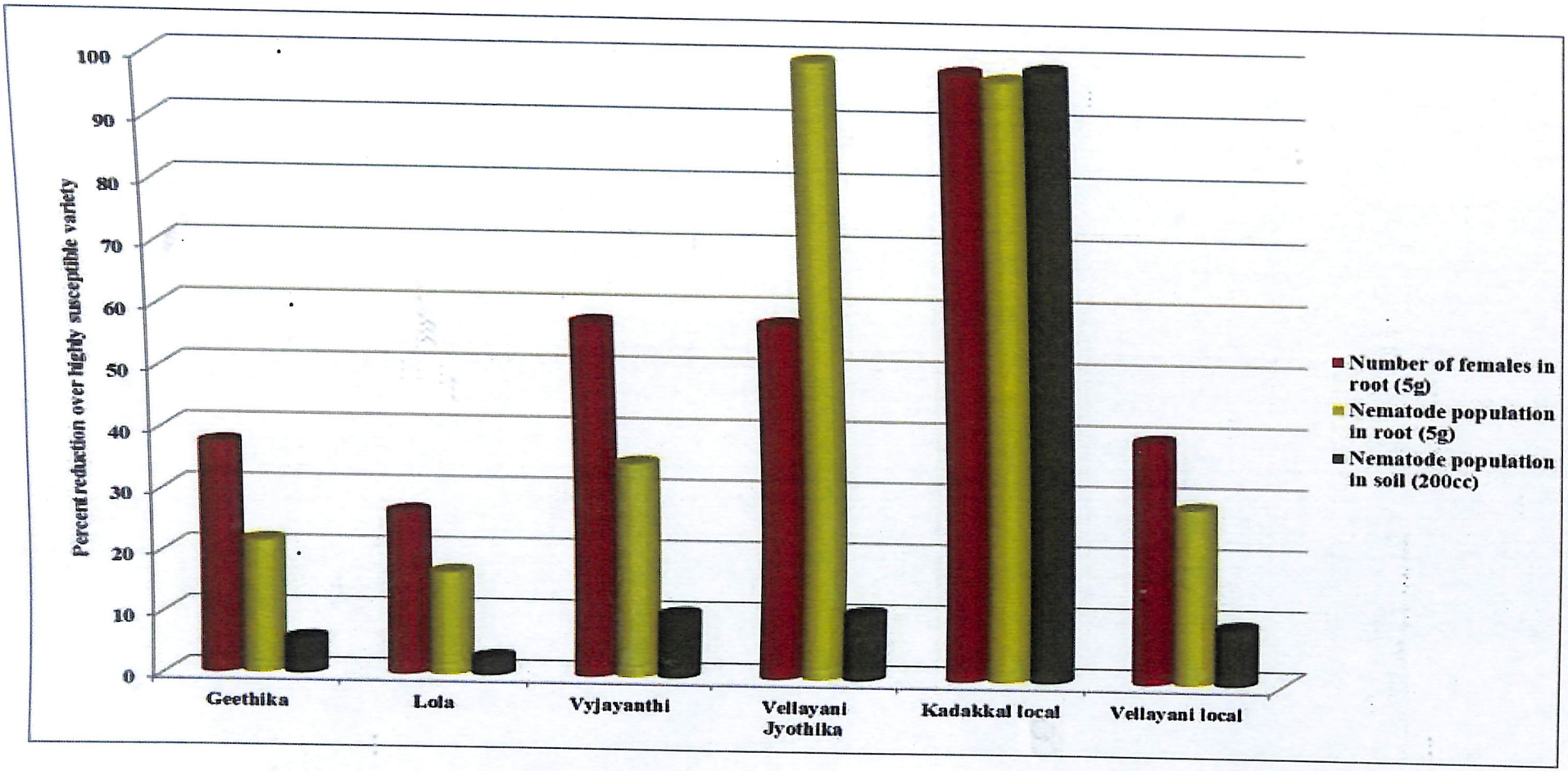


Fig 1. Response of cowpea varieties to population of *Meloidogyne incognita*

population in soil, root and number of females found to be highest in VS 50. The other varieties Vellayani Jyothika, Vyjayanthi, Geethika, Lola and local collection Vellayani local also showed 16.76 to 57.96 per cent reduction in nematode population in root and number of females compared to VS 50. Based on the data it is clear that nematode multiplication was least in Kadakkal local while VS 50 found to be the most susceptible variety. Tariq *et al.* (2016) reported reduced total nematode population in resistant varieties of fenugreek UM-72 and UM-178 against highly susceptible variety UM-90. Reproduction factor of *M. incognita* in varieties UM-72 and UM-178 recorded as 0.8 and 0.9 respectively while reproduction factor in highly susceptible variety, UM-90 was 13.4. Resistance to nematode infestation can be either due to pre-infection resistance where the nematodes cannot enter the roots due to the presence of toxic chemicals or post infection resistance in which nematodes are able to penetrate but fail to develop. Root exudates of plants play an important role for attracting and repelling nematodes (Huang, 1985).

The lowest mean number of galls (5.67 in 5 g roots) was recorded in Kadakkal local with root-knot index 1. KAU released variety, VS 50 recorded 300.67 galls 5g root^{-1}and root-knot index was 5. Number of galls in Kadakkal local was reduced significantly (98.12 per cent as compared to highly susceptible variety VS 50 (Fig 2). In other varieties such as Vellayani Jyothika, Vyjayanthi, Lola, Vellayani local and Geethika number of galls reduced to 28.60, 28.38, 25.43, 23.28 and 20.40 percent compared to VS 50. Kadakkal local found to be resistant and this finding is reported first time in this study. Similar resistance reactions in carrot, brinjal, ginger and african yam cultivars against *M. incognita* were reported by Arya and Tiagi (1982), Ravichandra *et al.* (1988), Eapen *et al.* (1988) and Mohandas *et al.* (1988). Das *et al.* (2008) observed significant difference in vacuolation between the resistant and susceptible cowpea genotypes. The large vacuoles in resistant cowpea roots were filled with hydrolases and toxins that deprived the nematodes of nutrients and led to giant cell collapse, whereas in susceptible roots nematode feeding did not cause the formation of large vacuoles. Seo *et al.* (2014) reported that during screening of carrot lines for resistance to *M. incognita* formation of well-developed giant cells was observed in susceptible lines after seven weeks of nematode inoculation and these

enlarged giant cells in the stelar region resulted formation of root knots. In resistant varieties instead of giant cells necrotic layers were observed around the nematodes which resulted in retardation of nematode growth.

With regard to number of egg masses in root the lowest number was recorded by Kadakkal local (2.33) and highest number was recorded in VS 50 (224.33). In the case of number of eggs in an eggmass also the lowest was recorded by Kadakkal local (63.33) followed by Vellayani Jyothika, Vyjayanthi, Vellayani local, Geethika, Lola and VS 50. Highest number was observed in VS 50 (147.00). The percentage reduction in production of eggs compared to the susceptible variety (VS 50) ranged from 23.59 to 56.92. The performance of Kadakkal local was significantly better than other varieties in reducing the number of galls, egg masses, eggs in egg mass (Fig 2). Similar results were reported by Nisha and Sheela (2015) in coleus and Patra and Nayak (2019) in tomato. The higher number of females, egg masses and eggs in eggmass in VS50 indicates its weakness in defense mechanism. They lack genes to stop penetration, development and reproduction of nematode. According to Olowe (2009) it may be due to low level of glycosides in plant tissue on which the enzymes glycosidase from nematodes may act upon to liberate free phenol for suppression of nematode reproduction and development.

Maximum number of rhizobium nodules was observed in resistant local collection Kadakkal local (22.67 nodules 5g root^{-1}). Highly susceptible variety VS 50 recorded 9.33 nodules 5g root^{-1}. Vyjayanthi, Vellayani Jyothika, Geethika, Vellayani local and Lola showed 78.67, 64.31, 57.23, 57.23 and 24.33 per cent increase in number of nodules respectively compared to VS 50. Reduction in number of nodules due to *M. incognita* infestation was already reported by several authors. Duponnois *et al.* (2000) reported that root knot nematodes reduced rhizobium nodules in *Acacia holosericea*. Izuogu *et al.* (2019) reported that number of rhizobium nodules was significantly higher in *M. incognita* resistant variety of cow pea IT89KD-288.

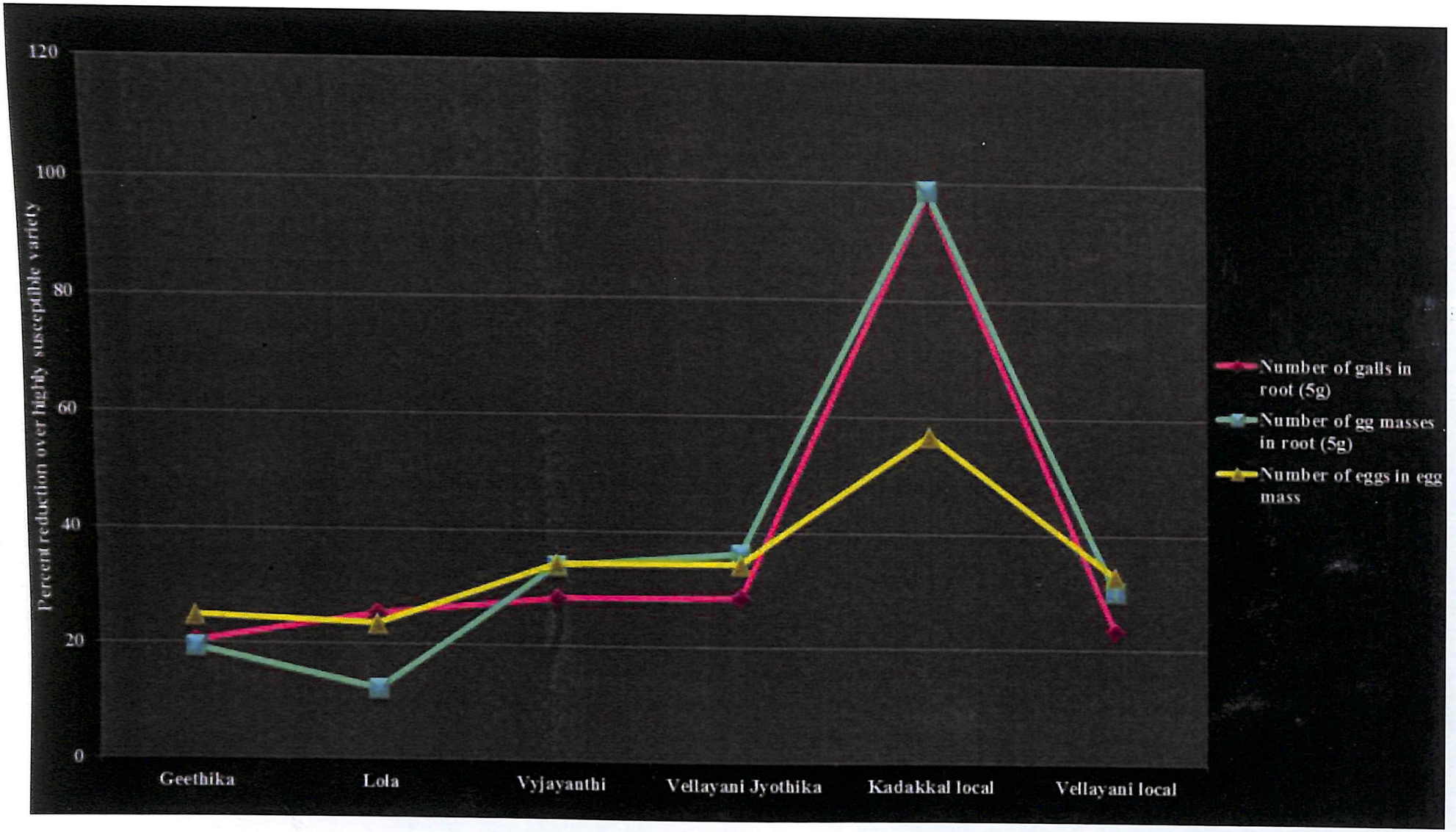


Fig 2. Response of cowpea varieties to number of galls and egg masses of *Meloidogyne incognita*

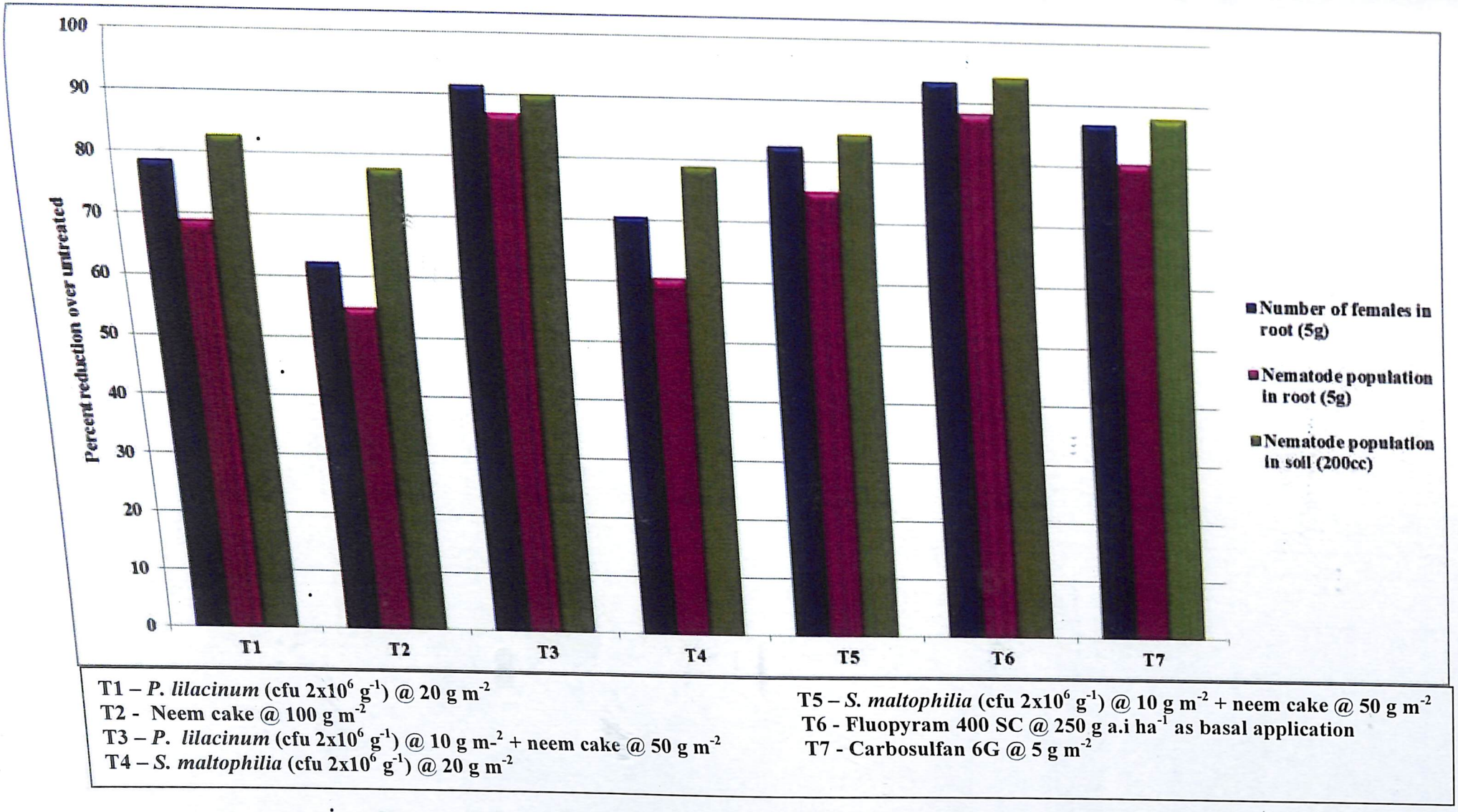
5.2. EVALUATION OF FLUOPYRAM AGAINST *M. INCOGNITA* IN COWPEA

Efficacy of green labelled fungicide fluopyram in managing *M. incognita* was conducted as pot culture experiment using susceptible variety VS 50. Different dosages *viz.* fluopyram 400 SC @ 500g a.i ha^{-1} as basal application, fluopyram 400 SC @ 500g a.i ha^{-1} as basal application + 500g a.i ha^{-1} 25 days after first treatment, fluopyram 400 SC @ 250g a.i ha^{-1} as basal application + 250g a.i ha^{-1} 25 days after first treatment, fluopyram 400 SC @ 250g a.i ha^{-1} as basal application were applied as soil drench to nematode infested soil. The initial population of nematodes ranged from 510 to 618 in 200 cc soil. Results presented in para 4.2 revealed that no nematode penetration was observed in roots of cowpea plants treated with fluopyram while in roots of untreated control plants, galls (257.75 in 5g root), females (218.75 in 5g root), eggmasses (227.75 in 5g root) and eggs in eggmass (130.25) were observed. In the case of nematode population in soil and root also, no nematodes were observed fluopyram treated plants while in untreated control plants it was 761.50 (200cc soil) and 222.75 (5g root). Number of rhizobium nodules was significantly lower in untreated plants (17.75) while in fluopyram treated plants it ranged from 24.25 to 27.5 in 5g roots of cowpea plants. As the nematode population in soil and root, number of galls, number of females, number of egg masses and number of eggs per egg mass being zero in all doses of fluopyram 400 SC, the lowest dose (250g ai ha^{-1}) was selected as effective dosage for managing *M. incognita* in cowpea (Plate 4). Fluopyram kill the nematodes by selectively inhibiting complex II of the mitochondrial respiratory chain, there by depletes the nematode's cellular energy (Broeksma *et al.*, 2014). The nematicidal property of fluopyram in tomato against *M. incognita* and *Rotylenchulus reniformis* was reported by Faske and Hurd (2015). Jones *et al.* (2017) reported that fluopyram performed well for root knot nematode control in lima beans as seed treatment or in-furrow application. Dahlin *et al.* (2019) reported that single application of Velam Prime 400 SC (fluopyram) @1 ppm a.i. pot^{-1} in tomato seedlings at planting time showed 92.00 percent reduction in *M. incognita* juveniles. By the nematicidal effect of fluopyram, number of *M. incognita* juveniles reduced from 43802 to 3566 and gall index reduced from 3.8 to 1.6. Average number of egg masses $plant^{-1}$ in fluopyram applied treatment recorded as

20.0 while 122.6 egg masses recorded in plant of control treatment. In this study, the dosage of fluopyram 400 SC against *M. incognita* in cowpea was standardized as 250g a.i ha^{-1} as basal application and it was reported first time.

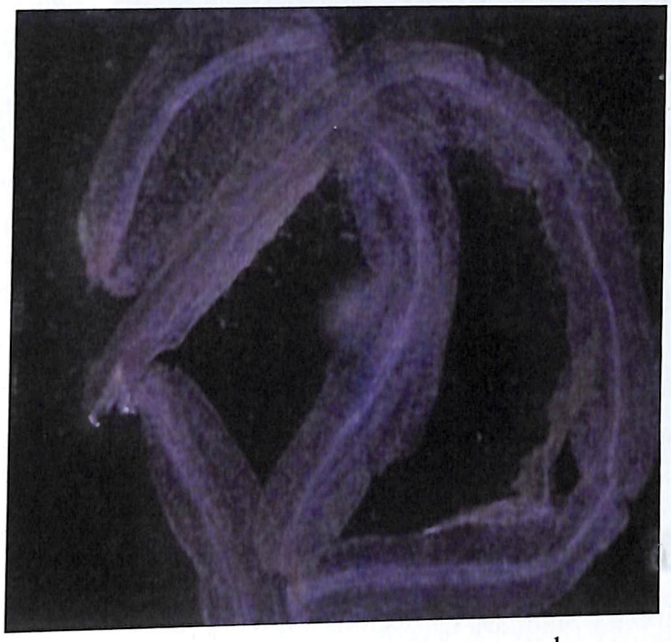
5.3. MANAGEMENT OF *M. INCOGNITA* IN COWPEA

A field experiment was conducted in Instructional Farm, Vellayani during 2019-20 to evaluate the effect of bioagents (*P. lilacinum* and *S. maltophilia*) and organic amendment (neem cake) in comparison with chemicals fluopyram and carbosulfan on management of *M. incognita* in cowpea. The highly susceptible variety, VS 50 obtained from the screening study was used for the management trial. The standardized dosage of fluopyram 400 SC @ 250 g a.i ha^{-1} was applied basally before sowing of seeds.The results presented in para 4.3.1 revealed that soil application of *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} found equally effective to basal application of fluopyram 400 SC @ 250 g a.i ha^{-1} in reducing the nematode population in soil and root. Effect of these two treatments was significantly superior to all other treatments giving 86.94 to 94.27 per cent reduction in nematode population over untreated (Fig 3). With regard to the number of females also, similar trend was observed with 91.48 to 93.39 per cent reduction over untreated. Regarding the total nematode population and reproduction factor also effect of combination treatment, *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} was statistically on par with fluopyram 400 SC @ 250g a.i ha^{-1}. Several workers reported the potential of *P. lilacinum* enriched neem cake in suppressing the population of *M. incognita* in crops *viz.* coleus (Nisha and Sheela, 2006), banana (Sundararaju and Kiruthika, 2009) and cabbage (Varghese, 2015). *P. lilacinum* being a heavy sporulator, incorporation with neem cake enhanced the multiplication and rapid establishment of the fungus. In the present study the reproduction factor was reduced to 0.25 in combination treatment of *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} while in untreated plants it was 2.97. Nisha and Sheela (2017) reported the nematode management potential of *P.lilacinum* in combination with farm yard manure as nursery and mainfield application in brinjal.

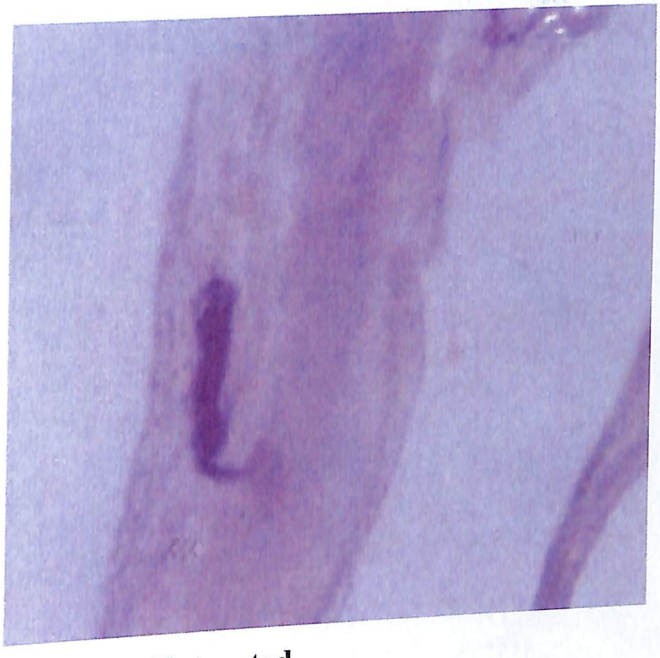


T1 – *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 20 g m^{-2}
T2 - Neem cake @ 100 g m^{-2}
T3 – *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}
T4 – *S. maltophilia* (cfu $2x10^6$ g^{-1}) @ 20 g m^{-2}
T5 – *S. maltophilia* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}
T6 - Fluopyram 400 SC @ 250 g a.i ha^{-1} as basal application
T7 - Carbosulfan 6G @ 5 g m^{-2}

Fig 3. Effect of different treatments on population of *Meloidogyne incognita* in cowpea



Fluopyram 400 SC @ 250 g a.i ha^{-1}



Untreated

Plate 5. Effect of fluopyram on the infection of *Meloidogyne incognita* in cowpea roots

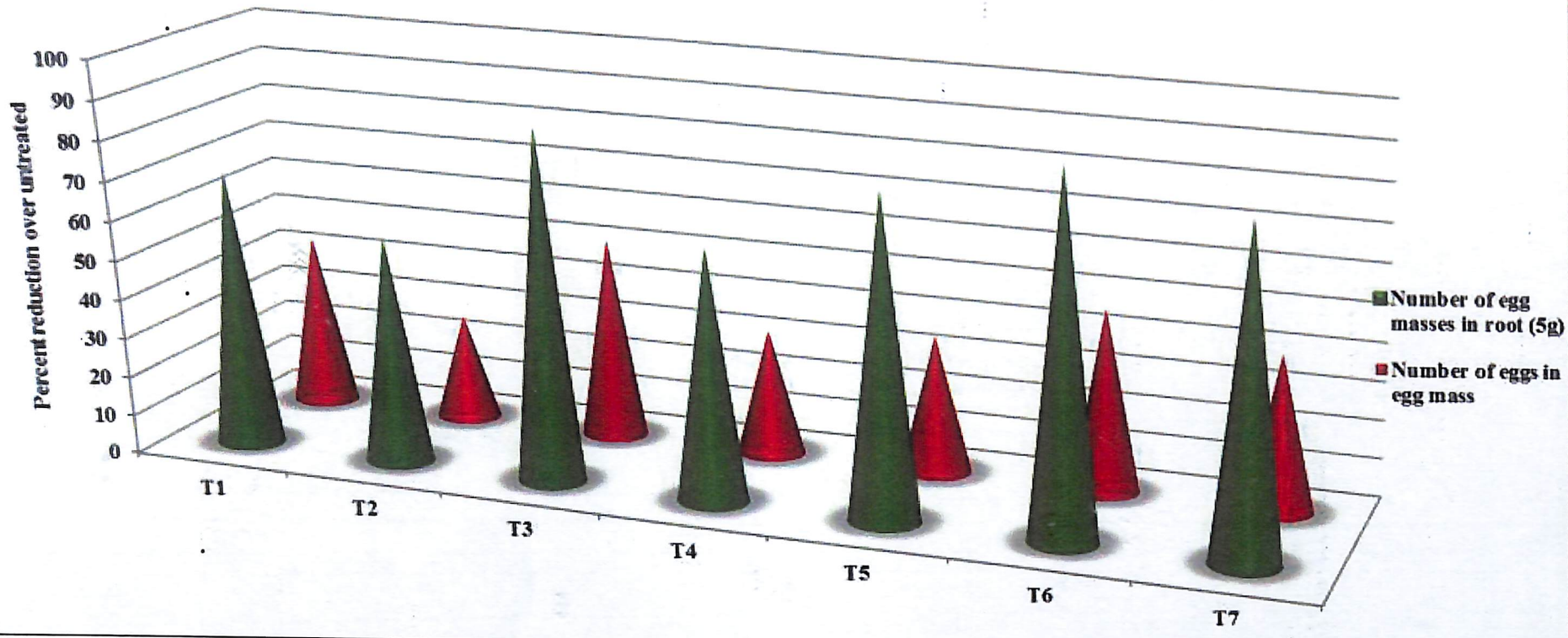
In the present study nematode suppression may be due to the added effect of neem cake and egg parasitic fungus, *P. lilacinum*. Mojumder and Pankaj (2002) reported nematicidal and nemastatic property of neem cake due to the presence of active principles *viz.* nimbidine, thionemone and limonoids. *S. maltophilia* @ 10 g m^{-2} + neem cake @ 50 g m^{-2} also found effective in reducing the nematode population in soil and root of cowpea plants giving 84.55 and 75.09 per cent reduction over untreated respectively. Regarding the number of females also soil application of *S. maltophilia* @ 10 g m^{-2} + neem cake @ 50 g m^{-2} showed significant reduction over untreated (82.43). Several researchers reported the combined effect of bacterial bioagents and neem cake in managing nematode population and increasing yield of crops. Sowmya *et al.* (2012) reported that application of *Pseudomonas putida* enriched with neem cake to field @ 20 g m^{-2} reduced population of *M. incognita* in soil (23.4 per cent over untreated) and root (23.25 per cent over untreated) in carrot. Singh (2013) also reported that combined application of *P. fluorescens* (10 kg ha^{-1}) with neem cake (1.5 t ha^{-1}) significantly reduced *M. incognita* population in the rhizosphere of eggplant from 1819.3 to 493.3 in 200 cc soil. Kar *et al.* (2018) reported that basal application of *P. fluorescens* @ 20 g m^{-2} + neem cake @100 g m^{-2} increased yield of cowpea.

Effect of *P.lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} found statistically on par with fluopyram 400 SC @ 250g a.i ha^{-1} in reducing the number of galls (87.13 to 89.53 per cent reduction over untreated), number of egg masses (88.45 to 90.84 per cent reduction over untreated) and number of eggs egg $mass^{-1}$ (46.80 to 51.73 per cent reduction over untreated) in cowpea roots (Fig 4). The outer layer of nematode egg is made up of chitin (Bird, 1997). *P. lilacinum* secretes chitinases and proteases by which it facilitates egg penetration (Morton *et al.*, 2004). *P. lilacinum* completely colonize the nematode egg and there by suppresses nematode population (Swarnakumari and Kalaiarasan, 2007).

Nematode suppression effect of the treatment combination of *P. lilacinum* and neem cake in this study may be due to the combined effect of the toxic metabolites produced by *P. lilacinum* and neem cake. Park *et al.* (2004) reported that metabolites

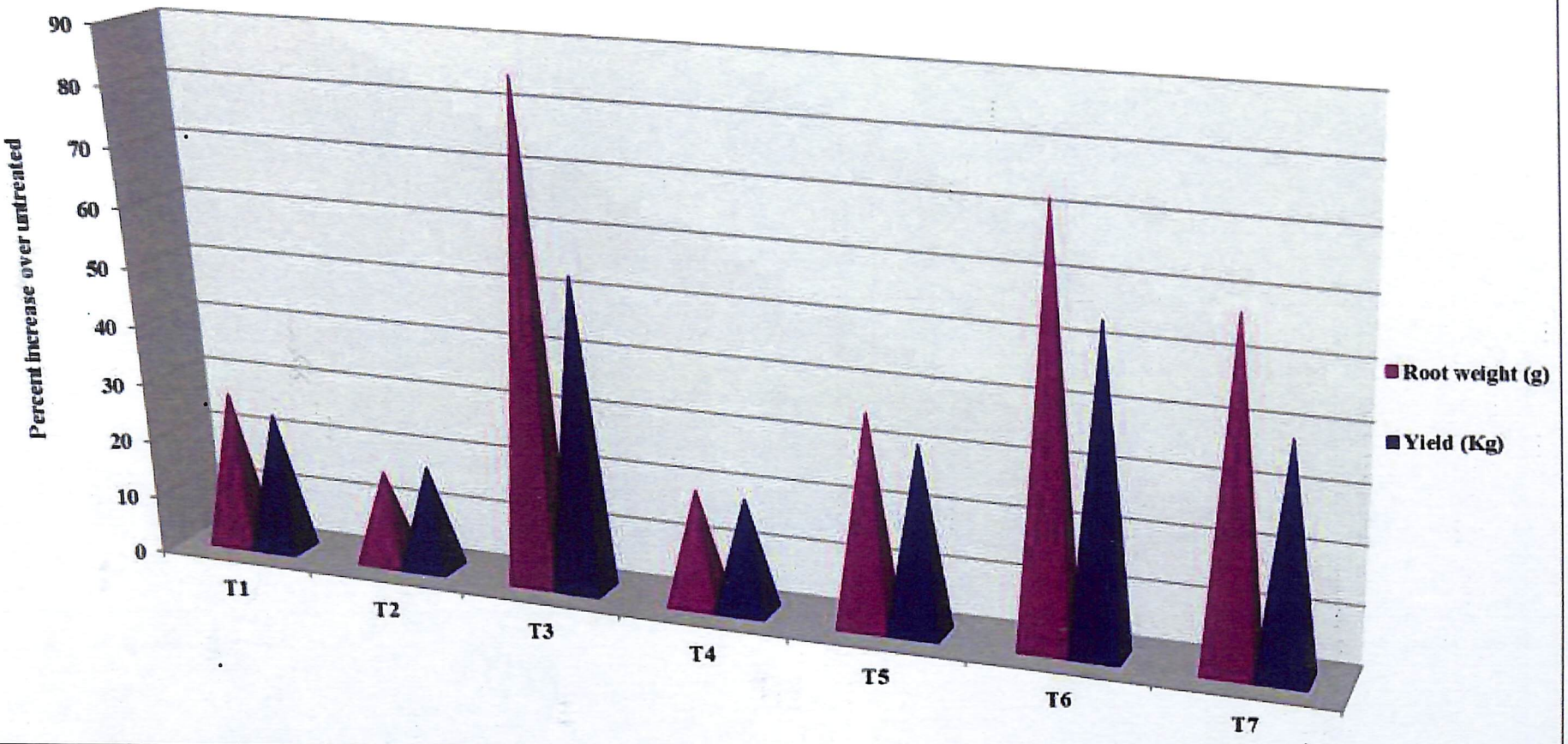
of *P. lilacinum* paecilotoxin and leusinostatins cause nematicidal effects on *M. incognita*. Microbial metabolites *viz.* volatile fatty acids, phenols, ammonia, amino acids etc. produced during decomposition of oilcake may be toxic to nematodes. Application of *S. maltophilia* @ 10 g m^{-2} + neem cake also found as effective as carbosulfan in reducing the number of galls (5g root) and egg masses (5g root) giving 81.03 to 82.58 per cent reduction over untreated. Application of *S. maltophilia*, *P.lilacinum* and neem cake alone also significantly reduced number of galls, eggs and eggmasses compared to untreated (57.24 to 72.37 percent). There are several reports of nematicidal property of *S. maltophilia, P.lilacinum* and neemcake. Seenivasan (2010) reported application of neem cake @ 500 kg ha^{-1} to coleus reduced the nematode population in soil by 31.20 per cent and reproduction factor of *M. incognita* was reduced to 3.30. Basal application of neem cake (1 t ha^{-1}) in cucumber field reduced the nematode population by 55.89 per cent (Devi and Das, 2016). Chormule *et al.* (2017) reported that basal application of neem cake @ 2 t ha^{-1} suppressed the *M. incognita* population in grape field. Number of galls in root was reduced by 18.67 per cent. Vishnu (2018) reported that soil drenching of *S. maltophilia* strain W2-7 (1×10^{7}cfu mL^{-1}) @ 50 mL pot^{-1}reduced *M. incognita* population in soil (72.69 per cent) and roots (82.23 per cent) in tomato under protected conditions. Hore *et al.* (2018) reported that drenching of Bio-Nematon 1.15% WP (*P. lilacinum*) @ 69 g a.i ha^{-1} into tomato rhizosphere at 500 mL ha^{-1} at the time of transplanting and thirty days after transplanting suppressed the *M. incognita* population (37.00 per cent reduction over untreated) and improve plant yield. Metwally *et al.* (2019) reported that application of *P. lilacinum* (1 x 10^{8} cfu g^{-1}) @ 10 ml pot^{-1} reduced (7.60 per cent reduction over untreated) the *M. incognita* population in soil and root of cowpea in pot culture under greenhouse condition. Reproduction factor of nematode was recorded as 4.2 and root knot index as 2.3. Here in this study, reproduction factor of *M. incognita* in *S. maltophilia*, *P. lilacinum* and neem cake treatments was 0.77, 0.61 and 0.84 respectively while in untreated it was 2.97.

All the treatments significantly improved the number of nodules in root, root weight and yield compared to untreated (Fig 5). The highest number of nodules (29.33 5g $root^{-1}$) was recorded in *P.lilacinum* (cfu $2x10^{6}$ g^{-1}) @ 10 g m^{-2} + neem cake



T1 – *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 20 g m^{-2}
T2 - Neem cake @ 100 g m^{-2}
T3 – *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}
T4 – *S. maltophilia* (cfu $2x10^6$ g^{-1}) @ 20 g m^{-2}
T5 – *S. maltophilia* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}
T6 - Fluopyram 400 SC @ 250 g a.i ha^{-1} as basal application
T7 - Carbosulfan 6G @ 5 g m^{-2}

Fig 4. Effect of different treatments on number of egg masses and eggs of *Meloidogyne incognita* in cowpea



T1 – *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 20 g m^{-2}
T2 - Neem cake @ 100 g m^{-2}
T3 – *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}
T4 – *S. maltophilia* (cfu $2x10^6$ g^{-1}) @ 20 g m^{-2}
T5 – *S. maltophilia* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2}
T6 - Fluopyram 400 SC @ 250 g a.i ha^{-1} as basal application
T7 - Carbosulfan 6G @ 5 g m^{-2}

Fig 5. Effect of different treatments on root weight and yield of cowpea at the time of harvest

@ 50 g m^{-2} and it was significantly superior to all other treatments. Similar trend was observed in root weight also. Percentage increase in root weight in these two treatments ranged from 72.55 to 84.87 over untreated. Incorporation of *P. lilacinum* enriched neem cake is basically a habitat management tactic which provided a better condition to soil microflora and fauna. The toxic metabolites of *P. lilacinum* and nematicidal principles released by the decomposition of neem cake may have contained the population of *M. incognita* which enhanced the root growth and nutrient uptake by cowpea plants. Increased nodulation in plants treated with *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} resulted increased conversion of nitrogen to nitrate which helped in improving the vigour of plants. Highest yield was recorded in plants treated with *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} and it was statistically on par with fluopyram 400 SC @ 250g a.i ha^{-1} giving (53.70 to 54.63 per cent over untreated). Effect of combined application of *P. lilacinum* and neem cake in improving the yield in cowpea reported in this study is in agreement with Nisha and sheela (2006) who reported that integration of soil solarization in nursery for 15 days with 150 guage LDPE film and main field application of *P. lilacinum* @ 15 g m^{-2} +neem cake @ 100 g m^{-2} protected coleus crop against *M. incognita* and improved plant growth parameters and yield (66.18 per cent increase over untreated). Effect of basal application of fluopyram 400 SC @ 250g a.i ha^{-1} in reducing nematode population and improving the nodulation, root growth and yield in cowpea is reported first time in this study.

Reisolation of bioagents at the time of harvest revealed that addition of organic substrate, neemcake increased the persistence of bioagents in soil. Final cfu of *P. lilacinum* in *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} treatment was 8.33×10^3 while it was 2.17×10^3 in *P. lilacinum* alone applied. Final cfu in *S. maltophilia* (2×10^6 cfu g^{-1}@ 10 g m^{-2} + neem cake @ 50 gm^{-2} treatment was 5.13×10^5 while it was 1.23×10^5 in *S. maltophilia* alone applied treatment. Organic amendments such as neem cake enhance growth and multiplication of biocontrol agents. This finding is in agreement with Kar *et al.* (2018) who reported increase of cfu in combination treatment of *P.lilacinum* and neem cake (2.78×10^5) compared to *P.lilcinum* alone ($2.11x10^5$) at harvest time in cowpea. They also reported that in

combination treatment of *P. fluorescens* with neem cake the cfu increased to 3.21×10^6 compared to *P. fluorescens* alone (2.74×10^6).

5.4 HARVEST TIME RESIDUES OF CHEMICALS

Residue of fluopyram and carbosulfan was found to be less than limit of quantification (LOQ) in cowpea pods, which were safe for consumption. Zhang *et al.* (2016) reported that seed treatment of rice by carbosulfan at 840 g a.i 100 kg seed^{-1} resulted residue of carbosulfan in brown rice as 0.05 mg kg^{-1} which was lower than maximum residue level. Matadha (2019) reported that soil drenching of Luna Experience 400SC (fluopyram 17.7% + tebuconazole 17.7%) at the time of tomato fruit setting resulted the residue of fluopyram in tomato fruit as 0.060 mg kg^{-1}which was below the maximum residue level. He also stated that fluopyram had less chance to enter into the food chain through the uptake of tomato fruits.

The findings of the present investigation clearly showed that Kadakkal local is *M. incognita* resistant cowpea variety which can be used in integrated nematode management strategy as plant resistance plays an important role in the effective management of root knot nematodes. In this study, the dosage of fluopyram 400 SC was standardized as @ 250g a.i ha^{-1} basal application for management of *M. incognita* in cowpea. No residues were detected in pods at the time of harvest. It can be recommended in hotspot areas of *M. incognita* infestation. Results of the management study highlighted that both *P. lilacinum* in combination with neem cake and fluopyram were the best treatments for recommendation in integrated nematode management strategy of cowpea. These two treatments significantly reduced the nematode population in cowpea rhizosphere and increased nodulation, root growth and yield. The treatment combination *P. lilacinum* enriched neem cake is eco-friendly as it will not contribute any toxic effect in soil and are not detrimental to the beneficial fauna. The left over population of nematode serve as a medium for the multiplication of *P. lilacinum* which will help in management of phytonematodes in a sustainable manner.

Summary

6. SUMMARY

The study entitled 'Management of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in vegetable cowpea' was conducted at Department of Nematology, College of Agriculture, Vellayani, Thiruvananthapuram during 2018-2020. The objectives of the study were to screen varieties for resistance and to evaluate efficacy of biocontrol agents, organic amendment and new nematicide fluopyram for the management of root-knot nematode in vegetable cowpea. The results obtained from the study are summarized in this chapter.

Seven varieties of vegetable cowpea (5 KAU released and 2 local) were screened for their resistance against *M. incognita* under pot culture condition in glass house of Department of Nematology. The varieties used for screening were Geethika, Lola, VS 50, Vyayanthi, Vellayani Jyothika, Kadakkal local and Vellayani local. Initial nematode population was 400 *M. incognita* juveniles 200cc soil^{-1}. Observations on nematode population in soil (200cc) and root (5g), number of galls (5g root), females (5g root), eggmasses (5g root), eggs eggmass^{-1} and nodules (5g root) were recorded 45 days after nematode inoculation.

The results revealed that performance of local collection Kadakkal local was significantly superior to all other varieties with lowest number of juveniles in 200 cc soil (7.33) and 5g root (6.33). The lowest number of galls (5.67 in 5g root) was recorded in Kadakkal local with lowest root-knot index of one. All other varieties (Geethika, Lola, VS 50, Vyayanthi, Vellayani Jyothika) and local collection Vellayani local found to be highly susceptible with gall index 5. Minimum number of females in 5g root (3.67) was recorded in Kadakkal local and it was significantly superior to all other varieties screened. In the case of number of egg masses root^{-1} (5g) and number of eggs in eggmass also lowest was recorded in Kadakkal local with mean number of 2.33 and 63.33 respectively. Based on nematode population in soil, root, number of females, number of egg masses and number of eggs in eggmass, performance of Kadakkal local found to be better than other varieties screened. Reproduction factor of *M. incognita* in Kadakkal local was 0.04 while in other varieties screened it ranged from 2.38 to 3.22. Maximum number of nodules (22.67 in 5g root) was recorded in

Kadakkal local while in VS 50 it was 9.33. Highly susceptible variety, VS 50 recorded maximum number of galls (300.67), egg masses (224.33) and eggs in eggmass (147.00). Population of *M. incognita* juveniles was also maximum in VS 50 with mean number of 799.33 in 200cc soil and 246.67 in 5g root.

Pot culture experiment was laid out in glass house condition to standardize the dosage of fluopyram for the management of *M. incognita* in cowpea. The treatments were fluopyram 400 SC @ 500g a.i ha^{-1} as basal application, fluopyram 400 SC @ 500g a.i ha^{-1} as basal application + 500g a.i ha^{-1} 25 days after first treatment, fluopyram 400 SC @ 250g a.i ha^{-1} as basal application + 250g a.i ha^{-1} 25 days after first treatment, fluopyram 400SC @ 250g a.i ha^{-1} as basal application and Untreated. Initial nematode population was 510 to 618 *M. incognita* juveniles 200 cc $soil^{-1}$. Plants were uprooted at different intervals and observations were recorded. Different stages of nematodes such as J2, J3, J4 and females were not observed in cowpea roots of fluopyarm applied treatments. Nematode penetration into roots and life cycle completion was observed only in untreated control plants. No galls, egg masses and females were found in fluopyram treated plants. High nematode infestation was observed in roots of untreated plants with 761.50 mean number of galls $root^{-1}$. Phytotoxicity symptoms were not observed in plants treated with different dosages of fluopyram 400SC. Fluopyram not affected the rhizobium nodule formation in root. Higher number of rhizobium nodules was observed in fluopyram treated plants compared to control. As there was no nematode penetration, development and reproduction in fluopyram treated plants, lower dosage of fluopyram 400 SC @ 250 g a.i ha^{-1} as basal application was selected as effective dosage for the management of *M. incognita* in cowpea.

Field experiment was conducted using susceptible variety (VS 50) to study the comparative effect of bio agents (*Purpureocillium lilacinum* and *Stenotrophomonas maltophilia*) and organic amendment (neem cake) in comparison with chemicals fluopyram and carbosulfan for the management of *M. incognita* in cowpea. Experiment was conducted in nematode infested field in Instructional farm, Vellayani. The initial population ranged from to 325 to 420 in 200 cc $soil^{-1}$. Experiment was laid

out in RBD with eight treatments and three replications. All the treatments were applied basally before sowing cowpea seeds. The results were assessed in terms of nematode population characteristics (number of galls, number of eggmasses, nematode population in soil and root) root weight, yield and number of nodules.

Results revealed that all the treatments significantly reduced the nematode population in soil and root compared to untreated. Percentage reduction in nematode population in soil and root over untreated ranged from 77.74 to 94.27 and 54.80 to 88.31 per cent respectively. The effect of treatment combination, *P. lilacinum* cfu 2×10^6 @ 10 g m^{-2} + neem cake @ 50 g m^{-2} was statistically on par with basal application of fluopyram 400 SC @ 250 g a.i ha^{-1} in reducing nematode population in soil and root giving 86.94 to 94.27 per cent reduction over untreated. Application of *S. maltophilia* cfu 2×10^6 @ 10 g m^{-2} + neem cake @ 50 g m^{-2} found as effective as *P. lilacinum* @ 20 g m^{-2} giving 82.82 to 84.55 per cent reduction in nematode population over untreated. Regarding nematode population in root also effect of these two treatments was statistically on par with 68.90 to 75.09 per cent reduction over untreated. Effect of carbosulfan found to be inferior to above two treatments giving 88.05 and 80.58 per cent reduction in nematode population over untreated in soil and root respectively. *S. maltophilia* @ 20 g m^{-2} and neem cake @ 100 g m^{-2} also recorded more than 50 per cent reduction in nematode population in soil and root. In the case of number of females, *P. lilacinum* cfu 2×10^6 @ 10 g m^{-2} + neem cake @ 50 g m^{-2} was statically on par with fluopyram 400 SC @ 250 g a.i ha^{-1} giving 91.48 to 93.39 per cent reduction over untreated. Reproduction factor of *M. incognita* in these two treatments ranged from 0.18 to 0.25 while in untreated plants it was 2.97.

With regard to number of galls in root, application of *P. lilacinum* cfu 2×10^6 @ 10 g m^{-2} + neem cake @ 50 g m^{-2} found to be equally effective to fluopyram 400 SC @ 250 g a.i ha^{-1} with mean gall index of 1.67 and 1.33 respectively while in untreated plants it was 5. Effect of *S. maltophilia* cfu 2×10^6 @ 10 g m^{-2} + neem cake @ 50 g m^{-2} was statistically on par with carbosulfan 6G @ 5 g m^{-2} and percentage reduction in number of galls in these four treatments ranged from 81.79 to 89.48. In the case of number of eggmasses also similar trend was observed. Application of

S. maltophilia, *P. lilacinum* and neem cake alone also significantly reduced number of galls, eggs and eggmasses compared to untreated (57.24 to 72.37 per cent).

Imposition of different treatments *viz. P. lilacinum, S. maltophilia*, neemcake, combination of bioagents (*P. lillacinum, S. maltophilia*) with neem cake, chemicals (fluopyram and carbosulfan) significantly reduced the nematode population characteristics of cowpea which was directly reflected in root weight and yield. Maximum root weight was recorded in plants treated with *P. lilacinum* cfu 2×10^6 @ 10 g m^{-2} + neem cake @ 50 g m^{-2} and it showed significant superiority to all other treatments. Regarding number of nodules also similar trend was observed. The number of nodules 5 g $root^{-1}$ ranged from 13.33 to 29.33 in plants treated while in untreated it was 9.33. With regard to yield, all the treatments showed statistically significant superiority over untreated. Highest yield was recorded in plants treated with *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} and it was statistically on par with fluopyram 400 SC @ 250 g a.i ha^{-1} giving (53.70 to 54.63 per cent over untreated).

Reisolation of bioagents at the time of harvest showed that addition of organic amendment, neem cake enhanced the multiplication of *P. lilacinum* and *S. maltophilia* which resulted in persistence of bioagents in soil upto harvest. Final cfu of *P. lilacinum* in *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} treatment was 8.33×10^3 while it was 2.17×10^3 in *P. lilacinum* alone applied. Final cfu in *S. maltophilia* (2×10^6 cfu g^{-1}@ 10 g m^{-2} + neem cake @ 50 g m^{-2} treatment was 5.13×10^5 while it was 1.23×10^5 in *S. maltophilia* alone treatment.

Residue of fluopyram and carbosulfan were evaluated in cowpea pods at the time of harvest by LCMS/MS techniques at the Pesticide Residue Research and Analytical Laboratory, Department of Agricultural Entomology, College of Agriculture, Vellayani. Residue was found to be less than limit of quantification (LOQ) in cowpea pods, which were safe for consumption.

These investigations highlighted the following results

- Vegetable cowpea variety Kadakkal local is resistant to root knot nematode, *Meloidogyne incognita* infection.
- Fluopyram 400 SC @ 250 g a.i ha^{-1} is the effective dosage for managing *M. incognita* in cowpea.
- Soil application of *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} can be recommended for management of *M. incognita* in organic cultivation of cowpea.
- The nematicide fluopyram 400 SC @ 250 g a.i ha^{-1} as basal application have no residue in cowpea pods at harvesting time.

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MANAGEMENT OF ROOT-KNOT NEMATODE, *Meloidogyne incognita* (KOFOID AND WHITE) CHITWOOD IN VEGETABLE COWPEA

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ABSTRACT

The study entitled 'Management of root-knot nematode, "*Meloidogyne incognita* (Kofoid and White) Chitwood in vegetable cowpea" was conducted at Department of Nematology, College of Agriculture, Vellayani, Thiruvananthapuram during 2018-2020. The objectives were to screen varieties for resistance and to evaluate efficacy of biocontrol agents, organic amendment and new nematicide fluopyram for the management of root-knot nematode in vegetable cowpea.

Seven varieties of vegetable cowpea (5 KAU released and 2 local) were screened for their resistance against *Meloidogyne incognita* in pot culture under glass house condition. The experiment was laid out in CRD with 7 treatments and 3 replications. The results revealed that local variety collected from Kadakkal was highly resistant to root-knot nematode with root-knot index 1. The local variety performed best in reducing the multiplication of nematodes. Lowest number of egg masses 5g root^{-1} (2.33), eggs egg mass^{-1} (63.33) and nematode population 200cc soil^{-1} (7.33) was observed in the local variety and it showed statistically significant variation compared to the KAU released varieties. Regarding the number of nodules 5g root^{-1} also the Kadakkal variety showed significant superiority (22.67 nodules 5g root^{-1}). KAU variety VS 50 was highly susceptible to *M. incognita* infestation with root-knot index 5. Highest number of egg masses 5g root^{-1}(224.33) and number eggs egg mass^{-1} (147.00) was recorded in VS 50.

Pot culture experiment was laid out in completely randomized design to standardize the dosage of fluopyram for the management of *M. incognita* in cowpea. The treatments were fluopyram 400 SC @ 500g a.i ha^{-1} as basal application, fluopyram 400 SC @ 500g a.i ha^{-1} as basal application + 500g a.i ha^{-1} 25 days after first treatment, fluopyram 400 SC @ 250g ai ha^{-1} as basal application + 250g a.i ha^{-1} 25 days after first treatment, fluopyram 400SC @ 250g a.i ha^{-1} as basal application, Untreated. All the dosages of nematicide were applied as soil drenching to the root knot nematode infected soil. Basal application of fluopyram 400 SC @ 250g a.i ha^{-1} was the effective dosage for managing *M. incognita* in vegetable cowpea. Phytotoxicity symptoms were not observed in any of the treatments. Nematode

penetration in roots and life cycle completion was observed in untreated control plants. *M. incognita* juveniles, adult female and male were not observed in roots of fluopyram treated cowpea plants. Galls and egg masses were observed in uprooted cowpea plant roots in untreated whereas in fluopyram applied treatments it was zero. Regarding final nematode population also, no nematodes were observed in soil samples were collected from fluopyram treated plants while in untreated control plants it was 761.5. Number of rhizobium nodules was significantly lower in untreated plants (17.75) while in fluopyram treated plants it ranged from 24.25 to 27.5 in 5g roots of cowpea plants.

Field experiment was conducted by using the susceptible variety (VS 50) to study the comparative effect of bio agents (*Purpureocillium lilacinum*) and organic amendment (neem cake) in comparison with chemicals fluopyram and carbosulfan. The experiment was laid out in RBD with 8 treatments and 3 replications. All the treatments significantly reduced nematode population in soil and root compared to untreated control. Effect of soil application of *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} found equally effective to basal application fluopyram 400 SC @ 250g a.i ha^{-1} in reducing the nematode population in soil (93.03 per cent reduction over untreated) and root (86.94 per cent reduction over untreated). Regarding yield also effect of these two treatments was statistically on par giving 53.70 to 54.63 per cent increase over untreated. Plants treated with *P. lilacinum* (cfu $2x10^6$ g^{-1}) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} showed significant superiority in number of nodules (29.33) in root (5g). Results on reisolation of bioagents at the time of harvest revealed that addition of organic substrate neemcake increased the persistence of bioagent ($8.33x10^3$ cfu g $soil^{-1}$) in soil. Residue of fluopyram and carbosulfan was found to be less than limit of quantification (LOQ) in cowpea pods, which were safe for consumption.

From this study, it is concluded that vegetable cowpea variety Kadakkal local is resistant to *M. incognita*. Soil application of *P. lilacinum* (cfu 2x106 g-1) @ 10 g m^{-2} + neem cake @ 50 g m^{-2} can be recommended for management of *M. incognita* in organic cultivation of cowpea.