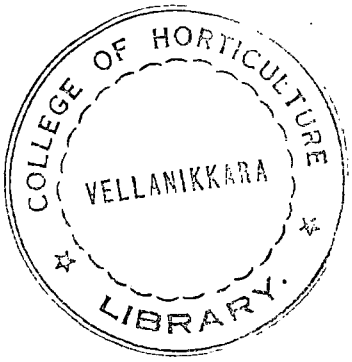
CONTROL OF ROOT-KNOT NEMATODE (Meloidogyne incognita Kofoid and White, Chitwood) INFESTING BLACK PEPPER (Piper nigrum L.) BY BACTERIAL PATHOGENS

BY
M.S.SHEELA



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D E C L A R A T I O N

I hereby declare that this thesis "Control of root-knot nematode (*Meloidogyne incognita* Kofoid and White, Chitwood) infesting black pepper (*Piper nigrum* L.) by bacterial pathogens" is a bonafide record of research work done by me during the course of research and that 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.

(M.S. Sheela.)

Vellanikkara,
4-12-1990.

C E R T I F I C A T E

Certified that this thesis, entitled "Control of root-knot nematode (*Meloidogyne incognita* Kofoid and White, Chitwood) infesting black pepper (*Piper nigrum* L.) by bacterial pathogens" is a record of research work done independently by Smt. M.S. Sheela under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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(M.S. SHEELA.)

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INTRODUCTION

1. INTRODUCTION

Pepper (*Piper nigrum* L.) is a seed spice known as King of Spices used all over the world in various forms. Kerala is the largest producer of this spice in India earning 55 per cent of the entire export earnings from spices. The total production of pepper in Kerala has shown a steady decrease from 27,680 tonnes during 1955-56 to 17,350 tonnes during 1984-85 in spite of the increase in the area of pepper from 86,490 to 1,08,835 hectares (Department of Economics and Statistics, 1988). The declining trend in the production of pepper during the last 30 years can be attributed to various factors including pests and diseases. Among the pests, nematode infestation has been observed as a serious problem threatening pepper production in the State (D'Souza *et al.*, 1970; Venkitesan, 1972).

Among the thirty six species of plant parasitic nematodes recorded in pepper, the root-knot nematode *Meloidogyne incognita* is of major importance and this is widely distributed in pepper gardens of Kerala and Karnataka States (Sundararaju *et al.*, 1979). It is the first nematode recorded on black pepper by Delacriox (1902) from Cochin-China and Butler (1906) from Wynad area. Moreover this nematode is often the only or one of the few nematode diseases of

pepper known to the farmer owing to the spectacular symptoms on root (root galls) and shoot system (dense yellowish discolouration of leaves as seen in Plate I).

In modern agriculture, bioagents play an important role in the control of many of the rampantly occurring pests and diseases. Although a wealth of natural enemies occur in the pepper soil ecosystem, their utilization in nematode control has not reached the desired level due to various reasons. However, taking advantage of their natural existence in soil ecosystem, the scope of exploiting their potential in the nematode management is quite encouraging and accumulation of knowledge of their occurrence and status as a biocontrol agent deserves prime consideration.

The development of a control strategy against the root-knot nematode using their natural enemies will be much more economical and desirable than the periodical application of toxic and hazardous nematicide chemicals. According to Mankau (1981) chemical control of nematodes, while effective in many cases, has always been a relatively crude practice, applying mainly general biocides to soil with little concern for their effects on natural nematode antagonists.

Among the natural enemies, bacteria, the potent and alternative long term biocontrol agent needs greater

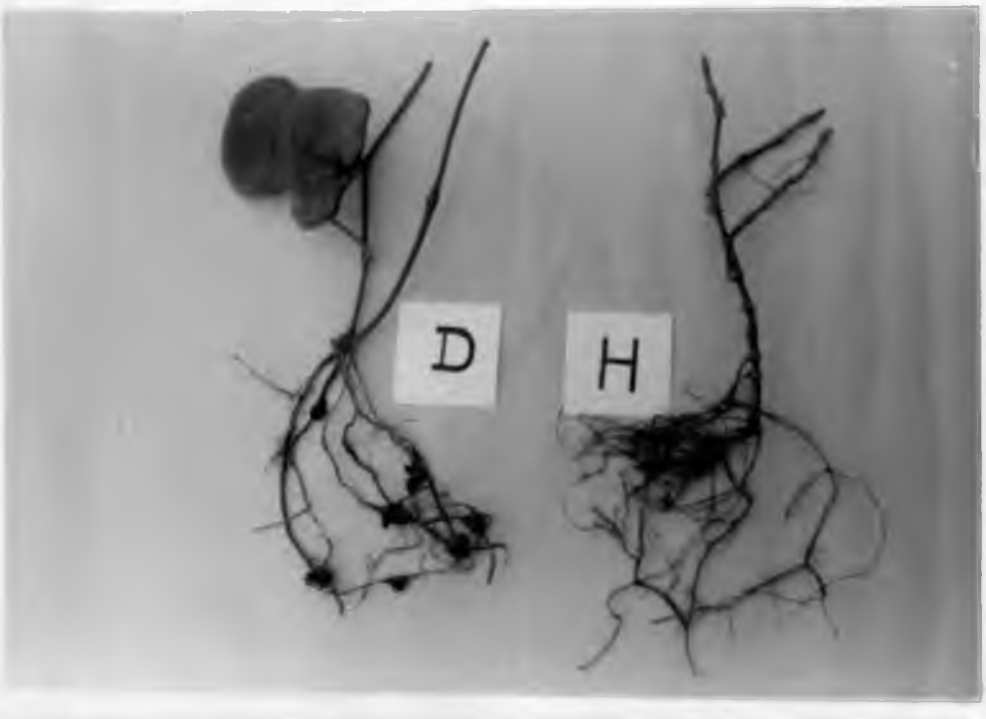
PLATE I

Symptoms of root-knot infestation on shoot and root system of pepper plant

H Healthy plant

D Diseased plant

PLATE I



emphasis and development. There are several reports of bacteria which are detrimental to root-knot nematode infection (Sayre and Wergin, 1977). Mankau (1981) reported and demonstrated Pasteuria penetrans exerted effective biological control of root-knot nematode. Recently Gokte and Swarup (1988a and b) reported the potentialities of the bacteria Bacillus pumilus, B. cereus and B. subtilis and Psuedomonas sp., as biocides against Anguina tritici, Heterodera cajani, H. avenae and M. incognita. Attempts made on microbial control of nematode in our country are very meagre. Hence this study was taken up, to work out the possibilities and potentialities of bacterial pathogens associated with root-knot nematode infesting balck pepper. The following aspects were studied in detail:

1. Survey of the bacteria in traditional pepper growing tracts

2. Identification, purification and maintenance of the organisms

3. Determining the pathogenicity of the organisms

4. Effect on plants and other higher animals

5. Development of an ideal medium for the mass production of the bacteria

6. Evaluation of the efficacy of the organisms in control of the nematode under pot culture conditions

7. The interaction of the bacteria and chemicals in an integrated approach, and

8. Effect on related genera of Meloidogyne spp.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Information available on various aspects related to the present investigation on control of root-knot nematode using bacterial pathogens have been briefly reviewed here.

2.1. Distribution of bacterial pathogens in the field

Thorne (1940) collected Pratylenchus pratensis (131 specimens) from soil and roots of cotton plants which included 87 individuals (66.4 per cent) parasitized by Duboscquia penetrans. Prasad et al. (1972) reported that though Bacillus thuringiensis was absent in a tomato field soil having a high level of root-knot nematode populations, it was present in soil with low level of incidence. Spaull (1981) surveyed 124 sugarcane fields in South Africa and found the infestation by B. penetrans at the rate of 6 per cent on Pratylenchus, 17 per cent on Helicotylenchus and 17 per cent on Scutellonema. Stirling and White (1982) reported the distribution of B. penetrans in vine yards more than ten year old having enough Meloidogyne females. The parasitism implicated the natural biological control of the nematode.

2.2. Mechanism of infection

Mankau (1975a) revealed the virulent disease of plant parasitic nematode caused by B. penetrans n. comb. The

spore is highly adhesive and become fairly attached to the nematode cuticle which it penetrates by enzymes.

Mankau and Imbriani (1975) reported that the spores of B. penetrans attach to nematodes and germ tube penetrate the cuticle and give rise to a spherical, dichotomously branched thallus which eventually breaks up and proliferates throughout the psuedocoelom of the nematode. The peripheral cells of the thallus then differentiate into spores which ultimately fill the body cavity. They also studied the sporangium morphogenesis of B. penetrans in Meloidogyne females and found that it was similar to that of the bacterial "milky disease" pathogens of insects B. popilliae and B. lentimorbus.

Mankau et al. (1976) studied the SEM observation on nematode cuticle penetration by B. penetrans. The sporangia attached to the lip region and most frequently to the anterior or neck region of nematode. When it was attached to the lip region of the nematode, it blocked the buccal opening and surrounding pits within which labial papillae apparently occur.

Imbriani and Mankau (1977) described the ultra structure and development of B. penetrans in Meloidogyne spp. with the help of a transmission electron

microscope. Host infection was from a germ tube from the cup-shaped sporangium containing the endospore. The prokaryotic vegetative cells contained septa formed by an ingrowth of the inner layer of the trilaminate cell wall and were associated with mesosomes. An exosporium which may function in attachment and host specificity surrounded the endospore.

According to Sayre and Wergin (1977) infection follows attachment of an endospore to the nematode and a germ tube penetrate the cuticle and mycelial colonies formed in the psuedocoelom. The sporulation is initiated when terminal cells of the mycelium enlarges to form sporangia and the spores are released when the nematode bursts.

Endo (1979) revealed the presence of the bacterium like microorganism in the anterior tissues of the larvae of Heterodera glycines especially in the sarcoplasm of the stylet protractor and median bulb muscles and in the highly reticulate and ribosome-rich tissues of the oesophageal glands.

Imbriani and Mankau (1979) studied the parasite in transmission electron microscopy and confirmed that infection of Meloidogyne by B. penetrans was by germ tube from the cup-shaped sporangium containing the endospore.

2.3. Factors affecting the infection

2.3.1. Temperature

Dutky and Sayre (1978) reported the thermal inactivation point of the spores in soil. Prevention of spore attachment required 130°C for one hour. After 30 minutes at 80°C, attachment occurred though no infection.

All stages in the process of infection of M. javanica by B. penetrans were favoured by temperature which was optimum for the nematode. The spores attached more readily to nematode at 22.5 to 30°C than at 15°C. The parasite also developed more quickly as temperature increased, completing its life cycle in 85 to 100 days at 20°C and in only 20 to 30 days at 30°C. At 30°C the parasite proliferated extensively through females before they reached maturity, at 20°C female often developed ovaries consisting egg before infection prevented further development (Stirling, 1981).

Maheswari et al. (1987a) reported that the bacterial spores of P. penetrans more readily encumbered on M. javanica juveniles at 30°C and 25.5 to 34°C than at 10 or 20°C.

2.3.2. Soil pore size and soil moisture level

Dutky and Sayre (1978) studied the thermal inactivation point of the spores in soil, soil pore size and moisture level. No correlation was found between the number of spores attached per larva and the soil pore size and soil moisture level.

Brown and Smart (1984) experimentally proved that the proportion of M. incognita second stage juveniles parasitized by B. penetrans was more in moistened soil than in air-dried soil and the spore attachment was enhanced by moisture.

2.3.3. Storage time and media

O'Brien (1980) found that storage time and media are the factors influencing the attachment of B. penetrans spores to nematode host, M. javanica. The interaction between spores and the cuticle of nematode may involve factors such as lectins and it is temperature dependent.

2.3.4. Host specificity

Mankau and Prasad (1977) tested the host specificity of B. penetrans against a number of plant parasitic nematodes. M. javanica, M. arenaria and M. incognita became heavily

infected than M. hapla or Pratylenchus scribneri under similar conditions but all of these species were good hosts.

The studies on the bacterial spore parasite, B.penetrans in green house condition using the cultures of M. incognita and P. brachyurus treated with A. ritzemabosi, D. dipsaci, D. triformis, T. claytoni, P. penetrans, P. brachyurus, M. incognita, M. hapla, M. javanica and Meloidoderita sp. showed that the spores of M. incognita became attached only to M. incognita, M. hapla and M. javanica. The spores of P. brachyurus became attached only to P. brachyurus (Dutky and Sayre, 1978).

Brown and Smart (1985) reported that B. penetrans inhibited penetration of M. incognita second stage juveniles (J_2) into tomato roots in the laboratory and green house. Spores get attached to the second stage juveniles of M. javanica, M. incognita and M. arenaria and a greater number of spores attached to M. incognita.

2.4. Nature of damage

2.4.1. Larval mortality and egg hatching

Prasad et al. (1972) reported that the heat stable exotoxin of Bacillus thuringiensis var. thuringiensis was

highly nematicidal whereas the spore crystal complex did not show any effect. The effectiveness of exotoxin was further confirmed with purified exotoxin, which showed 100 per cent mortality within 24 hr, ruling out the possibility of other bacterial metabolites.

Mankau and Prasad (1977) studied the infectivity of B. penetrans in Meloidogyne spp. Infection severely reduced the survival of second stage juveniles than other stages.

Rai and Rana (1979) studied the effectiveness of Beta-exotoxin of B. thuringiensis var. thuringiensis for the control of Meloidogyne spp. infesting brinjal. Beta-exotoxin produced by B. thuringiensis grown in acid hydrolysate of wheat and rice bran killed 95 and 85 per cent respectively of Meloidogyne sp. second stage juveniles after seven days. When bacterial culture was grown on farm yard manure the mortality was 72 per cent on seventh day.

Gokte and Swarup (1988b) reported that hundred per cent irreversible immobility was caused to Anguina tritici larvae within 48 hours after infection by some isolates of Bacillus spp., while there was 91 to 95 per cent immobility after exposure for six days using some other isolates of Bacillus spp. They also reported that nine bacterial populations,

in addition to Corynebacterium michiganense p.v. tritici which are associated with the yellow ear rot disease of wheat, in the nematode galls, collected from Punjab and Haryana States which showed larval mortality and immobility. The most effective isolates were Bacillus subtilis and B. pumilus.

2.4.2. Fecundity

Mankau (1975) reported that B. penetrans infected root-knot females which were dissected out from galls were generally smaller than healthy females and usually produced no eggs. B. penetrans infected nematodes do not lay eggs and eventually killed by the parasite whose spores are released into the soil upon disintegration of the host and remain for many years tolerant to desiccation, chemicals and adverse conditions (Mankau, 1981).

2.5. Effect on pesticides

Mankau and Prasad (1972) tested six nematicides at recommended field dosages, Nemagon (1, 2 dibromo 3 chloro-propane) was only slightly toxic while Telone (1, 3 dichloro propane + related chlorinated hydrocarbon), Temik (2 methyl - 2 (methyl thio) propionoldehydo-O-methyl carbamyl) oxime,

Furadan (2, 3 dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate), Nemacur (ethyl-4 (methyl thio) m-tolyl isopropyl-phosphoramidate) and Mocap (o-ethyl s,s-dipropylphosphoro-dithioate) had no noticeable effect on the endoparasite Duboscquia for the biological control of plant parasitic nematodes.

A synergistic reduction in root galling by M. incognita with Pasteuria penetrans and nematicides was reported by Brown and Nordmeyer (1985) in tomato seedlings under pot culture condition. It revealed that treatment of M. javanica infested soil with P. penetrans spores as well as aldicarb (1.5 ppm) or carbofuran (0.25 ppm) reduced galling by a factor of 10, much more than by bacterial or nematicide treatment alone. The nematicide may increase random nematode movement in soil, increasing the likelihood of contact with bacterial spores.

Maheswari et al. (1987b) also revealed the synergistic reduction of root galling in tomato roots by the addition of P. penetrans and nematicides. The biometric characters of plant was also improved by this treatment.

2.6. Efficacy with fungal pathogen

Dube and Smart (1987) found that M. incognita was controlled more effectively and yields of host plants

(tomato, tobacco, soyabean and Capsicum annum) were greater when Paecilomyces lilacinus and P. penetrans were applied together in field microplots than when either was applied alone.

Maheswari and Mani (1988) reported the combined efficacy of P. penetrans and P. lilacinus on the biocontrol of M. javanica. They found that simultaneous inoculation of P. penetrans (150 mg per kg of soil) and P. lilacinus (3.0 g per kg of soil) effectively controlled the M. javanica population in tomato.

2.7. Mass production of Bacillus penetrans

Root systems of the tomato plants containing large number of Meloidogyne females parasitized by B. penetrans was obtained by inoculating the plants with second stage juveniles infected with spores. These roots were air-dried and finely ground to produce a powdery material which was light, easily handled and stored. These preparations at the rate of 100 mg per kg gave 99 per cent infection of juveniles with B. penetrans spore (Stirling and Wachtel, 1980).

2.8. Effect of the pathogen on nematodes under pot-culture conditions

In microplot experiments, Mankau (1975b) used the following treatments: (a) Air dried soil containing

B. penetrans spores was placed in holes three inch wide and six inch deep, (b) Seedlings were grown in spore-infested soil and then transplanted into microplots, and (c) 2,40,000 larvae encumbered with spores were added to plots to a depth of 4 inches. When the soils were bio-assayed 11 months after cropping, 98 per cent of the larvae emerging from the treatment (a) were heavily encumbered with spores. In (b), only 53 per cent carried spores, but there were only a few spores per larva. In (c) 7 per cent were lightly infested.

For the control of root-knot nematode using B.penetrans under green house conditions, air-dried soil infested with spore of B. penetrans was planted with tomato seedlings to which 10,000 root-knot nematode larvae had been added. After 70 days, plants in the air-dried spore infested soil had greater dry-weights, more leaves and less root galling than plants in soil free of spores or growing in sterilized soil (Mankau, 1975b).

Birchfield and Antonopoulos (1976) reported that B. penetrans attached to the cuticle of the root-knot nematode larvae reduced a population of 2000 M. incognita per 500 m^3 soil to a population of 20 in three weeks. Three to twelve parasite spores were attached on the body of nematode larvae.

Soil incorporation or root dip with B. uniflagellatus spore powder was ineffective in controlling M. incognita (Gemrich and Vanderstreek, 1980).

When tomato plants growing in soil containing 0, 100, 200 or 400 mg of the preparation of B. penetrans per kg of dry soil were inoculated with juveniles of M. javanica, significantly fewer nematodes developed in the soils treated with B. penetrans. The number of females per root system decreased as the concentration of the pathogen increased and most of the females were diseased at the highest concentration of B. penetrans (Stirling, 1984). He also reporte that there was no significant difference in the number of juveniles hatched from primary roots in soil containing 0, 100, 200 or 400 mg of the preparation of B. penetrans per kg of dry soil. But it had a greater effect on the juveniles that migrated from the primary roots to infect other roots. These nematodes were invariably diseased and the number of juveniles hatched from secondary roots was reduced significantly.

Sharma (1985) and Bhattacharya (1987) recorded convincing reduction in cyst nematode populations, under pot culture conditions, with the use of Pasteuria penetrans and Glomus fasciculatum.

Gokte and Swarup (1988b) reported the larvicidal effect of B. subtilis, B. pumilus and two species of Pseudomonas on Anguina tritici. Seed treatment of the above isolates in wheat individually as well as in combination caused reduction in percentage of penetration of juveniles to the roots of wheat seedlings and the viability of the larvae.

Bhattacharya and Swarup (1988) revealed the parasitization of P. penetrans in the larvae of Heterodera and M. incognita. Mass multiplication of the bacterial inoculum was done on root-knot infested brinjal plants. Among the method of application, direct mixing of bacterial spore infested soil was found to be most effective against the nematode population and maximum reduction in cyst population was obtained when spore infected soil was incubated at 30°C before application.

Raj and Mani (1988) reported that P. penetrans reduced the multiplication of M. javanica in tomato by 48.4 per cent and 94.42 per cent at 250 mg and 1000 mg per kg inoculum levels respectively. After 60 days, respective increase of 17.67, 19.95 and 14.32 per cent in the fresh and dry weight of shoots and in the fresh weight of roots was recorded at the 1000 mg spore inoculum level.

Osman *et al.* (1988) studied the bioefficacy of two bacterial insecticide strains of *B. thuringiensis* (Dipel and SAN-415) as a biocontrol agent in comparison with nematicide Nemacur, on certain parasitic Nematoda. He found that two strains of *B. thuringiensis* in a green house experiment against the nematodes *M. javanica* and *Tylenchus semipenetrans* in tomato suppressed the population of both and also reduced the egg hatchability. These two preparations were more effective than the standard, Nemacur. Among these two bacterial insecticides SAN-415 was superior.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Survey on the occurrence of bacterial pathogens of root-knot nematode, M. incognita

3.1.1. Preliminary survey

A preliminary survey was carried out in the root-knot nematode infested pepper gardens in the Palode, Vellayani, Pampadumpara, Kumili, Vellanikkara, Marakkal, Kannara and Panniyur areas of the Kerala State, for detecting the presence of the association of bacteria. Twenty one root samples were collected from these eight locations.

3.1.1.1. Collection of root samples

Root samples were collected near root zone from the base of root-knot infested pepper plants. About 10 g of root (feeder roots) was collected at a depth of 15-30 cm. Root samples were packed in polythene bags with proper labels for further studies.

3.1.1.2. Processing of root sample

From each sample, 5 g of the root was taken in a plastic container and the roots were gently cleaned of any soil adhering to them by holding them in a stream of water under a tap. The clean roots were teased and the number of adult females and other developing stages were counted.

After this, the root bits were cut into small pieces and the larvae were extracted by incubation method (Young, 1954). After every 24 hr, nematode suspension was drawn out from the petri dish. This was continued till no nematode was obtained. The extracts were pooled together and counted the population.

3.1.1.3. Processing of root samples for bacterial isolation

Five g of the root was weighed out from each sample. These roots were washed free of soil and other dirt particles and examined under the stereobinocular microscope and the females and egg masses were dissected out under the microscope. Then the root bits were kept for extraction of larvae by incubation method (Young, 1954). The nematodes extracted as above (egg masses, larvae and adult females) were examined under the microscope for the symptoms of bacterial infection. The size, shape, colour ~~virulence~~ and motility of larvae and females were examined. After this the different stages having irregularities like immobility of juveniles, small size, shape, colour were picked out and surface sterilized with 0.1 per cent mercuric chloride solution. These were then washed in three changes of sterile distilled water and aseptically transferred to petri plates having sterile

Muller Hinton Agar medium (Plate II) having the following composition:

Muller Hinton Agar medium (MH)

Beef infusion	300 g
Casein hydrolysate	17.5 g
Starch	1.5 g
Agar agar	17.0 g
Distilled water	1000 ml
pH	7.4

3.1.1.4. Purification of bacterial isolates

The bacterial cultures obtained from the survey were purified initially by repeated streaking on nutrient agar medium. Pure cultures thus obtained from the streak plate were then transferred to Muller Hinton agar slants and preserved for further studies.

3.1.1.5. Studies on the preliminary characterisation of bacterial isolates

The bacterial isolates obtained were grouped according to their gram reaction (Hucker and Conn, 1923). By this procedure they were grouped into four groups as gram positive rods, gram negative rods, gram positive cocci and gram negative cocci.

PLATE II

Isolation of bacteria from the infected females and egg masses collected from pepper roots

1. Growth of bacteria B around the female stage F

2. Growth of bacteria around the egg masses and dispersal by apparently healthy juveniles

PLATE II

3.1.1.6. Maintenance of the bacterial cultures

The bacterial cultures thus grouped were sub-cultured on nutrient agar slants and incubated at 30 ± 2°C for 48 hours and stored at 5 - 10°C. It was sub-cultured as and when experiments were started. The age of the bacterial cultures at the time of treatments was adjusted to 48 hours.

3.2. Pathogenicity of the bacterial isolates on different life stages of M. incognita

3.2.1. Raising stock culture of M. incognita

Stock culture of M. incognita was raised from single egg mass collected from pepper vine roots after identifying the species by observing the perineal pattern and maintained on pepper vine plants in sterile soil in pots. Watering was done with sterile water. Further multiplication was done on pepper vine by collecting egg mass from the above stock culture and maintained separately. Sub-culturing was done periodically to ensure availability of sufficient larval population for the experimental purposes. Viable egg masses were collected from these plants having uniform age and size for pathogenicity studies. Nematode larval inoculum was prepared by collecting the fresh larvae emerged from viable egg masses collected from the roots of these pepper plants.

3.2.2. Preparation of bacterial suspension

Two day old bacterial culture was used for the preparation of the bacterial inoculum. The bacterial cells multiplied in nutrient agar medium was used. The bacterial cells were scrapped from the surface of the plates and they were suspended in sterile water. This bacterial suspension was shaken well and the strength was measured in terms of optical density with a Bausch and Lomb "Spectronic 20" colorimeter adjusted to a wave length of 470 n.m. The optical density (OD) of the bacterial suspension was adjusted to 1.5 first, then 1.0 and 0.5 by diluting it with sterile water.

The pathogenic effect of the bacterial isolates on the emergence of the larvae from egg mass and mortality of the larvae were studied. Twenty two bacterial isolates obtained during the preliminary survey were used for finding the effect on the emergence of the larvae from egg mass.

3.2.1.1. Effect on the emergence of the larvae from egg mass

For these studies, sterile cavity blocks with lids were used. *M. incognita* egg masses were collected from the pure culture maintained in pepper plants in sterile soil. The healthy egg masses having uniform size, colour and age were

surface sterilized with 0.1 per cent mercuric chloride solution. These were then washed in three changes of sterile distilled water. Then one egg mass each was aseptically transferred to a sterile cavity block having two ml sterile water. One ml of the bacterial suspension (22 isolates) prepared as mentioned above having O.D of 1.5, 1.0 and 0.5 was aseptically transferred into the cavity block having surface sterilized egg mass. These settings (PlateIII) were kept undistrubed for 10 days to observe the emergence of the larvae. Those of the surface sterilized egg masses kept in sterile distilled water in cavity blocks served as check treatment. There were three replications. Observations were recorded on the larvae emerged per egg mass from two days after treatment up to ten days.

3.2.1.2. Effect on larval mortality

All the different isolates of bacteria (22 Nos.) collected were used for determining their pathogenic effect on the mortality of the second stage juveniles of M.incognita in vitro.

The mortality effect was studied using sterile cavity blocks using bacterial cell concentration measured in terms of O.D levels of 1.5, 1.0 and 0.5 at three different periods

PLATE III

Cavity block with lid used for the evaluation of effect of bacteria in egg mass and larvae in sterile condition

PLATE III

like 48, 72 and 120 hours after treatment. Freshly hatched (24 hr old) second stage larvae of M. incognita were collected from viable egg masses from the roots of pure culture pepper plants maintained in sterile condition. The healthy larvae having uniform age were surface sterilized with 0.1 per cent mercuric chloride solution and washed in three changes of sterile distilled water. Then they were aseptically transferred to sterile cavity blocks with lid containing two ml sterile water. Two ml of the bacterial suspension prepared as mentioned above was also aseptically transferred to the cavity block containing 100 larvae in two ml of sterile water. These settings were kept undisturbed for five days to observe the mortality and immobility from 48 hours to 120 hours. Surface sterilized larvae kept in sterile distilled water served as control. Each treatment was replicated thrice. The mortality of the larvae were tabulated and corrected with natural mortality using Abbot's formula of corrected mortality percentage (Abbot, 1925).

$$\text{Abbot's corrected mortality percentage} = \frac{\text{Percentage survival in control (X)} - \text{Percentage survival in treatment (Y)}}{\text{Percentage survival in control (X)}} \times 100$$

3.3. Identification, purification and maintenance of the bacteria

The cultural and physiological characteristics of the bacterial isolates were studied following the procedures described in the 'Laboratory Methods in Microbiology' (Harrigan and McCance, 1966). The identification of bacteria was done at the Department of Microbiology, College of Veterinary and Animal Sciences, Mannuthy, following the method prescribed by Cowan (1974). Six bacterial isolates found effective in reducing the larval emergence from the egg mass and causing maximum larval mortality *in vitro* were identified up to the species level.

After the identification of bacterial isolates to species level, their distribution (location-wise) in major root-knot infested pepper gardens in the State were studied by carrying out a detailed survey.

3.4. Survey on the occurrence of six species of *Bacillus* associated with root-knot nematode in major pepper growing areas of Kerala

A detailed survey was carried out in the major pepper growing areas in the districts of Kannur, Ernakulam, Idukki, Kollam, Kottayam, Kozhikode, Pathanamthitta, Thiruvananthapuram, Thrissur and Wynad. A random sampling technique was

adopted in the survey. Thus 400 samples were collected from 120 locations covering ten districts.

Name of district	No. of locations	No. of samples collected
Kannur	30	100
Ernakulam	6	18
Idukki	14	50
Kollam	8	24
Kottayam	15	43
Kozhikode	10	40
Pathanamthitta	8	27
Thiruvananthapuram	8	30
Thrissur	6	18
Wynad	15	50
Total	120	400

Collection of root samples and processing were done as mentioned in para 3.1.1.1, 3.1.1.2 and 3.1.1.3.

3.4.1. Purification of bacterial isolates

The bacterial cultures (183 Nos.) obtained fro survey were purified initially as mentioned in para 3.1.1.4.

3.4.2. Studies on the preliminary characterisation of the bacterial isolates

These 183 isolates were grouped according to their gram reaction and ninety gram positive rods were selected for further detailed study. From these ninety, thirty nine isolates were sorted out after doing the first stage test for gram positive bacteria described by Cowan (1974). Then these bacterial isolates were identified up to the species level at the College of Veterinary and Animal Sciences, Mannuthy.

After the identification of bacterial isolates to species level, their distribution (location-wise) was marked out by plotting in the cadastral map.

The percentage occurrence of these six species in the different stages of *M. incognita* in the samples collected were worked out and presented.

3.5. Pathogenicity of different isolates of *Bacillus* spp.

The pathogenic effect of different isolates of six identified species of *Bacillus* were done separately *in vitro* to select out the most promising isolate of each species for detailed study.

Thirty nine bacterial isolates obtained during the survey were identified as follows:

Eight	isolates	as	B. subtilis
Twelve	,,		B. pumilus
Three	,,		B. coagulans
Four	,,		B. macerans
Seven	,,		B. circulans
Five	,,		B. licheniformis

3.5.1. Effect on the emergence of the larvae from egg mass

For these studies sterile cavity block method as described in para 3.2.1.1 was employed. Six different CRD experiments each were set up to evaluate this effect using three concentrations of bacterial cell suspension (prepared as described in para 3.2.2). The viable, uniform aged and sized egg masses collected from pepper roots were selected and used here. Each trial was replicated three to six times depending upon the number of isolates to be tested. The larval emergence were counted from two days after treatment up to ten days. The larvae emerged up to five days were given in the figure3. The data collected 10 days after treatment were tabulated and subjected to analysis of variance test.

3.5.2. Effect on larval mortality

All the different isolates of Bacillus (39) collected were used for determining their effect on the mortality of the second stage juveniles of M. incognita in vitro.

Larval mortality studies were done as described in para 3.2.1.2. Bacterial cell suspension having O.D of 1.5, 1.0 and 0.5 were used here also. The larval mortality was counted at 48, 72 and 120 hr after treatment. The treatment mortality was corrected with mortality in control using Abbot's formula. Altogether there were 54 CRD experiments to compare the isolates.

After comparing the ovicidal and larvicidal effect of the isolates of six species of Bacillus, one isolate each of the species was selected for further detailed study. Though the pigment producing bacteria having ovicidal and larvicidal effect was not used for further detailed study because of its feeble sporulation and variable gram reaction. It was also confined in forest areas and neglected pepper gardens revealing that this species may disappear easily by common agricultural practices.

3.6. Effect of the selected isolate of different species of Bacillus on root-knot nematode in vitro

The bacterial suspension of selected isolates of five species of Bacillus were prepared as described in para 3.2.2.

The viable bacterial cells were counted by serial dilution and pourplate method. Thus, B. subtilis (Pampadumpara isolate) 1.5×10^6 cells per ml, B. pumilus (Muzhoor isolate) 1.8×10^6 cells per ml, B. coagulans (Marakkal isolate) 1.2×10^6 cells per ml, B. macerans (Sulthanbathery isolate) 0.8×10^6 cells per ml and B. circulans (Kumili isolate) 0.9×10^6 cells per ml were obtained.

These bacterial suspensions were used for detecting their effect on the emergence of the larvae from the egg mass and larval mortality as done above. These suspensions were kept as stock solution for the next two experiments.

3.7. Effect of the selected isolates of different species of Bacillus on root-knot nematode in tomato plants

3.7.1. Sterilization of potting mixture and sand

Potting mixture was prepared by mixing sieved field soil (red loam), sieved sand and well decomposed farm yard manure in the ratio of 2:1:1. The potting mixture thus prepared and sand were filled separately in cloth bags and sterilized by autoclaving at 15 lb/sq.inch (1.05 kg/cm^2) pressure for one hr. The sterilized soil and sand filled in cloth bags are utilized for raising tomato seedlings, pepper rooted cuttings and other host plants of M. incognita for various experiments.

3.7.2. Preparation of bacterial inoculum

The bacterial suspension of selected isolates of five species of Bacillus were prepared as described earlier (para 3.6) was used for these studies. M. incognita second stage juveniles emerged from viable egg masses picked from pure culture plants of pepper raised in sterile soil were used for the inoculation.

Four week old tomato seedlings raised in sterile soil were used here. Ten ml of the bacterial suspension of each species of Bacillus (10^6 cells/ml) per pot (15 cm dia.) was inoculated into the soil just around the root zone after removing 2-3 cm depth of top soil. Two days after this M. incognita larvae at the rate of one larva per g of soil was added following the method described by Venkitesan and Setty (1977). Two control treatments were maintained here. One control having nematode alone and another check with nematode and carbofuran 500 ppm. Then the pots were kept in shade for proper establishment of the plants. Watering was done carefully without any draining out of water and soil particles.

Thus the treatments were as follows:

N + *B. subtilis* - 1.5×10^7 cells per pot

N + *B. pumilus* - 1.8×10^7 cells per pot

N + *B. coagulans* - 1.2×10^7 cells per pot

N + *B. macerans* - 0.8×10^7 cells per pot

N + *B. circulans* - 0.9×10^7 cells per pot

N *M. incognita* alone

N + carbofuran 500 ppm.

Forty five days after the nematode inoculation, plants were uprooted. The number of root-knots per plant and the number of nematodes in 100 ml soil were recorded. The data were tabulated and subjected to analysis of variance test.

3.8. Effect of the bacterial isolates on plants and higher animals (birds)

3.8.1. Effect of the bacterial isolates on crop plants

Pathogenicity of the bacteria was tested on betel vine, bhindi, brinjal, bitter gourd, tomato, *Erythrina*, drum stick and *Coleus* as these crops are favourable hosts of *M. incognita* and also seen in pepper gardens. It was assessed by four methods of transmission as detailed below. Observations on the stand of the crop, wilting, drying and any other disease symptoms were made daily up to three months. Four replicates were maintained for each experiment under each method of

transmission. The bacterial cell suspension containing the following viable cells per ml was used for all these four different methods of transmission study:

B. subtilis	-	1.5×10^6 cells per ml
B. pumilus	-	1.8×10^6 cells per ml
B. coagulans	-	1.2×10^6 cells per ml
B. macerans	-	0.8×10^6 cells per ml and
B. circulans	-	0.9×10^6 cells per ml.

Earthen pots of 15 cm diameter were filled with sterile potting mixture. Ten ml of the bacterial suspension was uniformly injected into the soil. Planting or sowing was done after two hours of adding the bacterial suspension at the rate of two seedlings, stem cuttings or seeds per pot. Soil mixed with ten ml of sterile water alone served as check. The plants were maintained for three months for recording the observations.

3.8.1.2. Seed treatment

Ten g seeds each of the test crops were thoroughly cleaned to remove all the foreign materials and soaked in 10 ml of the bacterial suspension by keeping for 12 hours. Seeds soaked in sterile water served as check. The treated seeds were sown in pots at the rate of three seeds per pot and replicated four times.

In case of betel vine, Coleus, Erythrina and drumstick, stem cuttings were used instead of seeds. The cuttings dipped in sterile water served as check. The treated cuttings were planted in pots 30 minutes after treatment at the rate of two cuttings per pot. Germination of seeds and cuttings was also observed.

3.8.1.3. Root treatment

Fifteen days old seedlings raised in sterile soil of each crop and thirty days old rooted cuttings were used for this test. The roots of the plants were washed thoroughly to remove all foreign materials. A small portion of the tips of the roots was cut and dipped in bacterial suspension for one hour and three seedlings per pot were planted. The roots of seedlings dipped in sterile water served as check.

3.8.1.4. Leaf treatment

One month old healthy plants were used for this purpose. Injury was made on the leaves by pin pricks. Inoculations were done with bacterial suspension soaked in sterile cotton plugs. A gentle swabbing with treated cotton plugs was done on the entire leaf for three times at five minutes interval. Injured spots on the leaf swabbed with

sterile distilled water in cotton plug served as check. Five leaves were treated in each plant.

3.8.2. Effect on higher animals (Birds)

Safety testing of the five bacterial isolates were carried out in two week old chicks having uniform weight and other growth characteristics. The bacterial isolates were maintained and multiplied in nutrient agar for 48 hours. The bacterial cells were scrapped from the surface of the solid media and it was suspended in sterile water. Then the bacterial cells were pelleted by centrifugation in an ultra-centrifuge at 12,000 G at 10°C for 20 minutes. These bacterial pellets were used for safety testing.

3.8.2.1. Oral administration

Five ml of the bacterial pellets having 10^8 cells/ml of each species of Bacillus were poured into the buccal cavity of the pre-starved chicks to avoid loss of bacterial cells. After one hour they were given the normal diet. This type of feeding was continued for ten consecutive days. The chicks were kept in five separate pens to avoid contamination. Six chicks per treatment were maintained in good condition and they were observed daily for one month for the expression of symptoms of bacterial infection such as chick fever, irritations in the skin and diarrhoea.

3.8.2.2. Subcutaneous injection

The bacterial cells pelletted as mentioned earlier were used for subcutaneous injection. One millilitre of the suspension prepared as mentioned above was injected to each chick below the two wings, subcutaneously. These chicks were caged in separate pens to avoid contamination and daily examined for the presence of any symptom of bacterial disease.

3.9. Growth of *Bacillus* spp. on liquid media for mass production

Five common bacteriological media were tested for the growth of the *Bacillus* spp. Growth of the bacteria in different liquid media was studied by turbidimetric method. 9.9 ml of each medium was poured in test tubes and autoclaved at 1.05 kg/cm^2 pressure for 30 minutes. After cooling, 0.1 ml of the bacterial suspension having 1.1×10^6 viable bacterial cells of each *Bacillus* spp. was added into the medium in the test tube. These tubes were shaked well and incubated at room temperature ($30 \pm 2^{\circ}C$) for varying periods ranging from 6 to 72 hours. The growth was measured by observing the O.D with a Bausch and Lomb "Spectronic 20" colorimeter adjusted to a wave length of 470 n.m. The growth of the bacteria was measured 6, 12, 24, 48 and 72 hours

after inoculation of respective bacterial suspension. Four replicates were maintained. The media used and their composition were as follows:

Nutrient broth

Peptone	5 g
Beef extract	3 g
Sodium chloride	2 g
Distilled water	1000 ml
pH	6.8

Muller Hinton medium

Beef infusion	300 g
Caesin hydrolysate	17.5 g
Starch	1.5 g
Distilled water	1000 ml
pH	7.4

Potato dextrose medium

Peeled potato	200 g
Dextrose monohydrate	20 g
Distilled water	1000 ml
pH	7.0

Glucose yeast extract medium

Glucose	10 g
Yeast extract	5 g
Peptone	5 g
Distilled water	1000 ml
pH	8.8

Lactose yeast extract medium

Lactose	10 g
Yeast extract	5 g
Peptone	5 g
Distilled water	1000 ml
pH	6.8

3.10. Evaluation of pathogenic effect of *Bacillus* spp. on root-knot nematode by pot culture studies

3.10.1. Preparation and sterilization of potting mixture

Potting mixture prepared as described earlier was used for this experiment also. This sterile potting mixture was filled three-fourth of the volume of black polythene bags (400 gauge thickness) having three holes for proper drainage. Sterile water was added to the maximum water holding capacity.

3.10.2. Preparation of nutrient solution

Nutrient solution recommended by Arnon and Hoagland (1940) was used for providing minor and trace elements for plants growing in pots.

3.10.3. Raising of rooted cutting of pepper vine

Stem cuttings with three nodes taken from mature vines of the variety Panniyur 1 were planted after treating them with IBA at 500 ppm to induce rooting in polythene bags containing sterile conditioned soil. At three to four leaf stage, those having good root system and uniform thickness were transplanted singly in small clay pot filled with sterilized soil.

The five bacterial isolates which indicated pathogenic effect in earlier tests were used. Ten ml each of the bacterial suspension was added per pot. Two days after this the root-knot nematode juveniles, one larva per ml of soil, were added to the pots, following the method adopted by Venkitesan and Setty (1977). Pots having root-knot nematode alone served as control and pots without bacteria and nematode served as absolute check. Each treatment was replicated three times. The list of treatments are given below.

Details of treatment

Root-knot nematode (1000 larvae)	(N) +	*B. macerans*	1.2×10^8	cells/pot - T_1
	N +	,,	1.2×10^6	,, - T_2
	N +	,,	1.2×10^5	,, - T_3
	N +	*B. pumilus*	1.1×10^8	,, - T_4
	N +	,,	1.1×10^6	,, - T_5
	N +	,,	1.1×10^5	,, - T_6
	N +	*B. circulans*	1.2×10^8	,, - T_7
	N +	,,	1.2×10^6	,, - T_8
	N +	,,	1.2×10^5	,, - T_9
	N +	*B. coagulans*	1.2×10^8	,, - T_{10}
	N +	,,	1.2×10^6	,, - T_{11}
	N +	,,	1.2×10^5	,, - T_{12}
	N +	*B. subtilis*	1.0×10^8	,, - T_{13}
	N +	,,	1.0×10^6	,, - T_{14}
	N +	,,	1.0×10^5	,, - T_{15}
Root-knot nematode (1000 larvae) alone			Control	- T_{16}
Without any organism			Absolute check	- T_{17}

All the plants were supplied with sterilized nutrient solution at the rate of 100 ml per pot at an interval of four weeks.

Observations were taken on number of leaves and length of vines on the day of treatment and at monthly intervals.

At the end of 3, 6, 12 and 18th month a set of plants was uprooted and length of vine, number of leaves, fresh top weight, fresh root weight, dry weight of shoot, number of galls, etc. were recorded. The bacteria was also re-isolated from different stages of M. incognita in root.

Root-knot count

The root system from each pot was carefully lifted by gentle tapping of the pots on all sides and bottom and removing the loose soil, the root was cleaned of adhering soil particles by gentle washing in water. These washings were collected, passed through 350 mesh sieve and preserved for nematode count. The clean root system was pressed gently between folds of blotting paper. The number of galls and fresh root weight were recorded in case of each plant. The number of galls was indexed using 1 to 5 scale. The egg masses obtained at eighteen month after treatment were picked and transferred to sterile cavity block containing 2 ml of distilled water. The number of larvae emerged was counted and subjected to analysis of variance test.

Population of nematode in soil

The soil from each pot was thoroughly mixed and a representative sample of 100 ml was collected and processed

for extracting the nematode following the method of Christie and Perry (1951) and the nematode extracted were estimated.

The inactive or dead larvae present in the soil were estimated by collecting 50 g of one more sample from each pot and by direct examination of the suspension collected after the sievings and the total nematodes computed for 100 ml based on the number recorded under each method.

Re-isolation of bacteria

The different stages (egg mass, larva, adult female) obtained in the roots were collected and washed in sterile water. They were then placed on the sterile petri plate containing the nutrient agar medium. These plates were incubated at 30 ± 2°C for 48 hours for the growth of the bacteria. The bacterial growth formed were then further streaked on another petri plate for single colony isolation. The colonies formed were then cross-checked with the characteristics of the original isolates.

After computing the final population of nematodes in each treatment, the reproductive rate and nematostatic value were arrived using the formula of Eissa and Moussa (1982) as given below.

$$\text{Rate of reproduction or Reproductive potential} = \frac{Pf + 1}{Pi + 1}$$

where Pi is the initial population and Pf is the final population.

$$\text{Nematostatic value} = \frac{\text{Rate of reproduction of treatment}}{\text{Rate of reproduction of check}} \times 100$$

3.11. Interaction with insecticides, nematicides and fungicides

Sensitivity of the bacteria to different pesticides were tested in vitro by the filter paper disc method. The stock solution of the pesticides were prepared in emulsified sterile water at different concentrations (at 125-2000 ppm). Sterilized filter paper discs of 10 mm diameter were dipped in appropriate dilutions of the pesticides. They were placed aseptically over the nutrient agar medium in petri plates which had been seeded with 24 hour old culture of the bacterial isolate. Sterilized filter paper discs dipped in emulsified sterile water were kept as checks for comparison. Three replicates each were maintained. The plates were incubated at 30 ± 2°C. The diameter of the zone of inhibition was measured after 24, 48 and 72 hours.

List of pesticides tested with their doses used in the *in vitro* sensitivity studies

Pesticides used		Dose in ppm
Nematicides/insecticides		
Methamsodium	Sodium N-methyldithio carbamate dihydrate	500 and 1000
Phorate	O,O-diethyl S-(ethyl thiomethyl phosphorothiolothionate	500 and 1000
Aldicarb	2-methyl-2(methylthio) propionaldehyde O-(methyl carbamoyl) oxime	500 and 1000
Carbofuran	2,3, dihydro-2,2 dimethyl 7 benzofuranyl methyl carbamate	500 and 1000
Formalin	Formaldehyde 40 per cent	500 and 1000
Insecticides		
HCH (BHC)	1,2,3,4,5,6-hexachlorocyclo-hexane	500 and 1000
Endosulfan	1,4,5,6,7,7-dexachlorc-8,9,10-trinor-born-5-Zn- 2,3-ylene-dimethyl sulphite	500 and 1000
Malathion	S 1,2 di (ethoxy carbonyl) ethyl dimethyl phosphorothiolothionate	500 and 1000
Quinalphos	O,O diethyl-O-(quinoxalinyl-(2)-thionophosphate	500 and 1000
Carbaryl	1-naphthyl-N-methyl carbamate	500 and 1000
Fungicides		
Agallol 3	(3% mercury) as methoxy ethyl mercury chloride	125, 250, 500, 1000 and 2000
Carbendazim	Methyl-1, H benzemedazol-2-ylcarbamate	125, 250, 500, 1000 and 2000
Copper oxy-chloride	$Cu\ Cl_2\ 3Cu\ (OH)_2$	125, 250, 500, 1000 and 2000
Maneb	Manganese ethylene bis dithiocarbamate	125, 250, 500, 1000 and 2000
Zineb	Zinc ethylene bis dithio-carbamate	125, 250, 500, 1000 and 2000
Ziram	Zinc dimethyl dithiocarbamate	125, 250, 500, 1000 and 2000

3.12. Effect of the bacterial isolates on related genera of Meloidogyne

The mortality effect induced by five bacterial isolates found potent on M. incognita second stage juveniles was studied on related genera of Meloidogyne namely Heterodera and Rotylenchulus. The mortality effect induced by these bacteria was studied using cavity block method in vitro at three inoculum levels. The mortality in the treatments were corrected with control mortality using Abbot's formula.

RESULTS

4. RESULTS

4.1. Preliminary survey

A preliminary survey was conducted to detect the bacterial pathogens encountered in the root-knot nematode Meloidogyne incognita. Twenty one samples were collected from eight locations, namely, Palode, Pampadumpara, Panniyur, Vellayani, Vellanikkara, Marakkal, Kannara and Kumili. The data are presented in Table 1. Results revealed that bacterial pathogens were associated with different life stages of M. incognita. From the egg mass, eleven bacterial isolates (gram positive rods) were obtained. From the female stage also eleven bacterial isolates (eight gram positive rods, one each of gram negative rod, gram positive cocci and gram negative cocci) were obtained. The second stage juveniles collected from Pampadumpara alone yielded one rod shaped bacterium showing gram positive reaction. Among the different bacterial isolates, one gram positive rod type was obtained both from the females and egg masses collected from Kumili.

4.2. Pathogenicity of the bacterial isolates on different life stages of M. incognita

4.2.1. Effect on the emergence of the larvae from the egg mass

Results are presented in Table 2. The effect of different bacterial isolates at the optical density of 1.5

Table 1. Distribution of *M. incognita* and the associated bacterial pathogens in root-knot infested pepper roots collected from different locations for the preliminary studies

Locations	Number of samples collected	Mean nematode population in root (5 g)			Bacteria isolated from		
		Female	Eggmass	2nd stage juvenile	Female	Eggmass	2nd stage juvenile
Palode	2	21	7	40	+R(1),+R(2)	+R(3)	--
Pampadumpara	3	2	2	56	+R(4)	+R(5)	+R(6)
PRS Panniyur	2	5	3	15	+R(7)	+R(8),+R(9)	--
Vellayani	1	4	2	3	--	+R(10)	--
Vellanikkara	1	21	2	11	+R(11)	--	--
Marakkal	5	12	4	29	+R(12),+C(14)	+R(13),+R(15)	--
Kannara	5	12	3	14	+R(16),-R(18), -C(20)	+R(17), +R(19)	--
Kumili	2	9	4	17	+R(21),+R(21)	+R(21),+R(22)	--

Figures in parentheses are the bacterial isolate numbers

+R - Gram positive rods
-R - Gram negative rods
+C - Gram positive cocci
-C - Gram negative cocci

reduced the larval emergence from 31 to 75 per cent. Among the twenty two, six isolates showed above 65 per cent reduction in larval emergence five days after treatment. Maximum reduction was exhibited by isolate 19 (75.4 per cent) followed by 6 (72.6 per cent), 21 (69 per cent), 13 (67.9 per cent), 5 (65.0 per cent) and 4 (65.0 per cent). Out of the six, isolates 19,13 and 5 were obtained from egg mass alone while isolate 19 was obtained from egg mass and females. Isolate 6 showed ovicidal effect and was also associated with the second stage juveniles of M. incognita.

Effect on the larval emergence from the egg mass treated with bacterial isolates, at the optical density of 1.0, also showed the similar results. The six bacterial isolates showed maximum reduction in the emergence of the larvae. They were isolate 13 (67.4 per cent), 6 (66.0 per cent), 19 (65.4 per cent), 4 (63.3 per cent), 21 (61.1 per cent) and 5 (56.3 per cent).

At the lowest concentration (OD 0.5), maximum reduction was seen in isolate 13 (57.8 per cent) followed by 5 (56.7 per cent), 21 (54.7 per cent) and 6 (53.1 per cent).

Thus the isolates 13, 5, 21 and 6 were selected for detailed study.

Table 2. Mean number of larvae of M. incognita which emerged from the eggmass five days after treatment with different bacterial isolates (mean of three replications)

Bacterial isolate numbers	Mean number of larvae emerged per eggmass and its percentage reduction over control at an optical density of					
	1.5		1.0		0.5	
1	83.3	35.3	94.3	27.0	106.0	17.6
2	86.7	32.6	96.0	25.0	105.3	18.2
3	85.7	33.4	93.3	28.0	106.3	17.4
4	44.7	65.3	47.0	63.0	67.7	47.4
5	44.3	65.6	56.3	56.0	55.7	56.7
6	35.3	72.6	44.0	66.0	60.3	53.1
7	84.7	34.9	97.3	24.0	111.7	13.2
8	87.3	32.2	92.6	28.0	98.3	23.6
9	77.7	39.6	85.0	34.0	105.7	18.0
10	85.3	33.7	99.0	23.0	117.3	9.8
11	88.7	31.1	100.0	22.0	116.3	9.9
12	88.0	31.6	97.6	24.0	113.3	11.9
13	41.3	68.0	42.0	67.4	54.3	57.8
14	83.0	35.5	98.3	24.0	114.0	11.4
15	88.0	31.6	107.7	16.3	126.7	2.6
16	81.3	36.8	94.3	26.7	107.3	16.6
17	85.7	33.4	95.9	25.6	113.3	11.9
18	89.0	30.8	94.7	26.4	109.3	15.1
19	31.7	75.4	44.6	65.3	73.3	43.0
20	87.0	32.4	99.7	22.5	119.3	7.3
21	39.7	69.2	50.0	61.1	58.3	54.7
22	55.0	57.3	66.3	48.4	103.3	19.7
Control					128.7	

4.2.2. Effect on larval mortality

Mean corrected larval mortality of the larvae observed at 120 hours after treatment with different bacterial isolates are given in Table 3.

The result showed that the larval mortality obtained after treatment with all the six bacterial isolates at the optical density of 1.5, was above 90 per cent. The isolate number 19 showed maximum larval mortality (93.3 per cent). This was followed by 13 (92.7 per cent), 21 (92 per cent), 6 (91.0 per cent), 4 (90.7 per cent) and 5 (90.3 per cent).

At the lower concentration (OD 1.0), six bacterial isolates showed 74.7 per cent to 86.9 per cent larval mortality. Maximum larval mortality was caused by isolate 21 (86.9 per cent) and it was followed by isolates 8 (84.1 per cent), 19 (80.7 per cent), 6 (78.4 per cent), 5 (76.0 per cent) and 4 (74.7 per cent).

At the lowest concentration (OD 0.5), also the effect of the above five bacterial isolates maintained their superiority. The isolates 21, 13, 5, 19 and 4 caused larval mortalities of 80.0, 75.3, 74.7, 72.7 and 69.7 per cent, respectively. Though the isolate 6 was obtained from second stage juveniles the larvicidal effect was not higher than those of other isolates. The isolates 6 and 8 obtained from

Table 3. Mean corrected larval mortality of *M. incognita* treated with different bacterial isolates observed at 120 hours after treatment (mean of three replications)

Bacterial isolate numbers	Mean corrected larval mortality per cent after treatment with bacterial isolates at an optical density of		
	1.5	1.0	0.5
1	57.7	39.7	38.0
2	34.7	41.0	26.7
3	35.0	35.3	22.7
4	90.7	74.7	69.7
5	90.3	76.0	74.7
6	91.0	78.4	65.7
7	34.3	41.3	39.0
8	89.7	84.1	65.3
9	83.3	70.7	60.0
10	40.0	34.0	37.0
11	33.3	36.0	30.7
12	37.0	40.0	29.3
13	92.7	73.3	75.3
14	54.3	43.0	40.3
15	75.0	50.0	42.7
16	73.3	69.0	64.0
17	67.0	51.7	43.3
18	65.7	44.3	48.3
19	93.3	80.7	72.7
20	48.7	41.3	37.3
21	92.0	86.9	80.0
22	80.0	74.3	60.0

second stage juveniles and egg masses from Pampadumpara were morphologically similar and also pigment producing but their ovicidal and larvicidal effects were not uniform.

The five bacterial isolates showing maximum larval mortality, in the three doses tried, isolates (21, 13, 5, 19 and 4) and isolates (13, 6, 5 and 21) giving minimum larval emergence were studied in detail.

4.3. Identification, purification and maintenance of the organism

Six bacterial isolates were found effective in reducing the emergence of the larvae from the egg mass and giving maximum larval mortality under *in vitro* condition were identified at Microbiology Department, College of Veterinary and Animal Sciences, Mannuthy, following the tests prescribed by Cowan (1974) and identified as follows:

Isolate number	4	*Bacillus subtilis*
,,	5	*B. pumilus*
,,	6	*B. licheniformis*
,,	13	*B. coagulans*
,,	19	*B. macerans*
,,	21	*B. circulans*

4.4. Distribution of the Bacillus spp. associated with root-knot nematode M. incognita in major pepper growing areas of Kerala

The distribution of Bacillus spp. in the different locations (districtwise) is given in Table 4 and Fig. 1.

The root samples (400 numbers) collected from ten major pepper growing districts of Kerala as mentioned in para 3.1 showed the wide distribution of root-knot nematode and the associated bacterial pathogens. On an average 4 to 150 M. incognita were present per five gram of root in various locations. The percentage of samples having infected mature females, egg masses and second stage juveniles ranged from 5 to 44, 12 to 38 and 0 to 6, respectively (Table 4).

4.4.1. Kannur district

B. pumilus was obtained from female stage, only collected from Pepper Research Station, Panniyur and Muzhoor. B. licheniformis was seen in egg mass collected from Pepper Research Station, Panniyur, Panniyur private holding and in females, egg masses and second stage juveniles from District Agricultural Farm, Thaliparamba.

4.4.2. Ernakulam district

B. subtilis and B. pumilus were obtained from the egg masses collected from Angamali. B. circulans was

Table 4. Distribution of root-knot nematode and the associated bacterial pathogens in pepper growing areas of Kerala

Locations	No.of samples collected	Mean nematode population in root (5g)				Bacteria isolated		
		Female	Eggmass	2nd stage juvenile	Total	Female	Eggmass	Larva
Cannore district								
PRS Panniyur B.I	4	3	2	11	16	+R(1)	+R(1)	+R(1)
,, II	4	1	0	4	5	--	--	--
,, III	4	6	2	12	20	+R,+R,+R	--	--
,, IV	4	4	1	10	15	+R(2),-R	+R(6)	+C
,, V	6	4	1	8	13	+R,+R	--	--
PRS young plantation and germplasm collection	3	0	0	0	0	--	--	--
Panniyur private holding	2	6	1	3	10	--	+R,+R(6)	--
Pukkot	4	15	7	13	35	--	--	--
Poovam	2	3	1	23	27	+R	--	--
DAF Thaliparamba B.I	4	8	2	22	32	+R(6)	+R(6)	--
,, II	4	1	0	5	6	--	+R	--
,, III	3	8	3	26	37	+R	--	--
,, IV	3	21	7	23	51	--	--	--
,, V	3	14	10	36	60	+R	+R	+R(6)
Thaliparamba private holding	2	0	0	4	4	--	--	--
Karimbam	1	3	1	41	45	--	+R	--
Chanakakundu	1	17	10	68	95	--	--	--
Muzhoor	4	4	1	5	10	+R(2)	+R	--
Thadikkadavu	4	4	1	11	15	--	--	--
Alakkode	2	15	9	18	32	--	--	--
Meenpatty	6	6	1	3	10	+R	+R	+R
Tellicherry	5	3	1	9	13	+R,+R	--	--
Kuppam	2	10	2	2	14	--	--	--
Mullenkolly	3	2	0	13	15	+R	+R	--
Kalikkadavu (north)	5	4	2	13	19	--	--	--
Kalikkadavu (south)	3	2	1	16	19	--	--	--
Karavachal	7	6	1	6	13	+R	+R	--
Payannur	1	0	0	0	0	+R,+R	--	--
Pazhayangadi	1	13	8	129	150	--	--	--
Puthur	1	13	4	49	66	--	--	--
Percentage of samples having infected nematodes						19	12	4
Ernakulam district								
Piravam	3	6	1	20	27	--	+R(5)	--
Alwaye	3	4	3	18	25	+R(5)	--	--
Kothamangalam	4	13	5	36	54	--	--	--
Moovattupuzha	4	12	5	35	52	--	--	--
Angamali	2	4	4	0	8	--	+R(2)+R(1)	--
Karukutti	2	19	10	40	69	--	--	--
Percentage of samples having infected nematodes						5	16	0
Idukki district								
Peerumedu	2	9	3	17	29	+R,+R(1)	+R,+R	--
Kumili	6	7	2	20	29	+R(1),+R(3)	+R,+R(3)	--
Kanjiramattam	2	0	0	0	0	--	+R(5),+R	--
Thodupuzha	5	11	5	27	43	+R(2)	--	--
Moolamattam	5	8	2	20	30	--	+R(2)	--
Pampadumpara	10	4	2	36	42	--	+R,+R	--
Elappara	3	13	4	21	38	--	+R(1),	-C
Neriamangalam	6	10	6	27	43	+R	+R(6)	--
Munnar	2	4	21	28	33	--	--	--
Vandiperiyar	1	11	2	23	36	--	+C,+C,+R	--
Nedumkandam	4	5	2	22	29	+R	--	--
Kattappana	1	8	0	54	62	+R	--	--
Karimpara	1	3	1	10	14	--	+R	--
Kalpadavu	2	2	0	15	17	+R	--	--
Percentage of samples having infected nematodes						--	--	--
Kollam district						14	34	4
Kadakkal	4	4	0	11	15	+C	--	--
Punaloor	3	5	1	19	24	--	+R	--
Adoor	3	4	1	9	14	+R	--	--
Kottarakkara	5	7	1	6	14	+R	+R(2),+R	--
Anchal	2	11	2	29	42	--	--	--
Chattanoor	2	8	3	26	37	+R	--	--
Ayoor	2	3	1	15	19	+R	--	--
Kulathoopuzha	3	9	0	12	21	+R(6)	--	--
Percentage of samples having infected nematode						33	16	0

Table 4 (Contd.)

Locations	No.of samples collected	Mean nematode population in root (5g)				Bacteria isolated		
		Female	Eggmass	2nd stage juvenile	Total	Female	Eggmass	Larva
Kottayam district								
Vaikom	2	7	3	22	32	+R(2)	—	—
Manimala	3	22	3	24	49	—	—	—
Kudukkachira	3	8	2	20	30	+R	+R	+R
Melekavumattam	3	6	1	13	20	+R,+R	+R	—
Palai	3	7	3	37	47	+C	—	+R(2)
RARS Kumarakom	1	0	0	—	—	—	—	—
Kumarakom private holding	3	2	0	4	6	—	—	—
DAF Kozha	5	3	1	17	21	—	—	—
Vazhoor	3	1	0	1	2	—	+R	—
Kanjirapally	3	7	1	30	38	—	—	—
Puthuppadiyil	2	4	1	19	24	—	—	—
Pallikallu	3	8	1	17	26	—	+R,+R	—
Kuravilangad	2	5	2	26	33	+R	—	+C
Kottayam	4	4	2	14	20	+C	+R(4),+C	—
Yettumannoor	3	1	0	9	10	+R	—	—
Percentage of samples having infected nematodes						18	16	6
Kozhikode district								
Alathur	5	5	2	12	19	+R,+R,+R	+R	—
Badagara	3	10	3	37	50	—	—	-C
Balusseri	4	9	1	8	18	+R	+R	—
Elathur	4	9	3	30	42	+R	+R,+R	—
Kuppadi	5	7	4	19	30	+R,+R	—	—
Mavoor	4	14	6	30	50	+R	—	—
Perambra	4	4	2	8	14	+R	—	—
Puthuppadi	5	16	5	9	30	+R(5)	—	—
Thariyode	3	10	3	37	50	—	—	—
Nadakkavu	3	1	0	5	6	—	—	—
Percentage of samples having infected nematodes						25	12	2.5
Pathanamthitta district								
Angadi	3	4	0	25	29	—	—	—
Chengannur	2	8	0	20	28	—	-C	—
Konni	5	12	5	39	56	+R(3)	—	—
Omallur	3	17	2	30	49	—	—	—
Pathanamthitta	3	8	1	34	43	+R	+R	—
Panthalam	3	26	2	35	63	+R	+R	—
Payyanamon	3	5	1	21	27	—	+R,-C	—
Ranni	5	14	5	18	37	—	+R(2)	+R(1)
Percentage of samples having infected nematodes						11	22	3

Locations	No.of samples collected	Mean nematode population in root (5g)				Bacteria isolated		
		Female	Eggmass	2nd stage juvenile	Total	Female	Eggmass	Larva
Thiruvananthapuram district								
Kallara	4	6	1	18	25	—	—	—
Kilimannoor	3	5	1	23	29	+R,+R	+R,+R	—
Nedumangad	3	7	2	19	28	—	+C,+R(4)	—
Palode	6	25	6	24	55	+R(2),+R,+R	+R,+R	—
Pangode	5	6	1	14	21	+R,+R	+R	+R
Vattappara	4	14	1	28	43	—	+R	—
Vellayani	2	2	1	8	11	—	+R(2)	—
Ulloor	1	4	0	21	25	—	—	—
Percentage of samples having infected nematodes						26.0	33.0	6.0
Trissur district								
Cherpu	2	3	1	23	27	+R(5)	—	+R
Kannara	5	12	3	14	29	+R,-R,-C	+R,+R(4)	—
Marakkal	5	12	4	29	45	+R,+C	+R(3),+R	—
Mannuthy	2	4	1	30	35	—	—	—
Nettisseri	2	18	7	17	42	+R(5)	+R	—
Vellanikkara	2	12	7	16	35	+R(2)	-R	—
Percentage of samples having infected nematodes						44	33	5
Wynad district								
HRS Ambalavayal								
B.I & II	2	33	2	9	44	+R(6)	+R(6)	—
,, III	6	9	0	28	37	+R(2)	-C	—
,, IV	5	12	3	17	32	+R,-C	-C	—
,, V	6	39	3	13	55	—	+R	+R
,, VIII	3	17	1	17	35	—	—	—
,, IX	4	78	12	33	123	+R,+R	+R,+R	—
,, X	2	50	7	40	97	+R,+R	+R,+R	—
,, XI	2	76	2	60	132	—	—	—
,, XII & XIII	3	108	7	146	256	+R,+R,-R	+R,+R,-C	—
,, XIV	4	13	4	17	44	+R,-C	+R,-C	—
Beenachi estate (coffee)	2	6	2	9	17	+R	+R	—
Beenachi (pepper) plantation	2	8	2	33	43	+R,+R	+R,+R	—
Ambalavayal forest area	3	7	4	25	36	+C	-R,+C	—
Sulthanbattery	3	17	2	59	78	+R(4)	—	—
Vadamaye	3	1	0	3	4	—	—	—
Percentage of samples having infected nematodes						36.0	38.0	2.0

Figures in parentheses are the serial numbers of bacteria identified - 1. Bacillus subtilis, 2. B. pumilis, 3. B.Coagulans, 4. B. macerans, 5. B. circulans and 6. B. licheniformis.

+R : Gram positive rods from a sample
-R : Gram negative rods from a sample
+C : Gram positive cocci from a sample
-C : Gram negative cocci from a sample

obtained from egg masses and females collected from Piravam and Alwaye, respectively.

4.4.3. Idukki district

B. subtilis was obtained from females collected from Perumede, Kumili and egg mass from Pampadumpara. B. pumilis was obtained from egg mass and females collected from Thodupuzha. B. coagulans was obtained from egg masses and females collected from Kumili. B. circulans was also obtained from two holdings from Kumili area. B. licheniformis was obtained from egg mass collected from Pampadumpara.

4.4.4. Kollam district

B. subtilis was obtained from females collected from Adoor and egg masses collected from Kottarakkara. B. pumilus was also obtained from egg masses collected from Kottarakkara. B. licheniformis was obtained from females collected from Kulathoopuzha.

4.4.5. Kottayam district

B. pumilus was obtained from the females, second stage juveniles collected from Vaikom and Palai, respectively. B. macerans was obtained from the egg masses collected from Kottayam.

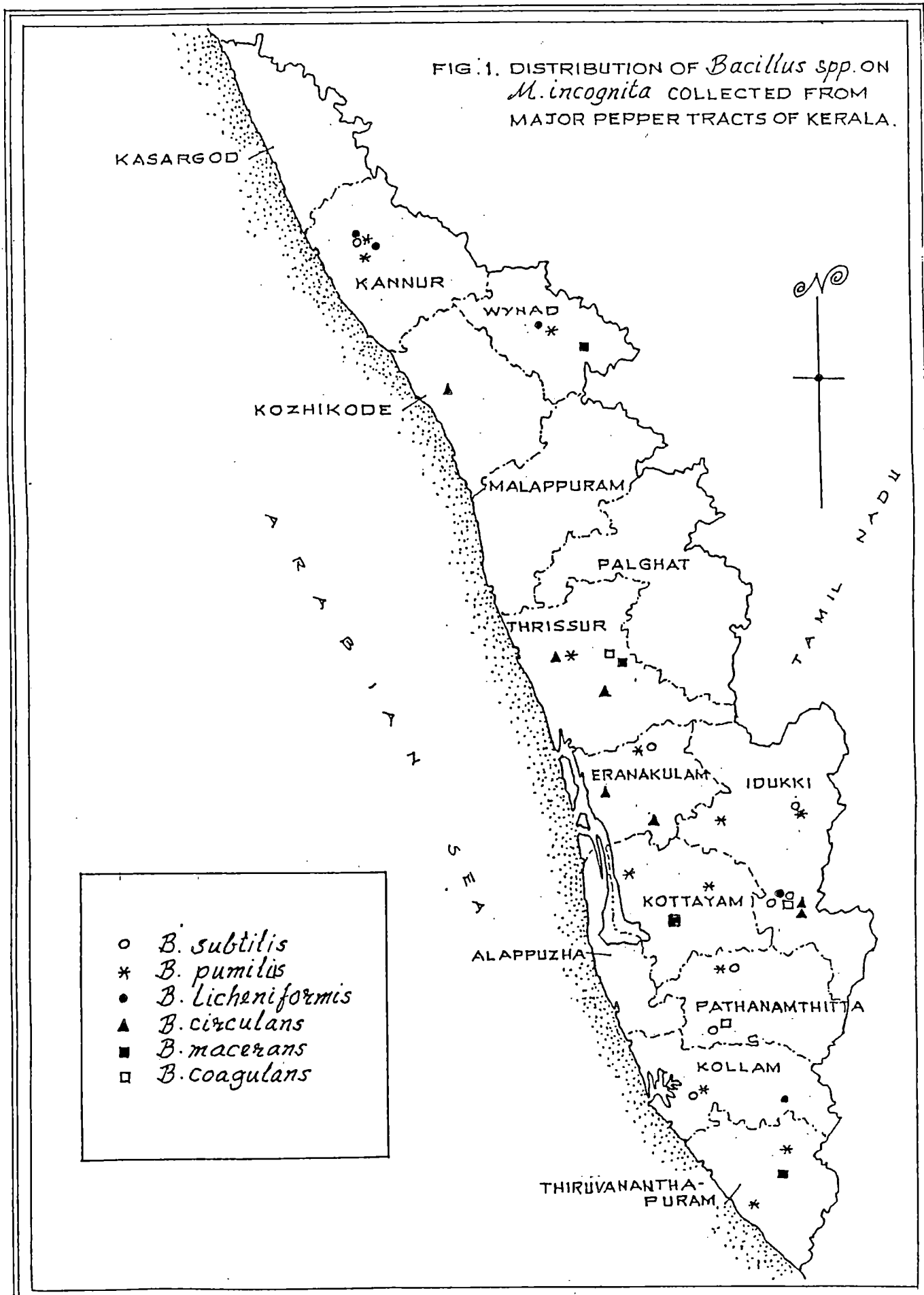


FIG. 1. DISTRIBUTION OF Bacillus spp. ON M. incognita COLLECTED FROM MAJOR PEPPER TRACTS OF KERALA.
KASARGOD
KANNUR
WYNAD
KOZHIKODE
MALAPPURAM
PALGHAT
THRISSUR
ERANAKULAM
IDUKKI
KOTTAYAM
ALAPPUZHA
PATHANAMTHITTA
KOLLAM
THIRUVANANTHA-PURAM
ARABIAN SEA
TAMIL NADU
B. subtilis
B. pumilus
B. licheniformis
B. circulans
B. macerans
B. coagulans

4.4.6. Kozhikode district

B. circulans was obtained from the egg mass collected from Puthuppadi.

4.4.7. Pathanamthitta district

B. subtilis was obtained from the second stage juveniles. B. pumilus was obtained from egg masses collected from Ranni. B. coagulans was obtained from the females collected from Konni.

4.4.8. Thiruvananthapuram district

B. pumilus was obtained from the females and egg masses collected from Palode and Vellayani, respectively. B. macerans was obtained from the egg masses collected from Nedumangad.

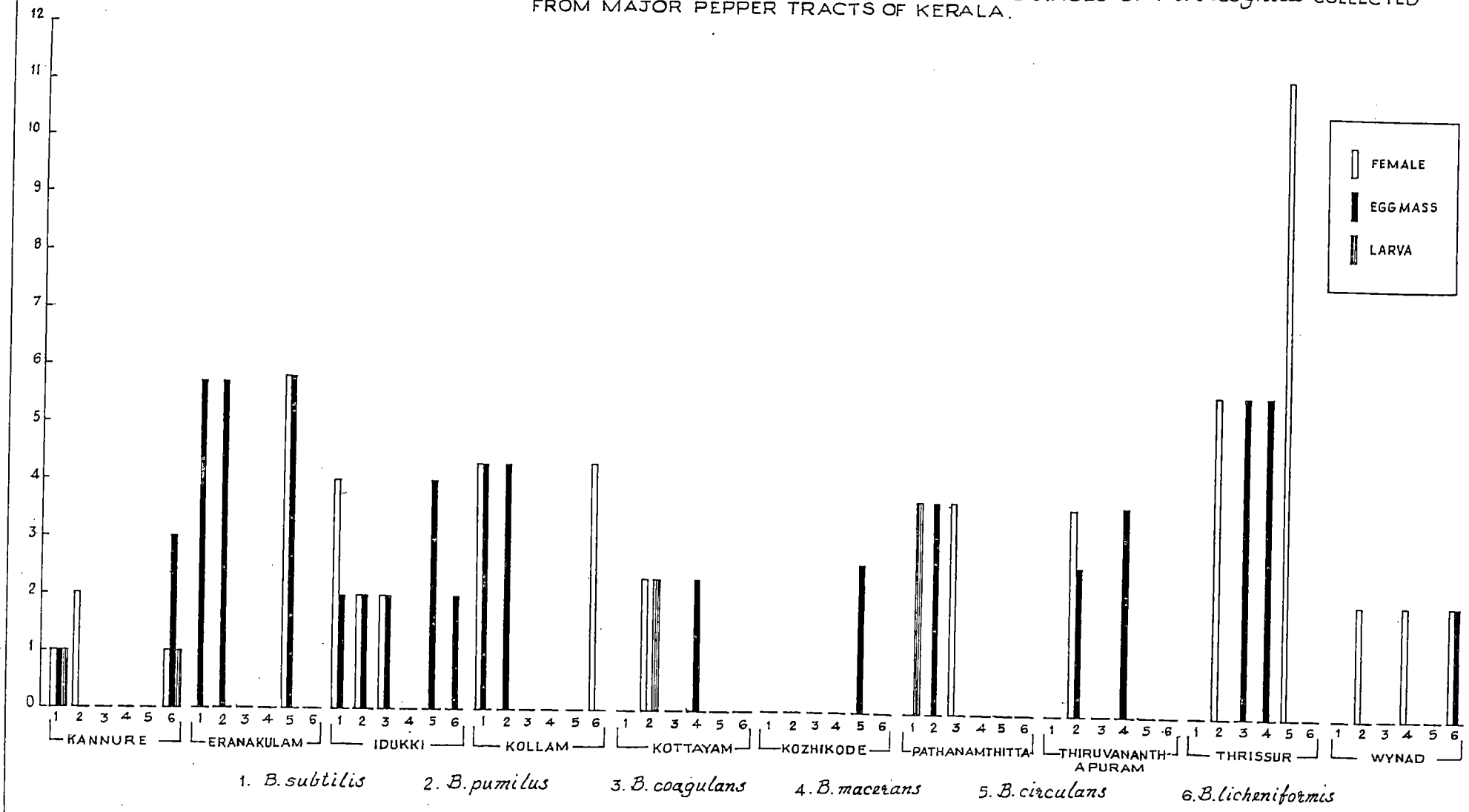
4.4.9. Thrissur district

B. pumilus was obtained from females collected from Vellanikkara. B. coagulans and B. macerans were obtained from egg masses collected from Marakkal and Kannara, respectively. B. circulans was obtained from the females collected from Cherpu and Nettisseri.

4.4.10. Wynad district

B. pumilus and B. macerans were obtained from females collected from Horticultural Research Station, Ambalavayal

FIG. 2. PERCENTAGE OCCURRENCE OF DIFFERENT SPECIES OF BACILLUS ON VARIOUS LIFE STAGES OF *M. incognita* COLLECTED FROM MAJOR PEPPER TRACTS OF KERALA.



and Sulthanbathery. B. licheniformis was obtained from the females and egg masses collected from HRS Ambalavayal.

The locationwise distribution of these six species of Bacillus are presented in Fig. 1.

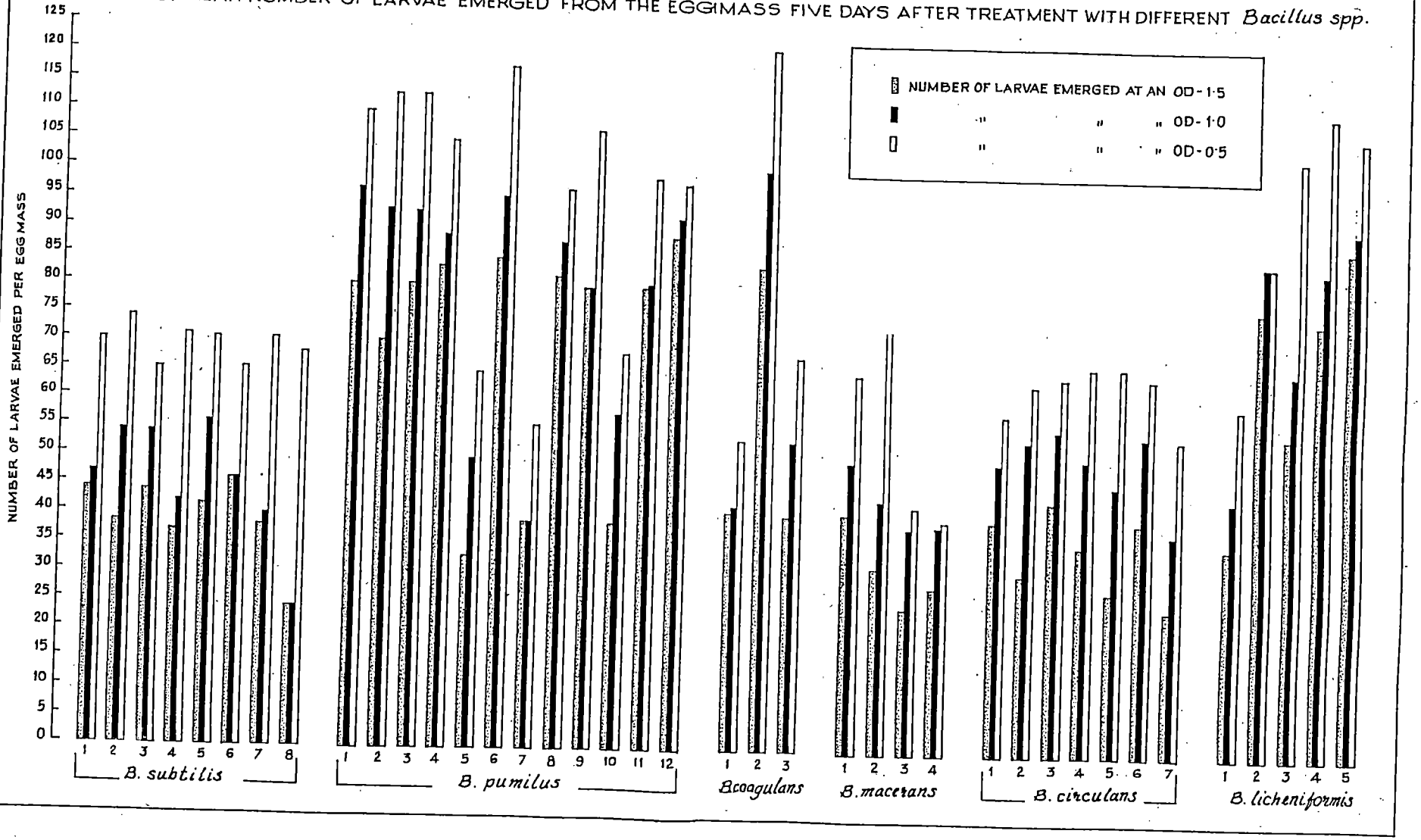
Thus B. subtilis was obtained from 8 locations, three isolates each from egg mass and females, one from second stage juvenile and one from all the stages. B. pumilus was obtained from 12 locations, 6 isolates from females, 4 from egg masses, one from second stage juveniles and one from both egg masses and females. Three isolates of B. coagulans were obtained from three locations, one from egg masses, one from females and one from egg masses as well as females. B. macerans was isolated from four locations, three from the egg masses and one from females. B. circulans was obtained from seven locations, three from the egg masses and four from females. B. licheniformis was isolated from five locations, two from the egg masses, one from the females, one from the egg masses and females and one from all the life stages. Percentage occurrence of these Bacillus spp. on different life stages are given in Fig. 2.

4.5. Pathogenicity of the selected isolates of Bacillus spp.

4.5.1. Effect on the emergence of the larvae from the egg mass

The results on this aspect are presented in Table 5 and Fig. 3.

FIG.3. MEAN NUMBER OF LARVAE EMERGED FROM THE EGGMASS FIVE DAYS AFTER TREATMENT WITH DIFFERENT *Bacillus spp.*



4.5.1.1. Bacillus subtilis

Results showed that all the eight isolates were statistically on par in reducing the emergence of the larvae from the egg masses ten days after treatment (DAT)

4.5.1.2. B. pumilus

The result showed that all the twelve isolates were statistically on par with reference to the number of larvae emerged from egg masses at three levels of treatment at 10 DAT.

4.5.1.3. B. coagulans

The results revealed that all the three bacterial isolates were statistically on par in reducing the emergence of the larvae from the egg mass 10 DAT. However, B. coagulans isolated from Marakkal showed maximum reduction in emergence of the larvae from the egg masses (Table 5).

4.5.1.4. B. macerans

The result showed that therewas statistical difference in the emergence of the larvae from the egg masses 10 DAT. B. macerans isolated from Kottayam showed maximum reduction which was on par with isolates from Kannara and Sulthanbathery.

Table 5. Mean number of larvae of M. incognita emerged from the eggmass ten days after treatment with different isolates of Bacillus spp.

Bacillus spp. tested	Locations	Mean number of larvae emerged from eggmass treated with an optical density of 1.5	1.0	0.5
B. subtilis	Panniyur (PRS)	112.6(10.6)	101.2(10.1)	120.2(11.0)
	Angamali	97.3 (9.9)	108.6(10.4)	112.2(10.6)
	Kumili	104.6(10.3)	106.2(10.3)	114.6(10.7)
	Pampadumpara	90.2 (9.5)	99.9(10.0)	103.8(10.2)
	Perumede	94.4 (9.7)	104.9(10.2)	109.3(10.5)
	Kottarakkara (1)	92.3 (9.6)	97.4 (9.7)	122.6(11.1)
	Adoor	95.0 (9.8)	97.7 (9.9)	104.7(10.2)
	Ranni	92.7 (9.6)	95.0 (9.8)	98.3 (9.9)
	C.D.	NS	NS	NS
B. pumilus	Panniyur (PRS)	109.8(10.5)	141.6(11.9)	159.8(12.6)
	Muzhoor	119.3(10.9)	118.3(10.9)	129.0(11.4)
	Angamali	97.7 (9.9)	119.3(10.9)	122.9(11.1)
	Thodupuzha	103.9(10.2)	98.3 (9.9)	105.2(10.3)
	Kottarakkara	95.7 (9.8)	105.2(10.3)	102.5(10.1)
	Vaikom	120.0(10.9)	111.6(10.6)	123.0(11.1)
	Palai	90.0 (9.5)	93.2 (9.7)	100.3(10.0)
	Ranni	92.3 (9.6)	98.3 (9.9)	106.0(10.3)
	Palode	95.7 (9.8)	97.3 (9.9)	107.3(10.4)
	Vellayani	86.9 (9.3)	99.1(10.0)	103.3(10.2)
	Vellanikkara	85.0 (9.2)	92.2 (9.6)	105.0(10.2)
	Ambalavayal	91.9 (9.6)	94.0 (9.7)	112.6(10.6)
	C.D.	NS	NS	NS
B. coagulans	Kumili	83.5 (9.1)	117.0(10.8)	120.3(11.0)
	Konni	89.5 (9.5)	104.9(10.2)	134.5(11.6)
	Marakkal	98.3 (9.9)	98.7 (9.9)	122.8(11.1)
	C.D.	NS	NS	NS
B. macerans	Kottayam	95.8 (9.8)	97.4 (9.9)	108.8(10.4)
	Nedumangad	140.3(11.8)	145.1(12.1)	163.0(12.8)
	Kannara	97.9 (9.9)	113.3(10.6)	116.8(10.8)
	Sulthanbathery	101.1(10.1)	107.5(10.4)	110.6(10.5)
	C.D.	(0.36)	(1.16)	(1.00)
B. circulans	Puthuppadi	93.0 (9.6)	104.6(10.2)	119.0(10.9)
	Alwaye	97.6 (9.9)	117.3(10.8)	119.3(10.9)
	Piravam	99.0 (9.9)	108.6(10.4)	113.6(10.7)
	Kumili (a)	91.1 (9.5)	94.7 (9.7)	118.9(10.7)
	Kumili (b)	92.9 (9.6)	114.3(10.7)	125.1(11.2)
	Cherpu	100.3(10.0)	101.1(10.1)	114.0(10.7)
	Nettisseri	95.3 (9.8)	98.7 (9.7)	122.6(11.1)
	C.D.	NS	NS	NS
B. licheniformis	Panniyur (Local)	88.3 (9.4)	120.8(11.0)	148.0(12.2)
	haliparamba	93.0 (9.6)	164.3(12.8)	126.5(11.2)
	ampadumpara	101.6(10.1)	108.4(10.4)	119.6(10.9)
	ulathoopuzha	106.6(10.3)	126.6(11.3)	136.4(11.7)
	mbalavayal	102.3(10.1)	116.3(10.8)	120.8(11.0)
	.D.	NS	(1.00)	(0.88)

Figures in parentheses are values after $\sqrt{x}$ transformation

NS : Not significant

This was the trend in all the three levels of treatment. Nedumangad isolate was inferior among the four isolates (Table 5).

4.5.1.5. B. circulans

The result revealed that all the seven isolates were statistically on par in reducing the larval emergence of M. incognita from the egg mass 10 DAT.

4.5.1.6. B. licheniformis

The result showed that at higher dose (OD 1.5) all the six isolates were statistically on par. At the lower dose (OD 1.0) maximum reduction was exhibited by Pampadumpara isolate followed by isolates obtained from Ambalavayal, Panniyur and Kulathoopuzha and these four were statistically on par. Isolate obtained from Thaliparamba was inferior to all the other four isolates. At the lowest dose also (OD 0.5) Thaliparamba isolate was inferior to other four isolates (Table 5).

4.5.2. Effect on the mortality of the larvae of M.incognita

4.5.2.1. Larval mortality at the optical density of 1.5

4.5.2.1.1. B. subtilis

The results are presented in Table 6. There was statistical significance between various isolates. The

isolate obtained from Pampadumpara showed maximum larval mortality and it was significantly superior to the other seven isolates which were on par. At 72 hours after treatment, there was no significant variations in the mortality caused by the isolates. At 120 hours after treatment there was significant variations. Maximum mortality was exhibited by isolate obtained from Pampadumpara followed by Perumede and Panniyur and the latter isolates were statistically on par.

4.5.2.1.2. *B. pumilus*

The result showed that there was statistically significant variations among the isolates 48, 72 and 120 hours after treatment (Table 6).

At 48 hours after treatment, Muzhoor isolate showed maximum larval mortality followed by Thodupuzha and these two were on par and significantly superior to other isolates.

At 72 hours after treatment also, Muzhoor isolate showed maximum larval mortality followed by Thodupuzha and these two were on par and superior to other ten isolates.

At 120 hours after treatment, Muzhoor isolate showed maximum larval mortality and it was significantly superior to other isolates (Table 6).

Table 6. Mean corrected mortality percentage of the larvae of M. incognita treated with Bacillus spp. obtained from different locations at an optical density of 1.5 (mean of three replications)

Bacillus spp. tested	Locations	Mean larval mortality percentage observed at different intervals (hours) 48	72	120
B. subtilis	Panniyur (PRS)	83.6(66.1)	86.0(68.0)	90.6(76.2)
	Angamali	79.2(62.9)	81.7(64.7)	86.8(68.7)
	Kumili	81.7(64.6)	83.7(66.1)	89.3(70.9)
	Pampadumpara	90.4(71.9)	91.7(73.2)	95.4(77.5)
	Perumede	81.0(64.1)	83.0(65.6)	92.4(74.3)
	Kottakkada	81.8(64.1)	85.5(67.6)	86.8(68.7)
	Adoor	78.0(62.0)	81.0(64.1)	84.7(66.9)
	Ranni	78.4(62.3)	83.4(65.9)	87.1(68.9)
	C.D.	(3.74)	NS	(5.06)
B. pumilus	Panniyur (PRS)	71.0(57.4)	72.0(58.0)	73.0(56.7)
	Muzhoor	87.0(68.9)	91.0(72.5)	95.4(77.5)
	Angamali	80.7(63.9)	82.3(65.1)	91.4(72.8)
	Thodupuzha	86.1(68.1)	88.8(70.4)	91.6(73.1)
	Kottarakkara	80.4(63.7)	83.0(65.7)	87.6(69.4)
	Vaikom	81.0(64.1)	83.4(65.9)	90.6(72.1)
	Palai	73.3(58.9)	78.0(62.0)	84.8(67.0)
	Ranni	76.0(60.7)	80.7(63.9)	84.3(66.7)
	Palode	75.1(60.0)	78.4(62.3)	82.7(65.4)
	Vellayani	77.4(61.6)	81.0(64.1)	87.0(68.9)
	Vellanikkara	81.0(64.1)	85.3(67.5)	88.4(70.0)
	Ambalavayal	73.7(59.1)	84.5(66.8)	90.4(71.9)
	C.D.	(2.5)	(3.65)	(3.70)
B. coagulans	Kumili	85.5(67.6)	90.7(71.9)	92.5(74.0)
	Konni	85.5(67.6)	93.4(75.0)	93.9(75.7)
	Marakkal	89.1(70.7)	91.4(72.9)	95.3(77.5)
	C.D.	NS	NS	NS
B. macerans	Kottayam	83.4(69.5)	84.9(67.2)	88.6(70.2)
	Nedumangad	90.4(71.9)	91.0(72.5)	93.4(75.1)
	Kannara	92.0(73.6)	94.0(75.8)	96.1(78.6)
	Sulthanbathery	93.4(75.1)	96.8(79.6)	98.0(82.0)
	C.D.	NS	(3.69)	(5.89)
B. circulans	Puthuppadi	86.1(68.1)	88.2(69.9)	92.0(73.5)
	Alwaye	83.4(66.0)	86.8(68.7)	94.3(76.2)
	Piravam	84.8(67.6)	87.0(68.9)	97.6(81.1)
	Kumili (a)	83.8(66.2)	87.5(69.3)	94.2(76.0)
	Kumili (b)	83.7(66.1)	87.4(69.2)	91.7(73.2)
	Cherpu	83.9(66.3)	89.6(71.2)	97.3(80.6)
	Nettisseri	81.7(64.6)	82.7(65.4)	86.0(68.0)
	C.D.	NS	NS	NS
B. licheniformis	Panniyur	87.4(69.2)	91.7(73.2)	94.0(75.8)
	Thaliparamba	75.3(60.2)	79.0(62.7)	83.4(65.9)
	Pampadumpara	81.0(64.2)	83.4(65.9)	90.0(71.7)
	Kulathoopuzha	85.5(67.6)	89.5(70.0)	92.3(73.8)
	Ambalavayal	90.1(71.6)	90.7(72.2)	92.3(73.9)
	C.D.	NS	NS	NS

Figures in parentheses are values after angular transformation

NS : Not significant

4.5.2.1.3. *B. coagulans*

The results showed that all the three isolates were statistically on par 48, 72 and 120 hours after treatment. However, at 48 and 120 hours after treatment isolate obtained from Marakkal showed highest larval mortality (Table 6).

4.5.2.1.4. *B. macerans*

The result revealed that all the four isolates were statistically on par 48 hours after treatment. At 72 hours after treatment, Sulthanbathery isolate showed its superiority and it was significantly superior to other three isolates. At 120 hours after treatment also it was superior but on par with Kannara isolate (Table 6).

4.5.2.1.5. *B. circulans*

The result revealed that all the seven isolates were statistically on par 48, 72 and 120 hours after treatment (Table 6).

4.5.2.1.6. *B. licheniformis*

The result revealed that all the five isolates were statistically on par 48, 72 and 120 hours after treatment. However, at 72 and 120 hours after treatment, Panniyur isolate showed maximum larval mortality followed by Ambalavayal isolate (Table 6).

4.5.2.2. Larval mortality at the optical density of 1.0

The results are presented in Table 7.

4.5.2.2.1. B. subtilis

The result showed that all the eight isolates were statistically on par 48, 72 and 120 hours after treatment.

4.5.2.2.2. B. pumilus

The result revealed that there was statistically significant difference among the isolates 48 and 72 hours after treatment (Table 7).

At 48 and 72 hours after treatment Vellayani (73.4), Kottarakkara (73.4), Muzhoor (73.0), Vaikom (71.7) and Angamali (71.5) isolates were on par and significantly superior to other seven isolates.

At 120 hours after treatment the effect of all the twelve isolates were statistically on par.

4.5.2.2.3. B. coagulans

The result showed that all the three isolates were statistically on par 48 and 120 hours after treatment.

At 72 hours after treatment, Marakkal isolate gave maximum larval mortality (73.7 per cent) and it was significantly superior to other two isolates.

Table 7. Mean corrected mortality percentage of the larvae of M. incognita treated with Bacillus spp. obtained from different locations at an optical density of 1.0 (mean of three replications)

Bacillus spp. tested	Locations	Mean mortality percentage observed at different intervals (hours) 48	72	120
B. subtilis	Panniyur (PRS)	67.0(54.9)	69.4(56.4)	74.7(59.8)
	Angamali	70.7(56.8)	74.7(59.8)	77.4(61.6)
	Kumili	70.0(56.7)	71.3(57.6)	75.3(60.2)
	Pampadumpara	72.4(58.3)	73.1(58.7)	75.1(60.0)
	Perumede	69.3(56.4)	72.7(58.5)	75.7(60.4)
	Kottarakkara	69.7(56.6)	72.0(58.0)	74.7(59.8)
	Adoor	70.0(56.8)	72.7(58.5)	75.7(60.4)
	Ranni	71.1(56.9)	74.1(59.4)	76.7(61.1)
	C.D.	NS	NS	NS
B. pumilus	Panniyur (PRS)	61.7(51.8)	64.0(53.1)	68.7(55.0)
	Muzhoor	73.0(58.9)	76.0(60.7)	80.0(63.4)
	Angamali	71.5(57.7)	75.1(60.1)	78.9(62.5)
	Thodupuzha	70.3(57.0)	72.2(58.5)	76.7(61.1)
	Kottarakkara	74.3(59.5)	77.0(61.3)	79.0(62.7)
	Vaikom	71.7(57.8)	74.7(59.8)	77.7(61.8)
	Palai	66.7(54.7)	71.0(57.4)	74.3(59.5)
	Ranni	68.7(55.9)	72.3(58.2)	75.0(60.0)
	Palode	69.3(56.4)	71.0(57.4)	74.0(59.3)
	Vellayani	74.3(59.5)	76.3(60.9)	78.4(62.2)
	Vellanikkara	68.7(55.9)	72.7(58.5)	77.7(61.8)
	Ambalavayal	69.0(56.2)	71.3(57.6)	76.0(60.7)
	C.D.	(2.35)	(2.54)	NS
B.coagulans	Kumili	65.7(54.1)	70.7(57.2)	73.3(58.9)
	Konni	66.4(52.7)	68.0(55.5)	71.3(57.6)
	Marakkal	69.3(56.4)	73.7(59.1)	76.0(60.7)
	C.D.	NS	(1.30)	NS
B. macerans	Kottayam	70.7(57.2)	73.3(58.9)	76.7(61.1)
	Nedumangad	73.7(59.1)	76.0(60.6)	80.7(63.9)
	Kannara	73.3(58.9)	74.4(59.6)	66.4(60.9)
	Sulthanbathery	70.3(56.9)	71.3(57.6)	73.3(58.9)
	C.D.	NS	(1.15)	(1.87)
B. circulans	Puthuppadi	70.4(56.9)	78.3(62.3)	86.9(68.8)
	Alwaye	71.0(57.4)	73.3(58.9)	80.7(63.9)
	Piravam	74.3(59.5)	78.4(62.3)	82.0(64.9)
	Kumili (a)	74.7(59.8)	80.0(63.4)	82.7(65.4)
	Kumili (b)	74.3(59.5)	76.7(61.2)	81.0(64.1)
	Cherpu	76.3(60.9)	78.7(62.5)	81.7(64.6)
	Nettissery	71.0(57.4)	73.7(59.1)	74.3(59.5)
	C.D.	(2.10)	(2.20)	(3.04)
B. licheniformis	Panniyur (Local)	70.3(56.9)	73.0(58.7)	78.4(62.3)
	Thaliparamba	67.1(54.9)	71.1(57.4)	73.7(59.1)
	Pampadumpara	71.1(57.5)	72.8(58.5)	74.4(59.6)
	Kulathupuzha	70.3(56.9)	75.4(60.2)	77.0(60.7)
	Ambalavayal	74.1(59.4)	76.7(61.1)	79.1(62.8)
	C.D.	NS	NS	NS

Figures in parentheses are values after angular transformation

NS : Not significant

4.5.2.2.4. B. macerans

The result showed that all the four isolates were statistically on par . . 48 hours after treatment. However, Nedumangad isolate showed maximum larval mortality. At 72 hours after treatment also, Nedumangad isolate showed its superiority but it was on par with Kannara isolate and was superior to other two isolates. At 120 hours after treatment also Nedumangad isolate was significantly superior to other three isolates (Table 7).

4.5.2.2.5. B. circulans

The results showed that there was significant differences among various isolates in causing the larval mortality . . 48, 72 and 120 hours after treatment.

At 48 hours, isolate obtained from Cherpu showed maximum larval mortality followed by two isolates from Kumili and Piravam. These four were statistically on par and superior to other three isolates.

At 72 hours after treatment, isolate obtained from Kumili, Cherpu, Puthupadi and Piravam were on par and statistically superior to other isolates. Isolate obtained from Puthupadi exhibited its superiority . . 120 hours after treatment. It was statistically superior to other six isolates (Table 7).

4.5.2.2.6. B. licheniformis

The results revealed that all the five isolates were statistically on par in their effect on the larval mortality observed 48, 72 and 120 hours after treatment. However, isolate obtained from Ambalavayal showed maximum mortality 48, 72 and 120 hours after treatment.

4.5.2.3. Larval mortality at the optical density of 0.5

The results are presented in Table 8.

4.5.2.3.1. B. subtilis

The result showed that the variations in the effects of the eight isolates on larval mortality were statistically significant at 48, 72 and 120 hours after treatment.

At 48 hours after treatment, isolate obtained from Perumede showed maximum larval mortality followed by isolates from Kottarakkara and Pampadumpara. These three were on par and significantly superior to other five isolates. At 72 hours after treatment isolate from Perumede showed its superiority and it was statistically superior than remaining seven isolates. But at 120 hours after treatment Pampadumpar isolate also showed higher larval mortality (75.0 per cent) and it was on par with Perumede isolate (73.3 per cent). These two isolates were superior than other six isolates.

4.5.3.2.2. *B. pumilus*

The results showed that at all the three period intervals, the larval mortality showed statistically significant variations.

At 48 hours, Kottarakkara isolate showed highest larval mortality. It was significantly superior to all the eleven isolates.

At 72 hours after treatment also, Kottarakkara isolate showed highest larval mortality followed by Ranni isolate and these two were on par and superior to other isolates.

At 120 hours after treatment also isolate from Kottarakkara showed maximum larval mortality (73.7 per cent) and it was followed by isolates from Ambalavayal (73.7 per cent), Vellanikkara (69.0), Vaikom (68.4), Ranni (68.3), Angamali (68.0), Vellayani (67.3) and Palode (67.0). These eight isolates were statistically on par (Table 8).

4.5.2.3.3. *B. coagulans*

The results revealed that all the three isolates were statistically on par in their effect on larval mortality observed 48 and 72 hours after treatment.

At 120 hours after treatment, isolate from Marakkal (75.3 per cent) showed maximum larval mortality. This

Table 8. Mean corrected mortality percentage of the larvae of M. incognita treated with Bacillus spp. obtained from different locations at an optical density of 0.5 (mean of three replications)

Bacillus spp. tested	Locations	Mean mortality percentage observed at different intervals (hours) 48	72	120
B. subtilis	Panniyur (PRS)	52.0(46.1)	56.7(48.8)	61.3(51.6)
	Angamali	58.0(49.6)	61.3(51.3)	68.3(53.3)
	Kumili	58.3(49.8)	61.3(51.3)	64.3(55.6)
	Pampadumpara	61.7(51.7)	71.0(57.6)	75.0(60.0)
	Perumede	67.3(55.1)	63.7(52.7)	73.3(59.1)
	Kottarakkara (1)	65.5(54.0)	65.0(53.7)	70.0(56.8)
	Adoor	58.3(49.8)	63.0(52.5)	68.3(55.8)
	Ranni	56.0(48.4)	61.0(51.4)	65.7(54.1)
	C.D.	(4.21)	(1.97)	(1.97)
B. pumilus	Panniyur (PRS)	43.3(41.2)	59.7(50.6)	64.0(52.9)
	Muzhoor	59.0(49.0)	56.0(48.4)	64.2(53.0)
	Angamali	54.3(47.5)	61.3(51.6)	68.0(55.6)
	Thodupuzha	57.0(49.0)	57.7(48.9)	64.0(53.1)
	Kottarakkara	65.7(54.1)	56.3(54.9)	73.7(57.4)
	Vaikom	58.3(49.8)	62.7(52.3)	68.4(55.9)
	Palai	54.3(47.5)	56.7(48.8)	61.7(51.7)
	Ranni	60.0(50.7)	64.0(53.1)	68.3(55.8)
	Palode	57.3(49.2)	61.7(51.7)	67.0(54.9)
	Vellayani	59.7(50.6)	62.7(52.3)	67.3(55.2)
	Vellanikkara	57.0(49.0)	63.0(52.6)	69.0(56.2)
	Ambalavayal	52.3(46.3)	62.3(52.1)	73.7(57.5)
	C.D.	(2.79)	(2.09)	(2.65)
B. coagulans	Kumili	50.7(45.4)	60.0(50.8)	68.0(55.6)
	Konni	57.0(49.0)	59.9(50.8)	61.0(51.0)
	Marakkal	56.3(48.6)	59.0(50.2)	75.3(60.3)
	C.D.	NS	NS	(1.83)
B. macerans	Kottayam	60.7(51.1)	62.3(50.0)	70.7(57.2)
	Nedumangad	62.0(51.9)	67.7(55.4)	72.7(58.5)
	Kannara	60.0(50.8)	63.7(52.9)	68.3(56.0)
	Sulthanbathery	61.0(51.3)	71.7(57.8)	76.7(61.1)
	C.D.	NS	(1.66)	(2.40)
B. circulans	Puthupadi	69.0(56.1)	70.3(57.0)	80.0(63.6)
	Alwaye	56.7(48.8)	62.7(52.3)	73.7(59.1)
	Piravam	67.0(54.9)	69.3(56.4)	64.7(55.6)
	Kumili (a)	67.0(54.9)	70.3(57.0)	72.7(58.5)
	Kumili (b)	68.7(55.9)	71.7(57.8)	74.7(59.8)
	Cherpu	68.3(55.7)	71.7(57.8)	67.7(55.8)
	Nettisseri	69.3(56.4)	64.0(53.1)	67.3(55.2)
	C.D.	NS	NS	NS
B. licheniformis	Panniyur (Local)	51.0(.6)	56.3(48.6)	75.7(60.5)
	Thaliparamba	51.3 .7)	56.0(48.5)	73.0(58.7)
	Pampadumpara	44.7(1.9)	62.0(52.0)	70.0(57.0)
	Kulathoopuzha	57.7(49.3)	59.7(50.6)	65.7(53.8)
	Ambalavayal	60.7(51.1)	64.7(53.8)	76.0(60.7)
	C.D.	(3.50)	NS	(3.47)

Figures in parentheses are values after angular transformation

NS : Not significant

isolate was statistically superior to two other isolates (Table 8).

4.5.2.3.4. *B. macerans*

The results showed that all the four isolates were statistically on par in their effect on larval mortality observed 48 hours after treatment.

At 72 and 120 hours after treatment, isolate from Kannara showed maximum larval mortality and it was higher than the mortality in other three treatments (Table 8).

4.5.2.3.5. *B. circulans*

The results revealed that all the seven isolates were statistically on par in their effect on larval mortality observed 48, 72 and 120 hours after treatment (Table 8).

4.5.2.3.6. *B. licheniformis*

The five isolates showed statistically significant differences in the larval mortality observed 48 hours after treatment. Maximum larval mortality was caused by isolate obtained from Ambalavayal (60.7 per cent) followed by Kulathoopuzha (57.7 per cent). These two were statistically on par and better than other isolates. At 72 hours after treatment also, Ambalavayal isolate showed maximum larval mortality but there was no statistical significance.

At 120 hours after treatment, there was statistical significance between isolates. The isolate obtained from Ambalavayal showed highest larval mortality (76.0 per cent) followed by Panniyur isolate (75.7 per cent), Taliparamba isolate (73.0 per cent) and Pampadumpara isolate (70.0 per cent). These isolates were on par.

Considering the effect on the egg mass and larvae at different doses and intervals the following isolates were selected for further detailed study:

B. subtilis - Pampadumpara isolate (egg mass)
B. pumilus - Muzhoor isolate (female)
B. coagulans - Marakkal isolate (egg mass)
B. macerans - Sulthanbathery isolate (female)
B. circulans - Kumili isolate (egg mass).

4.6. Effect of selected isolates of Bacillus spp. on the egg mass and larvae of M. incognita

The result showed that all the bacterial isolates reduced the emergence of larvae from the egg mass significantly. B. coagulans @ 1.2×10^6 cells per ml showed maximum reduction (66.0 larvae per egg mass) followed by B. subtilis @ 1.5×10^6 cells per ml (74.0 larvae per egg mass). These two treatments were statistically on par. B. circulans @ 0.9×10^6

Table 9. Effect of different species of Bacillus on the eggmass and larvae of M. incognita five days after treatment (mean of four replications)

Bacillus spp. tested (cells/ml)	Mean number of larvae emerged per eggmass	Mean larval mortality
	**	*
Bacillus subtilis (1.5×10^6)	73.75 (8.59)	81.50(65.46)
B. pumilus (1.8×10^6)	83.25 (9.10)	73.75(59.22)
B. coagulans (1.2×10^6)	65.75 (8.10)	73.50(59.07)
B. macerans (0.8×10^6)	90.75 (9.52)	75.50(60.35)
B. circulans (0.9×10^6)	79.5 (8.91)	76.75(61.18)
Control	147.25(12.13)	12.50(11.25)
C.D.	(0.68)	(8.80)

Figures in parentheses are values after *angular and **$\sqrt{x}$ transformations

cells per ml and B. pumilus @ 1.8×10^6 cells per ml caused reduction in larval emergence, and they were on par with B. subtilis and superior to B. coagulans (Table 9).

Mean larval mortality due to these selected isolates of Bacillus spp. showed that all the five species were statistically on par. The mean larval mortality ranged from 73.5 to 81.5 per cent (Table 9).

4.7. Effect of Bacillus spp. and carbofuran on root-knot nematodes in tomato plants

The results showed that the treatment with five species of Bacillus and carbofuran at 500 ppm reduced the root-knot production in tomato plant at 45 days after treatment. The treatment of B. circulans 0.8×10^7 cells/pot, B. macerans 0.9×10^7 cells/pot and carbofuran at 500 ppm were statistically on par in reducing the root-knot production. Rest of the treatments were on par and inferior to the above three (Table 10).

All the treatments reduced the nematode population in soil 45 days after treatment. Maximum reduction was obtained in B. subtilis treatment followed by B. coagulans, B.circulans and B. macerans. These four treatments were statistically on par and significantly better than B. pumilus and carbofuran (Table 10).

Table 10. Effect of different species of Bacillus and carbofuran on root-knot nematode in tomato - 45 days after treatment (mean of four replications)

Treatments	Bacterial cells per pot	Root-knot count per plant	Soil population per 100 ml
B. subtilis	1.5×10^7	13.00(3.77)	22.50(5.54)
B. pumilus	1.8×10^7	14.50(3.93)	19.50(4.52)
B. coagulans	1.2×10^7	14.25(3.77)	20.75(4.66)
B. macerans	0.8×10^7	6.50(2.68)	21.50(4.74)
B. circulans	0.9×10^7	5.75(2.52)	21.00(4.67)
Check (carbofuran 500 ppm)		8.00(2.99)	32.75(5.80)
Control (nematode alone)		45.75(6.81)	49.79(7.13)
C.D.		(0.80)	(0.48)

Figures in parentheses are values after $\sqrt{x}$ transformation

4.8. Pathogenicity of the bacterial isolates on crop plants and higher animals

4.8.1. Effect on crop plants

Pathogenicity of the bacteria, B.Subtilis ($1.5x10^6$cels/ml), B. pumilus ($1.8x10^6$), B. coagulans ($1.2x10^6$), B. macerans ($0.8x10^6$) and B. circulans ($0.9x10^6$) tested on eight common host plants representing different families was studied and results showed that the bacteria did not cause symptoms of bacterial disease or mortality of plants.

4.8.2. Effect on higher animals

Safety of the bacterial isolates (B. subtilis, B. pumilus, B. coagulans, B. macerans and B. circulans) on higher animals was studied through oral administration and sub-cutaneous injection on two week old chicken and expressions of bacterial infection leading to chick fever, irritation on the skin and diarrhoea were not noticed.

4.9. Growth of Bacillus spp. on different liquid media for mass production

The result obtained on growth of the different species of Bacillus studied in five different media viz. Nutrient broth (NB), Potato dextrose (PD), Muller Hinton (MH),

Table 11. Growth of Bacillus spp. on different liquid media observed at different intervals after inoculation (mean of four replications)

Media	Growth at 6hr	12hr	24hr	48hr	72hr
	B. subtilis				
Nutrient broth	0.292	0.340	0.390	0.390	0.856
Potato dextrose	0.018	0.075	0.291	0.291	0.668
Muller Hinton	0.079	0.354	0.410	0.410	0.692
Glucose yeast extract	0.128	0.133	0.140	0.140	0.649
Lactose yeast extract	0.032	0.067	0.117	0.336	0.557
C.D. at 5% level	0.030	0.039	0.066	0.069	0.141
	B. pumilus				
Nutrient broth	0.073	0.375	0.403	0.811	0.834
Potato dextrose	0.008	0.077	0.151	0.572	0.879
Muller Hinton	0.098	0.284	0.381	0.464	0.694
Glucose yeast extract	0.126	0.128	0.188	0.498	0.730
Lactose yeast extract	0.027	0.052	0.147	0.402	0.526
C.D. at 5% level	0.015	0.052	0.105	0.104	0.185
	B. coagulans				
Nutrient broth	0.048	0.391	0.463	0.720	0.760
Potato dextrose	0.085	0.122	0.315	0.686	0.935
Muller Hinton	0.081	0.353	0.507	0.508	0.645
Glucose yeast extract	0.178	0.183	0.288	0.839	0.960
Lactose yeast extract	0.072	0.103	0.287	0.520	0.565
C.D. at 5% level	0.029	0.052	0.153	0.106	0.100
	B. macerans				
Nutrient broth	0.127	0.352	0.383	0.383	0.724
Potato dextrose	0.041	0.203	0.388	0.388	0.714
Muller Hinton	0.072	0.075	0.331	0.336	0.766
Glucose yeast extract	0.130	0.130	0.197	0.197	0.915
Lactose yeast extract	0.052	0.185	0.185	0.185	0.510
C.D. at 5% level	0.041	0.038	0.039	0.059	0.084
	B. circulans				
Nutrient broth	0.149	0.344	0.390	0.654	0.716
Potato dextrose	0.027	0.060	0.309	0.671	0.769
Muller Hinton	0.076	0.382	0.502	0.549	0.769
Glucose yeast extr	0.125	0.152	0.169	0.671	0.841
Lactose yeast extract	0.061	0.098	0.221	0.480	0.620
C.D. at 5% level	0.036	0.049	0.099	0.068	0.298

Glucose yeast extract (GYE) and Lactose yeast extract (LYE), are presented in Table 11.

4.9.1. B. subtilis

The results showed that the growth of the bacterium at different intervals, showed significant variations. At six hours the growth was maximum in NB and it was superior to GYE, NB and LYE. The growth of the bacteria at 12, 24 and 48 hours showed that MH and NB were better media. The growth of the bacterium was maximum in NB and it was significantly superior to all other media at 72 hours. MH came next followed by PD and GYE and latter were statistically on par. Thus NB was found to be best suited medium for growing B. subtilis followed by PD and GYE.

4.9.2. B. pumilus

The growth on liquid cultures at various intervals showed statistically significant variations. Maximum growth was obtained in GYE which was significantly superior to all treatments six hours after inoculation. It was followed by MH and NB. These two were on par and inferior to GYE and superior to other two media. The growth at 12 and 24 hours showed maximum values in NB and MH. At 48 hours maximum growth was obtained in NB followed by PD. At 72 hours, PD gave maximum growth followed by NB and they were

statistically on par. This was followed by GYE and MH which were on par. NB was the best medium for the growth of this bacterium. Next best medium was PD. Considering the cost, PD may be ranked above.

4.9.3. B. coagulans

The results showed that in the initial period (6 hours) maximum growth of this organism was in GYE and it was statistically superior to all other media. The next best media were PD, MH and LYE. During the 12 hour period the growth in NB was found to be superior to all other media except MH and these two were on par. During the 48 hour period GYE showed maximum growth followed by NB and these two were significantly different. At 72 hour period also GYE gave maximum growth followed by PD and these two were statistically on par. Thus the test revealed that GYE is the best suited medium for growth of B. coagulans and it was followed by PD medium. Considering the cost, PD had to be chosen as the best.

4.9.4. B. macerans

The growth of the bacterium in different media at 6, 12, 24, 48 and 72 hours showed statistically significant variations (Table 11). Maximum growth was obtained in GYN

followed by NB, six hours after inoculation. GYE and NB media were significantly superior to the other three media. At 12 hours after inoculation, NB gave maximum growth. Next best media were PD and LYE. The growth was maximum in PD followed by NB and MH 24 hours after inoculation. The growth of these were statistically on par. At 48 hours after inoculation, the growth showed the same trend. At 72 hours after inoculation, maximum growth was in GYE followed by MH, NB and PD, and the latter four were statistically on par. For best growth of the organism, GYE medium was found to be the most suitable one. But PD was the cheapest medium.

4.9.5. B. circulans

At six hours, maximum growth was obtained in NB, followed by GYE and they were on par. At 12 hours, the bacterium showed maximum growth in MH followed by NB and these two were on par. Growth of the bacterium was maximum in MH followed by NB and PD at 24 hours. Here MH showed superiority to other two media and they were on par. At 48 and 72 hours, GYE showed maximum growth and it was on par with MH and PD. GYE medium was the best suited one for the growth of this bacterium followed by PD and MH. Considering the cost also, PD can be chosen as the best suited medium.

4.10. Evaluation of pathogenic effect of *Bacillus* spp. on root-knot nematode of pepper by pot culture studies

4.10.1. Nematode population in soil

The data recorded on the resultant nematode population in the soil, affected with five bacterial isolates at three doses observed 3, 6, 12 and 18 months after treatment are given in Table 12.

The data on nematode population collected three months after treatment showed significant reduction. All treatments except those which received bacterial dose of 1.2×10^5 cells per pot of *B. macerans* and 1.0×10^5 cells/pot of *B. subtilis* reduced the soil population significantly. The maximum reduction was observed in treatments where the bacterial cell concentration of 1.2×10^8 cells/pot of *B. circulans*, *B. coagulans*, 1.1×10^8 cells/pot of *B. pumilus* and 1.0×10^8 cells/pot of *B. subtilis* and they were significantly superior to other treatments.

The nematode population in soil after six months also showed significant differences among the treatments. The maximum reduction in nematode population was recorded in treatment with the bacterial cell concentration of 1.1×10^8 cells/pot of *B. pumilus* followed by 1.2×10^8 cells/pot of

Table 12. Effect of *Bacillus* spp. on *M. incognita* population in soil (100ml) collected from pepper root zone at different periods (mean of three replications)

Treatments (cells/pot)		Larval population at different periods (months) 3	6	12	18
N + *B. macerans*	(1.2×10^8)	30.3 (5.6)	22.0 (4.7)	37.6 (6.2)	30.0 (5.5)
,,	(1.2×10^6)	42.0 (6.6)	54.0 (7.4)	68.0 (8.3)	71.0 (8.5)
,,	(1.2×10^5)	69.0 (8.3)	86.0 (9.3)	68.0 (8.3)	71.7 (8.5)
N + *B. pumilus*	(1.1×10^8)	24.3 (5.0)	19.0 (4.5)	26.7 (5.9)	26.0 (5.2)
,,	(1.1×10^6)	54.3 (7.4)	70.0 (8.4)	51.7 (7.3)	45.7 (6.8)
,,	(1.1×10^5)	60.7 (7.8)	66.0 (8.2)	84.3 (9.2)	76.3 (8.8)
N + *B. circulans*	(1.2×10^8)	16.7 (4.1)	19.7 (4.5)	36.7 (6.1)	29.3 (5.5)
,,	(1.2×10^6)	28.7 (5.3)	29.3 (5.5)	49.0 (7.1)	48.0 (7.0)
,,	(1.2×10^5)	29.0 (5.5)	49.3 (7.1)	92.7 (9.7)	62.7 (8.0)
N + *B. coagulans*	(1.2×10^8)	23.0 (4.9)	23.0 (4.9)	52.3 (7.2)	28.0 (5.2)
,,	(1.2×10^6)	52.3 (7.3)	48.3 (7.0)	74.7 (8.7)	50.7 (7.0)
,,	(1.2×10^5)	67.0 (8.2)	72.7 (8.6)	93.7 (9.7)	76.0 (8.8)
N + *B. subtilis*	(1.2×10^8)	28.7 (5.4)	24.7 (5.0)	46.7 (6.9)	35.7 (6.1)
,,	(1.2×10^6)	42.0 (6.6)	43.7 (6.7)	53.3 (7.4)	52.3 (7.3)
,,	(1.2×10^5)	67.7 (8.3)	95.3 (9.8)	75.0 (8.7)	78.7 (8.9)
Nematode alone		92.0 (9.6)	111.7 (10.2)	82.3 (9.1)	85.0 (9.2)
Absolute check		0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
C.D. (0.05)		(1.36)	(1.75)	(0.89)	(1.44)

N : *M. incognita* 1000 larvae per pot

Figures in parentheses are values after $\sqrt{x+1}$ transformation

B. circulans, B. macerans and B. coagulans and 1.2×10^6 cells/pot of B. circulans. These treatments were statistically on par and superior to other treatments.

The observations on soil population after 12 months and 18 months also showed a significant variations. Maximum reduction in the soil population was noticed in B. pumilus treatment (1.1×10^8 cells/pot) followed by 1.2×10^8 cells/pot of B. circulans and B. coagulans and these treatments were statistically on par.

4.10.2. Nematode population in root

The data on the nematode population in the root (number of galls and root-knot index) after treatment with five Bacillus spp. at three dosage are given in Table 13.

The number of galls per g of root three months after treatment showed statistically significant variation. A concentration of 1.2×10^8 cells per pot of B. macerans, 1.1×10^8 cells per pot of B. pumilus, 1.2×10^8 and 1.2×10^6 cells per pot of B. coagulans reduced the nematode population in root considerably. These treatments were statistically on par and significantly better than control, the nematode alone treatment. But in the case of 1.2×10^5 cell concentration of all the Bacillus spp. reduced the nematode

Table 13. Effect of Bacillus spp. on M. incognita population in pepper roots (5 g) observed at different periods (mean of three replications)

Treatments	cells/pot	Number of galls (1) and root-knot index (2) at different periods (months)							
		3		6		12		18	
		(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
N + B.macerans	1.2×10^8	7.0(2.8)	1.0	12.0(3.5)	1.0	13.0(3.7)	1.0	17.0(4.2)	1.0
,,	1.2×10^6	13.7(3.3)	1.0	20.0(4.5)	1.0	19.0(4.4)	1.0	16.7(4.2)	1.0
,,	1.2×10^5	14.0(3.8)	1.0	25.3(5.1)	2.0	22.0(4.7)	1.0	24.7(5.1)	1.0
N + B.pumilus	1.1×10^8	6.7(2.8)	1.0	8.0(3.0)	1.0	20.0(4.6)	1.0	15.3(4.0)	1.0
,,	1.1×10^6	11.3(3.5)	1.0	16.3(4.2)	1.0	18.3(4.4)	1.3	22.7(4.8)	1.0
,,	1.1×10^5	10.5(3.4)	1.0	23.0(4.9)	1.0	25.7(5.2)	2.0	25.3(5.1)	2.0
N + B.circulans	1.2×10^8	7.0(3.0)	1.0	12.7(3.7)	1.0	29.3(5.5)	2.0	26.3(5.2)	2.0
,,	1.2×10^6	13.3(3.8)	1.0	17.7(4.3)	1.0	19.0(4.2)	1.0	25.0(5.1)	1.6
,,	1.2×10^5	10.3(3.4)	1.0	17.0(4.2)	1.3	34.7(5.9)	2.3	31.7(5.7)	2.0
N + B.coagulans	1.2×10^8	6.7(2.8)	1.0	13.3(3.7)	1.0	24.0(5.0)	2.0	26.7(5.3)	2.0
,,	1.2×10^6	6.0(2.6)	1.0	21.3(4.7)	1.0	24.7(5.1)	2.0	25.7(5.2)	2.0
,,	1.2×10^5	12.3(3.6)	1.0	29.0(5.5)	2.0	25.7(5.2)	2.0	26.3(5.2)	2.0
N + B.subtilis	1.0×10^8	9.3(3.1)	1.0	14.3(3.9)	1.0	23.0(4.8)	1.3	23.0(4.9)	2.0
,,	1.0×10^6	9.3(3.2)	1.0	25.0(5.1)	1.0	29.3(5.5)	2.0	29.0(5.4)	2.0
,,	1.0×10^5	11.6(3.6)	1.0	32.3(5.8)	2.0	31.7(5.7)	2.3	28.3(5.4)	2.3
Nematode alone (untreated)		14.7(3.9)	1.0	38.0(6.2)	2.3	38.7(6.3)	2.6	40.3(6.4)	2.6
Absolute check		0.0(1.0)	--	0.0(1.0)	--	0.0(1.0)	--	0.0(1.0)	--
C.D. (0.05)		(0.88)	--	(0.76)	--	(1.16)	--	(0.75)	--

N : M. incognita 1000 larvae per pot

Figures in parentheses are values after $\sqrt{x + 1}$ transformation

population in root, but their effects were not enough to get statistical significance.

The root-knot indices were uniform in all these treatments.

The number of galls recorded on sixth month also showed statistically significant variations. B. pumilus ($1.1x10^8$ cells per pot), $1.2x10^8$ cells per pot of B. macèrans, B. circulans and B. coagulans showed minimum number of galls and these treatments were on par and statistically superior to all other treatments. Root-knot indices also showed the same trend.

The data on the number of galls taken at twelfth month showed that the effect of the treatments $1.2x10^8$ cells per pot of B. macerans, $1.2x10^6$ cells per pot of B. circulans and B. macerans, $1.1x10^8$ cells per pot and $1.1x10^6$ cells per pot of B. pumilus, $1.2x10^5$ cells of B. macerans and $1.0x10^8$ cells per pot of B. subtilis were statistically on par in reducing the number of galls in pepper plants. But in terms of root-knot index, the treatments of B. macerans (all three cell concentrations), B. pumilus ($1.1x10^8$ cells/pot) and B. circulans ($1.2x10^6$ cells/pot) only maintained the superiority, giving minimum root-knot index of 1.0.

At the final stage the plants treated with $1.1x10^8$ cells per pot of B. pumilus showed minimum number of galls followed by B. macerans ($1.2x10^8$ and $1.2x10^6$ cells/pot) and these treatments were statistically on par and better than other treatments. But in terms of root-knot index, B. pumilus ($1.1x10^6$ cells/pot) and B. macerans ($1.2x10^5$ cells/pot) treatments showed minimum root-knot indices of 1.0 in addition to the above three treatments.

4.10.3. The reproductive factor (reproductive potential)

The data on the reproductive potential of M. incognita treated with five species of Bacillus are presented in Fig. 4. The reproductive factor (reproductive potential) calculated was used for computing the nematostatic value of the treatments.

4.10.4. Nematostatic value

The data on the nematostatic value of M. incognita treated with five species of Bacillus at three cell concentrations at 3, 6, 12, and 18 month intervals are given in Table 14.

The values computed for the different period intervals were found to be low when compared with that of the nematode alone treatment. The result at third month after treatment,

Table 14. Effect of *Bacillus* spp. on the nematostatic value of *M. incognita* infesting pepper vine observed at different periods (mean of three replications)

Treatments *Bacillus* spp. (cells/pot)		Nematostatic value observed at different periods (months) 3	6	12	18
N + *B.macerans*	1.2×10^8	32.6	31.5	31.3	51.3
,,	1.2×10^6	43.5	68.7	43.0	80.7
,,	1.2×10^5	76.0	61.3	70.3	83.7
N + *B.pumilus*	1.1×10^8	26.1	24.2	25.3	42.3
,,	1.1×10^6	58.5	79.1	55.1	60.0
,,	1.1×10^5	66.3	80.3	62.3	88.3
N + *B.circulans*	1.2×10^8	18.5	31.3	18.4	56.3
,,	1.2×10^6	31.5	42.2	30.3	60.7
,,	1.2×10^5	31.6	57.3	30.3	82.7
N + *B.coagulans*	1.2×10^8	25.1	33.4	24.3	52.7
,,	1.2×10^6	56.5	60.3	53.1	61.0
,,	1.2×10^5	72.8	83.1	67.7	86.7
N + *B.subtilis*	1.0×10^8	31.5	38.3	30.3	56.7
,,	1.0×10^6	45.7	54.0	42.7	65.7
,,	1.0×10^5	73.9	125.0	66.0	90.0
Nematode alone (untreated control)		100.0	100.0	100.0	100.0
Absolute check		--	--	--	--

N : *M. incognita* @ 1000 larvae per pot

revealed that B. circulans ($1.2x10^8$ cells per pot) treatment showed minimum value (18.5) followed by treatment with $1.2x10^8$ cells per pot of B. coagulans (25.1) and B. pumilus (26.1). Next came the lower doses of B. circulans (31.5 and 31.6) and higher dose of B. subtilis and B. macerans, having the nematostatic values of 31.5 and 32.6 respectively.

At sixth month, B. pumilus ($1.0x10^8$ cells per pot) treatment gave minimum value (24.2) followed by treatment with $1.2x10^8$ cells per pot of B. circulans (31.3) B. macerans (31.5) and B. coagulans (33.4).

The nematostatic value computed at twelfth month showed that the treatment of $1.2x10^8$ cells per pot of B. circulans gave minimum value (18.4) followed by B. coagulans (24.3), B. pumilus (25.3) and B. macerans (31.3).

Eighteen months after treatment, the lowest nematostatic value was given by B. pumilus ($1.1x10^8$ cells per pot) treatment (43.3) followed by the treatment of $1.2x10^8$ cells per pot of B. macerans (51.3).

4.10.5. Larval emergence from the egg mass

The mean number of larvae emerged from the egg masses of M. incognita collected at final stage (eighteen month after treatment) are given in Table 15.

Table 15. Mean number of larvae emerged from the eggmass of *M. incognita* collected at harvest (18 months after planting) (mean of three replications)

Treatments (cells per pot)		Number of larvae emerged per egg mass
B. macerans	1.2×10^8	56.3 (6.94)
	1.2×10^6	71.7 (8.30)
	1.2×10^5	75.3 (8.34)
B. pumilus	1.1×10^8	69.7 (7.15)
	1.1×10^6	97.7 (9.91)
	1.1×10^5	81.7 (8.97)
B. circulans	1.2×10^8	78.7 (8.89)
	1.2×10^6	99.7(10.03)
	1.2×10^5	101.0(10.04)
B. coagulans	1.2×10^8	122.3(10.84)
	1.2×10^6	111.0(10.57)
	1.2×10^5	100.3(10.07)
B. subtilis	1.0×10^8	96.0 (9.84)
	1.0×10^6	111.0(10.52)
	1.0×10^5	123.7(11.13)
Nematode alone		122.7(11.12)
C.D. (0.05)		(3.56)

Figures in parentheses are values after $\sqrt{x}$ transformation

The result showed that there was statistically significant variation among the different treatments. Minimum number of larvae per egg mass was emerged in the treatment with $1.2x10^8$cells per pot of B. macerans (56.3 larvae) followed by B. pumilus ($1.1x10^8$ cells per pot). These treatments were on par and significantly superior to nematode alone treatment. Treatment at lower doses of B. macerans ($1.2x10^6$ and $1.2x10^5$ cells/pot), B. circulans $1.2x10^8$, $1.2x10^6$ and $1.2x10^5$ cells per pot), two lower doses of B. pumilus ($1.1x10^6$ and $1.1x10^5$ cells per pot) and B. subtilis ($1.0x10^8$ cells per pot) were on par with above two treatments but not superior to nematode alone treatment.

4.10.6. Improvement in plant growth character due to the addition of the bacterial pathogen to pepper plants infested by M. incognita

4.10.6.1. Length of vine

The results are presented in Table 16. The vine length of pepper plants, exposed to bacteria and nematode though exhibited difference in measurement from the first month onwards, the data were not statistically significant. The vine length showed significant differences between the treated vines from ninth month onwards only.

Table 16. Effect of *Bacillus* spp. on length of vine (shoot) of *M. incognita* inoculated pepper plants observed at different periods (mean of three replications)

Treatments (cells/pot)	Increase in length of vine (cm) at different periods (months)							
	1	2	3	6	9	12	15	18
N + *B.macerans*								
1.2×10^8	3.0(1.9)	6.2	9.8	40.7	52.3	56.3	66.0	75.9
1.2×10^6	2.5(1.7)	4.3	7.5	26.3	33.7	39.0	44.3	52.0
1.2×10^5	1.8(1.6)	4.5	6.0	18.8	25.3	32.0	38.0	46.2
N + *B.pumilus*								
1.1×10^8	3.0(1.9)	5.2	11.7	44.0	48.3	57.3	61.8	78.0
1.1×10^6	1.8(1.6)	4.2	7.2	27.0	33.7	40.5	45.4	49.0
1.1×10^5	1.7(1.6)	4.0	5.5	25.2	30.3	37.0	41.3	47.2
N + *B.circulans*								
1.2×10^8	3.4(2.1)	6.7	11.5	44.2	52.3	57.7	63.0	74.4
1.2×10^6	1.8(1.6)	3.9	9.2	34.0	39.7	48.3	53.6	56.7
1.2×10^5	2.2(1.8)	4.3	8.5	21.8	26.5	32.3	38.5	42.3
N + *B.coagulans*								
1.2×10^8	3.7(2.2)	4.5	9.5	41.5	44.8	51.0	56.3	61.2
1.2×10^6	2.7(1.9)	4.0	6.9	27.2	30.3	38.2	44.3	49.0
1.2×10^5	2.0(1.7)	3.7	5.5	32.3	34.0	38.8	41.3	51.9
N + *B.subtilis*								
1.0×10^8	3.0(2.0)	4.3	9.2	30.0	34.7	42.3	49.0	57.8
1.0×10^6	2.8(1.9)	3.8	7.0	29.0	31.8	37.7	45.7	52.7
1.0×10^5	1.8(1.6)	3.8	6.3	28.3	31.3	36.7	43.2	50.0
Nematode alone (untreated control)	1.7(1.6)	3.5	4.2	23.5	30.0	37.0	41.0	46.5
Absolute check	5.0(2.4)	8.0	14.2	46.5	52.3	69.0	76.0	85.3
C.D. (0.05)	NS	NS	NS	NS	15.76	15.93	15.75	14.80

N : *M.incognita* @ 1000 larvae per pot NS : Not significant

Figures in parentheses are values after $\sqrt{x + 1}$ transformation

At the first month the growth of M.incognita infested plants treated with a concentration of $1.2x10^8$ cells/pot of B.coagulans showed 3.7 cm increase followed by B.circulans ($1.2x10^8$ cells/pot) with 3.4 cm. At a dose of $1.0x10^6$ cells per pot, the increase was maximum in plants treated with B. subtilis (2.8 cm) followed by B. coagulans (2.7 cm). In the case of other treatments, the lengths of vine in plants which received the bacterial dose of $1.2x10^5$, $1.1x10^5$ and $1.1x10^5$ cells per pot of all species were on par with nematode alone treatment (control).

In plants treated with a dose of $1.2x10^8$ cell per pot of B. circulans, the growth of the vine two months after treatment, was maximum. At the third month maximum vine growth was observed in those treated with B. pumilus ($1.2x10^8$ cells per pot) followed by B. circulans ($1.2x10^8$ cells per pot). At sixth month, the growth was maximum in plants treated with high dose of B. circulans followed by B. pumilus. The vine length showed significant difference between the bacteria treated vines over the check after nine months. At this stage, B. circulans ($1.2x10^8$ cells/pot) caused maximum increase in length followed by plant treated with B.macerans ($1.2x10^8$ cells/pot), B. pumilus ($1.1x10^8$ cells per pot), and B. coagulans ($1.2x10^8$ cells per pot). These treatments were on par with absolute check (without bacteria and nematode). Thus the data indicated that all the four species of Bacillus

were infective to the nematode parasite attacking pepper vine and they reduced the pathogenic effect of nematode resulting in better growth of vine. The plants under treatment of B. subtilis (1.0×10^8 cells) resulted in 34.7 cm increase in vine length which was the lowest compared to other bacterial applications. The plants under control recorded only 30 cm increase in vine length and these two treatments were statistically on par.

The data collected on vine length at twelfth month showed statistically significant differences among the treatments. Maximum increase in vine length was obtained in plants treated with B. circulans (1.2×10^8 cells/pot) followed by B. pumilus (1.1×10^8 cells/pot) and B. macerans (1.2×10^8 cells/pot) and they were on par with absolute check. All the other treatments were on par with the treatment in which nematode alone was added.

The result obtained at 15th month is presented in Table 16. The treatment with a dose of 1.2×10^8 cells/pot of B. macerans resulted in maximum increase in vine length (66 cm) followed by B. circulans (63 cm) and B. pumilus (61.8 cm). All the three treatments were on par with absolute check giving an indication of suppression of pathogenic effect of nematode. The other treatments were on par with the treatment in which nematode alone was added.

The final observations recorded at eighteenth month showed that a cell concentration of $1.1x10^8$ cells of B.pumilu treatment gave the maximum increase in vine length followed by B. macerans ($1.2x10^8$ cells) and B. circulans ($1.2x10^8$ cell These treatments were statistically on par with absolute check. The next best treatment was that of B. coagulans ($1.2x10^8$ cells) which was significantly inferior to the above three treatments and the absolute check. However, this treatment (B. coagulans $1.2x10^8$ cells) was superior to the nematode alone treatment, the control. All the other treatments were statistically on par and better than control.

4.10.6.2. Number of leaves

The results are presented in Table 17. The number of leaves of pepper plants infested with M. incognita after treatment with different species of Bacillus increased from one month after treatment onwards. Though there was difference in the increase in number of leaves on the vines up to six months, the data did not show significant differences statistically among the different doses of bacterial treatments. The maximum increase in number of leaves of pepper plant one month after treatment was noticed with B. macerans $1.2x10^8$ cells per pot (2.7).

Table 17. Effect of *Bacillus* spp. on number of leaves of *M. incognita* inoculated pepper plants observed at different periods (mean of three replications)

Treatments (*Bacillus* cells/pot)		Increase in number of leaves at different periods (months) 1	2	3	6	9	12	15	18
N + *B.macerans*	1.2×10^8	2.7(1.9)	2.7(1.9)	3.7(2.2)	12.3	18.7	25.3	26.7	32.0
,,	1.2×10^6	1.3(1.5)	2.3(1.8)	2.7(1.9)	8.7	11.3	14.0	25.7	27.3
,,	1.2×10^5	0.7(1.3)	1.7(1.5)	2.3(1.7)	9.3	12.0	15.7	19.7	23.0
N + *B.pumilus*	1.1×10^8	1.7(1.6)	3.0(2.0)	4.0(2.2)	13.0	15.0	21.0	25.3	28.0
,,	1.1×10^6	1.3(1.5)	1.7(1.6)	2.3(1.7)	7.7	11.0	15.3	19.0	22.3
,,	1.1×10^5	0.3(1.1)	1.0(1.4)	1.3(1.5)	8.7	12.0	15.7	19.0	21.3
N + *B.circulans*	1.2×10^8	2.0(1.7)	3.3(2.1)	4.0(2.2)	14.7	14.7	18.7	22.3	25.0
,,	1.2×10^6	0.6(1.3)	2.7(1.9)	3.0(2.0)	9.0	11.7	15.7	20.0	24.0
,,	1.2×10^5	0.3(1.1)	2.0(1.7)	2.7(1.9)	10.3	12.3	16.3	20.7	23.7
N + *B.coagulans*	1.2×10^8	1.0(1.4)	1.7(1.6)	2.3(1.8)	16.0	19.0	22.7	25.3	29.3
,,	1.2×10^6	1.0(1.4)	1.0(1.4)	2.0(1.7)	9.7	13.0	18.3	22.0	26.0
,,	1.2×10^5	0.7(1.2)	1.0(1.4)	1.7(1.6)	12.0	14.7	17.3	20.7	23.7
N + *B.subtilis*	1.0×10^8	2.0(1.7)	2.3(1.8)	3.0(2.0)	14.3	17.7	20.7	23.3	26.0
,,	1.0×10^6	1.0(1.4)	2.0(1.7)	2.7(1.8)	12.3	15.3	18.7	22.7	24.7
,,	1.0×10^5	0.7(1.3)	1.0(1.4)	1.3(1.5)	11.7	13.7	17.7	20.0	22.7
N alone (untreated control)		0.7(1.2)	0.7(1.3)	2.0(1.7)	11.7	14.7	18.3	19.0	20.0
Absolute check		2.7(1.9)	3.3(2.1)	6.0(2.6)	17.0	26.0	32.0	37.7	38.0
C.D. (0.05)		NS	NS	NS	NS	5.3	5.0	5.4	4.8

N : *M. incognita* @ 1000 larvae per pot NS : Not significant

Figures in parentheses are values after $\sqrt{x + 1}$ transformation

Two and three months after treatment B. circulans ($1.2x10^8$ cells per pot) showed maximum increase (3.3 and 4.0) and at six months after treatment, B. coagulans ($1.2x10^8$ cells per pot) gave maximum increase (16.0).

The data collected on the leaf production on vines during the ninth month revealed statistically significant difference among treatments. Maximum leaf production was seen in B. coagulans ($1.2x10^8$ cells per pot) followed by B. macerans ($1.2x10^8$ cells per pot), B. subtilis ($1.0x10^8$ cells per pot), B. pumilus ($1.1x10^8$ cells per pot) and B. circulans ($1.2x10^8$ cells per pot). These treatments were on par.

The increase in number of leaves produced 12 months after treatment also revealed statistically significant difference. The four species of Bacillus, B. macerans ($1.2x10^8$ cells per pot), B. coagulans ($1.2x10^8$ cells per pot), B. pumilus ($1.1x10^8$ cells per pot) and B. subtilis ($1.0x10^8$ cells per pot) showed maximum increase in leaf production and they were on par. However, leaf number in the treatment was less than the number of leaves in plants in absolute check.

At fifteenth month, the maximum leaf production was found in plants treated with B. macerans ($1.2x10^8$ cells per pot and $1.2x10^6$ cells per pot) followed by B. pumilus

($1.1x10^8$ cells per pot), B. coagulans ($1.2x10^8$ cells per pot and $1.2x10^6$ cells per pot), B. subtilis ($1.0x10^8$ cells per pot and $1.0x10^6$ cells per pot) and B. circulans ($1.2x10^8$ cells per pot). These treatments were on par and superior to nematode alone treatment and inferior to absolute check.

The final observations recorded 18th month after effecting the treatments showed significant variations. However, the leaf production was not on par with plants in absolute check; but they were higher than in plants treated with nematode alone. Addition of a dosage of $1.2x10^8$ cells per pot of B. macerans resulted in maximum increase in production of leaves followed by B. coagulans with $1.2x10^8$ cells per pot, B. pumilus with $1.1x10^8$ cells and a dosage of $1.2x10^6$ cells of B. macerans. However, effect of these five treatments was found to be on par in statistical analysis. Though all the other treatments recorded better leaf production in the plants, compared to the treatment nematode alone, they were found to be on par in statistical analysis.

4.10.6.3. Fresh weight of shoot

The fresh shoot weight of M. incognita infested pepper plants treated with the five species of Bacillus at different cell concentrations, recorded at 6, 12 and 18 months after

Table 18. Effect of *Bacillus* spp. on fresh weight of shoot, dry weight of shoot and fresh weight of root of *M. incognita* inoculated pepper plants observed at different periods (mean of three replications)

Treatments	(cells/pot)	Fresh wt.of shoot (g) at different periods (months)			Dry wt. of shoot (g) at different periods (months)			Fresh wt. of root (g) at different periods (months)		
		6	12	18	6	12	18	6	12	18
N + *B.macerans*	(1.2×10^8)	25.2	25.2	33.2	7.8	9.8	17.3	7.8	10.8	11.7
,,	(1.2×10^6)	24.2	20.3	32.2	7.0	7.3	13.8	7.0	9.7	6.2
,,	(1.2×10^5)	29.5	18.8	23.5	5.8	8.5	11.5	5.6	9.0	5.0
N + *B.pumilus*	(1.1×10^8)	33.3	27.7	41.0	6.5	8.0	19.0	6.0	9.5	11.7
,,	(1.1×10^6)	17.8	20.3	31.0	5.3	6.5	13.8	6.3	8.4	5.8
,,	(1.1×10^5)	16.5	27.8	16.8	4.5	7.0	7.8	6.5	7.8	4.7
N + *B.circulans*	(1.2×10^8)	40.2	21.5	48.7	8.8	33.5	17.2	8.8	11.7	9.4
,,	(1.2×10^6)	31.3	20.9	32.3	7.3	8.6	14.8	7.3	6.7	5.3
,,	(1.2×10^5)	19.0	37.3	32.8	4.5	8.3	14.3	7.5	5.2	6.3
N + *B.coagulans*	(1.2×10^8)	33.3	34.6	44.2	8.8	9.4	21.0	8.6	4.1	9.1
,,	(1.2×10^6)	25.5	26.4	24.3	5.3	8.0	11.8	8.3	7.2	3.9
,,	(1.2×10^5)	19.2	29.2	23.7	3.7	7.6	9.2	8.7	3.7	4.0
N + *B.subtilis*	(1.0×10^8)	32.2	30.8	40.7	9.7	9.7	16.2	9.7	6.5	9.3
,,	(1.0×10^6)	27.3	25.7	28.7	4.1	7.7	11.4	9.1	7.3	4.7
,,	(1.0×10^5)	21.2	31.3	26.0	4.3	6.5	9.3	8.3	7.0	3.9
Nematode alone (untreated control)		16.8	18.8	28.3	5.0	5.0	9.3	8.0	4.7	3.8
Absolute check (no organism)		52.8	45.5	49.7	10.6	12.9	24.2	10.6	12.3	15.3
C.D. (0.05)		11.3	8.7	13.7	3.9	NS	6.4	NS	3.9	3.1

N : *M. incognita* @ 1000 larvae per pot

NS : Not significant

treatment showed significant variations. The data are presented in Table 18.

At sixth month, the plants under treatment with a concentration of $1.2x10^8$ cells per pot of B. circulans showed maximum fresh shoot weight followed by the plants treated with B. pumilus ($1.1x10^8$ cells per pot), B.coagulans ($1.2x10^8$ cells per pot), B. subtilis ($1.0x10^8$ cells per pot) and B. circulans ($1.2x10^6$ cells per pot). The effect of these treatments were found to be statistically on par and superior to the other treatments and control but inferior to those plants under absolute check. Eventhough the plants in the treatments with a lower dose of bacteria recorded an increase in the weight of shoot considerably, the increase was not found to differ significantly in statistical analysis.

The observations recorded at 12th month revealed that plants treated with B. circulans ($1.2x10^8$ cells per pot) attained the maximum shoot weight among the treatments and it was on par with the shoot weights of plants under the absolute check and B. coagulans ($1.2x10^8$ cells per pot).

The final observations recorded after 18th month showed that the plants treated with $1.2x10^8$ cells per pot of B. circulans, B. coagulans and B. pumilus ($1.1x10^8$ cells per pot) had higher levels of shoot weight which were also

on par with that of absolute check. The data collected on the fresh shoot weight indirectly indicated that the three bacteria viz. B. circulans, B. coagulans and B.pumilus could induce a natural check on the pathogenic effect of nematode on the vines.

4.10.6.4. Dry weight of shoot

The data recorded are presented in Table 18. The data revealed that there was significant differences among treatments at sixth and eighteenth month. The maximum dry shoot weight of M. incognita infested pepper plants treated with bacterial pathogen was obtained in plants treated with B. subtilis ($1.0x10^8$ cells per pot) followed by B. circulans ($1.2x10^8$ cells per pot and $1.2x10^6$ cells per pot), B. coagulans ($1.2x10^8$ cells per pot) and B. macerans ($1.2x10^8$ cells per pot and $1.2x10^6$ cells per pot). The above treatments were on par with absolute check. The plants treated with $1.0x10^8$ cells of B. subtilis and $1.2x10^8$ cells per pot was the best treatment because this only was significantly superior to the control (nematode alone).

The data on dry weight of shoot recorded in plants at twelfth month did not show significant variations.

The dry weight of shoot recorded at eighteen month showed significant differences among the treatments. The

maximum weight was obtained in plants treated with B. coagulans ($1.2x10^8$ cells per pot) followed by B. pumilus ($1.1x10^8$ cells per pot) and these were on par with absolute check, giving an indication that these two treatments effected a reduction in the pathogenicity of M. incognita in pepper. The other treatments with three bacterial pathogens at high cell concentrations also increased the dry weight of shoot and they were on par with B. coagulans and B. pumilus. These three treatments, B. macerans, B. circulans and B. subtilis at higher cell concentration were significantly better than the nematode alone treatment but inferior to absolute check.

4.10.6.5. Fresh weight of root

The data relating to the fresh weight of root of pepper plants are presented in Table 18.

There was statistically significant variations among the treatments at 12 and 18 months after treatment.

The root weight of plants at sixth month after treatment with B. subtilis ($1.0x10^8$ cells/pot) and $1.2x10^8$ cells per pot of B. coagulans and B. circulans showed slight increase, but it was not enough to give statistical significance.

The root weight of plants under various treatments after 12 months showed that addition of B. circulans ($1.2x10^8$ cells/pot) resulted in maximum root weight, followed by the treatment with B. macerans ($1.2x10^8$ cells per pot), B. pumilus ($1.1x10^8$ cells per pot), two lower doses of B. macerans ($1.2x10^6$ and $1.2x10^5$ cells per pot) and B. pumilus ($1.1x10^6$ cells per pot). These treatments were on par with absolute check. The results indicated that addition of the above mentioned bacterial pathogens could reduce the root infestation by M. incognita.

The final observations recorded on the root weight at 18 month also revealed significant difference among treatments. In all the treatments the plants recorded better root weight than the control (nematode alone). B. macerans ($1.2x10^8$ cells per pot) and B. pumilus ($1.1x10^8$ cells per pot) increased the root weight considerably which were statistically on par. The root weight recorded in plants treated with B. circulans ($1.2x10^8$ cells per pot), B. subtilis ($1.0x10^8$ cells per pot) and B. coagulans ($1.2x10^8$ cells per pot) were inferior to B. macerans and B. pumilus but superior to control. All the five Bacillus spp. at lower concentrations were on par with nematode alone treatment.

4.11. Interaction with nematicides, insecticides and fungicides

4.11.1. Nematicides/insecticides

In vitro sensitivity of five species of *Bacillus* to nematicides are given in Table 19.

4.11.1.1. Metham sodium (Vapam)

The result showed that metham sodium was inhibitory to all the five species of bacteria tested namely *B. macerans*, *B. pumilus*, *B. circulans*, *B. coagulans* and *B. subtilis* at 500 to 1000 ppm levels from 24 hour exposure period onwards. Among the bacteria *B. macerans* was highly sensitive to this chemical, the diameter of the inhibition zone was maximum (43.7 mm) in this species (Plate IV).

4.11.1.2. Phorate (Thimet)

Phorate was non-inhibitory to all the *Bacillus* spp. except *B. coagulans*. At 500 ppm level, up to 24 hours it was non-inhibitory and afterwards showed slight inhibition. At 1000 ppm concentration, it inhibited the growth of *B. coagulans* from 24 hour onwards.

4.11.1.3. Aldicarb (Temik)

Aldicarb 500 ppm was inhibitory to all bacteria except *B. coagulans*. It inhibited the growth of the bacteria

PLATE IV

In vitro sensitivity of *B. macerans* to nematicides and granular insecticides used as nematicides, 72 hr after treatment

1	Control	
2	Metham sodium	1000 ppm
3	Carbofuran	1000 ppm
4	Phorate	1000 ppm
5	Aldicarb	1000 ppm
6	Formaldehyde	1000 ppm

PLATE IV

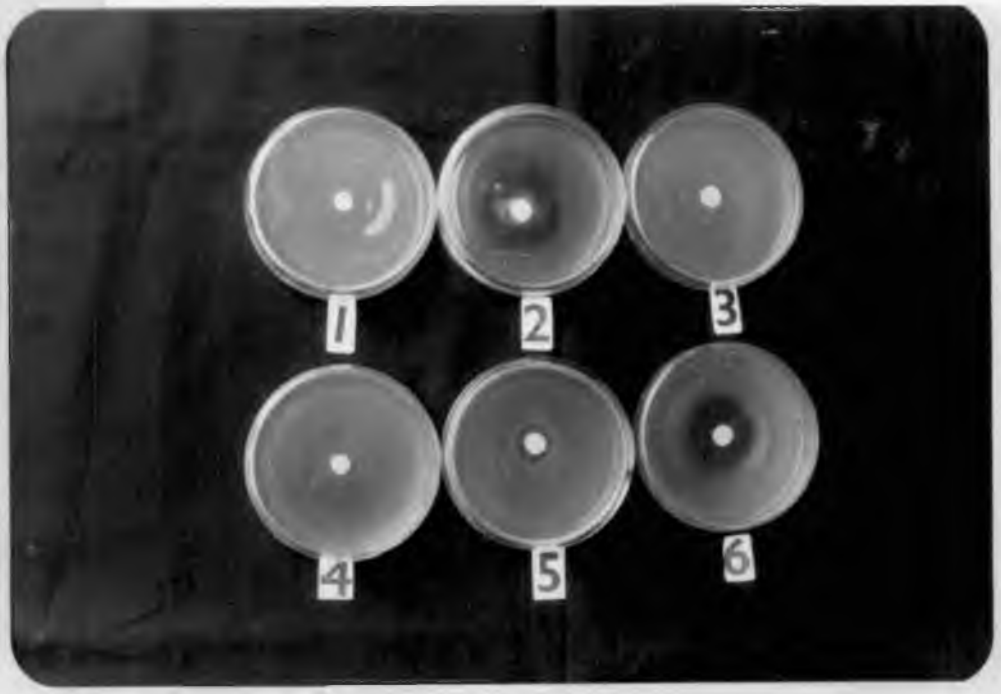


Table 19. *In vitro* sensitivity of *Bacillus* spp. to nematicides & insecticides observed at different periods (mean of three replications)

Nematicides/ insecticides	Diameter of inhibition zone in mm at different periods (hours)														
	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
	B. macerans			*B. pumilus*			*B. circulans*			*B. coagulans*			*B. subtilis*		
Methamsodium															
500 ppm	29.67	32.67	35.67	18.67	19.00	22.33	25.67	29.00	29.00	18.67	21.00	21.67	21.67	22.00	22.00
1000 ppm	39.67	43.67	43.67	19.67	21.00	23.00	26.00	38.33	40.00	22.67	29.00	29.00	25.33	25.00	26.00
Phorate															
500 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.00	12.67	0.00	0.00	0.00
1000 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.67	13.67	15.33	0.00	0.00	0.00
Aldicarb															
500 ppm	12.00	12.00	12.00	0.00	0.00	12.00	10.67	11.67	13.67	0.00	0.00	0.00	12.33	12.33	12.67
1000 ppm	12.00	12.00	12.00	12.00	13.83	15.67	12.00	13.00	15.67	0.00	12.00	12.00	13.33	14.00	14.00
Carbofuran															
500 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1000 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Formaldehyde															
500 ppm	18.33	20.00	21.67	10.00	10.00	10.00	25.33	26.67	39.00	17.00	17.67	23.67	26.67	26.67	27.00
1000 ppm	26.67	29.00	32.00	20.00	20.00	20.00	29.00	30.00	42.67	20.67	25.33	28.33	28.33	28.33	31.00
HCH															
500 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1000 ppm	0.00	0.00	0.00	10.67	10.67	10.67	0.00	11.33	17.67	10.50	12.00	14.00	0.00	0.00	0.00
Endosulfan															
500 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1000 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.67	10.50	12.00	12.00	0.00	0.00	0.00
Malathion															
500 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.50	0.00	0.00	0.00
1000 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.50	10.50	0.00	0.00	11.00
Quinalphos															
500 ppm	0.00	0.00	10.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.00
1000 ppm	12.33	13.33	15.67	10.50	10.50	10.50	0.00	0.00	11.00	0.00	10.50	11.00	0.00	10.50	12.00
Carbaryl															
500 ppm	0.00	0.00	10.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.50	10.87
1000 ppm	0.00	0.00	12.00	10.50	10.50	10.50	0.00	0.00	10.50	11.33	11.33	11.33	0.00	11.00	14.67

slightly at 1000 ppm level from 48 hour exposure period onwards. It was also non-inhibitory to B. pumilus at 500 ppm level up to 48 hours of exposure period.

4.11.1.4. Carbofuran (Furadan)

Carbofuran 500 and 1000 ppm were non-inhibitory to the five species of Bacillus tested. It was the safest nematicide chemical for the bacteria.

4.11.1.5. Formaldehyde (Formalin)

Formaldehyde 500 and 1000 ppm were highly inhibitory to all bacteria tested except B. pumilus. B. pumilus produced comparatively lesser inhibition area (20 mm diameter) than others. B. circulans was highly sensitive to this and produced an inhibition zone of 42.7 mm diameter.

4.11.1.6. Hexachlorohexane

HCH (BHC) at 500 ppm was non-inhibitory to the growth of B. pumilus, B. circulans and B. coagulans slightly. The diameter of the inhibition zone ranged from 10.7 to 11.0 mm in B. pumilus, 11.3 to 17.7 mm in B. circulans and 10.5 to 14.0 mm in B. coagulans.

4.11.1.7. Endosulfan

Endosulfan at 500 ppm and 1000 ppm were non-inhibitory to the bacteria except in B. coagulans. This bacterium showed slight inhibition at 1000 ppm level of endosulfan (Plate V).

PLATE V

In vitro sensitivity of *B. coagulans* to insecticides, 72 hr after treatment

1	Control	
2	HCH	1000 ppm
3	Malathion	1000 ppm
4	Endosulfan	1000 ppm
5	Quinalphos	1000 ppm
6	Carbaryl	1000 ppm

PLATE V

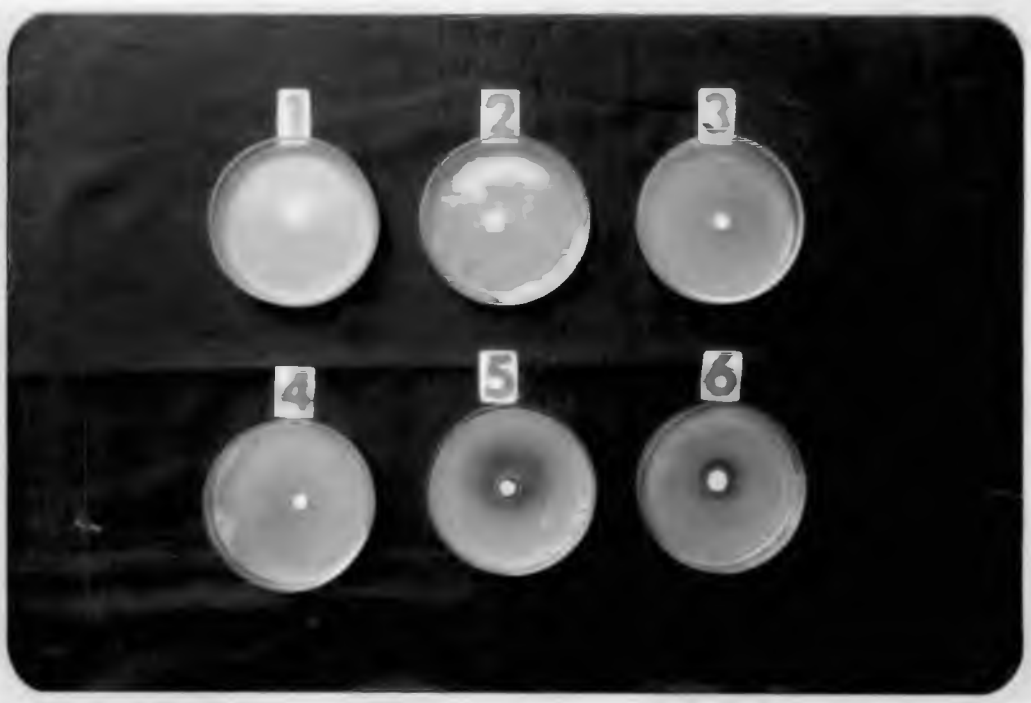
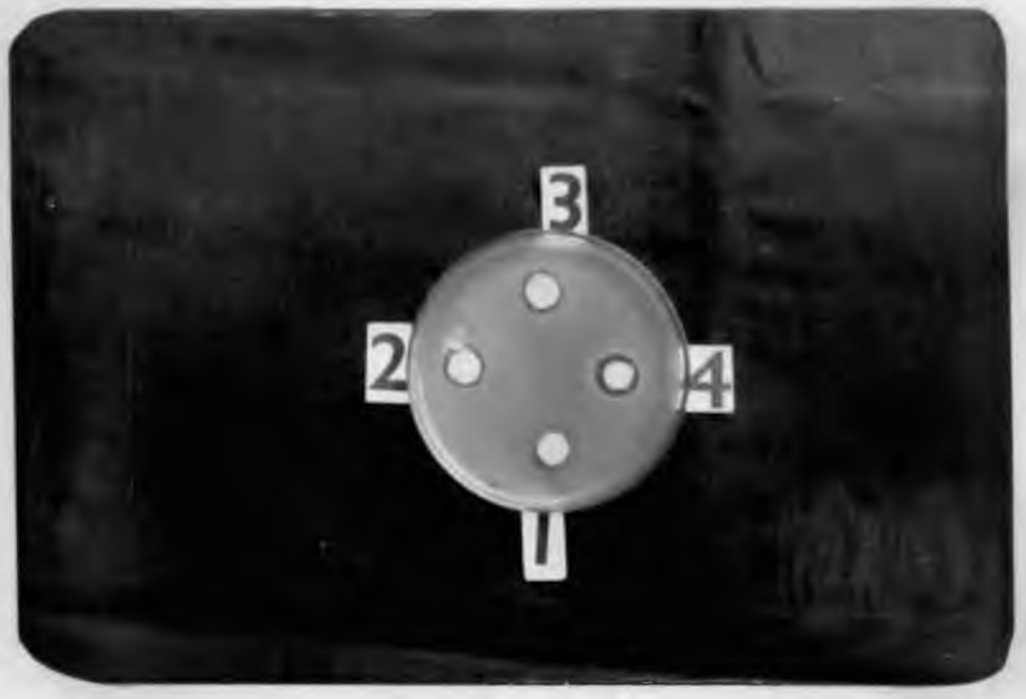


PLATE VI

In vitro sensitivity of *B. subtilis* to insecticides, 72 hr after treatment

1	Control	
2	HCH	500 ppm
3	Endosulfan	500 ppm
4	Carbaryl	500 ppm

PLATE VI



4.11.1.8. Malathion

Malathion 500 ppm was non-inhibitory to all the five species of Bacillus tested. At 1000 ppm level it slightly inhibited the growth of B. coagulans from 48 hour onwards.

4.11.1.9. Quinalphos

Quinalphos 500 ppm was found safe to the growth of most of the bacteria. B. macerans and B. subtilis showed slight inhibition at 72 hours after treatment. At 1000 ppm level it showed slight inhibition of the growth of all bacteria, and the diameter of the inhibition zone was maximum in B. macerans; it ranged from 12.3 to 15.7 mm from 24 to 72 hours after treatment.

4.11.1.10. Carbaryl

Carbaryl 500 ppm was non-inhibitory to the bacteria except B. macerans and B. subtilis. At 72 hours after treatment B. macerans showed slight inhibition (10.7 mm) and B. subtilis at 48 and 72 hours exposure period also showed slight inhibition (10.5 to 10.9 mm).

4.11.2. Fungicides

In vitro sensitivity of Bacillus spp. to fungicides was tested and the results are presented in Table 20 and Appendix VII.

Table 20. *In vitro* sensitivity of *Bacillus* spp. to fungicides observed at different periods (mean of three replications)

Fungicide in ppm levels	Diameter of incubation zone in mm at different periods (hours)														
	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Methoxy ethyl mercury chloride (Agallol)	*B. macerans*			*B. pumilus*			*B. circulans*			*B. coagulans*			*B. subtilis*		
125	13.33	14.00	14.00	15.17	15.75	17.00	10.83	12.67	13.00	0.00	0.00	0.00	12.50	12.67	13.00
250	14.00	14.67	15.00	18.00	18.00	18.00	12.00	14.00	14.00	0.00	0.00	0.00	15.00	15.00	15.00
500	16.83	17.00	18.00	17.00	19.00	20.00	14.33	16.00	17.00	11.00	11.00	11.00	16.33	17.00	18.00
1000	22.33	23.67	24.00	21.17	26.00	26.00	18.00	19.00	19.00	12.67	19.33	19.33	20.33	21.00	21.67
2000	25.00	25.00	26.33	25.00	27.50	30.00	21.50	23.33	20.00	24.00	24.17	24.17	24.00	24.33	25.67
Carbendazim (Bavistin)															
125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.00	0.00	0.00	0.00	0.00	0.00	14.00
2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.00	0.00	0.00	0.00	0.00	0.00	15.00
Copperoxichloride (Blitox)															
125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
500	0.00	0.00	13.00	0.00	0.00	0.00	0.00	11.00	12.00	0.00	0.00	0.00	0.00	0.00	0.00
1000	0.00	0.00	18.33	0.00	0.00	0.00	13.67	13.33	17.67	10.67	11.00	13.00	0.00	14.00	14.00
2000	0.00	0.00	21.00	0.00	0.00	22.00	14.33	14.67	18.00	14.00	14.33	15.33	0.00	15.00	15.67
Mancozeb (Dithane M_{45})															
125	15.33	16.00	16.00	12.00	12.50	15.00	0.00	0.00	0.00	0.00	0.00	0.00	13.00	13.33	13.67
250	15.67	16.00	16.00	16.33	17.50	18.00	0.00	10.83	12.00	0.00	0.00	0.00	15.00	15.00	15.00
500	16.00	16.50	17.00	17.00	17.50	21.00	12.00	14.00	15.00	0.00	0.00	0.00	18.67	19.00	19.00
1000	18.00	17.00	17.67	20.00	20.00	21.00	16.83	16.83	17.00	10.50	10.87	14.00	21.00	21.00	21.33
2000	19.00	20.00	20.33	21.17	20.33	22.00	17.00	17.00	18.00	11.00	11.33	15.33	23.00	24.00	24.00
Zineb (Dithane Z_{78})															
125	10.67	11.00	11.00	11.67	12.50	14.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
250	12.00	13.14	13.67	13.00	15.00	16.67	0.00	0.00	11.00	0.00	0.00	0.00	0.00	0.00	0.00
500	14.00	15.00	16.00	16.00	19.00	18.00	0.00	10.17	13.67	0.00	0.00	0.00	13.00	13.33	13.50
1000	15.00	16.00	18.17	19.33	21.10	21.00	11.67	11.67	14.13	12.00	12.00	16.00	15.33	16.00	16.33
2000	15.67	16.00	20.00	21.00	22.67	24.00	12.00	14.00	14.33	13.67	14.17	21.33	22.00	23.00	23.00
Ziram (Thride)															
125	12.00	12.00	13.00	13.00	14.00	17.00	0.00	10.50	11.00	12.33	12.50	13.00	12.00	12.00	12.33
250	15.33	16.33	17.00	15.33	16.00	21.00	11.00	12.33	12.33	13.67	13.85	14.33	15.33	16.00	16.00
500	16.33	17.14	17.33	16.67	18.00	28.00	14.67	15.00	15.00	16.67	17.00	18.00	18.00	18.00	18.00
1000	20.67	22.33	23.00	21.00	25.00	29.00	16.67	17.33	18.67	20.00	20.17	21.00	22.00	22.33	23.00
2000	24.33	26.00	23.00	25.33	28.00	30.00	18.33	22.67	19.33	24.33	26.00	26.67	23.67	24.00	24.33
C.D. (0.05)	0.85	0.80	0.44	0.71	2.29	0.49	0.89	0.80	0.77	0.43	6.13	0.43	1.34	0.86	2.51

4.11.2.1. Methoxyethylmercuric chloride

This mercuric fungicide inhibited the growth of bacteria *in vitro*, except *B. coagulans* at 125 and 250 ppm levels. At 500 ppm the fungicide produced a small inhibition zone having a diameter of 11 millimeter in *B. coagulans*. At higher levels the diameter of inhibition zone showed a statistically significant increase (Plate VII).

4.11.2.2. Carbendazim

This systemic fungicide was non-inhibitory to all bacteria at 500 ppm level. At 1000 and 2000 ppm levels, this chemical inhibited all bacteria except *B. circulans* and *B. subtilis*. The growth of *B. circulans* and *B. subtilis* were inhibited by this chemical at 1000 and 2000 ppm levels 72 hours after treatment.

4.11.2.3. Copperoxychloride

The copper fungicide was comparatively non-toxic at lower levels viz. 125 and 250 ppm. At 500 ppm level also this was non-inhibitory to all bacteria except *B. macerans* and *B. circulans*, which showed slight inhibition at 72 hours after exposure, and *B. circulans* at 48 hours and 72 hours after treatment, respectively (11 mm to 12 mm). At 1000 and 2000 ppm levels, this fungicide was inhibitory to *B.circulans*,

PLATE VII

In vitro sensitivity of *B. macerans* to fungicides, 72 hr after treatment

1	Control	
2	Methoxyethyl-mercuric chloride	250 ppm
3	Carbendazim	250 ppm
4	Copperoxychloride	250 ppm
5	Zineb	250 ppm
6	Maneb	250 ppm
7	Ziram	250 ppm

B. coagulans and B. subtilis, but the growth of B. macerans was inhibited only 72 hours after treatment. The growth of B. pumilus was inhibited at 2000 ppm level only, that too at 72 hours after treatment.

4.11.2.4. Maneb

This manganese based dithiocarbamate fungicide inhibited the growth of all bacteria except in 125 ppm level in case of B. circulans and 125, 250 and 500 ppm levels in case of B. coagulans.

4.11.2.5. Zineb

The zinc ~~and manganese~~ based dithiocarbamate fungicide at 1000 and 2000 ppm levels was inhibitory to all bacteria. But at 500 ppm level, it was inhibitory to all bacteria except B. coagulans. This fungicide at 125 and 250 ppm levels was non-inhibitory to B. circulans, B. coagulans and B. subtilis.

4.11.2.6. Ziram

This dithiocarbamate fungicide was not safe to the growth of bacteria tested. It inhibited the growth of bacteria at all treatment levels.

4.12. Effect of five Bacillus spp. on related genera of root-knot nematode

The data on the larvicidal effect of five Bacillus spp. to Heterodera oryzicola and Rotylenchulus reniformis are presented in Table 21.

4.12.1. Effect on H. oryzicola

The result showed that there was no significant variation among the treatments. B. circulans (1.2×10^8 cells per ml) showed maximum larval mortality (80.0 per cent) followed by B. macerans (75.7 per cent) and B. coagulans (75.0 per cent).

4.12.2. Effect on R. reniformis

The result showed that there was statistically significant variation among the treatments.

The treatment of 1.2×10^8 cells per ml of B. circulans gave maximum larval mortality (76 per cent) followed by B. macerans (74.3 per cent), B. pumilus (73.0 per cent) B. coagulans (73.0 per cent) and B. subtilis (73.0 per cent) and these five treatments were on par. Minimum larval mortality was caused by B. subtilis (1.0×10^5 cells per ml) with 60.3 per cent followed by same dose of B. coagulans with 61.0 per cent corrected larval mortality.

Table 21. Effect of different species of Bacillus on the larvae of Heterodera oryzicola and Rotylenchulus reniformis (mean of three replications)

Treatment (cells per ml)		Mean corrected larval mortality percentages 120 hr after treatment	
		H. oryzicola	R. reniformis
B. macerans	$1.2x10^8$	75.7(60.3)	74.3(59.5)
,,	$1.2x10^6$	70.3(56.9)	70.0(56.8)
,,	$1.2x10^5$	70.0(56.9)	65.0(53.7)
B. pumilus	$1.1x10^8$	74.0(59.3)	72.0(58.0)
,,	$1.1x10^6$	65.0(53.7)	73.0(58.7)
,,	$1.1x10^5$	65.3(54.0)	72.0(58.2)
B. circulans	$1.2x10^8$	80.0(63.4)	76.0(60.7)
,,	$1.2x10^6$	72.0(58.1)	69.0(56.5)
,,	$1.2x10^5$	70.0(56.8)	69.0(56.2)
B. coagulans	$1.2x10^8$	75.0(60.1)	73.0(58.7)
,,	$1.2x10^6$	72.0(58.2)	72.0(58.0)
,,	$1.2x10^5$	70.0(56.9)	61.0(51.4)
B. subtilis	$1.0x10^8$	72.0(58.0)	73.0(58.7)
,,	$1.0x10^6$	69.7(56.6)	72.0(58.1)
,,	$1.0x10^5$	69.0(56.3)	60.3(50.0)
C.D at 0.05 level		NS	(1.95)

NS: Not significant

Values in parentheses are angular transformation

DISCUSSION

5. DISCUSSION

The root-knot nematode Meloidogyne sp. is the first nematode recorded on black pepper by Delacriox (1902) from Cochin-China. Later Butler (1906) reported it from the Wynad area in Kerala. M. incognita has now been recognised as a serious problem in pepper plantations in Kerala threatening the productivity of vines. Among the antagonists of nematodes, bacteria are potential agents and the pathogen may assume importance in pest management programmes. So far no work of this nature has been reported from Kerala. In nematode management, the deleterious effects of fumigant and non-fumigant chemical pesticides have necessitated the exploitation of the bioagents. This emphasised the conservation and utilisation of the natural enemies in their habitat itself. Hence this investigation was carried out to generate information on the occurrence of bacterial pathogens of the root-knot nematode, M. incognita in the major pepper growing areas of the State and to assess the scope of utilising them for biocontrol of the nematode.

5.1. Preliminary survey

A preliminary survey, to know the prevalence and to gather information on the bacteria associated with

M. incognita, was conducted. Twenty two bacterial isolates were obtained. The pathogenic effect of these isolates on eggs and larvae revealed that six isolates were promising (para 4.2.1 and 4.2.2) and were identified. The bacteria identified were B. subtilis, B. pumilus, B. coagulans, B. macerans, B. circulans and B. licheniformis.

Among these B. subtilis and B. pumilus were reported earlier from India (Punjab and Haryana) by Gokte and Swarup (1988 a and b) on Anguina tritici, Heterodera avenae, H. cajani and Meloidogyne incognita. The association of the rest of the bacterial species are reported for the first time from the country on M. incognita. B. subtilis and B. pumilus are also not reported from M. incognita in the State.

5.2. Detailed survey

To ascertain the distribution of these six species of Bacillus, a random survey was carried out in major root-knot infested pepper growing areas located in ten districts. The survey indicated that root-knot nematode was found associated with pepper in almost all locations and that out of the 400 root samples collected, 340 had root-knot nematode infestation. Bacterial infection was detected in 137 samples. Out of these, 90 different isolates were gram positive rods.

Among these, thirty nine isolates were identified up to species level. B. subtilis was obtained from eight locations, B. pumilus from twelve locations, B. coagulans from three, B. macerans from four, B. circulance from seven and B. licheniformis from five locations (para 4.4). This type of survey for detecting the bacterial pathogens of M. incognita was not taken up in the Kerala State.

Among the six Bacillus spp., B. pumilus was the widely distributed species, obtained from nine of the ten districts surveyed, B. subtilis from five districts, B. coagulans from three districts, B. macerans, B. circulans and B. licheniformis from four districts each (para 4.4.1 to 4.4.6 and Fig. 1).

Another interesting observation made during the survey was the association of the six Bacillus spp. in different life stages, namely, egg mass, second stage juvenile and adult female while B. coagulans, B. macerans and B. circulans were obtained from egg masses and adult females only (Fig. 2)

The percentage occurrence of different species of Bacillus on various life stages of M. incognita is presented in Fig. 2. B. circulans showed maximum incidence on the females collected from Thrissur district (11.7 per cent).

The bacterial association was easily detected by the presence of bacteria attached to the cuticle of female and egg shell (Plate VIII). In infected egg mass all the larvae emerged were not immobilised and they move and spread the inoculum to other individuals as seen in Plate IV. Thus dispersal of the bacterial pathogen takes place effectively.

The nematode population in root samples did not show any significant difference, between the bacteria infected samples and non-infected samples. But Prasad and Tilak (1972) observed that B. thuringiensis was absent in tomato field soil supporting high population levels of root-knot nematodes while bacterium was present in soil with low level of nematode incidence.

5.3. Pathogenicity of the different isolates of Bacillus spp.

The pathogenic effect of different isolates of the Bacillus spp. on the emergence of the larvae from egg mass and larval mortality were studied at three different doses, O.D 1.5, 1.0 and 0.5 and concluded as follows.

B. subtilis

The effect of eight B. subtilis isolates were studied in detail and found that all the isolates were statistically

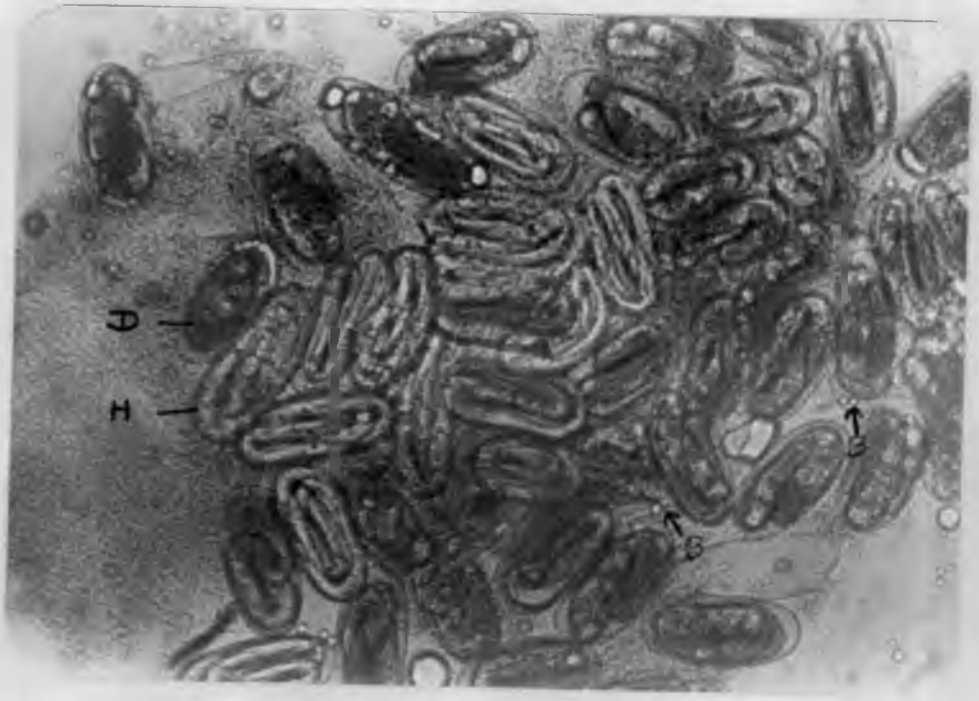
PLATE VIII

Attachment of bacterial cells on the cuticle of female and egg shell of *M. incognita* (B)

D Infected egg

H Healthy egg

PLATE VIII



on par in reducing the emergence of M. incognita larvae from egg mass (para 4.5.1.1). In the case of larval mortality at 1.5 O.D, Pampadumpara isolate showed its superiority (para 4.5.2.1.1). At 1.0 O.D all the isolates were on par. At 0.5 O.D Pampadumpara isolate was on par with Perumede isolate 120 hr after treatment. The variation of Pampadumpara and Perumede isolates from the others may be due to the differences in the ecological niche from where these two were isolated.

B. pumilus

The effect of twelve isolates of B. pumilus was uniform in the reduction in the emergence of the larvae from egg mass (para 4.5.1.2). In the case of larval mortality Muzhoor isolate showed statistically significant variation at 1.5 O.D. At 1.0 O.D Muzhoor isolate was on par with other four isolates (isolates collected from Vellavani. Kottarakkara, Vaikom and Angamali).

B. coagulans

The result presented in para 4.5.1.3 showed that the three isolates of B. coagulans were on par in reducing the emergence of the larvae from the egg mass at 10 DAT. In the case of larval mortality, all the isolates were on par at 1.5 O.D (para 4.5.2.1.3) and 1.0 O.D (para 4.5.2.2.3). At 0.5 O.D,

larval mortality observed at 48 and 72 hr after treatment were on par. At 120 hr after treatment, Marakkal isolate was superior to other two isolates. The superiority of this isolate at lower dose indicated its potency.

B. macerans

The effect of four isolates of B. macerans on the reduction in the emergence of the larvae from egg mass showed significant variation. Nedumangad isolate was inferior to other three at 1.5, 1.0 and 0.5 O.D (para 4.5.1.4). In the case of larval mortality at 1.5 O.D, Sulthanbathery isolate revealed its superiority at 72 hr after treatment. At 120 hr after treatment, it was on par with Kannara isolate. At 1.0 O.D Nedumangad isolate showed its superiority at 120 hr after treatment. At 0.5 O.D, Kannara isolate showed higher larval mortality than the other three isolates.

As mentioned in para 4.5.1.5, different isolates of B. circulans were uniform in reducing the larval emergence of M. incognita from the egg mass at 1.5, 1.0 and 0.5 O.D. In the case of larval mortality at 1.5 and 0.5 O.D, the effect was uniform. The difference in larval mortality at 1.0 O.D may be due to difference in the geographical habitat and stage specificity. Out of the seven, two were obtained from egg mass and five from the female. This type of work was not taken up earlier.

Considering the effect on the egg mass and larvae, one effective isolate each of the five species was selected for further detailed study. Though the pigment producing bacterium B. licheniformis obtained in the survey showed some larvicidal and ovicidal effects, it was not selected for further study, because the effects of the different isolates of this species were not uniform. It also showed feeble sporulation and gram reaction. Moreover the distribution of this species was confined to the forest areas and in some of the neglected pepper gardens. The restricted occurrence of this species might be due to the impact of the common agricultural practices. Out of the five selected isolates three were obtained from egg mass (B. subtilis, B. coagulans and B. circulans) and two from the female nematode (B. pumilus and B. macerans).

5.4. Effect of the different species of Bacillus on root-knot nematode in vitro

The emergence of the larvae from the egg mass revealed that B. coagulans exhibited maximum reduction followed by B. subtilis. These two were on par but superior to other three species. The superiority of these two species over the rest may be due to their preference for the egg stage of M. incognita. These two species were originally isolated

from the egg masses. In the larval mortality all the five species were on par (para 4.6). The observations made in this study also agreed with the pattern of mortality and immobility of the larvae due to bacterial association (B. subtilis, B. pumilus and B. cereus) as reported by Gokte and Swarup (1988).

5.5. Effect of the different species of Bacillus on root-knot nematode in vivo (tomato plants

The effect of five Bacillus spp. on M. incognita infested tomato plants showed that B. circulans @ 0.9×10^7 cells/pot and B. macerans @ 0.8×10^7 cells/pot and carbofuran 500 ppm were on par in reducing the nematode population in soil, but in the reduction of root-knot population, B. subtilis (1.5×10^7 cells/pot), B. coagulans (1.2×10^7 cells/pot), B. circulans (0.9×10^7 cells/pot) and B. macerans (0.8×10^7 cells per pot) were on par and statistically superior to B. pumilus (1.8×10^7 cells/pot) and carbofuran 500 ppm (para 4.7). This finding is in agreement with that of Mankau (1975) who reported that application of spores of B. penetrans to tomato plants infested with root-knot nematode showed less root galling than plants in soil free of bacterial spores.

5.6. Pathogenicity of the *Bacillus* spp. on crop plants and higher animals (birds)

The pathogenicity of the *Bacillus* spp. was tested against important host plants commonly infested by *Meloidogyne* spp. and also plants used as standards in pepper garden belonging to the families piperaceae (betel vine), malvaceae (bhindi), solanaceae (tomato), zingiberaceae (ginger), cucurbitaceae (bitter gourd), leguminaceae (*Erythrina*), moringaceae (drumstick) and labiatae (*Coleus*). None of these plants showed any symptom of phytotoxicity, withering and other bacterial diseases (para 4.8.1). It is known that gram positive spore forming bacteria do not generally infect plants and cause pathogenic effects. The observations from the above study are in agreement with the reports made by Burges (1982).

The pathogenicity of the bacteria on higher animals, if any, was assessed using chick as the test organism. The tests revealed that the bacteria were not injurious to birds (para 4.8.2). These *Bacillus* spp. may be parasitic and pathogenic to lower invertebrates (Burges, 1982). They cannot said to be truly saprozoic since these species parasitised the live nematode. Burges (1982) reported the safety of the *B. popilliae* group both for man and non-target

fauna. These spore forming bacteria are not reported as vertebrate pathogens. No allergies have been encountered among workers producing the organism. Spores fed to starlings and chickens had no adverse effect.

5.7. Effect of different media on the growth of the bacteria

The growth of the Bacillus spp. on liquid media of nutrient broth (NB), potato dextrose medium (PD), Muller Hinton medium (MH), glucose yeast extract medium (GYE) and lactose yeast extract medium (LYE) were studied in detail. It concluded that at initial period (6 hours) B. macerans, B. pumilus, B. coagulans and B. subtilis showed maximum growth in GYE medium and later the superiority was lost (para 4.9). This initial increase in growth of the bacteria may be due to the presence of glucose in the GYE medium, which is used as a germinant like dipcolinic acid and Penassy broth for Bacillus spp. except B. cereus (Aroson et al., 1986). But the growth at 48 and 72 hours are considered in selecting the best medium.

At 48 hours after inoculation, B. subtilis exhibited maximum growth in MH followed by NB and these were on par. At 72 hours, NB exhibited its superiority over the other media.

In the case of B. pumilus, NB showed its superiority at 48 hours after inoculation. But at 72 hours, PD, NB, MH and GYE were on par.

Growth of B. coagulans was maximum in GYE at 48 hours but at 72 hours GYE and PD were on par.

Growth of B. macerans was maximum in PD, NB and MH at 48 hours and at 72 hours GYE, MH, NB and PD were on par; thus PD was selected as the best suited medium.

Growth of B. circulans was maximum in GYE and PD at 48 hours and 72 hours after inoculation.

Thus NB was found as the best suited medium for B. subtilis, PD and NB for B. pumilus, GYE and PD for B. coagulans, PD, NB and GYE for B. macerans and GYE and PD for B. circulans. But considering the cost of media also, PD may be taken as the suitable medium to grow all the Bacillus spp. except B. subtilis. This aspect has been studied for the first time.

5.8. Evaluation of the bacteria in root-knot infested pepper plants in pot culture condition

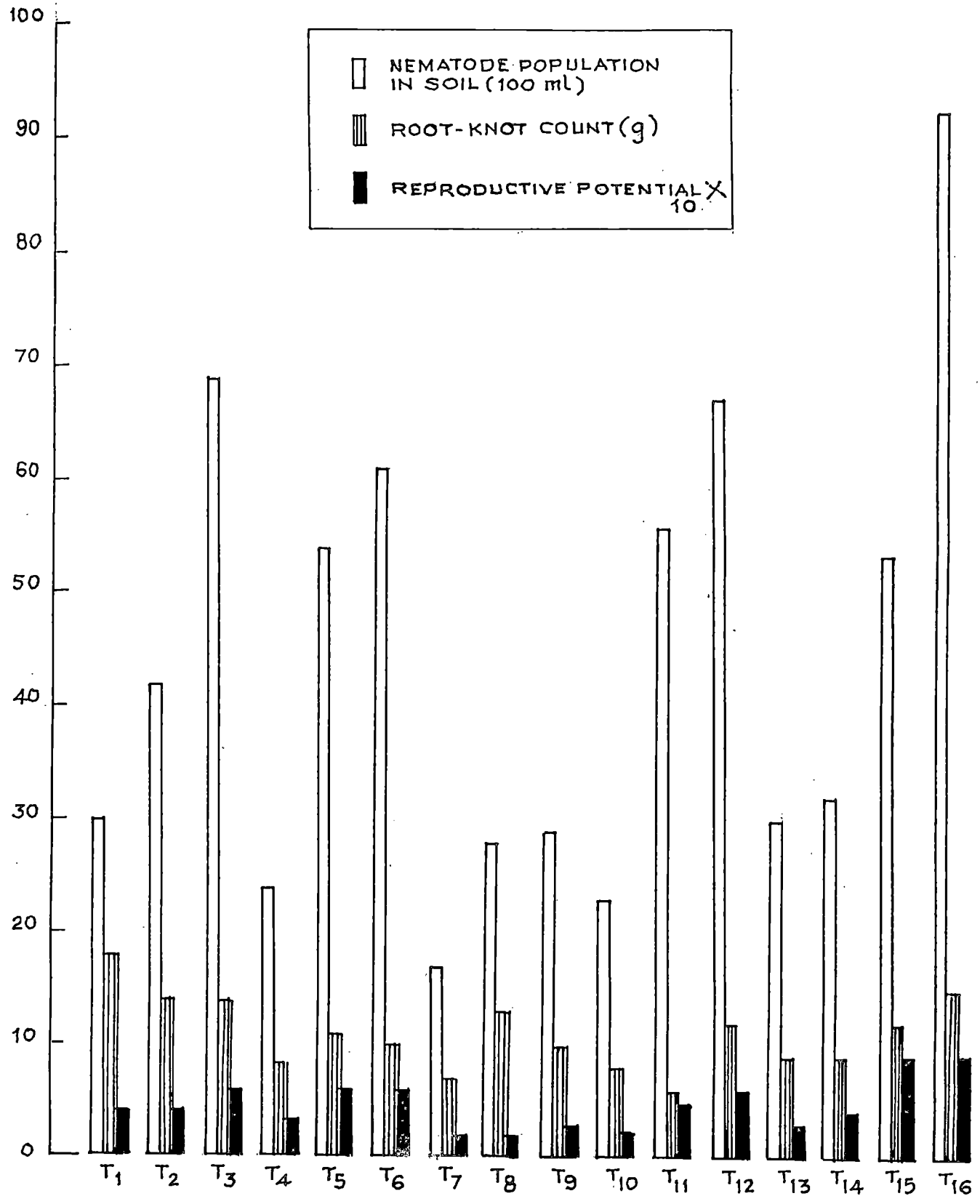
The experiment set up to study the improvement in plant growth characters due to the reduction of nematode

population in root and soil by the effect of addition of the bacterial pathogen to pepper plants infested with M. incognita gave clear indications that bacteria could suppress the pathogenicity of the nematode.

The nematode population in soil treated with the five bacterial pathogens observed at 3, 6, 12 and 18 months after treatment showed statistically significant reduction (para 4.10.1 and Fig. 4). The treatment of 1.1×10^8 cells per pot of B. pumilus, 1.2×10^8 cells per pot of B. circulans and B. macerans reduced the population significantly from third month onwards till the end of the experiment.

The nematode infestation in the root expressed in terms of number of galls per g of root and root-knot indices are mentioned in para 4.10.2 and Fig. 4. The reduction in root-knot incidence was high in B. macerans (1.2×10^8 cells per pot) and B. circulans (1.2×10^8 cells/pot). Gokte and Swarup (1988) reported the larvicidal effect of B. pumilus and B. subtilis on Anguina tritici, Meloidogyne spp. and H. avenae based on their experiment conducted ~~under~~ in vitro. The larvicidal effect of the bacterial pathogen tested in the present study may be the reason for the reduction of population of M. incognita in soil and subsequently in the root system. The above findings are similar to the

FIG. 4. EFFECT OF INOCULATIONS WITH *M. incognita* AND DIFFERENT SPECIES OF *Bacillus* ON THE NEMATODE POPULATION IN SOIL, ROOT-KNOT COUNT NEMATO STATIC VALUE.



observations reported by Mankau (1975a), on the reduction in gall production in tomato plants treated with air-dried B. penetrans spore infested soil in glass house condition, 70 days after treatment.

In the pepper plants which were inoculated with M. incognita treated with five species of Bacillus (para 4.10 and Fig. 5) there was drastic reduction in reproductive potential of the nematode from the sixth month onwards. These effects were more pronounced in the case of B. pumilus and B. circulans which resulted in higher level of reduction in reproductive potential from sixth month to end of the experimental period. At the end period of the experiment, the pathogenic ability of B. macerans was evident on plants even at the low dose of 1.2×10^6 cells/pot, which was on par with the dosage level of 1.1×10^8 cells/pot of B. pumilus, B. coagulans, B. circulans and 1.0×10^8 cells/pot of B. subtilis. But in terms of root-knot index, B. pumilus (1.1×10^6 cells/pot) and B. macerans (1.2×10^5 cells/pot) treatments showed minimum root-knot indices of 1.0 in addition to the above three treatments (para 4.10.2).

The reproductive factor and nematostatic value of various treatments computed (para 4.10.3, 4.10.4 and Fig. 4) revealed that B. circulans (1.2×10^8 cells per pot),

B. pumilus ($1.1x10^8$ cells per pot) and B. macerans ($1.2x10^8$ cells per pot) were effective in reducing the reproductive potential of M. incognita and thereby the reduction in nematostatic value. Eissa and Moussa (1982) reported that lowest nematostatic value for effective nematicide in the evaluation of nematode population characteristics in wheat.

The vine length and number of leaves of pepper plants treated with bacteria and nematode though exhibited increase from the first month onwards was not statistically significant (para 4.10.6). However, increase in length of vine in plants which received the bacterial load of $1.2x10^8$ cells/pot of B. circulans, B. macerans, B. coagulans, $1.1x10^8$ cells/pot of B. pumilus and $1.2x10^6$ cells/pot of B. circulans improved the growth equal to that of absolute check (without bacteria and nematode) at the ninth month and the superiority of the effect of the pathogens viz. B. circulans ($1.2x10^8$ cells/pot), B. pumilus ($1.1x10^8$ cells/pot) and B. macerans ($1.2x10^8$ cells/pot) was maintained up to the termination of the experiment (eighteen months) (Fig. 5). These results indicated the bio-suppression of the root-knot nematode by these pathogens under pot culture conditions.

The plants which received the bacterial dose of $1.2x10^8$ cells/pot of B. coagulans, B. macerans, $1.0x10^8$

cells/pot of B. subtilis and $1.2x10^8$ cells/pot of B. circulans showed statistically significant increase in leaf production from ninth month onwards up to eighteenth month (Table 17). This increase did not bring the plants on par with the plants in absolute check (without nematode and bacteria). Mankau (1975) had reported the improvement of leaf production in tomato plants treated with air-dried B. penetrans spores infested soil even 70 days after the treatments. In the present study, the increase in leaf production was not very prominent due to the introduction of five Bacillus spp. This is explicable on the basis of the variations in growth traits of pepper and tomato. The tomato plant is a seasonal short duration crop with fast growth characteristics while pepper is a perennial plant with slow growth characteristics.

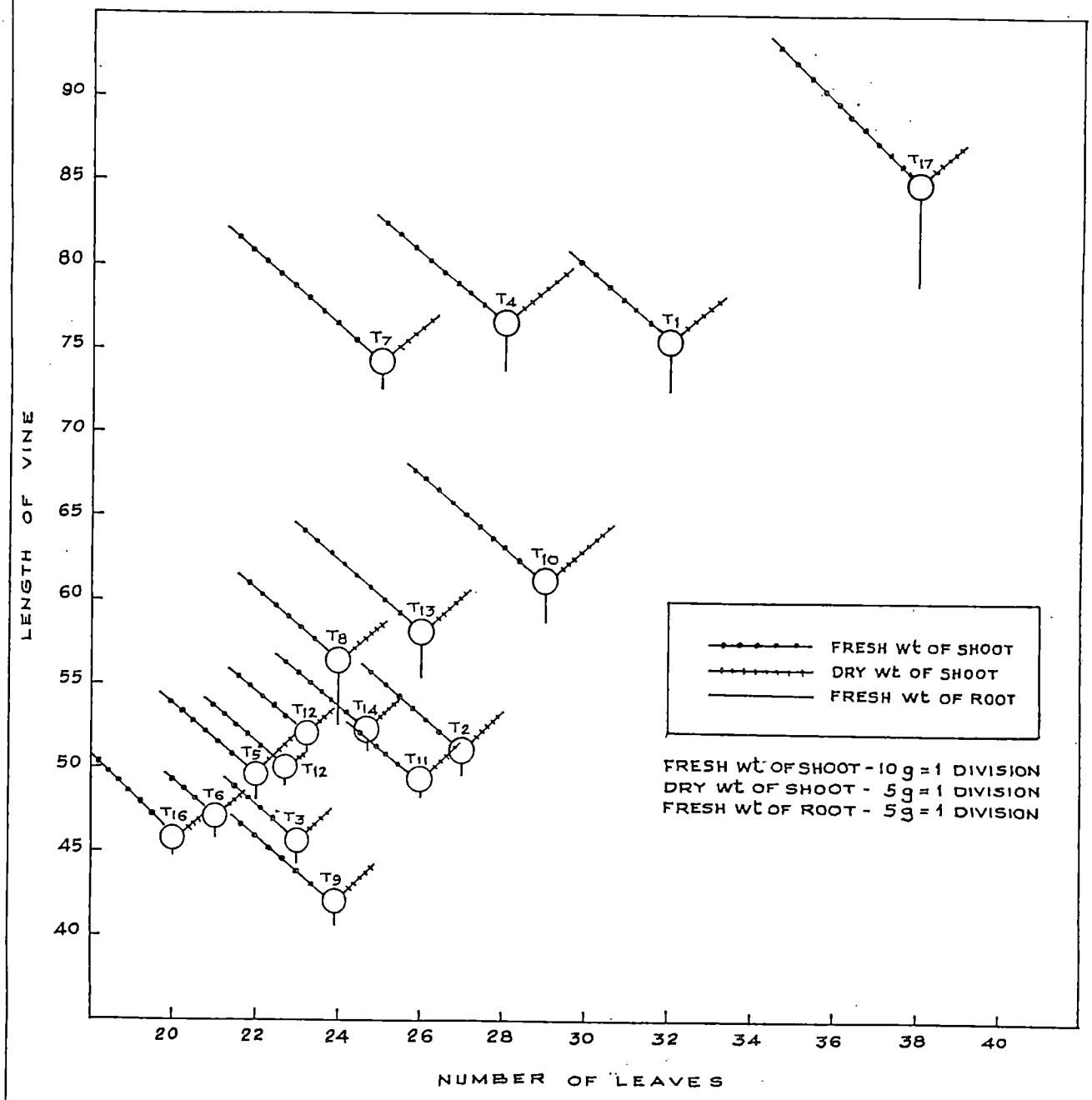
The fresh weight of pepper plants (shoot weight) treated with Bacillus spp. showed statistically significant variations among the different treatments from sixth month onwards. The plants which received the bacterial dose of $1.2x10^8$ cells/pot of B. circulans, B. coagulans and $1.1x10^8$ cells/pot of B. pumilus showed their superiority from sixth month after treatment. But at eighteenth month or on termination of the experiment, fresh top weight of plants treated with the above three bacterial pathogens came on par

with the plants in the treatment, absolute check (para 4.10.6.3). The above findings are in agreement with the observations made by Maheswari et al. (1987). They reported that the application of Pasteuria penetrans in combination with nematicides (aldicarb, carbofuran, miral, phorate and sebufos) improved the fresh weight of shoot of tomato plants under glass house condition. They also observed that the combined application was responsible for the improvement of the plant growth due to additive reactions than when compared with their individual effects.

The fresh root weight of pepper plants treated with Bacillus spp. showed significant variations from twelfth month. The results indicated that B. circulans, B. macerans and B. pumilus could reduce the root infestation by M. incognita and bring about healthy root system in plants as mentioned in para 4.10.6.5.

The dry weight of shoot of pepper plants recorded at sixth month showed that the treatments which received a bacterial dose of 1.0×10^8 cells/pot of B. subtilis, 1.2×10^8 cells per pot of B. circulans and 1.2×10^8 cells/pot of B. coagulans and 1.2×10^6 cells/pot of B. macerans were statistically on par with the dry shoot weight of plants in absolute check (para 4.10.6.4). Among the three species,

FIG. 5. EFFECT OF INOCULATION WITH *M. incognita* AND *Bacillus spp.* ON THE DIFFERENT GROWTH PARAMETERS OF PEPPERVINE 18 MONTHS AFTER TREATMENTS.



B. macerans ranked first as this bacterium with its lower cell concentration also improved the weight of shoot of pepper plant even at sixth month after treatment. However, at the end period of the experiment, treatment with B. coagulans (1.2×10^8 cells/pot) and B. pumilus (1.1×10^8 cells/pot) resulted in the dry shoot weight of treated plants equal to the plants in the treatment, absolute check, indicating that these bacterial pathogens could reduce the effect of M. incognita on pepper.

5.9. Interaction of the bacteria with nematicides, insecticides and fungicides

Pesticides are being used extensively for the control of pests and diseases of pepper. Hence a study on the compatibility or sensitivity of the bioagents to common pesticides was felt necessary.

The in vitro-sensitivity of the Bacillus spp. to nematicides revealed that among the common fumigants and non-fumigant chemicals tested, carbofuran was the safest chemical which did not show any inhibitory effect on the bacteria (para 4.11.1.4). Phorate was the next best which showed slight inhibition on B. coagulans (para 4.11.1.2). Metham sodium, formaldehyde and aldicarb showed a high degree of growth inhibition on the bacteria (para 4.11.1.1, 4.11.1.5 and Plate IV) and therefore cannot be used along

with these bio-agents. The general biocide nature of these fumigants (metham sodium and formaldehyde) and non-fumigant (aldicarb) may be the reason for the inhibition of the growth of the bacteria. Mankau and Prasad (1972) reported that among the six nematicides used at recommended field dosages, DBCP was slightly toxic while DD, aldicarb, carbofuran, phenamiphos and ethoprop had no noticeable effect on the endoparasite Dubscquia which could be considered for the biological control of plant parasitic nematodes.

Among the insecticides tested, endosulfan and malathion were found safer for the growth of bacteria. Malathion is a relatively safer chemical among organophosphates and endosulfan a member of the cyclodiene group is reported to be safe to parasites, predators and pollinators. The results of the test (mentioned in para 4.11.1.6, 4.11.1.7, 4.11.1.8, 4.11.1.9 and 4.11.1.10, and Plate V and VI) have clearly shown that all the chemicals at 500 ppm level (just lower than the recommended dose) was non-inhibitory to the bacteria tested and this indicated that the aerial application of this chemical is not likely to hinder the soil fauna.

The results of the test on in vitro-sensitivity of Bacillus spp. to fungicides presented in 4.11.2.1. to

4.11.2.6 and Plate VII revealed that the mercuric fungicide, methoxy ethyl mercuric chloride inhibited the growth of all bacteria except B. coagulans at 125 and 250 ppm levels. At higher dose, the diameter of inhibition zone showed statistically significant increase. The systemic fungicide, carbendazim was non-inhibitory to the growth of all bacteria up to 500 ppm level. At 1000 and 2000 ppm level this chemical was inhibitory to all bacteria except B. circulans and B. subtilis. Copper oxychloride was non-inhibitory to the growth of all bacteria up to 250 ppm level. B. macerans and B. circulans were sensitive to this chemical at 500 ppm level onwards. But B. pumilus was not sensitive to this chemical up to 1000 ppm level. The zinc and manganese based dithiocarbamate fungicides were highly inhibitory to all the bacteria tested. This aspect has been studied for the first time in these bacteria.

5.10. Effect of five Bacillus spp. on related genera of root-knot nematode

The data on the larvicidal effect of five Bacillus spp. to H. oryzicola are presented in para 4.12.1. The larval mortality caused by the bacteria at different doses were not statistically significant, but corrected mortality ranged form 69 to 79 per cent. Larval mortality caused by

B. pumilus and B. subtilis on H. avenae and H. cajani was reported by Gokte and Swarup (1988) but for the rest of the species this is reported for the first time in the State as well as in the country.

The data on the effect of five Bacillus spp. to Rotylenchulus sp. are presented in para 4.12.2. The result revealed that the treatment with 1.2×10^8 cells per ml of B. circulans, B. macerans, B. pumilus, B. coagulans and B. subtilis showed maximum effect and the corrected larval mortality ranged from 73 to 76 per cent. The mortality effect of this nematode has been studied for the first time.

In the present study the experiments were limited to eighteen months. Tests were carried out with individual bacterium and the combinations of different species could not be tested. In nature there is possibility of occurrence of combination of different bacterial species, which may interact with each other. In the survey, two instances of concurrent incidence of B. pumilus and B. subtilis were observed.

Since these bacterial pathogens were found distributed in different geographical areas (Fig. 1), ecological requirements of each species have to be precisely studied and

established so that while recommending them as bio-control agents, suitability can be assessed in relation to the ecological situation in each location.

SUMMARY

6. SUMMARY

6.1. Survey on the occurrence of bacterial pathogens

Twenty one samples were collected from eight locations namely Palode, Pampadumpara, Panniyur, Vellayani, Vellanikkara Marakkal, Kannara and Kumili for detecting the presence of the association of bacteria. Twenty two isolates were obtained (19 gram positive rods, one each of gram negative rod, gram positive cocci and gram negative cocci). The effect of these isolates were studied on the egg mass and larvae and promising six were selected for identification. Identification up to species level was done by following the procedure described by Cowan (1974) and conformed its identity at the College of Veterinary and Animal Sciences, Mannuthy. They are *Bacillus subtilis*, *B. pumilus*, *B. coagulans*, *B. macerans*, *B. circulans* and *B. licheniformis*.

6.2. Distribution of the *Bacillus* spp. associated with root-knot nematode *M. incognita* in major pepper growing areas of Kerala

To study the occurrence of these six identified species survey was conducted in major pepper growing districts, namely, Kannur, Ernakulam, Idukki, Kollam, Kottayam, Kozhikode, Pathanamthitta, Thiruvananthapuram, Thrissur and Wynad. Four

hundred root samples were collected from these ten districts. Three hundred and forty samples yielded root-knot nematode in root samples. Bacterial infection was detected only from 137 samples. Out of these 90 different isolates were gram positive rods. Among this thirty nine isolates were identified up to species level. Eight isolate as B. subtilis, twelve isolate as B. pumilus, three as B. coagulans, four as B. macerans, seven as B. circulans and five as B. licheniformis. Their percentage occurrence at different life stages were worked out separately. The pigment producing bacteria B. licheniformis was not taken for further detailed study because of its feeble sporulation and gram reaction. Its occurrence was also confined to root-knot nematodes collected from forest ecosyste

6.3 Pathogenicity of the different isolates of Bacillus spp.

The different isolates of five species of Bacillus obtained in the survey were tested separately for their effect on the emergence of the larvae from the egg mass and larval mortality. Thus one most effective isolate from each of the five species was selected for detailed study. They are:

B. subtilis - Pampadumpara isolate (egg mass)
B. pumilus - Muzhoor isolate (female)
B. coagulans - Marakkal isolate (egg mass)
B. macerans - Sulthanbathery isolate (female)
B. circulans - Kumili isolate (egg mass).

6.4. Effect of different species of Bacillus on root-knot nematode in vitro

The emergence of the larvae from the egg mass showed that B. coagulans showed maximum reduction followed B.subtilis These two were on par and superior to other three species, while in the larval mortality all the five species were on par. These two effects were assessed in vitro by cavity block method.

6.5. Effect of the selected isolates of different species of Bacillus on root-knot nematode in tomato plant

The treatments of B. circulans at 0.9×10^7 cells per pot and B. macerans at 0.8×10^7 cells per pot and carbofuran 500 ppm (check) were on par in reducing the nematode population in soil but in the reduction of root-knot population in root, B. subtilis at 1.5×10^7 cells per pot, B. coagulans 1.2×10^7 cells per pot, B. circulans 0.9×10^7 cells per pot and B. macerans at 0.8×10^7 cells per pot were on par and statistically superior to B. pumilus (1.8×10^7 cells/pot) and carbofuran 500 ppm.

6.6. Effect of the bacterial isolates on plants and higher animals (birds)

Pathogenicity of five Bacillus spp. on eight host plants representing eight different families did not produce symptom of any bacterial disease.

Safety testing of the bacteria (B. subtilis, B. pumilus, B. coagulans, B. macerans and B. circulans) was done by oral administration and subcutaneous injection of two week old chicks. There was no expression of common bacterial infection, leading to chick fever, irritations on the skin and diarrhoea.

6.7. Growth of the Bacillus spp. on different media for mass production

The growth of the five Bacillus spp. were studied in five different liquid media namely nutrient broth, potato dextrose, Muller Hinton, glucose yeast extract, lactose yeast extract. The experiment revealed that GYE and PD were the best suited medium for B. coagulans. Nutrient broth is the best suited medium for B. subtilis, PD and NB for B. pumilus, PD, NB and GYE for B. macerans and GYE and PD for B.circulans. But considering the cost of media also, PD is observed to be the suitable medium to grow all the Bacillus spp. except B. subtilis.

6.8. Evaluation of pathogenic effect of different Bacillus spp on root-knot nematode of pepper by pot culture studies

The different inoculum levels of the five Bacillus spp. were prepared and applied to pepper root zone two days prior to nematode inoculation. Biometric observations were recorded prior to treatments and at monthly intervals. Three replica-

tions each were uprooted at 3, 6, 12 and 18 months after treatment and recorded the nematode population in root and soil. Highest dose of B. pumilus, B. macerans, B. circulans and B. coagulans were statistically on par in reducing the population in soil in various period interval.

Highest dose of B. macerans, B. circulans, B. pumilus and B. coagulans were statistically on par in reducing the nematode population in root. The root-knot index computed also showed the same trend.

The reproductive factor and nematostatic value for five species showed that B. circulans, B. pumilus and B. macerans showed minimum value concluding effective management of M. incognita.

The biometric characters (length of vine, number of leaves, fresh weight of shoot and root, and dry weight of shoot) also improved by this bacteria.

6.9 In vitro sensitivity of five Bacillus spp. to pesticides

In vitro sensitivity studies of the bacteria were conducted by filter paper disc method. Various groups of pesticides at different concentrations were tried. Among the nematicides and insecticides used as nematicides metham sodium and formaldehyde were highly toxic to all the five

species tested. Phorate was non-inhibitory to the growth of all Bacillus spp. except B. coagulans. In carbamate group, aldicarb 500 ppm was inhibitory to all bacteria except B. coagulans while carbofuran was non-inhibitory to all species of Bacillus tested.

Among the insecticides, HCH, endosulfan, malathion, quinalphos and carbaryl were studied. All these up to 500 ppm levels were non-inhibitory to all bacteria.

The result indicated that systemic fungicides carbendazim and copper fungicide, copperoxychloride were non-inhibitory to the growth of the bio-agents tested up to 500 ppm level.

6.10. Effect of bacteria on related genera of M. incognita

The trial to study the effect of these bacteria (B. subtilis, B. pumilus, B. coagulans, B. macerans and B. circulans) on related genera like H. oryzicola concluded that 1.2×10^8 cells per ml of these bacteria caused 70 to 80 per cent larval mortality. In the case of R. reniformis a cell concentration of 1.2×10^8 cells per ml of B. circulans, B. macerans, B. pumilus, B. coagulans and B. subtilis showed 73 to 76 per cent larval mortality.

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* Original not seen

APPENDICES

APPENDIX I

Summary of Analysis of Variance

Table 5. Larval emergence

Source	df	MS		
		OD-1.5	OD-1.0	OD-0.5
B. subtilis				
Treatment	7	0.40	0.17	0.50
Error	16	0.97	0.54	0.45
B. pumilus				
Treatment	11	1.05	1.49	1.76
Error	24	0.48	0.90	0.96
B. coagulans				
Treatment	2	0.20	1.19	0.17
Error	15	1.49	0.53	0.91
B. macerans				
Treatment	3	5.88**	5.15*	6.20**
Error	20	0.08	0.94	0.70
B. circulans				
Treatment	6	0.12	0.46	0.83
Error	14	0.72	0.93	0.85
B. licheniformis				
Treatment	4	0.61	3.40**	2.38
Error	15	0.42	0.43	0.33

* Significant at 5 per cent

** Significant at 1 per cent

APPENDIX II

Summary of Analysis of Variance - Larval mortality

Source	df	Table 6. OD-1.5 MS			Table 7. OD-1.0 MS			Table 8. OD-0.5 MS		
		48 h	72 h	120 h	48 h	72 h	120 h	48 h	72 h	120 h
B.subtilis										
Treatment	7	3281.40**	20.65	38.22*	2.85	3.32	4.60	20.48*	15.14*	15.14*
Error	16	4.68	58.80	8.54	2.95	3.08	3.39	5.93	1.29	1.29
B.pumilus										
Treatment	11	45.29*	59.82**	79.48**	14.53**	14.87*	14.60	31.84**	39.74**	36.67*
Error	24	2.20	4.72	4.84	1.95	2.28	6.30	2.76	1.55	2.49
B.coagulans										
Treatment	2	18.39	16.35	17.72	21.06	19.28**	6.11	32.08	114.88**	126.91**
Error	15	9.67	7.27	8.39	12.20	1.12	1.90	11.13	2.08	2.22
B.macerans										
Treatment	3	97.40**	187.22**	194.40**	5.33	9.62**	25.40*	1.46	11.05**	12.96*
Error	20	7.80	9.36	23.80	1.87	0.91	2.41	1.67	1.90	3.97
B.circulans										
Treatment	6	3.39	4.90	65.68	4.84*	8.90*	19.03*	7.34	12.45	8.20
Error	14	22.82	19.74	32.00	1.44	1.63	3.08	8.73	12.20	2.94
B.licheniformis										
Treatment	4	64.29	9.92	4.94	7.92	6.47	8.89	44.90*	14.24	22.15*
Error	10	35.32	21.33	9.40	7.88	4.97	4.78	3.69	9.42	3.62

* Significant at 5 per cent

** Significant at 1 per cent

APPENDIX III

SUMMARY OF ANALYSIS OF VARIANCE

Table 9. Effect on eggs and larvae

Source	df	MS	
Treatment	5	8.16**	1569.12**
Error	18	0.21	46.20

** Significant at 1 per cent

APPENDIX IV

Summary of Analysis of Variance

Table 10. Nematode population

Source	df	MS	
Treatment	6	8.46**	3.62**
Error	21	0.31	0.11

** Significant at 1 per cent

APPENDIX V

Summary of Analysis of Variance

Table 11. Growth on different media at different periods (hours)

Source	df	MS				
		6	12	24	48	72
B. subtilis						
Treatment	4	0.154	0.081*	0.046**	0.100*	0.047*
Error	15	0.042	0.001	0.002	0.005	0.009
B. pumilus						
Treatment	4	0.010*	0.078**	0.065**	0.101**	0.076**
Error	15	0.000	0.001	0.005	0.005	0.015
B. coagulans						
Treatment	4	0.010*	0.071**	0.044	0.079**	0.121**
Error	15	0.000	0.001	0.010	0.005	0.004
B. macerans						
Treatment	4	0.010**	0.051*	0.039**	0.039**	0.084*
Error	15	0.000	0.001	0.002	0.002	0.003
B. circulans						
Treatment	4	0.007*	0.086**	0.075*	0.028**	0.027
Error	15	0.001	0.001	0.002	0.011	0.039

* Significant at 5 per cent

** Significant at 1 per cent

APPENDIX VI

Table 12. Nematode population in soil

Source	df	Mean square			
		3M	6M	12M	18M
Treatment	16	12.41**	17.14**	12.82**	12.70**
Error	34	0.67	1.11	0.29	0.73

Table 13. Nematode population in root

Source	df	Mean square			
Treatment	16	1.47**	4.38**	3.94**	4.12**
Error	34	0.28	0.21	0.20	0.47

Table 15. Number of larvae emerged from egg mass

Source	df	Mean square
Treatment	16	17.73*
Error	34	4.61

Table 16. Length of vine

Source	df	Mean square							
		1M	2M	3M	6M	9M	12M	15M	18M
Treatment	16	0.16	4.40	20.4	214.4	259.1**	334.78**	334.48**	506.8**
Error	34	0.17	2.57	19.3	128.7	90.2	92.23	90.17	79.6

Table 17. Number of leaves

Source	df	Mean square							
		1M	2M	3M	6M	9M	12M	15M	18M
Treatment	16	0.19	0.20	0.27	22.2	42.96	39.00	63.00	51.08
Error	34	0.08	0.15	0.15	12.4	10.08	9.00	10.45	8.38

Table 18.

		Fresh weight of shoot			Dry weight of shoot		
Source	df	Mean square			Mean square		
Treatment	16	270.5**	158.2**	258.5**	12.33*	122.1	61.3**
Error	34	46.1	27.1	67.7	3.21	96.5	14.9
		Fresh weight of root					
Treatment	16	13.5*	19.6**	34.8*			
Error	34	5.6	5.6	3.5			

Table 21 Larval mortality

Source	df	Mean square	
		H. oryzicola	R. reniformis
Treatment	14	17.39	24.19**
Error	30	95.84	1.37

* significant at 5% level ** Significant at 1% level M: Month

APPENDIX VII

Summary of Analysis of Variance

Table 20. In vitro sensitivity to fungicides

Source	df	MS		
B.macerans				
Treatment	50	103.10**	112.32**	120.01**
Error	74	0.27	0.24	0.07
B.pumilus				
Treatment	40	47.01**	64.18**	250.65**
Error	59	0.19	1.92	0.90
B. circulans				
Treatment	50	172.81**	142.57**	120.24**
Error	74	0.52	0.30	0.24
B.coagulans				
Treatment	50	210.19**	188.27**	279.95**
Error	74	0.07	13.99	0.07
B.subtilis				
Treatment	50	172.51**	198.14**	74.15**
Error	74	0.66	0.27	2.35

** Significant at 1 per cent

CONTROL OF ROOT-KNOT NEMATODE (*Meloidogyne incognita* Kofoid and White, Chitwood) INFESTING BLACKPEPPER (*Piper nigrum* L.) BY BACTERIAL PATHOGENS

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ABSTRACT OF A THESIS
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ABSTRACT

A survey was carried out to study the occurrence and association of bacterial pathogens of the root-knot nematode Meloidogyne incognita infesting the black pepper (Piper nigrum L) covering ten major pepper growing districts namely Kannur, Eranakulam, Idukki, Kollam, Kottayam, Koshikode, Pathanamthitta, Thiruvanandapuram, Thrissur and Wynad.

The survey revealed that six species of bacteria were associated with the nematode namely, Bacillus subtilis, B. pumilus, B. coagulans, B. macerans, B. circulans and B. licheniformis. B. subtilis, B. pumilus and B. licheniformis were found associated with egg masses, second stage juvenile and adult females while B. coagulans, B. macerans and B. circulans were obtained from egg masses and adult females only.

These six Bacillus spp. showed ovicidal and larvicidal effect against M. incognita.

The pathogenic effect tested with the six Bacillus spp. on M. incognita on tomato as host plant showed that B. circulans (0.9×10^7 cells per pot) and B. macerans (0.8×10^7 cells per pot) compared with treatment of carbofuran 500 ppm were on par in reducing the larval stage of the nematode population in soil. However among the five species all were

equal except B. subtilis ($1.8x10^7$ cells per pot) and carbofuran treatment in reducing the root-knot population in roots.

Eight common host plants of root-knot nematode were tested for pathogenicity with the five Bacillus spp., B. subtilis ($1.5x10^6$ cells/ml), B. pumilus ($1.8x10^6$ cells/ml) B. coagulans ($1.2x10^6$ cells per ml), B. macerans ($0.8x10^6$ cells per ml) and B. circulans ($0.9x10^6$ cells per ml). None of these plants showed any symptom of pathogenesis, withering or disease.

Safety testing of these Bacillus spp. carried out by oral administration and subcutaneous injection, on two week old chicks, did not produce any bacterial infection indicating that itwas not injurious.

Among the different media tested for mass production and for the growth of these Bacillus spp., nutrient broth was observed to be the best medium for B. subtilis, potato-dextrose and nutrient broth media for B. pumilus, glucose yeast extract and potato dextrose for B. coagulans and B. circulans, potato dextrose, nutrient broth and glucose yeast extract media for B. macerans. However potato dextrose medium for mass production of all these Bacillus spp. except B. subtilis, considering the cost factor.

In the experiment to study the suppression of nematode infestation in root and soil, by the bacterial pathogen on pepper plants indicated significant pathogenic ability on the nematode. The plants which received the bacterial load of $1.1x10^8$ cells per pot of B. pumilus, $1.2x10^8$ cells per pot each of B. circulans and B. macerans reduced the nematode population in soil from third month onwards till the end period of the experiment. However in respect of suppression of root-knot count in roots, B. macerans ($1.2x10^8$ cells per pot) and B. circulans ($1.2x10^8$ cells per pot) were found effective.

Nematode population characteristics were studied by computing the nematostatic values of various treatments. B. circulans ($1.2x10^8$ cells/pot), B. pumilus ($1.1x10^8$ cells per pot) and B. macerans ($1.2x10^8$ cells per pot) treatment gave minimum value, concluding the maximum reduction in the reproductive potential (reproductive factor) of M. incognita.

In vitro sensitivity of the Bacillus spp. to nematicides revealed that among the common fumigant and non-fumigant insecticides and nematicides tested, carbofuran was the safest chemical which had no inhibitory effect on bacteria. Phorate, malathion and endosulfan were slightly inhibitory. Metham sodium, formaldehyde and aldicarb

showed high degree of growth inhibition on the bacteria and therefore cannot be used along with these bioagents.

The test on the *in vitro* sensitivity of *Bacillus* spp. to fungicides, showed that, systemic fungicide carbendazim and copper fungicide copper oxychloride up to 500 ppm, were safe to the five species, indicating that these fungicides were safe to use in combination with these bioagents in the integrated management of root-knot nematode.

The effect of these bacteria (*B. subtilis*, *B. pumilus*, *B. coagulans*, *B. macerans* and *B. circulans*) was studied on related genera like *Heterodera oryzicola* revealed that at 1.2×10^8 cells per ml of these bacteria caused 70 to 80 per cent larval mortality. In the case of *Rotylenchulus reniformis* a cell concentration of 1.2×10^8 cells per ml of *B. circulans*, *B. macerans*, *B. pumilus*, *B. coagulans* and *B. subtilis* showed maximum larval mortality (73 to 76 per cent).