

ALLELOCHEMICALS FOR THE MANAGEMENT OF *Meloidogyne incognita*

(Kofoed and White, 1919) Chitwood, 1949 ON TOMATO AND BRINJAL

Thesis submitted in part fulfillment of the requirements for the award of the Degree
of **DOCTOR OF PHILOSOPHY (Agriculture) in PLANT NEMATOLOGY**
to the Tamil Nadu Agricultural University, Coimbatore – 641 003.

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2001

CERTIFICATE

This is to certify that the thesis entitled "**ALLELOCHEMICALS FOR THE MANAGEMENT OF *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 ON TOMATO AND BRINJAL**" submitted in partial fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT NEMATOTOLOGY** to the Tamil Nadu Agricultural University, Coimbatore - 3 is a record of *bonafide* research work carried out by **Mr. I. CANNAYANE** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

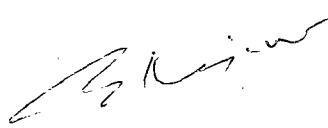
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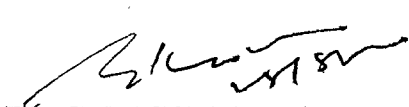
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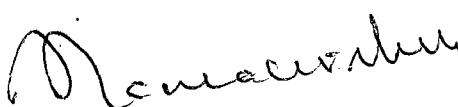
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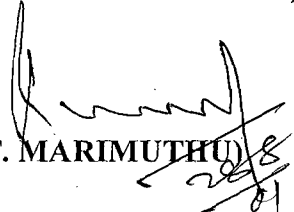
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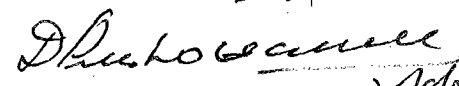
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Acknowledgement

ACKNOWLEDGEMENTS

I render my foremost thanks to **OUR ALMIGHTY** for his blessing in accomplishing the task successfully.

I feel privileged and elated to keep on record the deep sense of gratitude and heartfelt thanks to my beloved chairman who had been instrumental in this venture, **Dr. G. RAJENDRAN**, Professor and Head, Dept. of Nematology for the steadfast inspiration, meticulous care, surpassing guidance who conceived, detailed and shaped the problem by providing insights and indomitable lead with much care and affection throughout the study.

I would like to place on record my utmost reverence and indebtedness to the members of my advisory committee, **Dr. S. RAMAKRISHNAN**, Associate Professor of Nematology for his valuable suggestions, guidance, sustained encouragement, keen interest, friendly help and meticulous care throughout the study period and **Dr.T. MARIMUTHU**, Dean (PG.Studies), TNAU and **Dr.D.PURUSHOTHAMAN**, Professor of Microbiology for their suggestions, inspiring guidance during the study period.

I extend my heartfelt thanks to **Dr. M. SIVAKUMAR**, Associate Professor, **Dr. S. SUBRAMANIAN** and Tmt. **B. ANITA**, Asst. Professors for their meticulous guidance, constructive criticism, constant and unfathomable help during the course of my investigation. I express my profound sense of gratitude to **Dr (Tmt.) RAJESWARI SUNDARABABU**, Professor, **Tmt. K. POORNIMA** and **Tmt. B. SHANTHI**, Asst. Professors for their valuable advices at various stages of this study.

I express my special thanks to **Dr. P.L. LAKSHMANAN**, Prof. and Head (Rtd.) **Dr. S. KUMAR**, Associate Professor and **Dr. E.I. JONATHAN**, Professor, **Dr. S. SANKARANARAYANAN**, Scientist (SBI), **Dr. K. DEVARAJAN**, **Dr. S.B. NIRMAL JOHNSON**, Asst. Professors, **Mr. M.P. MANI**, SRF and **S. RAMAKRISHNAN**, Scientist (CTRI) for their valuable suggestions during the period of research.

I extend my heartfelt thanks to my classmates **J. GULSAR BANU** and **N. SEENIVASAN** and Senior and Juniors **N. SWARNA**, **J. JAYAKUMAR**, **P. KALAIARASAN**, **UMA**, **SENTHAMIZH**, and **SENTHIL**.

I feel immense pleasure in extending my heartfelt thanks to Nantha, Johnee, Senthil, Prabu, Vakesh, Velu, Raja, Babu, Anand, Senthil, Anbazhagan, Ragu, Rama, Subu, Raj, KP, Deva, Sudha, Djohny, Kandan, Sateesh, Ashok, Mari, Ramesh, Ravi, Segar, Raja and Anitha for their love, care, moral support and affection. Without them this could have been an another everest to conquer.

Words fail me to express my gratitude to my parents, beloved brother and sisters for their encouragement and devoted interest shown on me, which played a vital role in completing the study successfully.



(CANNAYANE. I)

Abstract

ABSTRACT

ALLELOCHEMICALS FOR THE MANAGEMENT OF *Meloidogyne incognita*

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By

I. CANNAYANE

Degree : Doctor of Philosophy (Agriculture)
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2001

The efficacy of different plant extracts were evaluated against root-knot nematode, *Meloidogyne incognita* (Race 3) under laboratory, glasshouse and microplot conditions.

Among the fifty plant extracts, *Annona squamosa* (leaf), *Tagetes erecta* (root) and *Bauhinia scandens* (leaf) were most effective in preventing hatching of eggs and causing juvenile mortality of *M. incognita*. Comparatively, these three plant extracts showed better performance with lower LC₅₀ and LT₅₀ values. Penetration of *M. incognita* juveniles into tomato and brinjal roots was reduced by the application of *A. squamosa*, *T. erecta* and *B. scandens* extracts and most significantly by *A. squamosa* extract.

Seed treatment with extracts of *A. squamosa*, *T. erecta* and *B. scandens* at 60 per cent concentration for 1 hr was found to influence the plant growth parameters of

tomato and brinjal positively. Maximum inhibition of *M. incognita* was observed in treatment with *A. squamosa*.

Combination of root dip, foliar spray and soil application of plant extracts (*A. squamosa*, *T. erecta* and *B. scandens*) proved to be the best in improving plant stand and to increase the yield of both tomato and brinjal. Among the three methods of its application, root-dip ranked first in its effectiveness. Combined application of all the three methods reduced root-knot index, number of females and egg masses/g root, number of eggs/egg mass and soil nematode population followed by root dip + foliar spray. In both the crop plants, *A. squamosa* proved to be the best among the three plant extracts in restricting the development of *M. incognita*.

The best performing plant extract, *A. squamosa* was also tested against nematode fungal complex involving *M. incognita* and *Fusarium oxysporum* f. sp. *lycopersici* in tomato and *M. incognita* and *Rhizoctonia solani* in brinjal. The maximum effect was achieved when the plant extract was applied by all the three methods of application.

Integrating the application of *A. squamosa* extract with *Paecilomyces lilacinus*, castor cake and carbofuran significantly reduced the development of *M. incognita* and increased the plant growth both in tomato and brinjal.

A. squamosa extracts when applied by all the three methods increased the total phenol, polyphenol oxidase, peroxidase, phenylalanine ammonia lyase and free aminoacids in tomato and brinjal.

The inhibitory principles against *M. incognita* found in *A. squamosa* leaf have been identified as terpenoids, phenols and saponins. A protein portion was isolated from *B. scandens* and *T. erecta*.

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Introduction

CHAPTER I

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) and brinjal (*Solanum melongena* L.) are the most important and popular vegetables. These crops enjoy wide distribution both in tropical and temperate countries. Tomato is grown in an area of 29.15 lakh ha throughout the world with a total production of 6.85 crore tonnes per annum (FAO, 1994). In Tamil Nadu, about 2.32 lakh tonnes of raw tomatoes are produced annually from 21,055 ha (Singhal, 1999). The world production of brinjal is about 8.98 lakh MT from 5.56 lakh ha (FAO, 1994). Brinjal occupies 6,879 ha in Tamil Nadu with a production of 64,374 tonnes (Director of statistics, 1991).

Though the area under vegetable crop is steadily increasing at the rate of around three per cent per annum, the productivity is quite low as compared to world levels. Both biotic and abiotic stresses are responsible for the low level of production.

Tomato and brinjal are being affected by a number of nematodes, fungi and insects. Sasser (1954) reported that the major nematode damaging these crops is the root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949. Yield loss due to this nematode ranges from 39.70 to 46.92 per cent in tomato (Bhatti and Jain, 1977; Reddy, 1985; Darekar and Mhase, 1988) and 32.73 to 80.00 per cent in brinjal (Darekar and Mhase, 1988; Saxena *et al.*, 1989).

Ogoshi (1975) and Bora (1977) reported about the association of fungus with *M. incognita* in tomato (*Fusarium oxysporum* f.sp.*lycopersici*) and brinjal (*Rhizoctonia solani*) which cause more damage to both the crops than either pathogen alone.

Thus it is imperative that the management of root-knot nematode disease of tomato and brinjal is of utmost importance for economic crop production. In the past, use of chemical pesticides increased rapidly where the production methods were intensified to increase the agricultural output and where different crops were introduced into new agroecological zones. The utility of synthetic chemical nematicides has become questionable at present owing to the reasons like exorbitant cost, non availability during time of need, health hazards involved in their use, residue problems especially in the freshly consumable crops like vegetables and pollution of environment. The above reasons have necessitated the choice for alternative measures to manage nematode pests. Other conventional methods of nematode management like cultural, physical and plant resistance have technical and operational limitations of one kind or the other.

The concept of allelopathy in modern agriculture has now attained wider applications than earlier. Its relevance in plant protection is all the more important since future pest management strategies will rely heavily upon non-chemical methods. Allelochemicals (Prohibitins) are plant chemicals (secondary metabolites) that may be beneficial or inhibitory to other organisms. In the latter case, the effect is called allelopathy. The term 'allelopathy' though not commonly used in nematological literature, the significance of its principles and practices in nematode control has been known since the birth of nematology. Being eco-friendly, it is considered as one of the feasible and practicable methods of nematode management.

The development of new, safer and effective agrochemicals as nematicides and growth promoters is important. It is obvious that synthetic chemicals are important in controlling plant parasitic nematodes, fungi and other insects and weeds, but in judging the

efficacy of a method, it is essential to assess its non target toxicity, including health and environmental hazards. The literatures abound with reports that synthetic chemicals affect non target organisms and the environment. Thus, plant protection needs more attention. It is important to note that many allelochemicals are known to inhibit the germination, growth and multiplication of weeds, fungi, insects including nematodes (Tattersfield and Potter, 1940; Rice, 1984; Bijloo, 1965; Barbosa *et al.*, 1999; Srivastava *et al.*, 2000; Ray *et al.*, 2000) which is an essential feature of all herbicides, fungicides, insecticides and nematicides, respectively. Therefore allelochemicals can be an alternative to synthetic chemicals because they are safer to use and easily biodegradable. The present investigations were taken up to exploit the utility of various plant extracts for the management of *M. incognita* in tomato and brinjal with the following objectives.

- i. Preliminary screening of fifty locally available plants to select the most effective ten plants for testing on hatching and mortality of *Meloidogyne incognita* and to estimate LC_{50} and LT_{50}
- ii. To study the effect of most effective three plant extracts on penetration and management of *M. incognita* and to study the best performing plant extract for its effectiveness in the management of nematode fungal disease complex in tomato and brinjal.
- iii. Integrated management of *M. incognita* in tomato and brinjal with plant extract, oilcake, bioagent and nematicide.
- iv. To identify and isolate the allelochemicals from the selected plants.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum* Mill.) and brinjal (*Solanum melongena* L.) are highly susceptible to the root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949. During the past two decades nematode control has undergone spectacular changes regarding concepts and management techniques, largely due to advances in the understanding of the nematode biology and innovations in pest management approaches. The picture of nematode control through chemical is gradually changing for obvious reasons. One of the promising alternative is the use of plants and plant products for nematode control.

In view of the above concepts a study was undertaken to manage the root-knot nematode, *M. incognita* on tomato and brinjal. A brief review on the effect of plant extracts on hatching, mortality and management of root-knot nematode through plant products by seed treatment, seedling root-dip and soil applications are furnished.

The effect of plant leaf extracts against plant parasitic nematodes have been studied by many workers and proved that most of the tested plant species has nematicidal and nematostatic properties (Vijayalakshmi *et al.*, 1979; Ramakrishnan *et al.*, 1995; Joymati *et al.*, 1998; Qamar *et al.*, 1998).

2.1. Effect of plant extracts against plant-parasitic nematodes *in vitro* bioassays

Mohammed *et al.* (1981) reported that, the extracts of leaves of *Delphinium ajacis* L, *Urtica urens* and *Eminium intortum*, flowers of *Papaver rhoeas* L, fruits of *Citrullus colocynthis* L. Kuntze and *Xanthium strumarium* L. and seeds of *Paganum harmala*, *Brassica arvensis* L, *Lepidium draba* L and *Cephaloria syriaca*, showed nematicidal

properties against *Tylenchulus semipenetrans* Cobb, 1913. Leaf extracts of *U. urens* and seed extracts of *C. syriaca* were the most effective.

Preliminary screening of some weed shrubs conducted by Nandal and Bhatti (1983) revealed that 14 plant extracts caused more than 50 per cent mortality of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 at 1 : 20 dilution.

Methanolic leaf extracts of *Ixora carnea*, *Tagetes patula* L., *Lawsonia alba* Lamk and *Datura stramonium* L. caused 75–100 per cent larval mortality of *Tylenchulus semipenetrans* Cobb, 1913 and *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935 at 1:5 dilution, in *in vitro* experiment conducted by Kumari *et al.* (1986).

Goswami and Vijayalakshmi (1986 b) studied the effect of *Argemone mexicana* L., *Datura metel* Sims, *Euclyptus globosus* Labil and *Phyllanthus niruri* Hook. and non edible cakes of *Calophyllum inophyllum* L, *Madhuca indica* JF.Gmel and *Shorea robusta* Gaerth. on the hatching of *M. incognita*.

Jain *et al.* (1986) studied the allelochemical effects of six leaf extracts and found that all the extracts reduced hatching of *M. incognita* with increased emergence at greater dilution. Eggs treated with *C. procera* leaf extract showed poor hatching when transferred to water after 24 hr indicating its nematicidal activity.

According to Nandal and Bhatti (1986), juvenile hatching of *M. javanica* was less after 3, 6 and 9 days exposure to leaf extracts of *C. procera*, *D. stramonium*, *Ricinus communis* and *Xanthium strumarium*.

Mojumder and Goswami (1987) reported that aqueous extract of *C. gigantea* and *Cuscuta reflexa* showed 100 per cent juvenile mortality of *M. incognita* and complete inhibition of hatching at higher concentrations (0.5 and 0.25 N).

Subramanian and Selvaraj (1988) tested *Tagetes patula* L. leaf extracts (1:1, 1:5, 1:20, 1:50 and 1:1000 dilutions) against *Radopholus similis* (Cobb, 1893) Thorne, 1949. All nematodes were killed or inactivated after four hr. in 1:1 and 1:5 dilutions and the same effect was seen after 48 hours in the 1:10 and 1:20 dilutions also. However, at 1:5 and 1:100 dilutions the nematicidal effect was found reduced.

Out of eleven plant extracts tested under laboratory conditions, leaf extracts of *Crotalaria procera*, *C. gigantea*, *Datura stramonium* and *Leucaena leucocephala* caused a high mortality of second stage juveniles of *M. incognita* at 500 and 1000 ppm concentrations (Mani and Chitra, 1989).

Pathak *et al.* (1989) observed that the aqueous leaf extracts of *Parthenium* sp., *Catharanthus roseus*, *Argemone mexicana* L., *D. stramonium* L and *Brassica campestris* L. significantly suppressed the egg hatch of *M. incognita* when compared with distilled water. *Parthenium* sp., was the most effective followed by *C. roseus*, *A. mexicana*, *D. stramonium* and *B. campestris*.

Ahmad *et al.* (1990) observed that standard concentrations of seven plant extracts including *C. procera* and *Ipomoea fistulosa* caused 100 per cent mortality within 12 hr. Ahmad and Karim (1991) evaluated ten different indigenous plants, and found that the extracts of *I. fistulosa* (leaf) and *Plumeria rubra* (leaf and bark) were more toxic to the juveniles of *M. javanica*.

Goswami and Vijayalakshmi (1990) observed that the larval hatch from egg masses of *M. incognita* soaked in aqueous extracts of *Cassia fistula* L, *Acacia arabica*, (Lamk.) Willd, *A. nilotica* (L.) Willd, *Eclipta alba* Hassk, *Swertia chirata* Buch-Ham, *D. metel* and *Argemone mexicana* exhibited inhibitory effect.

Subramanian and Vadivelu (1990) reported the inhibition in hatching and mortality of the juveniles of *M. incognita* as greatest in 1:5 and 1:10 diluted root extract of *C. spectabilis* Juss followed by 1:5 diluted leaf extract of the same. The respective percentage of juvenile mortality over control was 79, 63 and 48 while percentage inhibition of hatching was 86, 83 and 70 respectively.

More than 50 per cent population of the *M. incognita* was killed even at the lowest concentration of the leaf extracts (S/60) of *Azadirachta indica*, *C. procera* and *R. communis* after 24 hr, as observed by Khanna (1991). Leaf extracts of *A. indica* gave maximum (100 %) inhibition of egg hatching and larval mortality of *M. incognita* followed by *Melia azaderach* (Ahmad *et al.*, 1991).

Awan *et al.* (1992) obtained maximum larval mortality of *T. semipenetrans* with *A. indica* leaf extracts followed by *D. metel* and *C. procera*. With increased exposure time and concentration of extract, increased juvenile mortality was observed.

According to Haque *et al.* (1993), crude extracts of *Prosopis juliflora* L., *P. glandulosa* L. and *P. cineraria* L. caused mortality of *M. javanica* juveniles. Philip *et al.* (1993) observed that the leaf extracts of *C. gigantea*, *Carica papaya* and *Euphorbia synadenium* completely suppressed the emergence of *M. incognita* at S/10 concentration in laboratory studies.

Inhibition of juvenile emergence from the egg sacs of *Heterodera cajani* Koshy, (1967) was observed by Devi and Gupta (1995), when egg sacs were dipped in extracts of neem, mustard, mahua and linseed cakes for 24 and 48h.

Prasad *et al.* (1995) observed that concentrations of *C. gigantea* leaf extracts from 25 – 100 per cent inhibited hatching and gave 100 per cent juvenile mortality at all

exposure times ranged from 24 to 72 hrs. No egg hatching of *M. javanica* was observed by Jain and Gupta (1995) in leaf extracts of neem and eucalyptus at 1:5, 1:10 and 1:20 dilutions and less hatching at 1:40 dilution.

Among the leaf extracts of 11 plants, *T. erecta* and *Eucalyptus* were very effective in inhibiting the egg hatch of *M. incognita* and *M. javanica* and *M. arenaria* (Neal, 1889) Chitwood, 1949 and greater inhibition was observed in *C. procera* extract at 1:2 and 1:4 dilutions (Hussaini *et al.*, 1996).

Ethanol extracts of *Annona squamosa*, *Cocculus pendulus*, *D. fasthosa* and *S. surattense* produced 100 per cent mortality of *M. javanica* juveniles after 72 hr exposure (Abid *et al.*, 1997). Aqueous extracts of neem seed kernel and neem seed coat totally inhibited the hatching of *M. incognita* eggs at all concentrations tried (Reshmi and Mojumder, 1998).

Petroleum ether and ethyl alcohol extracts of *Mentha longifolia*, *Resmarines officinalis* (rose mary), *Origanum majorana* (sweet majorana) and *Thymus vulgaris* exhibited toxic effects on *M. incognita*, the per cent mortality was governed by extract concentration and exposure time (Hashim *et al.*, 1999).

Joymati *et al.* (1999 a) observed 85 per cent *M. incognita* juveniles mortality when the juveniles treated with 2/100 concentration of *Vitex trifolia*. Seed extract of *Momordica dioica* had the greatest nematocidal activity against *M. incognita* followed by leaf and stem extracts. Egg hatch was inversely proportional to the concentration and directly proportional to the exposure time (Joymati *et al.* 1999 b).

In vitro studies on hatching and mortality of juveniles of *M. incognita* indicated a reduction in hatching and an increase in juvenile mortality when treated with aqueous leaf

extracts of *Murraya koenigii*, *Jasminum sambac*, *Citrus aurantiifolia*, *Rauwolfia serpentina*, *Zizyphus jujuba*, *Hibiscus rosa-sinensis* and *Justicia gaudurosa* (Padhi and Behera, 2000).

2.2. Effect of plant extracts against penetration of plant-parasitic nematodes

Vijayalakshmi and Goswami (1987) reported that *M. incognita* juvenile penetration into tomato roots was inhibited to a greater extent when the egg masses were treated with extracts of *C. gigantea*.

Nandal and Bhatti (1987) tested the effect of the extracts of *C. procera*, *Ricinus communis*, *Xanthium strumarium* and *D. stramonium* and found that at higher doses (30 and 40g/kg soil), the juvenile penetration of *M. javanica* in brinjal roots was completely prevented upto six weeks. Even at 20 g/kg, juvenile penetration was prevented upto two weeks. Nandal and Bhatti (1990) again tested the effect of powdered form of leaves of the above four plants. The authors observed that all the four doses tested viz., 10, 20, 40 and 80 g/kg of river sand, reduced the juvenile penetration of *M. javanica* in brinjal roots from 97 to 100 per cent upto six weeks.

According to Reshmi and Mojumder (1998), root penetration of juveniles of *M. incognita* in chickpea was adversely affected by the aqueous extracts of neem seed kernel and seed coat treatment. Latif *et al.* (1999) observed that neem cake, neem oil and *C. procera* extracts effectively reduced root penetration of *M. incognita* juveniles in cowpea plants, and the cake is being more effective.

2.3. Effect of plant products on plant-parasitic nematodes by seed treatment.

Seed dressing with latex collected from *C. gigantea*, *C. procera*, *Euphorbia nerrifolia* and *E. milli* brought about significant reduction in the population of the reniform nematode, *Rotylenchulus reniformis*, with a corresponding increase in plant growth of some vegetables like tomato, brinjal and bhendi (Siddiqui and Alam, 1988 a and b). They also reported that seed dressing with latex of *C. gigantea*, *C. procera* and *Euphorbia* sp. significantly inhibited *Tylenchorhynchus brassicae* (Siddiqui, 1961) multiplication on cabbage and cauliflower and improved the plant growth.

Khan and Hussain (1988) tested the efficacy of neem leaf extract as seed treatment of cowpea and found that it not only controlled *M. incognita* and *R. reniformis* but also the disease complex involving these nematodes and the root rot fungus, *Rhizoctonia solani*.

Kathirvel *et al.* (1992) reported that seed treatment of okra with mahua oil and pinnai oil @ 3 per cent concentration effectively controlled the *M. incognita* invasion. In a trial conducted by Anver and Alam (1992), seed treatment with latex of *C. procera* resulted in significant control of *M. incognita* and *R. reniformis*, singly or concomitantly with corresponding increase in plant growth (length and weight of plants, number of pods, chlorophyll content of leaves, water absorption capacity of roots and root nodulation) of pigeonpea (*Cajanus cajan*) cv. Prabhat line and chickpea (*Cicer arietinum*) cv. K-850.

Seed soaking in aqueous extracts of aak (*C. procera*) and datura (*D. stramonium*) resulted in reduction in number of cysts of *Heterodera avenae* in wheat and increased plant height (Dalal *et al.*, 1995).

Sebastian and Gupta (1996) reported that chickpea seeds coated with latex extracted from *C. gigantea* and *Euphorbia pulcherrima* significantly reduced soil population of *Pratylenchus thornei* Sher and Allen, (1953).

2.4. Effect of plant products on plant parasitic nematodes by seedling bare root-dip treatment

Husain *et al.* (1984) observed that root-dip treatment of egg plant seedlings with margosa and marigold leaf extracts and mustard cake considerably reduced the development of *M. incognita* as compared to chenopodium oil and groundnut cake.

Root-dip of tomato seedlings with water extracts of oilcakes of *Arachis hypogaea*, *S. indicum*, *B. rapa* and *Madhuca india* significantly reduced the root knot nematode infestation and increased the plant growth. Among all treatments, extracts of *B. rapa* was the most effective (Vijayalakshmi and Goswami, 1987). Siddiqui and Alam (1988 c) found that many plant products are effective even at little quantity when used as bare root-dip treatments.

Bare root-dip treatment of tomato seedlings with undecomposed and decomposed extracts of castor leaves and cakes significantly reduced the root-knot development in pre-infected seedlings and in those which were inoculated with juveniles of *M. incognita* after root-dip treatment (Akhtar and Alam, 1990). Aubergine seedlings treated with extracts of *I. fistulosa* suppressed *M. javanica* population and enhanced plant growth, as reported by Ahmad and Karim (1990).

Akhtar *et al.* (1992) reported that seedling root-dip treatment with extracts of *Melia azedarachta* and *C. procera* gave significant reduction in root-knot development caused by

M. incognita in tomato and chilli, besides these plant extracts also improved the plant stand.

Bare root-dip treatment of tomato seedlings in aqueous leaf extracts of *R. communis* and *C. procera* @ 5 and 10 per cent increased the plant growth parameters and reduced the root-knot index and soil population of *M. incognita* (Rao *et al.*, 1999).

2.4. Effect of plant products on plant-parasitic nematodes by foliar application

Egunjobi and Onayemi (1981) tested the effect of neem leaf extract by foliar application on maize and found that it efficiently controlled *P. brachyurus* proving that the extract was systemic in function. In another trial conducted by Prasad *et al.* (1988), neem oil efficiently controlled *Pratylenchus indicus* Das, 1960 in upland rice, when given as foliar application.

Neem products *viz.*, neem bitter, repelin, wellgrow and neem seed kernel proved to be beneficial in decreasing the root-knot disease incidence by *M. incognita* and promoted the growth of tomato, with neem bitter indicating better action in different methods of treatments like seed treatment, bare root-dipping of seedlings and foliar spraying. (Dash and Padhi, 1998).

2.5. Effect of plant products against plant-parasitic nematodes by soil application

Goswami and Vijayalakshmi (1986 a) conducted pot culture trials for testing the nematicidal properties of *Andrographis paniculata*, *Calendula officinalis*, *Enhydra fluctuans* and *Solanum khasianum* against *M. incognita* in tomato and reported that all plant materials reduced galls and nematode population, of which *C. officinalis* and *E. fluctuans* being more effective.

Application of chopped and shade dried calotropis leaves @ 2.5 t/ha to the betelvine field heavily infested with plant parasitic nematodes resulted significant reduction in soil population and increased the yield of betelvine (Sivakumar and Marimuthu, 1986).

According to Kaliram and Gupta (1989), neem leaf extract applied @ 40 g/kg of soil showed improvement in plant growth and reduction in number of galls caused by *M. javanica* infecting cowpea. In wheat, the number of cockles caused by *A. tritici* was zero/plant, on treatment with *A. indica* leaf extracts in microplots (Qamar *et al.*, 1989).

Siddiqui and Alam (1989) found that soil amended with chopped plant parts of water hyacinth (*E. crassipes*) effectively controlled *M. incognita* and *R. reniformis* infecting tomato and egg plant.

Plant extracts of aak (*C. procera*) and naffati (*I. fistulosa*) applied to tomato nursery, increased the seedling height, fresh shoot weight and decreased the population of *M. javanica* and *M. incognita* by 25.5 per cent and 36.5 per cent respectively (Patel *et al.*, 1993).

Leaf extracts of *A. indica* and *Eucalyptus tereticornis* were found to be the most effective treatments in reducing the number of galls and egg masses of *M. javanica* and increased the growth of host plant, tomato (Vats and Nandal, 1993). According to Mukhtar *et al.* (1994), leaf extracts of *A. indica*, *M. azaderach* and *D. alba* enhanced the growth of tomato and reduced the disease severity by *M. incognita*, with *A. indica* extracts being the most effective.

Powdered and sun dried neem leaves at 5 per cent w/w were found suitable for the control of the nematode, *Aphelenchoides compositicola* Franklin, 1957 in mushrooms as reported by Khanna and Sharma (1994).

Abid *et al.* (1995) observed that the dry leaf powder, seed powder and seed cake of *A. indica* @ 2, 4 and 6g per pot size showed better plant growth and reduced the gall formation by *M. javanica* on okra as compared to control, with maximum effect in oil cakes. Highest plant growth and lowest number of galls of *M. javanica* occurred with 6g dried *A. indica* leaves/ kg soil in tomato cv.HS 101 and 10 and 15g leaves/ kg soil in aubergine cv .Br-112 (Walia and Gupta, 1995). Acid extract of neem cake at different dilutions enhanced the growth of cowpea and also decreased the population build up of *M. incognita*, as observed by Alagumalai *et al.*(1995).

It was reported by Singh *et al.* (1995) that a maximum increase in yield and decrease in cyst population of *Heterodera avenae* Wollenweber, 1924 infesting wheat was recorded by application of neem leaves @ 5, 10 and 20 g/kg soil followed by aak (*C. procera*) and datura (*D. stramonium*).

Alam *et al.* (1995) observed that treatment with extract of dhorkami (*I. fistulosa*) alone and in combination with carbofuran 3G gave effective control of *M. javanica* on aubergine and improved plant growth.

According to Ahmad *et al.*(1996), soil amendment with dried leaves of *C. procera* @ 25g/kg soil showed better results in reducing population of *M. javanica* on egg plant and controlled root- knot disease.

Soil amendment with leaf extracts of neem, calotropis and datura showed a significant increase in the growth of tomato plants which was found to be positively

correlated with the increasing concentration of extract and negatively correlated with the population of *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961 (Firoza and Maqbool, 1996).

Ramakrishnan *et al.* (1997 a) reported that among the various botanicals, *M. azadirach* (80 g/pot) recorded maximum shoot length, fresh shoot weight, pod yield of okra and highest reduction in root-knot index and nematode population of *M. incognita* followed by *A. indica* leaves.

It was reported by Jain and Trivedi (1997) that soil amendment with leaf powders at 1g, 2g and 4g per pot with *C. procera* showed a marked decline in number of root galls, eggs/egg mass and final nematode population indicating a decrease in severity of disease caused by *M. incognita* infecting chickpea.

Rao *et al.* (1997) observed that the combination of the use of 5 or 10 per cent aqueous extract of neem cake as seed treatment and soil drenching under field conditions was found as effective as the application of carbofuran at 2kg a.i./ha for the management of *M. incognita* on okra. According to Haque *et al.* (1997) soil amendment with *C. procera* leaves @ 0.5 and 1.0 per cent (w/w) significantly reduced the infection of *M. incognita* on mungbean (*Vigna radiata*) and *C. procera* leaf powder at low dosage (0.5% w/w) increased the root nodulation.

Amending *Tagetes* sp. leaves to soil significantly increased plant growth in tomato and brinjal and reduced the number of galls, egg masses and final soil nematode population of *M. incognita* (Walia and Gupta, 1997).

Ritzinger and McSorley (1998) reported that addition of fresh/dried shoots of *R. communis*, *B. oleracea*, *Sesamum indicum*, *Sorghum bicolor*, *Mucuna deeringiana* and

Zinnia elegans decreased the *M. arenaria* in okra. The best result was obtained when soil amended with 4 g of castor and *M. deeringiana* fresh or dried leaves.

Bougainvillia spectabilis, *T. patula*, *R. communis* and *Cannabis sativa* leaves were tested against *M. incognita* infesting tomato under greenhouse conditions (Khanna and Sharma, 1998). *T. patula* proved to be the best one among the plants in terms of reduction in nematode count as well as gall index, besides it promoted the growth of plants. *B. spectabilis* was found to be the least effective against nematodes.

Das (1999) reported that addition of chopped shoots of latex bearing plants, *C. papaya*, *Artocarpus heterophyllus*, *Ficus carica*, *F. elastica*, *F. glomerata*, *I. fistulosa* and *Nerium odorum* significantly reduced *M. incognita* and root-knot development and improved the growth of aubergine and tomato. Among the plants tested *F. elastica* was found to be the most effective in reducing nematode population both in soil and root.

Kotova *et al.* (1999) concluded that out of 26 plant products tested for the control of *M. incognita* infesting tomato, a preparation from the sap of *Nasturtium officinale* (water cress) and *Armoracia rusticana* (horse radish) reduced the nematode infestation by 60 per cent. Application of neem cake, neem oil and extracts of *C. procera* to cowpea plants enhanced plant growth variables, and decreased the number of galls per plant caused by *M. incognita* with the maximum effect recorded in neem cake (Latif *et al.*, 1999).

Incorporating a mixture of *Parkia biglobosa* seed extract and goat manure @ 30 g/4.5 kg soil, inhibited the growth and development of *M. incognita* under greenhouse. Combined application of above treatments also supplied nutrients to the plants and enhanced growth of tomato (Umar and Jada, 2000).

2.6. Effect of plant extracts on pathogenic fungi in tomato and brinjal

The plant derivative has been demonstrated to possess excellent fungicidal properties in the recent years. Several higher plants and their constituents have shown success in plant disease control (Appleton and Tansey, 1975; Fries, 1973; Misra and Dixit, 1976; Shekhawat and Prasad, 1971; Spencer *et al.*, 1957; Dixit *et al.*, 1983; Srivastava and Lal, 1997; Rajappan *et al.*, 1997; Singh, 1999).

Ashrafuzzaman *et al.* (1990) observed that the leaf extracts of mango, *A. squamosa* and *Citrus grandis* and lemon grass (*C. citratus*) oil completely inhibited the growth of *R. solani* *in vitro*. The mycelial growth and sclerotial germination of *Thanatephorus cucumeris* were effectively inhibited by the water extracts of *Allium sativum* and *Bougainvillea spectabilis* (Lakshmanan *et al.*, 1990). The leaf extracts of *Leucaena leucocephala* and *T. erecta* significantly inhibited the mycelial growth of *R. solani* *in vitro* (Miah *et al.*, 1990).

Shukla and Dwivedi (1990) found that *A. cepa* (bulb), *A. sativum* (bulb), *Embllica officinalis* (leaf), *Lawsonia alba* (leaf), *O. canum* (leaf), *Piper betle* (leaf) and *Rauwolfia serpentina* (root) had fungitoxic effect on *R. solani*. Tewari (1990) documented that aqueous and ethanol extracts of *Amorphophallus bulbifer*, *Michelia champaca* and *Zizyphus jumuba* were effective against *R. solani*.

Kumar and Tripathi (1991) found that leaf extract of *Eupatorium cannabinum* completely inhibited the mycelial growth of *R. solani* and *S. rolfsii* even at a minimum dilution of 1:1. The leaf extracts of *P. betle*, *O. sanctum*, *N. arbor-tristis* and *C. limon* were effective in reducing the growth of *R. solani* *in vitro* and extracts of *P. betle* and *O. sanctum* were effective in controlling the spread of the pathogen *in vivo* (Tewari and

Nayak, 1991). Crude ethanolic extract of *Polyalthia longifolia* (2.5 %) completely inhibited the radial growth of *R. solani* (Mishra and Tewari, 1992).

The leaf extracts of *Ipomoea cornea*, *P. longifolia*, *Pithacolibium dulce* and neem seed kernel extract were highly inhibitory to the mycelial growth, sclerotial production and sclerotial dry weight of *R. solani* (Senthil Kumar, 1993).

Ezhilan *et al.* (1994) reported that the cold water extracts of pinnai (*Calaphyllum inophyllum*) cake, *Prosopis juliflora* and *Thevetia peruviana* completely inhibited the sclerotial production at 10 per cent concentration. Uma (1994) observed that palmarosa oil (0.1%), neem oil (3%), bulb extract of *A. sativum* (10%) and 10 per cent leaf extract of *P. longifolia* totally inhibited the sclerotial germination of *R. solani* *in vitro*.

Ansari (1995) demonstrated that between the leaves or seed of ajwain (*Trachispermum ammi*), lemon grass (*Cymbopogon citratus*), tulsi (*Ocimum* sp.), mentha (*Mentha* sp.), *Rauwolfia* sp., lawsone (*Lawsonia inermis*) and samhalu (*Vitex trifolia*) tested, ajwain (seed) and tulsi (leaves) extracts exhibited fungicidal activity. The spray of ajwain and tulsi extracts at 1:20 dilutions reduced the disease incidence by 72.25 and 69.58 per cent respectively.

Sundararaj *et al.* (1996) reported that the cold water extracts of *A. sativum*, *P. juliflora*, *Gynandropsis pentaphylla*, *Leucos aspera* and *Vitex negundo* significantly inhibited the radial growth of *R. solani* *in vitro*. Water extract of *Agave americana*, *Cassia fistula* *Eucalyptus teriticornis* and *V. negundo* were most effective in reducing mycelial growth of *Fusarium oxysporium* f.sp. *zingiberi* (Sharma, 1998).

Root- exudates and extracts of garlic, onion and tomato cv. Castle-rock (resistant to fusarium wilt) were more effective in reducing the radial growth of *F. oxysporium* f.sp.

lycopersici and *F. oxysporium* f.sp *cepa* (Awad *et al.*, 1997). *In vitro* screening of the plant extracts, *A. cepa* and *A. sativum* against *R. solani* resulted 40.9 and 57.9 per cent inhibition in mycelial growth of fungus (Meena *et al.*, 2000).

2.7. *Paecilomyces lilacinus*, a bioagent for the management of nematodes

P. lilacinus was first observed in association with nematode eggs by Lysek (1976). The spores of *P. lilacinus* germinate and penetrate into the eggs within the cysts of *Glabodera pallida* and egg masses of *M. incognita acrita* in 10 to 12 days and eventually destroyed the embryo. The fungus penetrated mature females of root-knot nematodes through anus or vulva and *G.pallida* cysts through the vulva and exposed neck region (Jatala *et al.*, 1979).

Application of *P. lilacinus* to micro plots infested with *M. incognita* at a dosage of 4g increased the yield of soybean by 172 per cent and of winter vetch by 218 per cent and its suppressive action persisted in the subsequent crops also (Dube and Smart, 1987).

The biocontrol agent brought about 36 and 20 per cent gall inhibition, respectively, in tomato when treated with *P. lilacinus* at a dosage of 20 g and 10 g/plot (0.76 m-diam) using infested wheat grain. The greatest control of *M. incognita* was achieved when the fungus was applied at 10 days before or at planting of tomato (Cabanillas and Barker, 1989).

The spores of *P. lilacinus* mixed with 5 and 10 per cent neem cake extracts and applied to soil gave 58.2 and 61.9 per cent egg mass parasitization, respectively in egg-plants inoculated with *M. incognita*, which indicated that neemcake extract supported the sporulation of the fungus (Rao and Reddy, 1994).

Khan and Saxena (1996) reported that the *P. lilacinus* significantly reduced the root galling by *Meloidogyne* spp. which resulted in improvement of plant growth of tomato in microplot. *P. lilacinus* reduced *M. incognita* population in soil and root, parasitized the nematode eggs and reduced the root galling in tomato in a microplot experiment (Martez *et al.*, 1996).

Among the eight biocontrol agents tested, culture filtrates of *Verticillium chlamydosporium* and *P. lilacinus* at 100 per cent caused 77.5 and 74.5 per cent mortality of *M. incognita*, respectively (Sharma, 1999). At a dosage of 4 g/kg soil, *P. lilacinus* increased plant growth and yield of *Piper belle* and reduced the *M. incognita* infestation. The effect of fungus and carbofuran were comparable (Hazarika *et al.*, 2000).

M. incognita egg hatching was suppressed to the extent of 62.08 per cent by spore suspension of *P. lilacinus* after 18 days of inoculation (ShuHong and XueBiao, 2000).

2.8. Oil cakes for the management of nematodes

The oil seed cakes as organic amendments are known to reduce the nematode population in soil. The water soluble fractions of cakes as well as their mixture with soil are recorded to affect various nematodes. Many oil cakes like groundnut, castor and mahua have shown to decrease nematode population in treated soil (Singh and Sitaramaiah, 1966, 1970).

The oilseed cake of castor bean (*R. communis*) was effective in controlling *M. incognita* and inhibiting egg hatch, as it contained a specific protein 'Recin' which had adverse effect on the nematode larvae and eggs (Hackney and Dickerson, 1975).

In brinjal, oil cakes of castor, mustard, mahua and groundnut reduced the root-knot nematode population (Singh *et al.*, 1988) and cotton, linseed, mustard and sesamum cakes suppressed the root-knot development (Saifullah and Zulfiquir, 1990)

In an experiment with oil cakes, mustard and karanji significantly reduced the *M. incognita* penetration on tomato and both oilcakes also improved the growth of the plant (Goswami and Meshran, 1991).

Akhtar and Alam (1993) in their review reported that oil cakes, sawdust, sugarcane bagasse, farm yard manure and green manure effectively controlled the plant parasitic nematodes. Thakur and Darekar (1995) tested the effectiveness of oil seed cakes of castor, karanji and mustard against *M. incognita* in brinjal. Among the oil cakes karanji cake @ 44 g/plant resulted 44.9 and 47.5 per cent reduction in galling and soil population of *M. incognita*.

In a field trial, oil cakes of castor, groundnut, mustard and rocket salad were found to be highly effective against *M. incognita* and *R. reniformis* in chickpea than organic amendment as compost. All the oil cakes improved the growth of plant by reducing number of galls and soil population. The beneficial effect of all the above oil cakes persisted even in the subsequent crop of tomato (Anver and Alam, 2000).

2.9. Allelochemicals

Plants synthesize a variety of compounds and several of them have been implicated in allelopathy. The compounds involved in allelopathic interactions are called allelochemicals/toxins. These are secondary plant metabolites of relatively simple structure and generally are low or medium molecular weight substances. Those associated with allelopathic activity are mostly either volatile terpenoids or phenolic compounds

excreted from the leaf, stem, flower and root of plants to enter the environment. Other allelopathic agents include organic acids, lactones, naphthaquinones, anthraquinones, polyacetylenes, benzoic acid derivatives, coumarins, flavonoids, tannins, steroids, alkaloids, cyanohydrins etc., (Mathela, 1994).

The compounds isolated and identified as nematocidal principles from plants are chemically diverse ranging from simple straight chain acids, phenols, terpenoids, alkaloids, organosulphurous compounds and polyacetylenes etc., (Devakumar, 1994). The allelochemicals isolated from various plants effective against root-knot nematodes have been listed in Table 1.

2.9.1. Allelochemicals from *Annona* and *Tagetes*

2.9.1.1. *Annona* spp.

The family Annonaceae (custard-apple family) find place in the main of aromatic trees and shrubs of tropical origin. The chemistry of plants of the family Annonaceae has been little investigated. The leaves of *A. squamosa* have a heavy disagreeable odour and the seeds contain a highly acrid principle fatal to insects (Lindley, 1860).

Callan and Tutin (1911) isolated a steam-volatile essential oil, uncrystallizable alkaloid, fatty acids, myricyl alcohol, sitosterol and anonol (member of phytosterol glucosides). An alkaloid, anonaine was isolated from the bark of *A. reticulata* (Santos, 1930 and 1932) and *A. squamosa* (Reyes and Santos, 1931). Manske (1938) also isolated the alkaloid anolobine from the bark of closely allied genus *Asimina triloba*,

Barger and Sargent (1939) made studies on the alkaloids of *Artabotrys suavezens* (Annonaceae) and isolated three alkaloids viz., Artabotrine (0.19%), Suaveloline (0.0013%) and Artabotrinine (0.012%).

Table 1. Nematicidal compounds in plants

Sl.No.	Plant	Family	Active principle	Test nematode	Reference
1	<i>Acacia auriculiformis</i> Acunn.	Mimosoideae	Saponins, A, B; Acaciaside	<i>M. incognita</i>	Sinhababu <i>et al.</i> (1992)
2	<i>Asparagus officinalis</i> L.	Liliaceae	Asparagusic acid	<i>M. hapla</i>	Takagusi <i>et al.</i> (1975)
3	<i>Eragrostis curvula</i> Nees	Poaceae	Pyrocatechol	<i>Meloidogyne</i> sp.	Paxton (1977)
4	<i>Ocimum sanctum</i> L.	Lamiaceae	Eugenol	<i>M. incognita</i>	Chatterjee <i>et al.</i> (1982)
5	<i>Nicotiana tabacum</i> L.	Solanaceae	Nicotine	<i>M. incognita</i>	Davis and Rich (1987)
6	<i>Xanthium strumarium</i> L.	Asteraceae	Oleic acid, 4-dihydroxycinnamic acid	<i>M. incognita</i>	Ghosh and Sukul (1992)

2.9.1.2. *Tagetes* spp.

Marigold (*Tagetes* spp.) is a member of Asteraceae family rich in many secondary compounds. Uhlenbroek and Bijloo (1958 and 1959) reported the isolation and structure of a nematicidal compound occurring in tagetes roots as 5-(3-buten-1-ynyl) 2, 2'-bithienyl which were effective against *Globodera* Skarbilovich, 1959 and *Pratylenchus* Filipjev, 1936. In the next year the same authors have isolated α -terthienyl from tagetes roots and tested against *Ditylenchus* Filipjev, 1936 and *Anguina* Scooli, 1777.

Gupta and Bhandari (1974) studied chemical composition of *T. erecta* flowers and found to contain d-limonene (31.4%), Ocimene (18.0%), linalool (13.6%), α -d-phellandrene (3.5%), linalyl acetate (11.6%), tagetone (4.0%), n-nonylaldehyde (6.0%) and 1:8 cineol (11.9%). In addition, lutein acylated with fatty acids and a non-volatile chemical constituent was reported by Philip and Berry (1975).

Baslas and Singh (1980) extracted yellow essential oils by steam distillation from flowers of *T. erecta* and identified α -pinene, β -pinene, dipentene, menthol and geraniol along with d-limonene, tagetone, linalool, 1:8 cineole and linalyl acetate.

T. minuta was reported to possess acyclic, monocyclic and bicyclic monoterpenes, sesquiterpenes, flavonoids, thiophenes and aromatics (Rodriguez and Mabry, 1977) and are effective deterrents of nematodes (Grainge and Ahmed, 1988). Nematicidal compounds like thiophene and α -terthienyl obtained from the roots of *Tagetes* sp. showed toxic effect against *M. incognita* (Castro and Munoz, 1982)

The bioactive extracts of different plant parts of *Tagetes* sp exhibited nematicidal, fungicidal and insecticidal activities. The nematicidal activity of roots is attributed to thienyls. The biocidal components of the essential oils from flowers and leaves are

terpenoids. Piccaglia *et al.* (1998) have identified the occurrence of lutein and lutein ester contents in different types of *T. patula* and *T. erecta*.

Ray *et al.* (2000) isolated α -sesquiphellandrene, β -sesquiphellandrene, myristoleic acid and triecosane from flowers of *T. erecta* and also found that purified myristic acid caused 87.6 per cent juveniles mortality of *M. incognita* at a dosage of 1000 $\mu\text{g m}^{-1}$ after 24 hrs.

2.9.2. Allelochemicals from other plants

Several allelochemicals isolated from different plants were evaluated against nematodes. *Artemisia* spp. are reported to contain various types of essential oils and sesquiterpene lactone including santonin which possess nematicidal properties (Sukul, 1992; Nin *et al.*, 1995).

Barbosa *et al.* (1999) reported that triacylglycerols, β -sitosterol, L-3,4-dihydroxyphenylalanine, stigmasterol, daucosterol, allantoin from *Mucuna aterrima* @ 5 $\mu\text{g m}^{-1}$ caused 2.1 to 74.4 per cent mortality of *M. incognita*.

Purification of the methanol extract of *Knema hookeriana* (Sumatran rainforest plant) led to the identification of two phenolics 3-undecylphenol and 3-(8Z-trideceny)-phenol. These compounds were equally effective against *Bursaphelenchus xylophilus* (Alen *et al.*, 2000).

Koenimbine and koenidin from *Murraya koenigii* had high degree of nematicidal activity against *M. incognita* with LT_{50} value of 587.5 and 56.3 respectively (Srivastava *et al.*, 2000). They also identified two essential oils, β -caryophyllene (29.0 %) and β -gurjunene (21.0 %) and suggesting the antinemic activity of oils being highly polar

to penetrate the nematode cuticle. Latif *et al.* (2000) identified quassinoid and chapparinone and chapparinone showed high degree of larval mortality of *M. incognita* at 10000 ppm.

2.10. Biochemical changes

Biochemical alterations in plants infected by *M. incognita* was established by Tayal and Agarwal, (1982); Ganguly and Dasgupta, (1987); Ravichandra and Setty, (1989) and Sharma *et al.* (1990).

2.10.1. Phenols

Phenol metabolism and hypersensitivity in plants are resistant mechanisms against the invading pathogenic organisms. Johnson and Schaal (1957) reported higher concentration of chlorogenic acid and ortho-dihydroxy phenols in the periderm of tubers of scab resistant variety of potato.

It has been reported that polyphenols and polyphenol oxidase together in an oxidative process resulted in browning and products in browning reaction. Phenolics have been reported to accumulate in the roots of apple parasitized by *Pratylenchus penetrans* (Pitcher *et al.*, 1960). Ortho-dihydroxy phenols were reported to be more reactive on infestation and upon oxidation they yielded quinones which were more toxic to the invading organism (Farkas and Kirby, 1962).

Giebel (1970) reported that phenol accumulation and products of phenol oxidation are associated with cellular browning in solanaceous crops infected by *Heterodera rostochiensis*. Alam *et al.* (1980) reported that the plants treated with organic amendment, oilcakes of mahua, castor, neem, mustard and groundnut contained greater concentration of

phenol than the untreated ones. The increase in total free phenols was ranged between 12.96 to 151.85 per cent in tomato, 13.73 to 81.37 per cent in egg plant and 15.91 to 209.09 per cent in chilli.

Singh *et al.* (1983) observed that the tomato plants grown from seeds treated with oilcakes have higher amount of total free phenols, ortho-dihydroxy phenols compared to untreated plants. Amending the soil with neem cake or sawdust supplemented with fertilizers increased the phenolic content in tomato plants which helped the plants to reduce nematode infestation (Singh *et al.*, 1985).

In the study of nematicidal activity of some phenolic compounds against *M. incognita*, Mahajan *et al.* (1985) found that transcinnamic acid, pyrogallol, 2-OH naphthoic acid and ethyl gallate suppressed the egg hatch of *M. incognita*.

Mahmood and Siddiqui (1993) found that root-dip treatments of phenolic compounds *viz.*, catechol, hydroquinone, phloroglucinol, pyrogallol and orcinol for 30 and 60 min. in different concentrations reduced the multiplication of *Rotylenchulus reniformis* and improved the growth of tomato.

2.10.2. Enzymes

Studies on involvement of enzymes in host-pathogen relationship are important in infestation/infection by any plant pest and changes in plant as affected by nematodes (Benedic and Hildebrand, 1958; Diener, 1960; Hanks and Feldman, 1966; Owens and Specht, 1966).

The enzyme peroxidase play a vital role in alleviating free radical toxicity in plant tissues. Following the entry of nematode an increased peroxidase levels in infested plants suggested a greater *de nova* synthesis of peroxidase since nematode exudates do not

contain peroxidase (Glasiov *et al.*, 1967). It was also observed that the glucose present in sugarcane destroy the mRNA which in turn induced the activity of peroxidase.

Huang *et al.* (1971) reported that *M. incognita* infections of tomato resulted in increased peroxidase activity in the host tissue during the development of second stage larvae and adult females but not third and fourth larval stages. They also reported that *M. incognita* infections on tomato resulted in the appearance of peroxidase enzymes that were not detected in the absence of the nematode.

Increase in polyphenol oxidase after the entry of nematode may be attributed to a triggered phenol oxidation process induced by pathogen (Maraite, 1973). It was reported that one of the isoenzymes of the host was of pathogen origin.

Ganguly and Dasgupta (1980) purified IAA- oxidase from the nematode infested resistant variety (SL-120) which was more active than that of susceptible variety (Pusa ruby) of tomato. The IAA-oxidase isozymes were heat stable. Bajaj *et al.* (1985) reported that *M. incognita* induced an increase of peroxidase activity in resistant tomato cultivars but a significant decrease in susceptible cultivars.

Molinari (1991) detected isoperoxidases in resistant and susceptible tomato roots uninfected and infected by *M. arenaria*, but the peroxidase activities and cellular locations were induced differently in resistant and susceptible cultivars by nematodes.

The activity rates of the oxidative enzymes peroxidase and polyphenol oxidase showing significantly higher values in the resistant tomato genotypes of parental cultivars than in susceptible ones was also reported by Hassan *et al.* (1994).

Rajasekhar *et al.* (1997) observed the gradual decrease of super oxide dismutase in the resistant tomato (Mangla) than susceptible cultivar (Pusa ruby) consequent to

M. incognita penetration but there was corresponding increase in the production of superoxide free radicals (O_2) affecting the biology of the invading nematode.

Pankaj *et al.* (1998) observed the increased activity of Ascorbic Acid Oxidase (AAO) in roots of resistant tomato (SL 120, Hisar lalit and NR-7) than susceptible varieties of Pusa ruby and Pusa gaurav. The importance of acid phosphatase in resistance to *M. incognita* infesting tomato was purified by Ganguly *et al.* (2000). The activity of acid phosphatase increased significantly in resistant (Nemamukt) variety than susceptible variety (Pusa ruby) of tomato.

Many researches documented the changes in peroxidase, polyphenol oxidase, AAO and phenylalanine ammonia lyase activity in relation to nematode resistance in plants (Ganguly and Dasgupta, 1979 and 1988; Ganguly *et al.*, 1991; Sirohi and Dasgupta, 1993; Zacheo and Zacheo, 1988; Zacheo *et al.*, 1995).

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1. Maintenance of pure culture of root-knot nematode, *M. incognita*

The inoculum required for raising the pure culture was obtained from tomato (*Lycopersicon esculentum* Mill.) plants raised in the glasshouse in sterilized condition. Roots with conspicuous galls were selected, washed gently and thoroughly in water and examined for the presence of egg masses under microscope. Galls that showed the protruding bodies of the mature females covered with gelatinous matrix were dissected and the egg masses kept individually in the embryo cups, half filled with water. Perineal pattern of the female was prepared for confirmation (Taylor and Sasser, 1978) of the species as *M. incognita* (Race - 3). The egg masses collected were utilized for raising pure culture.

Three weeks old tomato seedlings (cv Co 2) were transplanted in five-kg capacity earthen pots, filled with steam sterilized pot mixture. Single egg masses were collected in fresh distilled water and the larvae emerged from each individual egg mass in suspension was utilized for inoculation to start pure culturing. The suspension was inoculated in the soil at the base of the tomato plants 15 days after transplanting. The pots were maintained at the glasshouse and regularly watered with tap water passed through 325-mesh sieve. From this stock culture, well-developed egg masses were carefully teased out and transferred to Petri dish containing adequate amount of distilled water and incubated under laboratory conditions. The larvae that hatched out were used for the experiments.

3.2. Estimation of nematode population in soil and root

Soil nematode population was estimated by processing the soil samples, using Cobb's wet sieving method (Cobb, 1918) and the nematodes recovered by modified Baermann funnel method (Schindler, 1961). Nematode population in roots was counted by staining the roots with acid fuchin-lactophenol (McBeth *et al.*, 1941). Quantity of soil and roots used for estimation of nematode population were specified under the respective experiments.

3.3. Gall index

Gall index (GI) were graded on 0-5 scale rating after Taylor and Sasser (1978).

GI (Galls/root system)	Score/Rating	Disease intensity
0	0	Disease free
≥ 2	1	Very mild
≥ 15	2	Mild
≥ 25	3	Moderate
≥ 75	4	Severe
≥ 100	5	Very severe

3.4. Preparation of plant extracts

The freshly collected plant materials (Table 2) were washed with tap water and then with changes with distilled water. These were ground in a pestle and mortar by adding 80 per cent ethanol @ one ml per g of sample (Narwal, 1996). The extract was expressed by squeezing through two layers of muslin cloth; subsequently through Whatman No.1 filter paper and finally passed through Seitz filter to eliminate bacterial

Table 2. Plants screened *in vitro* against *M. incognita* juveniles

Sl. No	Scientific name	Common/vernacular name	Family	Parts used
1	<i>Abrus precatorius</i> L.	Kundumani	Papilionaceae	Seeds
2	<i>Acacia coincinna</i> DC	Shikakai	Mimosoideae	Pod
3	<i>Adhatoda vesica</i> Nees	Aduthoda	Acanthaceae	Leaf
4	<i>Aegle marmelos</i> Corr.	Vilvum	Rutaceae	Leaf
5	<i>Allium cepa</i> (L.) Wild	Onion	Lilaceae	Bulb
6	<i>Annona squamosa</i> L.	Custard apple/Seetha pazham	Annonaceae	Leaf
7	<i>Bauhinia scandens</i> L.	Geranium tree	Leguminosae	Leaf
8	<i>Bougainvillea spectabilis</i> Wild	Bougainvillea	Nyctaginaceae	Leaf
9	<i>Bryophyllum</i> sp.	Ranakalli	Crassulaceae	Leaf
10	<i>Capcicum annuum</i> L.	Chilli	Solanaceae	Fruit
11	<i>Carica papaya</i> L.	Papaya	Caricaceae	Leaf
12	<i>Centella asiatica</i> L.	Vallarai	Umbelliferae	Leaf
13	<i>Coccinia indica</i> Wight & Am	Kovai	Cucurbitaceae	Fruit
14	<i>Curcuma domestica</i> Val.	Turmeric	Zingiberaceae	Rhizome
15	<i>Cuscuta reflexa</i> Roxb.	Dodder	Convolvulaceae	Plant
16	<i>Cynodon dactylon</i> (L.) Pers.	Hariyali	Poaceae	Leaf
17	<i>Datura metal</i> L.	Datura/Umathai	Solanaceae	Seeds
18	<i>Colocasia esculenta</i> Schott.	Eddoe	Araceae	Leaf
19	<i>Eclipta alba</i> Hassak.	Karisalanganni	Compositae	Leaf
20	<i>Eucalyptus globulus</i> Labill	Eucalyptus	Myrtaceae	Leaf

Conti..

21	<i>Ficus elastica</i> Roxb.	Rubber	Moraceae	Bark
22	<i>Euphorbia hirta</i> L.	Amman paccharisi	Euphorbiaceae	Leaf
23	<i>Ixora coccinea</i> L.	Idlipoo	Rubiaceae	Leaf
24	<i>Jatropha multifida</i> L.	Coral plant	Euphorbiaceae	Leaf
25	<i>Leucos aspera</i> Sparg.	Thumbai	Labiatae	Leaf
26	<i>Punica granatum</i> L.	Pomegranate	Punicaceae	Seeds
27	<i>Mentha viridis</i> L.	Pudina\Mint	Labiatae	Leaf
28	<i>Mimosa pudica</i> L.	Thottasuringi	Mimosoideae	Leaf
29	<i>Momordica charantia</i> L.	Bitter gourd	Cucurbitaceae	Seeds
30	<i>Murraya koenigii</i> Spr.	Curry leaf	Rutaceae	Leaf
31	<i>Nerium oleander</i> L.	Arali	Apocynaceae	Leaf
32	<i>Nicotiana tabacum</i> L.	Tobacco	Solanaceae	Leaf
33	<i>Ocimum sanctum</i> L.	Thulasi	Labiatae	Leaf
34	<i>Parthenium hysterophorus</i> L.	Parthenium	Asteraceae	Leaf
35	<i>Phyllanthus niruri</i> L.	Keezhanelli	Euphorbiaceae	Leaf
36	<i>Piper nigrum</i> L.	Pepper	Piperaceae	Seeds
37	<i>Plumaria alba</i> L.	Pagoda tree	Apocynaceae	Leaf
38	<i>Polyalthia longifolia</i> Benth & Hook, F.	Polyalthia/ Netilingam	Annonaceae	Leaf
39	<i>Pithecolobium dulce</i> L.	Kodukapuli	Mimosoideae	Seeds
40	<i>Ricinus communis</i> L.	Castor	Euphorbiaceae	Seeds

Conti...

41	<i>Solanum nigrum</i> L.	Manathakkali	Solanaceae	Leaf
42	<i>S. xanthocarpum</i> Sch. Swardle	Kandangathiri	Solanaceae	Leaf
43	<i>Tagetes erecta</i> L.	Marigold	Compositae	Root
44	<i>Tamarindus indica</i> L.	Puli/tamarind	Caesalpinoideae	Seeds
45	<i>Tecoma stans</i> L.	Tecoma/Thanga arali	Bignoniaceae	Leaf
46	<i>Thespesia populnea</i> Cav.	Poovarasu	Malvaceae	Leaf
47	<i>Thuja orientalis</i> L.	Thuja	Coniferae	Leaf
48	<i>Vitex negundo</i> L.	Nochi	Verbanaceae	Leaf
49	<i>Zingiber officinale</i> Rosc.	Ginger	Zingiberaceae	Rhizome
50	<i>Zizyphus jujuba</i> L.	Seema elanthai	Rhamnaceae	Leaf

contamination. This formed the standard plant extracts solution (100 %). The extract was further diluted to required concentration (20, 40, 60 and 80 %) by adding 80 per cent ethanol.

3.4.1. Effect of plant extracts on nematode mortality

To study the effect of plant extracts on larval mortality ten ml of the each standard extracts and a an equal amount of ethanol (80 %) as control were taken in 5 cm diameter sterilized Petri dishes, and the solvent was evaporated to near dryness. One hundred freshly hatched juveniles of *M. incognita* suspension in 10 ml water was added to each Petri dishes containing the residue. The contents were mixed thoroughly and the Petri dishes were covered with lid. Each treatment was replicated thrice. These Petri dishes containing juveniles of test nematode were kept in a BOD incubator at $27\pm 1^{\circ}$ C (Nandal and Bhatti, 1983). Observations on juvenile mortality were recorded after 48, 72 and 96 hr of exposure by counting the live and dead larvae under a stereoscopic binocular microscope (Prasad, 1990). The per cent mortality was calculated. The criteria for selection of nematicidal plants were the highest per cent larval mortality at minimum period of exposure time.

From the primary screening of fifty plant extracts, ten most effective plant extracts were selected based on the criteria fixed in the above screening. Different concentrations of the selected ten plant extracts viz., 20, 40, 60 and 80 per cent were prepared. The effect of different concentration of each extract on the mortality of *M. incognita* was determined as described earlier at three exposure timings of 12, 24 and 36 hr. The data gathered were

subjected to arc sine transformation and analyzed statistically by applying factorial completely randomized design.

3.4.2. Effect of plant extracts on hatching

Ten ml of the different concentrations (20, 40, 60 and 80 %) of the selected ten plant extracts were pipetted into 5 cm sterilized Petri dishes along with equal amount of ethanol as control and allowed to evaporate. Five healthy and uniform size egg masses were freshly dissected out from the heavily infested tomato roots with *M. incognita* and were transferred after surface sterilization with 0.1 per cent aqueous solution of streptomycin sulphate to avoid contamination to each dishes containing the residue with 10 ml of sterile water. Three replications were taken for each treatment and maintained at $26\pm 1^{\circ}\text{C}$ (Rao *et al.*, 1986). After 3, 6 and 9 days after exposure to the extracts the number of hatched larvae was counted under a stereoscopic binocular microscope. After each count, a new extract suspension was added to the Petri dishes. The data were subjected to square root transformation and analyzed statistically by adopting factorial completely randomized design.

3.5. Multiplication of the pathogenic fungi

The fungi, *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani* were obtained from the Department of Plant Pathology, TNAU, Coimbatore were sub cultured on Potato Dextrose Agar (PDA) medium (Ricker and Ricker, 1936) for 10 days at $29\pm 1^{\circ}\text{C}$. Mycelial discs were cut out (5 mm) from the advancing zones of the colony on PDA and were transferred to Potato Dextrose Broth (PDB) for mass multiplication (Ricker and

Ricker, 1936). Required quantity of mycelial mat was taken from the PDB, blended and used for inoculation.

3.5.1. Antifungal activity of plant extracts (*Annona squamosa*, *Bauhinia scandens* and *Tateges erecta*) - Poisoned food technique (Schmitz, 1930)

Three most effective plant extracts were selected from the fifty plants screened for further study. The standard plant extract solution (100 %) was prepared as already described. Calculated quantity of each extract was mixed with warm sterilized potato dextrose agar (PDA) medium after evaporating the extract and thoroughly mixed just before plating so as to get the specified concentration of the plant extracts (60 %). Twenty ml of the mixture was immediately plated into a sterilized Petri dish, gently swirled and allowed to solidify. A 10 mm diam culture disc of the pathogen cut from seven days old PDA culture by means of a sterilized cork borer was placed on center of the medium. All these were carried out under aseptic conditions. The plates were incubated at room temperature ($28 \pm 2^\circ \text{C}$) for 5 days. The PDA medium without incorporating plant extracts served as control. Five replications were maintained for each treatment. The radial growth of the mycelium was measured. The results were expressed as per cent growth inhibition over the control.

3.6. Culturing of *Paecilomyces lilacinus*

The fungus, *P. lilacinus* isolated by the Department of Nematology, TNAU, Coimbatore was sub cultured in test tubes on Potato Dextrose Agar (PDA) and incubated at room temperature ($28 \pm 1^\circ \text{C}$) for 10 days. Sorghum grains were washed and soaked in water for 12 hrs. These grains were then filled in half of 500 ml flasks and were

autoclaved for 30 minutes at 15 lb pressure. The fungus was then inoculated to the sorghum grains under a laminar air flow chamber. The flasks were tumbled daily to maintain uniform growth of fungus and to prevent the seeds from sticking together (Mani *et al.*, 1989). After 14 days, one gram of seed was taken washed in distilled water and the number of conidia present was counted in a haemocytometer. One gram of grain was found to contain 10^6 conidia.

3.7. Effect of plant extracts (*A. squamosa*, *B. scandens* and *T. erecta*) on penetration of *M. incognita* in tomato and brinjal

Twenty days old tomato (cv. CO 2) and brinjal (cv. CO 1) seedlings were planted @ two seedlings per pot of 250g capacity containing steam sterilized pot mixture after treating with plant extracts (40 and 60 %). Seedlings were thinned to one/pot five days after planting and then inoculated with *M. incognita* @ 1 J₂/g soil.

On 1, 2, 3, 4 and 5 days after inoculation, the plant root system was removed intact and stained with acid fuchion lactophenol and the number of nematodes entering into the roots was recorded and per cent parasitization was calculated. Each treatment was replicated four times and the data recorded were statistically analyzed after suitable transformations.

3.8. Effect of seed treatment with plant extracts (*A. squamosa*, *B. scandens* and *T. erecta*) against *M. incognita* in tomato and brinjal

Seeds treated with the plant extracts of *A. squamosa*, *B. scandens* and *T. erecta* for one hr. were sown in seedling pan and maintained in glasshouse to study the efficacy of plant extracts. Each plant extract was tested at 60 per cent concentration.

Tomato and brinjal seeds were treated with the residues of the above said plant extracts, after evaporating the 50 ml of the solvent and then the residue was dissolved in 50 ml of distilled water. To that, one drop of Teepol was added to enhance the stickiness of the substances and allowed to remain for an hr. After soaking, the seeds were allowed to dry and sown in seedling pan (30 cm-diam) containing soil infested with *M. incognita* @ 1 J₂ /g soil. An untreated control was also maintained. Each treatment was replicated five times. Observations on plant growth parameters, number of galls and final soil nematode population per 250 g soil, were made at the termination of experiments, 25 days after sowing.

3.9. Effect of seedling root-dip, foliar spray and soil application of plant extracts (*A. squamosa*, *B. scandens* and *T. erecta*) against *M. incognita* in tomato and brinjal

To study the effect of the plants extract (60 %) from *A. squamosa*, *B. scandens* and *T. erecta* in different methods of application and their combinations against root-knot nematode, *M. incognita* on tomato and brinjal, three pot culture experiments each on tomato and brinjal were carried out under glasshouse conditions.

Treatments

- T₁ : Seedling root-dip (SRD) for one hr
- T₂ : Foliar spray (FS) at planting
- T₃ : Soil application (SA) at planting
- T₄ : SRD + FS
- T₅ : SRD + SA
- T₆ : FS + SA
- T₇ : SRD + FS + SA
- T₈ : Untreated control

Tomato and brinjal seeds were surface sterilized with 0.1% mercuric chloride for 3 min and washed thrice with distilled water. The seeds were sown in a seedling pan containing steam sterilized soil. Twenty days old tomato and twenty five days old brinjal seedlings were given with the respective treatment separately for three plant extracts and transplanted in earthen pots containing 3 kg of steam sterilized pot mixture @ 2 seedlings per pot and subsequently thinned to one seedling/pot. Five days after transplanting *M. incognita* second stage juveniles (J₂) were inoculated at the rate of 1 J₂/g of soil. Each treatment was replicated three times in Randomized block design. The experimental data was statistically analyzed.

Observations

The following observations were recorded on 135 and 150 days after planting (DAP) of tomato and brinjal respectively.

- i. height of the shoot (cm)
- ii. length of the root (cm)
- iii. fresh weight of shoot and root (g)
- iv. gall index
- v. number of females/g root
- vi. number of egg masses/g root
- vii. number of eggs/egg mass
- viii. final soil nematode population (250 g soil)

3.10. Management of nematode fungal disease complex in tomato and brinjal with plant extract (*A. squamosa*).

To study the effect of best performing plant extract, *A. squamosa* against nematode-disease complex involving *M. incognita* with *Fusarium oxysporum* f.sp. *lycopersici* in tomato and *M. incognita* with *Rhizoctonia solani* in brinjal, three experiments were conducted under glasshouse conditions. The same eight treatments as mentioned in the above study of 3.9 were adopted here also and 2.0 g of *F. oxysporum* f.sp. *lycopersici* for tomato and 2.0 g of *R. solani* for brinjal as soil inoculation were made simultaneously at the time of nematode inoculation. An untreated control with nematode and fungus alone were also included in the treatment schedule for comparison. There were totally ten treatments replicated thrice were maintained till harvest. The observations were taken in the same way as described above.

3.11. Integrated management of *M. incognita* with plant extract, bioagent, oilcake and carbofuran in tomato and brinjal under microplot conditions

A microplot experiment was carried out to study the compatibility of the selected plant extract (*A. squamosa*) with bioagent (*P. lilacinus*) and oilcake (castor) and carbofuran against *M. incognita* in tomato

Tomato seedlings were transplanted in 1 m² microplot filled with 10 kg of field soil after sterilization. Five days after transplanting *M. incognita* juveniles were inoculated @ 1 J₂/g soil and the following treatments were given. Each treatment was replicated three times. Samples were taken periodically to assess the nematode population.

Treatments

T₁ - Seedling root-dip (1 hr) + foliar spray at 30 DAP with *A. squamosa* extract (60 %)

T₂ - *P. lilacinus* @ 10 g/m²

T₃ - Castor cake @ 10 g/m²

T₄ - T₁ + T₂

T₅ - T₁ + T₃

T₆ - T₁ + T₂ + T₃

T₇ - T₁ + carbofuran 3G @ 1.75 g/m²

T₈ - Carbofuran 3G @ 3.5 g/m²

T₉ - Untreated control

The plots were regularly irrigated and maintained till harvest. Soil and root samples were taken to assess the soil nematode population, number of egg masses/g root, per cent parasitized egg masses and colony forming units (cfu's) of the *P. lilacinus* at the time of harvest. The soil and root populations were assessed as described earlier. The data gathered were subjected to analysis of variance.

The same procedure was adopted to study the compatibility of *A. squamosa* extract with *P. lilacinus*, castor cake and carbofuran against *M. incognita* in brinjal under microplot (1 m²) conditions.

3.12. Histopathology of *M. incognita* infested tomato and brinjal roots treated with *A. squamosa* extract

The nematode infested tomato and brinjal roots at 40 days after inoculation were taken and washed free of soil particles under running water and cut into 1 cm bits and fixed in formalin acetic acid (FAA) followed by Tertiary Butyl Alcohol (TBA) dehydration

(Johanson, 1961). The root bits were then infiltrated with paraffin wax (58° C m.pt) and embedded in the wax of same melting point. Ribbons of 12 μ thickness were cut with a rotary microtome (Spencers, USA), stained in safranin and counter stained with fast green. Dewaxing was done with xylene and mounted in DPX mount. The details of giant cells were observed under a compound microscope.

3.13. Biochemical analysis

3.13.1. Preparation of ethanol extract

Fresh tomato and brinjal root samples (100 mg) were chopped into small bits and immersed in 10 ml of 80 per cent ethanol on boiling water bath for 10 min and later cooled in running tap water. The tissues were then crushed in a pestle and mortar and the macerate was squeezed through two layers of cheesecloth. The extract was collected in a beaker. The residue was again extracted in 80 per cent hot ethanol and squeezed through cheesecloth. Both the extracts were pooled together and this extract was centrifuged in 3000 rpm for five minutes and the supernatant was collected. This was allowed to evaporate completely. The residue was dissolved in 10 ml of distilled water and this solution was used for further analysis.

3.13.1.1. Estimation of free aminoacids

The estimation of amino acid was carried out by the method described by Spies, (1955). A quantity of 0.1 ml of ethanol extract (tomato and brinjal) was taken in a test tube and the volume was made up to one ml with distilled water. To this five ml acid ninhydrin reagent was added and kept in a boiling water bath for 12 minutes. Then it was cooled under running water to room temperature and the absorbance was recorded at

570 nm. The result was expressed as mg of free aminoacids/g of fresh tissue expressed in proline equivalent.

3.13.1.2. Estimation of total phenols

The ethanol extract (tomato and brinjal) was used to estimate the total phenols (Bray and Torphy, 1954). One ml of the extract was taken in two test tubes and the volume was made up to three ml with distilled water. Then one ml of 1M Folin-Ciocalteu reagent was added to each test tube followed by the addition of two ml of 20 per cent sodium carbonate solution. The tubes were kept in a boiling water bath for 15 min and then cooled and diluted to 10 ml with distilled water. This resulted in the development of deep blue colour, whose intensity was measured at 725 nm. From standard curve prepared (Catechol) the amount of phenol was estimated. The phenol content was expressed as ciocalteu equivalents in mg per g weight of fresh tissue.

3.13.2. Assay of enzymes in tomato and brinjal

For assaying the induced proteins, the tomato and brinjal root samples were collected from the plants (grown in glasshouse) which were treated with *A. squamosa* extract.

3.13.2.1. Enzyme extraction

The tomato and brinjal root tissues were obtained from *A. squamosa* treated tomato and brinjal plants and immediately homogenized with liquid nitrogen. One g of powdered sample was extracted with 2 ml of 0.1 M sodium citrate buffer (pH 5.0) at 4 °C. The homogenate was centrifuged for 20 min at 10000 rpm. The supernatant used as crude

enzyme extract for assaying. Phosphate buffer 0.1 M (pH 7.0) was used for the extraction (Sadasivam and Manickam, 1991).

3.13.2.2. Assay of Peroxidase (PO)

Both the tomato and brinjal homogenate was centrifuged at 15,000 rpm for 10 min at 4 °C and the supernatant was immediately used for enzyme assay. Peroxidase activity was assayed using guaiacol as a hydrogen donor as previously reported by Rathmell and Sequiera, (1974). Exactly, 1.5 ml of phosphate buffer, 1.5 ml of 0.05M pyrogallol, 100µl of enzyme source were taken as a reaction mixture. Finally, 100 µl of hydrogen peroxide was added and the change in absorbance was read at 470 nm. Peroxidase activity was expressed as change in absorbance/min/g of fresh tissue.

3.13.2.3. Assay of Phenylalanine ammonia lyase (PAL)

The PAL assay was carried out as per the method described by Brueske (1980). The assay mixture (tomato and brinjal) containing 300µl of enzyme, 1.2 ml of 0.1M borate buffer (pH 8.8) and 1.5 ml of 12 mM L-phenylalanine in a test tube and incubated for 30 min. The reaction was arrested by adding 0.5 ml of 1M trichloroacetic acid and incubated at 37°C for 5 min. The blank contains 0.3 ml of crude enzyme extract and 2.7 ml of 0.1 M borate buffer (pH 8.8) and measured the absorbance at 290 nm and used an extinction coefficient of $9630\text{m}^{-1}\text{cm}^{-1}$ for trans cinnamic acid in 0.1 M borate buffer (pH 8.8). The absorbance 9630 is equal to 1 mol/l/min or the absorbance is 0.963, the product formed is 100 nmol/ml/min. The enzyme activity was expressed as µmol of cinnamic acid/min/g fresh tissue.

3.13.2.4. Assay of Polyphenol oxidase (PPO)

The PPO activity was determined as per the procedure given by Mayer *et al.* (1965). The reaction mixture consisted of 1.5 ml of 0.1 M phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction 200 µl of 0.01 M catechol was added and the activity was expressed as the changes in absorbance at 495 nm min⁻¹g⁻¹ fresh tissue. For all biochemical assays, the samples were collected at 0, 1, 2, 4, 6 and 8 days after the *M. incognita* inoculation.

3.14. Isolation and identification of allelochemicals in *A. squamosa*, *B. scandens* and *T. erecta*

3.14.1. Separation of the protein and non-protein part

The best performing plant extracts among the fifty test plants were *A. squamosa* (leaf), *B. scandens* (leaf) and *T. erecta* (root). Hence, they were tested to find out whether the protein or the non-protein part helped in the inhibition of hatching and juvenile mortality.

Weighed quantity of 40 g fresh samples was ground in 100 ml distilled water with pestle and mortar. The water extract was filtered through a four ply muslin cloth in order to remove the plant debris and then centrifuged for 5 minutes at 4000 rpm kept at 4° C . The supernatant solution was separated and to that 52.3 g of ammonium sulphate was added to enhance the precipitation of proteins. It was kept overnight in a refrigerator and then centrifuged at 10,000 XG at 4° C for 20 min. The supernatant was the non-protein part and it was transferred to separate flasks. The sediments settled at the bottom were the protein part. This was dissolved in equal quantity of sterile distilled water and transferred to

dialysis tubes and tied up tightly. Dialysis was done to remove the ammonium sulphate from the sample. The dialysis tube was kept in a beaker filled with water which was frequently changed for every 6 hrs. for one day, to enhance faster dialysis and stored in a refrigerator. From the protein and non-protein parts, 1ml each was taken for hatching study to check which part is inhibitory in nature.

3.14.1.1. Hatching and juvenile mortality

From the protein part, 1ml was pipetted out into five Petri dishes and 9ml of distilled water was added to it. One egg mass or 100 J₂ of *M. incognita* was transferred into each Petri dish. Likewise, from the non-protein part, 1ml was added with 9 ml of distilled water and an egg mass or 100 J₂ of *M. incognita* was kept in each Petri dish. They were maintained for 5 days at 26±1° C and 24 hr at 27±1° C to observe the effect of protein or non-protein part on hatching and juvenile mortality respectively.

3.14.2. Isolation and identification of allelochemicals in *B. scandens* and *T. erecta*

3.14.2.1. Sample Preparation for SDS-PAGE

As the protein part was inhibitory to nematodes in *B. scandens* and *T. erecta* the protein profile was estimated by SDS-PAGE method.

The samples (1 g) were weighed and it was ground well in 3 ml 0.1 M phosphate buffer (pH 7.0). Grinding was done on an ice tray and then it was transferred to eppendorf tubes. They were centrifuged in a microfuge at 10,000 XG for 15-20 min. The supernatant that had the protein part was taken in separate eppendorf tubes, labeled and stored at -20° C. The protein content of it was estimated by Bradford's method.

3.14.2.2. Protein estimation by Bradford's method (Bradford, 1976)

3.14.2.3. Preparation of Bradford dye

Coomasie brilliant blue G 250 weighing 100mg was dissolved in 50ml of ethanol (90 %) and 100ml of orthophosphoric acid. The volume was made upto 200ml with sterile water and stored in a brown bottle at 4⁰ C.

3.14.2.4. Standard preparation with Bovine Serum Albumin (BSA)

Bovine serum albumin 100 mg was dissolved in 100ml water (1mg / ml). From this, 10ml was taken and diluted to 100ml with distilled water. This gave 10mg/100 ml, which was equal to 0.1mg/ml. From this solution, 0.1, 0.2, 0.3, 0.4, 0.5ml were taken and was made up to 0.5 ml with distilled water. To each concentration 2.5ml dye was added. Distilled water was taken for about 0.5 ml and added with the dye, which served as the blank. Samples were also taken to about 0.5 ml and added with the dye. Then the protein content was estimated in a spectrophotometer at 595nm. With the standard values, a standard curve was drawn. The sample value was plotted and the concentration of the protein calculated.

3.14.2.5. Poly acrylamide–sodium dodecyl sulphate slab gel electrophoresis (SDS – PAGE) of proteins (Laemmli, 1979)

Separating gel buffer

1.875M Tris – Hcl	22.7 g
Distilled water	100 ml

Stacking gel buffer

0.6M Tris.HCl	7.26 g
Distilled water	100 ml

Polymerizing agents:

- i) Ammonium per sulphate (APS) 10% = 1.0 g / 10 ml was prepared freshly everytime before use.
- ii) TEMED – Tetra Methyl Ethyl Diamine – used fresh from refrigerator

Stock acrylamide solution

About 30 g of acrylamide and 0.8 g bisacrylamide was added to a total volume of 100ml of distilled water and stored at 4⁰ C in dark. It was kept for not more than 30 days as acrylamide gradually hydrolyses to acrylic acid and ammonia.

Electrode Buffer

0.05M Tris	12g
0.192M Glycine	28.8g
0.1% SDS	2g
Distilled water	2 l

Sample buffer

Tris Hcl buffer pH 6.8	5ml
SDS	0.5 g
Sucrose	5 g
Mercapto ethanol	0.25 ml
Bromophenol blue (0.5% w/v solution in water)	1ml
Distilled water	10 ml.

Sodium dodecyl sulphate (10%)

This solution was stored at room temperature after preparation.

Protein staining solution

Coomassie brilliant blue	100 mg
Methanol	40 ml
Acetic acid	10 ml
Distilled water	50 ml

The dye was dissolved in methanol and it was prepared fresh every time before use.

Destaining solution

Methanol	40 ml
Acetic acid	10 ml
Distilled water	50 ml

Standard marker protein used as such given by Bangalore Genei (Medium range)

3.14.2.6. Methodology

The glass plates and the spacers were thoroughly cleaned and assembled properly by holding them with bull dog clips. It was clamped in an upright position. Agar 2 per cent was melted in a boiling water bath and then applied around the edges of the spacers to hold them in place and sealed.

Separating gel mixture was prepared for a chamber of 18 x 9 x 0.1cm holding 30 ml of separating gel by mixing the following chemicals.

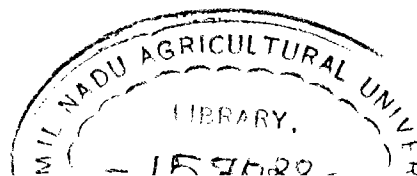
Reagents	Volume taken for separating gel (12 %)
Acrylamide	3.0 ml
Distilled water	2.5 ml
Tris (8.8)	1.875 ml
SDS (10 %)	75 μ l
APS (10 %)	25 μ l
TEMED	5 μ l
Total	7.5 ml

After preparation, the above mixture was carefully poured between the glass plates. Distilled water was layered on the top of the gel and left undisturbed for 30 – 60 min for polymerization.

Stacking gel mixture was then prepared as follows.

Reagents	Volume taken for stacking gel (4%)
Acrylamide	0.65 ml
Distilled water	3.25 ml
Tris (6.8)	1.25 ml
SDS (10.1)	50 μ l
APS (10%)	25 μ l
TEMED	5 μ l
Total	5.0 ml

The water from the top of separating gel is removed and the stacking gel mixture was poured into the chamber. Then a comb was inserted from the top for creation of wells and the set up was kept undisturbed for 30-60 min. After the polymerization of the



stacking gel, the comb was removed without disturbing the shape of the wells. The positions of the wells were marked with a marker pen to avoid confusion about the location of the wells. Then the gel was installed carefully in the electrophoresis apparatus and clipped properly. Electrode buffer was poured and the air bubbles trapped at the bottom of the gel were removed.

Samples were made ready by that time by following suitable extraction procedures. One g of *B. scandens* (leaf) and *T. erecta* (root) were weighed and ground well in a mixture of 1.5ml of potassium di-hydrogen phosphate buffer (pH 7.0) and 0.1g of PVP on an ice tray. It was then transferred to eppendorf tubes and centrifuged in a microcentrifuge at 10,000 XG for 20 min at 4⁰ C. The supernatant solutions were taken and the protein concentration in sample was adjusted using the sample buffer in such a way that the same amount of protein was present / unit volume.

The sample solution was heated in boiling water for 2-3min to ensure complete interaction between protein and SDS. The sample solution was carefully injected into the sample wells, seeing the position of the wells. The presence of bromophenol blue in the sample buffer facilitated easy loading of the sample. Similarly standard marker was also loaded. The connections of the apparatus were given and the knob was turned to 10-15mA for the initial 10-15 min for the sample to travel through the stacking gel. The run was then continued at 30 mA until the bromophenol blue that reached the bottom of the gel, for about 3 hr.

After the run was complete, the gel was carefully removed between the plates and was immersed in the staining solution overnight with uniform shaking. The protein part absorbed the Coomassie brilliant blue.

The gel was then transferred to a suitable container with at least 200 – 300 ml of the destaining solution. It was kept in a shaker for uniform shaking. For every half an hr, the destainer solution was changed to faster destaining. The dye that was not bound to the proteins was removed. Destaining process was stopped when the bands were clearly seen with the colourless background of the gel. The gel was photographed. The Rf values were measured. A standard graph was plotted with Rf values of marker protein and from it, the molecular weight of the samples were calculated.

3.14.3. Isolation and identification of allelochemicals in *A. squamosa* using Thin Layer Chromatography (Srivastava and Srivastava, 1991)

The non-protein part of *A. squamosa* (leaf) extract was found to have nematocidal properties. Thin Layer Chromatography (TLC) was suggested to isolate and identify the allelochemicals present in the sample.

Fresh plant material weighing 5 g was cut into small pieces and ground well after drying in oven at 69° C for 48 hours. This was tied in a filter paper and kept in the soxhlet apparatus. Extract was done with benzene, and the crude extract was collected and the residue was again used for ethanol and then for ethyl acetate and finally the residue were extracted with petroleum ether. In each case the crude solvent extract was collected and used for further study.

3.14.3.1. Preparation of TLC plates

Glass plates of 20 x 20 cm were cleaned with detergent and rinsed well with water. After drying, it was wiped well with tissue paper, the glass plates were then soaked in benzene. Ten TLC plates were prepared in this way. Silica gel (80 g) was weighed and

transferred to a wide mouthed conical flask and 175 ml of water was added to it and shaken thoroughly for 30 seconds. This slurry was applied on the glass plate uniformly with the help of a spreader with which the thickness of the layer was adjusted to 0.25mm. The plates coated with silica gel slurry was dried in air at room temperature and then oven dried at 100° C for 2 hr for activation.

The sample was spotted on the test plate with the help of a micropipette, without disturbing the silica gel layer. The sample applied at the bottom of the plate was marked with a pin.

The solvent mixture (225 ml) containing methanol and chloroform (1:9); acetic acid and chloroform (1:9) was taken in a separate tank. Then the TLC plates spotted with samples and placed inside the tank and tightly covered with the lid. When the solvent front reached the top of the plate, the plates were removed and air dried and sprayed with the required spray reagent and the R_f values were calculated. The compounds present in the samples were then identified.

3.14.3.2. Screening for different compounds

TLC plates were placed in Iodine tank and left for 15-20 min. this visualized most of the compounds present on the plates (Horborne, 1976).

3.14.3.2.1. Terpenoids

TLC plates were sprayed with vanillin-H₂SO₄ reagent and the terpenoids were apparently visible after heating to 110° C for 5 min. Presence of red, purple, blue, green and grey spots indicated the presence of unsaturated terpenoids.

Vanillin-H₂SO₄ reagent - This reagent was prepared by using 10 per cent vanillin in concentrated H₂SO₄. A mixture of concentrated H₂SO₄ and ethanol was prepared in the

ratio of 2:1 (v/v). About 10 gm of vanillin powder was added to 100 ml of the above solution. This was prepared fresh every time before use.

3.14.3.2.2. Phenols

TLC plates were sprayed with Diazotized sulphanilic acid reagent. Development of pink and purple colour indicates the presence of phenolics.

Diazotized sulphanilic acid reagent – This was prepared by dissolving 25 g sulphanilic acid in 125 ml of 10 per cent sodium nitrite. This mixture was added drop by drop to 60 ml of 8 M HCl in ice cold condition. After 10 min. filtered and washed with cold water and then with ethanol, followed by ether.

3.14.3.2.3. Saponins

A small amount of all the four crude extracts were poured in test tubes and to it 2 ml of water was added. The tubes were shaken vigorously and the formation of persistent foam was observed. This indicated the presence of saponins. A drop of methyl alcohol was added further to enhance the formation of foam.

3.14.3.3. Preparative TLC

Most of the compounds are visualized in the ethanol extract, compared to other solvents and reacted with vanillin H_2SO_4 reagent, indicating the presence of terpenoids in *A. squamosa* leaf. TLC plates were spotted only with ethanol extract of the sample for the second time to confirm the previous result. After the solvent system reached the top of the plates, three bands were sprayed with the respective spray reagent covering the rest of the bands with a thick plastic sheet. After development of the compounds their corresponding places in the other bands were scrapped off. This scrapped off material was dissolved in 9 ml ethanol and used for mortality study as described earlier to confirm their effectiveness.

3.15. Statistical analysis

The data obtained from various experiments were analyzed statistically adopting the procedure described by Finney (1962) and Panse and Sukhatme (1967).

Experimental Results

CHAPTER IV

EXPERIMENTAL RESULTS

4.1. Preliminary screening of plant extracts against *M. incognita* juveniles, *in vitro*

Fifty plant extracts (100 %) belonging to 34 families screened against *M. incognita* juveniles under *in vitro* conditions revealed the possession of higher level of nematocidal property in almost all plant species (Table 3). The larval mortality was directly correlated with the exposure period of the test plant extracts. Among the fifty plant extracts tried, 100 per cent mortality was observed in *A. squamosa*, *B. scandens*, *C. papaya*, *T. erecta*, *T. indica* and *V. negundo* within 48 hr of exposure to the extracts. More than 95 per cent of juveniles were dead in *A. precatorius*, *E. globulus*, *P. niruri* and *T. orientalis* extracts in the same exposure period of 48 hr and it was increased to 100 per cent after 72 hr of exposure. The rest of the plant extracts showed more than 80 per cent mortality except *M. charantia*, *F. elastica*, *Z. jujuba*, *Z. officinale*, *J. multifida*, *P. nigrum*, *I. coccinea* and *P. alba* with 60 to 79 per cent mortality at the lowest period of exposure (48 hr). All the test plant extracts caused more than 80 and 90 per cent mortality on *M. incognita* juveniles when exposed to 72 and 96 hr of exposure period, respectively (Table 3).

4.2. Effect of selected ten plant extracts on hatching of *M. incognita* eggs.

From the preliminary screening of different plant extracts, ten most effective plant extracts were tested for their effect on hatching of *M. incognita* eggs at different concentrations (20, 40, 60 and 80 %) and at three intervals (3, 6 and 9 days) under *in vitro* conditions (Table 4). The study markedly revealed a linear relationship between the concentration of the extract and the number of eggs hatched.

Table 3. Preliminary screening of plant extracts against *M. incognita* under *in vitro* conditions

Sl.No	Plant species	Per cent juvenile mortality after exposure for (hr)		
		48	72	96
1	<i>A. precatorius</i>	98.00	100.00	100.00
2	<i>A. coincinna</i>	80.67	96.00	100.00
3	<i>A. vesica</i>	93.00	98.33	100.00
4	<i>A. marmelos</i>	94.33	100.00	100.00
5	<i>A. cepa</i>	90.00	100.00	100.00
6	<i>A. squamosa</i>	100.00	100.00	100.00
7	<i>B. scandens</i>	100.00	100.00	100.00
8	<i>B. spectabilis</i>	86.00	95.00	99.67
9	<i>Bryophyllum sp.</i>	91.00	100.00	100.00
10	<i>C. annuum</i>	91.33	100.00	100.00
11	<i>C. papaya</i>	100.00	100.00	100.00
12	<i>C. asiatica</i>	86.67	95.33	100.00
13	<i>C. indica</i>	88.33	94.00	100.00
14	<i>C. domestica</i>	92.00	100.00	100.00
15	<i>C. reflexa</i>	85.33	91.67	100.00
16	<i>C. dactylon</i>	93.67	100.00	100.00
17	<i>D. metal</i>	90.33	98.33	100.00
18	<i>Dioscoria sp.</i>	88.67	93.33	98.33
19	<i>E. alba</i>	80.00	90.00	100.00

20	<i>E. globulus</i>	99.00	100.00	100.00
21	<i>E. hirta</i>	90.00	100.00	100.00
22	<i>F. elastica</i>	78.67	85.00	93.33
23	<i>I. coccinea</i>	62.00	82.33	90.00
24	<i>J. multifida</i>	75.00	89.67	96.33
25	<i>L. aspera</i>	96.00	100.00	100.00
26	<i>M. viridis</i>	90.67	100.00	100.00
27	<i>M. pudica</i>	86.00	91.33	96.67
28	<i>M. charantia</i>	79.00	87.00	94.33
29	<i>M. koenigii</i>	90.00	100.00	100.00
30	<i>N. oleander</i>	94.67	100.00	100.00
31	<i>N. tabacum</i>	80.00	92.00	85.00
32	<i>O. sanctum</i>	94.00	100.00	100.00
33	<i>P. hysterophorus</i>	90.00	100.00	100.00
34	<i>P. niruri</i>	97.67	100.00	100.00
35	<i>P. nigrum</i>	74.33	88.67	90.33
36	<i>P. dulce</i>	86.67	90.33	97.67
37	<i>P. alba</i>	60.00	80.66	94.33
38	<i>P. longifolia</i>	94.67	100.00	100.00
39	<i>P. granatum</i>	92.33	100.00	100.00
40	<i>R. communis</i>	90.00	100.00	100.00
41	<i>S. nigrum</i>	91.00	100.00	100.00
42	<i>S. xanthocarpum</i>	94.00	100.00	100.00

43	<i>T. erecta</i>	100.00	100.00	100.00
44	<i>T. indica</i>	100.00	100.00	100.00
45	<i>T. stans</i>	90.67	100.00	100.00
46	<i>T. populnea</i>	86.33	92.67	100.00
47	<i>T. orientalis</i>	96.67	100.00	100.00
48	<i>V. negundo</i>	100.00	100.00	100.00
49	<i>Z. officinale</i>	75.33	80.33	92.00
50	<i>Z. jujuba</i>	78.00	95.33	97.33
51	Control	0.00	0.00	0.00

In treatment concentration interaction, complete inhibition in egg hatch was seen in *A. squamosa*, *T. erecta* at higher concentration of 80 per cent. This was followed by *B. scandens* and *T. orientalis* with 4.78 and 65.44 eggs hatched in the same concentration. A maximum of 232.11 eggs hatched in *T. indica* was followed by *E. globulus*, *A. precatorius*, *C. papaya* and *P. niruri* with 229.56, 224.78, 220.67 and 214.44 eggs hatched respectively, at 20 per cent concentration (Fig. 1). About 181.11 eggs were hatched out from the egg masses treated with *T. orientalis* extract at 20 per cent concentration and this was on par with *V. negundo* (at 40 %) and *T. indica* (at 80 %)

In treatment days interaction eggs hatched out was lowest in *A. squamosa*, *T. erecta* and *B. scandens* in the ascending order after three days and the same trend was observed in six and nine days after exposure to the extracts. In extracts of *T. orientalis*, *V. negundo* and *C. papaya* about 89.75, 117.25 and 118.83 eggs hatched out after 3 days of incubation and were found to be on par with each other. This trend slightly fluctuated in six days as the number of larvae hatched in *C. papaya* (197.67) was found to be on par with *A. precatorius* (177.23). Maximum of 285.83 eggs hatched in *T. indicus* followed by *E. globulus* with 239.25 eggs hatched out in nine days after exposure to the extracts (Table 4).

In case of days concentration interaction, all combinations were found to differ statistically from each other. Maximum reduction in hatching was noticed in three days at the higher concentration of 80 per cent with 84.36 larvae hatched out which was found to be significantly superior over the rest of the treatments. The next best response was in six days at the same concentration with 132.21 eggs hatched and the highest number of 266.06 eggs hatched out after nine days at the lowest concentration of 20 per cent (Table 4).

Among the plant extracts attempted, minimum hatching was recorded in *A. squamosa*, *T. erecta* and *B. scandens* extracts with 23.19, 28.03 and 35.22 respectively. The next best treatments with reduced hatching were recorded in *T. orientalis* (136.22), *P. niruri* (160.58) and *V. negundo* (161.72) but the last two treatments were found to be on par with each other. Maximum hatching of 211.44 eggs was observed in *T. indica* as against 564.11 in control (Table 4).

Marked difference in hatching was also observed between different concentrations of plant extracts. As a whole, number of egg hatching was reduced when the concentration was increased. Among the different concentrations tested, extract at 80 per cent was found to be superior over other concentrations with the lowest being 130.36 eggs hatched followed by 155.82 and 184.52 hatched eggs in 60 and 40 per cent concentration respectively. While maximum egg hatching was recorded in 20 per cent concentration (204.88). In all the ten extracts, concentration at 80 per cent concentration showed significant reduction in hatching while an increased emergence of larvae from eggs at 20 per cent concentration were observed in all the treatments irrespectively (Table 4).

Reasonably, number of days of incubation had a profound effect on egg hatching. Different plant extracts treated with egg masses incubated for different days showed a minimum of 115.55 eggs hatched in three days followed by 168.52 in six days and the maximum of 222.77 eggs hatched in nine days of incubation (Table 4). Thus it revealed a linear relationship between the number of days of incubation and inhibition of egg hatching. Wholly, there exhibit a significant difference between the different interactions *viz.*, treatment concentration interaction, treatment days interaction and concentration days interaction.

Table 4. Effect of most effective plant extracts on hatching of *M. incognita* eggs

Treatments	Number of larvae hatched							
	Concentration (%)				Mean	Days		
	20	40	60	80		3	6	9
T ₁	224.78	192.22	167.44	135.44	179.97	139.67	177.25	223.00
	(14.94)	(13.81)	(12.87)	(11.56)	(13.29)	(11.75)	(13.25)	(14.89)
T ₂	53.11	32.44	7.22	0.00	23.19	13.75	23.58	32.21
	(7.19)	(5.64)	(2.18)	(0.71)	(3.93)	(2.60)	(4.00)	(5.20)
T ₃	67.33	53.89	15.89	4.78	35.47	20.33	35.08	51.00
	(8.11)	(7.28)	(3.25)	(1.26)	(4.97)	(3.19)	(5.00)	(6.74)
T ₄	220.67	202.00	159.44	124.33	176.61	118.83	179.67	231.33
	(14.74)	(14.13)	(12.46)	(11.01)	(13.09)	(10.78)	(13.34)	(15.14)
T ₅	229.56	196.89	161.22	134.78	180.61	133.33	169.25	239.25
	(15.06)	(13.92)	(12.59)	(11.52)	(13.27)	(11.48)	(12.94)	(15.40)
T ₆	214.44	165.67	147.44	114.78	160.58	125.33	157.67	198.75
	(14.58)	(12.83)	(12.08)	(10.65)	(12.54)	(11.11)	(12.48)	(14.02)
T ₇	57.89	44.33	9.89	0.00	28.03	17.94	26.92	39.20
	(7.51)	(6.62)	(2.54)	(0.71)	(4.34)	(2.99)	(4.30)	(5.72)
T ₈	232.11	232.78	205.67	175.22	211.44	159.96	188.58	285.83
	(15.20)	(15.09)	(14.19)	(13.09)	(14.39)	(12.59)	(13.70)	(16.88)
T ₉	181.11	171.11	127.22	65.44	136.22	89.75	118.92	200.00
	(13.17)	(12.89)	(11.09)	(7.96)	(11.28)	(9.21)	(10.74)	(13.88)
T ₁₀	208.56	174.22	148.44	115.67	161.72	117.25	164.75	203.17
	(14.38)	(13.13)	(12.08)	(10.65)	(12.56)	(10.71)	(12.78)	(14.19)
T ₁₁					564.11	335.00	612.00	745.55
					(23.45)	(18.30)	(24.74)	(27.30)
Mean	204.88	184.52	155.82	130.36		115.55	168.52	222.77
	(13.49)	(12.62)	(10.80)	(9.22)		(9.52)	(11.57)	(13.58)

Concentration (%) x days

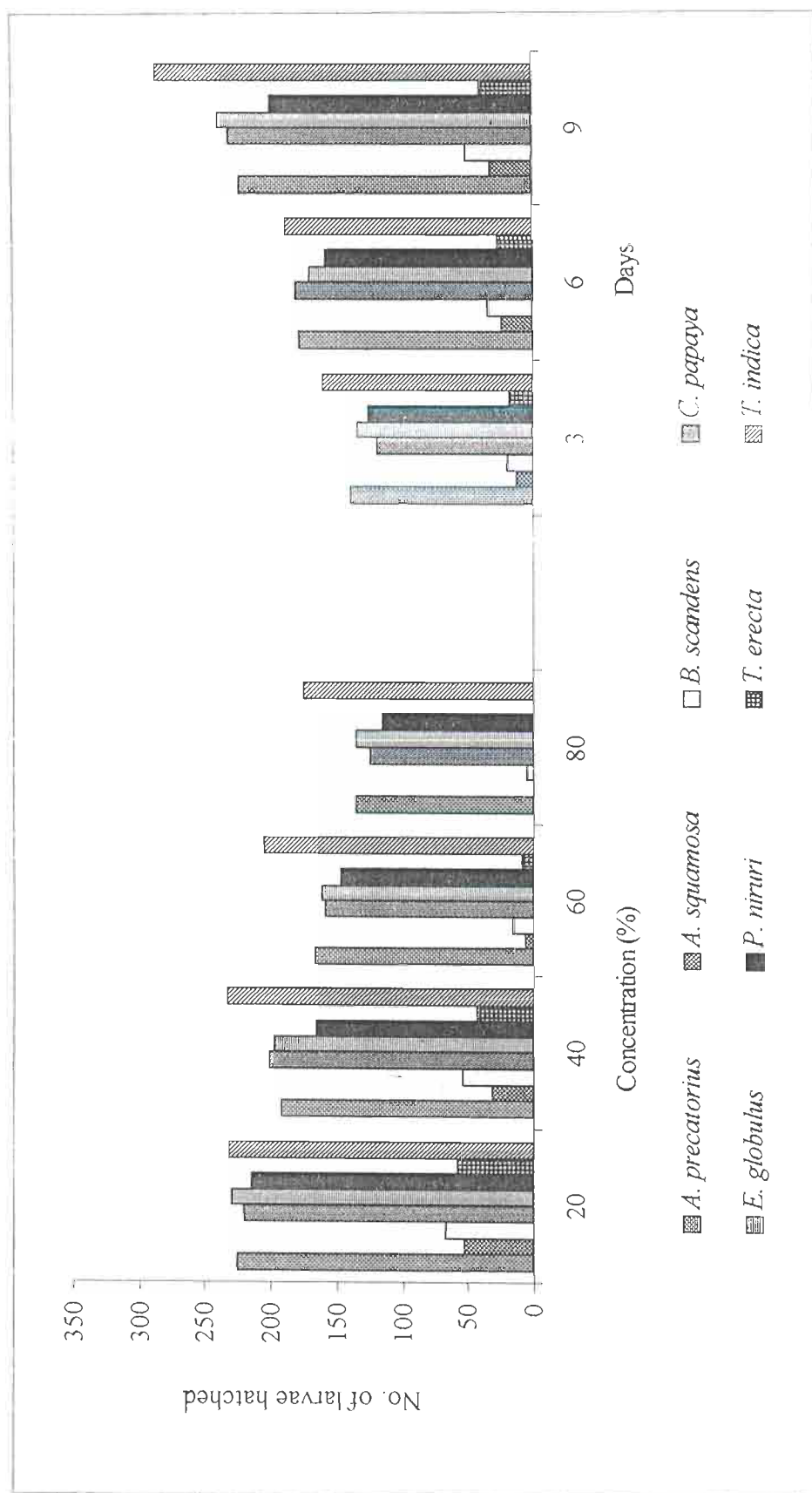
Concentration (%) /		20	40	60	80
Days					
3		149.36	128.12	100.36	84.36
		(11.64)	(10.69)	(8.29)	(7.44)
6		199.21	183.82	158.82	132.21
		(13.28)	(12.62)	(11.19)	(9.18)
9		266.06	241.61	208.27	176.06
		(15.53)	(14.54)	(12.91)	(11.45)

Figures in the parenthesis are $x + 0.5$ transformed values

CD	T	(0.07)	T x C	(0.13)
	C	(0.04)	T x D	(0.11)
	D	(0.03)	C x D	(0.07)
	Control Vs Rest		(0.23)	

Treatments T_1 - *A. Precatorius* T_6 - *P. niruri* T_2 - *A. squamosa* T_7 - *T. erecta* T_3 - *B. scandens* T_8 - *T. indica* T_4 - *C. papaya* T_9 - *T. orientalis* T_5 - *E. globulus* T_{10} - *V. negundo*

Fig. 1. Effect of selected plant extracts on *M. incognita* egg hatching



4.3. Effect of selected plant extracts on mortality of *M. incognita* juveniles

The data presented in Table 5 depict that there has been a regular and gradual increase in the juvenile's mortality with an increase in concentration of the different plant extracts and its period of exposure.

In treatment concentration interaction, 100 per cent juvenile mortality was observed in *A. squamosa* and *T. erecta* extracts at 80 per cent concentration followed by *B. scandens*, *T. indica* and *V. negundo* recorded 90 per cent mortality at the same concentration (Fig. 2). The same trend in juvenile mortality was exhibited by 60 and 40 per cent concentration of the test plant extracts. The treatments with *C. papaya*, *E. globulus* and *V. negundo* under 20 per cent concentration were found to be on par with each other. Lowest juvenile mortality of 26.22 per cent was recorded in *P. niruri* at 40 per cent concentration and 20.44 per cent in *T. orientalis* at 20 per cent concentration (Table 5).

Statistically significant difference was noticed between the different treatment hr interactions. *A. squamosa*, *T. erecta* and *B. scandens* at 36 hr recorded maximum of 90.5, 86.75 and 82.25 per cent mortality respectively. In contrast, lowest juvenile mortality of less than 50 per cent observed in *P. niruri* and *T. orientalis* after 36 hr; *A. precatorius* and *V. negundo* at 12 hr and *P. niruri* at 24 hr were found to be on par with 48.42, 49.42 and 49.5 per cent juvenile mortality respectively.

In the concentration hr interaction, all the treatments were found to exhibit significant variation between their concentration and hr except 60 per cent at 12 hr and 40 per cent at 24 hr which were on par with each other. In the highest concentration (80 %) after 36 hr maximum of 86.64 per cent juvenile mortality was recorded followed by 60 and

Table 5. *In vitro* screening of most effective plant extracts against *M. incognita* juveniles

Treatments	Per cent juveniles mortality							
	Concentration (%)				Mean	Hours		
	20	40	60	80		12	24	36
T ₁	34.00	57.11	62.89	83.78	59.44	48.42	58.44	71.50
	(35.29)	(49.18)	(52.66)	(66.56)	(50.92)	(43.98)	(50.27)	(58.52)
T ₂	56.11	81.78	97.00	100.00	83.72	74.08	86.53	90.50
	(48.60)	(66.06)	(84.01)	(89.72)	(73.10)	(63.87)	(74.83)	(77.59)
T ₃	37.33	63.67	77.67	99.67	69.58	58.42	68.08	82.25
	(37.34)	(53.24)	(63.23)	(88.24)	(60.51)	(52.64)	(59.50)	(69.40)
T ₄	30.67	50.67	68.44	89.11	59.72	46.67	58.42	74.08
	(33.28)	(45.38)	(56.38)	(72.49)	(51.88)	(43.01)	(50.60)	(62.03)
T ₅	30.11	53.00	66.44	85.89	58.86	46.00	58.08	72.50
	(32.86)	(46.77)	(55.08)	(68.54)	(50.81)	(42.50)	(50.18)	(59.76)
T ₆	27.56	26.22	55.33	79.22	47.08	36.67	49.50	55.08
	(31.00)	(30.42)	(48.16)	(63.31)	(43.22)	(36.66)	(44.77)	(48.24)
T ₇	49.44	70.67	91.11	100.00	77.81	68.42	78.25	86.75
	(44.71)	(57.55)	(76.06)	(89.72)	(67.01)	(59.88)	(66.66)	(74.49)
T ₈	33.89	58.33	75.65	94.56	65.58	53.00	65.42	78.33
	(35.25)	(49.93)	(61.19)	(79.12)	(56.37)	(47.24)	(55.59)	(66.28)
T ₉	20.44	31.44	46.22	73.33	42.81	27.75	43.75	37.08
	(26.04)	(33.54)	(42.74)	(59.47)	(40.45)	(30.60)	(41.11)	(49.63)
T ₁₀	30.55	53.89	71.00	91.67	61.78	49.42	60.67	75.25
	(33.06)	(47.30)	(57.94)	(74.80)	(53.27)	(44.74)	(52.25)	(62.83)
T ₁₁					0.00	0.00	0.00	0.00
					(0.29)	(0.29)	(0.29)	(0.29)
Mean	31.83	49.71	64.70	81.57		46.26	57.02	67.58
	(32.52)	(43.61)	(54.34)	(68.39)		(42.31)	(49.64)	(57.19)

Concentration x hour				
Concentration (%) /	20	40	60	80
Hour				
12	19.73 (24.82)	36.79 (35.69)	52.67 (45.54)	75.85 (63.19)
24	28.88 (30.95)	52.39 (45.27)	64.58 (54.01)	82.21 (68.33)
36	46.88 (41.78)	59.94 (49.86)	76.85 (63.44)	86.64 (73.63)

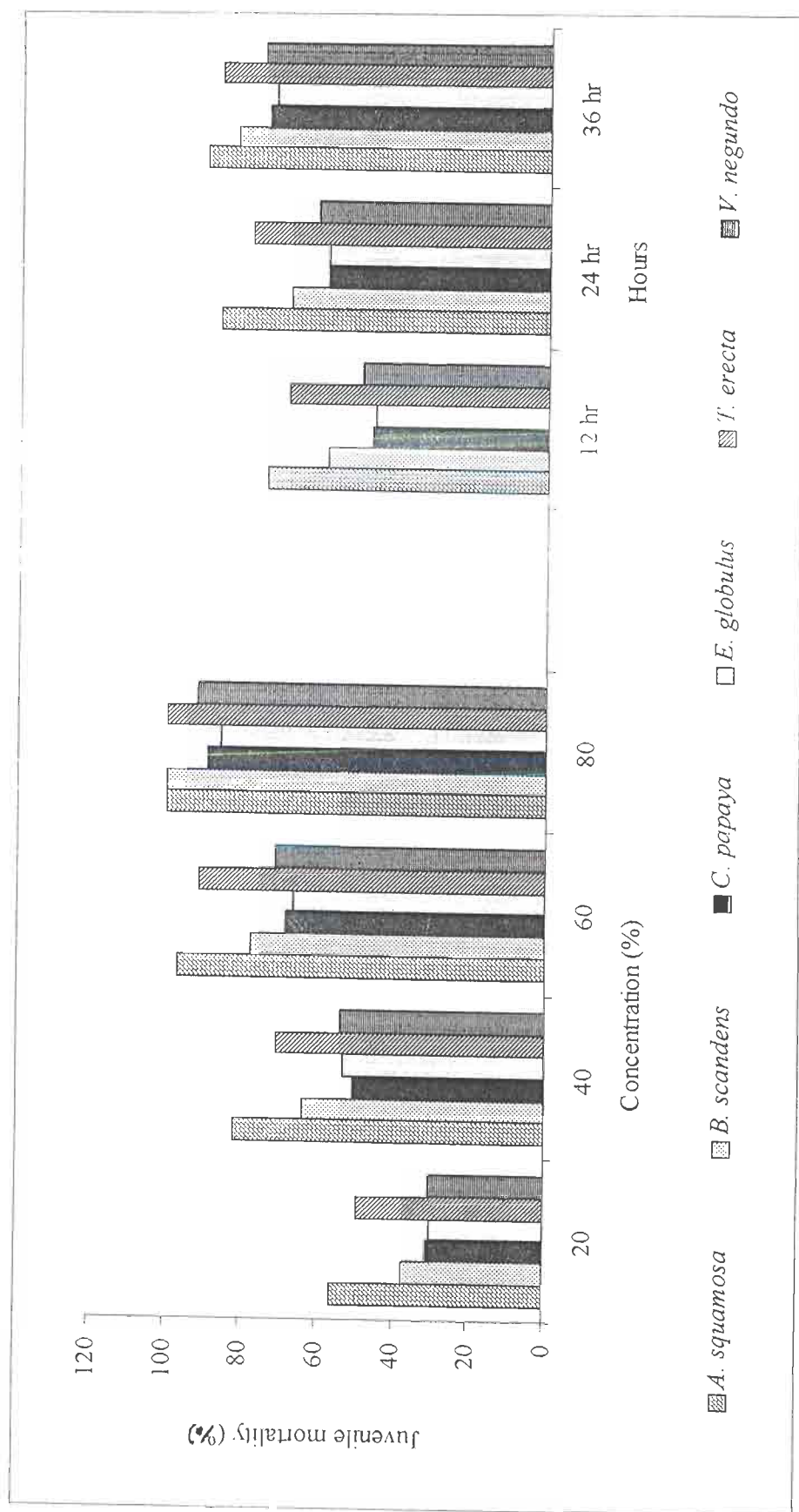
Figures in the parenthesis are arc sine transformed values

CD	T	(0.75)	T x C	(1.51)
	C	(0.45)	T x H	(1.31)
	H	(0.39)	C x H	(0.79)
	Control Vs Rest		(2.61)	

Treatments

T1 - <i>A. Precatorius</i>	T6 - <i>P. niruri</i>
T2 - <i>A. squamosa</i>	T7 - <i>T. erecta</i>
T3 - <i>B. scandens</i>	T8 - <i>T. indica</i>
T4 - <i>C. papaya</i>	T9 - <i>T. orientalis</i>
T5 - <i>E. globulus</i>	T10 - <i>V. negundo</i>

Fig. 2. Effect of selected plant extracts on *M. incognita* juvenile mortality



40 per cent concentration recording 82.21 and 75.85 per cent mortality after 24 and 12 hr of exposure period, respectively. While less than 50 per cent mortality was recorded in 20 per cent concentration after 36 hr and 40 per cent concentration after 12 hr and the lowest of 19.73 per cent larval mortality was recorded in 20 per cent concentration after 12 hr of exposure to the extracts (Table 5).

It was inferred that among the plant extracts tested, *A. squamosa*, *T. erecta* and *B. scandens* had high degree of nematocidal effect both in inhibiting emergence of juveniles from eggs and causing mortality of *M. incognita* juveniles.

4.4. Comparative efficacy of plant extracts against *M. incognita*

4.4.1. Median Lethal Concentration (LC₅₀)

The non-overlapping of fiducial limits (Table 6) reflected significant variations among the efficacy of plant extracts. *A. squamosa* extract was the most effective with lowest LC₅₀ value of 25.57 while *T. orientalis* was the least effective with highest LC₅₀ value (63.97). The next in their order of effectiveness was *T. erecta*, *B. scandens* and *E. globulus* with LC₅₀ value of 28.90, 36.93 and 40.24 respectively (Fig. 3). *T. indica* and *C. papaya* were not statistically different with LC₅₀ of 45.84 and 45.00 respectively, as seen by the overlapping of the fiducial limits.

4.4.2. Median Lethal Time (LT₅₀)

A. squamosa extract registered the lowest LT₅₀ value of 8.62 proving its effectiveness against *M. incognita* juveniles within short time compared to the other plant extracts (Table 7). The next plant extract was *T. erecta* (LT₅₀: 9.42) and *B. scandens* (LT₅₀: 13.33). There were no statistical difference existed between *E. globulus* and *A. precatorius* in the LT₅₀ values registering 15.71 and 15.68 respectively as evident by the

Table 6. Probit analysis of Concentration – Mortality response of *M. incognita* juveniles to plant extracts

Sl.No.	Plant extracts	No.of juveniles	χ^2 *	Slope 'b'±SE	LC ₅₀ (%)	Fiducial Limits	
						Lower	Upper
1	<i>A. precatorius</i>	100	22.743	3.1202 ± 0.2755	43.55	17.0688	70.0526
2	<i>A. squamosa</i>	100	16.824	3.7003 ± 0.3332	25.57	09.3904	36.5523
3	<i>B. scandens</i>	100	32.043	4.0051 ± 0.3112	36.93	10.7796	58.8850
4	<i>C. papaya</i>	100	29.944	3.5026 ± 0.2874	45.00	15.2668	78.1212
5	<i>E. globulus</i>	100	24.383	3.5886 ± 0.2908	40.24	17.1394	61.2386
6	<i>P. niruri</i>	100	41.750	3.4608 ± 0.2952	54.54	08.6476	66.5269
7	<i>T. erecta</i>	100	24.650	3.5246 ± 0.3061	28.90	05.9176	44.4190
8	<i>T. indica</i>	100	25.147	3.4381 ± 0.2870	45.84	20.0486	74.2593
9	<i>T. orientalis</i>	100	55.678	3.8903 ± 0.3326	63.97	07.2694	69.5524
10	<i>V. negundo</i>	100	23.350	3.7118 ± 0.2296	43.31	21.4864	64.8957

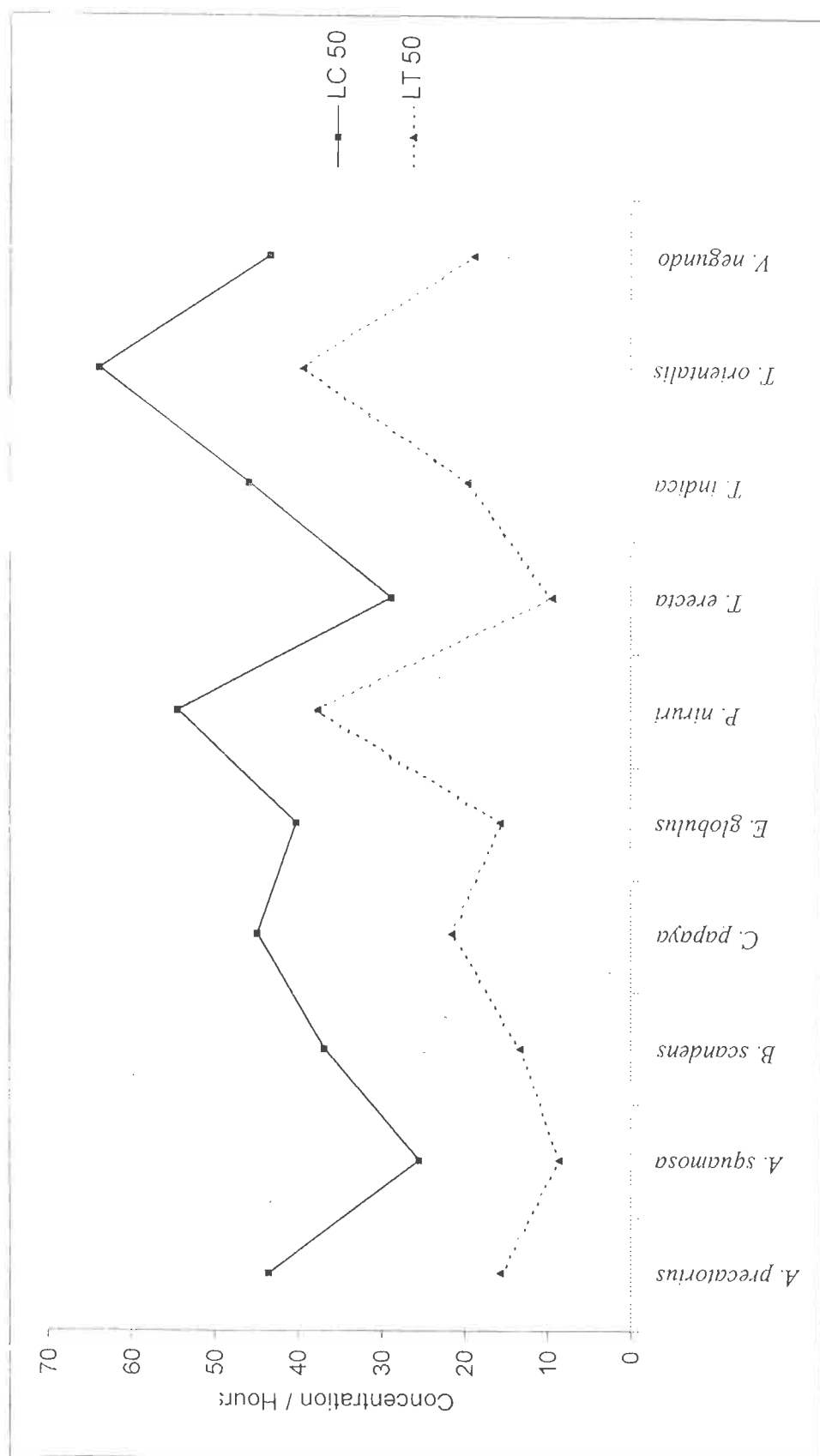
* All lines are significantly a good fit (P 0.05)

Table 7. Probit analysis of Time – Mortality response of *M. incognita* juveniles to plant extracts

Sl.No.	Plant extracts	No. of juveniles	χ^2 *	Slope 'b'±SE *	LT ₅₀ (hr)	Fiducial Limits	
						Lower	Upper
1	<i>A. precatarius</i>	100	0.327	1.2650 ± 0.3742	15.68	08.7232	20.4195
2	<i>A. squamosa</i>	100	0.651	2.5242 ± 0.4576	08.62	05.3946	11.0136
3	<i>B. scandens</i>	100	0.657	1.7262 ± 0.3844	13.33	08.6181	16.6516
4	<i>C. papaya</i>	100	0.150	1.8448 ± 0.3807	21.48	17.5451	26.0940
5	<i>E. globulus</i>	100	0.009	1.5230 ± 0.3771	15.71	10.4159	19.6965
6	<i>P. niruri</i>	100	0.300	1.3217 ± 0.3845	37.73	28.3205	82.8419
7	<i>T. erecta</i>	100	0.145	1.5584 ± 0.3926	09.42	04.1111	12.9906
8	<i>T. indica</i>	100	0.210	1.6473 ± 0.3775	19.56	15.0725	24.0663
9	<i>T. orientalis</i>	100	0.008	2.0495 ± 0.4168	39.42	31.9369	59.1314
10	<i>V. negundo</i>	100	0.213	1.5586 ± 0.3764	18.90	114.0516	23.4685

* All lines are significantly a good fit (P 0.05)

Fig. 3. Probit analysis of concentration and time – mortality response of *M. incognita* juveniles to plant extracts



fiducial limits. The rest of the plant extracts registered LT_{50} values ranging from 18.90 to 37.73 (Fig. 3). Least effect was seen in *T. orientalis* extract with highest LT_{50} value (39.42).

4.5. Effect of plant extracts (*A. squamosa*, *B. scandens* and *T. erecta*) on penetration of *M. incognita* in tomato and brinjal

Number of nematodes penetrating the roots were reduced with an increase in the concentration of the plant extracts and increased with an increase in the number of days of incubation.

Among the three plant extracts viz., *A. squamosa*, *B. scandens* and *T. erecta* with two concentrations (40 and 60 %) the maximum inhibition in penetration was recorded in *A. squamosa* at 60 per cent concentration showing 6.10 and 4.55 per cent penetration. It was followed by *T. erecta* at the same concentration recording 10.75 and 8.90 per cent penetration in tomato and brinjal respectively (Table 8). Maximum number of *M. incognita* juveniles was recorded in roots of plants treated with *B. scandens* at 40 per cent concentration showing 40.15 and 36.00 per cent penetration in tomato and brinjal respectively (Plate 1). In the control plants highest juveniles were recorded both in tomato (63.35 %) and brinjal (57.60 %).

There was a remarkable difference existed in penetration with increase in number of days of incubation (Fig. 4). On the first day, lowest population of *M. incognita* juveniles was noticed both in tomato (8.93 %) and brinjal (7.68 %). But there was a marked difference in penetration on 3rd day after inoculation showing 26.82 per cent in tomato and 22.75 per cent of juveniles in brinjal (Table 9). The increase in penetration between 4th and 5th day was only 6.64 per cent in tomato and 5.82 per cent in brinjal.

Table 8. Effect of plant extracts on penetration of *M. incognita* juveniles in tomato

Plant extracts	Concentration (%)	Per cent juveniles penetrated - Days After Inoculation (DAI)					Mean
		1	2	3	4	5	
<i>A. squamosa</i>	40	3.25 (10.26)	9.25 (17.68)	16.75 (23.54)	24.00 (29.11)	30.75 (33.58)	16.80 (22.83)
	60	1.50 (6.93)	4.00 (12.23)	7.25 (15.89)	8.50 (16.94)	9.25 (18.02)	6.10 (14.00)
	40	9.25 (17.68)	23.25 (28.82)	45.75 (42.85)	57.00 (48.92)	65.50 (54.13)	40.15 (38.48)
<i>B. scandens</i>	60	4.00 (11.50)	10.50 (18.36)	19.50 (26.16)	32.75 (34.72)	41.00 (39.82)	21.55 (26.11)
	40	6.25 (14.43)	14.00 (22.16)	24.00 (29.22)	39.00 (38.56)	46.25 (42.85)	25.90 (29.44)
	60	2.75 (9.44)	6.75 (15.03)	10.50 (19.02)	15.25 (22.77)	18.50 (25.43)	10.75 (18.34)
Control		35.50 (36.57)	50.25 (42.29)	64.00 (53.61)	78.00 (61.64)	89.00 (69.94)	63.35 (52.81)
Mean		8.93 (15.26)	16.88 (22.37)	26.82 (30.04)	36.36 (36.10)	43.00 (40.54)	

CD (P=0.05) t : 0.60

d : 0.51

t x d : 1.34

Table 9. Effect of plant extracts on penetration of *M. incognita* juveniles in brinjal

Plant extracts	Concentration (%)	Per cent juveniles penetrated -- Days After Inoculation (DAI)					Mean
		1	2	3	4	5	
<i>A. squamosa</i>	40	3.00 (10.04)	7.75 (16.66)	11.25 (19.58)	20.25 (26.22)	26.00 (30.82)	13.65 (20.66)
	60	1.25 (6.69)	3.25 (10.25)	4.75 (12.48)	6.00 (14.77)	7.50 (16.10)	4.55 (12.10)
	40	8.75 (17.20)	20.50 (26.74)	41.25 (39.96)	50.50 (45.15)	59.00 (50.33)	36.00 (35.88)
<i>B. scandens</i>	60	3.25 (10.25)	8.50 (17.44)	15.00 (22.78)	26.50 (31.31)	34.75 (36.27)	17.60 (23.61)
	40	5.00 (13.18)	11.25 (19.58)	20.50 (26.74)	36.75 (37.61)	41.00 (39.96)	22.90 (27.41)
	60	2.00 (7.40)	5.25 (13.42)	7.50 (16.14)	13.50 (21.76)	16.25 (24.15)	8.90 (16.57)
Control		30.50 (33.52)	42.25 (40.69)	59.00 (50.33)	73.25 (55.24)	83.0 (66.10)	57.60 (49.18)
Mean		7.68 (14.04)	14.12 (20.69)	22.75 (26.86)	32.40 (33.15)	38.22 (37.68)	

CD (P=0.05) t : 0.52

d : 0.52

t x d : 1.38

Fig. 4. Effect of *A. squamosa*, *B. scandens* and *T. erecta* on penetration of *M. incognita* in tomato

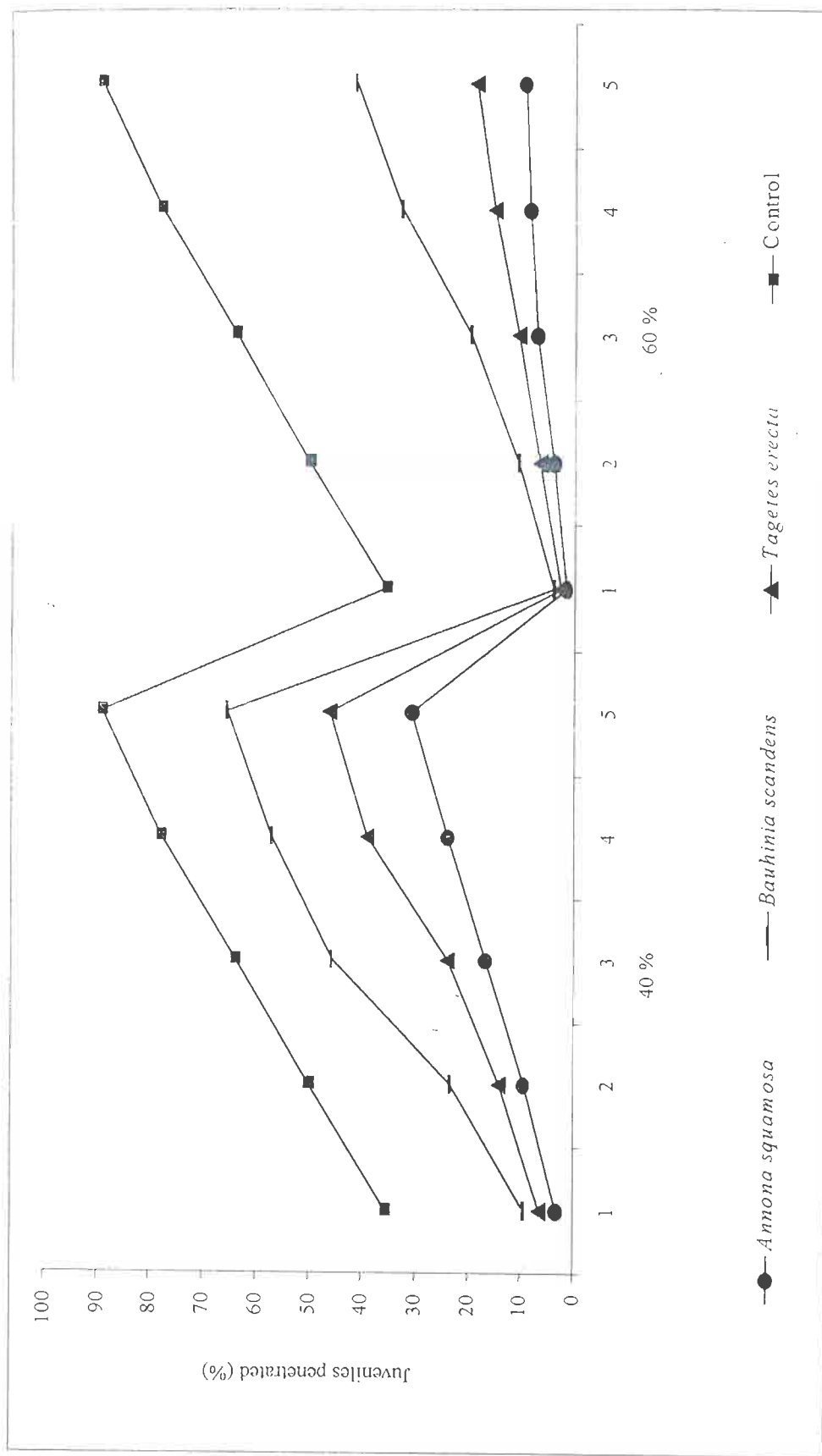
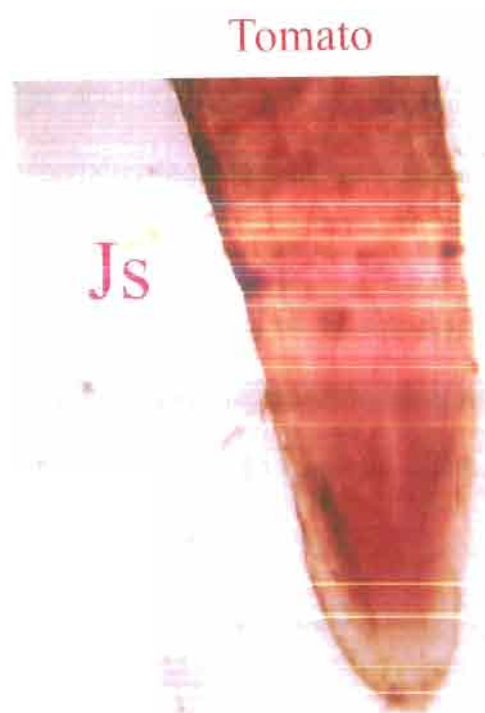


Plate 1. Penetration of *M. incognita* juveniles in tomato and brinjal roots



In respect of concentration and days interaction, *A. squamosa* extract at 60 per cent recorded 1.5 and 1.25 per cent penetration in tomato and brinjal on the 1st day and this slowly increased to 7.25 and 9.25 per cent in tomato and 4.75 and 7.50 per cent in brinjal on 3rd and 5th day respectively. The next best was *T. erecta* at the same concentration showing 2.75, 10.50 and 18.50 per cent penetration of juveniles in tomato and it was 2.00, 7.50 and 16.25 per cent in brinjal on 1st, 3rd and 5th day after inoculation respectively. While juveniles penetrated the roots of both tomato and brinjal was maximum in plants treated with *B. scandens* extract at 40 per cent concentration and it was 65.50 per cent in tomato and 59.00 per cent in brinjal on 5th day after inoculation. In the untreated control plants root-knot nematode juveniles penetrated up to 35.50 per cent on 1st day and 89.00 per cent on 5th day in tomato; 30.50 per cent on 1st day and 83.00 per cent on 5th day in brinjal. In general the density of *M. incognita* juveniles penetrating the roots of tomato plants was comparatively higher than brinjal (Fig. 5)

4.6. Effect of plant extracts (*A. squamosa*, *B. scandens* and *T. erecta*) on the growth of *F. oxysporum* f.sp. *lycopersici* and *R. solani* *in vitro*

Among the plant extracts tested *in vitro* against the growth inhibition of pathogenic fungi viz., *F. oxysporum* f. sp. *lycopersici* and *R. solani*, *A. squamosa* extract dominated in inhibition of the growth of test fungi (Plate 2). About 85.4 per cent growth inhibition of *F. oxysporum* f.sp. *lycopersici* was registered when the media was amended with *A. squamosa* extract. The next best plant extracts were *T. erecta* and *B. scandens* with 79.5 and 61.0 per cent reduction over control respectively (Table 10).

With regard to *R. solani*, *A. squamosa* recorded maximum inhibition of 76.8 per cent over control followed by *T. erecta* (65.6 %) and *B. scandens* (47.8 %). Among the

Fig. 5. Effect of *A. squamosa*, *B. scandens* and *T. erecta* on penetration of *M. incognita* in brinjal

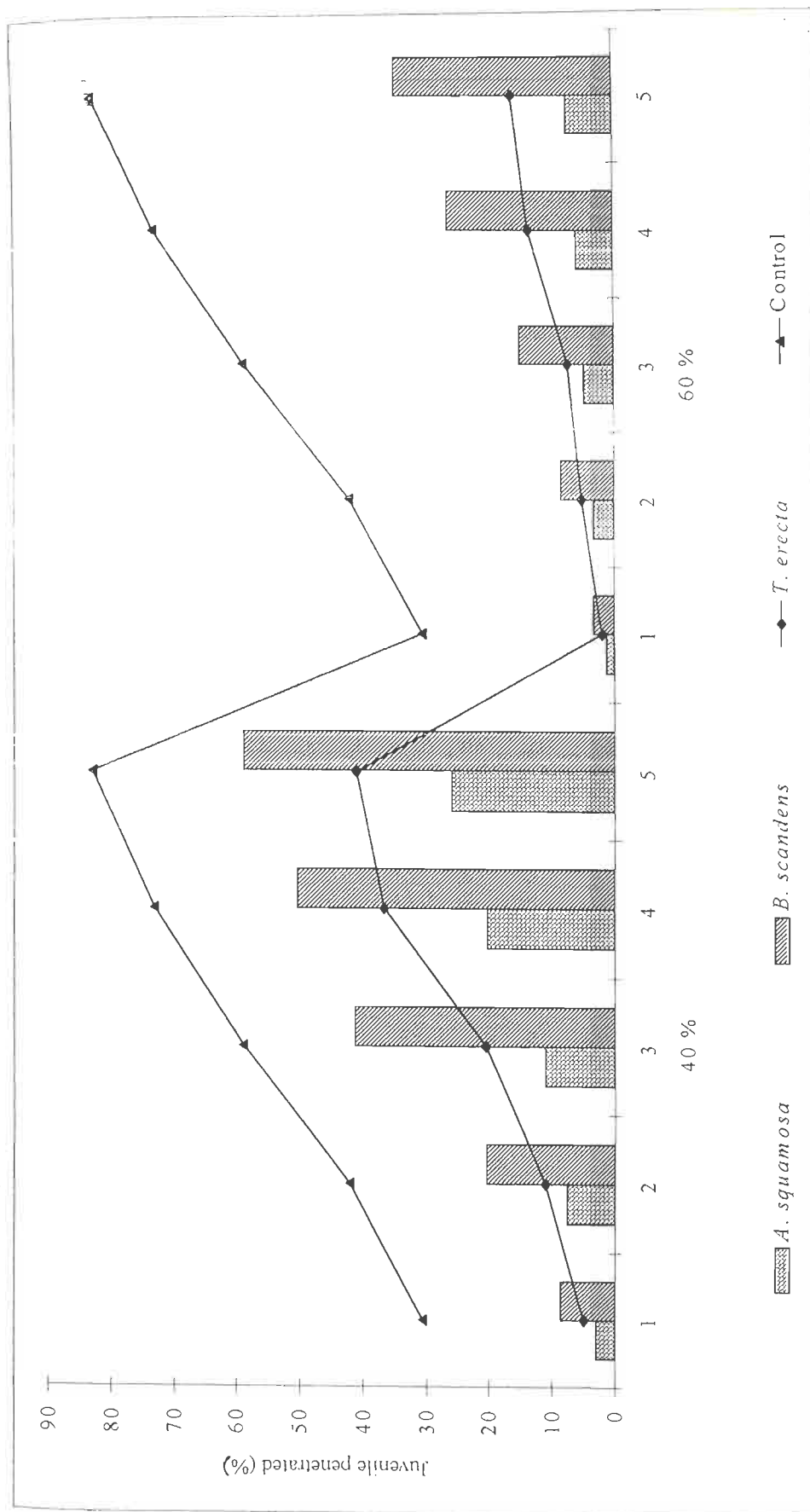


Plate 2. Effect of plant extracts on growth of *F. oxysporum* f.sp. *lycopersici* and *R. solani*

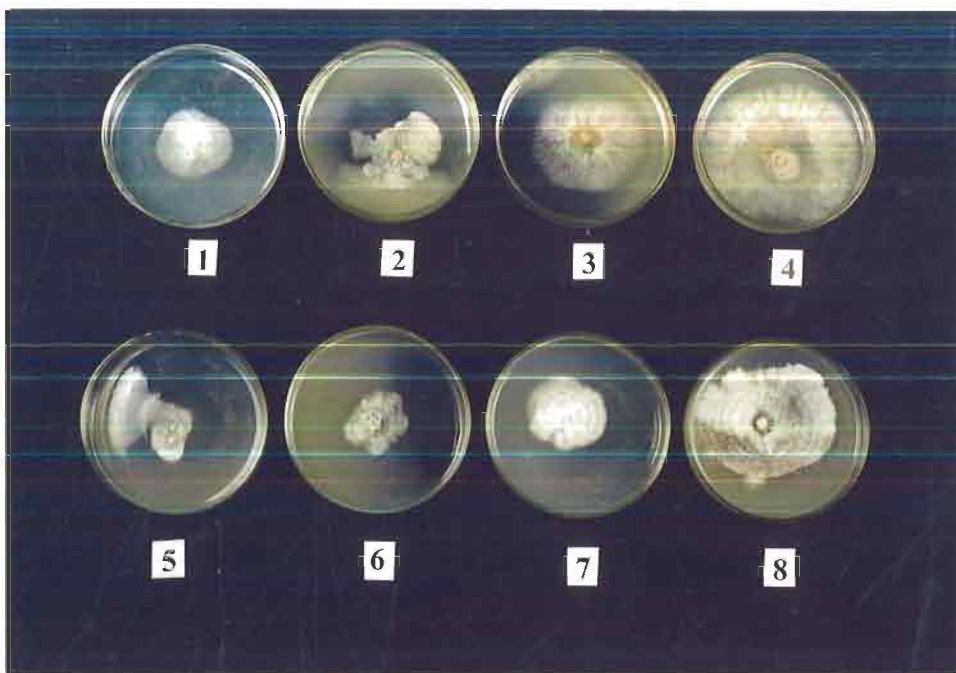
R. solani

1. *A. squamosa*
2. *T. erecta*
3. *B. scandens*
4. Control

F. oxysporum f.sp. *lycopersici*

5. *A. squamosa*
6. *T. erecta*
7. *B. scandens*
8. Control

Plate 2



plant extracts tested, maximum growth inhibition was recorded in *A. squamosa* and the least was registered in *B. scandens* extracts against both fungi. With regard to the susceptibility nature of both pathogenic fungi, *F. oxysporum* f.sp. *lycopersici* was found to be more susceptible than *R. solani* (Table 10).

4.7. Effect of seed treatment with plant extracts (*A. squamosa*, *B. scandens* and *T. erecta*) against *M. incognita* on tomato and brinjal

4.7.1. Tomato

Seeds treated with plant extracts from *A. squamosa*, *B. scandens* and *T. erecta* had significant effect in improving the plant growth parameters (Plate 3). Among the plant growth parameters analysed, shoot length (16.6 cm), root length (8.4 cm) and seedling weight (28.1 g) were increased and ranked first in seeds treated with *A. squamosa* extract. This was followed by *T. erecta* and *B. scandens* in respect of all the growth parameters. Similarly penetrating ability of *M. incognita* juveniles, gall formation and inherent soil nematode population was drastically reduced with all three plant extracts when compared with untreated control. Minimum number of galls (12.3) and soil nematode population (268.2) was registered in *A. squamosa* extract and the least reduction of root-knot nematode development were noticed only in *B. scandens* extract (Table 11).

4.7.2. Brinjal

Of all the plant extracts as treatment, *A. squamosa* recorded 14.5 cm shoot length followed by *T. erecta* and *B. scandens* with 12.8 and 9.0 cm respectively as against 7.5 cm in control (Table 12). Root length of seedlings recorded in *A. squamosa* treatment was 7.0 cm and this was on par with *T. erecta* (6.8 cm). But *B. scandens* registered lowest root length of 4.4 cm. The same trend of increase in seedling weight was observed in

Table 10. Effect of plant extracts on the growth of *F. oxysporum* f.sp. *lycopersici* and *R. solani*

Sl.No.	Plant extracts	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>		<i>R. solani</i>	
		(mm)	Per cent inhibition	(mm)	Per cent inhibition
1	<i>A. squamosa</i>	11.6	85.4	20.0	76.8
2	<i>T. erecta</i>	16.2	79.5	29.6	65.6
3	<i>B. scandens</i>	30.8	61.0	45.0	47.8
4	Control	79.0		86.2	
	CD (P=0.05)	2.03		3.15	

Table 11. Seed treatment with plant extracts against *M. incognita* in tomato

Treatments	Shoot length (cm)	Root length (cm)	Seedling weight gm/10 seedlings	Number of Galls	Soil population/ 250 gm soil
<i>A. squamosa</i>	16.6	8.4	28.1	12.3	268.2
<i>B. scandens</i>	11.0	7.5	17.8	24.6	376.2
<i>T. erecta</i>	13.5	8.2	21.5	20.0	315.0
Control	9.3	6.0	12.0	35.2	660.4
CD (P= 0.05)	0.92	0.50	1.17	2.14	9.42

Table 12. Seed treatment with plant extracts against *M. incognita* in brinjal

Treatments	Shoot length (cm)	Root length (cm)	Seedling weight gm/10 seedlings	Number of Galls	Soil population/ 250 gm soil
<i>A. squamosa</i>	14.5	7.0	23.6	10.6	243.6
<i>B. scandens</i>	9.0	4.4	14.0	23.5	334.2
<i>T. erecta</i>	12.8	6.8	18.2	16.0	270.0
Control	7.5	3.8	9.5	29.6	610.8
CD (P= 0.05)	0.85	0.44	1.03	2.00	7.31

Plate 3. Seed treatment with plant extracts against *M. incognita* on tomato

T₁: *A. squamosa*

T₂: *T. erecta*

T₃: *B. scandens*

T₄: Control

Plate 4. Seed treatment with plant extracts against *M. incognita* on brinjal

T₁: *A. squamosa*

T₂: *T. erecta*

T₃: *B. scandens*

T₄: Control

Plate 3



Plate 4



A. squamosa, *T. erecta* and *B. scandens* with 23.6, 18.2 and 14.0 g respectively. The plant extracts evaluated influenced the gall formation and soil nematode population markedly, with the lowest being *A. squamosa* and the highest with *B. scandens* (Plate 4). Maximum number of galls (29.6) and soil nematode population (610.8) was registered in the untreated control plants.

4.8. Effect of different methods of application of plant extracts against *M. incognita* in tomato and brinjal

4.8.1. *A. squamosa*

4.8.1.1. Tomato

4.8.1.1.1. Plant growth parameters

In general, different method of application of *A. squamosa* extract like seedling bare-root-dip, foliar spray, soil application and their combination enhanced the shoot height ranging from 45.7 cm to 70.5 cm (Plate 5). Among the different method of application, combined application of seedling bare root-dip + foliar spray + soil application recorded the maximum shoot height over control (150 %) and was followed by seedling bare root-dip + foliar spray, seedling root-dip + soil application and foliar spray + soil application recording 125.6, 115.6 and 113.2 per cent increase over control. However the effect of these treatments were on par with each other. Apart from these an individual application of the *A. squamosa* extract as seedling root-dip, foliar spray and soil application also recorded 95.1, 80.2 and 62.1 per cent increase in shoot length, respectively (Table 13).

An enhancement of 295.0 per cent over control in shoot weight of the plants was observed in combined application of all the three methods. This response was proved

Table 13. Effect of *A. squamosa* extract against *M. incognita* on tomato - Plant growth parameters

Treatments	Shoot				Root				No of fruits	Yield, g/plant	Per cent Increase
	Height (cm)	Per cent increase	Weight (g)	Per cent increase	Length (cm)	Per cent increase	Weight (g)	Per cent increase			
T1	55.0	95.1	85.6	114.0	22.8	107.2	30.8	120.0	4.3	274.0	114.9
T2	50.8	80.2	80.0	100.0	18.3	66.4	29.1	107.9	4.0	268.0	110.2
T3	45.7	62.1	60.7	51.8	15.0	36.4	20.8	48.6	3.0	200.7	57.5
T4	63.6	125.6	134.2	235.5	35.5	222.7	51.3	266.5	8.6	400.6	214.2
T5	60.8	115.6	118.5	196.3	32.7	197.3	49.0	250.0	8.3	385.0	201.9
T6	60.1	113.2	106.0	165.0	25.0	127.3	39.9	185.0	6.0	315.1	147.1
T7	70.5	150.0	158.0	295.0	44.6	305.5	56.4	302.9	9.0	480.5	276.9
T8	28.2		40.0		11.0		14.0		2.6	127.5	
CD (P=0.05)	4.54		7.23		5.07		3.13		0.51	17.40	

T7: Root-dip + Foliar spray + Soil application

T4: Root-dip + Foliar spray

T1: Root-dip

T8: Untreated control

T2: Foliar spray

T5: Root-dip + Soil application

T3: Soil application

T6: Foliar spray + Soil application

Plate 5. Effect of *A. squamosa* against *M. incognita* on tomato – Shoot

T₁: Root-dip (RD)

T₂: Foliar spray (FS)

T₃: Soil application (SA)

T₄: RD + FS

T₅: RD + SA

T₆: FS + SA

T₇: RD + FS + SA

T₈: Untreated control

Plate 6. Effect of *A. squamosa* against *M. incognita* on tomato – Root

Plate 5



Plate 6



significant over other treatments. On the contrary, seedling bare root-dip alone recorded 114.0 per cent increase and it was on par with foliar spray (100 %). But soil application gave the lowest per cent increase over control (60.7 %).

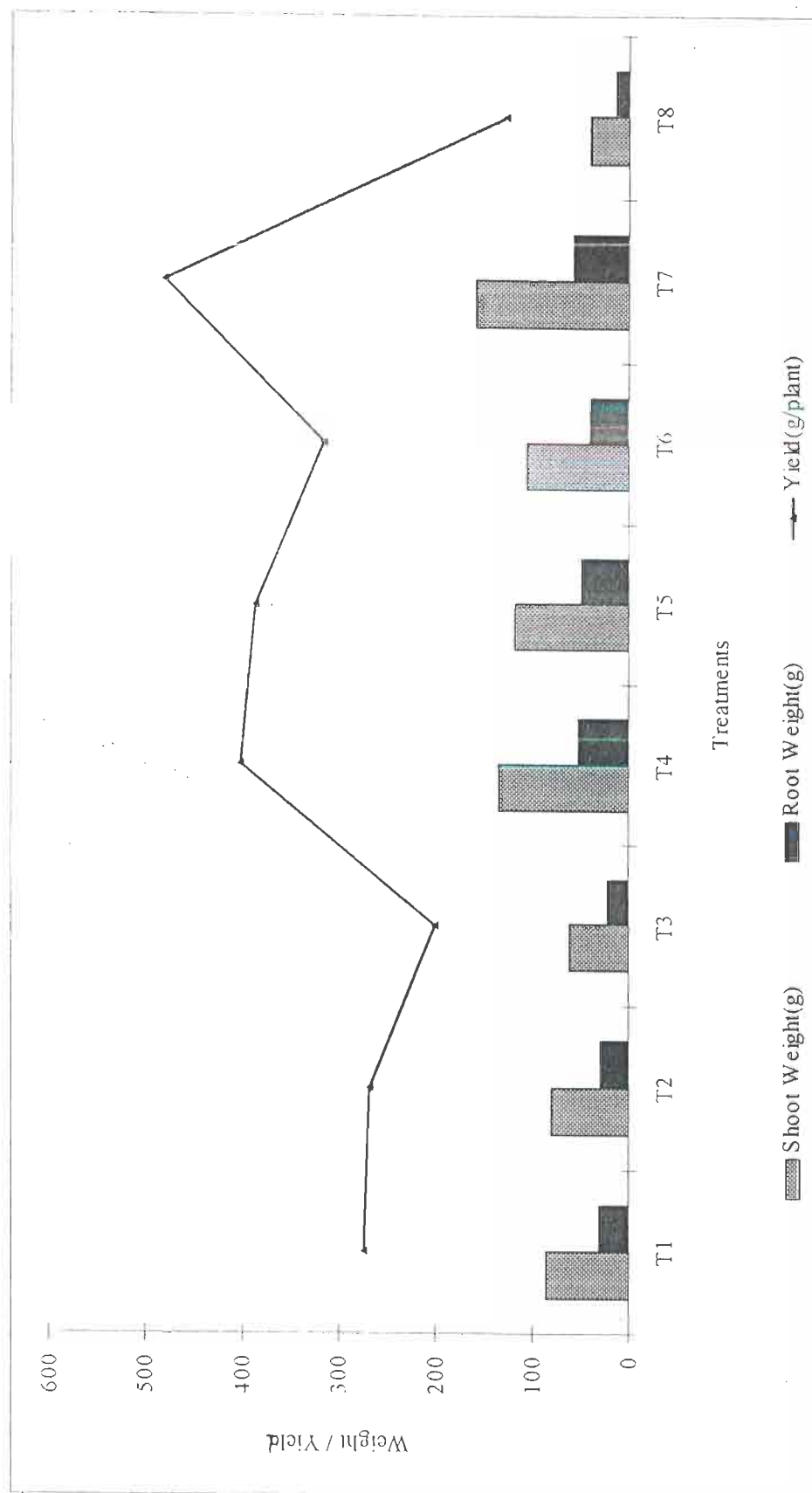
In combined application of the three methods, a maximum of 305.5 per cent increase in root length and 302.9 per cent increase in root weight over untreated control was registered (Plate 6). This was followed by seedling root-dip + foliar spray and seedling root-dip + soil application and it was on par in their effect. Individual application of the extract as seedling root-dip and foliar spray registered 107.2 and 66.4 per cent increase in root length and 120.0 and 107.9 per cent increase in root weight respectively and they found to be on par with each other. The increase in root length (36.4 %) and weight (48.6 %) was lowest in the method of soil application.

Yield parameters like number of fruits and yield was also maximum in the combined application of all the three methods registering 9.0 and 480.5g respectively, which remained significantly superior. The next best came the seedling root-dip + foliar spray recording and seedling root-dip + soil application registering 8.6 and 8.3 number of fruits and 400.6 385.0 g fruit yield respectively, but the difference between these two method of application is not significant. Whereas single method of seedling root-dip recorded 274.0 g yield and found to be on par with foliar spray of the extract (Fig. 6). The lowest number of fruits (3.0) and fruit yield (200.7 g) was obtained in soil application of *A. squamosa* extract as against 2.6 fruits and 127.5 g fruit yield in control.

4.8.1.1.2. Nematode population

The nematode population both in root and soil was significantly reduced by all the treatments. Reduction in gall index ranged from 1.3 to 3.6 by different treatments,

Fig. 6. Effect of *A. squamosa* against *M. incognita* on tomato – Plant growth and yield



recording lowest in the combination of three methods and highest in the individual method of soil application. Gall index in rest of the treatments ranged from 1.6 to 3.0 (Table 14).

Combined application made through root-dip + foliar spray + soil application reduced the number of females/g of root to the extent of 79.0 per cent and remained superior among the different method of application. Root-dip + foliar spray was the next best with 73.8 per cent decrease and this was closely nearer to root-dip + soil application with 71.0 per cent decrease over untreated control. Whereas individual method of root-dip alone decreased 44.4 per cent in number of females/g root and its performance on par with foliar spray (41.8 %). While the lowest reduction in number of females/g root was recorded in soil application with only 20.9 per cent.

A similar behaviour was also noticed with respect to number of egg masses/g root and number of eggs/egg mass. The reduction in number of egg masses (85.0) and eggs/egg mass (71.1) was maximum in combined application of three methods and was followed by root-dip + foliar spray and root-dip + soil application expressing 64.1 and 61.6 per cent reduction in number of egg masses/g root and 63.4 and 62.5 per cent reduction in number of eggs/egg mass respectively and in both observations these treatments were on par. Least reduction in the above parameters was noticed in plants, which received the extracts through soil application alone.

This pattern was slightly altered in the case of soil nematode population. Root-dip + soil application was found to be on par with root-dip + foliar spray and foliar spray + soil application, but there exist significant difference between the last two treatment combination (Fig. 7). However, maximum reduction in soil nematode population (63.1 %) was registered in combined application of all the three methods. Individual application of

Table 14. Effect of *A. squamosa* extract against *M. incognita* on tomato - Nematode population

Treatments	Root knot index	No. of females /g root	Per cent decrease	No. of egg masses/g Root	Per cent decrease	No of eggs /egg mass	Per cent decrease	Soil population (250 g soil)	Per cent decrease
T1	3.0	67.0	44.4	36.0	43.7	122.0	45.7	301.6	26.7
T2	3.0	70.3	41.8	38.0	40.7	127.3	43.2	310.6	24.3
T3	3.6	95.3	20.9	51.0	20.3	167.6	25.4	340.3	17.2
T4	1.6	31.6	73.8	23.0	64.1	82.3	63.4	195.0	52.6
T5	1.9	35.0	71.0	24.6	61.6	84.3	62.5	201.3	51.0
T6	2.6	52.0	56.8	32.0	50.0	108.6	51.7	208.6	49.4
T7	1.3	25.3	79.0	9.6	85.0	65.0	71.1	152.0	63.1
T8	5.0	120.6		64.0		224.0		411.0	
CD (P=0.05)	0.28	5.03		3.90		8.10		11.41	

T7: Root-dip + Foliar spray + Soil application

T4: Root-dip + Foliar spray

T1: Root-dip

T8: Untreated control

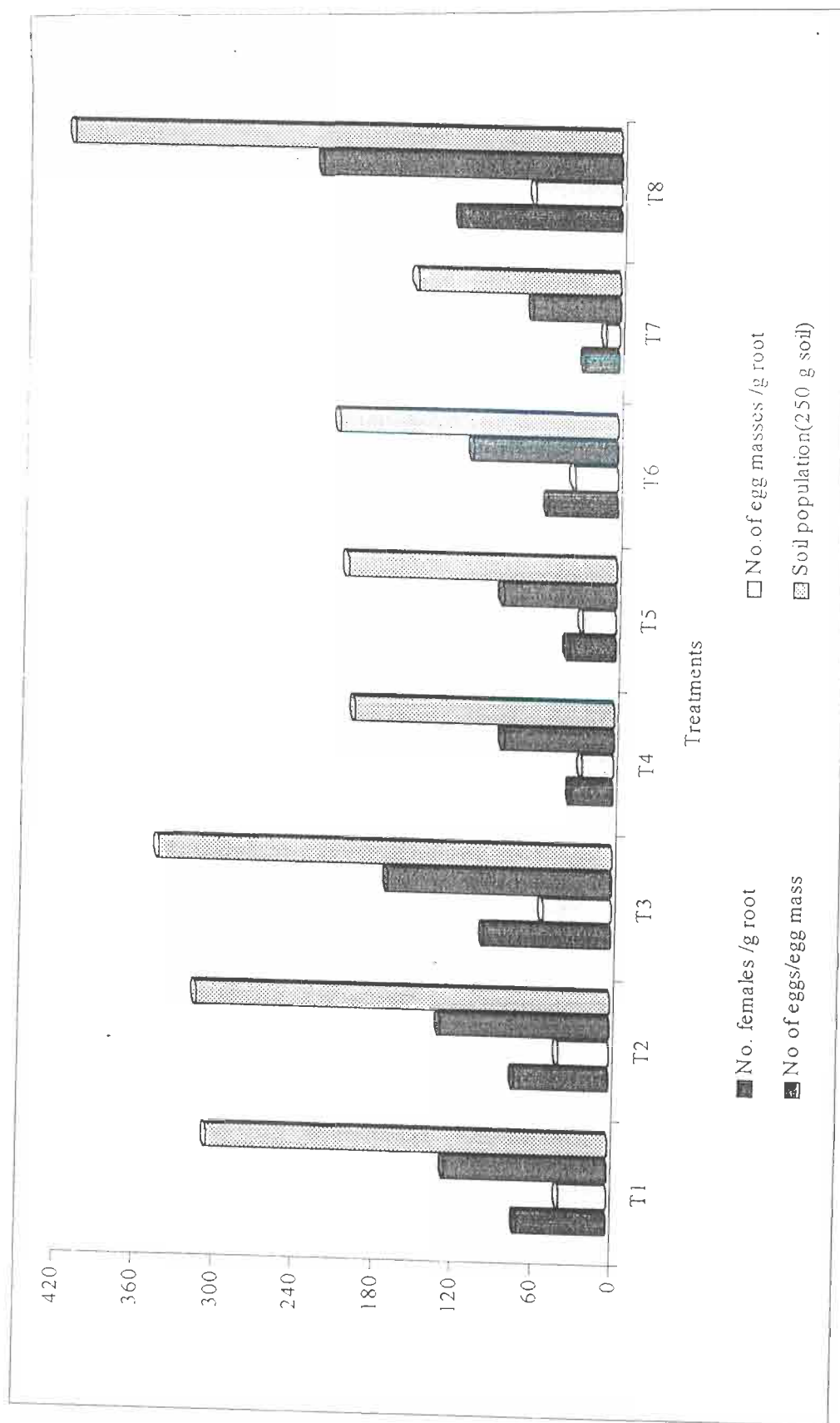
T5: Root-dip + Soil application

T2: Foliar spray

T6: Foliar spray + Soil application

T3: Soil application

Fig. 7. Effect of *A. squamosa* against *M. incognita* on tomato – Nematode population



the *A. squamosa* extract as root-dip recorded 26.7 per cent and it was on par with foliar spray in reducing soil nematode population (24.3 %). Maximum soil nematode population was registered in plants treated with extract through soil.

4.8.1.2. Brinjal

4.8.1.2.1. Plant growth parameters

When different method of application of *A. squamosa* extract like seedling bare-root-dip, foliar spray, soil application and their combination was attempted, an enhancement in the shoot height ranged from 40.8 cm to 60.0 cm (Plate 7; Fig. 8). Among the different method of application, combined application of seedling bare root-dip + foliar spray + soil application recorded the maximum increase in shoot height (140 %) over untreated control, followed by seedling bare root-dip + foliar spray, seedling root-dip + soil application and foliar spray + soil application recorded 120.4, 114.4 and 106.8 per cent increase respectively. All these treatments were on par with each other. When individual application of the *A. squamosa* extract as seedling root-dip, foliar spray and soil application was imposed, an increase in shoot length of 90, 70.4 and 63.2 per cent, respectively was noticed (Table 15).

A significant increase in shoot weight of plants (278.6 %) over control was noticed in the combined application of all the three methods. This was followed by seedling root-dip + foliar spray and seedling root-dip + soil application. When treatments were imposed individually, seedling root-dip alone recorded highest per cent increase of 104.9 and it was on par with foliar spray (97.4 %). Soil application gave the lowest per cent increase over control (48.1 %).

Fig. 8. Effect of *A. squamosa* against *M. incognita* on brinjal – Plant growth and yield

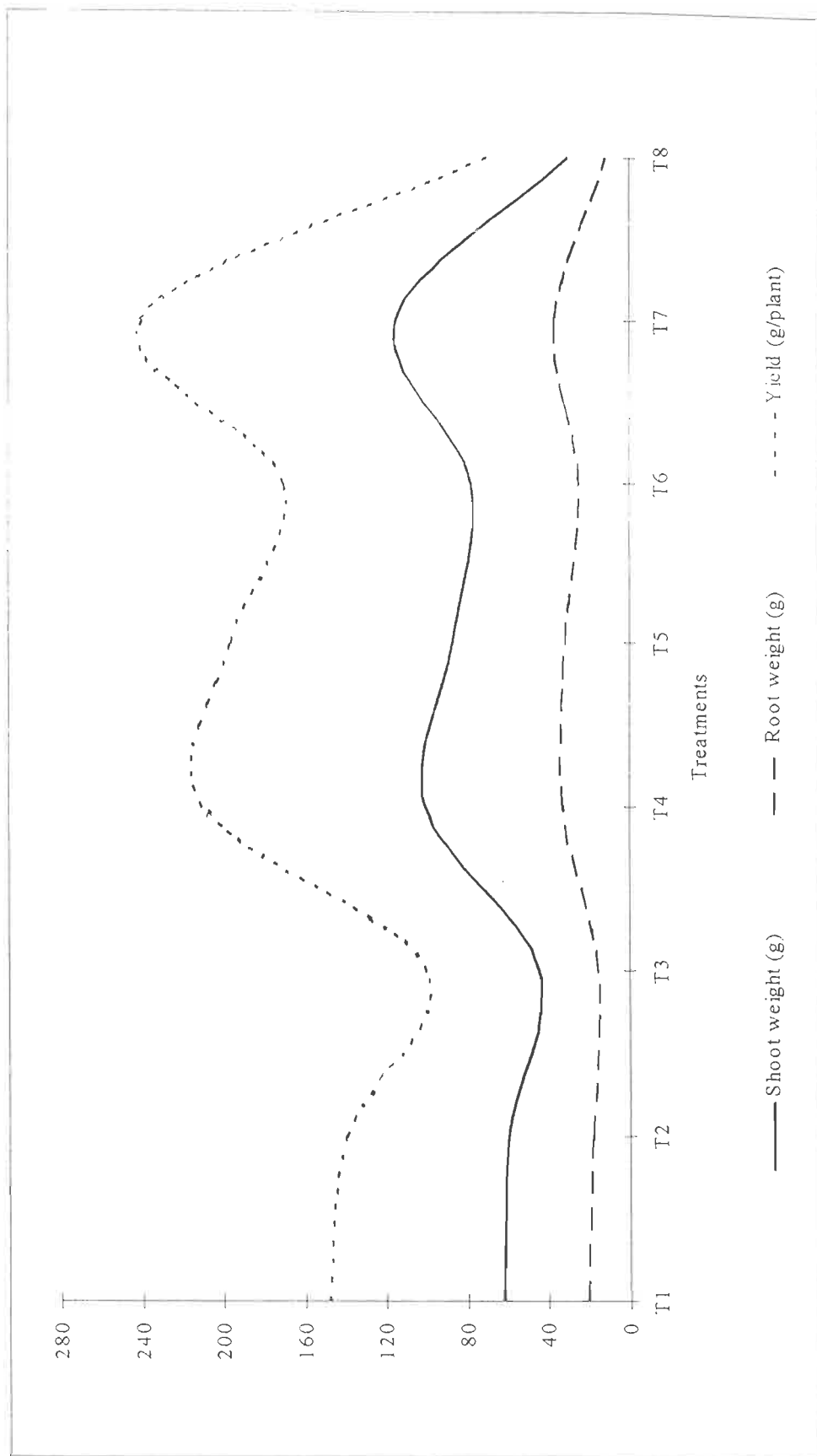


Plate 7. Effect of *A. squamosa* against *M. incognita* on brinjal – Shoot

T₁: Root-dip (RD)

T₂: Foliar spray (FS)

T₃: Soil application (SA)

T₄: RD + FS

T₅: RD + SA

T₆: FS + SA

T₇: RD + FS + SA

T₈: Untreated control

Plate 8. Effect of *A. squamosa* against *M. incognita* on brinjal – Root

Plate 7



Plate 8



Maximum per cent increase over untreated control was registered for length (239.2 %) and weight (208.3 %) of the roots in combined application of all the three methods (Plate 8). The next best treatments, which seemed to be on par, was seedling root-dip + foliar spray and seedling root-dip + soil application for both the parameters. In case of individual application of the extract as seedling root-dip and foliar spray, an increase of 75.7 and 58.9 per cent in root length and 71.6 and 54.2 per cent in root weight was noticed respectively. In soil application, both the parameters accounted for a least increase of 44.8 per cent and 31.7 per cent respectively.

Important yield parameters like number of fruits and yield was significantly higher in the combined application of all the three methods registering 7.0 and 240.0 g respectively. Though, seedling root-dip + foliar spray (5.0 and 210.70 g) and seedling root-dip + soil application (5.0 and 196.50 g) reside as the next best treatments for both the parameters, they were statistically non-significant. Whereas seedling root-dip alone recorded 147.8 g yield and found to be in concordance statistically with foliar spray of the extract (Fig. 8). The lowest number of fruits (2.6) and fruit yield (100.0 g) was obtained in the method of soil application of the extract as against 2.0 fruits and 70.4 g fruit yield in control.

4.8.1.2.2. Nematode population

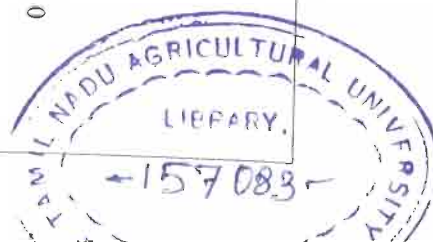
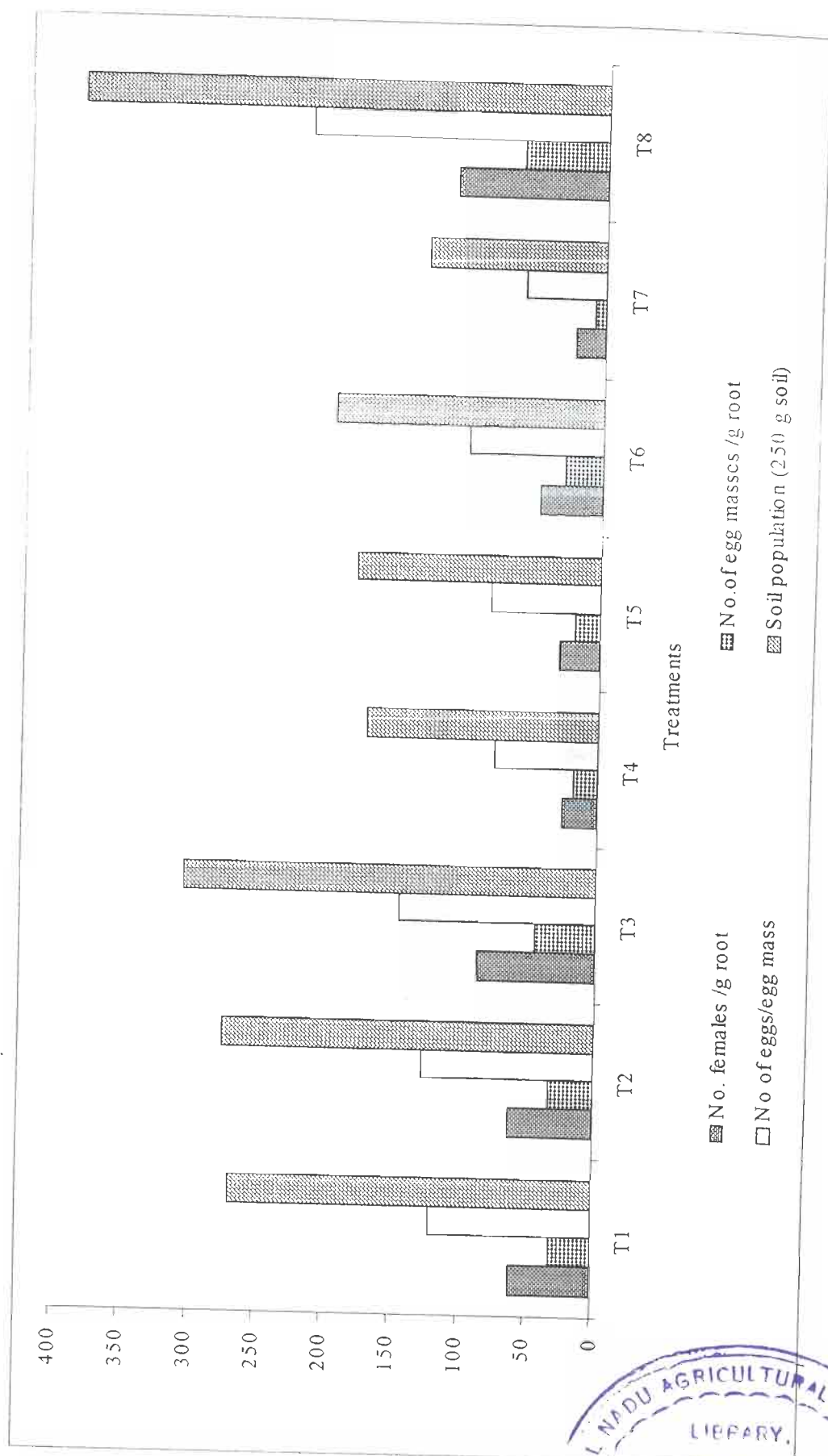
All the treatments reduced the density of nematodes significantly both in root and soil. This drastic reduction in gall index accounted for 1.3 to 3.6, recording the lowest in the combination of three methods and the highest in the individual soil application, while gall index in rest of the treatments ranged between 1.6 to 2.9.

Root-dip + foliar spray + soil application reduced the number of females/g of root to the extent of 81.4 per cent and remained superior among all the treatments. Root-dip + foliar spray was the next best with 76.2 per cent decrease and this was closely related to root-dip + soil application with 72.8 per cent decrease over untreated control (Table 16). In case of individual application, root-dip alone decreased 45.4 per cent in number of females/g root and it was on par with foliar spray (43.3 %). The lowest reduction in number of females/g root was recorded in soil application with only 21.8 per cent.

The same trend was also noticed with respect to number of egg masses/g root and number of eggs/egg mass. Maximum of 86.9 and 74.1 per cent reduction in number of egg masses and eggs/egg mass respectively was observed in combination of three methods of application. It was followed by root-dip + foliar spray and root-dip + soil application expressing 70.7 and 69.0 per cent reduction in number of egg masses/g root and 64.7 and 63.2 per cent reduction in number of eggs/egg mass respectively. Both these parameters in these treatments were on par. Least reduction in the above parameters was noticed in plants, which received the extract as soil application alone.

This pattern was slightly found to be altered with regard to soil nematode population. Root-dip + soil application was on par with foliar spray + soil application. However maximum reduction of soil nematode population (66.2 %) was registered in combined application of all the three methods. Individual application of the *A. squamosa* extract as root-dip recorded 30.4 per cent reduction and was on par with foliar spray (28.8 %). Maximum soil nematode population in the treatments was registered in pots treated with extract only through soil application (Fig. 9).

Fig. 9. Effect of *A. squamosa* against *M. incognita* on brinjal – Nematode population



4.8.2. *B. scandens*

4.8.2.1. Tomato

4.8.2.1.1. Plant growth parameters

Among the different method of application, combined application of seedling bare root-dip + foliar spray + soil application recorded the maximum increase in shoot height (111.6 %) over control, followed by seedling bare root-dip + foliar spray, seedling root-dip + soil application and foliar spray + soil application recording 100.0, 89.0 and 72.8 per cent increase over untreated control respectively. However the first two combined method of application were on par with each other. Individual application of the *A. squamosa* extract as seedling root-dip, foliar spray and soil application recorded 64.9, 46.4 and 37.5 per cent increase in shoot length, respectively (Table 17; Plate 9).

Shoot weight of plants in the combined application of all the three methods registered highest per cent increase (243.7) over control. Seedlings root-dip alone recorded 65.0 per cent increase and it was on par with foliar spray (56.6 %). Whereas soil application gave lowest per cent increase over control (33.7 %).

Of all the three methods a profound increase in root length and weight registered a maximum of 250.50 and 281.60 per cent over untreated control in combined method of application. This was followed by seedling root-dip + foliar spray and seedling root-dip + soil application (Plate 10). The effect of individual method of application as seedling root-dip (79.3 %) or as foliar spray (66.4 %) was similar and their effect was on par with each other. However, soil application gave the lowest per cent increase of root length (18.8 %) and root weight (25.0 %) comparatively.

Plate 9. Effect of *B. scandens* against *M. incognita* on tomato – Shoot

T₁: Root-dip (RD)

T₂: Foliar spray (FS)

T₃: Soil application (SA)

T₄: RD + FS

T₅: RD + SA

T₆: FS + SA

T₇: RD + FS + SA

T₈: Untreated control

Plate 10. Effect of *B. scandens* against *M. incognita* on tomato – Root

Plate 9



Plate 10



Even the parameters that determine the yield components like number of fruits and yield was maximum in the combined application registering 7.3 and 350.30 g respectively. This response was followed by seedling root-dip + foliar spray and seedling root-dip + soil application recording 6.0 and 310.0 g and 5.6 and 295.5 g respectively, but with no significant difference in yield component parameters. Whereas seedling root-dip alone recorded 215.0 g yield and found to be on par with foliar spray of the extract (210.6 g). The lowest number of fruits (3.0) and fruit yield (151.3 g) was obtained in soil application of *B. scandens* extract as against 2.0 fruits and 125.0 g fruit yield in control.

4.8.2.1.2. Nematode population

The multiplication of nematode both in root and soil was significantly reduced by all the treatments. Maximum gall index was recorded in the control with 5.0, while the lowest (1.3) in the combination of three methods. Gall index in rest of the treatments ranged from 2.0 to 4.3.

Root-dip + foliar spray + soil application reduced the number of females/g of root to the extent of 69.5 per cent. Root-dip + foliar spray was the next best with 67.5 per cent decrease and this was on par with root-dip + soil application (63.2 %). Root-dip alone decreased number of females/g root by 34.5 per cent and was on par with foliar spray (31.5 %). The lowest reduction (13.8 %) in number of females/g root was recorded in soil application. (Table 18).

The same trend was also noticed with respect to number of egg masses/g root and number of eggs/egg mass. The per cent reduction in number of egg masses (79.6 %) and eggs/egg mass (46.6 %) was maximum in combination of three methods of application.

Least reduction in the number of egg masses/g root (14.3 %) and number of eggs/egg mass (15.2 %) was noticed in plants, which received soil application alone.

The same pattern of influence prevailed on soil nematode population with a maximum reduction (54.3 %) in combined application of all the three methods (Fig. 10). Whereas individual applications of the *B. scandens* extract as root-dip recorded 23.3 per cent reduction and was on par with foliar spray (21.7 %).

4.8.2.2. Brinjal

4.8.2.2.1. Plant growth parameters

In general, different method of application of *B. scandens* extract like seedling bare-root-dip, foliar spray, soil application and their combination enhanced the shoot height ranging from 31.5 cm to 50.0 cm (Plate 11). Among the different method of application, combined application of seedling bare root-dip + foliar spray + soil application recorded the maximum shoot height increase (100 %) over control, followed by seedling bare root-dip + foliar spray, seedling root-dip + soil application and foliar spray + soil application recording 71.2, 60.0 and 56.0 per cent increase over untreated control respectively. Individual application of the *B. scandens* extract as seedling root-dip, foliar spray and soil application recorded 42.4, 36.4 and 26.0 per cent increase in shoot length, respectively (Table 19).

Shoot weight of plants in the combined application of all the three methods registered 230.6 per cent increase over control and was also significantly superior over other treatments. In contrast, seedlings root-dip alone recorded 34.0 per cent increase and it was on par with foliar spray (27.2 %). Whereas soil application gave lowest per cent increase over control (20.3 %).

Fig. 10. Effect of *B. scandens* against *M. incognita* on tomato – Nematode population

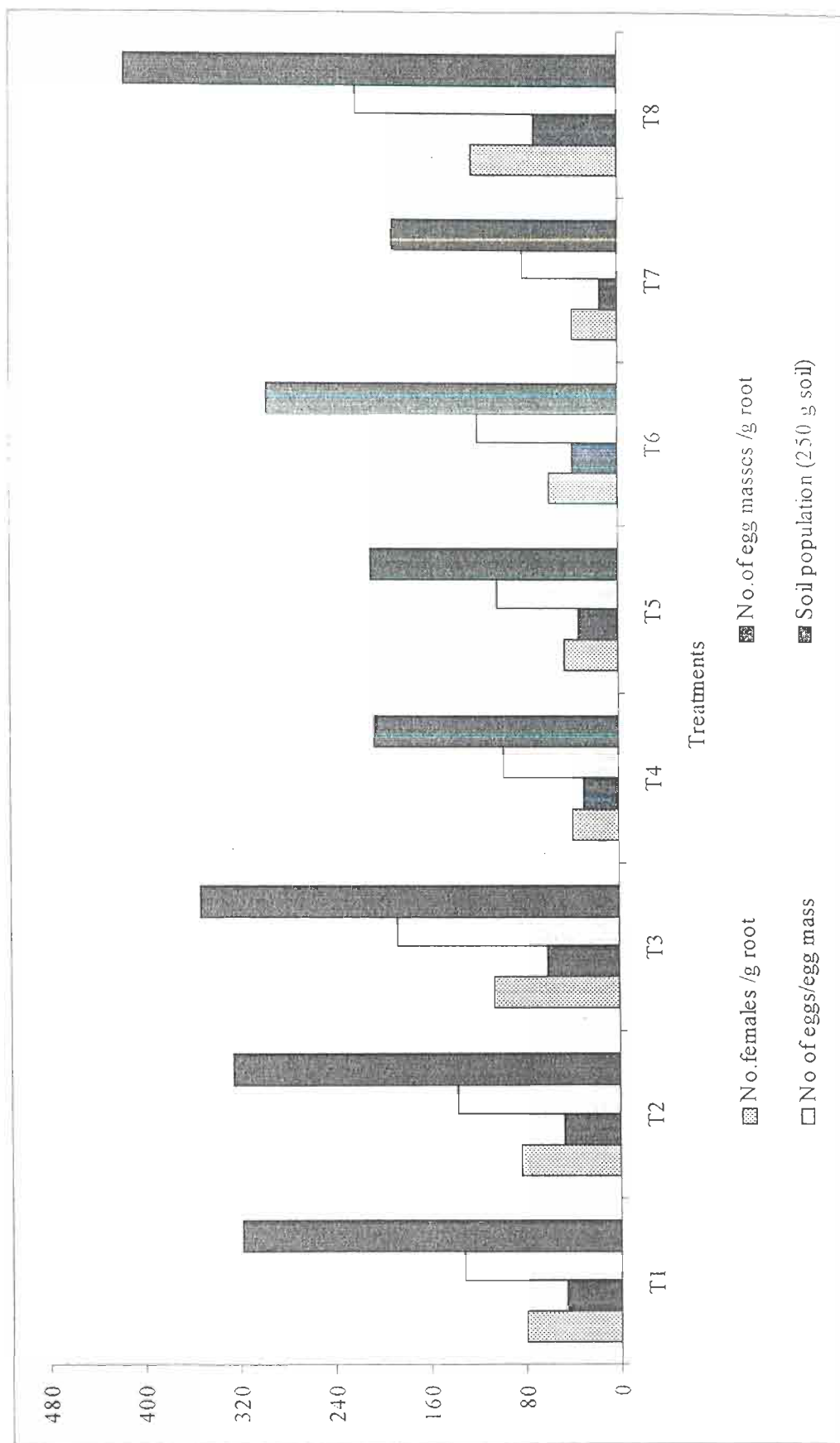


Plate 11. Effect of *B. scandens* against *M. incognita* on brinjal – Shoot

T₁: Root-dip (RD)

T₂: Foliar spray (FS)

T₃: Soil application (SA)

T₄: RD + FS

T₅: RD + SA

T₆: FS + SA

T₇: RD + FS + SA

T₈: Untreated control

Plate 12. Effect of *B. scandens* against *M. incognita* on brinjal – Root

Plate 11



Plate 12



Root length and weight registered a maximum of 142.8 and 174.7 per cent increase over untreated control, respectively in combination of three methods. The next best was seedling root-dip + foliar spray and seedling root-dip + soil application and in both the parameters these two treatments were on par. Individual application of the extract as seedling root-dip and foliar spray registered 51.4 and 26.2 per cent increase in root length and 63.5 and 45.8 per cent increase in root weight respectively and found to be on par with each other. The lowest increase in root length and weight was observed in soil application of *B. scandens* extract (Plate 12).

Yield parameters like number of fruits and yield was also maximum in the combined application of all the three methods registering 5.6 and 200.0 g respectively. Next best was the seedling root-dip + foliar spray recording 4.0 and 179.0 g and seedling root-dip + soil application registering 3.3 number of fruits and 170.0 g yield with no significant difference. Whereas seedling root-dip alone recorded 120.0 g yield and found to be on par with foliar spray of the extract (117.4 g). The lowest yield parameters were recorded in soil application of *B. scandens* extract as against 1.3 fruits and 73.5 g fruit yield in control.

4.8.2.2.2. Nematode population

The development of nematodes both in root and soil was significantly reduced by all the treatments. Gall index ranged from 1.3 to 4.0 by different treatments, recording the lowest in the combination of three methods and the highest in the individual soil application. Gall index of 5.0 was recorded in the untreated control plant.

Root-dip + foliar spray + soil application reduced the number of females/g of root to the extent of 74.2 per cent. Root-dip + foliar spray was the next best with 73.6 per cent

decrease and root-dip + soil application with 70.8 per cent decrease in number of females/g root over untreated control (Table 20). Root-dip alone decreased the number of females/g root by 39.5 per cent and it was on par with foliar spray (38.4 %). The lowest reduction in number of females/g root was recorded in soil application with only 18.8 per cent decrease over untreated control.

The same trend was also noticed with respect to number of egg masses/g root and number of eggs/egg mass. Maximum of 81.6 and 67.2 per cent reduction in number of egg masses and eggs/egg mass respectively was observed in combination of three method of application. The lowest reduction in the above observation was noticed in plants, which received the extract as soil application alone (Fig. 11).

Effect of root-dip + soil application was found to be on par with root-dip + foliar spray in the reduction of soil nematode population. However, maximum reduction of soil nematode population (59.3 %) was registered in combined application of all the three methods. Individual application of the *B. scandens* extract as root-dip recorded 25.2 per cent reduction. Maximum soil nematode population was registered in pots treated with extract as soil application.

4.8.3. *T. erecta*

4.8.3.1. Tomato

4.8.3.1.2. Plant growth parameters

Wholly, different method of application of *T. erecta* extract like seedling bare-root-dip, foliar spray, soil application and their combination enhanced the shoot height ranging from 45.8 cm to 68.5 cm. Among the different method of application, combined application of seedling bare root-dip + foliar spray + soil application recorded the

Table 20. Effect of *B. scandesn* extract against *M. incognita* on brinjal - Nematode population

Treatments	Root knot index	No.of females/g root	Per cent decrease	No.of egg masses/g root	Per cent decrease	No of eggs/egg mass	Per cent decrease	Soil population (250 g soil)	Per cent decrease
T1	3.0	71.0	39.5	38.0	41.5	128.3	40.2	292.0	25.2
T2	3.6	72.3	38.4	40.6	37.6	130.0	39.4	300.3	23.1
T3	4.0	95.3	18.8	53.0	18.5	160.6	25.0	320.0	18.0
T4	2.0	31.0	73.6	22.0	66.2	85.3	60.2	184.6	52.6
T5	2.6	34.3	70.8	24.0	63.1	90.0	58.0	188.3	51.7
T6	2.9	52.3	55.4	35.0	45.2	108.6	49.3	262.0	32.8
T7	1.3	30.3	74.2	12.0	81.6	70.3	67.2	158.6	59.3
T8	5.0	117.3		65.0		214.3		390.0	
CD (P=0.05)	0.46	5.11		3.45		7.05		7.65	

T7: Root-dip + Foliar spray + Soil application

T8: Untreated control

T4: Root-dip + Foliar spray

T5: Root-dip + Soil application

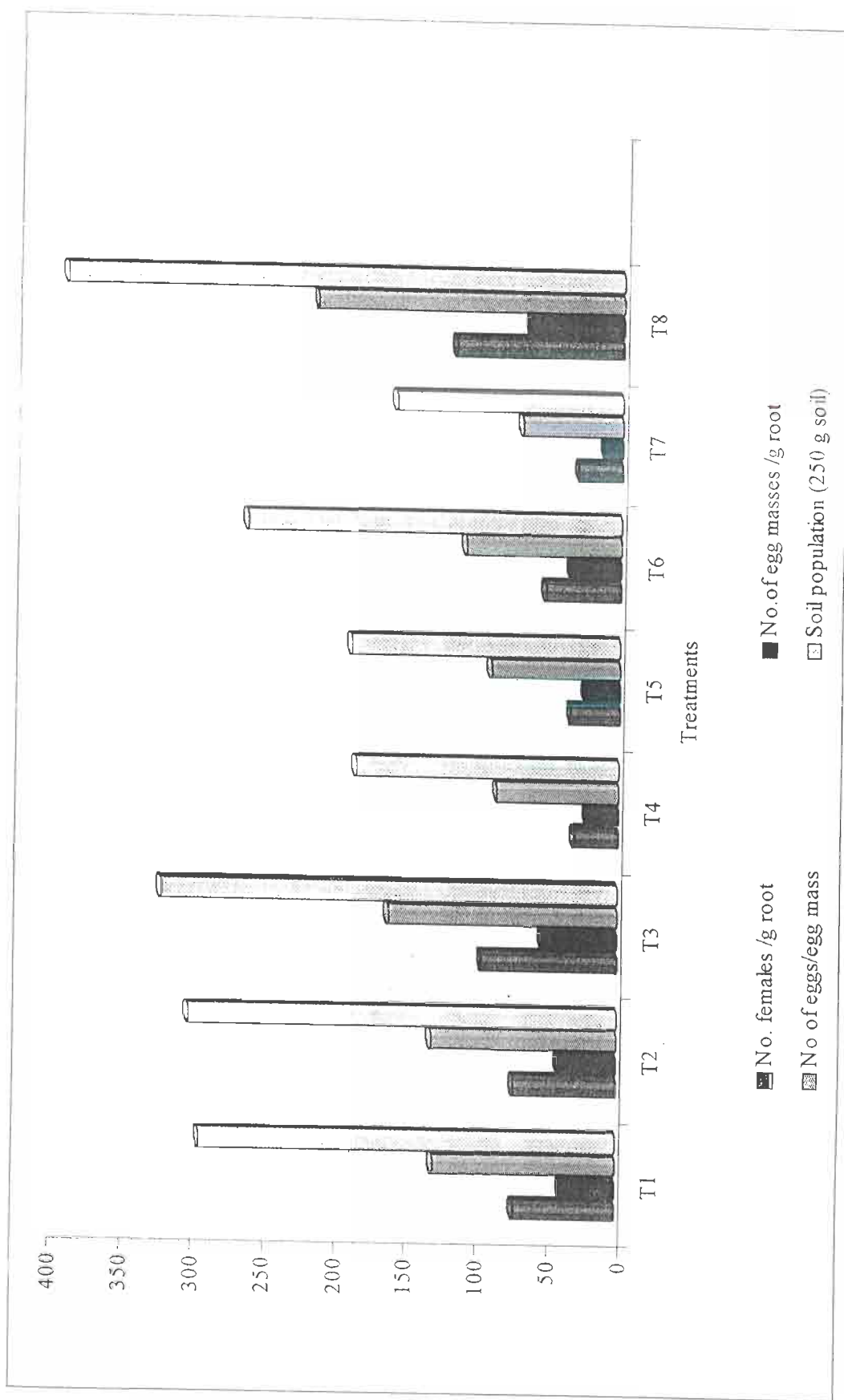
T6: Foliar spray + Soil application

T1: Root-dip

T2: Foliar spray

T3: Soil application

Fig. 11. Effect of *B. scandens* against *M. incognita* on brinjal – Nematode population



maximum shoot height increase (133.8 %) over control. Seedling bare root-dip + foliar spray, seedling root-dip + soil application and foliar spray + soil application recorded 106.2, 99.0 and 90.5 per cent increase over untreated control respectively (Plate 13). Apart from these an individual application of the *T. erecta* extract as seedling root-dip, foliar spray and soil application also recorded 72.7, 63.8 and 56.3 per cent increase in shoot length, respectively.

Shoot weight of plants in the combined application of all the three methods registered 263.9 per cent increase over control followed by seedling root-dip + foliar spray (229.4 %) and seedling root-dip + soil application (179.8 %). Seedlings root-dip alone recorded 95.4 per cent increase and soil application gave the lowest per cent increase in shoot weight over control. In combination of three methods, a maximum of 257.50 per cent increase in root length and 287.87 per cent increase in root weight over untreated control was registered (Table 21). Individual application of extract as seedling root-dip and foliar spray registered 80.5 and 59.3 per cent increase in shoot length and 87.8 and 75.6 per cent increase in root weight respectively and they found to be on par with each other. The lowest increase in root length (20.4 %) and root weight (56.5 %) was observed in soil application (Plate 14).

The parameters that determine the yield components like number of fruits and yield was higher in combined application registering 8.0 and 412.5 g respectively. This response was followed by seedling root-dip + foliar spray recording 7.0 and 370.0 g and seedling root-dip + soil application registering 7.0 and 360.0 g respectively, but with no significant difference. Whereas seedling root-dip alone recorded 235.0 g yield and found to be on par with foliar spray (226.0 g). The lowest number of fruits (3.0) and fruit yield

Plate 13. Effect of *T. erecta* against *M. incognita* on tomato – Shoot

T₁: Root-dip (RD)

T₂: Foliar spray (FS)

T₃: Soil application (SA)

T₄: RD + FS

T₅: RD + SA

T₆: FS + SA

T₇: RD + FS + SA

T₈: Untreated control

Plate 14. Effect of *T. erecta* against *M. incognita* on tomato – Root

Plate 13



Plate 14



(177.6g) was obtained in soil application as against 2.6 fruits and 122.0 g fruit yield in control.

4.8.3.1.3. Nematode population

The development of nematode both in root and soil was significantly reduced by all the treatments. Gall index ranged from 1.3 to 3.9 in different treatments, recording lowest in the combination of three methods and highest in the individual method of application of extract through soil. Maximum gall index was registered in the untreated control plants (5.0).

Root-dip + foliar spray + soil application reduced the number of females/g of root to the extent of 78.2 per cent and remained superior among all the treatments. Root-dip + foliar spray was the next best with 72.0 per cent decrease and root-dip + soil application with 70.2 per cent over untreated control (Table 22). Whereas root-dip alone reduced the number of females/g root by 42.2 per cent and its effect was on par with foliar spray (39.6 %). While the lowest reduction (18.1 %) in number of females/g root was recorded in the application of extract through soil.

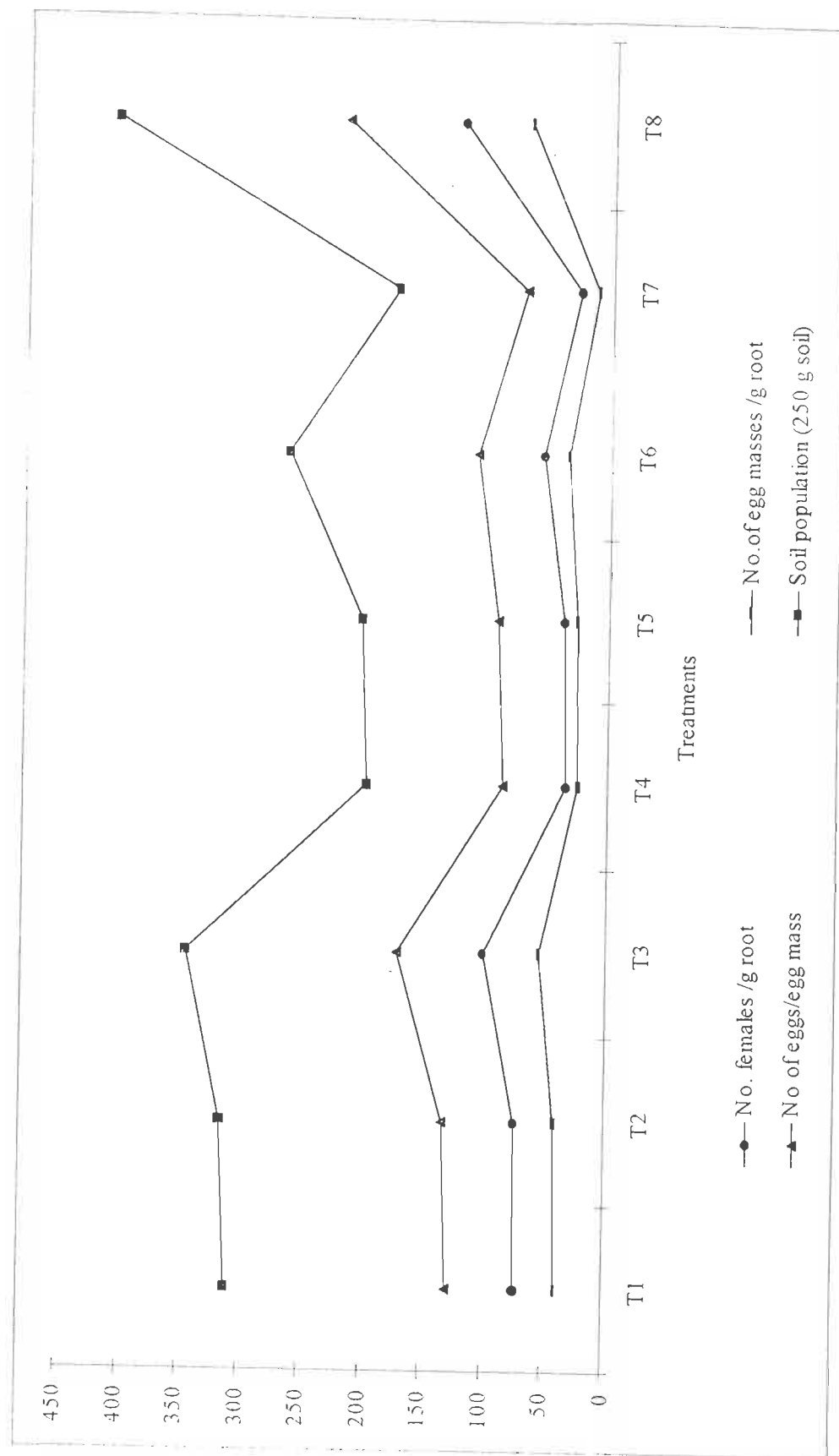
Maximum of 81.9 and 67.4 per cent reduction in number of egg masses and eggs/egg mass respectively was observed in combination of three method of application and was followed by root-dip + foliar spray and root-dip + soil application expressing 62.5 and 60.5 per cent reduction in number of egg masses/g root and 60.6 and 58.3 per cent reduction in number of eggs/egg mass respectively and these treatments were on par in reducing number of egg masses and eggs/egg mass (Fig. 12). Least reduction in the above nematode counts was noticed in plants, which received the extract as soil application alone.

Table 22. Effect of *T. erecta* extract against *M. incognita* on tomato - Nematode population

Treatments	Root knot index	No. of females/g Root	Per cent decrease	No. of egg masses/g root	Per cent decrease	No of eggs/egg mass	Per cent decrease	Soil population (250 g soil)	Per cent decrease
T1	3.0	73.0	41.2	39.3	42.5	129.0	41.1	310.0	24.0
T2	3.3	75.0	39.6	42.3	38.1	134.0	38.8	316.3	22.5
T3	3.9	101.6	18.1	55.6	18.6	172.0	20.1	345.0	15.4
T4	1.6	34.6	72.0	25.6	62.6	86.3	60.6	198.0	51.5
T5	2.0	37.0	70.2	27.0	60.5	91.3	58.3	203.6	50.1
T6	2.0	55.0	55.7	35.0	48.8	110.0	49.8	265.0	35.1
T7	1.3	27.0	78.2	12.3	81.9	71.3	67.4	175.3	57.0
T8	5.0	124.0		68.3		219.0		408.0	
CD (P=0.05)	0.39	5.14		3.80		8.23		9.66	

T1: Root-dip	T4: Root-dip + Foliar spray	T7: Root-dip + Foliar spray + Soil application
T2: Foliar spray	T5: Root-dip + Soil application	T8: Untreated control
T3: Soil application	T6: Foliar spray + Soil application	

Fig. 12. Effect of *T. erecta* against *M. incognita* on tomato – Nematode population



Maximum reduction in soil nematode population (63.1 %) was registered in combined application of all the three methods while root-dip + soil application was found to be on par with root-dip + foliar spray. Individual application of the *T. erecta* extract as root-dip recorded 24.0 per cent reduction and was on par with foliar spray (22.5 %). Maximum soil nematode population was registered in pots treated with extract as single method of soil application.

4.8.3.2. Brinjal

4.8.3.2.1. Plant growth parameters

When different method of application of *T. erecta* extract like seedling bare-root-dip, foliar spray, soil application and their combination attempted, an enhancement in shoot height was ranging from 33.8 cm to 57.6 cm (Plate 15). Among the different method of application, combined application of seedling bare root-dip + foliar spray + soil application recorded the maximum shoot height increase (117.4 %) over control. The next best treatment was seedling bare root-dip + foliar spray with 90.6 per cent increase in shoot height. Individual application of *T. erecta* extract as seedling root-dip, foliar spray and soil application recorded 60.4, 38.1 and 37.6 per cent increase in shoot length, respectively (Table 23).

A significant increase in shoot weight of plant (247.3 %) over control was noticed in combined application. This was followed by seedling root-dip + foliar spray and seedling root-dip + soil application. When treatments were imposed individually, seedling root-dip alone recorded 65.6 per cent increase comparatively and it was on par with foliar spray (55.2 %). While at the least, soil application gave the lowest per cent increase over control (34.5 %).

Plate 15. Effect of *T. erecta* against *M. incognita* on brinjal – Shoot

T₁: Root-dip (RD)

T₂: Foliar spray (FS)

T₃: Soil application (SA)

T₄: RD + FS

T₅: RD + SA

T₆: FS + SA

T₇: RD + FS + SA

T₈: Untreated control

Plate 16. Effect of *T. erecta* against *M. incognita* on brinjal – Root

Plate 15



Plate 16



Per cent increase over untreated control with regard to length (215.0 %) and weight (229.20 %) of the roots was maximum in combined application. The next best treatments which seemed to be on par was seedling root-dip + foliar spray and seedling root-dip + soil application for both the parameters. In case of individual application of extract as seedling root-dip and foliar spray, an increase of 60.0 and 44.0 per cent and 66.4 and 56.4 per cent in root length and weight was noticed respectively (Plate 16).

Important yield parameters like number of fruits and yield was significantly higher in the combined application of all the three methods registering 6.3 and 215.5g respectively. Though seedling root-dip + foliar spray (94.3 and 200.0 g) and seedling root-dip + soil application (4 and 192.5 g) reside as the next best treatments for both the parameters they were statistically non-significant. Whereas seedling root-dip alone recorded 135.6 g yield and found to be on par with foliar spray of the extract. The lowest number of fruits (2.0) and fruit yield (94.1 g) was noticed with *T. erecta* extract application through soil.

4.8.3.2.1. Nematode population

All the treatments significantly reduced the density of nematodes both in root and soil. This drastic reduction in gall index ranged from 1.3 to 3.6, recording the lowest in the combination of three methods and the highest in the individual soil application. While gall index in rest of the treatments ranged from 1.9 to 3.6 as against 5.0 in the untreated control (Table 24).

Root-dip + foliar spray + soil application reduced the number of females/g of root to the extent of 78.1 per cent and stands first among all the treatments. Root-dip + foliar spray with 74.6 per cent decrease was the next best. Method of application of extract as

root-dip alone decreased 42.8 per cent of number of females/g root and on par with foliar spray (40.3 %) in their effect. The lowest reduction was recorded in soil application with only 20.1 per cent decrease in number of females/g root.

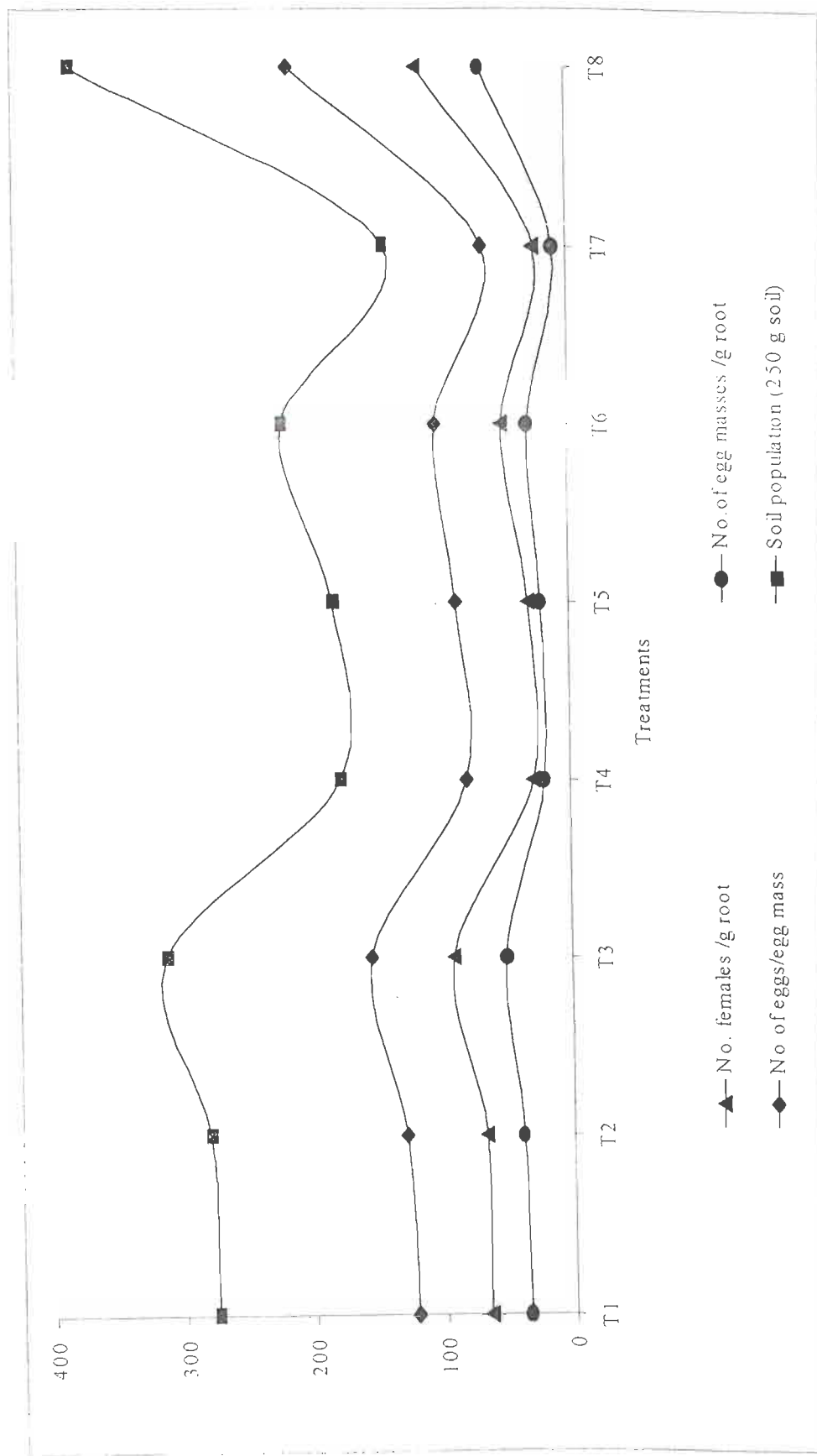
The same trend was also noticed in respect to number of egg masses/g root and number of eggs/egg mass. Highest per cent reduction in number of egg masses (83.5 %) and eggs/egg mass (69.4 %) was observed in combination of three methods of application and was followed by root-dip + foliar spray and root-dip + soil application registering 68.1 and 65.5 per cent reduction in number of egg masses/g root and 62.3 and 58.9 per cent reduction in number of eggs/egg mass respectively and for both these parameters the above treatmental effect were on par (Fig. 13). Least reduction in the above parameters was noticed in plants, which received the extract as soil application alone.

With regard to soil nematode population, the maximum reduction of soil nematode population (62.9 %) was registered in combined application of all the three methods. However, root-dip + soil application was found to be on par with root-dip + foliar spray. Individual application of the *T. erecta* extract as root-dip recorded 27.6 per cent reduction and was on par with foliar spray (26.3 %). The extract applied through soil had little effect in the reduction of nematodes (312.6 nematodes/250 g soil).

4.9. Effect of *A. squamosa* extract against nematode-disease complex in tomato and brinjal.

4.9.1. Effect of *A. squamosa* on *M. incognita* and *F. oxysporum* f.sp. *lycopersici* disease complex in tomato.

Fig. 13. Effect of *T. erecta* against *M. incognita* on brinjal – Nematode population



4.9.1.1. Plant growth parameters

Combination of root-dip + foliar spray + soil application of the extract recorded the maximum shoot height of 59.5 cm which was significantly superior over other treatments. Root-dip + foliar spray was the next best (55.7 cm) exhibiting no statistical difference when compared to the effect of root-dip + soil application method (55.0 cm). Root-dip alone registered 44.0 cm plant height and it was on par with foliar spray (42.7 cm). Untreated control with nematode and fungus alone registered 30.1 and 29.0 cm as against 27.3 cm in their combination (Plate 17).

The similar influence of *A. squamosa* extract was seen in increasing shoot weight and root length with maximum of 113.0 g and 24.5 cm in the combination of three methods and 46.0 g and 13.1 cm in soil application respectively (Plate 18). Root-dip + soil application recorded 25.6 g of root weight and was on par with foliar spray (24.0 g) (Table 25).

Number of fruits (7.0) and yield (325.0 g) of tomato plants treated with root-dip + foliar spray + soil application was the maximum and statistically superior over other treatments. Following this treatment the yield recorded was higher in root-dip + foliar spray (285.8 g). Among the treatments, soil application of extract recorded the lowest yield of 140.5 g. Whereas nematode and fungus alone received plants registered 120.6 and 114.4 g respectively as against 94.0 g in nematode + fungus inoculated plant.

4.9.1.2. Nematode population

All the treatments with leaf extract of *A. squamosa* reduced the development of *M. incognita*. Minimum root-knot index of 1.3 was observed in plants treated with this extract as root-dip + foliar spray + soil application followed by root-dip + foliar spray

Table 25. Effect of *A. squamosa* extract against *M. incognita* and *F. oxysporum* f. sp. *lycopersici* disease complex in tomato - Plant growth parameters

Treatments	Shoot				Root				No of fruits	Yield g/plant	Per cent increase
	Height (cm)	Per cent increase	Weight (g)	Per cent increase	Length (cm)	Per cent increase	Weight (g)	Per cent increase			
T1	44.0	61.2	54.5	65.1	15.4	62.2	19.6	90.3	3.0	180.6	92.2
T2	42.7	56.4	52.0	57.6	15.0	57.9	18.5	79.6	2.3	172.0	83.0
T3	35.6	30.1	46.0	39.4	13.1	37.9	15.2	47.6	2.3	140.5	49.5
T4	55.7	104.1	88.2	167.3	21.5	126.4	30.0	191.3	5.0	285.8	204.1
T5	55.0	101.5	85.0	157.6	20.0	110.5	25.6	148.5	4.6	260.3	176.9
T6	48.1	76.2	67.5	104.6	17.0	78.9	24.0	133.1	3.6	216.5	130.4
T7	59.5	118.0	113.0	242.4	24.5	157.8	33.0	220.4	7.0	325.0	245.7
T8	30.1	10.3	42.6	29.1	11.4	20.0	13.1	27.2	2.0	120.6	28.3
T9	29.0	6.3	40.0	21.2	11.0	15.8	12.5	21.4	2.0	114.4	21.7
T10	27.3		33.0		9.5		10.3		2.0	94.0	
CD (P=0.05)	1.82		4.72		1.22		1.94		0.34	15.22	

T1: Root-dip, T2: Foliar spray, T3: Soil application, T4: Root-dip + Foliar spray, T5: Root-dip + Soil application, T6: Foliar spray + Soil

application, T7: Root-dip + Foliar spray + Soil application, T8: Nematode alone, T9: Fungus alone and T10: Nematode + fungus

Plate 17. Effect of *A. squamosa* against *M. incognita* and *F. oxysporum* f.sp. *lycopersici* disease complex in tomato – Shoot

1. Root-dip
2. Root - dip + Foliar Spray
3. Root - dip + Foliar Spray + Soil application
4. Untreated control

Plate 18. Effect of *A. squamosa* against *M. incognita* and *F. oxysporum* f.sp. *lycopersici* disease complex in tomato – Root

Plate 17



Plate 18



(1.6). Whereas maximum root knot index of 5.0 was recorded in plant inoculated only with nematodes and 4.6 in nematode + fungus inoculated untreated plants.

A broad range of number of females (19.0 to 75.0/g root) was noticed in all the treatments as against 102/g root in combination of both the organisms inoculated plant (Table 26). The least number of females was seen in combined application of all the three methods (81.4 %) followed by root-dip + foliar spray (74.3 %). Number of egg masses/g root and number of eggs/egg mass was also minimum in plants treated by the method of root-dip + foliar spray + soil application with 86.8 and 74.5 per cent reduction and was followed by root-dip + foliar spray with 72.2 and 66.7 per cent reduction over untreated and combined inoculation of both the organisms respectively. Lowest reduction in number of egg masses/g root (26.2 %) and number of eggs/egg mass (31.6%) was registered in soil application of the *A. squamosa* extract.

Soil nematode population (66.3 %) recorded in combined application was the lowest of all the three methods of application. Root-dip + foliar spray registered 56.9 per cent reduction and found to be on par with root-dip + soil application. On a whole individual method of application of the extract recorded 27.7 to 23.1 per cent reductions in soil nematode population (Fig. 14). In all the above observations, root-knot nematode multiplication was less in the presence of the pathogenic fungus, *F. oxysporum* f.sp. *lycopersici*.

Table 26. Effect of *A. squamosa* extract against *M. incognita* and *F. oxysporium fsp lycopersici* disease complex in tomato - Nematode

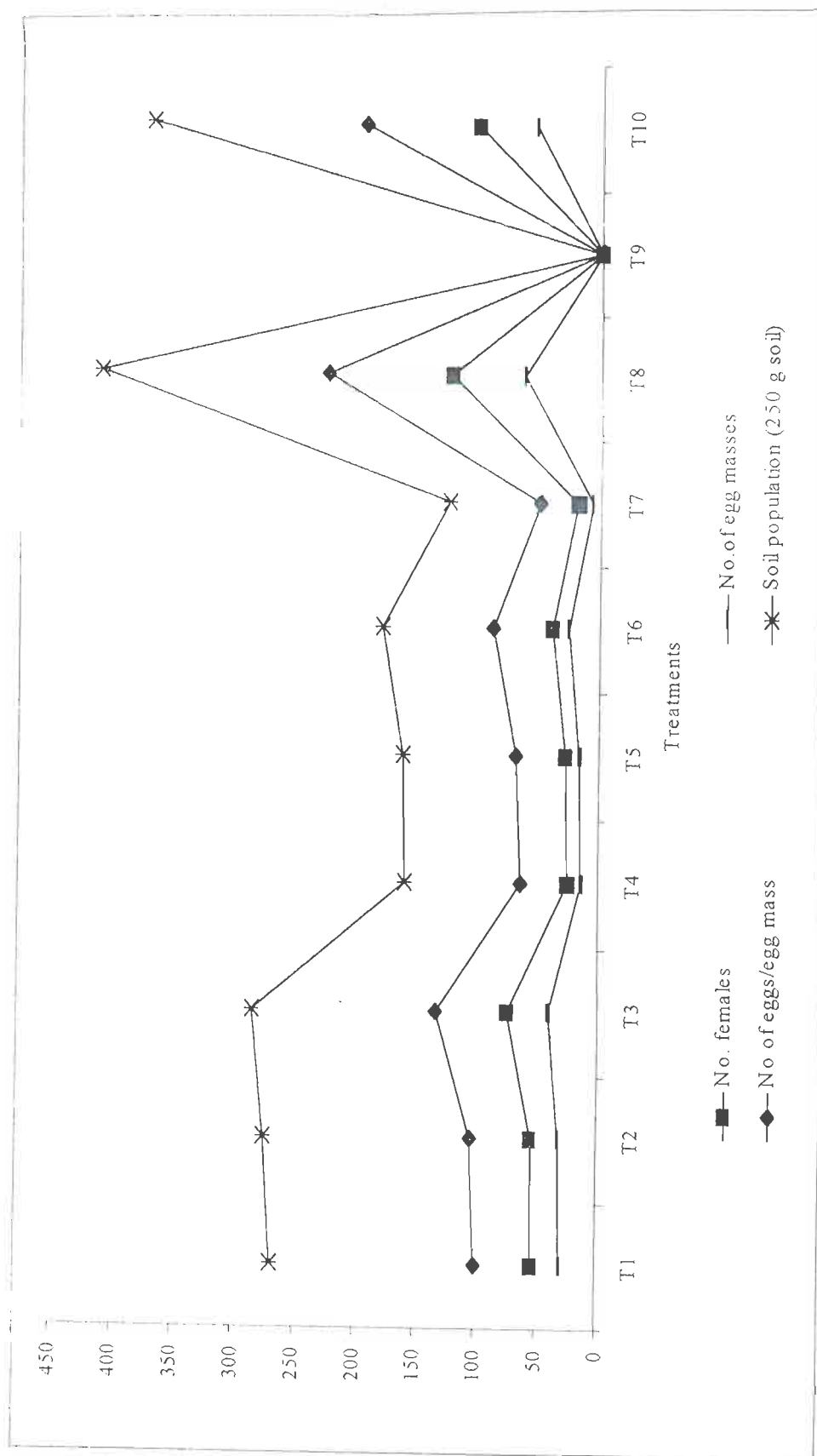
population

Treatments	Root knot index	No. of females/g root	Per cent decrease	No. of egg mass/g root	Per cent decrease	No of eggs/egg mass	Per cent decrease	Soil population (250 g soil)	Per cent decrease
T1	2.6	53.3	47.8	30.0	45.5	100.0	49.0	268.0	27.7
T2	2.9	55.0	46.1	32.0	41.8	104.3	46.8	275.3	25.8
T3	3.0	75.0	26.4	40.6	26.2	134.0	31.6	285.0	23.1
T4	1.6	26.3	74.3	15.3	72.2	65.3	66.7	160.0	56.9
T5	2.0	28.0	72.6	16.6	69.8	69.0	64.8	163.0	56.1
T6	2.3	40.0	60.8	26.0	53.0	88.6	54.8	180.0	51.4
T7	1.3	19.0	81.4	7.3	86.8	50.0	74.5	125.0	66.3
T8	5.0	123.6	+17.5	63.6	+13.5	226.0	+13.3	413.0	+11.4
T9	-	-	-	-	-	-	-	-	-
T10	4.6	102.0		55.0		196.0		370.6	
CD (P=0.05)	0.26	4.63		3.02		6.51		8.22	

T1: Root-dip, T2: Foliar spray, T3: Soil application, T4: Root-dip + Foliar spray, T5: Root-dip + Soil application, T6: Foliar spray + Soil

application, T7: Root-dip + Foliar spray + Soil application, T8: Nematode alone, T9: Fungus alone and T10: Nematode + fungus

Fig. 14. Effect of *A. squamosa* against *M. incognita* and *F. oxysporum* f.sp. *lycopersici* disease complex in tomato – Nematode population



4.9.2. Effect of *A. squamosa* on *M. incognita* and *R. solani* disease-complex in brinjal

4.9.2.1. Plant growth parameters

Shoot length of the plant was maximized (40 cm) in the combination of root-dip + foliar spray + soil application of the extract which was in addition significantly superior over other treatments (Plate 19). This was followed by root-dip + foliar spray (38.4 cm) but with no statistical difference to root-dip + soil application (37.1 cm). When the extract was treated individually as root-dip and foliar spray, they accounted for 33.6 and 36.5 cm respectively. Untreated control with nematode and fungus alone registered the shoot length of 25.2 and 24.1 cm as against 22.1 cm in their combination (Table 27).

The effect of *A. squamosa* extract in increasing shoot weight and root length was maximum (84.0 and 22.9 cm) in the combination of three methods and it was 31.2 g and 12.5 cm in soil application respectively (Plate 20). Root-dip + soil application recorded 24.3 g of root weight and was on par with foliar spray + soil application (23.0 g).

Maximum number of fruits (5.3) and yield (142.6 g) of tomato has registered in plant treated with root-dip + foliar spray + soil application. The next highest yield was recorded in root-dip + foliar spray (125.6 g). Among the treatments, the lowest yield was recorded in soil application of the extract showing 75.5 g. Untreated control with nematode and fungus alone registered 60.2 and 57.0 g respectively as against 50.1 g in nematode + fungus application.

4.9.2.2. Nematode population

In general, the treatments with leaf extract of *A. squamosa* reduced the development of *M. incognita*. Minimum root-knot index of 1.3 was observed in plants treated with extract as root-dip + foliar spray + soil application followed by root-dip +

Table 27. Effect of *A. squamosa* extract against *M. incognita* and *R. solani* disease complex in brinjal - Plant growth parameters

Treatments	Shoot				Root				No of fruits	Yield g/plant	Per cent increase
	Height (cm)	Per cent increase	Weight (g)	Per cent increase	Length (cm)	Per cent increase	Weight (g)	Per cent increase			
T1	33.6	52.1	37.3	35.6	14.2	57.8	17.8	74.6	2.0	88.1	75.9
T2	36.5	42.5	36.0	30.9	13.5	50.0	16.9	65.7	2.0	80.0	59.7
T3	28.0	26.7	31.2	13.5	12.5	38.9	14.5	42.2	1.9	75.5	40.7
T4	38.4	73.8	64.8	135.7	19.4	115.6	24.3	138.3	3.3	125.6	150.7
T5	37.1	67.9	58.4	112.4	16.6	84.4	23.0	125.5	3.0	114.0	127.5
T6	35.0	58.4	50.0	81.8	16.0	77.8	20.1	97.1	2.3	103.2	105.9
T7	40.0	80.9	84.0	205.5	22.9	154.4	28.0	174.5	5.3	142.6	185.2
T8	25.2	14.1	33.1	20.4	12.0	33.3	13.4	31.4	1.6	60.2	20.6
T9	24.1	9.1	31.8	15.7	11.4	26.7	12.8	25.5	1.6	57.0	13.6
T10	22.1		27.5		9.0		10.2		1.3	50.1	
CD (P=0.05)	1.53		2.30		1.75		2.36		0.30	10.26	

T1: Root-dip, T2: Foliar spray, T3: Soil application, T4: Root-dip + Foliar spray, T5: Root-dip + Soil application, T6: Foliar spray + Soil application, T7: Root-dip + Foliar spray + Soil application, T8: Nematode alone, T9: Fungus alone and T10: Nematode + fungus

Plate 19. Effect of *A. squamosa* against *M. incognita* and *R. solani* disease complex in brinjal – Shoot

1. Root-dip
2. Root - dip + Foliar Spray
3. Root - dip + Foliar Spray + Soil application
4. Untreated control

Plate 20. Effect of *A. squamosa* against *M. incognita* and *R. solani* disease complex in brinjal – Root

Plate 19

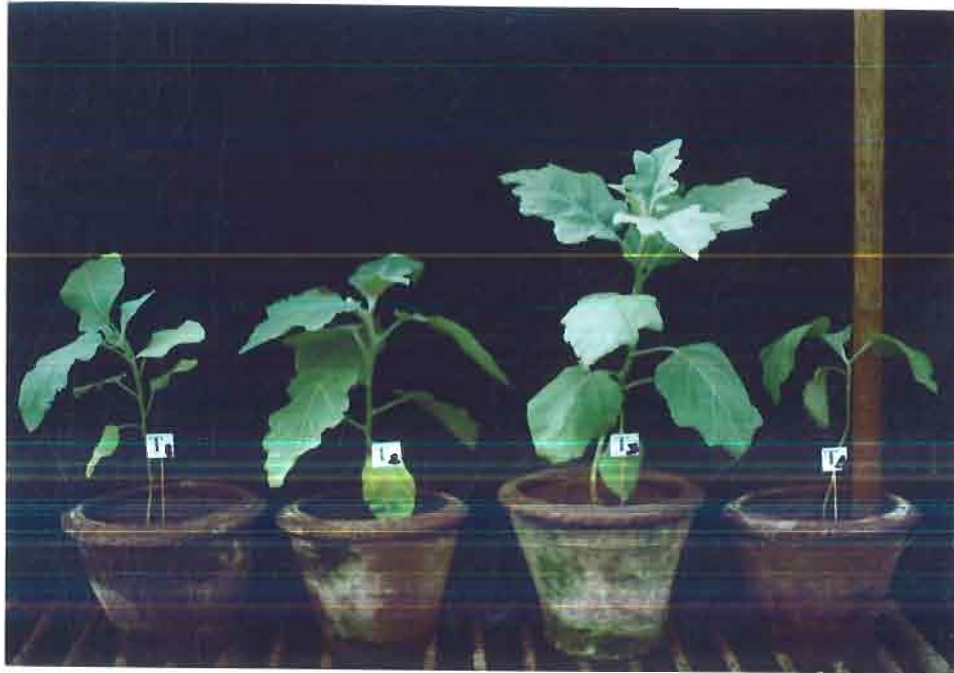


Plate 20



foliar spray (1.6). Maximum root knot index of 5.0 was observed in the plants inoculated only with nematodes and 4.6 in nematode + fungus inoculated untreated plants (Table 28).

The number of females were found to be in the wide range from 16.3 to 611.3 per g root in the treatments as against 97.6 in the combination of both the organisms inoculated plant (Fig. 15). The least number of females was seen in combined application of all the three methods (83.3 %) followed by root-dip + foliar spray (78.5 %). Number of egg masses/g root and number of eggs/egg mass was also minimum in plants treated with extract by the method of root-dip + foliar spray + soil application with 88.1 and 78.9 per cent reduction and this was followed by root-dip + foliar spray with 78.1 and 72.5 per cent reduction over untreated and combined inoculation of both the organisms respectively. Lowest reduction in number of egg masses/g root (27.1 %) and number of eggs/egg mass (40.3 %) was registered in soil application of the *A. squamosa* extract.

The density of soil nematode population (67.7 %) was minimum in combined application of all the three methods. Root-dip + foliar spray registered 59.0 per cent reduction and found to be on par with root-dip + soil application. Individual method of application of the extract recorded 38.2 per cent in root-dip and 23.1 per cent reductions in soil application of the extract.

4.10. Integrated management of *M. incognita* in tomato and brinjal with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran.

4.10.1. Tomato

4.10.1.1. Plant growth parameters

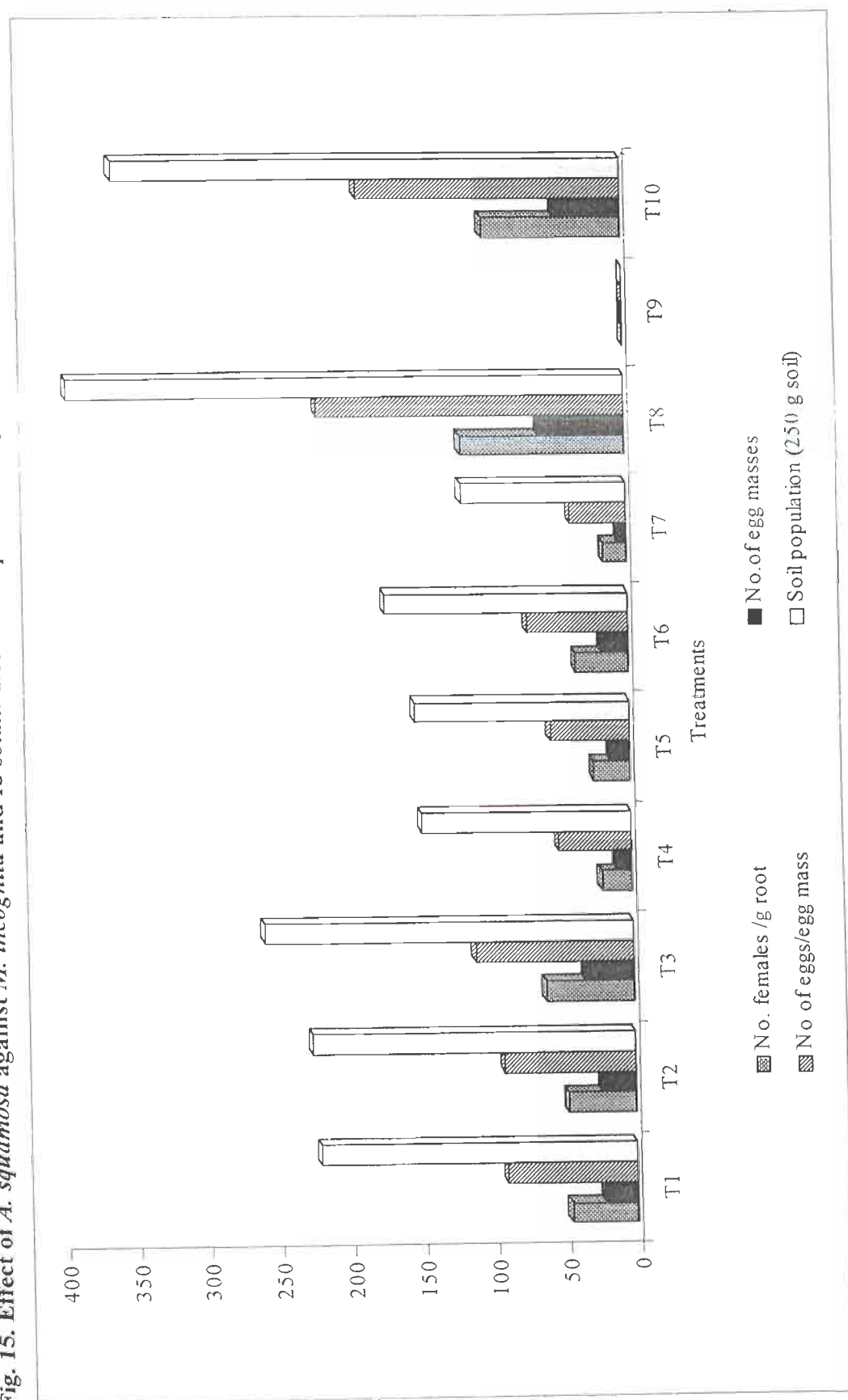
Shoot and root weight was significantly influenced by the application of the plant extract, *A. squamosa* extract alone and in combination with bioagent (*P. lilacinus*), oilcake

Table 28. Effect of *A. squamosa* extract against *M. incognita* and *R. solani* disease complex in brinjal - Nematode population

Treatments	Root knot index	No. of females/g Root	Per cent decrease	No. of egg mass/g root	Per cent decrease	No of eggs/egg mass	Per cent decrease	Soil population (250 g soil)	Per cent decrease
T1	2.6	45.6	53.3	22.0	53.2	90.3	51.3	220.3	38.2
T2	2.6	47.0	51.8	23.3	50.5	91.0	50.9	225.0	36.9
T3	3.0	61.3	37.2	34.3	27.1	110.6	40.3	257.6	27.7
T4	1.6	21.0	78.5	10.3	78.1	51.0	72.5	146.3	59.0
T5	2.0	25.3	74.1	13.6	71.1	55.3	70.2	150.6	57.7
T6	2.3	37.0	62.1	18.0	61.7	70.6	61.9	170.0	52.3
T7	1.3	16.3	83.3	5.6	88.1	39.0	78.9	115.3	67.7
T8	5.0	115.3	+15.5	60.0	+21.7	215.0	+14.0	390.6	+9.7
T9	-	-	-	-	-	-	-	-	-
T10	4.6	97.6	47.0	185.3	7.22	3.01	6.34	356.3	6.34
CD (P=0.05)	0.22	4.31	3.01	7.22	6.34	3.01	6.34	356.3	6.34

T1: Root-dip, T2: Foliar spray, T3: Soil application, T4: Root-dip + Foliar spray, T5: Root-dip + Soil application, T6: Foliar spray + Soil application, T7: Root-dip + Foliar spray + Soil application, T8: Nematode alone, T9: Fungus alone and T10: Nematode + fungus

Fig. 15. Effect of *A. squamosa* against *M. incognita* and *R. solani* disease complex in brinjal – Nematode population



(castor) and carbofuran (Plate 21). Maximum shoot and root weight was recorded in *A. squamosa* + *P. lilacinus* + castor cake with 86.7 and 138.1 per cent increase over untreated control respectively. This was followed by the combined application of *A. squamosa* + castor cake (75.5 and 120.4 %) and found to be on par with the *A. squamosa* + *P. lilacinus* application (Table 29). When the *A. squamosa* extract was applied in combination with carbofuran @ 1.75 g/m diam, it registered 53.5 per cent increase in shoot weight and 111.1 per cent increase in root weight and in addition it was on par with carbofuran alone @ 3.5 g/ m diam. *A. squamosa*, *P. lilacinus* and castor cake alone recorded 41.1, 34.4 and 43.3 per cent increase in shoot weight and 87.4, 66.2 and 87.7 per cent increase in root weight over untreated control respectively. Maximum of 5.85 kg fruits/plot was harvested from the plot treated with plant extract + bioagent + oil cake. Next to this treatment the higher yield was registered in *A. squamosa* + castor cake treatment (4.65 kg). *A. squamosa* + carbofuran gave 3.75 kg/plot and was on par with carbofuran alone (3.86 kg). The lowest yield was observed in the plot treated with *P. lilacinus* alone with 3.32 kg/plot as against 2.175 kg in the untreated control plot (Fig. 16).

4.10.1.2. Nematode population

Minimum root knot index of 2.0 was recorded in the combined application of *A. squamosa* + *P. lilacinus* + castor cake. *A. squamosa* + carbofuran treatment was found to be on par with carbofuran alone registering the gall index of 3.20 and 3.00 respectively. Individual application *A. squamosa* registered root knot index of 3.50 as against 3.75 in *P. lilacinus*. Highest root knot index of 4.75 was observed in the untreated control plant. (Table 30).

Treatments

T₁ - Seedling root-dip (1 hr) + foliar spray at 30 DAP with *A. squamosa* extract (60 %)

T₂ - *P. lilacinus* @ 10 g/m²

T₃ - Castor cake @ 10 g/m²

T₄ - T₁ + T₂

T₅ - T₁ + T₃

T₆ - T₁ + T₂ + T₃

T₇ - T₁ + carbofuran 3G @ 1.75 g/m²

T₈ - Carbofuran 3G @ 3.5 g/m²

T₉ - Untreated control

Table 29. Integrated management of *M. incognita* in tomato with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran - Plant growth parameters

Treatments	Shoot weight (g)	Per cent increase	Root weight (g)	Per cent increase	Yield, kg/plot	Per cent increase
T1	230.5	41.1	169.2	87.4	3.390	55.9
T2	219.0	34.4	150.0	66.2	3.320	52.7
T3	233.6	43.3	169.5	87.7	3.410	56.8
T4	280.8	71.8	194.4	115.3	4.635	113.1
T5	286.1	75.5	199.0	120.4	4.650	113.8
T6	304.3	86.7	215.6	138.1	5.850	169.0
T7	250.1	53.5	180.6	111.1	3.750	72.4
T8	257.6	58.5	191.8	112.4	3.860	77.5
T9	163.0		90.3		2.175	
CD(P=0.05)	8.42		6.13		0.21	

Table 30. Integrated management of *M. incognita* in tomato with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran - Nematode and fungus population

Treatment	Soil Nematode population (250 g soil) and CFUs of the fungus (x 10 ⁴)						Root knot index	No. egg masses/g root	Per cent parasitized egg masses
	45 DAP			90 DAP					
	Nematode	Fungus	Nematode	Nematode	Fungus	Fungus			
T1	142.0		213.3	310.3			3.50	34.15	
T2	154.3	8.0	224.0	330.3	16.3	17.6	3.75	39.13	24.0
T3	135.0		210.0	308.0			3.45	33.62	
T4	110.0	8.6	166.3	201.6	17.6	20.3	2.42	16.13	30.3
T5	107.3		165.0	198.0			2.31	14.60	
T6	94.6	10.0	140.0	175.3	24.0	26.3	2.0	9.00	56.3
T7	85.0		194.0	295.6			3.20	27.71	
T8	81.0		190.3	288.0			3.00	25.52	
T9	194.6		311.0	380.0			4.75	45.33	
CD(P=0.05)	5.61		7.33	10.41			0.20	2.51	

Fig. 16. Integrated management of *M. incognita* on tomato and brinjal with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran - yield

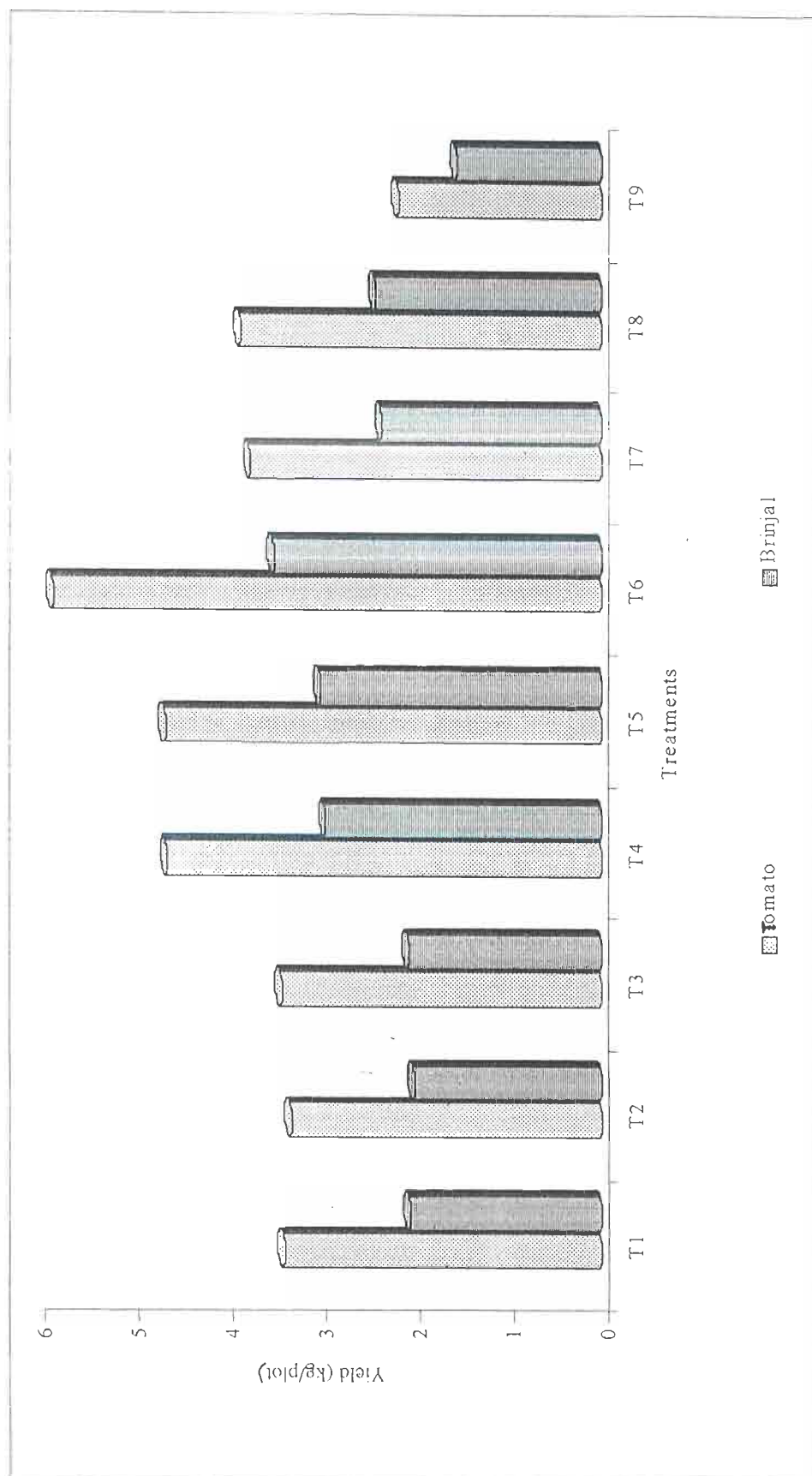


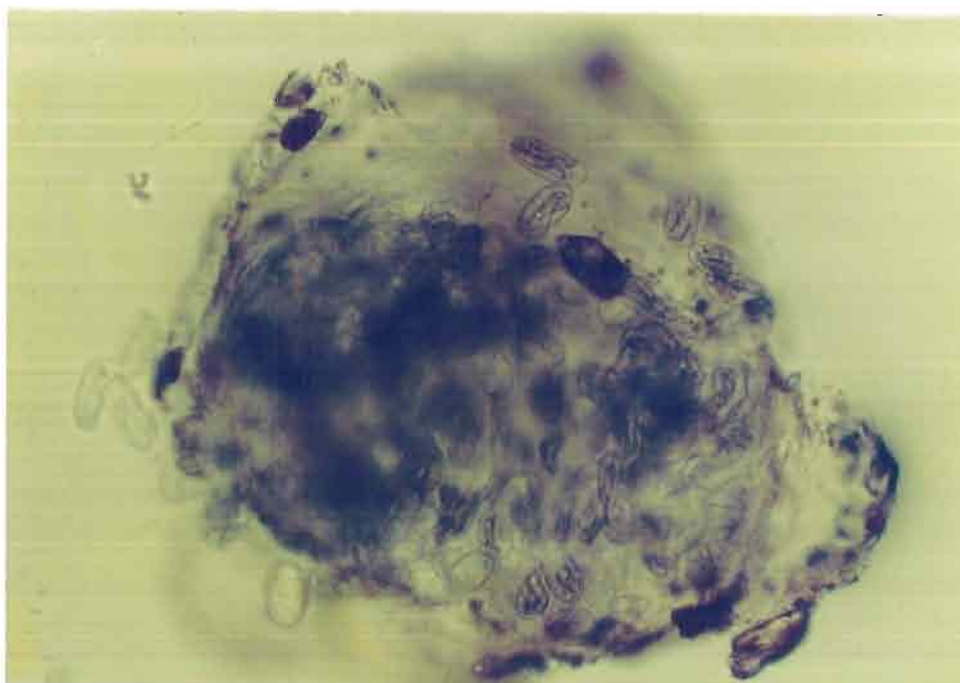
Plate 21. Integrated management of *M. incognita* on tomato with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran – microplot view

Plate 22. Colonization of *M. incognita* egg mass by *P. lilacinus*

Plate 21



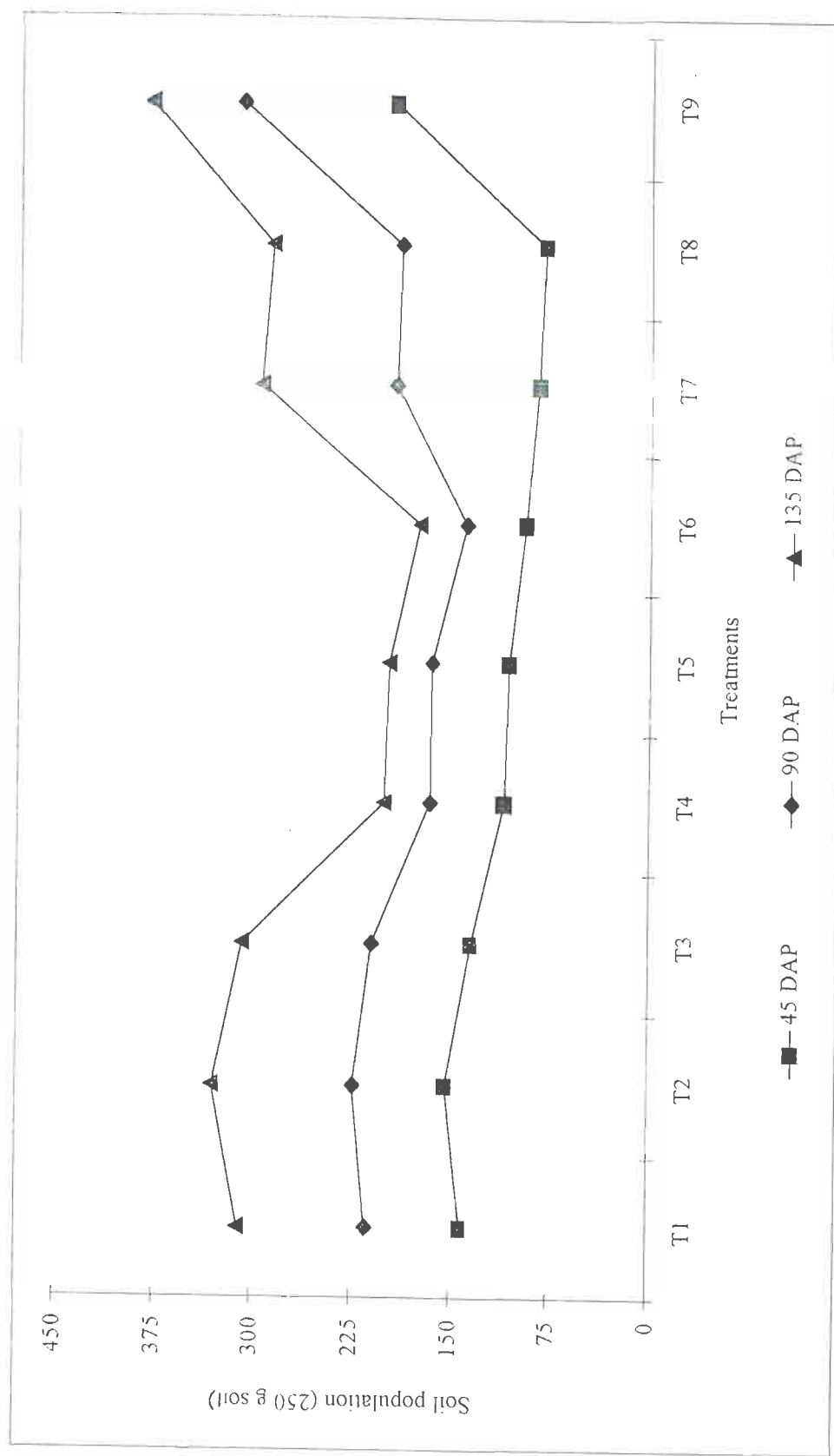
Plate 22



The egg mass production was also reduced due to the treatments and it ranged between 9.0 to 39.13 per cent. About 80.2 per cent reduction in egg mass production was observed in combination of three treatments which followed by *A. squamosa* + castor cake (67.8 %) and *A. squamosa* + *P. lilacinus* (64.5 %). *A. squamosa* in combination of half dose of carbofuran (1.75 g/m diam.), registered 38.9 per cent reduction and showed no statistical difference with full dose of carbofuran (3.5 g/m²), which registered 43.7 per cent reduction. Whereas in their individual application the egg masses recorded was lowest in castor cake followed by *A. squamosa* and the highest in *P. lilacinus* treated plants.

At 45 days after planting, lowest population (81.0) was recorded in carbofuran alone treated plants which accounted for 58.4 per cent reduction over untreated plants (194.6 nematodes/250 g soil). Maximum of 154.3 nematodes was registered in *P. lilacinus* alone treated plants. In combination with half dose of carbofuran, *A. squamosa* registered 56.3 per cent reduction. After 90 days, combination of all the three treatments had lowered nematode population (140.0/250 g soil). *A. squamosa* alone recorded 213 nematodes/250 g soil (32.5 %) while the maximum of 311 nematodes was registered in the control plot (Fig. 17). Carbofuran alone recorded 38.8 per cent reduction in soil nematode population and when *A. squamosa* combined with half dose of carbofuran 37.6 per cent reduction in nematode population was observed. Among the treatments, the lowest reduction in soil nematode population was registered in sole application of *P. lilacinus* (27.9 %). A similar pattern of reduction in final soil nematode population was observed at harvest, 135 days after planting with 53.9 per cent reduction in *A. squamosa* + *P. lilacinus* + castor cake. Carbofuran alone in full dose gave 24.2 per cent reduction and in combination *A. squamosa* + half dose of carbofuran registered 22.2 per cent reduction in soil nematode

Fig. 17. Integrated management of *M. incognita* on tomato with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran – Soil nematode population



and was on par with each other. While lowest reduction in soil nematode population was achieved only with *P. lilacinus* at all intervals.

4.10.1.3. Bioagent

The bioagent, *P. lilacinus* used in this study was found to parasitize the *M. incognita* egg masses in various degrees with maximum of 56.3 per cent parasitization in *A. squamosa* + *P. lilacinus* + castor cake application and this was followed by 30.3 and 24.0 per cent in *A. squamosa* + *P. lilacinus* and *P. lilacinus* alone, respectively (Plate 22 a and b). Comparatively *P. lilacinus* with castor cake was found to multiply faster than with *A. squamosa*. A maximum of 10×10^4 colony forming units (cfu) were isolated from *A. squamosa* + *P. lilacinus* + castor cake treated soil than 8.6×10^4 cfu along with *A. squamosa* at 45 days after planting. There was a similar pattern of increase in the cfu of fungus both at 90 and 135 days after planting with 24×10^4 and 26.3×10^4 cfu in *A. squamosa* + *P. lilacinus* + castor cake received soil and 17.6×10^4 and 20.3×10^4 cfu in *A. squamosa* + *P. lilacinus* treatment respectively (Table 30). In contrast, its individual application responded poorly in the multiplication of the fungus in terms of cfu.

4.10.2. Brinjal

4.10.2.1. Plant growth parameters

Shoot and root weight was significantly improved by the application of the *A. squamosa* extract alone and in combination with bioagent, oil cake and carbofuran. Maximum shoot and root weight was recorded in *A. squamosa* + *P. lilacinus* + castor cake with 79.5 and 100.5 per cent increase over untreated control respectively (Plate 23). This was followed by the combined application of *A. squamosa* + castor cake (71.3 and 85.7 %) and found to be on par with the *A. squamosa* + *P. lilacinus* application. When the

**Plate 23. Integrated management of *M. incognita* on brinjal with *A. squamosa*,
P. lilacinus, castor cake and carbofuran – microplot view**

Plate 23



A. squamosa extract was applied in combination with half dose of carbofuran @ 1.75 g/m diam. it registered 66.4 per cent increase in shoot weight and 79.0 per cent increase in root weight and in addition it was on par with the treatment of full dose of carbofuran alone @ 3.5 g/m diam. Application of *A. squamosa*, *P. lilacinus* and castor cake individually recorded 56.2, 50.4 and 58.7 per cent increase in shoot weight and 43.6, 33.0 and 48.3 per cent increase in root weight over untreated control respectively. Maximum fruit weight of 3.5 kg/m diam. was harvested from the plants treated with plant extract + bioagent + oil cake. The next best yield was registered in *A. squamosa* + castor cake treatment (3 kg). Combined application in the treatment of *A. squamosa* + carbofuran gave 2.35 kg/m diam. and was on par with carbofuran alone (2.41 kg). The lowest yield was observed in the plot treated with *P. lilacinus* alone with the fruit weight of 2 kg/plot as against 1.55 kg in the untreated control plot (Table 31; Fig. 16).

4.10.2.2. Nematode population

The highest root knot index of 4.5 was observed in the untreated control plant. While it was minimum with 1.75 root-knot index in the combined application of *A. squamosa* + *P. lilacinus* + castor cake. *A. squamosa* + half dose of carbofuran treatment was found to be on par with full dose of carbofuran alone registering the gall index of 3.13 and 3.0 respectively.

The egg mass production was also reduced due to various treatments and it ranged between 7.18 to 36.62 (Table 32). About 82.7 per cent reduction in egg mass production was observed in combination of three treatments which was followed by *A. squamosa* + castor cake (68.7 %) and *A. squamosa* + *P. lilacinus* (65.6 %). The reduction in egg mass production between *A. squamosa* + half dose of carbofuran (41.3 %) and full dose of

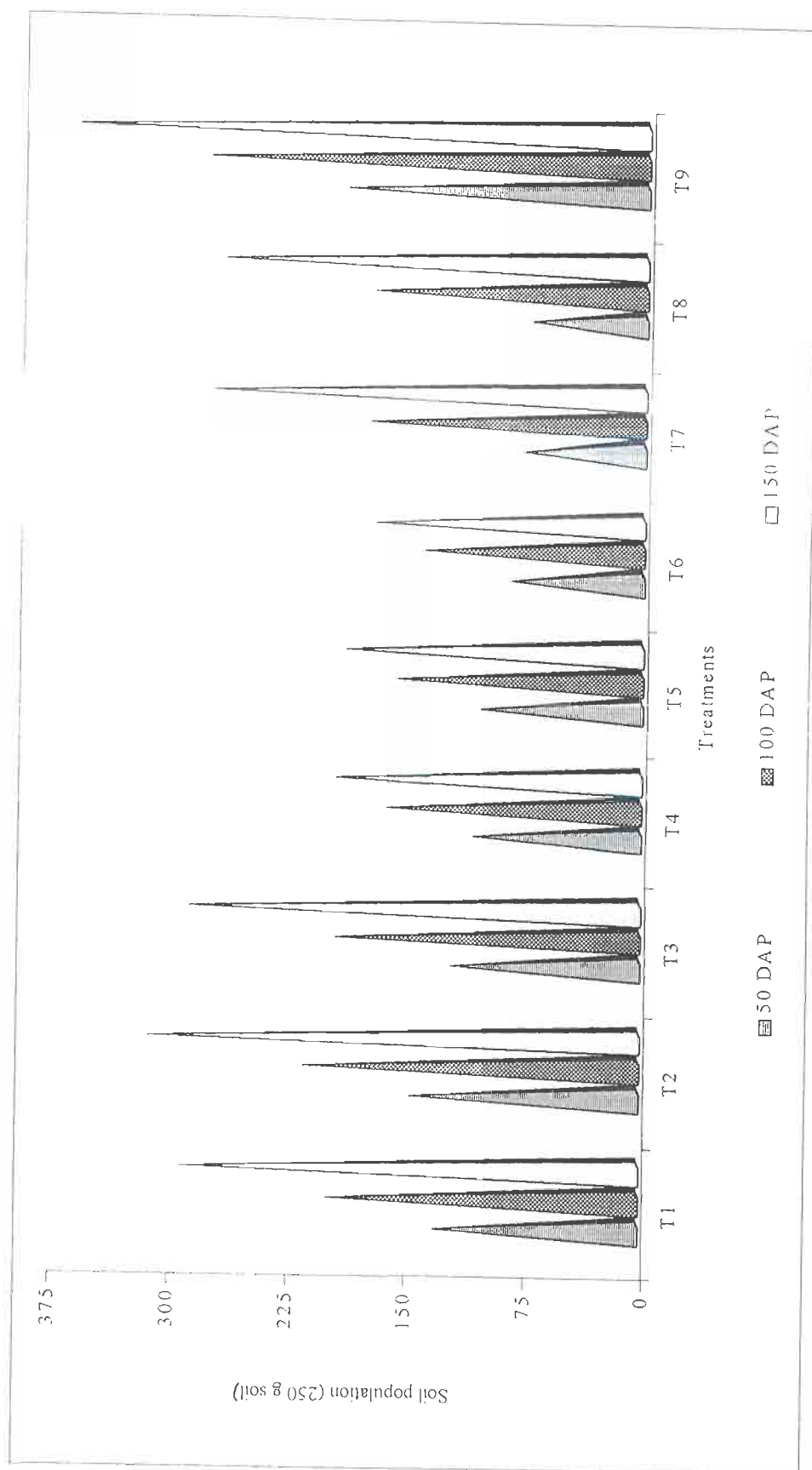
Table 31. Integrated management of *M. incognita* in brinjal with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran
- Plant growth parameters

Treatments	Shoot weight (g)	Per cent increase	Root weight (g)	Per cent increase	Yield kg / plot	Per cent increase
T1	221.0	56.2	100.5	43.6	2.048	32.2
T2	212.8	50.4	93.1	33.0	2.000	29.0
T3	224.5	58.7	103.8	48.3	2.060	32.9
T4	240.0	69.9	128.2	83.2	2.950	90.3
T5	242.3	71.3	130.0	85.7	3.000	93.6
T6	274.0	79.5	140.3	100.5	3.500	125.9
T7	235.5	66.4	125.3	79.0	2.350	51.6
T8	237.0	67.5	126.0	80.0	2.410	55.5
T9	141.5		70.0		1.550	
CD(P=0.05)	6.61		5.02		0.18	

carbofuran (44.6 %) was not significant. In case of individual application, the number of egg masses recorded was lowest in castor cake and it was highest in *P. lilacinus* treated plants.

Application of carbofuran singly accounted for 62.1 per cent reduction recorded the lowest nematode population (71.3) over untreated plants, (188.3 nematodes/250 g soil) at 50 days after planting. Maximum population of 143.0 nematodes with 24.1 per cent reduction was registered in *P. lilacinus* alone. In combination with half dose of carbofuran, *A. squamosa* registered 60.2 per cent reduction as against 62.1 per cent reduction with full dose of carbofuran. After 100 days, combination of all the three treatments had the lowest number of nematode population (136.6/250 g soil). *A. squamosa* alone recorded 195.6 nematodes/250 g soil (28.9 %) while in the control plot 275.0 nematodes were recorded (Fig. 18). Carbofuran alone recorded 38.0 per cent reduction in soil nematode population whereas the per cent reduction of nematode population was 37.4 when *A. squamosa* combined with half dose of carbofuran. Among the treatments, the lowest reduction in soil nematode population was registered in sole application of *P. lilacinus* (23.3 %). The same trend of reduction in final soil nematode population was observed at harvest, 150 days after planting with 53.0 per cent reduction in *A. squamosa* + *P. lilacinus* + castor cake. Carbofuran alone in full dose gave 26.1 per cent reduction and in combination of *A. squamosa* with half dose of carbofuran registered 24.5 per cent reduction in soil nematode and was on par with each other. While lowest reduction in soil nematode population was obtained only with *P. lilacinus* at all period of observation made at three intervals.

Fig. 18. Integrated management of *M. incognita* on brinjal with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran – Soil nematode population



4.10.2.3. Bioagent

In the present study the bioagent, *P. lilacinus* was found to parasitize the *M. incognita* egg masses at varying level with maximum of 64.0 per cent parasitization in *A. squamosa* + *P. lilacinus* + castor cake application and minimum of 27.0 per cent in *P. lilacinus* alone. The egg parasitic fungi *P. lilacinus* with castor cake potentially multiplies faster when compared to *P. lilacinus* + *A. squamosa*. A maximum of 13×10^4 colony forming units (cfu) were developed from *A. squamosa* + *P. lilacinus* + castor cake applied soil than 10.3×10^4 cfu along with *A. squamosa* at 50 days after planting. The same trend of increase in the cfu of fungus was seen both at 100 and 150 days after planting with 26.3×10^4 and 29.6×10^4 cfu in *A. squamosa* + *P. lilacinus* + castor cake applied plots and 21.6×10^4 and 22×10^4 cfu in *A. squamosa* + *P. lilacinus* treatment respectively. In contrast, its individual application yielded the lowest cfu of the fungus, *P. lilacinus* (Table 32).

4.11. Histopathology of *M. incognita* infested roots of tomato and brinjal plants treated with *A. squamosa* extract

The penetration of the second stage juveniles of *M. incognita* were found to occur mainly behind the root tip. The microtome (Spencers, USA) sections of 12 μ thickness of *M. incognita* infested roots of both tomato and brinjal plants untreated with *A. squamosa* extract revealed the formation of conspicuous and well formed giant cells of spherical to slightly elongated shape numbering 4 to 6. The cytoplasmic granules and the nuclei are condensed either at the center of the cells or at the inner wall of the cells leaving clear zones in the giant cells (Plate 24). The length of giant cells ranged from 95 to 170 μ and

Table 32. Integrated management of *M. incognita* in brinjal with *A. squamosa*, *P. lilacinus*, castor cake and carbofuran - Nematode and fungus population

Treatment	Soil nematode population (250 g soil) and CFUs of the fungus (x 10 ³)						Root knot index	No. egg masses/g root	Per cent parasitized egg masses
	50 DAP			100 DAP					
	Nematode	Fungus	Nematode	Fungus	Nematode	Fungus			
T1	128.3		195.6		288.3		3.31	32.45	
T2	143.0	9.0	211.0	21.6	309.0	21.0	3.50	36.62	27.0
T3	118.6		191.0		283.0		3.20	31.15	
T4	105.0	10.3	159.6	21.6	191.3	22.0	2.31	14.31	32.6
T5	100.3		153.0		186.0		2.16	13.00	
T6	82.6	13.0	136.6	26.3	168.3	29.6	1.75	7.18	64.0
T7	75.0		172.0		270.0		3.13	24.36	
T8	71.3		170.6		264.6		3.00	23.00	
T9	188.3		275.0		357.6		4.5	41.50	
CD(P=0.05)	5.04		6.72		9.04		0.16	1.94	

Plate 24. Histopathology of *M. incognita* infested tomato and brinjal roots treated with *A. squamosa* extract

A and B: Sections of untreated tomato (A) and brinjal (B) roots showing varied sizes and shapes of giant cells with matured *M. incognita* female

C and D: *A. squamosa* treated tomato (C) and brinjal (D) roots with smaller size giant cells and lignified tissues

n: nematode

gc: giant cells

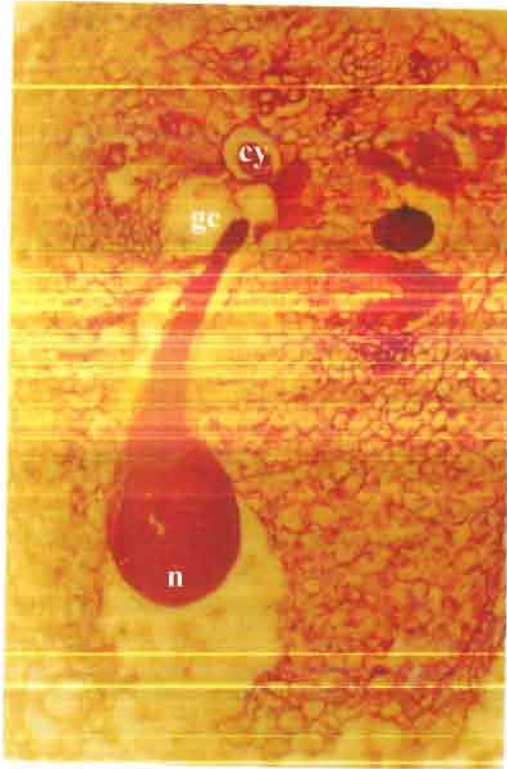
cy: cytoplasm

x: xylem vessels

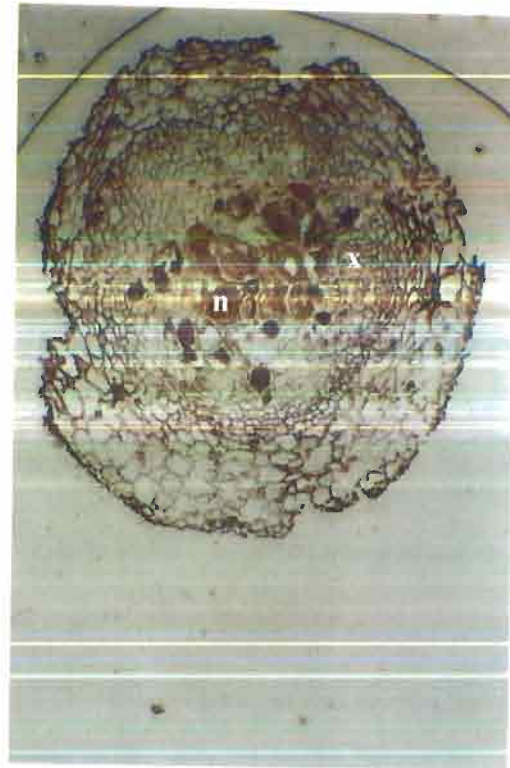
nc: necrosis

Plate 24. Histopathology of *M. incognita* infested tomato and brinjal roots treated with *A. squamosa* extract

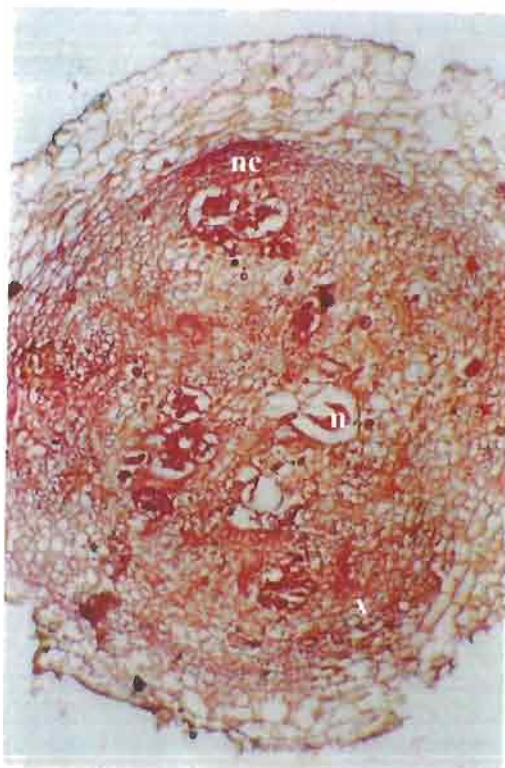
A. Tomato



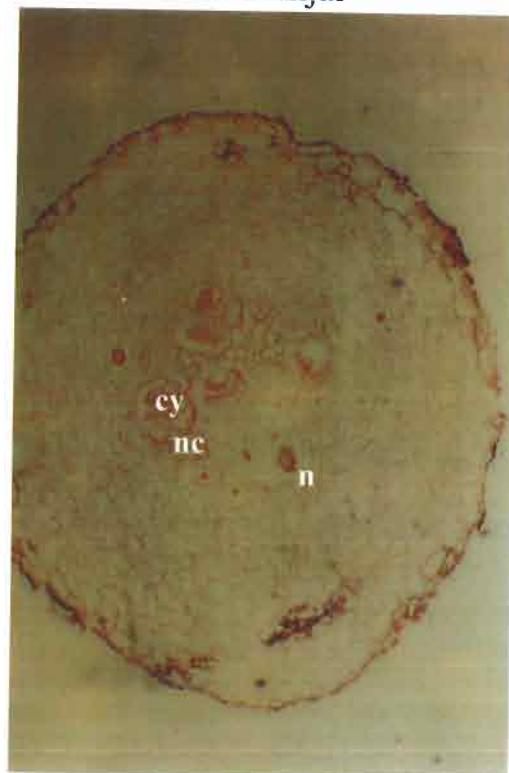
B. Brinjal



C. Tomato



D. Brinjal



the width ranged from 55.2 to 134.6 μ and contains 8 to 15 nuclei in tomato and 88 to 167 μ length and 50 to 115.4 μ width in brinjal with 7 to 13 nuclei. The xylem and phloem vessels are pushed to one side of the root cortex thereby offering a residence for giant cells. The egg masses were partially embedded in the root cortex.

A. squamosa treated root sections of both tomato and brinjal roots showed that the larval penetrations directs some cellular disturbances leading to necrosis in epidermal and stelar regions. In circumstances when the nematode deliberately rules out this situation, small giant cells with less number of nuclei were formed instead of large single multinuclear giant cell (Plate 24). The length and width of giant cells ranged from 53 to 136 and 36 to 75.2 μ in tomato and 50 to 121.6 μ and 29 to 67.3 μ in brinjal, respectively and the giant cells with less number of nuclei, ranged from 5 to 10 in tomato and 4 to 8 in brinjal when compared to the untreated control roots. Nematodes were found trapped in the cortical region without accompanying giant cell. The immature stages of the *M. incognita* were confined within the necrotic cells in the stelar region.

4.12. Biochemical analysis of tomato and brinjal plants treated with *A. squamosa* extract

4.12.1. Free Aminoacid

An increase in amino acid content in different treatments ranged between 194.7 to 28.9 in tomato at 6 days after nematode inoculation, with maximum under root-dip + foliar spray + soil application and minimum under soil application of the *A. squamosa* extract (Table 33). However, in brinjal 292 and 200 per cent increase in amino acid content at six days after inoculation was recorded with root-dip + foliar spray + soil application and root-

Table 33. Changes in free aminoacid* content in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa* extract.

Treatments	Tomato								Brinjal									
	Days after inoculation																	
	0	1	2	4	6	8	0	1	2	4	6	8	0	1	2	4	6	8
T1	0.44	0.56	0.70	0.72	0.75	0.71	0.18	0.26	0.44	0.51	0.53	0.47	0.17	0.24	0.43	0.48	0.49	0.43
T2	0.42	0.51	0.66	0.67	0.69	0.65	0.15	0.19	0.31	0.39	0.38	0.31	0.28	0.39	0.65	0.72	0.75	0.70
T3	0.36	0.39	0.45	0.46	0.49	0.40	0.25	0.34	0.61	0.69	0.71	0.66	0.22	0.26	0.48	0.52	0.50	0.42
T4	0.58	0.74	0.91	0.95	1.01	0.98	0.46	0.58	0.83	0.96	0.98	0.95	0.11	0.13	0.25	0.26	0.25	0.22
T5	0.55	0.71	0.89	0.92	0.96	0.91												
T6	0.43	0.46	0.51	0.54	0.55	0.48												
T7	0.62	0.80	0.97	1.03	1.12	1.01												
T8	0.32	0.35	0.41	0.41	0.38	0.35												

* mg of free aminoacids/g of fresh root tissue expressed in proline equivalent

CD (P=0.05) t 0.033 0.020
 d 0.025 0.013
 t x d 0.074 0.053

dip + foliar spray treatments respectively. Among the treatments, a minimum increase in soil application of the *A. squamosa* extract was observed at all the time of intervals (Fig. 19). However, there was gradual increase in amino acid activity between period in days both in tomato and brinjal

4.12.2. Changes in total phenol content

A study was made whether the induction of phenol content was due to the treatment with *A. squamosa* extract. The data presented in table 34 showed that there was significant induction at 1st day after nematode inoculation and increased up to 6th day. Root-dip + foliar spray + soil application and root-dip + foliar spray showed 100.9 and 97.26 per cent increase in the content of phenol over control in tomato and the same was 114.1 and 105.8 per cent in brinjal (Fig. 20) at 6 days after nematode inoculation. In both plants, increase in total phenol content was lowest in soil application of the *A. squamosa* extract, thus revealing significant differences between treatments at different time of intervals

4.12.3. Activity of Peroxidase

Induction of peroxidase in tomato and brinjal plants was noticed due to the treatment with *A. squamosa*. The table 35 showed that maximum peroxidase activity was observed at 6 days after pathogen inoculation in all the treatments. Root-dip + foliar spray + soil application, root-dip + foliar spray and root-dip + soil application showed 47.2, 42.5 and 38.6 per cent increase in tomato and 48.0, 44.1 and 39.3 per cent increase in brinjal over control at six days after nematode inoculation (Fig. 21). There was a significant difference in peroxidase activity between treatments at different time intervals.

Table 34. Changes in total Phenol* content in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa* extract.

Treatments	Tomato								Brinjal									
	Days after inoculation																	
	0	1	2	4	6	8	0	1	2	4	6	8	0	1	2	4	6	8
T1	57.38	88.41	93.43	95.23	97.15	94.53	59.14	91.32	95.74	99.62	100.15	98.24	59.14	91.32	95.74	99.62	100.15	98.24
T2	53.26	88.20	92.20	94.65	96.40	93.53	54.23	90.65	95.16	99.13	100.11	97.24	54.23	90.65	95.16	99.13	100.11	97.24
T3	50.65	85.21	88.81	91.90	94.45	92.81	54.11	85.15	88.13	93.71	94.05	91.11	54.11	85.15	88.13	93.71	94.05	91.11
T4	73.63	121.60	131.73	133.73	136.90	132.63	76.37	130.13	139.50	146.23	149.27	145.67	76.37	130.13	139.50	146.23	149.27	145.67
T5	72.13	115.91	119.48	121.16	125.46	121.76	75.56	128.44	132.72	138.05	140.32	133.14	75.56	128.44	132.72	138.05	140.32	133.14
T6	65.35	109.25	113.67	119.08	122.31	120.68	68.84	117.85	121.06	125.81	129.36	121.71	68.84	117.85	121.06	125.81	129.36	121.71
T7	83.93	126.15	132.21	134.02	139.41	139.17	89.26	134.27	147.36	154.52	155.27	149.33	89.26	134.27	147.36	154.52	155.27	149.33
T8	33.50	63.73	67.75	72.48	69.4	71.60	36.10	65.02	69.44	76.19	72.54	65.32	36.10	65.02	69.44	76.19	72.54	65.32

* mg of phenol/g of fresh root tissue expressed in catechol equivalent

CD (P=0.05) t 1.34 1.61
 d 1.20 1.49
 t x d 2.94 3.42

Table 35. Changes in Peroxidase* activity in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa* extract.

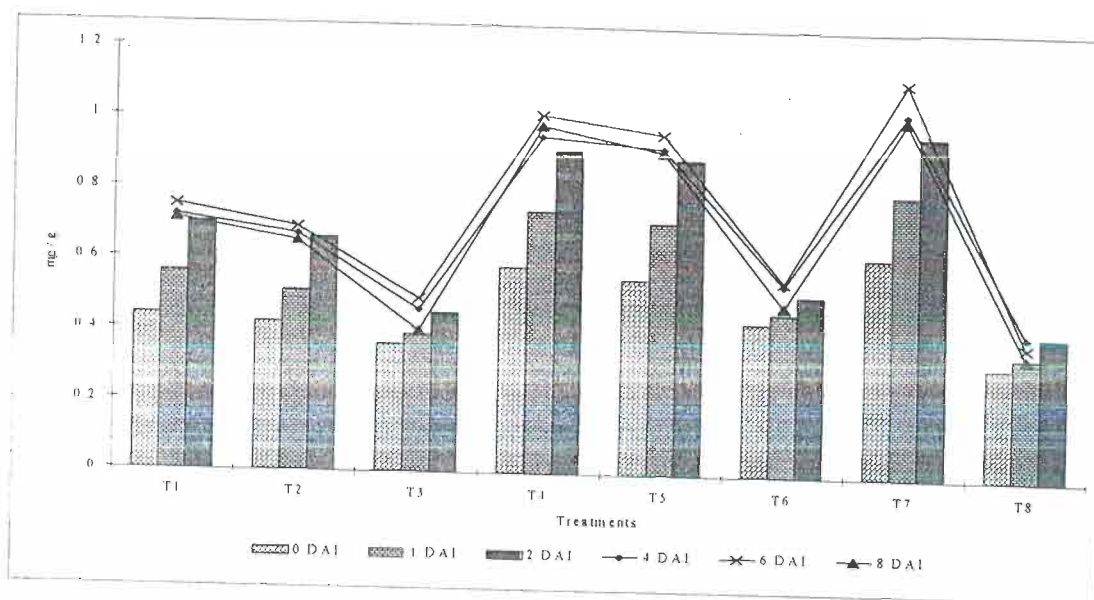
Treatments	Tomato									Brinjal								
	Days after inoculation																	
	0	1	2	4	6	8	0	1	2	4	6	8						
T1	7.79	8.53	8.99	9.85	10.90	10.15	9.21	9.54	9.82	10.61	11.36	10.67						
T2	7.45	8.09	8.49	9.36	10.15	9.47	9.04	9.50	9.67	10.04	10.66	10.41						
T3	6.73	7.44	8.74	9.32	9.86	9.20	8.25	8.63	8.91	9.45	10.31	9.11						
T4	8.40	9.34	10.15	11.58	12.33	11.13	10.64	11.04	11.89	12.75	13.58	13.12						
T5	8.31	9.226	10.20	11.06	11.99	10.89	10.22	10.96	11.64	12.33	13.14	12.77						
T6	7.68	8.74	9.46	10.32	10.87	9.09	9.76	10.11	10.52	10.91	11.32	10.95						
T7	8.62	9.87	10.87	12.05	12.73	11.24	10.92	11.75	12.54	13.49	13.95	13.61						
T8	6.2	6.57	7.41	8.13	8.64	8.35	7.75	7.83	8.52	9.11	9.43	8.72						

• Changes in absorbance/min/g fresh root tissue at 470 nm

CD (P=0.05)	t	0.17	0.21
d		0.11	0.18
t x d		0.34	0.47

Fig. 19. Changes in amino acid content in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa*

Tomato



Brinjal

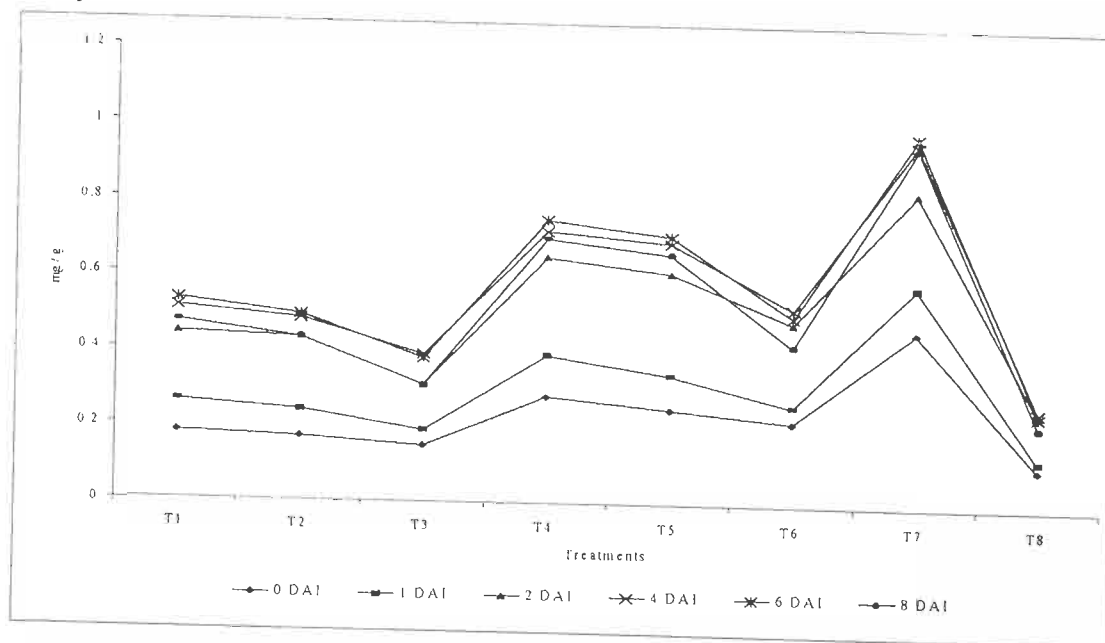
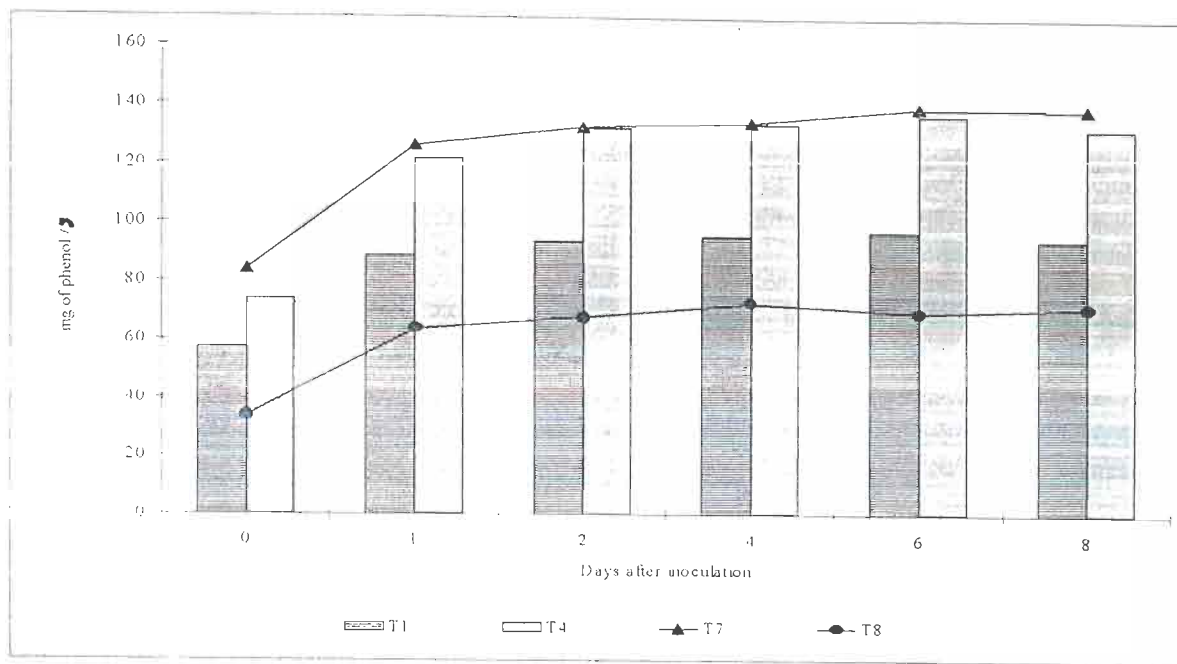


Fig. 20. Changes in total phenol content in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa*

Tomato



Brinjal

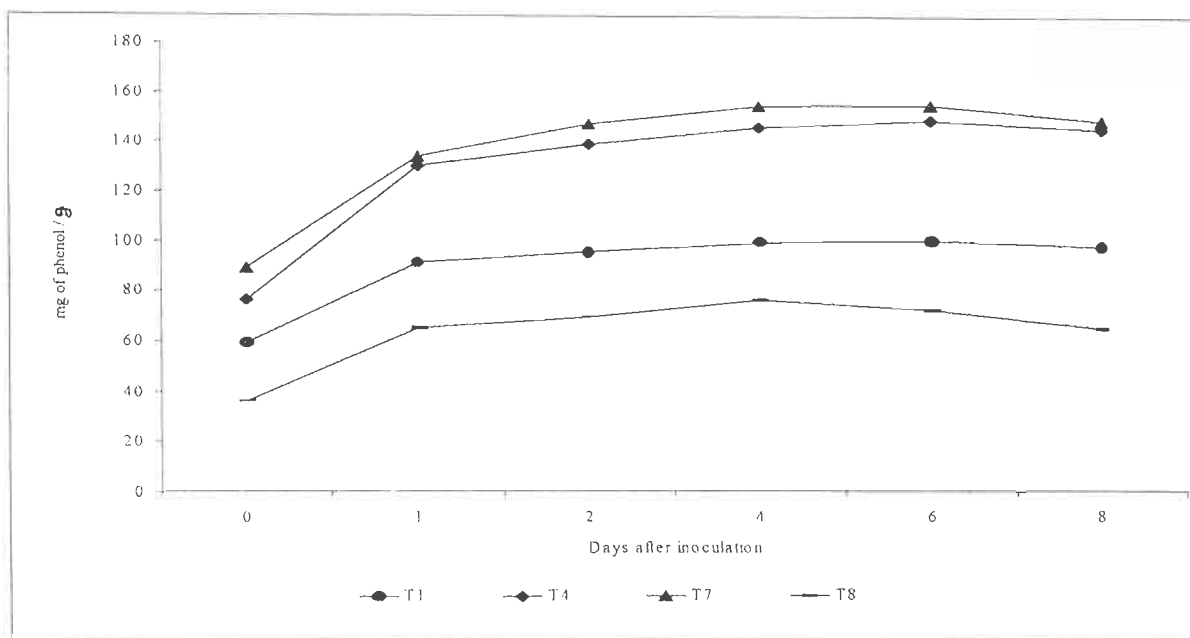
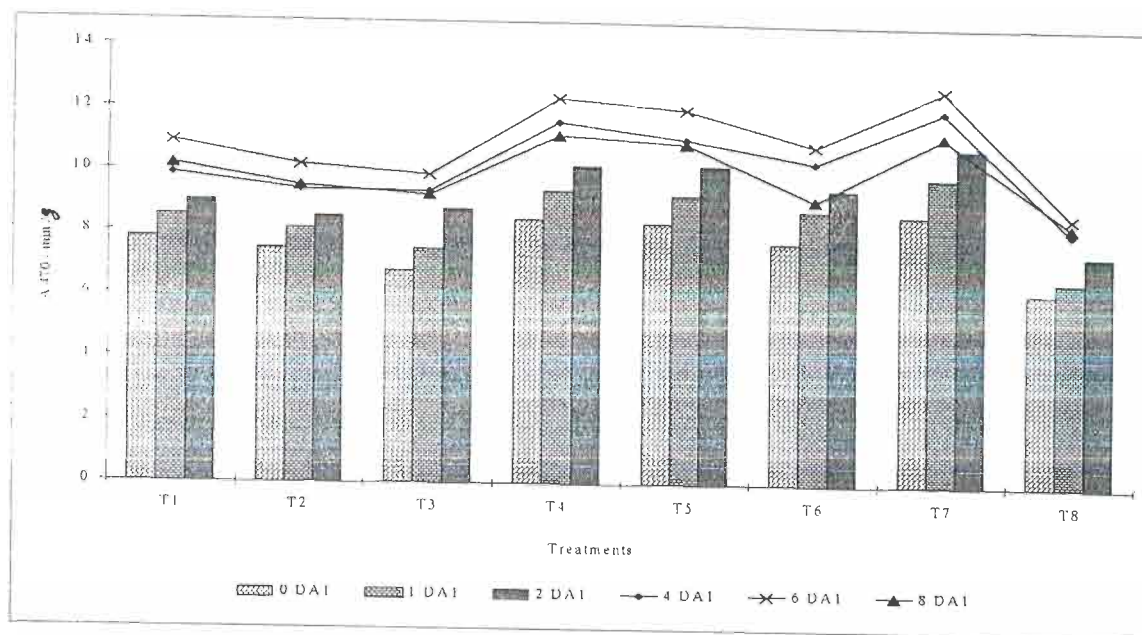
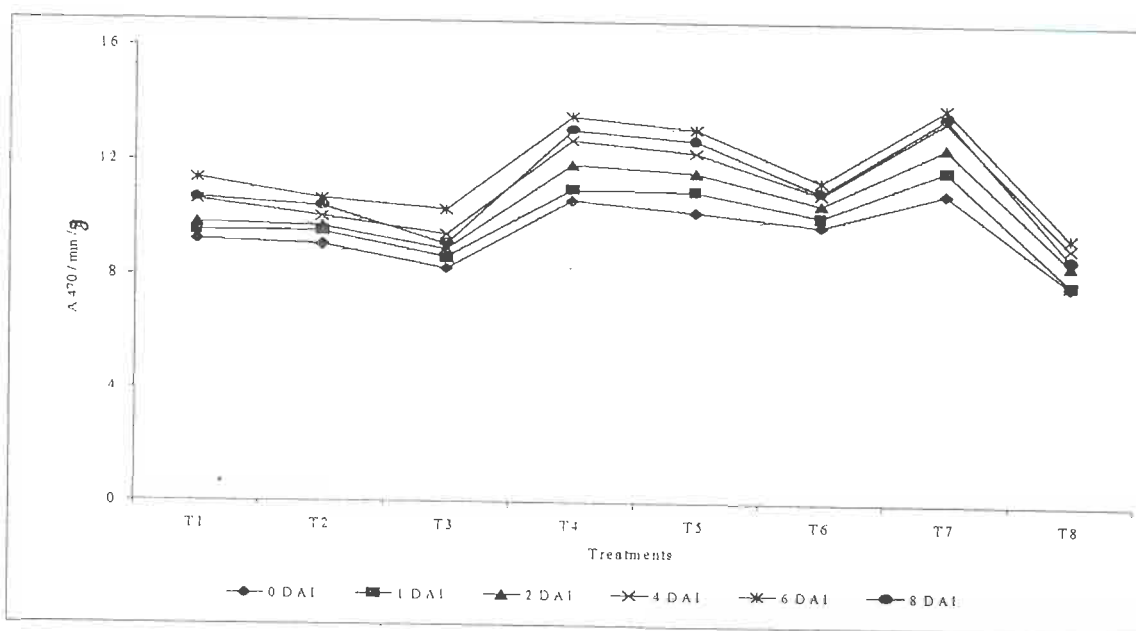


Fig. 21. Changes in peroxidase activity in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa*

Tomato



Brinjal



In comparison with other biochemical factors, peroxidase increase was also low in soil application of the *A. squamosa* extract.

4.12.4. Activity of Phenylalanine ammonia-lyase (PAL)

It was inferred that application of *A. squamosa* extract led to an increase in PAL activity in different treatments. Root-dip, root-dip + foliar spray and root-dip + foliar spray + soil application had 89.6, 151.7 and 155.2 per cent increase respectively, over control at six days after nematode inoculation in tomato (Table 36). The same trend of increase in PAL activity was also observed in brinjal also with a maximum increase in the combination of three methods (164.8 %). But it was minimum with 21.6 per cent increase over control in soil application alone (Fig. 22). However, there exist significant differences between treatments and time intervals.

4.12.5. Activity of Polyphenol oxidase (PPO)

Application of *A. squamosa* extract led to an increase in PPO activity in different treatments. The data presented in table 37 depicts that PPO activity was higher at six days after nematode inoculation. Moreover, the root-dip + foliar spray + soil application showed 154.4 per cent increase in tomato and 231.3 per cent increase in brinjal over control at six days after nematode inoculation. While individual application of the *A. squamosa* extract, root-dip, foliar spray and soil application registered 85.9, 77.2 and 71.9 per cent increase in tomato and 63.7, 57.5 and 26.2 per cent in brinjal over untreated control plant (Fig. 23).

Table 36. Changes in Phenylalanine ammonia lyase* activity in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa* extract.

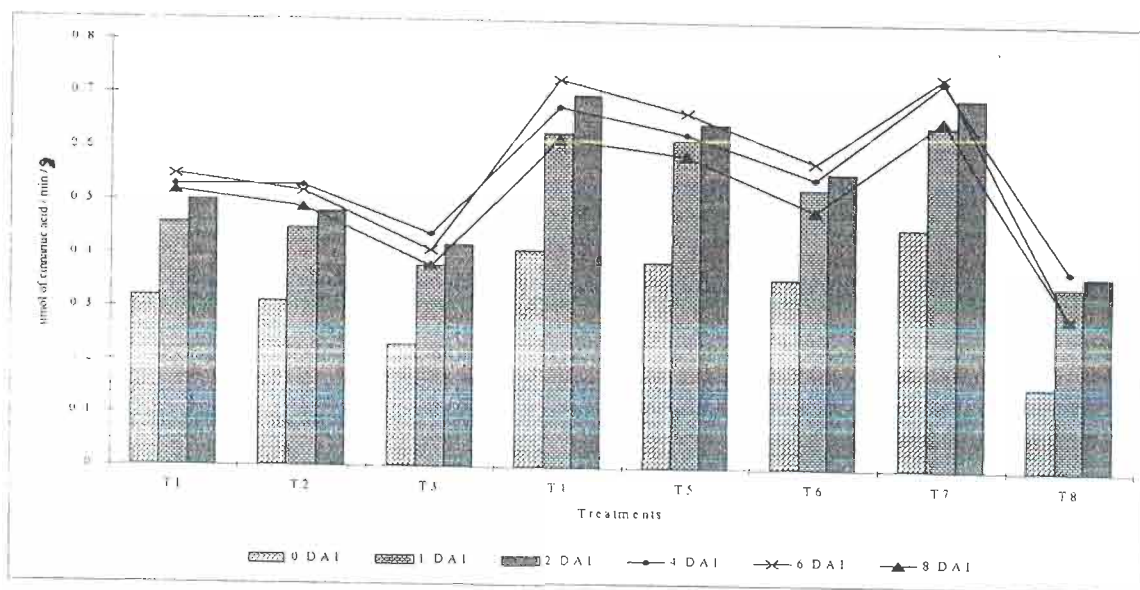
Treatments	Tomato								Brinjal									
	Days after inoculation																	
	0	1	2	4	6	8	0	1	2	4	6	8	0	1	2	4	6	8
T1	0.32	0.46	0.50	0.53	0.55	0.52	0.35	0.42	0.65	0.67	0.67	0.63	0.35	0.42	0.65	0.67	0.67	0.63
T2	0.31	0.45	0.48	0.53	0.52	0.49	0.30	0.39	0.61	0.64	0.63	0.58	0.30	0.39	0.61	0.64	0.63	0.58
T3	0.23	0.38	0.42	0.44	0.41	0.38	0.21	0.25	0.48	0.49	0.45	0.37	0.21	0.25	0.48	0.49	0.45	0.37
T4	0.41	0.63	0.70	0.68	0.73	0.62	0.45	0.57	0.82	0.88	0.90	0.85	0.45	0.57	0.82	0.88	0.90	0.85
T5	0.39	0.62	0.65	0.63	0.67	0.59	0.40	0.51	0.77	0.79	0.81	0.74	0.40	0.51	0.77	0.79	0.81	0.74
T6	0.36	0.53	0.56	0.55	0.58	0.49	0.38	0.44	0.66	0.68	0.69	0.61	0.38	0.44	0.66	0.68	0.69	0.61
T7	0.46	0.65	0.70	0.73	0.74	0.66	0.49	0.63	0.89	0.95	0.98	0.94	0.49	0.63	0.89	0.95	0.98	0.94
T8	0.16	0.35	0.37	0.38	0.29	0.29	0.19	0.26	0.41	0.45	0.37	0.31	0.19	0.26	0.41	0.45	0.37	0.31

* Changes in absorbance/min/g fresh root tissue at 290 nm

CD (P=0.05) t 0.030
 d 0.022
 t x d 0.071

Fig. 22. Changes in phenylalanine ammonia lyase activity in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa*

Tomato



Brinjal

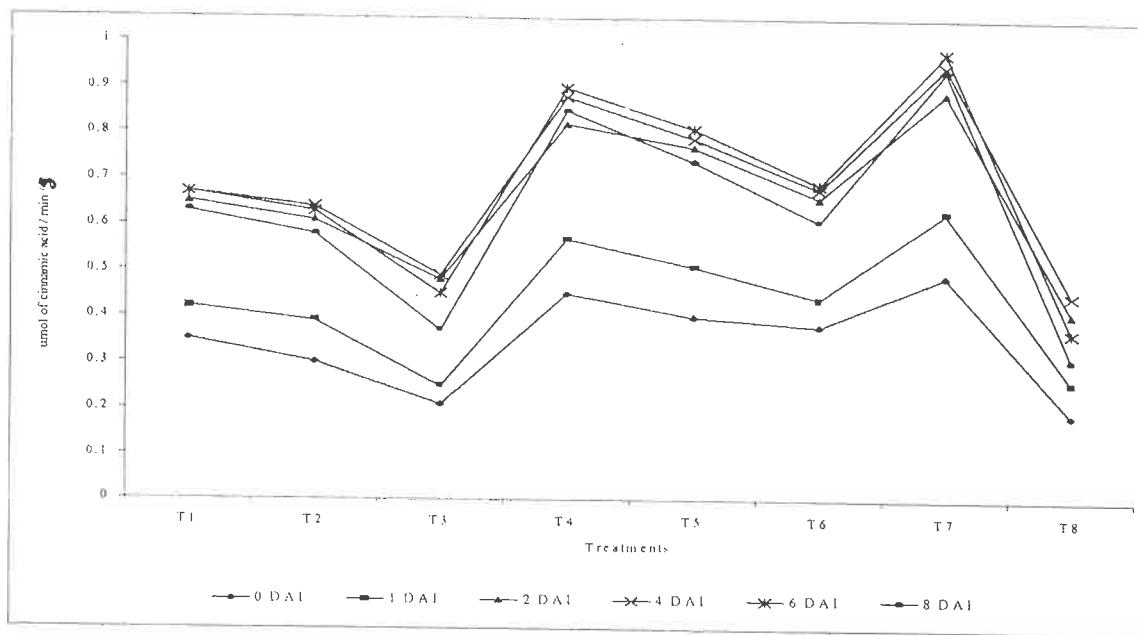
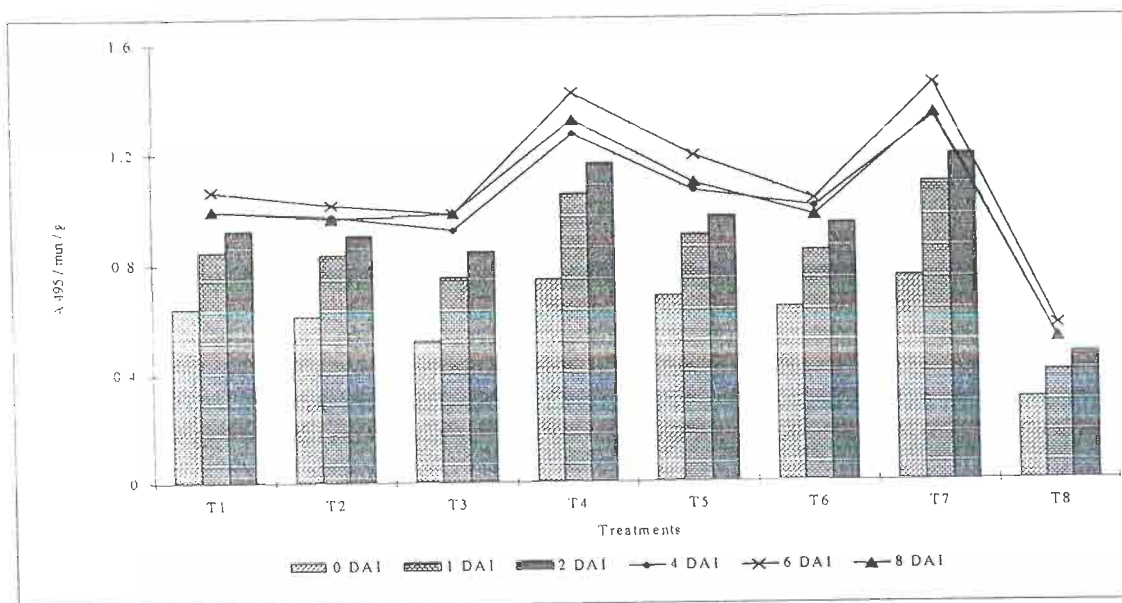
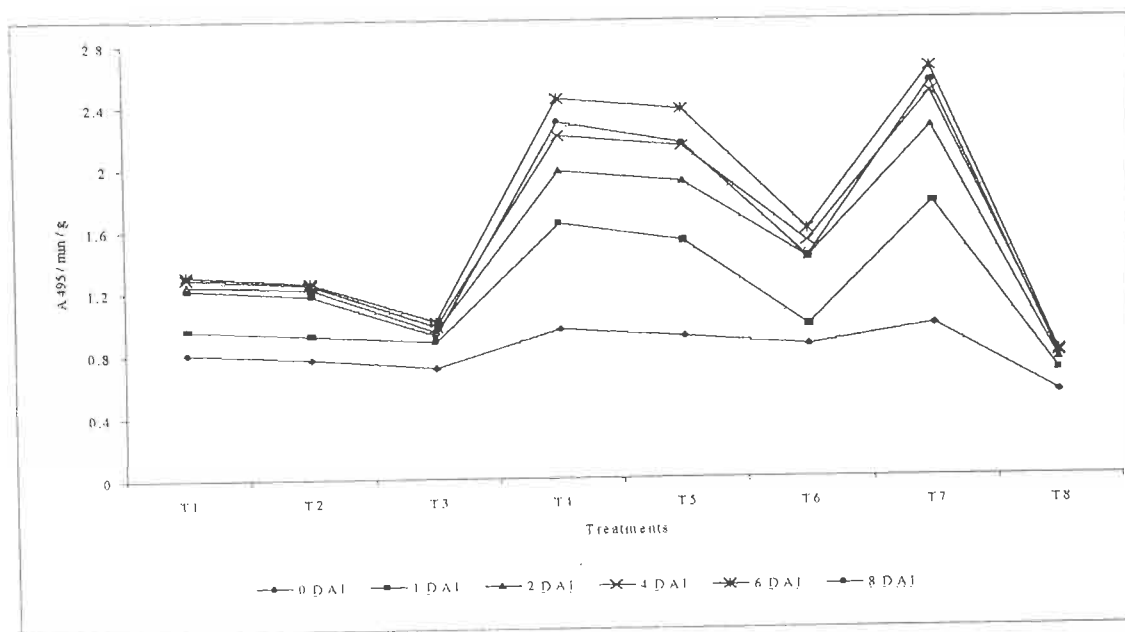


Fig. 23. Changes in polyphenol oxidase activity in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa*

Tomato



Brinjal



4.13. Allelochemicals

4.13.1. Isolation and identification of allelochemicals in *B. scandens* and *T. erecta* by SDS-PAGE method

It has been often observed that each plant part has different protein profile, which is responsible for its nature of action, such observations are well reflected on their biochemical characteristics. Figure 24 shows an alteration in the steady state protein profile and their respective Rf values and molecular weight of the proteins are given in table 38. It is clear from these figures, the protein alterations ranged in molecular weight from as low as 6.4 KDa to as high as 98.8 KDa. The presence of 98.8, 82, 68, 54, 52.6, 47.4, 47.12 and 6.4 KDa proteins was observed in *B. scandens* leaf and *T. erecta* root except 52.6 and 6.4 KDa proteins, on the other hand alteration in level (enhanced expression) of certain specific polypeptides were found to be unique to *T. erecta* root, for instance, accumulation of 82, 68, 54, 47.4 and 47 KDa proteins (Plate 25).

4.13.2. Isolation and identification of allelochemicals in *A. squamosa* by Thin Layer Chromatography (TLC)

The *A. squamosa* extract prepared in benzene, ethanol, ethyl acetate and petroleum ether showed a varying degree of nematocidal activity against *M. incognita* juveniles with 43.4, 96.7, 14.5 and 66.5 per cent mortality after 24 hr of exposure to the extract.

4.13.2.1. Terpenoids

The test sample, *A. squamosa* leaf was extracted in four organic solvents viz., benzene, ethanol, ethyl acetate and petroleum ether to identify the nematocidal principle present in it. Among the organic solvents tried ethanol extract showed comparatively more number of compounds with Rf values of 0.22, 0.31, 0.51, 0.59 and 0.74 (Table 39) when

Table 38. Rf values and molecular weight of proteins present in *B. scandens* and *T. erecta*

Sl.No.	Rf value	Mol. weight (Kda)	<i>B. scandens</i>	<i>T. erecta</i>
			leaf	root
1	0.8	98.8	+	+
2	2.0	82.0	+	+
3	3.0	68.0	+	+
4	4.0	54.0	+	+
5	4.1	52.6	+	-
6	4.5	47.4	+	+
7	5.3	47.0	+	+
8	6.0	12.0	+	+
9	6.4	6.4	+	-

+ Present

- Absent

Table 39. Rf values of allelochemicals in *A. squamosa* leaf extract sprayed with Vanillin H₂SO₄ reagent on TLC plate

No. of spots	Spray reagent	Solvent system used			
		Benzene	Ethanol	Ethyl acetate	Petroleum ether
1		0.14	0.22	-	0.19
2	Vanillin H ₂ SO ₄ reagent	-	0.31	-	0.34
3		-	0.51	-	0.51
4		-	0.59	-	-
5		-	0.74	-	-

Fig. 24. Zymogram of protein bands in *B. scandens* leaf and *T. erecta* root

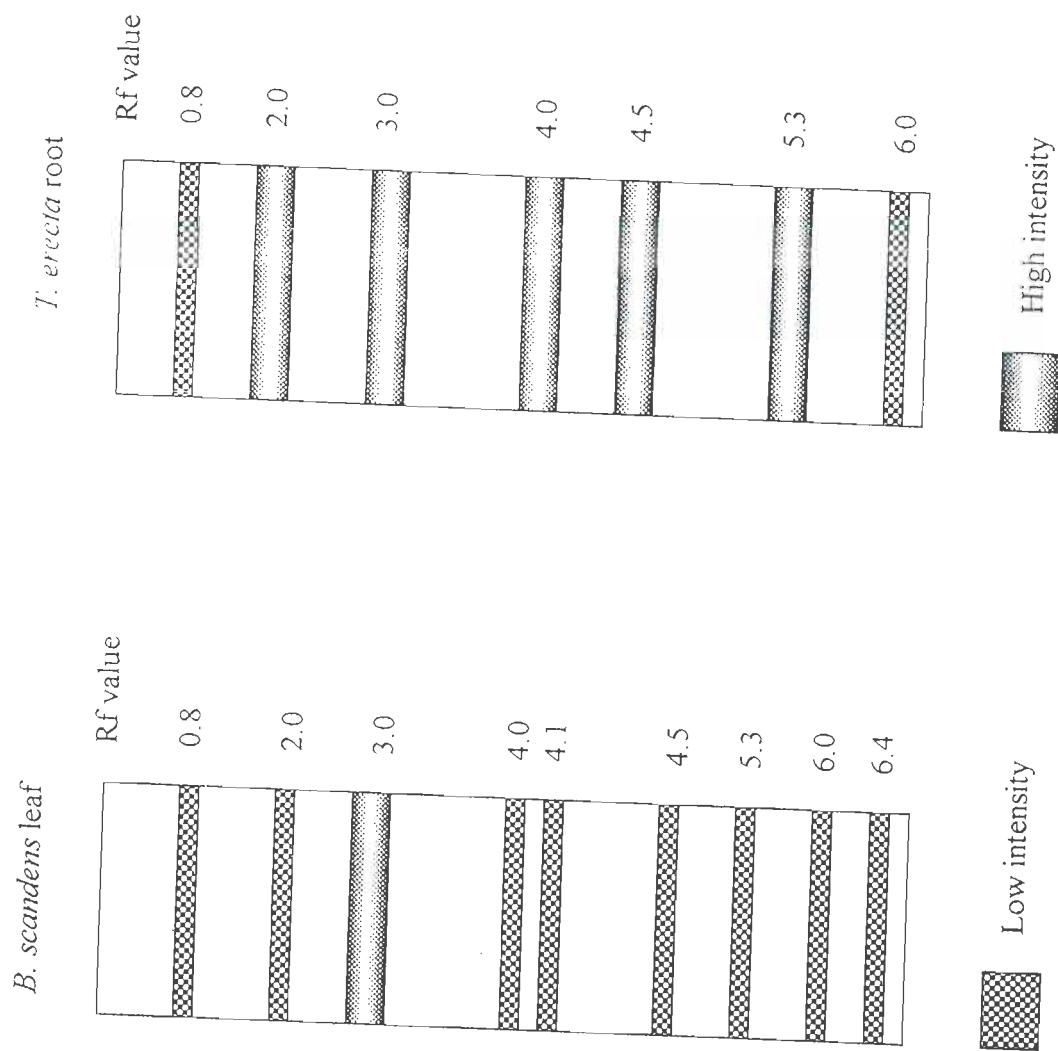


Plate 25. SDS – PAGE analysis of crude proteins in *B. scandens* leaf and *T. erecta* root

1. *B. scandens* leaf
2. *T. erecta* root

Plate 26. TLC plate showing terpenoids in *A. squamosa*

1. Benzene
2. Ethanol
3. Ethyl acetate
4. Petroleum ether
5. Benzene
6. Ethanol
7. Ethyl acetate
8. Petroleum ether

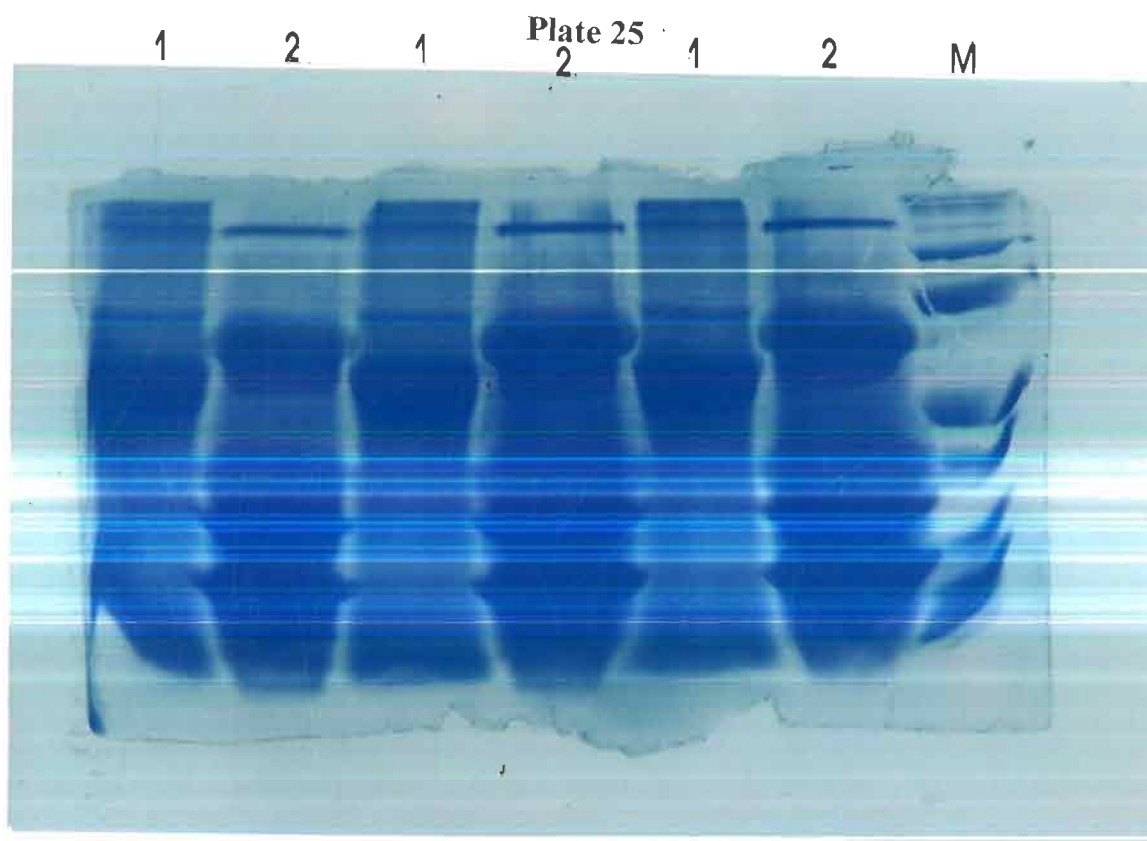


Plate 26



sprayed with Vanillin-H₂SO₄ reagent for terpenoids (Plate 26). These spots were then scraped correspondingly in portions not sprayed and dissolved in ethanol and tested against *M. incognita* juveniles and eggs. The compounds with Rf values of 0.74, 0.59 and 0.51 were most effective to cause mortality of juveniles and to inhibit egg hatching when compared to the compounds with Rf values of 0.31 and 0.22.

4.13.2.2. Phenols

When the ethanol extract sprayed with Diazotized sulphanilic acid reagent for the identification of phenols it showed two compounds with Rf values of 0.36 and 0.42. Phenols were also tested against *M. incognita* juveniles and eggs as in the terpenoids and showed strong larvicidal and ovicidal action.

4.13.2.3. Saponins

A. squamosa extract when tested for the presence of saponins, it reacted positively by forming the foam and confirm the presence of saponins.

The above results revealed the presence of these allelochemicals in *A. squamosa*, *B. scandens* and *T. erecta* responsible for its detrimental effect on root-knot nematode, *M. incognita*. Further, the test plant possessing some other unidentified secondary metabolites may be also explained as possible reason for its adverse effect on *M. incognita*.

Discussion

CHAPTER V

DISCUSSION

The results obtained on various aspects like effect of different plant extracts on hatching, larval mortality and management of root-knot nematode on tomato and brinjal by different method of application of plant extracts, their induced biochemical changes in plants, histopathology of *M. incognita* infested roots treated with plant extract, isolation of allelochemicals in the most effective plants of *A. squamosa*, *T. erecta* and *B. scandens* are discussed.

5.1. *In vitro* nematicidal effect of plant extracts

Out of the 50 plants tested against the root-knot nematode, *M. incognita*, *A. squamosa*, *T. erecta* and *B. scandens* extracts were found to be more toxic to *M. incognita* and to inhibit the emergence of juveniles.

Numerous similar reports about the nematicidal properties of different plants are available in the literature. The efficacy of *C. longa*, *E. alba*, *A. sativum*, *A. marmelos* and *O. basilicum* extracts against juveniles of nematodes including *M. incognita* studied by several workers and proved that these plant extracts were nematicidal even at reduced concentration (Pillai *et al.*, 1975; Chatterjee *et al.*, 1982; Ramakrishnan *et al.*, 1995 and 1999).

Absolute mortality of *M. incognita* juveniles had been reported with calotropis leaf extracts (Mojumder and Goswami, 1987; Ahmad and Karim, 1990). The finding of Goswami and Vijayalakshmi (1986 a) agrees with the present result, as different leaf extracts reduced hatching of *M. incognita*. Hasan and Jain (1984), Rao *et al.* (1986) and

Pathak *et al.* (1989) reported *Parthenium* sp. to be effective in reducing the hatching of *M. incognita*. Bhatti (1988) vouched to the efficacy of *P. hysterophorus* plant extract in checking the hatching of the cereal cyst nematode, *Heterodera avenae*.

The experiments conducted by Mishra and Prasad (1973) and Mishra *et al.* (1989) showed an adverse effect of neem cake extract on hatching of root-knot nematode. Gowda and Setty (1979) found that *M. incognita* egg masses soaked in 1:10 dilution of water extract of neem cake for 24 hr significantly reduced hatching. Experiments conducted by Chatterjee *et al.* (1982) reported that the aqueous extracts of *O. sanctum* and *O. basilicum* leaves killed *M. incognita* larvae within a short period of 160 minutes and that the essential oils, eugenol and linalool present in leaves were responsible for the nematocidal effect.

Extracts of *A. squamosa*, *C. pendulus*, *D. fastuosa* and *S. surattense* produced 100 per cent mortality of *M. javanica* juveniles after 72 hr of exposure (Abid *et al.*, 1997). These workers have also proved that the toxicity of the leaf extracts to nematodes increased with increasing concentration of the extract and the exposure period. Similarly, the egg hatch was inversely proportional to concentration of extract and directly proportional to the exposure time as observed in the present study.

The water extract of marigold plant inhibited juvenile hatch (Walia and Gupta, 1997) and also root extracts were more effective than extracts from aerial parts and this differences in activity may be due to differences in concentrations of nematocidal thiophenes between plant parts (Uhlenbroek and Bijloo, 1958). These results justifying the results of the present study wherein 100 per cent inhibition was seen with the ethanol extract of *Tagetes* root. All these findings were in consonance with the present results.

Similarly, the nematocidal effect of *Tagetes* spp. leaf, root and flower extract was observed by several workers on the egg hatching and mortality of root-knot, cyst and citrus nematodes (Husain and Mashood, 1975 a; Kumari *et al.*, 1986; Siddiqui and Alam, 1988 a and b; Subramanian and Selvaraj, 1988; Sasanelli and Divito, 1991; Sharma and Trivedi, 1995)

The trend of the present result indicated decrease in hatching of *M. incognita* with increased concentration of plant extracts. Goswami and Vijayalakshmi (1986 b) and Jain *et al.* (1986) also reported a linear relationship between the concentration of plant extract and number of larvae hatched out through their experiments.

A. squamosa and *T. erecta* at 80 per cent concentration caused 100 per cent suppression of hatching. Panday and Haseeb (1988) have also observed highest inhibition in *M. incognita* egg hatching in the standard dilution of the shoot extract of *G. superba*. Hasan and Jain (1984) found 100 per cent inhibition of hatching egg masses kept in 1:10 and 1:25 concentration of the plant extracts and at 2500 and 5000 ppm of Parthenin and Saponin, the bitter principles from *P. hysterophorus*.

In general, larval mortality was higher with increase in concentration of ethanol extracts of different plant parts and exposure period. This result agrees with conclusions of Goswami and Vijayalakshmi (1990), Mani *et al.* (1986) and Chhabra *et al.* (1988). In the present study, among different treatments *A. squamosa* recorded the highest per cent of juvenile mortality followed by *T. erecta* and *B. scandens*. Nematicidal property of *Tagetes* spp. extracts prepared in petroleum ether was found effective against root-knot nematode (Castro and Munoz, 1982; Munoz *et al.*, 1982). Vijayalakshmi *et al.* (1979)

recorded 100 per cent mortality of *M. incognita* with *C. procera*, *E. alba*, *A. indica*, *C. sativa* and *M. piperita*.

T. indica, *V. negundo*, *A. precatorius* and *E. globulus* was found as second in the order of efficacy against *M. incognita* in the laboratory experiment conducted in the present study. Chhabra *et al.* (1988) also found the difference in efficacy of leaf extracts with *L. leucocephala*, *P. deltoides*, *L. camera* and *E. hybrida*. Subramanian and Vadivelu (1990) observed 79.0, 63.0 and 48.0 per cent mortality of *M. incognita* with 1:5 and 1:10 dilutions of root extract and 1:5 dilution of leaf extract of *C. spectabilis*.

Among the different concentrations tried, 80 per cent recorded the highest per cent mortality followed by 60 and 40 percent and least was in 20 per cent. In the treatment concentration interaction, 100 per cent mortality was seen in *A. squamosa* and *T. erecta* at 80 % concentration followed by 99.67 per cent in *B. scandens*. This result is in accordance with the findings of Siddiqui and Alam (1985) who had recorded 100 per cent mortality of *M. incognita* in 'S' (100 %) concentration of margosa fruit after 24 hr and in the same concentration of margosa leaf, flower and bark after 48 hr. The 'S' concentration of root extract of *G. superba* was reported to give 100 per cent mortality of *M. incognita* after 12 hr (Pandey and Haseeb, 1988).

Lethal activity of methanol extracts of *Q. bidwillii* was found effective against *M. incognita* (Latif *et al.*, 2000). They also isolated chapparinone as the active principle and estimated the LC₅₀ values of 47.0 ppm for *M. incognita*. LC₅₀ value of 4.4µg/ml was estimated against *Caenorhabditis elegans* with oleanolic acid from *O. gratissimum* leaves (Njoku *et al.*, 1997). Experiment conducted by Mishra and Prasad (1973) revealed that, on

the basis of LC₅₀ values, aqueous extract of neem cake was the most toxic to second stage juveniles of *M. incognita*, followed by mahua cake.

In the present study the antinemic activity of leaf extracts of the test plants can be attributed to the presence of nematotoxic chemicals (Prohibitins) such as flavones, flavonoids, flavonic glycosides, steroids, alkaloids, terpenoids and thiophenes etc.

Different groups of naturally occurring phytochemicals from plant juices, or their parts and extracts with organic solvents or root leachings having nematocidal or nematostatic compounds were in record (Gommers, 1973; Miller *et al.*, 1973; Sukul *et al.*, 1974).

Many chemical compounds are known to occur in plants. Some of them like fatty acids (Tarjan and Cheo, 1956), phenols (Hasan and Saxena, 1974), alkaloids (Husain and Masood, 1975 b) and phenolic glucosides (Clark *et al.*, 1959) have been proved toxic to nematodes. Mortality of second stage larvae of *M. incognita* by different plant extracts tested can be attributed to the presence of some of these chemicals.

Presence of fatty acids, myricyl alcohol, sitosterol, anonol and Anonaine in the leaves, bark and seeds of *Annona* spp. (Callan and Tutin, 1911; Santos, 1930), alkaloids viz., α -tetrathienyl derivatives, thiophene, d-limonene, ocimene, linalool, α -d-phellandrene, linalyl acetate, tegetone, n-nonylaldehyde, cinceol, α -pinene, β -pinene, dipentine, menthol and geraniol in flower, leaf, stem and roots of *Tagetes* spp. (Uhlenbroek and Bijloo, 1959; Gupta and Bhandari, 1974; Baslas and Singh, 1980; Philip and Berry, 1975; Tripathy and Gupta, 1991), palmitic, stearic, linoleic acids in *Datura* spp. (Badami and Patil, 1975) and koenimbine and koenidin from *M. koenigii* (Srivastava *et al.*, 2000) had been reported to possess biocidal action.

Essential oils, β -caryophyllene and β -gurjunene was isolated from *M. koenigii* by Srivastava *et al.* (2000) and suggesting the antinemic activity of oils being highly polar to penetrate the nematode cuticle.

Uhlenbroek and Bijloo (1958 and 1959) isolated two thiophenic compounds, viz., α -tetrathienyl and 5-(-3-buten-1-yny 1)-2, 2'-bithienyl, with strong *in vitro* nematicidal activity from a variety of *T. erecta*. These compound also possesses the characteristics of membrane distortion and inactivation of nematodes enzymes (Foote, 1976; Spikes, 1977; Ito, 1978). Suatmadji (1969) suggested that the nematicidal principle in marigold be made up of several components in addition to thiophenes. This result further substantiates the toxic effect of the leaves/roots against root-knot nematodes.

5.2. Effect of Plant extracts against *M. incognita* in tomato and brinjal

Botanicals as seed protectant were tested to evaluate their relative efficacy in protecting them from nematodes in the soil, enhancing germination and establishment of seedlings. Seed treatment with *A. squamosa*, *B. scandens* and *T. erecta* extracts reduced the entry of *M. incognita* in tomato and brinjal roots and also improved the growth of seedlings.

The effect of seed treatment with different plant products against *M. incognita* in tomato and brinjal were in record (Mojumder and Goswami, 1987; Mojumder and Mishra, 1991). Saxena and Poornima (1995) concluded that the compounds present in the extract adhering to seed might have direct action on the nematode when they come in contact in the soil, at the same time plants absorb the water-soluble fractions of the compounds present on the seeds, imparting resistance to nematode

attack. Therefore in the present study also seed treatment of plant extracts (*A. squamosa*, *B. scandens* and *T. erecta*) proved effective in controlling *M. incognita* in tomato and brinjal even in the heavily infested soil.

The results obtained by Siddiqui and Alam (1990) showed that seed dressing with nimbin (a terpenoid from *A. indica*) contributed to the improvement of plant growth in tomato, egg plant and okra infested by *M. incognita*, *R. reniformis* in cabbage and cauliflower infested by *T. brassicae*. Improved plant growth of cowpea coupled with nematicidal property against *M. incognita*, *R. reniformis* and also against fungal pathogen, *R. solani* have been demonstrated by Khan and Hussain (1988) due to seed treatment with neem cake.

According to Siddiqui and Alam (1987) the effect of seed treatment with plant products/nematicide on nematodes might have been due to the leaching of the nematicidal compounds from seed into rhizosphere of plants which may act directly as a chemical nematicide or the plants grown from treated seeds had acquired some resistance or tolerance against the nematodes. The findings of Treulove *et al.* (1977) revealed that the advantages of introducing a protective chemical into seeds were (i) the material inside the seed concentrate at the site of attack, (ii) a precise calculated, control rate of chemical can be introduced into the soil thereby it might limit the amount of pesticide and minimize environmental pollution.

The present results of different methods of application of the plant extracts, bare root-dip, foliar spray and soil application of *A. squamosa*, *B. scandens* and *T. erecta* extract proved to be best in increasing plant growth and also reducing *M. incognita* population

both in root and soil. This profound influence of application of plant extracts in improving the plant growth and reducing the nematode population and to inducing unfavorable environment for developing nematode could be attributed to increased inoculation load of the extracts applied in combination of all the three methods (seedling root-dip, foliar spray and soil application) as compared to the lower inoculation of the extracts in the individual method of application. The reduction in nematode population in seedling bare root-dip might be, perhaps due to the absorption of toxic principles of plant extracts by roots, which render even, the susceptible plant highly unfavorable to *M. incognita*. The extracts may have acted against the nematode by way of changing root conditions inimical to them or by means of some chemicals absorbed by the roots or by way of some chemical reactions triggered by these extracts causing resistant to cells against the invasion and development of the pathogens as suggested by Bell (1981) and Giebel (1982). The findings of Husain *et al.* (1984) were fall in line with the present results. They have observed the effect of root-dip of egg plant seedlings with margosa, marigold leaf extract, aldicarb, mustard cake or carbofuran which considerably reduced the development of *M. incognita*.

Vijayalakshmi and Goswami (1987) reported significant increase in plant growth and reduction in *M. incognita* infestation in tomato seedlings dipped in water extracts of oil cakes of *A. hypogaea*, *A. indica*, *S. indicum*, *B. rapa* and *Madhuca indica*. Oils of chalmogra (*Hydrocarpus kurzii*), neem, karanj (*Pongamia glabra*) and polanga (*Calophyllum inophyllum*) were also found to be effective in preventing the larval penetration and gall formation by *M. incognita* in tomato. Among the three methods like seedling root-dip, wet seed dressing and soil treatment tried by Prandhan *et al.* (1989) with some organic oils, the first two methods were reported to be relatively more effective.

The damaging effects of *M. incognita* on tomato were checked to an appreciable extent through bare root-dip treatment with oilcakes of castor and mustard (Abid and Maqbool, 1991).

Organic amendments are highly effective against plant parasitic nematodes. However, in many cases they required in bulk amounts to achieve satisfactory results. Some workers have found out that many plant products are effective even in small amounts when used as bare root-dip treatment (Siddiqui and Alam, 1988 c). Akhtar and Alam (1990) have demonstrated similar results with bare root-dip of tomato in extracts of oil cake and leaves of castor. The reduction in root-knot development due to root-dip treatment could also be attributed to unfavorable root conditions causing poor penetration and later retardation in biological activities such as feeding and/or reproduction of the nematode as suggested by Bunt (1975).

Foliar spray with the commercial formulation of *Annona* spp., namely Annona 72 EC, Annona 36 EC and Neem 36 EC significantly reduced the pigeonpea pod borer, *Helicoverpa armigera*. The efficacy of Annona in combination with neem was highly significant (Das *et al.*, 2000). As stated by Verma and Anwar (1995) and Walia and Gupta (1997). *T. erecta* also improved the plant growth, inhibited the nematode population and gall index in to the present study.

Christensen (1998) pointed out that acetylenes and thiophenes from higher plants are having strong biocidal activity against fungi and nematodes. The primary mechanism of action of thiophenes seems to be a photodynamic disruption of membranes, involving singlet oxygen, whereas aliphatic acetylenes appear to have a predominantly non-photodynamic (oxygen-independent) mechanism of action. Aromatic acetylenes

exhibit both a photodynamic and a non-photodynamic mechanism of action. The biological activities of acetylenes and thiophenes against a wide range of organisms suggest that they may have a protective role within the plant.

From the present investigations it is clear that the extracts of *A. squamosa*, *B. scandens* and *T. erecta* reduced the gall index, egg mass production and number of eggs per egg mass by *M. incognita* both in tomato and brinjal. This may be due the reason that the plants have acquired some induced systemic resistant to the *M. incognita* and reduced its multiplication. Systemic acquired resistance response occurs in plants as a result of physical injury, parasite infection or application of foreign substances (Ogallo and McClure, 1995; 1996; Roy *et al.*, 1995). Induced systemic resistance was also observed by Sukul *et al.* (2001) in tomato and mulberry plants treated with *Acacia auriculiformis* and *Artemisia nilagirica* extracts as foliar spray at extreme dilution against *M. incognita*.

M. incognita being an sedentary endoparasite, the closer contact probably allows permeation of toxic compounds from (ruptured) plant tissue into *M. incognita* since many compounds, especially smaller apolar molecules, easily penetrate the body of nematodes could had deleterious effect in its development and reproduction as suggested by Marks *et al.* (1968), Haverkate *et al.* (1972) and Castro and Thomason (1973). Starr (1979) detected peroxidase isozymes in females of *Meloidogyne* spp. similar to those of the host plant.

5.3. Effect of plant extracts against nematode-disease complex in tomato and brinjal

Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic unlike chemical fungicides (Misra and Dixit, 1977; Singh *et al.*, 1986; Dubey, 1991). The extracts of plants also

exhibited marked effect on germination of fungal spores and some plant parts were also recommended to control the disease (Dixit *et al.*, 1983; Pandey *et al.*, 1983).

Extracts of *A. squamosa*, *B. scandens* and *T. erecta* inhibited the growth of *F. oxysporum* f. sp. *lycopersici* and *R. solani*. Hethelyi *et al.* (1986) found anti-microbial activity of five secondary compounds in *T. minuta*: beta-ocimene, dihydrotagetone, tagetone, ocimenone (Z) and ocimenone (E) when tested on 40 strains of fungi, the essential oil of *T. minuta* had 100 per cent inhibitory effect on the test fungi. Antifungal activity of *B. scandens*, *B. variegata*, *B. vahlii*, *B. purpurea* and *B. acuminata* leaf residual solid prepared in ethanol were tested against *R. oryzae* showed inhibition in fungal growth and sporulation (Hajra and Chatterjee, 1996).

5.4. Integrated management of *M. incognita* in tomato and brinjal with plant extract, bioagent, oil cake and nematicide

Amending the soil with the non-edible oilseed cake, castor @ 10 g/m² along with *A. squamosa* (seedling root-dip for 1 hr + foliar spray at 30 DAP) at 60 per cent concentration and *P. lilacinus* @ 10 g sorghum grain colonized fungus per m² had reduced root galling, eggs/egg mass, root-knot index and increased shoot and root growth and yield of tomato and brinjal. The *M. incognita* population reduction as a result of amending soil with oil cakes may be due to the release of toxic substances like ammonia (Khan *et al.*, 1974 a), fatty acids (Sitaramaiah and Singh, 1978 a), phenols and amino acids (Alam *et al.*, 1977 and 1979). The decomposing oilcakes also increase the host resistance (Alam *et al.*, 1980, Haseeb, 1983) and predacious and parasitic activity fungi including *P. lilacinus* in the soil (Kirmani, 1977; Singh *et al.*, 1980 b). The results obtained during

the present studies also are in conformity with those reported by Khan *et al.* (1974 a) and Thakur and Darekar (1995). Sitaramaiah and Singh (1978) also reported that the phenolic compounds released in the soil during the process of decomposition of neem cake may play an important role in controlling root-knot nematodes through larval mortality and by rendering the host roots less attractive to the nematodes.

Application of organic amendments including various oilcakes have been reported to reduce the population of plant parasitic nematodes in soil and at the same time improve the plant growth and increase yield of tomato, okra (Linford *et al.*, 1938; Johnson, 1959; Gowda and Setty, 1973; Ramakrishnan *et al.*, 1997 b). Singh and Sitaramaiah (1967) studied extensively on the effect of organic amendments on nematodes are in accordance with the present findings. They had noted better and increased plant growth of tomato and brinjal by the application of organic amendments and bioagent. Gowda and Setty (1973) also reported the efficacy of castor, groundnut, honge, neem and niger cake in reducing the galling by root-knot nematode and increasing the shoot and root weight of tomato. Bhattacharya and Goswami (1987) observed, groundnut cake to be more effective than neem cake in reducing root-knot nematode, *M. incognita* population in tomato and that effect of groundnut cake was more in unsterilised soil than in sterilized soil. Zaki and Bhati (1989) found leaves of *C. procera*, *R. communis* and *A. indica* to increase the growth of tomato in terms of height and weight thus confirming the phytotherapeutic effects of these plant leaves on *M. javanica*.

In the present study root-knot index, number of females, number of egg masses, number of eggs and soil population was also reduced in the combination treatment of *A. squamosa* and castor cake. This result is in corroboration with the findings of Singh and

Sitaramaiah (1966) who had reported that the application of margosa, castor and peanut cake 0.2 per cent w/w reduced the root galls of okra and tomato caused by *M. incognita*. The highest reduction in population of stylet bearing nematodes were reported in neem cake and nemagon followed by mahua, mustard, groundnut, castor cake and DD (Alam *et al.*, 1974). Application of neem cake was found to reduce the population of *M. incognita* in tomato (Gowda and Setty, 1973; Darekar *et al.*, 1990 a), tomato, egg plant and chili (Alam *et al.*, 1980) and *T. semipenetrans* in khagzi lime (Mani, 1988).

In amended soil due to rapid root development, replacement of diseased root with newer ones and better and prolonged utilization of nutrients by enhanced root system is possible and thus might help in reducing the effect of nematode injury to plants (Sitaramaiah, 1987). Ram and Gupta (1982) reported maximum reduction in gall number in the chickpea plant infected with *M. javanica* in the combination of neem leaves, potassium and/or aldicarb.

In the present study when carbofuran (1.75 g/plot) combined with *A. squamosa* extract (root-dip + foliar spray) reduced the development of *M. incognita* in tomato and brinjal and also boosted the plant growth. Overman and Jones (1975) reported that carbofuran was better in reducing the root galls and root-knot nematode population in tomato. Field trials conducted by Coelho *et al.* (1984) showed that aldicarb at 4 kg a.i/ha was the most effective in controlling *M. javanica* on potato, whereas, the highest dosage of carbofuran (4 Kg a.i/ha) was found as most effective against *P. brachyurus*. Bost (1985) observed that application of phenamiphos or aldicarb to cotton fields infected with *R. reniformis* reduced the nematode population. Aldicarb at 2 kg a.i/ha gave the best control of *M. incognita* on okra (Sultan and Singh, 1987). Sheela and Nair (1988)

concluded that carbofuran or aldicarb caused significant increase in shoot weight of okra, while, aldicarb increased root weight. Thakar *et al.* (1988) observed that phenamiphos and carbofuran at 3 kg a.i/ha increased plant growth.

In a field trial, Siddiqui and Alam (1999) observed that application of oilcakes of castor, groundnut, mustard, rocket salad and carbofuran and aldicarb improved the plant growth of brinjal and reduced the reproduction of *M. incognita*. The beneficial effects of all the above treatments persisted even in the subsequent crop of tomato.

The efficacy of *Polygonum hydropiper*, *Ageratum* sp., *Mikania* sp., rice straw and water hyacinth and two antagonistic fungi, *P. lilacinus* and *Gliocladium virens* were evaluated against *M. graminicola* under field conditions by Das *et al.* (1999) and found to significantly reduces the nematode population. The botanicals also supported the multiplication of the antagonistic fungi.

The bare root-dip treatment of tomato seedlings in castor leaf extract mixed with *P. lilacinus* spores significantly increased the plant growth and reduced the root-knot index and nematode population in comparison to the single application of the either agent. (Rao *et al.*, 1999). In the present findings also combination of *P. lilacinus* with *A. squamosa* bare root-dip treatment decreased the nematode population both in the root and soil. *A. squamosa* extract at 60 per cent concentration increased mycelia growth, sporulation and propagule density of *P. lilacinus*, which was shown by the cfu of the fungus. The increased density of bioagent might help in colonizing the roots of tomato and brinjal plants and offered enhanced biological protection with concomitant increase in parasitization of eggs of *M. incognita* as observed by Cabannillas *et al.* (1989) and Rao and Reddy (1994) in tomato.

From these results, it can be concluded that ethanolic extracts of plant parts contain nematicidal compounds and further investigations are required to identify and characterize the active nematicidal compounds present in different plants for effective control of plant parasitic nematodes.

5.5. Histopathology of *M. incognita* infected tomato and brinjal roots treated with *A. squamosa* extract

Histopathology of plant extracts treated tomato and brinjal root sections showed that there was delay in the development of *M. incognita* and produced only less number of giant cells with less number of nuclei when compared with untreated root sections. In the untreated root tissues *M. incognita* induced multinucleate dense cytoplasmic giant cells in the stelar regions. The giant cells were associated with abnormal xylem and phloem cells.

The most important criteria for successful development of the root-knot nematode is the production of multinucleate giant cells. The increased number and size of giant cells in the untreated root by *M. incognita* indicated the amount of damage caused and also the extent of susceptibility of the host to the nematode. Sosa-Moss *et al.* (1983) reported that *M. arenaria* induced extensively lobed giant cells. This kind of reaction was also noticed in the present investigation with *M. incognita* in tomato and brinjal.

Roots treated with plant extracts showed reduced number and size of giant cells. Similarly small giant cells were produced in *Cucumis* spp. resistant to *M. incognita acrita* (Fassuliotis, 1970) and mung bean and soybean resistant to *M. incognita* (Hussaini and Seshadri, 1976; Kaplan *et al.*, 1979). Hypersensitive reaction was observed in roots of both the crops treated with the plant extracts. The presence of hypersensitivity in the damaged tissue/cells were earlier reported by several workers in many crops, root-knot

nematode resistant sweet potato (Krusberg and Nielsen, 1958; Giamalva *et al.*, 1963), soybean (Dropkin and Nelson, 1960), cotton (Brodie *et al.*, 1960), cowpea (Lakshmanan, 1981) and tomato (Paulson and Webster, 1972; Taylor, 1975). In the present investigation, observations of less number and smaller size giant cells might be due to the presence of systemic resistance in the tomato and brinjal roots as induced by the application of the plant extracts.

5.6. Biochemical changes in *M. incognita* infested tomato and brinjal plants treated with *A. squamosa* extract

Phenolic compounds are known to play a major role in the defense mechanism of plants against various external and infectious agents. Distinct correlation between the degree of plant resistance and the phenolics present in plant tissue has been observed by Pitcher *et al.* (1960), Troll and Rhode (1966) and Brueske and Dropkin (1973). In the present study increased phenols content was recorded in *A. squamosa* treated tomato and brinjal plants. Singh *et al.* (1980 a) found that roots of tomato plants developing from castor, mustard, neem, mahua and groundnut cake treated seeds contained higher amount of phenols than in nematicide treatments or untreated control. It is possible that phenol content might provide resistance to nematode attack either by repelling the larvae or by adversely affecting the development of larvae that entered into roots (Singh *et al.*, 1983). Singh *et al.* (1985) reported that amending soil with neem cake or sawdust supplemented with fertilizers increased the phenolic content, which helped in reducing the nematode infestation. Experiments conducted by Ramachandran (1986) revealed the presence of highest phenolic content in neem cake treated tomato plants. Jonathan (1988) reported

maximum amount of total phenols in bhendi plants grown in the soil amended with neem leaves and pressmud compared to untreated check.

In the present study, amount of free aminoacids was more in tomato and brinjal plants treated with *A. squamosa* extract when compared to the untreated control. The increase in free aminoacids may lead to protein increase in protein content as suggested by Feldman and Hanks (1964), to be either directly from a stimulation in nitrogen metabolism of the host, or indirectly from oxidation of certain phenols by the host to form quinones that combine with and thereby inactivate some proteins. Alam *et al.* (1980) showed an increase in concentration of phenols, aminoacids, protein and carbohydrate in roots of tomato, egg plant and chilli treated with oilcakes of mahua, castor, neem, mustard and groundnut. Significant increase in the phenols and aminoacid content in roots of egg plant treated with oilcakes of sunflower and peanut were reported by Singh and Singh (1988 b).

The enzyme peroxidase (PO) oxidises a wide variety of compounds in plants by hydrogen peroxide. The invasion of nematodes into plant roots appears to lead to an increase of peroxidase in infected roots of host during the early stages (Gregory and McClure, 1978, Ibrahim, 1991). Mohanty *et al.* (1986) reported a similar trend in peroxidase activity in resistant cowpea plants inoculated with *M. incognita*. Significantly higher peroxidase enzyme was detected in *M. incognita* resistant tobacco and tomato tissues by Shukla and Charaborty (1988)

Veech and Endo (1969) and Giebel *et al.* (1971) have stated that peroxidase is bound to the plant cell walls and that it is associated with peroxidation of phenolic substances during the formation of lignin and suberin cell wall. Asada *et al.* (1975), Goldberg and Catesson (1985) and Kavers and Gasper (1985) reported that lignin forms as a direct response to infection or wounding in plants. Giebel *et al.* (1971) observed the "barrier" in cells with higher peroxidase activity at the beginning of giant cell formation which later disappears. The above observations suggest that peroxidase are produced by plants as the first reaction against nematode invasion.

It was speculated that reduced level of PO isozymes could be one of the reasons to explain susceptibility to nematodes (Ganguly and Dasgupta, 1979). In the present investigation also low level of PO was observed in the untreated plant which showed maximum *M. incognita* multiplication. Whereas the tomato and brinjal plants treated with plant extract showed higher induction of PO with minimum *M. incognita* reproduction.

About 95 per cent of the oxygen taken up by the higher plants are consumed *via* cytochrome oxidases which are sensitive to cyanide. The other 5 per cent is utilized by ascorbic acid oxidase (AAO) and polyphenol oxidase (PPO), which are insensitive to cyanide poisoning (Huang, 1985). The role of AAO, PPO and ascorbic acid and cyanide resistant respiration imparting root-knot resistant has been well documented by various workers (Arrigoni, *et al.*, 1977; Zacheo *et al.*, 1980; Hassan *et al.*, 1994).

The nematode penetration into the host plant leads to the production of superoxide anions in resistant cultivars and in susceptible cultivars these were scavenged by the enhanced superoxide dismutase (SOD) activity. In the resistant cultivars, the relative

inactivity of SOD produce increased superoxide anions, which might be the possible cause of cell necrosis and the hypersensitive reaction (Zacheo and Zacheo, 1988).

The present study with the application of the plant extract increased the activity of enzymes *viz.*, PO, PPO, phenylalanine ammonia lyase both in tomato and brinjal when compared to the untreated control. This may be due to the capacity of *A. squamosa* extract to induce the activity of the enzymes, which impart resistant to the *M. incognita*.

5.7. Isolation and identification of allelochemicals

5.7.1. Allelochemicals in *T. erecta* and *B. scandens*

Marigold (*T. erecta*) is a member of Asteraceae family rich in many secondary compounds. Nematicidal compounds 5-(3-buten-1-ynyl) 2, 2'-bithienyl and α -terthienyl were the first nematicidal principles isolated from *Tagetes* sp. roots by Uhlenbroek and Bijloo (1958 and 1959). Further, alpha pinene, β -pinene, dipentine, menthol and geraniol, d-limonene, tagetone, linolool, 1:8 cineole and linalyl acetate was also reported to present in *Tagetes* sp. (Baslas and Singh, 1980).

Vasudevan *et al.* (1997) suggested that the (i) bioactive extracts of different plant parts of *Tagetes* sp. exhibited nematicidal, fungicidal and insecticidal activities, (ii) the nematicidal activity of roots is attributed to thienyls and (iii) the biocidal components of the essential oils from flowers, leaves and root are the terpenoids. Piccaglia *et al.* (1998) have identified the occurrence of lutein and lutein ester contents in different types of *T. patula* and *T. erecta*. Ray *et al.* (2000) isolated α -sesquiphellandrene, β -sesquiphellandrene, myristoleic acid and triecosane from flowers of *T. erecta* and also purified myristic acid.

Bauhinia spp. is a tropical plant belonging to the family Leguminaceae, found in the hills and plains of India. Its species, *B. scandens* is used as snake repellent, *B. vahlii* as fodder, *B. variegata* as antibacterial, antifungal, antiprotozoal and antiviral (Aswal *et al.*, 1984). Leguminous plants constitute a larger number of pyrrolizidine alkaloids (Warren, 1970). *B. scandens* being a leguminous plant has every possibility of constituting the pyrrolizidine.

From the present investigation it was identified that protein part was also found to have nematocidal action in *B. scandens* and *T. erecta*.

A number of biochemical and physiological changes have been associated with infection of pathogen. These include synthesis of novel proteins and enhanced expression of specific antagonistic proteins (Bol *et al.*, 1990; Bowles, 1990; Dangl *et al.*, 1996). A protein is classified as Systemic Acquired Resistance (SAR) protein when its presence or activity correlates tightly with maintenance of the resistance state (Neuenschwander *et al.*, 1996).

From the result it was observed that 6.4 and 52.6 KDa proteins were observed only in *B. scandens* leaf, but enhanced expression of 82, 68, 54, 47.4 and 47 KDa proteins was observed in *T. erecta* root. This enhanced expression of proteins may trigger SAR in the treated plants. The relative expression of level of SAR genes vary between different plant species as coated by Ryals *et al.* (1992). Because the SAR genes are strongly expressed when resistance is maintained, the encoded proteins could contribute to resistance. In support of the present investigation the enhanced expression of proteins may triggered the defence system of tomato and brinjal plants to *M. incognita* infection. Furthermore, synergistic activity has been found for over expression of Pathogenesis-related proteins with increase in resistance to pathogen infection (Alexander *et al.*, 1993).

5.7.2. Allelochemicals in *A. squamosa*

The family Annonaceae (custard-apple family) consists aromatic trees and shrubs of tropical origin. The leaves of *A. squamosa* have a heavy disagreeable odour and the seeds contain a highly acrid principle fatal to insects (Lindley, 1860).

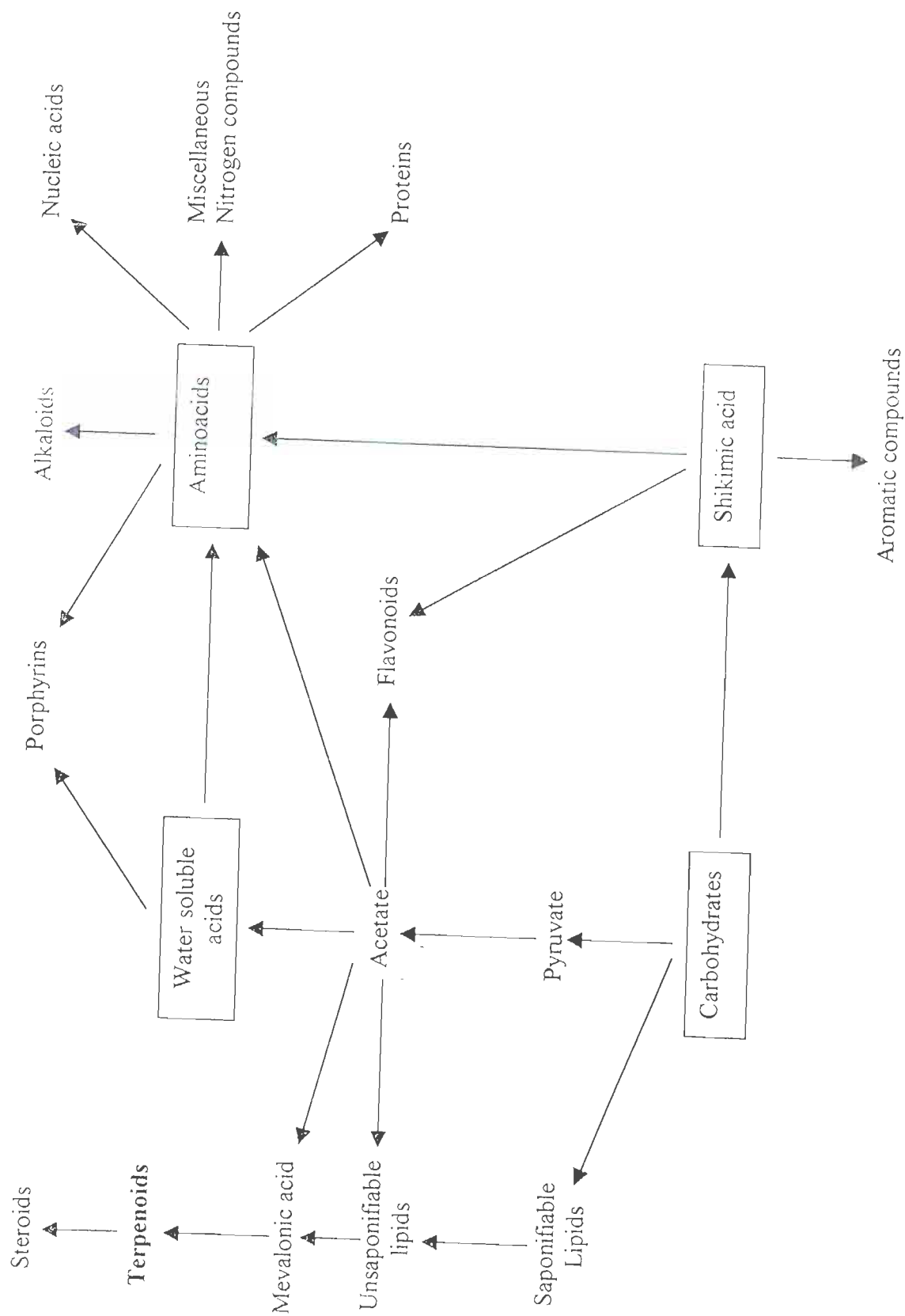
Callan and Tutin (1911) isolated a steam-volatile essential oil, uncrystallizable alkaloid, fatty acids, myricyl alcohol, sitosterol and anonol (member of phytosterol glucosides). An alkaloid, *Anonaine* was isolated from the bark of *A. squamosa* (Timurti, 1924), *A. reticulata* (Santos, 1930) and *A. squamosa* (Reyes and Santos, 1931). Barger and Sargent (1939) made studies on the alkaloids of *Artabotrys suaveolens* (Annonaceae) and isolated three alkaloids, Artabotrine, Suaveloline and Artabotrine.

Apart from the above said compounds, terpenoids, phenols and saponins were isolated in the present investigation from the leaves of *A. squamosa*. These compounds also showed strong nematicidal effect against second stage juveniles of *M. incognita* and adding some more credits to the custard apple tree, *A. squamosa*.

Mathela (1994) pointed out that the terpenes have allelochemic properties because of (i) their presence in various phases of interaction, (ii) presence in the aerial parts and capable of forming vapour clouds of volatiles around the plants, (iii) presence in the soil surrounding the plants, (iv) ability to remain in the dry soil until the soil micro-organisms decompose, and (v) being able to be transported into plant cells through the waxy coatings of seeds or roots.

These allelochemicals originate either through the acetate or shikim pathways. The major biogenetic pathways leading to the formation of v allelochemicals are given in figure 25 as suggested by Mathela (1994).

Fig. 25. General metabolic pathways of allelochemicals biosynthesis



Summary

CHAPTER VI

SUMMARY

The root-knot nematode, *Meloidogyne incognita* is a potential pest of tomato and brinjal and is established to be one of the major causes for limiting crop production. Hence, it is needless to state that suitable management method is essential for successful cultivation. With the above facts in view, studies were undertaken to know the effect of various plant extracts on hatching and mortality of *M. incognita*. Attempts were also made to evaluate the effect of different methods of application of plant extracts against root-knot nematode, nematode disease complex and biochemical changes, integrating the most effective plant extract with bioagent, oilcake and nematicide against *M. incognita* in tomato and brinjal, isolation and identification of allelochemicals in the test plants were summarized.

1. Inhibition in egg hatching and high degree of larval mortality was caused by *Annona squamosa*, *Bauhinia scandens*, *Tagetes erecta*, *Thuja orientalis* and *Phyllanthus niruri* even in the 20 per cent concentration of the extracts, out of fifty plant extracts of 20, 40, 60 and 80 per cent concentration screened against *M. incognita*.
2. In the comparative efficacy of plant extracts, *A. squamosa*, *T. erecta* and *B. scandens* showed reduced LC₅₀ value of 25.57, 28.90 and 36.93 per cent and LT₅₀ value of 8.62, 9.42 and 13.34 hr respectively.
3. *A. squamosa* and *T. erecta* recorded lowest penetration of *M. incognita* juveniles into the roots of tomato with 9.25 and 18.50 per cent and 7.50 and 16.25 per cent in brinjal at 60 per cent concentration of the plant extracts on 5th day after inoculation.

While *B. scandens* showed the maximum penetration of juveniles in both crops. In addition all these plant extracts inhibited the growth of test fungi, *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* *in vitro*.

4. Seed treatment of tomato (cv. Co 2) and brinjal (cv. Co 1) with *A. squamosa* extract registered the maximum plant growth parameters, minimum number of galls and soil nematode population, followed by *T. erecta* and *B. scandens*. Their combined application as root-dip + foliar spray + soil application proved to be the best in improving plant stand and also increased the yield of both tomato and brinjal.
5. In tomato and brinjal lowest root-knot index, number of females and egg masses/g root, number of eggs/egg mass and soil nematode population were recorded in the method of combined application of the plant extracts.
6. The best performing plant extract, *A. squamosa* was also tested against nematode-disease complex involving *M. incognita* with *F. oxysporum* f. sp. *lycopersici* in tomato and *M. incognita* with *R. solani* in brinjal, proving its maximum efficacy when all the three methods of application was combined.
7. Histopathology of *M. incognita* infested roots of tomato and brinjal plants treated with *A. squamosa* extract showed smaller size giant cells with less number of nuclei.
8. Integrated application of *A. squamosa* extract with *Paecilomyces lilacinus*, castor cake and carbofuran against *M. incognita* in tomato and brinjal exhibited pronounced effect on plant growth and yield. Root-knot index and number of egg masses/g root was minimum in plants treated with *A. squamosa* + *P. lilacinus* + castor cake in both tomato and brinjal.

9. Combined application of root dip + foliar spray + soil application of *A. squamosa* extract showed maximum induction of total phenol, polyphenol oxidase, peroxidase, phenylalanine ammonia lyase and aminoacid in both tomato and brinjal.
10. Allelochemicals like terpenoids, phenols and saponins were isolated and identified from the *A. squamosa* leaf in the present investigation. These compounds exerted strong nematocidal action against *M. incognita*.
11. A protein portion has been identified to be the active nematocidal principle from *B. scandens* and *T. erecta* and their molecular weight of different proteins were calculated.

References



REFERENCES

- Abid, M. and M.A. Maqbool. 1991. Effect of Bare root-dip treatment in oilcakes and neem leaf extracts on the root-knot development and growth of tomato and egg plant. **Pak. J. Nematol.**, **9**: 13-16.
- Abid, M., M.I. Choudhary., M.A. Maqbool and A.U. Rhaman. 1997. Preliminary screening of some plants for their nematicidal activity against *Meloidogyne javanica*. **Nematol. Medit.**, **25**: 155-157.
- Abid, M., M.J. Zaki and M.A. Maqbool. 1995. Neem derivatives for the control of root-knot nematode, *Meloidogyne javanica* on okra. **Pak. J. Phytopath.**, **7**: 212-214.
- Ahmad, H.U., M.R. Karim and M.S.A. Khan. 1990. Effect of some indigenous plant extracts on juvenile mortality of *Meloidogyne javanica*. **Int. Nematol. Network Newsletter.**, **7**: 5 -7.
- Ahmad, M.U and M.R. Karim. 1991. Effect of ten indigenous plant extracts on root-knot nematode of brinjal. **Bangladesh J. Pl. Path.**, **7**: 5-8.
- Ahmad, M.U. and M.R. Karim. 1990. Effects of extract of indigenous plants on galling and growth of brinjal infected with *Meloidogyne javanica*. **Int. Nematol. Network Newsletter.**, **7**: 3- 4.
- Ahmad, R., M. Tariq., N. Javed and M.I. Haq. 1991. Effect of extracts of various plants on egg hatching and larval mortality of root-knot nematode. **Pak. J. Phytopath.**, **3**: 38-42.
- Ahmad, R., M.Z. Shaheb., M.I. Haq., N. Javed., M.A. Dogar and M. Khan. 1996. Effect of soil amendment with *Calotropis procera* for the control of *Meloidogyne javanica* infection of egg plant. **Pak. J. Nematol.**, **14**: 55-59.
- Akhtar, M. and M. M. Alam. 1993. Utilization of waste materials in nematode control: A review. **Bioresource Technol.**, **45**: 1-7.

- Akhtar, M. and M.M. Alam. 1990. Effect of bare-root dip treatment with extracts of castor on root-knot development and growth of tomato. **Nematol. Medit.**, **18**: 53-54.
- Akhtar, M., A. H. Want and M. M. Alam. 1992. Control of root-knot nematode with bare-root dip in leaf extracts of persian lilac and calotropis. **Curr. Nematol.**, **3**: 41-44.
- Alagumalai, K., M. Thiruvalluvar., N. Nagendran and P.Ramaraj. 1995. Effect of acid extract of neem cake on the population build up of *Meloidogyne incognita*. **Environ. Ecol.**, **13**: 275 – 277.
- Alam, K.S., M.U. Ahmed., I. Hussain and A.K.M.A. Haque. 1995. Efficacy of dholkami (*Ipomoea fistulosa*) and furadon to control root-knot nematode of brinjal. **Bangladesh J. Pl. Path.**, **11**: 33-35.
- Alam, M.M., A.M. Khan and S.K. Saxena. 1974. Control of phytonematodes with oilcake amendments in spinach field. **Indian J. Nematol.**, **4**: 239-240.
- Alam, M.M., M. Ahmed and A.M. Khan. 1980. Effect of organic soil amedments on the growth and chemical composition of tomato, egg plant and chilli and their susceptibility to attack by *Meloidogyne incognita*. **Plant and Soil**. **57**: 231-236.
- Alam, M.M., S.A. Siddiqui and A.M. Khan. 1977. Mechanism of control of plant parasitic nematodes as a result of application of organic amendments to the soil. III. Role of phenols and aminoacids in the roots. **Indian. J. Nematol.**, **7**: 27-31.
- Alam, M.M., S.A. Siddiqui and A.M. Khan. 1979. Mechanism of control of plant parasitic nematodes as a result of the application of organic amendments to the soil. V. Role of phenolic compounds. **Indian J. Nematol.**, **9**: 136-142.
- Alen, Y., S. Nakajima., T. Nitoda., N. Baba., H. Kanzaki and K. Kawazu. 2000. Two antinematodal phenolics from *Knema hookeriana*, a Sumatran rainforest plant. **Biosciences**, **55**: 300-303.

- Alexander, D., C. Glascock., J. Pear., J. Stinson., P. Ahl-Goy., M. Gut-Rella., R.M. Goodman and J. Ryals. 1993 a. Systemic acquired resistance in tobacco: Use of transgenic expression to study the functions of pathogenesis-related proteins. *In: Advances in molecular genetics of plant-microbe interactions. Vol.3.* (Eds. E.W. Nester and D.P.S. Verma), Kluwer Academic Publishers, The Netherlands, pp. 527-533.
- Ansari, M.M. 1995. Control of sheath blight of rice by plant extracts. **Indian Phytopath.**, **48**: 268-270.
- Anver, S. and M.M. Alam. 1992. Effect of latex seed dressing on interacting root-knot and reniform nematodes. **Afro Asian J. Nematol.**, **2**: 17-20.
- Anver, S. and M.M. Alam. 2000. Organic management of concomitant *Meloidogyne incognita* and *Rotylenchulus reniformis* on Chickpea. **Allelopathy J.**, **7**: 79-84.
- Appleton, J.A. and M.R. Tansey. 1975. Inhibition of growth of 200 pathogenic fungi by garlic extract. **Mycologia.** **67**: 882-885.
- Arrigoni, O., R.L. Arrigoni and G. Calabrese. 1977. **Federation of European Biochem. Soc.**, **81**: 135-138.
- Asada, Y., T. Oguchi and I. Matsumoto. 1975. Lignin formation in fungus infected plants. **Rev. Plant Protc. Res.**, **8**: 104.
- Aswal, B.S., D.S. Bhakuni., A.K. Goel., K. Kar and B.N. Mehrotra. 1984. Screening of indian plants for biological activity-part XI. **Indian J. Exp. Biol.**, **22**: 487-504.
- Ashrafuzzaman, M.H., A.R. Khan and A.R. Howlider. . 1990. *In vitro* effect of lemon grass oil and crude extracts of some higher plants on *Rhizoctonia solani*. **Bangladesh J. Pl. Path.**, **6**: 17-18.
- Awad, A.G.H., A.M.E. El-Toony., M.F.I. Tadrous and M.A.I. Khalil. 1997. Efficacy of root exudates and extracts of tomato, garlic and onion on *Fusarium oxysporum* f.sp. *lycopersici*, *F. oxysporum* f.sp. *cepa* and *Meloidogyne incognita*. **Arab Univ. J. Agrl. Sci.**, **5**: 105-120.

- Awan, M.N., N. Javed., R. Ahmad and M.I. Haq. 1992. Effect of leaf extracts of four plant species on larval mortality of citrus nematode (*Tylenchulus semipenetrans*, Cobb) and citrus plant growth. **Pak. J. Phytopath.**, 4: 41-45.
- Badami, R.C. and K.P. Patil. 1975. Minor seed oils: Physico-chemical characteristics and components acids of four seed oils. **J. Oil Technol. Assoc.**, 7: 79-81.
- Bajaj, K.L., P. Singh and R. Mahajan. 1985. Changes induced by *Meloidogyne incognita* in superoxide dismutase, peroxidase and polyphenol oxidase activity in tomato roots. **Biochem. Physiol. Pflanz.**, 180: 543-546.
- Barbosa, L.C.A., F.F. Barcelos., A.J. Dsemuner and M.A. Santos. 1999. Chemical constituents from *Mucuna aterrima* with activity against *Meloidogyne incognita* and *Heterodera glycines*. **Nematropica**. 29: 81-88.
- Barger, G. and L.J. Sargent. 1939. The alkaloids of *Artabotrys suaveolens*. **J. Chem. Soc.**, 129: 991-999.
- Baslas, R.K. and A.K. Singh. 1980. Chromatographic separation of essential oil of *Tagetes erecta*. **Indian Perfumer**. 24: 163-165.
- Bell, A.A. 1981. Biochemical mechanisms of disease resistance. **Ann. Rev. Pl. Physiol.**, 32: 21-81.
- Benedict, W.G. and R.A. Hildebrand. 1958. The application of chromatographic methods to study the susceptibility of soybean to stem canker. **Canadian J. Bot.**, 38: 155-163.
- Bhattacharya, D. and B.K. Goswami. 1987. A study of the comparative efficacy of neem and groundnut oilcakes against root-knot nematode, *Meloidogyne incognita* as influenced by microorganisms on sterilised and unsterilised soil. **Indian J. Nematol.**, 17: 81-83.
- Bhatti, D.S. 1988. Utilization of toxic plants for control of nematode pests of economic crops. **Final Technical Report, April 1, 1983 to March 31, 1989**.

- Bhatti, D.S. and R.S. Jain. 1977. Estimation of loss in okra, tomato, brinjal due to *Meloidogyne incognita*. **Indian J. Nematol.**, 7: 37-41.
- Bijloo, J.D. 1965. The "Pisum test": a simple method for the screening of substances on their therapeutic nematocidal activity. **Nematologica**. 11: 643-644.
- Bol, J.G., H.J.M., Linthorst and B.J.C. Cornelissen. 1990. Plant pathogenesis-related proteins induced by virus infection. **Ann. Rev. Phytopath.**, 28: 113-138.
- Bora, T. 1977. *In vitro* and *in vivo* investigations on the effect of some antagonistic fungi against the damping-off disease of egg plant. **J. Turkish Phytopath.**, 6: 17-23.
- Bost, S.C. 1985. Evaluation of nematicides for control of the reniform nematode on cotton. **American Phytopath. Soc.**, 40: 97-98.
- Bowles, D.J. 1990. Defense-related proteins in higher plants. **Ann. Rev. Biochem.**, 59: 873-907.
- Bradford, M.M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. **Ann. Biochem.**, 72: 248-254.
- Bray, G.C. and W.V. Torphy. 1954. Analysis of phenolic compounds of interest in metabolism. **Meth. Biochem. Anal.**, 1: 27-52.
- Brodie, B.B., L. A. Brinkerhoff and F.B. Struble. 1960. Resistance to the root-knot nematode, *Meloidogyne incognita acrita* in upland cotton seedlings. **Phytopath.**, 50: 673-677.
- Brueske, C.H. 1980. Phenylalanine ammonia lyase in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita*. **Physiol. Pl. Path.**, 16: 406-414.
- Brueske, C.H. and V.H. Dropkin. 1973. Free phenols and root necrosis in nematex tomato infected with the root-knot nematode. **Phytopath.**, 63: 329-334.

- Bunt, J.A. 1975. Effect and mode of action of some systemic nematicides. **Meded Landb Hogesch. Wageningen**, 75: 1-128.
- Cabanillas, E. and K.R. Barkar. 1989. Impact of *Paecilomyces lilacinus* inoculum level and application time on control of *Meloidogyne incognita* on tomato. **J. Nematol.**, 21: 115-120.
- Cabanillas, E., K.R. Barker. and L.A. Nelson. 1989. Growth of isolates of *Paecilomyces lilacinus* and their efficacy in biocontrol of *Meloidogyne incognita* on tomato. **J. Nematol.**, 21: 164-172.
- Callan, T. and F. Tutin. 1911. Chemical examination of the leaves of *Annona muricata*. **Pharm. J.**, 33, 743.
- Castro, C.E. and I.J. Thomason. 1973. Permeation dynamics and osmoregulation in *Aphelenchus avenae*. **Nematologica**. 19: 100-108.
- Castro, C.O. and C.L. Munoz. 1982. Natural derivatives in the roots of *Tagetes jalisciencis*. **Revista Latinoamericana de Quimica**. 13: 36-37.
- Chatterjee, A., N.C. Sukul., S. Laskar and S. Ghoshmajumdar. 1982. Nematicidal principles from two species of Lamiaceae. **J. Nematol.**, 14: 118-120.
- Chatterjee, A., N.C. Sukul., S. Laskar and S.G. Majoumdar. 1982. Nematicidal principles from two species of Lamiaceae. **J. Nematol.**, 14: 118-120.
- Chhabra, H.K., P.S. Grewal and A. Singh. 1988. Efficacy of some plant extracts on root-knot nematodes (*Meloidogyne incognita*). **J. Tree Sci.**, 7: 24-25.
- Christensen, L.P. 1998. Biological activities of naturally occurring acetylenes and related compounds from higher plants. **Recent Res. Develop. Phytochem.**, 2: 227-257.
- Clark, R. A., J. Kuc., R.E. Henze and F.W. Quacken. 1959. The nature and fungitoxicity of an aminoacid, addition product of chlorogenic acid. **Phytopath.**, 49: 594-598.

- Cobb, N.A. 1918. Estimating the nematode population of soil. **U.S. Dept. Agr. Tech. Circ.**, 1: 1-48.
- Coelho, S.M.B.M., H.O.A.S.M. Filho and J.F. Franco. 1984. Effect of systemic insecticides-nematicides in control of nematodes on potato. **Revista de agricultura**. 59: 127-136.
- Dalal, H.R., A.S. Taya and B.P. Rana. 1995. Studies on seed soaking in aqueous leaf extracts of some plants for management of *Heterodera avenae* in wheat. **Indian J. Nematol.**, 25: 1-3.
- Dangl, J.L., R.A. Dietrich and M.H. Richberg. 1996. Death don't have no mercy: Cell death programs in plant-microbe interactions. **Plant Cell**. 8: 1793-1807.
- Darekar, K.S., N.L. Mhase and S.S. Shelke. 1988. Assessment of yield loss due to root-knot nematode, *Meloidogyne incognita* race-3 in tomato, brinjal and bitter gourd. **Int. Nematol. Network Newsletter.**, 5: 7-9.
- Darekar, K.S., N.L. Mhase and S.S. Shelke. 1990 a. Effect of placement of non-edible oil seed cakes on the control of root-knot nematodes on tomato. **Int. Nematol. Network Newsletter.**, 7: 5-7.
- Das, D., M.K. Saikia and D.K. Sarmah. 1999. Comparative efficacy of botanicals and antagonistic fungi for the management of rice root-knot nematode, *Meloidogyne graminicola*. **Int. J. Trop. Agri.**, 17: 287-290.
- Das, N. 1999. Nematicidal effect of organic soil amendments of some latex bearing plants on root-knot nematode, *Meloidogyne incognita*. **Crop Res.**, 18: 299-300.
- Das, N.D., G.R.M. Sankar and K.K. Biswas. 2000. Field evaluation of botanical and biopesticides against pod borer, *Helicoverpa armigera* on pigeonpea. **Ann. Pl. Protec. Sci.**, 8: 233-236.
- Dash, B. and N.N. Padhi. 1998. Relative efficacy of neem products against root-knot nematode on tomato. **Indian J. Nematol.**, 28 : 163-167.

- Davis, E. and J.R. Rich. 1987. 1987. Nicotine content of tobacco roots and toxicity to *Meloidogyne incognita*. **J. Nematol.**, **19**: 23-29.
- Devakumar, C. 1994. Nematicidal principles of plant origin. *In*: **Nematode pest management in crops** (Eds. D.S. Bhatti and R.K. Walia), CBS Publishers, New Delhi, pp.165-189
- Devi, S. and P. Gupta. 1995. Larval emergence from egg sacs of *Heterodera cajani* in extracts of cakes in various media and their effect on cowpea. **Indian J. Nematol.**, **25**: 190-193.
- Diener, R.T.O. 1960. Free aminoacids and amides in healthy and virus infected cherry and peach leaves. **Phytopath.**, **50**: 141-145.
- Director of Statistics, 1991. Quarterly Statistical Abstract of Tamil Nadu, March, 153 p.
- Dixit, S.N., N.K. Dubey and N.N. Tripathi. 1983. Fungitoxic essential oils vis-à-vis disease control. *In*: **Recent advances in Plant Pathology** (Eds. A. Husain., K. Singh., B.P. Singh and V.P. Agnihotri), Print House, Lucknow. 521 p.
- Dropkin, V.B. and P.E. Nelson. 1960. The histopathology of root-knot nematode infections in soyabeans. **Phytopath.**, **50**: 442-447.
- Dube, B. and Jr.C. Smart. 1987. Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. **J. Nematol.**, **19**: 222-227.
- Dubey, R.C. 1991. Fungicidal effect of essential oils of three higher plants on sclerotia of *Macrophomina phaseolina*. **Indian Phytopath.**, **44**: 241-243.
- Egunjobi, O.A. and S.O. Onayemi. 1981. The efficacy of water extract of neem (*Azadirachta indica*) leaves as a systemic nematicide. **Nigerian J. Pl. Protec.**, **5**: 70-74.
- Ezhilan, G.J., V. Chandrasekar and V. Kurucheve. 1994. Effect of six selected plant products and oil cakes on the sclerotial production and germination of *Rhizoctonia solani*. **Indian Phytopath.**, **47**: 183-185.

- FAO, 1994. Food and Agricultural Organization, Quarterly Bulletin of Statistics, 162 p.
- Farkas, G.L and Z. Kiraby. 1962. Role of phenolics compard in the physiology of plant diseases and disease resistance. **Phytopath.**, **44**: 105-150.
- Fassuliotis, G. 1970. Resistance of *Cucumis* spp. to the root-knot nematode, *Meloidogyne arenaria acrita*. **J. Nematol.**, **2**: 174-178.
- Feldman, A.W. and R.W. Hanks. 1964. Quantitative changes in the free aminoacids in roots of healthy *Radopholus similis* infected and recovered grape fruit seedlings. **Phytopath.**, **54**: 1210-1215.
- Finney, D.J. 1962. **Probit analysis**. Cambridge University Press, Cambridge, New York, 318 p.
- Firoza, K. and M.A. Maqbool. 1996. Effect of plant extracts in the control of *Helicotylenchus dihystra*. **Pak. J. Nematol.**, **14**: 61-66.
- Foote, C.S. 1976. Photosensitized oxidation and singlet oxygen, consequences in biological systems. *In: Free Radicals in biology. Vol II.* (Ed. W.V. Pryor), New York, Academic Press, pp.85-133.
- Fries, N.C. 1973. Effect of volatile organic compounds on the growth and development of fungi. **Trans. Br. Mycol. Soc.**, **60**: 1-21.
- Ganguly, A.K. and D.R. Dasgupta. 1987. Comparison of protein and some enzymes from galled and non galled parts of the same root system of tomato cultivar Pusa Ruby infected with root-knot nematode, *Meloidogyne incognita*. **Indian J. Nematol.**, **17**: 343-345.
- Ganguly, A.K. and D.R. Dasgupta. 1979. Sequential development of peroxidase and IAA oxidase activities in relation to resistant and susceptible responses in tomatoes to the root-knot nematode, *Meloidogyne incognita*. **Indian J. Nematol.**, **9**: 189-204.

- Ganguly, A.K. and K.R. Dasgupta. 1988. Superoxide dismutase activity in resistant and susceptible cowpea cultivars inoculated with root-knot nematode, *Meloidogyne incognita*. **Indian J. Nematol.**, **18**: 322-325.
- Ganguly, A.K. and D.R. Dasgupta. 1980. Purification and some properties of peroxidase from resistant and susceptible varieties of *Lycopersicon esculentum* infested with root-knot nematode, *Meloidogyne incognita*. **Indian J. Nematol.**, **10**: 189-204.
- Ganguly, A.K., M. Sreedhar and S.C. Swain. 2000. Characterization of partially purified acid phosphatase from susceptible and resistant cultivars of tomato against root-knot nematode *Meloidogyne incognita*. **Ann. Pl. Protec. Sci.**, **8**: 51-56.
- Ganguly, A.K., R. Raman and D.R. Dasgupta. 1991. Qualitative and quantitative changes in protein in cowpea inoculated with root-knot nematode, *Meloidogyne incognita* Race – 1. **Indian J. Nematol.**, **21**: 113-122.
- Ghosh, T. and N.C. Sukul. 1992. Nematicidal principles in the leaves of *Xanthium strumarium*. **Environ. Ecol.**, **10**: 963-968.
- Giamalva, M.J., W.J. Martin and T. P. Hernandez. 1963. Sweet potato varietal reaction to species and races of root-knot nematodes. **Phytopath.**, **53**: 1187-1189.
- Giebel, J. 1982. Mechanism of resistance to plant nematodes. **Ann. Rev. Phtopath.**, **20**: 257-279.
- Giebel, J. 1970. Phenolic content in roots of some solanaceae and its influence on IAA-oxidase activity as an indicator of resistance to *Heterodera rostochiensis*. **Nematologica**. **16**: 22-32.
- Giebel, J., J. Krenz and A. Wilski. 1971. Localisation of some enzymes in roots of susceptible and resistant potatoes infected with *Heterodera rostochiensis*. **Nematologica**. **17**: 29-33.

- Glasiov, K.T., K.R. Gayler and J.C. Waldron. 1967. Effects of auxin and gibberellic acid on the regulation of enzyme synthesis in sugarcane stem tissue. Proceedings of 6th International conference on plant growth substances. 483-442 pp.
- Goldberg, R.L. and A.M. Catesson. 1985. Localization of some enzymes in roots at susceptible and resistant potatoes infected with *Heterodera rostochinesis*. **Nematologica**. 17: 23-33.
- Gommers, F.J. 1973. Nematicidal Principles in Compositae. Dissertation, Agricultural University, Wageningen, The Netherlands, 73 p.
- Goswami, B.K. and N.J. Meshram. 1991. Studies on comparative efficacy of mustard and karanji oil seed cakes with a nematicide, carbofuran against root-knot nematode, *Meloidogyne incognita* on tomato. **Indian J. Nematol.**, 21: 66-70.
- Goswami, B.K. and K. Vijayalakshmi. 1986 a. Nematicidal properties of some indigenous plant materials against root-knot nematodes, *Meloidogyne incognita* on tomato. **Indian J. Nematol.**, 16: 65-68.
- Goswami, B.K. and K. Vijayalakshmi. 1986 b. Efficacy of some indigenous plant materials and non-edible oil seed cakes against *Meloidogyne incognita* on tomato. **Indian J. Nematol.**, 16: 280-281.
- Goswami, B.K. and K. Vijayalakshmi. 1990. Studies on the effect of some plant and non edible oil seed cake extracts on larval hatching of *Meloidogyne incognita*. **J. Res. Assam Agri. Univ.**, 8: 62-64.
- Gowda, D.N. and K.G.H. Setty. 1973. Studies on comparative efficacy of various organic amendments on the control of root-knot nematodes of tomato. **Mysore J. Agric. Sci.**, 7: 419-423.
- Gowda, D.N. and K.G.H. Setty. 1979. *In vitro* studies of neem cake (*Azadirachta indica* Juss.) extract on hatching of eggs and survival of larvae of root-knot nematode (*Meloidogyne incognita* Chitwood). **Curr. Res.**, 8: 78-79.

- Grainge, M. and S. Ahmed. 1988. **Handbook of Plants with Pest-control Properties**. Willey, New York, 203 p.
- Gregory, R.N. and A.M. McClure. 1978. Peroxidase and 6-Phosphogluconate dehydrogenase in resistant and susceptible cotton infected by *Meloidogyne incognita*. **J. Nematol.**, **10**: 34-39.
- Gulsar Banu, J. and S. Vadivelu. 1995. Efficacy of seed soaking with nematicides or botanicals for the control *Meloidogyne incognita* infesting tomato. **Indian J. Nematol.**, **25**: 108-109.
- Gupta, Y.S. and K.S. Bhandari. 1974. Chemical examination of essential oil from the flowering tops of *Tagetes erecta*. **Indian Perfumer**. **2**: 29-33.
- Hackney, R.W. and O.J. Dickerson. 1975. Marigold, castorbean and chrysanthemum as control of *Meloidogyne incognita* and *Pratylenchus alleni*. **J. Nematol.**, **7**: 84-90.
- Hajra, A.G and P. Chatterjee. 1996. Screening of *Bauhinia* spp. for fungicidal, bactericidal and insecticidal properties. *In: Alleopathy in Pests Management for Sustainable Agriculture*. (Eds. S.S. Narwal and P. Tauro), Scientific Publishers, Jodhpur, India, pp. 309-313.
- Hanks, R.W. and A.W. Feldman. 1966. Quantitative changes in free and protein aminoacids in leaves of healthy, *Radopholus similis* infected and recovered grape fruit seedlings. **Phytopath.**, **56**: 261-264.
- Haque, E.S., M. Abid., V. Sultana., J. Ara., A. Saltar and A. Ghaffar. 1993. Use of *Prosopis* sp in the control of root-knot disease of okra. **Pak. J. Nematol.**, **14** : 101-106.
- Haque, S.E., V. Sultana., M. Abid., J. Ara and A. Ghaffar. 1997. Use of *Calotropis procera* and microbial antagonists in the control of *Meloidogyne* root-knot on mung bean. **Pak. J. Phytopath.**, **9**: 108-110.

- Hasan, N. and S.K. Saxena. 1974. Effect of different concentrations of commonly occurring plant phenolic compounds on the larval hatching of *Meloidogyne incognita* (Kofoid and White) Chitwood. 1st Ann. Meet. Soc. Adv. Bot., Mecrut, pp 27-28.
- Hasan, N. and R.K. Jain. 1984. Biototoxicity of *Parthenium hysterophorus* extracts against *Meloidogyne incognita* and *Helicotylenchus dihystera*. **Nematol. Medit.**, 12: 239-242.
- Haseeb, A. 1983. Studies on the changes in roots of egg plant (*Solanum melongena* L.) resulting from infection of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood *Rhizoctonia solani* Kuhn, Complex. Ph.D. Thesis, Aligarh Muslim University, Aligarh, India.
- Hashim, E.F., K.A.A. Seham and A.A. Kheir. 1999. Nematicidal activity of some Labiateous plant extracts on *Meloidogyne incognita*. **Ann. Agrl. Sci.**, 44: 447-457.
- Hassan, H.M., A.M.K. Aliah., I.K.A. Ibrahim and M.M. Badr. 1994. Free aminoacids and oxidative enzymes in infested roots of tomato genotypes resistant and susceptible to *Meloidogyne incognita*. **Nematol. Medit.**, 22: 179-183.
- Haverkate, F., J.W. Brevoord and A. Verloop. 1972. Automatic recording of uptake of pesticides and related compounds by small plant parasites. I. Description of the method. **Pesticide Sci.**, 3: 1-11.
- Hazarika, K., P.K. Dutta., L. Saikia and S.C. Deka. 2000. Biological control of root-knot nematode in betelvine using parasitic fungus, *Paecilomyces lilacinus*. **Crop Res.**, 19: 338-342.
- Hethelyi, E., B. Danos and P. Tetenyi. 1986. GC-MS analysis of the essential oils of four *Tagetes* species and the anti-microbial activity of *Tagetes minuta*. **Flav. Fragr. J.**, 1: 169-173.
- Horborne, J.B. 1976. **Phytochemical methods**, Chapman and Hall, London, 33 p.

- Huang, C.W., L.H. Lin and S.P. Huang. 1971. Changes in peroxidase isoenzymes in tomato galls induced by *Meloidogyne incognita*. **Nematologica**, 17: 460-466.
- Huang, J.S. 1985. Mechanism of resistance to root-knot nematodes. *In: Advanced treatise on Meloidogyne*, Vol I (Eds: J.N. Sasser and C.C. Carter) North Carolina State Univ. Graphics, pp 165-174.
- Husain, S.I. and A. Masood. 1975 a. Effect of some plant extracts on larval hatching of *Meloidogyne incognita* (Kofoid and White) Chitwood. **Acta Bot. Indica.**, 3: 142-146.
- Husain, S.I. and A. Masood. 1975 b. Nematicidal action of plant extracts on plant parasitic nematodes. **Geobios**, 2: 74-76.
- Husain, S.I., R. Kumar, T. Khan and A. Titov. 1984. Effect of root-dip treatment of egg-plant seedlings with plant extracts, nematicides, oilcakes extracts and anthelmintic drugs on plant growth and root-knot development. **Pak. J. Nematol.**, 2: 79-83.
- Hussaini, S.S. and A. R. Seshadri. 1976. Resistance in some mung bean (*Vigna radiata*) varieties and breeding lines to the root-knot nematode, *Meloidogyne incognita*. **Indian J. Nematol.**, 6: 131-137.
- Hussaini, S.S., R.V.V.P. Rao and H.K. Pandu. 1996. Toxicity of water soluble leaf extracts against larvae and egg masses of three *Meloidogyne* species. **Indian J. Nematol.**, 26: 23 -31.
- Ibrahim, K.S. 1991. Peroxidase isoenzymes from *Meloidogyne spp.* cultured on different hosts. **Revue Nematol.**, 14: 335-344.
- Ito, T. 1978. Cellular and subcellular mechanisms of photodynamic action: The $^1\text{O}_2$ hypothesis as a driving force in recent research. **Photochem. Photobiol.**, 28: 493-508.

- Jain, C. and P.C. Trivedi. 1997. Nematicidal activity of certain plants against root-knot nematode, *Meloidogyne incognita* infecting chickpea, *Cicer arietinum*. **Ann. Pl. Protec. Sci.**, **5**: 171 -174.
- Jain, R.K. and D.C. Gupta. 1995. Effect of a few plant extracts on hatching of root-knot nematode (*Meloidogyne javanica*) eggs. **Indian J. Nematol.**, **25**: 107-108.
- Jain, U., S. Datta, P.C. Trivedi and B. Tiagi. 1986. Allelochemic effects of some plants on hatching in *Meloidogyne incognita*. **Indian J. Nematol.**, **16**: 275-276.
- Jatala, P., R. Kaltenbach and M. Bowngel. 1979. Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes. **J. Nematol.**, **11**: 303.
- Johanson, H.J. 1961. **Plant Microtechnique**. New York, McGraw Hill, 311 p.
- Johnson, G. and E.A. Schaal. 1957. Chlorogenic acid and other ortho-dihydric phenols in scab resistant Russet Burbank and scab susceptible Triumph potato tubers of different maturities. **Phytopath.**, **47**: 253-255.
- Johnson, L.F. 1959. Effect of addition of organic amendments to soil on root-knot of tomatoes. Preliminary Report. **Plant Dis. Repr.**, **43**: 1059-1062.
- Jonathan, E.I. 1988. Effect of salinity on the root-knot nematode (*Meloidogyne incognita* (Kofoid and White, 1919) Chitwood (1949) in Bhendi (*Abelmoschus esculentus* L.). Moench. Dissertation Submitted for M.Sc., (Ag.) degree to the Tamil Nadu Agricultural University, Coimbatore, India.
- Joymati, L., C. Dhanachand and L.S. Devi. 1998. Effect of plants extracts on *Meloidogyne incognita*. **Indian J. Nematol.**, **28**: 225-230.
- Joymati, L., L. Sobita., N. Mohilal and C. Dhanachand. 1999 a. Evaluation of the nematicidal action of a plant *Vitex trifolia* against *Meloidogyne incognita* Race - 2. **Ann. Pl. Protec. Sci.**, **7**: 212-251.

- Joymati, L., M. Shyamkesho and C. Dhanachand. 1999 b. Nematicidal activity of *Momordica dioica* on egg hatching and larval mortality of *Meloidogyne incognita*. **J. Mycol. Pl. Path.**, **29**: 205-208.
- Kaliram, S.A. and D.C. Gupta. 1989. A note on the efficacy of fresh neem leaf extract in the control of *Meloidogyne javanica* infecting chick pea (*Cicer arietinum*). **Indian J. Nematol.**, **19**: 96 -98.
- Kaplan, D.T., I.J. Thomason and S.D. Van Gundy. 1979. Histological study of the compatible and incompatible interaction of soybeans and *Meloidogyne incognita*. **J. Nematol.**, **11**: 338-343.
- Kathirvel, M., G. Balasubramanian, M., Gopalan and C.V., Sivakumar. 1992. Effect of seed treatment with botanicals and chemicals for the control of root-knot nematode, *Meloidogyne incognita* infesting okra, *Abelmoschus esculentus* L. **Indian J. Plant Prot.**, **20**: 191-194.
- Kavers, C. and T.H. Gasper. 1985. Soluble membrane and wall peroxidase phenylalanine ammonia lyase and lignin changes in relation to nitrification of carnation tissues *in vitro*. **J. Pl. Physiol.**, **118**: 41-48.
- Khan, A.M., M.M. Alam and R. Ahmed. 1974 b. Mechanism of the control of plant parasitic nematodes as a result of application of oilcakes to the soil. **Indian J. Nematol.**, **4**: 93-96.
- Khan, M.W., M.M. Alam, A.M. Khan and S.K. Saxena. 1974 a. Effect of water soluble fractions of oilcakes and bitter principles in neem on some fungi and nematodes. **Acta Bot. Indica.**, **2**: 120-128.
- Khan, T.A. and S.I. Hussain. 1988. Studies on the efficacy of seed treatment with pesticides, oil cakes, neem leaf and culture filtrate of *Paecilomyces lilacinus* for the control of diseases caused by the presence of *Rotylenchulus reniformis*, *Meloidogyne incognita* and *Rhizoctonia solani* - either individually or concomitantly on cowpea. **Indian J. Nematol.**, **18** : 192-198.

- Khan, T.A. and S.K. Saxena. 1996. Comparative efficacy of *Paecilomyces lilacinus* in the control of *Meloidogyne* spp. and *Rotylenchulus reniformis* on tomato. **Pak. J. Nematol.**, **14**: 111-116.
- Khanna, A.S. and N.K. Sharma. 1994. Efficiency of neem leaf powder as an additive in management of mushroom nematodes. **Indian J. Hill Farming.**, **7**: 114-115.
- Khanna, A.S. and N.K. Sharma. 1998. Phytotherapeutic effect of some indigenous plants/nematicides on *Meloidogyne incognita* infesting tomato. Proceedings of national symposium on rational approaches in nematode management for sustainable agriculture, Anand, India. pp. 35.
- Khanna, A.S. 1991. *In vitro* studies on some plant extracts as nematicides against *Meloidogyne incognita*. **Curr. Nematol.**, **2**: 199-200.
- Kirmani, M.R. 1977. Studies on the role of the fungi from decomposed oilcakes on root-knot development on egg plant, *Solanum melongena*. Ph.D. Thesis, Aligarh Muslim University, Aligarh, India.
- Kotova, V.V., E.G., Kozar, P.F., Konokov, and A.A. Shesteperov. 1999. Use of the properties of antagonistic plants to control plant parasitic nematodes. **Biologia**. **3**: 24-32.
- Krusberg, L.R. and L.W. Nielsen. 1958. Pathogenesis of root-knot nematodes to the Porto Rico variety of sweet potato. **Phytopath.**, **48**: 30-39.
- Kumar, A. and S.C. Tripathi. 1991. Evaluation of the leaf juice of some higher plants for their toxicity against soil borne pathogens. **Plant and Soil**. **132**: 297-301.
- Kumari, R., K.K. Verma., K.S. Dhindsa and D.S. Bhatti. 1986. *Datura*, *Ipomea*, *Tagetes* and *Lawsonia* as control of *Tylenchulus semipenetrans* and *Anguina tritici*. **Indian J. Nematol.**, **16**: 236-240.

- Laemmli, U.K. 1979. Manual on techniques in molecular Biology (Proteins) 1986 workshop held at the Department of Biochemistry , Tamil Nadu Agricultural University, Coimbatore, India, pp.16.
- Lakshmanan, P., S. Mohan and R. Jeyarajan. 1990. Antifungal properties of some plant extracts against *Thanatephorus cucumeris*, the causal agent of collar rot disease of *Phaseolus aureus*. **Madras Agric. J.**, 77: 1-4.
- Lakshmanan, P.L. 1981. Studies on resistance in cowpea (*Vigna unguiculata* (L.) Walp) to *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1940. Ph.D. Dissertation. Tamil Nadu Agricultural University, Coimbatore, India. 124 p.
- Latif, Z., L. Craven., T.G. Hartley., B.R. Kemp., J. Potter., M.J. Rice., R.D. Waigh and P.G. Waterman. 2000. An insecticidal quassinoid from the new Australian species *Quassia* sp. aff. *bidwillii*. **Biochem. Systema. Eco.**, 28: 183-184.
- Latif, Z.H., R. Ahmad and M.I. Haq. 1999. Effect of seed treatments with neem cake, neem oil and latex of oak on the germination of cowpea and its vulnerability to root-knot nematode (*Meloidogyne incognita*). **Pak. J. Phytopath.**, 11: 52-55.
- Lindley, J. 1860. **The vegetable kingdom**, 421 p.
- Linford, M.B., F. Yap and J.M. Oliveira. 1938. Reduction of soil population of the root-knot nematode during decomposition of organic matter. **Soil Sci.**, 45: 121-141.
- Lysek, H. 1976. Autodehelminthization of soil in low land deciduous forests. **Universitatis Palackianae Olomucensis Facultatis Medicae**. 41: 73-106.
- Mahajan, R., P. Singh and L. Bajaj. 1985. Nematocidal activity of some phenolic compounds against *Meloidogyne incognita*. **Revue Nematol.**, 8: 161-164.

- Mahmood, I. and Z.A. Siddiqui. 1993. Effect of phenolics on the growth of tomato and reproduction of *Rotylenchulus reniformis*. **Nematol. Medit.**, **21**: 97-98.
- Mani, A. 1988. Effect of leaf and oilcake amendments on growth of kagzi lime seedlings and multiplication of *Tylenchulus semipenetrans* Cobb. **Geobios.** **7**: 61-62.
- Mani, A. and K.C. Chitra. 1989. Toxicity of certain plant extracts to *Meloidogyne incognita*. **Nematol. Medit.**, **17**: 43-44.
- Mani, A., I.R. Murthy., P.K. Rao and V.D. Prasad. 1989. Growth of *Paecilomyces lilacinus* on natural substrates and its efficacy against citrus nematode, *Tylenchulus semipenetrans*. **J. Biol. Control.**, **3**: 59-61.
- Mani, A., S.N. Ahmed, P.K. Rao and V. Dakshinamurthi. 1986. Plant products toxic to the citrus nematode, *Tylenchulus semipenetrans* Cobb. **Int. Nematol. Newsletter.**, **3**: 14-15.
- Manske, R.H.F. 1938. Anolobine an alkaloid from *Asimina triloba* Dunal. **Canad. J. Pharm.**, **57**: 339-348.
- Maraite, H. 1973. Changes in the polyphenol oxidases and peroxidase in muskmelon (*Cucumis melo* L.) infected by *Fusarium oxysporum* f.sp. *melonis*. **Physiol. Pl. Path.**, **3**: 29-49.
- Marks, C.F., I.J. Thomason and C.E. Castro. 1968. Dynamics of the permeation of nematodes by water, nematocides and other substances. **Experimental Parasitol.**, **22**: 321-337.
- Martez, J.L., N. Acosta., C. Betancourt., N. Vicente and R. Rodriguez-Kabana. 1996. Biological control of *Meloidogyne incognita* in tomato in Puerto Rico. **Nematropica.** **26**: 143-152.
- Mathela, C.S. 1994. Allelochemicals in medicinal and aromatic plants. **In: Allelopathy in Agriculture and Forestry** (Eds. S.S. Narwal and P. Tauro), Scientific Publishers, Jodhpur, India, pp. 213-228.

- Mayer, A.M., E. Harel and R.B. Shaul. 1965. Assay of catechol oxidase, a critical comparison of methods. **Phytochem.**, **5**: 783-789.
- McBeth, C.W., A.L. Taylor and A.L. Smith. 1941. Note on staining nematodes in root tissues. **Proc. Helminth.Soc.Wash.**, **8**: 26.
- Meena, B., V. Ramamoorthy and M. Muthusamy. 2000. Control of rice sheath blight using oils, oil cakes, bulb and seed extracts. **J. Ecobiol.**, **12**: 87-91.
- Miah, M.A.T., A.V. Ahmed., N.R. Sharma., A. Alli and S.A. Miah. 1990. Antifungal activity of some plant extracts. **Bangladesh J. Bot.**, **19**: 5-10.
- Miller, P.M., N.C. Turner and H. Tomlinson. 1973. Toxicity of leaf and stem extracts to *Tylenchorhynchus dubius*. **J.Nematol.**, **5**: 173-177.
- Mishra M. and S.N.Tewari. 1992. Toxicity of *Polyalthia longifolia* against fungal pathogens of rice. **Indian Phytopath.**, **45**: 59-61.
- Mishra, S.D. and S.K.Prasad. 1973. Effect of water extracts of oilcakes on the second stge larvae of *Meloidogyne incognita* at different concentrations and exposure times. **Indian J. Ent.**, **35**: 104-106.
- Mishra, S.D., M.M. Haque, V. Mojumdar and B.K. Goswami. 1989. Efficacy of aqueous extract of neem seed cake on egg hatching and penetration of *Meloidogyne incognita*. **Neem Newsletter**, **6**: 6-7.
- Misra, S.B and S.N. Dixit. 1976. Fungicidal spectrum of leaf extracts of *Allium sativum*. **Indian Phytopath.**, **29**: 448-455.
- Misra, S.B and S.N. Dixit. 1977. Fungicidal properties of *Clematis gouriana*. **Indian Phytopath.**, **30**: 577-579.
- Mohammed, H.Y., S.I. Husain and A.J. Al-Zarari. 1981. Effect of plant extracts of some poisonous plants of Iraq on mortality of citrus nematode, *Tylenchulus semipenetrans* Cobb. **Acta Bot. Indica.**, **9**: 198-200.

- Mohanty, K.C., A.K. Ganguly and D.R. Dasgupta. 1986. Development of peroxidase activities in susceptible and resistant cultivars of cowpea inoculated with the root-knot nematode, *Meloidogyne incognita*. **Indian J. Nematol.**, **16**: 252-256.
- Mojumder, V. and B.K. Goswami. 1987. Effect of aqueous extracts of madar (*Calotropis gigantea*) and amarbel (*Cuscuta reflexa*) on larval mortality, hatching from egg masses and subsequent penetration into tomato roots. **Ann. Agrl. Res.**, **8**: 285-289.
- Mojumder, V. and S.D. Mishra. 1991. Effect of aqueous extracts of neem seeds on hatchability of eggs and penetrability of hatched juveniles of *Meloidogyne incognita* into roots of mung bean. **Curr. Nematol.**, **2**: 117-120.
- Molinari, S. 1991. Induction of isoperoxidases in resistant and susceptible tomato cultivars by *Meloidogyne incognita*. **J. Nematol.**, **23**: 254-258.
- Mukhtar, T., R.R. Ahmad, M.I. Haq and N. Javed. 1994. Effect of leaf extracts of some plants on *Meloidogyne incongnita*. **Pak. J. Phytopath.**, **6**: 35-37.
- Munoz, C.L., C.O. Castro., C.R. Lopez., A.R. Arisa., F. Pignant and J. Calzada. 1982. Potential nematicides from new natural sources, *Tagetes*. **Ingenieria Y Ciencia Quimica**. **6**: 158-160.
- Nandal, S.N. and D.S. Bhatti. 1983. Preliminary screening of some weed shrubs for their nematicide activity against *Meloidogyne javanica*. **Indian J. Nematol.**, **13**: 123-127.
- Nandal, S.N. and D.S. Bhatti. 1986. Influence of four plant extracts on the hatching of *Meloidogyne javanica* and invasion of host roots. **Nematol. Medit.**, **14**: 291-94.
- Nandal, S.N. and D.S. Bhatti. 1987. Effect of weed and shrub extracts on penetration and gall formation by *Meloidogyne javanica* on brinjal. **Nematol. Medit.**, **15**: 159-162.

- Nandal, S.N. and D.S. Bhatti. 1990. Efficacy, persistence and field application potential of some weed shrubs for controlling *Meloidogyne javanica* on brinjal. **Nematol. Medit.**, **18**: 113 -115.
- Narwal, S.S. 1996. Suggested methodology for allelopathy laboratory bioassays. *In: Allelopathy in Agriculture and Forestry* (Eds. S.S. Narwal and P. Tauro), Scientific Publishers, Jodhpur, India, pp. 255-266.
- Neuenschwander, U., K. Lawton and J. Ryals. 1996. Systemic acquired resistance. *In: Plant-microbe interactions. Vol.1.* (Eds. G. stacey and N.T. Keen), Chapman and Hall, New York, pp. 81-106.
- Nin, S., P. Arfaoli and M. Bosetto. 1995. Quantitative determination of some essential components of selected *Artemisia absinthium* plants. **J. Essen. Oil Res.**, **7**: 271-277.
- Njoku, C.J., L. Zeng., I.U. Asuzu., N.H. Oberlies and J.L. McLaughlin. 1997. Oleanolic acid, a bioactive component of the leaves of *Ocimum gratissimum* (Lamiaceae). **Int. J. Pharmacognosy.**, **35**: 134-137.
- Noel, G. R. and A. Micheal. 1978. Peroxidase and 6 phosphogluconate dehydrogenase in resistant and susceptible cotton infested by *Meloidogyne incognita*. **J. Nematol.**, **10**: 34-39.
- Ogalllo, L.L. and M.A. McClure; 1995. Induced resistance to *Meloidogyne hapla* by other *Meloidogyne* species on tomato and pyrethrum plants. **J. Nematol.**, **27**: 441-447.
- Ogalllo, L.L. and M.A. McClure; 1996. Systemic acquired resistance and suceptibility to root-knot nematodes in tomato. **Phytopath.**, **86**: 498-501.
- Ogoshi, A. 1975. Grouping of *Rhizoctonia solani* Kuhn and their perfect stages. **Rev. Pl. Protec. Res.**, **8**: 93-103/
- Overman, A.J. and J.P. Jones. 1975. Carbofuran in a systemic approach to pest management in tomato. **Nematropica.** **5**: 26-27.

- Owens, R.G. and H.N. Specht. 1966. Biochemical alterations induced in host tissue by root-knot nematodes. **Contrib. Boyce Thompson Inst.**, **23**: 181-198.
- Padhi, N.N. and G. Behera. 2000. Evaluation of nematicidal potential in ten indigenous plant species against *Meloidogyne incognita*. **Indian Phytopath.**, **53**: 28-31.
- Pandey, R. and A. Haseeb. 1988. Studies on the toxicity of extract of certain medicinal plants to root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. **Indian J. Pl. Path.**, **6**: 184-186.
- Pandey, R.S., S.N. Bhargava., D.N. Shukla and D.K. Dwivedi. 1983. Control of Pestalotia fruit rot of guava by leaf extracts of two medicinal plants. **Revista Mexicana De Fitopatologia**. **2**: 15-16.
- Pankaj, A.K., Ganguly., A. Shrohi and V. Singh. 1998. Ascorbic acid oxidase (AAO) activity in roots of resistant and susceptible tomato cultivars infested with *Meloidogyne incognita*. **Indian J. of Nematol.**, **28**: 91-92.
- Panse, V.G. and P.V.E. Sukhatme. 1967. **Statistical methods for Agricultural workers**. Indian Council of Agricultural Research, New Delhi, 381 p.
- Patel, D.J., H.V. Patel., S.K. Patel and B.A. Patel. 1993. Nematicidal properties of some plant materials for the management of root-knot nematodes in tomato nursery. **Indian J. Pl. Protec.**, **21**: 242-244.
- Pathak, K.N., R.P. Nath and A.K. Jha. 1989. Effect of some aqueous leaf extracts on larval hatch of root-knot nematode (*Meloidogyne incognita*) *in situ* conditions. **National Academy Science Letter**, **12**: 17-18.
- Paulson, R.E. and J.M. Webster. 1972. Ultrastructure of the hypersensitive reaction in roots of tomato, *Lycopersicon esculentum* L. to infection by the root-knot nematode, *Meloidogyne incognita*. **Physiol. Pl. Path.**, **2**: 227-234.
- Paxton, J.D. 1997. Phytoalexins. *In: Active defence mechanism in plants* (Ed. R.K.S. Wood), Plenum, New York, pp. 344-346.
- Philip, T. and J.W. Berry. 1975. Nature of lutein acylation in marigold flower (*Tagetes erecta*). **J. Food Sci.**, **40**: 1089-1095.

- Philip, T., Govindiah, A.K. Bajpai and R.K. Datta. 1993. Effect of certain leaf extracts on hatching and mortality of root-knot nematode, *Meloidogyne incognita*, infesting mulberry. **Indian J. Sericulture.**, **32**: 37- 41.
- Piccaglia, R., M. Marotti and S. Grandi. 1998. Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. **Industrial Crops and Products.** **8**: 45-51.
- Pillai, S.N., M.V. Desai and H.M. Shah. 1975. Nematicidal properties of turmeric. **Indian Phytopath.**, **28**: 128-129.
- Pitcher, R.S., Z.A. Patrick and W.B. Moutain. 1960. Studies on the host parasite relations of *Pratylenchus penetrans* (Cobb) to apple seedlings. **Nematologica.** **5**: 309-314.
- Pradhan, P., S.N. Das and D.K. Dora. 1989. Effect of some organic oils on the infectivity of root-knot nematode (*Meloidogyne incognita*) infecting tomato. **Indian J. Nematol.**, **19**: 162-165.
- Prasad, D. 1990. Bioefficacy of synthetic pyrethroids against root-knot nematode, *Meloidogyne incognita* larvae. **Ad. Plant Sci.**, **3**: 131-135.
- Prasad, J.S., M.S. Panwar., Y.S. Rao and S. Rajamani. 1988. Control of root-lesion nematode, *Pratylenchus indicus* Das, 1960 in rainfall upland rice. **Indian J. Nematol.**, **18**: 117-118.
- Prasad, S., B.P. Singh and N. Singh. 1995. Effect of weed plant extracts on larval mortality, hatching and penetration of root-knot nematode, *Meloidogyne incognita*. **Weed News.** **2**: 24-27.
- Qamar, F., M. Saeed, A. Khan and N. Seema. 1989. Control of anguinosus of wheat using plant extracts. **Pak. J. Scientific and Indus. Res.**, **32**: 260-261.
- Qamar, F., M.A. Kalhor and Y. Badar. 1998. Anthelmintic properties of some indigenous plants. **Hamdard Medicus.** **41**: 115-117.

- Rajappan, K., V. Mariappan and A. A. Kareem. 1997. Effect of dried leaf extract of *Ipomoea* on rice sheath rot pathogen and beneficial microorganisms. **Indian Phytopath.**, **50**: 329-331.
- Rajasekhar, S.P., A.K. Ganguly and S.C. Swain. 1997. Quantitative changes in superoxide dismutase, catalase and peroxidase with reference to resistance in tomato to *Meloidogyne incognita*, **Indian J. Nematol.**, **27**: 79-85.
- Ram, K. and D.C. Gupta. 1982. Efficacy of plant leaves, nematicides and fertilizers alone and in combination against *Meloidogyne javanica* infected chickpea (*Cicer arietinum* L.). **Indian J. Nematol.**, **12**: 221-225.
- Ramachandran, P. 1986. Studies on the management of the nematode (*Meloidogyne incognita*) and disease (*Rhizoctonia solani*) of tomato (*Lycopersicon esculentum* Mill). Dissertation submitted for M.Sc., (Ag.) Degree to the Tamil Nadu Agricultural University, Coimbatore, India.
- Ramakrishnan, S., C.R. Gunasekaran and S. Vadivelu. 1995. Effect of various leaf extracts on root-knot nematode *Meloidogyne incognita* (Kofoid and White 1919) Chitwood. 1949. **J. Root Crops.**, **21**: 43-45.
- Ramakrishnan, S., C.R. Gunasekaran and S. Vadivelu. 1997 a. Control of *Meloidogyne incognita* on okra through botanicals as soil amendments. **Indian J. Nematol.**, **27**: 24-27.
- Ramakrishnan, S., C.R. Gunasekaran and S. Vadivelu. 1997 b. Efficacy of organics in control of *Meloidogyne incognita* on okra. **Indian J. Nematol.**, **27**: 74-78.
- Ramakrishnan, S., C.R. Gunasekaran, S. Vadivelu and S.M. Viswanath. 1999. Effect of various aqueous leaf extracts on the larval mortality of *Meloidogyne incognita*, infesting tobacco. **Tob. Res.**, **25**: 37-39.
- Rao, C.V., A. Mani and P. K. Rao. 1986. Effect of plant products on egg hatch and larval mortality of *Meloidogyne incognita*. Proc. Indian Acad.Sci., 4th July. pp. 397-401.

- Rao, M.S. and P.P. Reddy. 1994. A method for conveying *Paecilomyces lilacinus* to soil for the management of root-knot nematodes on eggplant. **Nematol. Medit.**, **22**: 265-267.
- Rao, M.S. P.P. Reddy and M. Nagesh. 1999. Bare root dip treatment of tomato seedlings in caloptropis or castor leaf extract mixed with *Paecilomyces lilacinus* spores for the management of *Meloidogyne incognita*. **Nematol. Medit.**, **27**: 323-326.
- Rao, M.S., P.P. Reddy and M. Nagesh. 1997. Effective use of neem cake extract for the management of root-knot nematodes infecting okra (*Abelmoschus esculentus*). **Pest Mgt. Horti. Ecosystems.**, **3**: 95-99.
- Rathmell, W.G. and L. Sequiera. 1974. Soluble peroxidase in fluid from the intercellular spaces of tobacco leaves. **Pl. Physiol.**, **53**: 317-318.
- Ravichandra, N.G. and K.G.H. Setty. 1989. Histopathology and histochemistry of resistant and susceptible brinjal cultivars infested with *Meloidogyne javanica* and three races of *M. incognita*. **Indian Phytopath.**, **42**: 331.
- Ray, D., D. Prasad and R.P. Singh. 2000. Chemical examination and antinemic activity of marigold (*Tagetes erecta* L.) flower. **Ann. Pl. Protec. Sci.**, **8**: 212-217.
- Reddy, D.D.R. 1985. Analysis of crop loss in tomato due to root-knot nematode, *Meloidogyne incognita*. **Indian J. Nematol.**, **15**: 55-59.
- Reshmi, B. and V. Mojumder. 1998. Effect of aqueous extracts of neem – based pesticides on hatching and penetration of juveniles of *Meloidogyne incognita* in chickpea. **Indian J. Nematol.**, **28**: 210-212.
- Reyes, F.R. and A.C. Santos. 1931. Isolation of anonaine from *Annona squamosa* Linn. **Philipp. J. Sci.**, **44**: 409.

- Rice, E.L. 1987. Allelopathy: An overview. *In: Allelochemicals, Role in agriculture and forestry* (Ed. G.R. Waller), Vol. 330, American Chemical Society, Washington, pp. 8-22.
- Rice, E.L. 1984. **Allelopathy**, Academic Press, New York, 254 p.
- Ricker, A.J. and R.S. Ricker. 1936. **Introduction to Research on Plant Disease**. Saint Louis Jone's Swift Co., New York, 117 p.
- Ritizinger, C.H.S.P. and R. McSorley. 1998. Effect of fresh and dry organic amendments on *Meloidogyne arenaria* in greenhouse experiments. **Nematropica**. **28**: 173-185.
- Rodriguez, E. and T.J. Mabry. 1977. Tageteae – Chemical review. *In: The biology and chemistry of the Compositae*. (Eds. V.H. Heywood., J.B. Harborne and B.L. Turner), Academic press, London, pp. 164-183.
- Roy, D., S.P. Sinhababu and N.C. Sukul. 1995. Root-knot nematode extract increases growth of plants and reduces nematode infection. **Environ. Eco.**, **13**: 775-779.
- Ryals, J., E. Ward and J.P. Metraux. 1992. Systemic acquired resistance: An inducible defense mechanism in plants. *In: Inducible plant proteins: Their biochemistry and molecular biology* (Ed. J.L. Wray), Cambridge University Press, UK, pp. 205-229.
- Sadasivan, S. and A. Manickam, 1991. **Biochemical methods for agricultural sciences**, Wiley Eastern Ltd., New Delhi, 246 p.
- Saifullah, S.A. and M. Zulfiquir. 1990. Promising control of root-knot nematodes (*Meloidogyne* spp.) of tomato through organic amendments. **Sarhad J. Agri.**, **6**: 417-420.
- Santos, A.C. 1932. Anonaine. **Philipp. J. Sci.**, **47**: 357.
- Santos, A.C. 1930. Alkaloid from *Annona reticulata* Linn. **Philipp. J. Sci.**, **43**: 561.

- Sasanelli, N. and M. Divito. 1991. The effect of *Tagetes* spp. extracts on the hatching of an Italian population of *Globodera rostochiensis*. **Nematol. Medit.**, **19**: 135-137.
- Sasser, J.N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (*Meloidogyne* spp.). **Univ. Md. Agric. Exp. Sta. Bull. A.**, **77**: 30 p.
- Saxena, G.C., Y.P. Singh and P.K. Deshmukh. 1989. A survey of nematodes associated with brinjal in Goa district of Madhya Pradesh. **J. Phytological Res.**, **2**: 105-107.
- Saxena, S.K. and S. Poornima. 1995. **Neem in management of plant nematodes**. 125 p.
- Schindler, A.F. 1961. A simple substitute for a Baermann funnel. **Plant Dis. Repr.**, **45**: 747-748.
- Schmitz, H. 1930. **Poisoned food technique**. Industrial Engin.Chem.Analyst, Editon II, pp 361-363.
- Sebastian, S. and P. Gupta. 1996. Evalaution of three plant lattices as seed treatment against *Pratylenchus thornei* on chick pea. **Int. Chickpea and Pigeon pea Newsletter.**, **3**: 39-40.
- Senthil Kumar.R. 1993. Studies on the management of sheath blight of rice insighed by *Rizoctonia solani* Kuhn. M.Sc., (Ag) thesis, Tamil Nadu Agricultural University, Coimbatore, India, 183 p.
- Sharma, B.K. 1998. Antifungal properties of biocontrol agents and plants extracts against causal fungi of yellows and rhizomes rot of ginger. **J. Biol. Control.**, **12**: 77-80.
- Sharma, D.D. 1999. Effect of culture filtrates of biocontrol agents on larval mortality of *Meloidogyne incognita*, in comparison with Rugby 10G. **Indian J. Sericulture.**, **38**: 152-154.

- Sharma, J.L., P.C. Trivedi, M.K. Sharma and B. Tiagei. 1990. Alteration in proline and phenol content of *Meloidogyne incognita* infected brinjal cultivars. **Pak. J. Nematol.**, **8**: 33-38.
- Sharma, W. and P.C. Trivedi. 1995. Nematicidal and nematostatic response of aqueous extracts of certain plants of semi-arid niche. **Curr. Nematol.**, **6**: 43-53.
- Sheela, M.S. and K.K.R. Nair. 1988. Control of root-knot nematode in bhendi (Okra) using different granular nematicides. *In: Integrated Pest Control: Progress and Perspectives*. (Eds. N. Mohandas and G. Koshy), Naya Prokash Publishers, New Delhi, pp. 73-81.
- Shekhawat, P.S. and R. Prasad. 1971. Antifungal properties of some plant extracts I. Inhibition of spore germination. **Indian Phytopath.**, **24**: 800-802.
- ShuHong, C. and G. XueBiao. 2000. Suppression of the MCWA 18 isolate of *Paecilomyces lilacinus* on *Meloidogyne javanica*. **Chinese J. Biol. Cont.**, **16**: 78-80.
- Shukla, R.P. and R.S. Dwivedi. 1990. Toxic activity of some higher plants against sheath blight pathogen of rice. *Proc. Int. Sym. Rice Res.*, New Frontiers, 253 p.
- Shukla, Y.M. and M.K. Charaborty. 1988. Biochemical studies on response of tobacco and tomato plants to root-knot nematode infection. **Tob. Res.**, **14**: 43-50.
- Siddiqui, M.A. and M.M. Alam. 1988 c. Control of root-knot and reniform nematodes by bare-root dip in leaf extracts of margosa and persian lilac. **Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz**. **95**: 138-142.
- Siddiqui, M.A. and M.M. Alam. 1999. Integrated management of plant parasitic nematodes with oilcakes, nematicides and ploughing. **Pak. J. Nematol.**, **17**: 129-136.

- Siddiqui, M.A. and M.M. Alam. 1985. Further studies on the nematode toxicity of margosa and persian lilac. **Neem Newsletter**, **2**: 43-470
- Siddiqui, M.A. and M.M. Alam. 1987. Efficacy of seed dressing with extracts of neem and persian lilac against *Meloidogyne incognita* and *Rotylenchulus reniformis*. **Nematol. Medit.**, **15**: 399-403.
- Siddiqui, M.A. and M.M. Alam. 1988 a. Effect of latex seed dressing on *Rotylenchulus reniformis* and plant growth of some vegetables. **Nematol. Medit.**, **16**: 129-130
- Siddiqui, M.A. and M.M. Alam. 1989. Possible utilization of a Noxious weed in nematode control. **Biol. Wastes.**, **22**: 181-188.
- Siddiqui, M.A. and M.M. Alam. 1990. Control of root-knot, reniform and stunt nematodes with nimbin seed dressing. **Nematol. Medit.**, **18**: 19-22.
- Siddiqui, M.A. and M.M. Alam. 1988 b. Effect of seed dressing with plant latex on *Tylenchorhynchus brassicae* and plant growth of cabbage and cauliflower. **Pak. J. Nematol.**, **6**: 65-71.
- Singh, D., R.S. Dahia and M.R. Dalal. 1995. Nematicidal efficacy of some plant leaves to *Heterodera avenae* in wheat. **Indian J. Nematol.**, **25**: 2-5.
- Singh, G. 1999. Studies on the biocidal activities of certain essential oils. **J. Medi. Arom. Pl. Sci.**, **21**: 1119-1130.
- Singh, R.N., I.R. Sindhu and K. Gupta. 1986. Effect of leaf exudate and extract of spinach on some phylloplane fungi. **Acta Bot. Indica.**, **14**: 104-110
- Singh, R.S. and K. Sitaramaiah. 1966. Incidence of root-knot of okra and tomatoes on oilcakes amended soil. **Plant Dis. Repr.**, **50**: 668-672.
- Singh, R.S. and K. Sitaramaiah. 1967. Effect of decomposing green leaves, saw dust and urea on the incidence of root-knot of okra and tomato. **Indian Phytopath.**, **20**: 348-355.

- Singh, R.S. and K. Sitaramaiah. 1970. Control of plant parasitic nematodes with organic soil amendments. **Pests and News Summaries**, **16**: 287-297.
- Singh, S.P. and V. Singh. 1988. Evaluation of oilcakes and nematicides for the control of *Meloidogyne incognita* infecting egg plants. **Indian J. Nematol.**, **18**: 366-368.
- Singh, S.P., A.M. Khan and S.K. Saxena. 1980 a. Effect of nematicides and oilcakes separately and in mixture on plant parasitic nematodes. **Geobios**, **7**: 111-113.
- Singh, S.P., M. Ahmed, A.M. Khan and S. Saxena. 1980 b. Effect of seed treatments with certain oilcakes or nematicides on the growth of tomato and on rhizosphere population of nematodes and fungi. **Nematol. Medit.**, **8**: 193-198.
- Singh, S.P., V. Pant, A.M. Khan and S.K. Saxena. 1983. Attractiveness of *Meloidogyne incognita* larvae to roots of tomato and changes in biochemical content of plants as affected by oilcakes and nematicides. **Nematol. Medit.**, **11**: 115-118.
- Singh, S.P., V. Pant, A.M. Khan and S.K. Saxena. 1985. Changes in the phenolic contents, related rhizosphere microflora and nematode population in tomato inoculated with *Meloidogyne incognita* as a result of soil amendment with organic matter. **Indian J. Nematol.**, **15**: 197-201.
- Singhal, V. 1999. **Indian Agriculture**. Indian economic data research centre, New Delhi, pp. 197-198.
- Sinhababu, S.P., D. Roy., N.D. Sukul and S.B. Mahato. 1992. Nematicidal principle from *Acacia auriculiformis*. In: **Allelopathy in Agroecosystem**, (Eds. P. Tauro and S.S. Narwal), Haryana Agric. Univ. Hisar, India, pp. 181-183.
- Sirohi, A. and D.R. Dasgupta. 1993. Mechanism of resistance in cowpea to the root-knot nematode *Meloidogyne incognita* race – 1. I. Early induction of phenylalanine ammonia lyase and chlorogenic acid. **Indian J. Nematol.**, **23**: 31-41.

- Sitaramaiah, K. and R.S. Singh. 1978 b. Role of fatty acids in margosa cake applied as soil amendment in control of nematodes. **Indian J. Agrl. Sci.**, **48**: 266-270.
- Sitaramaiah, K. 1987. Cultural control of nematodes. *In: Problems and progress in economic phytonematology* (Eds. D.S. Bhatti and R.K. Walia) Directorate of Publication, Haryana Agrl. Univ. Hisar, pp. 233-245.
- Sitaramaiah, K. and R.S. Singh. 1978 a. Effect of organic amendments on phenolic content of soil and plant and response of *Meloidogyne javanica* and its host to related compounds. **Pl. Soil.**, **50**: 671-679.
- Sivakumar, M. and T. Marimuthu. 1986. Efficacy of different organic amendments against phytonematodes associated with betelvine (*Piper betle* L.). **Indian J. Nematol.**, **16**: 280-281.
- Sosa-Moss, C., K.R. Barker and M.E. Daykin. 1983. Histopathology of selected cultivars of tobacco infected with *Meloidogyne* sp. **J. Nematol.**, **15**: 392-397.
- Spencer, D.M., J.N. Topps and R.L. Wain. 1957. Fungistatic properties of plant tissue. An antifungal substance from the tissue of *Vicia faba*. **Nature**, **179**: 651-662.
- Spies, J.R. 1955. *In: Methods in Enzymology*. (Eds. S.P. Colowick and W.O. Kaplan) Academic Press, pp. 467-468.
- Spikes, J.D. 1977. Photosensitization. *In: The Science of Photobiology*. (Ed. K.C. Smith), Plenum Press, New York, pp. 87-112.
- Srivastava, A. K. and B. Lal. 1997. Studies on biofungicidal properties of leaf extract of some plants. **Indian Phytopath.**, **50**: 408-411.
- Srivastava, S., D. Prasad and R.P. Singh. 2000. Antinemic studies of the chemical constituents of the leaves of *Murraya koenigii* (L.) Spreng. **Ann. Pl. Protec. Sci.**, **8**: 183-186.

- Srivastava, V.K. and K.K. Srivastava. 1991. **Introduction to chromatography: Theory and practice**, Chand and Company Ltd., New Delhi, 154 p.
- Starr, J.L. 1979. Peroxidase isoenzymes from *Meloidogyne* spp. and their origin. **J. Nematol.**, **11**: 1-5.
- Suatmadji, R.W. 1969. **Studies on the effect of *Tagetes* species on plant parasitic nematodes**. (Eds. H. Veenman and N.V. Zones). Export Bureau Publication, The Netherlands, 132 p.
- Subramanian, S. and P. Selvaraj. 1988. Effect of *Tagetes patula* L. leaf extract on *Radopholus similis* (Cobb, 1893) Thorne 1949. **Indian J. Nematol.**, **18**: 337-338.
- Subramanian, S. and S.Vadivelu. 1990. Effect of *Crotalaria spectabilis* extracts on *Meloidogyne incognita*. **Int. Nematol. Newsletter.**, **7**: 8-9.
- Sukul, N.C. 1992. Plants antagonistic to plant parasitic nematodes. **Indian Rev. Life Sci.**, **12**: 23-52
- Sukul, N.C., P.K. Das and G.C. De. 1974. Nematicidal action of some edible crops. **Nematologica**, **20**: 187-191.
- Sukul, N.C., S.P. Sinhababu., S.C. Datta and A. Sukul. 2001. Nematotoxic effect of *Acacia auriculiformis* and *Artemisia nilagirica* against root-knot nematodes. **Allelopathy J.**, **8**: 65-72.
- Sultan, M.S. and I. Singh. 1987. Efficacy of some nematicides for the control of root-knot nematode (*Meloidogyne incognita*) infesting okra. **J. Res.**, **24**: 243-246.
- Sundararaj, T., V. Kurucheve and J. Jayaraj. 1996. Screening of higher plants and animal faeces for the fungitoxicity against, *Rhizoctonia solani*. **Indian Phytopath.**, **49**: 398-403.
- Takagusi, M., Y. Yachida., M. Anetai., T. Masamune and K. Kegasawa. 1975. Identification of asparagusic acid as a nematicide occurring naturally in the roots of *Asparagus*. **Chemical Letters**. 43-44.

- Tarjan, A.C. and P.A. Cheo. 1956. Nematicidal value of some fatty acids. Bull. Rohde Island. Agrt. Exp. Stn. No. 112, 41 p.
- Tattersfield, F. and C. Potter. 1940. The insecticidal properties of certain species of *Annona* and of an Indian strain of *Mundulea sericea*. **J. Appl. Biol.**, **27**: 262-273.
- Tayal, M.S. and M.S. Agarwal. 1982. Biochemical alterations in galls induced by *Meloidogyne incognita*. Some hydrolysing enzymes and related chemical metabolites. **Indian J. Nematol.**, **12**: 379-382.
- Taylor, A.L. and J.N. Sasser. 1978. **Biology, Identification and control of root-knot nematode (*Meloidogyne* spp.)**, Cooperative Publication, Dept. of Plant Pathology, NCSU and USAD, Raleigh, North Carolina, 111 p.
- Taylor, D.P. 1975. Observations on a resistant and a susceptible variety of tomato in a field heavily infested with *Meloidogyne* in Senegal. **Cah. Orstom. Ser. Biol.**, **10**: 239-245.
- Tewari, S.N. 1990. Toxic effect of a few botanicals on the three fungal pathogens of rice. In: **Botanical Pesticides in IPM**. Proc. National Sym, 21-22 January, CTRI, Rajahmundry, India, pp. 397-403.
- Tewari, S.N. and M. Nayak. 1991. Activity of four plant leaf extractants against three fungal pathogens of rice. **Tropical Agri.**, **68**: 373-375.
- Thakar, N.A., H.R. Patel, C.C. Patel and M.S. Vora. 1988. Comparative efficacy of *Azolla* and nematicides in management of root-knot nematode in tomato. **Indian J. Nematol.**, **18**: 115.
- Thakur, S.G. and K.S. Darekar. 1995. Effect of some non-edible oilseed cakes against root-knot nematode, *Meloidogyne incognita* and growth parameters of brinjal. **Curr. Nematol.**, **6**: 21-26.
- Toida, Y., S. Keereevan and N. Tangjitsomkid. 1994. Effects of addition of organic materials on population density of *Meloidogyne javanica* attacking mung bean in Thailand. **Japanese J. Nematol.**, **23**: 90-94.

- Trimurti, N. 1924. An alkaloid from *Annona squamosa* leaves. **J. Indian Inst. Sci.**, 7, 232-237
- Tripathy, C.L. and K.K. Gupta. 1991. Plant phenolics of *Tagetes erecta*. **Fitoterapia**. 62: 91-92.
- Troll, J. and R.A. Rhode. 1966. Pathogenicity of *Pratylenchus penetrans* and *Tylenchorhynchus claytoni* on turf grasses. **Phytopath.**, 56: 995-998.
- Truelove, B., R. Rodriguez-Kabana and P.S. King. 1977. Seed treatment as a means of preventing nematode damage to crop plants. **J. Nematol.**, 9: 326-330.
- Uhlenbroek, J.H. and J.D. Bijloo. 1959. Investigations of nematicides. II. Structure of a second nematocidal principle isolated from *Tagetes* roots. **Recueil des Travaux Chimiques des Pays-Bas**. 78: 382-390.
- Uhlenbroek, J.H. and J.D. Bijloo. 1958. Investigations on nematicides. I. Isolation and structure of a nematocidal principle occurring in *Tagetes* roots. **Rocueil des Travaux Chimiques des Pays-Bas**. 77: 1004-1008.
- Uma, N. 1994. Studies on the management of sheath blight of rice incited by *Rhizoctonia solani* Kuhn. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore. 183 p.
- Umar, I. and M.Y. Jada. 2000. The efficacy of mixtures of two organic amendments (Parkia seeds and goat manure) on the control of root-knot nematodes (*Meloidogyne incognita*) on tomato (*Lycopersicon esculentum*). **Global J. Pure and Appl. Sci.**, 6: 177-180.
- Vasudevan, P., S. Kashyap and S. Sharma. 1997. *Tagetes* sp. a multipurpose plant. **Bioresource Technol.**, 62: 29-35.
- Vats, R. and S.N. Nandal. 1993. Optimization of dipping periods of tomato seedlings in plant extracts for the management of root-knot nematode, *Meloidogyne javanica*. **Curr. Nematol.**, 4: 161-164.

- Veech, J.A. and B.Y. Endo. 1969. The histochemical localisation of several enzymes of soybeans infected with the root-knot nematode. *Meloidogyne incognita acrita*. **J. Nematol.**, 1: 264-276.
- Verma, A.C. and A. Anwar. 1995. Integrated nematode management by using leaves of medicinal plants in pointed gourd (*Trichosanthes dioica*) field. **Indian J. Nematol.**, 25: 5.
- Vijayalakshmi, K. and B.K. Goswami. 1987. Effect of root dip treatment of tomato seedlings with aqueous extracts of some oilcakes on root-knot nematode infestation. **Ann. Agric. Res.**, 8: 168-171.
- Vijayalakshmi, K., S.D. Mishra and S.K. Prasad. 1979. Nematicidal properties of some indigenous plant materials against second stage larvae of *Meloidogyne incognita*. **Indian J. Nematol.**, 9: 81.
- Walia, K.K. and D.C. Gupta. 1997. Management of root-knot nematode, *Meloidogyne javanica* on vegetable crops with *Tagetes* sp. **Indian J. Nematol.**, 27: 18-23.
- Walia, K.K. and D.C. Gupta. 1995. Neem: An effective biocide against *Meloidogyne javanica* attacking vegetable crops. **Pl. Dis. Res.**, 10: 59-61.
- Warren, F.L. 1970. *In: The Alkaloids, Vol 12* (Ed. R.H.F. Manske), Academic Press, New York, 246 p.
- Zacheo, G and T.B. Zacheo. 1988. Involvement of superoxide dismutase and superoxide radicals in the susceptible and resistant tomato plants to *Meloidogyne incognita* attack. **Physiol. Mol. Pl. Path.**, 32: 313-322.
- Zacheo, G., T.B. Zacheo., A. Arrigoni and O. Arrigoni. 1980. Mitochondrial superoxide dismutase and peroxidase activity in susceptible and resistant tomato plants infected by *Meloidogyne incognita*. *Europ. Soc. Nematologists, 16th Int. Nematol. Symp.*, Bari, Itali, 20-21.

- Zacheo, G., T.B.Zacheo., D. Pacoda., C. Orlando and R.D. Durbin. 1995. The association between heat-induced susceptibility of tomato to *Meloidogyne incognita* and peroxidase activity. **Physiol. Mol. Pl. Path.**, **46**: 491-507.
- Zaki, F.A. and D.S. Bhatti. 1989. Phytotherapeutic effect of some plant leaves on *Meloidogyne javanica* infecting tomato plants. **Nematol. Medit.**, **17**: 71-72.

