

P-ISSN: 2349-8528  
 E-ISSN: 2321-4902  
 IJCS 2018; 6(3): 2757-2761  
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 Received: 22-03-2018  
 Accepted: 29-04-2018

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## Bio-nematicidal effect of *Azadirachta indica*, against *Meloidogyne incognita* in tomato

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### Abstract

A pot experiment under screen house conditions was carried out to manage the root-knot nematode, *Meloidogyne incognita* in tomato by using different neem leaves @ 5, 10, 20 and 30 g/kg soil along with chemical as well as untreated checks. Soil was autoclaved and inoculated with root-knot nematode (1000 J/kg soil). Neem leaves were incorporated to the soil in pot as treatment wise and a waiting period of ten days was given for decomposition of neem leaves before transplanting of tomato seedling. The results revealed that all plant growth parameters of tomato improved while the nematode reproduction factors were suppressed significantly in case of neem leaves and chemical checks as compare to untreated inoculated check. Among the doses neem leaves 30 g/ kg soil were found to be effective in suppressing galling (38) and final population in soil (170) followed by 20 g/ kg soil in improving plant growth of tomato and reducing reproduction of root knot nematode.

**Keywords:** *Meloidogyne incognita*, *Azadirachta indica*, tomato

### Introduction

Tomato, *Lycopersicon esculentum* Mill. is an important vegetable crop grown worldwide for its edible fruits, responsible for correcting malnutrition in third-world countries like India. Tomatoes are consumed in the form of juice, paste, ketchup, puree, soup, etc. Fruits, besides containing the essential amino acid like tryptophan, also have citric and malic acids in appreciable amounts. Tomato contains a glucoalkaloid ‘tomatine’ which is used as precipitating agent for cholesterol. The estimated world production of tomato is about 125.02 million tons and the total area under its cultivation is about 45.5 lakh ha. Indian contribution to the annual world production was 10.26 million tons, with an area of 5.72 lakh ha in 2006 (Mane *et al.*, 2010) [20]; however, a slightly increased 17.50 million tons in the recent years FAO, STAT (2012) [10]. Andhra Pradesh ranked first for tomato production. Despite great production rate globally, productivity of this vegetable is far below expectation particularly in India as compared to other countries, which could mainly be due to improper and inadequate supply of nutrients, disease incidence, and lack of adoption of new improved production technologies.

There are several constraints on the successful cultivation of tomato. Nematode alone causes about 20.6% loss in yield worldwide (Sasser, 1989) [26]. Subramaniyan, Rajendran, and Vedivelu (1990) [33] reported yield loss of 42.05–54.42% due to *Meloidogyne incognita* from India. Jain *et al.*, (1994) [17] reported 47.3 and 71.9% yield loss in vegetable crops due to *M. javanica* and *M. incognita*, respectively. The crop is greatly affected with root-knot nematodes in all parts of India and elsewhere (Siddiqui & Shaukat, 2003; Sikora & Fernández, 2005) [30, 32] and difficult crop pests to control (Chitwood, 2002) [8] because they have high reproduction rates (Ananhirusalee, *et al.*, 1995) [3]. Nematode-infected plants generally show foliar symptoms like nutrient deficiency, particularly nitrogen (Birat, 1963; Good, 1968) [6, 13], yield losses in tomato due to root-knot nematode *Meloidogyne* spp. ranges from 35 to 50% in India (Jain, 1991; Jonathan *et al.*, 2001) [16, 18] and as high as 85% globally (Sasser, 1979; Taylor & Sasser, 1978) [25, 34]. Several plants, belonging to different botanical families, contain principles possessing nematicidal or nematostatic properties (Gommers, 1981; Grainge and Ahmed, 1988) [12, 14]. Investigations on extracts from various indigenous plants and neem (*Azadirachta indica* A. Juss.) products have revealed that some of them are effective against insects and nematodes (Holyoke and Reese, 1987; Byomakesh *et al.*, 1998; Nanjegowda *et al.*, 1998; Khanna and Sharma, 1998; Sharma, 2000) [15, 7, 23, 19, 28] and commercial formulations of them

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are already available. Neem-based formulations are known to have nematicidal potential, particularly against plant parasitic nematodes (Alam, 1990; Mojumder, 1995) [2, 22].

The fresh extracts of fruits, leaf bark, root and gum of neem inhibited hatching of *Meloidogyne incognita* (Siddiqui and Alam, 1985) [19]. Therefore, investigations were undertaken to evaluate the efficacy of the fresh neem leaves applied as soil application, on plant status, root gall index and final nematode density of the root-knot nematode *Meloidogyne incognita* (Kofoid *et al* White) Chitwood in tomato.

## Materials and Methods

### Raising of tomato seedlings

Tomato seeds (cv. Selection-7) were given pre-sowing treatment with Bavistin (0.1%) + Indofil M-45 (0.25%) to control damping off. Seedlings were raised from these treated seeds in a nursery on the departmental farm.

### Rearing of *Meloidogyne incognita*

Egg masses of *M. incognita* were isolated from infected tomato roots and placed singly in Petri dishes containing distilled water. The second stage juveniles emerging from each single egg mass were inoculated on seedlings of brinjal (*Solanum melongena* L.) cv. Pusa Purple Long, grown in earthen pots containing 4,000 g steam sterilized soil (soil : sand : FYM in the ratio of 1 : 1 : 1). The plants were maintained in a screen-house for six month to allow the reproduction of the nematode. This nematode culture was used for the experiments.

### Assay Procedure

A soil mixture (soil: sand in the ratio of 1:1) was sterilized by autoclaving. According to treatment neem leaves @ 5, 10, 20 and 30 gm/pot were mixed in soil. This mixture was used to fill 1000 cm<sup>3</sup> plastic pots sterilized by dipping in 5% formalin for 5 minutes. After filling pot soil were watered and waiting period 15 days for decomposition of leaves. Carbofuran 0.1 g a.i. /kg soil, untreated inoculated and untreated uninoculated check also maintained. One four week-old healthy tomato seedling was transplanted into each pot. Seedlings were grown under screen-house conditions. Fifteen days after transplanting, each pot was inoculated with 1000 freshly hatched second stage juveniles of *M. incognita*. The egg masses were isolated from infected brinjal roots and placed singly in Petri dishes containing distilled water. The second stage juveniles emerging from egg masses were collected after three days and combined for use. Observations on plant status, *viz.*, shoot length; dry shoot weight and dry root weight were recorded at the end of the experiment. The final nematode populations of second stage juveniles per 200 cc of soil were counted after extraction by the Cobb decanting and sieving technique.

## Results and Discussion

### Effect on plant status

Plant height increased with the increase in concentration of all tested dosage of neem leaves and the increase was significant at all concentrations (Table I). Nematode inoculated plants treated with neem leaves @ 30gm/pot concentration attained the maximum shoot lengths of 34.5 cm, followed by plants treated with neem leaves @ 20gm/pot with a shoot lengths of 28.5 cm. Mean dry shoot weight increased with the increase in concentration of the neem leaves and the increase was significant for all treatments. Neem leaves @ 30gm/pot

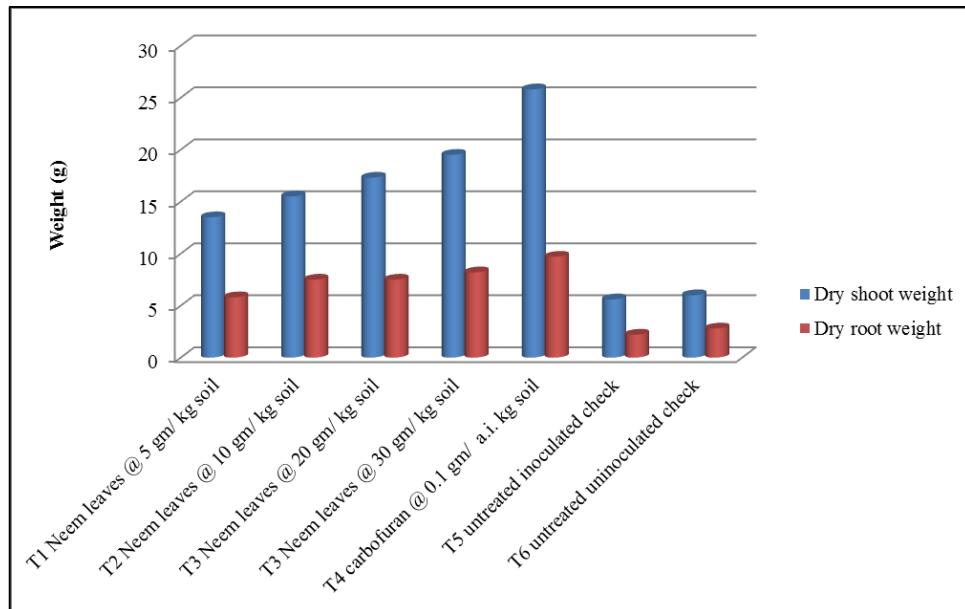
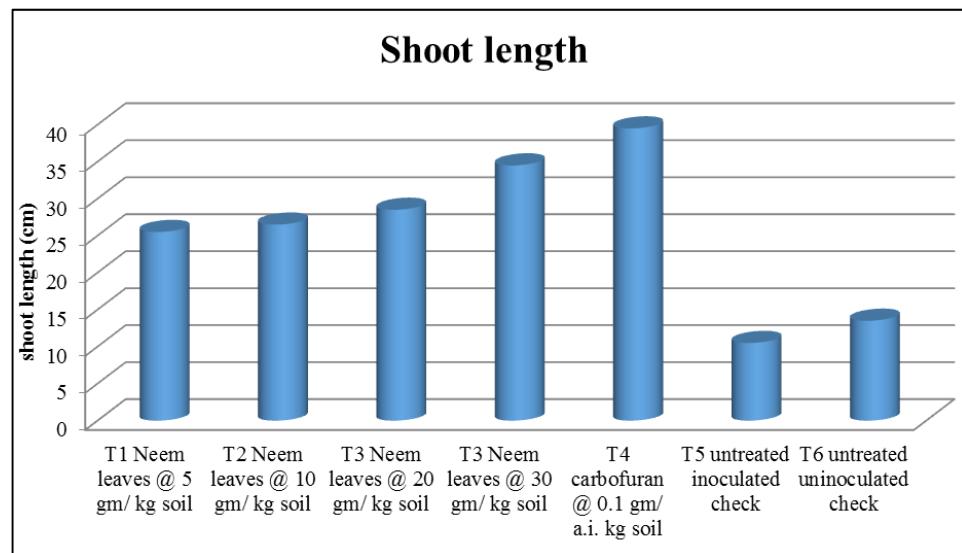
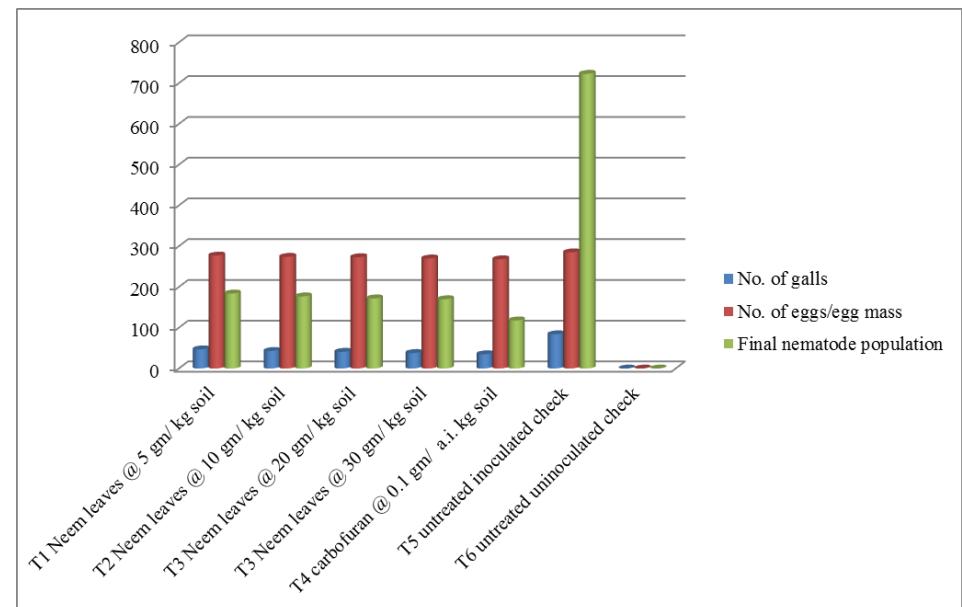
treated plants had the highest mean dry shoot weight of 19.5 g as compared to 5.6 g in the control, followed by mean dry shoot weights of 17.3 g in neem leaves @ 20gm/pot. Data indicated (Table 1) that significant increase in dry root weight was observed with the increase in concentration of the neem leaves. Mean dry root weight was 8.2 g in plants treated with the neem leaves 30gm/ pot concentration, as compared to the average dry root weight of 2.2 g of plants untreated uninoculated.

### Effect on population of *M. incognita*

All neem leaves dosage greatly suppressed the soil population density of *M. incognita* and the suppression was greater the higher the concentration of the nematicide. The differences in the number of nematodes/200 cc soil were significantly decrease nematode populations in increasing dose of neem leaves. The lowest nematode population was observed when treating with neem leaves 30gm/pot and carbofuran check. Plants treated with carbofuran (35) and neem leaves 30gm/pot (38) had the lowest mean of galls, followed by neem leaves 20gm/ pot (41) treated plants. Neem leaves @ 5 gm/pot treated plants showed least degrees of root gall suppression. This indicates that, although all neem concentration reduced the infestation by *M. incognita* significantly, the degree of their efficacy varied significantly. At 30gm/pot neem leaves (38) was the most effective. The maximum root galls of the untreated inoculated plants (84) as compared to other treatment.

*Azadirachtin* is the main active content of neem and is reported very effective and target specific to controlling insects and nematodes pests of the various crops. The findings of the present study can be correlated with of Egumnjobi & Afolami (1976) [19] where they have reported the successful use of neem leaves extracts in nematode control. Mishra (1999) [21] states that the neem formulations are most effective control of root knot nematode *Meloidogyne* sp. as compared to other botanicals. Akhtar and Malik (2000) [1], Siddiqui & Alam (2001) [31], they have reported that phenols, amino acids, aldehydes and fatty acids are release from neem which is antagonistic to root-knot nematodes. Our results are supported by the study of Siddiqui & Alam, (2001) [31]; Ravishankar & sharma (2005) [24]; Ganai, *et al* (2014) [11]; Satyandra *et al* (2011) [27]; Babu & Rana (2012) [5] Archana & Prasad (2014) [4] states that organic amendments of soil using dried poultry litter, municipal refuse, oil cakes of ground nut, neem mustard & neem products (which are commercial available in market) have been found effective in the control of *Meloidogyne incognita*.

In present study thus it may be concluded that changes in protein after infection are related to defence action, because abnormal metabolites are produced in adjacent non-infected tissues. Such metabolites accumulated in infected tissues and are toxic to parasites and inhibit their growth and penetration. The metabolites released from the chemical constituents of neem (*Azadirachtin*, salannin, limonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones) stimulated the plant cells to release abnormal metabolites which repel the nematodes from the uninfected cells of plant. However, the green leaves are rich in *azadirachtin*, salannin, meliantrol and nimbin (Jacobson, 1990; National Research Council, 1992). So the use of neem products stimulated and changes the physiology of plant cells and tissue to repel the nematode parasites.

**Fig 1:** Effect of neem leaves on plant growth parameters (dry shoot and root weight in g)**Fig 2:** Effect of neem leaves on plant growth parameters (shoot length in cm)**Fig 3:** Effect of neem leaves on nematode reproduction in tomato

**Table 1:** Effect of neem leaves on plant growth parameters and *Meloidogyne incognita* in tomato

Treatments	Shoot Length	Dry shoot weight	Dry root weight	Number of galls per root	Number of eggs per egg masses	Final Nematode Population
T1 Neem leaves @ 5 gm/ kg soil	25.5	13.5	5.8	47 (6.9)	277 (16.5)	184 (13.5)
T2 Neem leaves @ 10 gm/ kg soil	26.5	15.5	7.5	43 (6.5)	274 (16.5)	177 (13.3)
T3 Neem leaves @ 20 gm/ kg soil	28.5	17.3	7.5	41 (6.4)	273 (16.5)	172 (13.1)
T3 Neem leaves @ 30 gm/ kg soil	34.5	19.5	8.2	38 (6.2)	270 (16.4)	170 (13.0)
T4 carbofuran @ 0.1 gm/ a.i. kg soil	39.5	25.8	9.7	35 (5.9)	268 (16.3)	118 (10.9)
T5 untreated inoculated check	10.5	5.6	2.2	84 (9.2)	285 (16.8)	723 (26.9)
T6 untreated uninoculated check	13.5	6.0	2.8	0 (1.0)	0 (1.0)	0 (1.0)
CD	1.911	2.038	1.157	0.156	0.055	0.101
SEm	0.913	0.973	0.552	0.053	0.019	0.034

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