# Effect of distillery effluent on root-knot nematode *Meloidogne incognita* in growth of Tomato and Brinjal

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**Abstract:**

Two important vegetable crops tomato and brinjal are highly vulnerable to the root-knot nematode, *Meloidogne incognita.* These plants are regularly planted crop after crop without any soil treatment, crop rotation or biofertilizer etc. to control nematode population. This has lead to high increase of nematodes in the soil and consequent decrease in the crop size, fruiting and quality of fruits. Hence these studies were undertaken to see if the nematode population can be reduced by using treated distillery effluent. Growth parameters of tomato plant and brinjal treated with different percent levels of diluted distillery effluent after *M.incognita* were observed with control. The maximum percent decrease of shoot length over control was recorded in the nematode infected plant which was not treated with distillery effluent and minimum was found in treated plant with 2% dilution of effluent. The shoot fresh weight gradually increased in 2.5% effluent treatment level. The fresh root length was 2% and the root length decreased in the nematode inoculated and treated plants. In case of root fresh weight showed that the weights in all the treatments were almost similar. Fresh root weight of the plants was decreased in nematode inoculated and no distillery effluent. In Brinjal (nematode inoculated), shoot length was highly decreased over control. In *M.incognita* inoculated plants showed that, decrease in shoot length, shoot fresh weight, root length and root fresh weight.

***Key words:*** M.incognita, Brinjal, tomato, nematode

## 1.Introduction

Tomato, *Lycospersicum esculentum L.* being a rich source of vitamins and minerals is an important vegetable crop. Andhra Pradesh stands second to Orissa in production of tomato in India. Andhra Pradesh is the largest grower of tomato and the major tomato growing belts are Rangareddy, Mahabubnagar, Prakasam, Vishakhapatnam and Chittoor (SNX, 2006). The tomato is grown throughout the year and is prone to various biotic and abiotic stresses. Brinjal (*Solanum melongena* L.) is an important and indigenous vegetable crop in India. The major brinjal producing states are Uttar Pradesh, Orissa, Bihar, Assam, West Bengal, Maharashtra, Gujarat, Andhra Pradesh, and Karnataka (ICMR, 2008). The root-knot nematode damage is more harmful to seedlings than to older plants. The affected plants show the development of galls on the roots. The plants become stunted and the leaves show chlorotic symptoms. Fruiting is adversely affected. Among the plant parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.) are capable of reproducing on over 2,000 species of plants and are responsible for approximately 50% of overall nematode damage. After hatching from eggs, second-stage juveniles invade roots of host plants and migrate intercellularly to differentiating vascular regions. The symptoms of nematode infection are the formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting, mineral deficiency, weak and poor yielding plants (Abad *et al.*, 2003; Abbasi *et al.*, 2008). The various species of *Meloidogyne* induce major morphological and physiological changes within roots, attack nearly every crop sown where not only yields are greatly affected but quality is also reduced.

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## 2.ATERIALS AND METHODS

### Host plants

Tomato, *Lycopersicon esculentum* L. and brinjal, *Solanum melongena* L. were selected as host plant for the present study. These crops being highly pathogenic to root- knot nematode *Meloidogyne incognita.* A common methodology was followed for the sterilization of soil mix to be used in all the experiments, maintenance of stock culture of nematodes and for the extraction of nematodes from soil and roots.

### Soil mix:

Red soil and farmyard manure were mixed in the ratio of 2:1:1 and sterilized by solarization. Soil mix was exposed to hot sun and by evening piled up. Soil was mixed thoroughly and spread out to heat and the process was continued regularly. After a week soil was checked for the presence of nematodes if any, and stored in a heap, covered with a polyethylene sheet and used for subsequent experiments.

### Maintenance of stock culture of *Meloidogyne incognita*

Pure culture of *M. incognita* raised from a single egg mass progeny and maintained on tomato seedlings (*Lycopersicon esculentum L.)*. Single egg mass from adult female was isolated and placed in 5 ml of sterile water in a glass vial. Adult females from the egg masses was isolated and teased out from the galled tissues. A regular perennial pattern made from females was examined, for further confirmation of the species (Franklin, 1962).

Second stage juveniles hatched from egg mass in the glass vial was poured into holes made in soil around tomato seedlings, raised in sterilized soil mix in clay pots kept in the glass house. The aerial stem parts were cut off at the soil level, leaving the roots undisturbed in the soil. New tomato seedlings were planted regularly for continuous maintenance of stock cultures. As and when the nematodes required for experimental purposes tomato and brinjal plants were carefully uprooted from the soil and roots was cleaned in running water. Egg masses picked out from galls on their roots placed in small Petri dish containing filtered water and hatched out juveniles (J2) were collected and used for the experimental purposes.

### Extraction and estimation of nematode population

### Extraction of nematode from soil

Extraction of nematode from soil samples were carried out following Cobb's sieving and decanting technique (Cobb, 1918). 250 cc of soil was soaked in water for 5 mins in a plastic basin and mixed thoroughly disintegrate soil aggregates. The supernatant containing the nematodes passed through a series of test sieves of mesh 20, 60, 100, and 350 µ. Final suspension retained over 350 µ mesh and collected in a beaker.

From the final soil suspension nematodes extracted by the centrifugal flotation technique (Caveness and Jensen, 1955). The nematode suspension in water was centrifuged for 5 mins at 3000g to sediment nematodes along with the residual soil. Supernatant water was discarded and sugar solution of a specific gravity 1.18 (484 g sugar/ 1 of water) was added to the residue in the tube, mixed thoroughly with a glass rod and will be centrifuged again at 3000 g for 1minute, where nematodes float in the solution and other debris settle down. The supernatant containing nematodes were poured over 350µ mesh sieve, washed rapidly in running tape water. The clear nematode suspension in the sieve was collected, labeled and stored at 4ºC.

### Estimation of Nematodes from Roots of Tomato and Brinjal

Nematodes from the host roots of tomato and brinjal was extracted by a modified Whitehead and Hemming (1965) technique. A modified method (Nirmala 1993), was adopted to extract nematodes, where a simple inexpensive plastic net ring device is used instead of regular wire mesh which gets rusted and needs to be replaced frequently. The plastic net ring is easy to fabricate using locally available materials like polypropylene rings and plastic nets.

Roots were placed in 25ml of water and blended in warming blender, at a slow speed to macerate the roots and resulting suspension poured through a facial tissue paper supported on plastic net rings. The ring was then placed in Petri-dish containing water just enough to cover the roots in the net rings the nematode from the roots passed through the tissue paper and collected in water in the Petri-dish.

**Estimation of nematode population**

Finally, clear nematode suspension extract were poured into a rectangular plastic counting dish, with sloping slides and graduated bottom to facilitate easy counting. Counting was carried out using stereoscopic microscope (A/O stereo star zoom).

**Results**

**Tomato**

Effects on growth parameters of tomato plant (30DAI) treated with different percent levels of diluted distillery effluent after *M.incognita* inoculation are recorded in the table and figures. The maximum percent decrease of shoot length over control was recorded in the nematode infected plant (-18.8) not treated with distillery effluent and minimum was found in treated plant (- 7.37) with 2% dilution of effluent. Thus, it can be seen that as the percentage of dilution of distillery effluent increased from 10 to 2 (Table 1; Fig.1) the shoot length also increased. The maximum shoot length was recorded in 2.5% distillery effluent been 67.13 cm. the minimum shoot length can be noted in the nematode inoculated treatment with no distillery effluent. Thus, it can be said that 2.5% distillery effluent is more standardized for control of *M.incognita* in the tomato plant. The shoot fresh weight gradually increased from 0.65 to 50.65%. The shoot fresh also showed the maximum in 2.5% effluent treatment level. In case of nematode inoculated treatment with no effluent there is a sudden drop in shoot fresh weight.

The fresh root length was also recorded and the maximum was in 2% effluent treatment being 16.68 cm. Table.1 and Fig.1 showed depicts gradual increase in root length from 5to 2% effluent treatment. In this case a sudden drop in the root length can be noted in the nematode inoculated and treated plants. In case of root fresh weight in all the treatment were almost similar ranging from 5.18 – 5.56 while there is a drop in the fresh weight of the roots in the plants where nematode was inoculated and no distillery effluent was given.

**Brinjal**

Effect on growth parameters of brinjal [30 days after inoculation (30DAI)] with *M.incognita* and treated with various dilution of distillery effluent are recorded (Table. 2; Fig. 2). The maximum percent decreased of shoot length over control was recorded in the non-treated plants with distillery effluent and maximum was found in 10% treated distillery effluent (28.38) with the decreased dilution from 10 – 2.5 the shoot length was less over control table 2 (Fig. 2). Root length of 2.5% dilution was more (13.75 cm) than the control (10.5 cm) and *M.incognita* inoculated (8.5 cm). Root fresh weights were found to be more or less same when compared to control, increased fresh weight (4.78 g) and increased shoot fresh weight (28.45g) were found in 2.5% dilution compared to control and *M.incognita* inoculation. In *M.incognita* inoculated plant there was a drop in shoot length (34.75 cm) shoot fresh weight (15.50g), root length 8.5 cm and root fresh weight 3.7g (Table.2).

**Table-1: Tomato growth parameters of crop infested with *M. incognita***

| Parameters | % effluent treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | Nematode inoculated | 2.0 | 2.5 | 3.30 | 5 | 10 |
| Shoot length (cm) | 52.36 | 42.50 | 48.50 | 67.13 | 65.10 | 58.50 | 49.60 |
| Shoot fresh weight (g) | 30.40 | 25.40 | 30.60 | 45.80 | 34.40 | 38.50 | 30.40 |
| Root length (cm) | 15.50 | 11.75 | 16.68 | 16.24 | 16.37 | 15.24 | 14.75 |
| Root fresh weight (g) | 5.25 | 4.00 | 5.18 | 5.25 | 5.23 | 5.33 | 4.33 |

**Fig. 1**

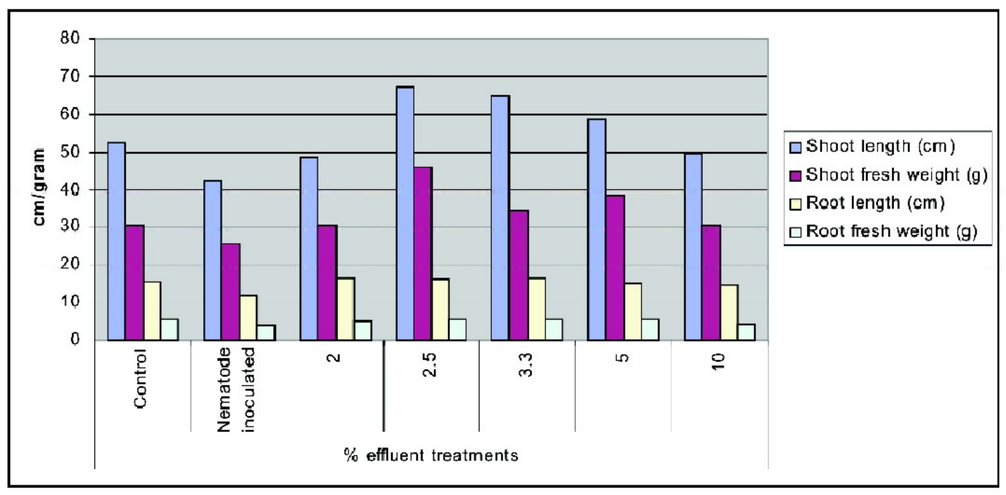
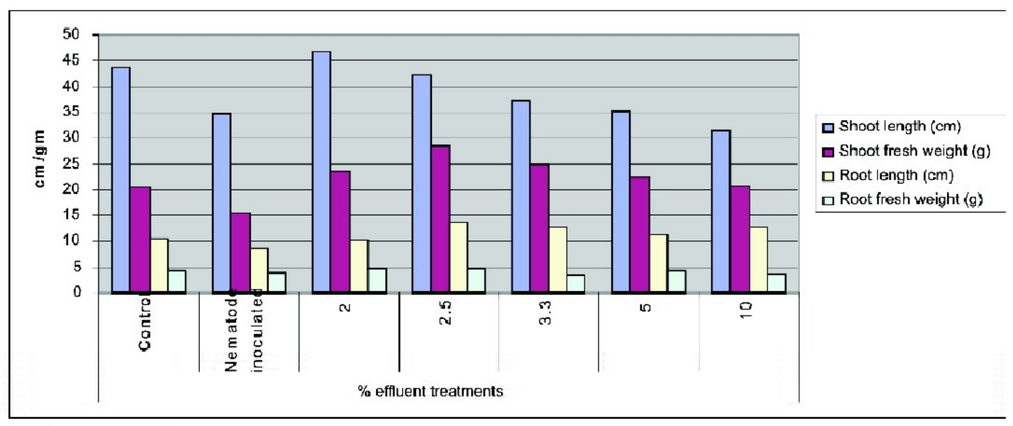



**Table-2: Brinjal growth parameters of crop infested with *M. incognita***

| Parameters | % effluent treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | Nematode inoculated | 2.0 | 2.5 | 3.30 | 5 | 10 |
| Shoot length (cm) | 43.75 | 34.75 | 46.75 | 42.25 | 37.25 | 35.28 | 31.33 |
| Shoot fresh weight (g) | 20.50 | 15.50 | 23.45 | 28.45 | 24.86 | 22.50 | 20.65 |
| Root length (cm) | 10.50 | 8.50 | 10.24 | 13.75 | 12.75 | 11.24 | 12.68 |
| Root fresh weight (g) | 4.25 | 3.75 | 4.68 | 4.78 | 3.33 | 4.25 | 3.64 |

**Fig. 2**




**Discussion**

The nematode inserts its stylet into the plant cell cytoplasm and induces nuclear division without cytokinesis, creating multinucleate giant cells that

nurture the developing worm. Infection is associated with the reprogramming of plant cell development rather than host cell death (Caillaud *et al.,* 2008). *M. incognita* infection causes plant defense genes to become either promptly suppressed or transiently induced, in contrast to incompatible interactions, which immediately induce and sustain expression of defense genes (Panstruga, 2003). *M. incognita* is an obligatory sedentary parasite that reproduces by mitotic parthenogenesis. Among the plant parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.) are capable of reproducing on over 2,000 species of plants (Sasser and Freckman, 1987) and are responsible for approximately 50% of overall nematode damage. The various species of *Meloidogyne* induce major morphological and physiological changes within roots, attack nearly every crop sown where not only yields are greatly affected but quality is also reduced (Sasser, 1980). Addition of phosphorus or *Glomus sp* significantly increased the shoot and root dry weight and phosphorus content and the addition of phosphorus, nemacur or *Glomus sp* significantly decreased the gall number in plants infected with *M. incognita*. Addition of phosphorus significantly decreased the mycorrhizal infection percentage, whereas, the addition of nemacur did not affect it. Shoot and root dry weight and phosphorus content were significantly increased, whereas, the gall number significantly decreased, when the mycorrhizal infection preceded the nematode infection. The results indicated that tomato growth could be improved by the addition of mycorrhiza, which could also be used as a control for root-knot nematodes and *M. incognita* formed conspicuous galls in roots of tomato plants and reduced the shoot and root dry weights and phosphorus content of shoots and results indicated that tomato growth could be improved by the addition of mycorrhiza, which could also be used as a control for root-knot nematodes (Mirghani and Elsheikh, 1996).

Mani *et al.,* (1986) determined that a mixture of long-chain alkanes was responsible for the toxicity of *Fusarium solani* cultures to the root-knot nematode, *Meloidogyne incognita*, a major pathogen that attacks hundreds of crop and ornamental plant species. Although not isolated specifically from a nematode-antagonistic fungus strain, several commercially available mycotoxins commonly produced by *Fusarium* species were tested and found to be nematicidal to *Meloidogyne javanica* at low concentrations (Ciancio, 1995). Hawes and Pueppke (1986) postulated that root border cells, which are detached or sloughed from the root cap, may offer roots protection from pathogenic nematodes by acting as decoys dispersed in the soil around the elongating root tip. This is an attractive hypothesis, as very large numbers of cells may be detached during the life of a root tip (Hawes *et al.,* 1998), and the cells remain functioning in the soil for up to 7 days after detachment (Vermeer and McCully, 1982).

Shoot length, shoot fresh weight, root length and root fresh weight of tomato infested with *M.incognita* treated with 10, 5, 3.3, 2.5 and 2% dilution of effluent showed increased values on comparison with control and *M.incognita* inoculated. 2.5% dilution was very effective than all other dilutions. Shoot length, shoot fresh weight, root length and root fresh weight were recorded 67.13 cm, 453.8 g, 16.24 cm, and 5.25 g for 2.5% dilution and 42.5 cm, 25.4 g, 11.75 cm and 4.00 g. for *M.incognita inoculated* condition. Treated distillery effluent (spent wash) can be classified as a dilute liquid organic fertilizer, with high potassium content. It contains many essential micro and macro elements viz. Ca, K, Mg, P, S, N, Fe, Mn, Cu, and Zn. The research work carried out in many countries has proved its use as irrigation water. The increase in shoot length, shoot fresh weight, root length and root fresh weight were due to addition of effluent rich in micro and macro elements. Therefore, effluent after suitable treatment, are ideally suited as liquid manure for irrigation (Tauro, 1988). The growth parameters of plant such as shoot length, shoot fresh weight, root length and root fresh weight of brinjal infested with *M.incognita* and treated with various dilutions (10, 5, 3.3, 2.5, and 2%) of treated distillery effluent showed slight variations over nematode infected plants at 30 DAI. Among the various dilutions 2.5% was effective over control. Shoot length, shoot fresh weight, root length and root fresh weight of 2.5% dilutions were 46.75 cm, 28.45 g, 13.75 cm and 4.68 g over control was found to be very effective over control (43.75 cm 20.50 g, 10.50 cm, and 4.25 g).

The nematode inoculated plant showed reduction in these parameters compared to the treated.

**4.Conclusion**

The distillery effluent reduces nematode population due to its high nutrient and organic content. Proper dilutions of the effluent increased plant and root growth. This improves the ability of the plant for better fruiting.

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