

## ORIGINAL ARTICLE



# Nematode extract-induced resistance in tomato against *Meloidogyne incognita*

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## Abstract

**Objective:** Present study was carried out to establish the biocontrol potentiality of nematode extract on *Lycopersicon esculentum* (Tomato) Pusa Ruby variety infected with *Meloidogyne incognita* (Kofoid & White) Chitwood nematode. **Methods/Statistical analysis:** We examine in vitro test, phytotoxicity test, in vivo test, in vivo glasshouse bioassay test, PAL (Phenylalanine ammonia lyase) extraction test to identify the effect of nematode extract on tomato plant. One way analysis of variance, ANOVA test is performed in this experiment. **Findings:** The result of in vitro laboratory bioassay showed that application of nematode extract safe for second-stage juveniles (J<sub>2</sub>) of *M. incognita*. The result of in vivo test revealed that nematode extract increased growth of inoculated plants in terms of shoot length, shoot weight and root length as compared with inoculated untreated plants. Application of nematode extract showed reduction in root gall number and number of nematode eggs in inoculated roots. PAL (Phenylalanine ammonia lyase) activity increased in roots of nematode extract treated plants. Root protein content was greater in inoculated untreated plants compared to treated groups. **Application:** This is the first study to control plant parasitic nematode *M. incognita* with nematode extract. In the future it will minimize the global crop loss.

**Keywords:** Biocontrol; Nematode; PAL activity; Root gall

## 1 Introduction

Management of plant parasitic nematodes are more difficult than any other pests because they generally inhabit the 'O' horizon of soil, so several types of management practices have been developed yet, management with nematode extract is one of the new methods identified in our laboratory. Several years back, chemical nematicides were effectively applied but due to their toxic effect on human health and environment they were withdrawn from the market<sup>(1)</sup>. About 2000 plant species are susceptible to plant nematode infection and they cause approximately 5% of global crop loss<sup>(2)</sup>. Tomato, *Lycopersicon esculentum* (Kofoid & White) Chitwood is an important vegetable crop and is infected by root-knot nematode, *Meloidogyne incognita* in tropical and subtropical countries

like India<sup>(3)</sup>. Nematologists around the world are working significantly to identify and learn to manipulate natural enemies of nematodes, so that they can be used as biological control agents. Several types of bacteria and fungi have been isolated from nematode populations that were apparently being kept at low levels by natural enemies. Nematologists around the world have been able to use them to reduce the percentage of populations under laboratory conditions but success at the field level is low. Several culture-dependent methods were established to isolate nematode-associated soil bacteria and it was discovered that J<sub>2</sub> of *M. hapla* established a very specific subset of bacterial community from different soils<sup>(4)</sup>. Under the influence of salicylic acid (SA) which acts as an endogenous signal for the activation of certain plant defense responses, including pathogenesis-related (PR) gene expression and establishment of greater resistance<sup>(5)</sup>. Phenyl-propanoid pathway is the source of endogenous SA concentration in plants and is most probably synthesized from trans-cinnamic acid catalysed by phenylalanine ammonia lyase (PAL). In this experiment we discuss the possibility of nematode control with nematode extract in tomato plants as a new research area.

## 2 Materials and Methods

### 2.1 Test materials

Tomato plants with *Meloidogyne incognita* (Kofoid & White) Chitwood infection were collected from a tomato farm in Santiniketan, Birbhum, West Bengal, India and single egg mass was used to establish a population on tomato (*Lycopersicon esculentum*) cv. PusaRuby variety in the Glass house at Visva Bharati University, WB, India.

### 2.2 In vitro test with salicylic acid on *M. incognita* juveniles

Active J<sub>2</sub> obtained from egg masses were kept in cavity blocks containing sterile tap water, each block containing 110 + 20 juveniles. To assess the effect of SA, the sterile tap water was removed by pipette and immediately replaced by 1 ml of 10 mM SA. Two cavity blocks containing only sterile tap water served as negative controls. Mortality of the nematodes at room temperature (26°C to 28°C) was recorded hourly for 6 h.

### 2.3 Phototoxicity test

Salicylic acid and nematode extracts were applied separately as a foliar spray on tomato cv. Pusa Ruby plants at the concentration of 2.76 mg/plant and 2 mg/plant respectively. The treated plants did not show any toxic effects due to the spraying of SA and nematode extract. Tomato cv. Pusa Ruby plants were susceptible to *M. incognita* was used for the experiment.

### 2.4 In vivo glass house bioassay

Aseptically germinated seeds of tomato cv. Pusa Ruby were sown, one per pot, in pots (32 cm diam.) containing a sterilized mixture of clay soil and compost (2:1 v/v). The pots were divided into six groups of 10 each. The groups were: non-inoculated, untreated; inoculated, untreated; non-inoculated and treated with SA; inoculated and treated with SA; non-inoculated and treated with nematode extract; inoculated and treated with nematode extract. In foliar spray groups, SA and NE were applied as a foliar spray by an atomizer, each plant receiving 2.76 mg/plant and 2 mg/plant respectively. Plants in the non-inoculated untreated and inoculated untreated groups received an equal volume of distilled water spray. After 24h, inoculated groups were inoculated with 2400±275 *M. incognita* J<sub>2</sub>. Treatments were repeated 3 days after inoculation at the same dose. The plants were regularly watered and the experiment was conducted outdoors at an ambient atmospheric temperature (26±2°C) and humidity (73±5%).

### 2.5 Phenylalanine ammonia lyase (PAL) extraction

At 4 days after inoculation, PAL was extracted from the roots of tomato plants. From each group three plants were uprooted and their fresh roots were separately taken, mixed and chopped into pieces. One hundred mg of roots from each group were homogenized in 25mM borate – HCL buffer, pH 8.8, 5mM 2- Mercaptoethanol (400μl<sup>-1</sup>) at 4°C. Following centrifugation at 12000rpm for 20min at 4°C, the supernatant was used as enzyme source. The activity of PAL was assessed using the method of Brueske<sup>(6)</sup> according to Sadasivam and Manickam<sup>(7)</sup> by measuring the release of trans-cinnamic acid from L- Phenylalanine. Data were analysed by ANOVA (P=0.05) and means are presented with the standard error of the difference between means of ten replicates.

## 2.6 Plant growth and nematode assessments

At 40 days after inoculation, shoot length, shoot weight, length of longest root, root weight, number of root galls and number of eggs per g of root were recorded. Nematode eggs were extracted from the roots with the sodium hypochlorite method<sup>(2)</sup>. Three samples of root pieces were taken at random from each plant and the total protein in each sample was determined by the Folin-phenol method<sup>(8)</sup>.

## 2.7 Statistical analysis

Statistical analysis (by one way analysis of variance, ANOVA) was performed to test differential effects among the treatments using MS-Excel software. P values are obtained from the ANOVA table. The value  $p < 0.001$  implies significant difference among the treatments at 0.1% level and means are presented with the standard error of the difference between means of ten replicates.

## 3 Results

Nematodes survived as well in 2mg NE *in vitro* as in the controls, with only 2.3% and 3.2% mortality after 6h. Both NE and SA increased growth of inoculated plants in terms of shoot length, shoot weight and root length as compared with inoculated untreated plants ( Table 1). The results further reveal that spraying with NE did have direct influence on plant growth. Root mass was greater in inoculated untreated plants compared to the non-inoculated plants. PAL activity increased in the roots of tomato sprayed with SA, irrespective of nematode inoculation. Root protein was greater in inoculated untreated plants compared with inoculated and treated plants ( Table 1). Inoculated untreated plants showed more enzyme activity than NE treated plants. Root protein content was not positively correlated with PAL activity, irrespective of nematode inoculation and SA-treatment.

**Table 1.** Plant growth, protein content and nematode infestation of tomato sprayed with SA and nematode extract at 24h before and 72h after inoculation with  $2400 \pm 275$  second stage juveniles of *M. incognita* (40 days after inoculation)

Treatment*	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Root galls	Eggs per g root	PAL activity in root ( $\mu\text{g/g}$ )	Root protein conc. (mg/g)
NU	40	41.8	26	11.6	-	-	13.83	4.2
IU	31.6	31.6	21.5	20	699.5	4993.3	20.74	8.5
N SA	34.1	33.3	25.5	13.3	-	-	20.53	4.3
I SA	32.5	37.5	26	15.8	519.1	3663	21.03	4.7
N NE	39.6	35.8	26.1	12.5	-	-	14.26	4.5
I NE	33.1	33.3	26.5	13.3	470.8	3223.3	13.84	4.6
SED	1.86	2.7	1.3	2.3	45.0	1.6	0.08	0.152
P	<0.001	0.011	0.005	0.016	<0.001	<0.001	<0.001	<0.001

\*Dashes(-) indicate no root galls or eggs in this group. NU, non-inoculated untreated; IU, inoculated and untreated; NSA, non-inoculated and treated with SA at 2.76 mg/plant; ISA, inoculated and treated with SA at 2.76 mg/plant; NNE, non-inoculated and treated with nematode extract at 2 ml / plant; INE, inoculated and treated with nematode extract at 2 ml / plant. Means of seven replicates.

## 4 Discussion

Several types of economic damages occur worldwide due to plant nematode infections. Plant parasitic nematodes are harmful to crop growth and development depending on population density. There are several practices identified to reduce the effect, such as biocontrol bacteria<sup>(9)</sup>, essential oils such as citral, menthol<sup>(10)</sup> etc. but treatment with nematode extract is a new era. Treatment with *Arthrobotrys oligospora* and SA treatment also control nematodes such as *Meloidogyne javanica*<sup>(11)</sup>. It was also proved that application of ascorbic acid (AS) and dipotassium hydrogen phosphate (DKP) also control *Meloidogyne incognita*<sup>(12)</sup>. Application of *Trichoderma* in split-root system of tomato plants reduce the density of *Meloidogyne incognita*<sup>(13)</sup>. In tomato plants, application of several bio control agents induces PR gene, generally PR-1b which acts as a marker of systemic acquired resistance and helps to increase plant immune system<sup>(14)</sup>. In the present study, it has been demonstrated that tomato plants treated with nematode extract enhanced resistance against infection by *M. incognita* and improved plant growth. It appears from the data that treatment with NE was more effective than SA treatment. Infected roots had greater enzyme activity in all the plants studied. It is very much likely that increased PAL activity in SA treated plants interferes with juvenile at the time of root penetration which may not be the cause in case of NE-treated plants. Treatment of host plants with NE at the time

of root penetration reduced subsequent nematode reproduction assessed at 40 days after inoculation. We showed that NE did not kill infective juveniles *in vitro*. Since less number of juveniles were recovered from the nematode extract-treated roots at 40 days after inoculation, it may be that enhanced PAL activity interfere with J<sub>2</sub> at the time of root penetration. Treatment of host plants with nematode extract at the time of root penetration reduced subsequent nematode reproduction, assessed 40 days after inoculation. In this experiment we found that nematode extract treated plants effectively increased resistance to nematodes. It is, therefore, likely that improved growth and reduced protein content of NE-treated nematode-infected plants was a result of the reduction in root-knot reproduction in NE-treated plants.

## 5 Conclusion

In conclusion, it is evident that complete elimination of nematodes is not possible; the main goal is to manage their number of population below damaging levels. It is still not yet been possible understand the interacting mechanism between nematode extract-root/leaf interaction leads to induce systemic resistance against plant-parasitic nematodes but it has been suggested in plants nematode-extract-induced systemic resistance triggers a signal transduction pathway that is different from other common pathogens or bacterial or chemical induced pathways.

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