Vol. 21, No. 1, pp. 225-233. International Journal of Nematology June, 2011

# Biochemical and morphological response of selected germplasm of tobacco to inoculation with *Meloidogyne incognita*

**Mujeebur Rahman Khan, Ziaul Haque and M. Arshad Anwer**

**Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh-202002, India**

E-mail: mrkhan777in@yahoo.co.in

**Abstract.** Five tobacco cultivars (RK-10 P3, RK-12 P3, RK-13 P4, RK-18 P8 and RK-26 P3) were assessed against *Meloidogyne incognita* in pot experiments. The cultivar response at the nematode inoculation level of 2000 $J_2$/plant, was studied on the total phenol (TP), salicylic acid (SA), chlorophylls and carotenoid content of leaves, and plant growth characters.The gall and eggmass indices (on a 0-5 scale, where 5=>100 galls/egg masses/root system) were 3.0 and 2.66 on cv. RK-18 P8, followed by 2.66 and 2.0 (cv. RK-10 P3), 2.33 and 1.66 (cv. RK-26 P3) 2.0 and 1.33 (cv. RK-13 P4) and 1.33 and 1.0 (cv. RK-12 P3), respectively. Final population of root-knot nematode increased, being highest on the cv. RK-18 P8 ($P \leq 0.001$) followed by cvs. RK-10 P3, RK-26 P3, RK-13 P4 and RK-12 P3. Inoculation with the nematode resulted in 13% (cv. RK-18 P8), 16% (cv. RK-10 P3), 17% (cv. RK-26 P3), 21% (cv. RK-13 P4,) and 32% (cv. RK-12 P3, $P \leq 0.001$) increases in the TP content of leaves. SA concentration in tobacco leaves increased in cvs. RK-18 P8, RK-10 P3, RK-26 P3 (9-15%, $P \leq 0.01$) and in cv. RK-13 and RK-12 P3 (18-20%, $P \leq 0.001$) in comparison to uninoculated cultivars. Total chlorophyll content of leaves in response of inoculation with *M. incognita* decreased in tobacco cvs. RK-18 P8 (20%, $P \leq 0.001$), RK-10 P3 (14%, $P \leq 0.01$) and RK-26 P3 (9%, $P \leq 0.05$. Reduction in chlorophyll a and b in these cultivars varied 11-18%. Total carotenoid contents of tobacco leaves decreased significantly in cvs. RK-18 P8 and RK-10 P3 ($P \leq 0.05$). Significant and greater decrease in plant growth variables was recorded in the cultivars in which increase in TP and SA was lower and decrease in chlorophyll and carotenoids was greater. The study has revealed that greater synthesis of TP and SA may form the basis of cultivar response to *M. incognita*. The cv. RK-12 P3 in which the greatest increase in the SA (20%) and TP (32%) was recorded, did not exhibit a significant decrease in plant growth variables and leaf pigments ($P \leq 0.05$). Similarly to cv. RK-18 P8 exhibited the greatest reduction in the plant growth and biomass (24-29%) showed lowest increase in TP (13%) and SA (9%). The study has revealed that among the five cultivars of tobacco screened, cv. RK-12 P3 showed considerable degree of tolerance against *M. incognita* which could be exploited commercially.

**Keywords.** Biomass, carotenoids, chlorophyll content, cultivars, *Meloidogyne incognita*, plant response, salicylic acid, tobacco germplasm, total phenols.

## INTRODUCTION

Root-knot (*Meloidogyne* spp.) is the major disease problem in tobacco cultivation throughout the world and may cause up to 60% loss (Charles *et al*., 2005). *M. incognita* has been the most frequently associated species. Plants react to pathogen attack through a variety of active and passive defence mechanisms (Garcia *et al.,* 2001). As soon as the plant detects the presence of the invading microorganism, one or more defence mechanisms are triggered to restrict the growth of the pathogen and ultimately to destroy it (Dixon *et al*., 1994; Benhamou, 1996).

Phenolic compounds and salicylic acid are among the most influential and widely distributed secondary products in the plant and each has a different action in the defence of plants against pathogen attack (Nicholson and Hammerschmidt, 1992; Takahama and Oniki, 1992). In general, phenolic acids and salicylic acid are present in a very low concentration in healthy plants. However, upon

infection with pathogens their concentration increases considerably. In incompatible interactions the accumulation of these compounds is restricted to a few cells in the close vicinity of the invading pathogen, accompanying hyper-sensitive response (HR), which results in necrosis of both plant cell and pathogen, thus preventing further proliferation of the pathogens (Staswick *et al*. 1992). However the severity of infection and defence mechanism varies with the cultivar. In view of the essential role of phenolic compounds and salicylic acid in the development of pathogen resistance in plants, the present study was undertaken to evaluate the response of some tobacco cultivars to *M. incognita* with regard to biochemical parameters (total phenol, salicylic acid content, chlorophyll a and b and carotenoid) and morphological characters (root-knot, eggmass index, soil population, plant growth and biomass). Salicylic acid and phenols play a vital role in the host reaction to pathogen including nematodes. Hence these biochemicals were measured to ascertain the reaction of tobacco cultivars to *M. incognita.*

## MATERIALS AND METHODS

The germplasm of tobacco (*Nicotianum tobaccum* L.) consisting of five cultivars: RK-10 P3, RK-12 P3, RK-13 P4, RK-18 P8, RK-26 P3, was procured from the Central Tobacco Research Institute (CTRI), Rajamundri, Andhra Pradesh, India. Seedlings of all the tobacco cultivars were raised in 25 cm clay pots filled with 2 kg autoclaved mixture of soil and farm yard manure (3:1).

### Preparation of nematode inoculum

Infected root samples of eggplant were collected from the nematode culture bed. Roots were rinsed with distilled water, thereafter females and eggmasses from the galled tissue were excised. The species of *M. incognita* (Chitwood) Kofoid and White was confirmed using perennial pattern technique (Hartman and Sasser, 1985) of ten females excised from the root system. The egg masses were incubated on a coarse sieve lined with two layers of tissue paper and put in a Baermann's funnel filled with an adequate amount of water at 25$\pm$2$^{o}$C for a week. The hatched juveniles were collected from the funnel every 24 h and stored in water inside a refrigerator. Nematodes in the suspension were counted in a counting dish under stereomicroscope (Khan, 2008) and standardized to 2000 larvae/5 ml suspension.

### Plant culture

Clay pots (25 cm diameter) were filled with 2 kg autoclaved soil and compost (3:1). Nematode suspension at 2000 $J_2$/pot was added to the top soil of the pot. A day after inoculation, three to four tobacco leaf seedlings at 4-weeksold of cvs. RK-10 P3, RK-12 P3, RK-13 P4, RK-18 P8 and RK-26 P3, were transplanted at the centre of a pot and watered immediately. Six replicates were maintained for each treatment, plants from three pots were harvested 15 days after inoculation for biochemical analysis. The remaining three pots were harvested at 4 months after inoculation for plant growth variables. Plants were arranged in a complete randomized block design in an area receiving uniform sunlight. Plants were grown for four months. During this period, they were regularly observed for any visible symptoms attributed to the pathogen. At harvest, four months after inoculation, plants were flooded with water to facilitate root recovery, root-knot and eggmass index (0-5 scale), soil population of nematodes and length, fresh and dry weight were determined. Dry weight was determined by drying the plants in a hot air oven at 60°C for 2 days. Estimation of leaf pigments, total phenol contents and salicylic acid (SA) was done after 15 days of inoculation

### Root-knot symptoms

At harvest roots were examined to count galls and egg masses on a 0-5 scale (Taylor and Sasser, 1978):

**0**=0 galls/egg masses/root system.

**1**=1-2 galls/egg masses/root system.

**2**=3-10 galls/egg masses/root system.

**3**=11-30 galls/egg masses/root system.

**4**=31-100 galls/egg masses/root system.

**5**=>100 galls/egg masses/root system.

### Soil population of *Meloidogyne incognita*

The population level of *M. incognita* was determined at harvest using Cobb's decanting and sieving method (modified) followed by Baermann's funnel technique (Khan, 2008). Nematode suspensions collected from the Baermann funnel were examined in a counting dish under stereomicroscope to count nematode larvae and the population was determined per kg soil.

### Estimation of total phenol (TP) contents and salicylic acid (SA)

Total phenol (TP) and salicylic acid (SA) contents of 1 g leaf sample from each of the five tobacco plants were assayed 15 days after nematode inoculation. The samples for TP assay were homogenized in 10 ml of 80% methanol and agitated for 15 minutes at 70°C (Zieslin and Ben Zaken, 1993). The leaf samples from the three plants of each cultivar and treatment were processed separately. One millilitre of methanol extract (1 ml) was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1N), and the solution was measured at 725 nm in a spectrophotometer (UV 2450, Shimadzu Japan). Catechol was used as the standard. The amounts of TP were expressed as µg catechol/g fresh leaf (Sharma and Sain, 2005).

For SA assay, the 1 g leaf sample was cut into small

**Table 1.** Effects of *Meloidogyne incognita* on gall index (GI), egg mass index (EMI), soil population ($J_2$/kg), fresh weight (g) and dry weight (g) on tobacco cultivars.

| Cultivars | Inoculationon level ($J_2$/pot) | [α]Gall index (GI) (0-5) | [β]Egg mass index (EMI) (0-5) | [γ]Soil population ($J_2$/kg) | Plant length (cm) | | Fresh weight (g) | | Dry weight (g) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Shoot | Root | Shoot | Root | Shoot | Root |
| **RK-18 P8** | 0.0 | 0.0 | 0.0 | 0.0 | 60.83 | 20.02 | 57.53 | 16.61 | 11.11 | 3.42 |
| | 2000 | 3.0 [h] | 2.66 [m] | 5814 [r] | 45.7*** | 14.3*** | 42.5*** | 12.8*** | 8.2*** | 2.6*** |
| **RK-10 P3** | 0.0 | 0.0 | 0.0 | 0.0 | 55.3 | 18.1 | 52.3 | 15 | 10 | 3.1 |
| | 2000 | 2.66 [g] | 2.0 [l] | 5421[q] | 47.16** | 15.3** | 43.6** | 12.17*** | 8.31** | 2.53*** |
| **RK-26 P3** | 0.0 | 0.0 | 0.0 | 0.0 | 32.1 | 18.1 | 30.4 | 15.3 | 5.5 | 2.2 |
| | 2000 | 2.33 [f] | 1.66 [k] | 4354 [p] | 28.4* | 15.62** | 26.5** | 13.2*** | 4.8** | 1.9*** |
| **RK-13 P4** | 0.0 | 0.0 | 0.0 | 0.0 | 41.4 | 23.9 | 38.4 | 20.9 | 8.2 | 2.6 |
| | 2000 | 2.0 [e] | 1.33 [j] | 3842 [o] | 38.5 | 21.8 | 34.2* | 18.5* | 7.4* | 2.3* |
| **RK-12 P3** | 0.0 | 0.0 | 0.0 | 0.0 | 48.30 | 16.40 | 49.80 | 13.20 | 10.30 | 2.80 |
| | 2000 | 1.33 [d] | 1.0 [i] | 2213 [n] | 45.3 | 15.90 | 48.00 | 12.80 | 10.00 | 2.6 |
| **LSD** | P ≤ 0.05 | 0.239 | 0.267 | 6.287 | 8.729 | 0.753 | 4.860 | 1.086 | 0.869 | 0.14 |
| | P ≤ 0.01 | 0.803 | 0.802 | 21.138 | 11.967 | 1.0330 | 6.663 | 1.489 | 1.192 | 0.19 |
| | P ≤ 0.001 | 1.202 | 1.347 | 31.708 | 16.288 | 1.4061 | 9.069 | 2.026 | 1.622 | 0.25 |
| **F-value** | Cvs. (df=4) | 20.42 [c] | 10. 90 [b] | 8.22 [b] | 20.36 [c] | 185.90 [c] | 51.75 [c] | 87.75 [c] | 101.0 [c] | 128.58 [c] |
| | Nematode (df=1) | - | - | - | 8.09 [a] | 367.80 [c] | 103.8 [c] | 67.12 [c] | 41.11[c] | 348.16 [c] |
| | Cvs. x Nematode (df=4) | - | - | - | NS | 18.74 [b] | 17.52 [b] | 5.19 [b] | 3.40 [a] | 16.16 [b] |

Each value is the mean of three replicates, values followed by *(P≤0.05), **(P≤0.01) and ***(P≤0.001) are significantly different from the control (uninoculated) otherwise not significant at P≤0.05. F values followed by [a](P≤0.05), [b](P≤0.01) and [c](P≤0.001) are significant otherwise not significant (NS) at P≤0.05. [α, β] values followed by different superscript letters are significantly different from each other (P≤0.05), [γ] values followed by different superscript letters are significantly different from each other (P≤0.001).

pieces of 0.5-1.0 cm and soaked in water overnight. Water-leaf solution was filtered through Whatman filter paper no. 1 and extracted in ethyl acetate. The ethyl acetate fraction was taken and sodium sulphate was added to remove moisture. The filtrate was evaporated to dryness in a water bath. Methanol (10 ml) was added to the dried sample and the absorbance of the solution was read in a spectrophotometer (UV 2450, Shimadzu Japan), at 306 nm (Shane and Kowblansky, 1968). A standard curve of SA was prepared by making SA solutions of different concentrations in methanol (0, 10, 20, 30, 40, 50, 100 ppm). Absorbance was plotted and the best fit line passing through the origin was drawn. From the standard curve, the concentration of SA in the sample was calculated according to the formula of (Lowery *et al*., 1951).

**Figure 1.** Correlation between disease (gall index) and percent change in phenolic contents, salicylic acid, total chlorophyll and carotenoid of tobacco cultivars subjected to inoculation of *Meloidogyne incognita* (2000 $J_2$).

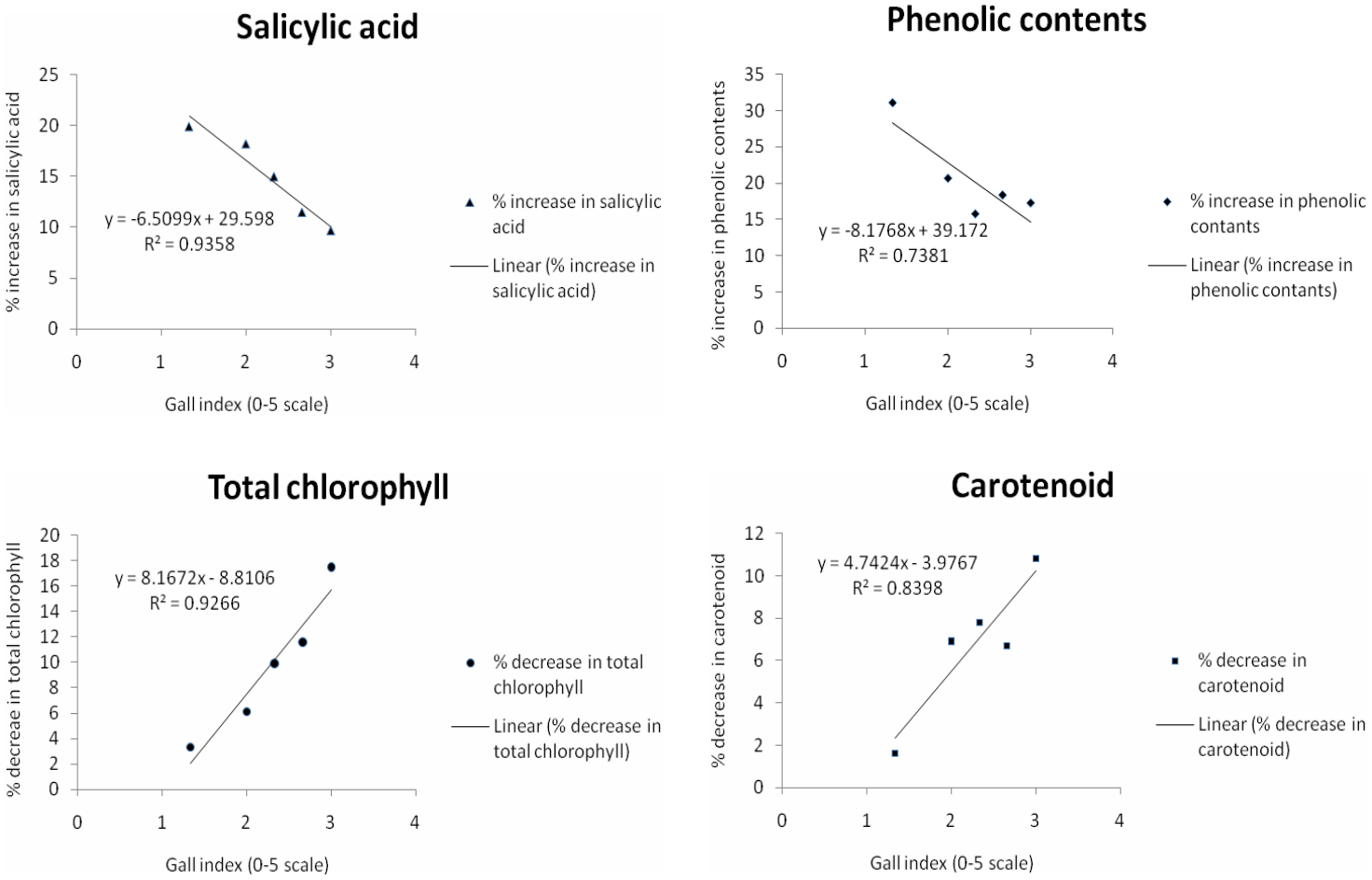


**Table 2.** Effects of *Meloidogyne incognita* on leaf pigments, salicylic acid, and total phenolic contents of leaf of tobacco cultivar.

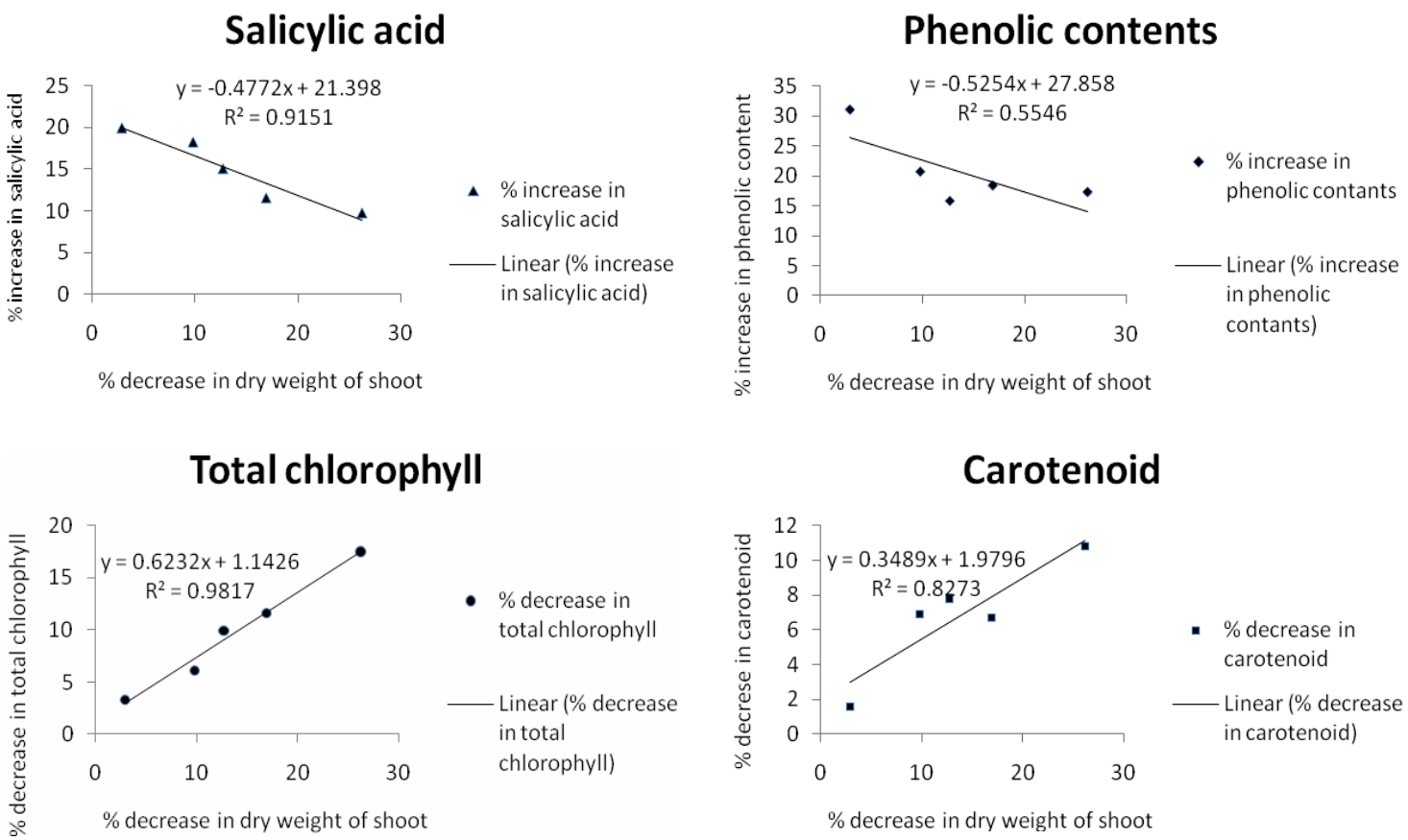
| Tobacco Cultivars | Inoculation on level ($J_2$/pot) | Phenolic contents (µg catechol $g^{-1}$ FW) | Salicylic Aaid (ppm/g FW) | Chlorophyll 'a' (mg/g FW) | Chlorophyll 'b' (mg/g FW) | Total chlorophyll (mg/g FW) | Carotenoids (mg/g FW) |
|---|---|---|---|---|---|---|---|
| **RK-18 P8** | 0.0 | 86.5 | 13.55 | 1.041 | 0.654 | 1.695 | 0.139 |
| | 2000 | 101.5** | 14.86* | 0.849*** | 0.549** | 1.398*** | 0.124* |
| **RK-10 P3** | 0.0 | 81.5 | 12.57 | 1.028 | 0.999 | 2.027 | 0.134 |
| | 2000 | 96.5** | 14.02* | 0.897** | 0.894* | 1.791* | 0.125* |
| **RK-26 P3** | 0.0 | 91.5 | 14.20 | 1.002 | 0.889 | 1.891 | 0.128 |
| | 2000 | 106.5*** | 16.33** | 0.894* | 0.810* | 1.704* | 0.118 |
| **RK-13 P4** | 0.0 | 96.5 | 12.79 | 0.799 | 0.731 | 1.530 | 0.131 |
| | 2000 | 116.5*** | 15.12*** | 0.746 | 0.690 | 1.436 | 0.122 |
| **RK-12 P3** | 0.0 | 96.5 | 14.04 | 0.888 | 0.822 | 1.710 | 0.123 |
| | 2000 | 126.5*** | 16.84*** | 0.851 | 0.802 | 1.653 | 0.121 |
| **LSD** | P ≤ 0.05 | 9.535 | 0.593 | 2.498 | 0.0795 | 0.141 | 0.0153 |
| | P ≤ 0.01 | 13.071 | 0.813 | 3.425 | 0.109 | 0.193 | 0.0210 |
| | P ≤ 0.001 | 17.791 | 1.106 | 4.662 | 0.148 | 0.263 | 0.0286 |
| **F-value** | Cvs. (df=4) | 39.95 [c] | 36.81[c] | 32.80 [c] | NS | 70.99 [c] | NS |
| | Nematode (df=1) | 219.26 [c] | 143.62 [c] | 8.76 [b] | NS | 34.16 [c] | 6.00 [a] |
| | Cvs.x Nematode (df=4) | 10.23 [b] | 3.33 [a] | NS | NS | 3.89 [a] | NS |

Each value is the mean of three replicate, values followed by *(P≤0.05), **(P≤0.01) and ***(P≤0.001) are significantly different from the control (uninoculated) otherwise no significant at P≤0.05. F values followed by [a](P≤0.05), [b](P≤0.01) and [c](P≤0.001) are significant otherwise not significant, (NS) at P≤0.05.
FW= fresh weight

## Estimation of leaf pigments

Total chlorophyll and carotenoids contents of leaf were estimated by grinding 1 g of fresh leaves from interveinal areas of 15 days old tobacco plants in 40 ml of 80% acetone with the help of mortar and pestle. The suspension was decanted in a Buchner funnel having two Watman filter paper No. 1. The filtration was done with the help of a suction pump. The residue was ground thrice by adding acetone. The suspension was decanted in the Buchner funnel and filtered by a vacuum pump. Lastly,the mortar and pestle were rinsed with acetone and the resultant solution was transferred in Buchner funnel and filtered. The filtrate was transferred to 100 ml volumetric flask and the volume was made up to the capacity by adding acetone. The optical density (O.D.) of the filtrate was read in a spectrophotometer at 470 nm for carotenoids (MacLachlan and Zalik, 1963) and 645 and 663 nm for total chlorophyll contents (Arnon, 1949).

**Fig. 2.** Correlation between percent decrease in shoot dry weight and percent change in phenolic contents, salicylic acid, total chlorophyll and carotenoid of tobacco cultivars subjected to inoculation of *Meloidogyne incognita* (2000 $J_2$).




### Statistical analysis

The experiment was conducted during two consecutive years. The data obtained during the two years were statistically identical; hence results are based on the experiment conducted during the second year because there was greater precision and perfection due to the experience gained in the first year. All data were subjected to ANOVA using SPSS 11.0 for Windows. Means were then separated using the least significant difference (LSD) test at $P \leq 0.05$, 0.001 and 0.001. The F-values were calculated and compared with the table value at $P \leq 0.05$, 0.01 and 0.001.

## RESULTS

### Symptoms of root-knot

Tobacco cultivars inoculated with 2000 $J_2$ of *M. incognita* exhibited stunted growth and pale green foliage, especially in the cv. RK-18 P8. The characteristic symptoms of galls appeared on the roots. The gall and eggmass indices (on a 0-5 scale) were 3.0 and 2.66 (RK-18 P8), followed by 2.66 and 2.0 (cv. RK-10 P3), 2.33 and 1.66 (cv. RK-26 P3), 2.0 and 1.33 (cv. RK-13 P4), and 1.33 and 1.0 (cv. RK-12 P3), respectively. F values for gall and eggmass indices were significant at $P \leq 0.01$ (Table I).

### Soil nematode population

The soil population of *M. incognita* increased over time, and was recorded at the highest in the tobacco cv. RK-18 P8 and lowest in RK-12 P3. F-value for soil population was highly significant at ($P \leq 0.001$, Table I).

### Plant growth and biomass

Inoculation with *M. incognita* resulted in significant suppression in the length, fresh weight and dry weight of root

and shoot of tobacco cv. RK-18 P8 ($P \leq 0.001$) and RK-10 P3 ($P \leq 0.01$and $P \leq 0.001$) in comparison to uninoculated control (Table I). The greatest decrease in the variables considered was recorded in cv. RK-18 P8 (24-29%) and RK-10 P3 (15-19%). The lowest significant decrease was recorded in the cv. RK-13 P4, for fresh and dry weight of root and shoot ($P \leq 0.05$). The cv. RK-12 P3 did not exhibit significant decrease in any plant growth and biomass variables at $P \leq 0.05$. The ANOVA has revealed significant F values for length, fresh and dry weight of shoot and root for all cultivars (Table I).

### Phenol and salicylic acid contents of leaf

Total phenol content of leaves of *M. incognita* inoculated plants increased by 13% (cv. RK-18 P8 $P \leq 0.05$) to 32% (cv. RK-12 P3, $P \leq 0.001$) in comparison to uninoculated control (Table 2). Due to inoculation with nematode, SA concentration in tobacco leaves increased from 9-20%, being greatest in cv. RK-12 P3 (18-20%, $P \leq 0.001$) over control. F-value of all three sources for salicylic acid was significant at $P \leq 0.001$ (Table II).

### Chlorophylls and carotenoid contents of leaf

Total chlorophyll content of leaves in response of inoculation with *M. incognita* decreased in tobacco cultivars. By 9% (RK-26 P3, $P \leq 0.05$) to 20% (RK-18 P8, $P \leq 0.001$). Reduction in chlorophyll a and b in these cultivars varied from 11-18%. Highest decrease in carotenoid contents was observed in cvs. RK-18 P8 (10.8%, $P \leq 0.05$) and least in cv. RK-12 P3 (1.6%). Overall effect of the nematode inoculation and cultivars was significant for total chlorophyll ($P \leq 0.01$ and 0.001), chlorophyll a ($P \leq 0.01$), and carotenoids ($P \leq 0.05$). Individual and interactive effects were significant for chlorophyll (Table 2).

## DISCUSSION

The tobacco cultivars inoculated with 2000 $J_2$ of *M. incognita* exhibited stunted growth and mild yellowing of leaves. The characteristic root-knot galls developed on the tobacco cvs. RK-18 P8, RK-10 P3, RK-26 and RK-13 P4, in particular due to inoculation with *M. incognita.* The varied reaction of tobacco germplasms to root-knot nematode has been reported earlier (Opperman *et. al*, 1994). Eggmass production of the nematode was however, less than that of the gall formation indicating that the host could not provide optimal nutrition to the nematode because of the deteriorating health status of the susceptible host.

Prominent galls developed on the cvs. RK-18 P8 and RK-10 P3, indicating their susceptibility to the pathogen. *M. incognita* multiplies rapidly in the root zone of susceptible plants. Nematode-induced mature galls contained more than one female or male. The number of eggs layed by obese female varies widely, but on average it ranged from 400-500; however, it has been found that one female *Meloidgyne* spp. may lay a maximum of 2000 eggs (Lucos, 1975). Highest soil population of the nematode was recorded on the cv. RK-18 P8 which exhibited the greatest number of galls and eggmasses. This was apparently due to the stimulatory effect of the root exudates of the cultivars. Root-knot nematodes are important pests of solanaceous crops and induce significant reduction in growth and yield as was observed in the present study (Khan and Akram, 2000). The nematode infection resulted in significant suppression of the plant growth and dry matter production of tobacco cvs. RK-18 P8 and RK-10 P3. Shepherd and Barker (1990) have reported a 50-60% decrease in the leaf yield of tobacco due to the root-knot infestation.

Chlorophylls are considered as the basic unit for photosynthesis as these pigments absorb light and transfer to cell organelles for $CO_2$ fixation (Wallace, 1987). On susceptible cultivars, mild yellowing of leaves was observed which gradually became pronounced with the advancement of plant age. At 3-4 months old, the entire foliage of susceptible cultivars became discernibly yellowish (cv. RK-18 P8) whereas the foliage of the resistant cultivar (RK-12 P3) remained healthy. The study has revealed that the chlorophyll pigments are highly sensitive to alteration in host physiology induced by *M. incognita*. The resulting water stress was due to damage to roots and development of galls caused by the nematode. The chlorophyll and carotenoids molecules get denatured and consequently the leaf contents were reduced, with subsequent decreased photosynthetic activity reflecting in a lower biomass production (Khan and Khan, 1987). This is shown by the linear regression between percent decrease in dry shoot weight, and decrease in chlorophyll ($r^2$=0.98) and carotenoid ($r^2$=0.82) contents (Fig. 2).

The total phenol content of leaves of all five cultivars increased in the nematode-inoculated plants but varied greatly. Increase in the phenol of cv. RK-18 P8, which was found highly susceptible to the nematode was lowest whereas the increase was greatest in cv. RK-12 P3, which expressed resistance to *M. incognita.* This is also discernable from the linear regression analysis done between disease (gall index) and phenolic contents ($r^2$=0.73, Fig. 1). This indicates that the phenolic compounds also contribute to the defence of plants against nematode attack also (Nicholson and Hammerschmidt, 1992; Hammond Kosack and Jones, 1996). In general, these compounds act as phytoalexins and are present in very low concentrations in healthy plants. However, upon infection with the pathogens, their concentration increases considerably as found in the present study. Salicylic acid (SA) has also been implicated as one of the key components in the signal transduction pathway leading to plant resistance to various pathogens (Ryals *et al*., 1996; Wobbe and Klessig, 1996). Highest concentration of SA was recorded in the cvs. RK-12 P3, which did not exhibit galls and plant growth reduction, whereas lowest SA increase occurred in cvs. RK-18 P8, RK-10 P3 and RK-26 P3, which showed susceptibility to *M. incognita.* The tolerant and susceptible reaction of tobacco cultivars against root-knot

nematode was apparently due to the varied synthesis of SA as shown by the linear regression analysis ($r^2$=0.93, Fig. 1). In tobacco, systemic acquired resistance activation by SA, results in a significant reduction of disease symptoms caused by *M. incognita* (Ganguly *et. al.,* 1998; Nandi *et. al.,* 2000) It has also been found that galls induced by nematode in inoculated plants is accompanied by the accumulation of SA at the root site as well as in the phloem fluid and in healthy uninoculated leaves (Malamy *et al*., 1990; Enyedi *et al*., 1992; Summermatter *et al*., 1995). F-values were much greater for salicylic acid than total phenols, indicating the greater role of the former in the disease tolerance.

The study has revealed that among the five cultivars of tobacco screened, cv. RK-12 P3 showed a considerable degree of tolerance against *M. incognita* which can be exploited commercially. The expressed tolerance can be considered as a reliable character as it was based on the biochemical parameter i.e. greater synthesis of phenol and SA in response of the nematode inoculation. A crop rotation system substituting four other tobacco cultivars (RK-18 P8, RK-10 P3, RK- 26 P3, RK-13 P4) is suggested to the growers in the nematode infested areas. However, for future commercial use, multilocational field trials may be needed to test reliability of the tolerance reaction of the cv. RK-12 P3

**Acknowledgement.** The aid of the Director, CTRI, Rajamundri, Andhra Pradesh (India) in supplying the tobacco germplasm is gratefully acknowledged.

## LITERATURE CITED

**Arnon, D. 1949.** Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* **24,** 1- 15.

**Benhamou, N. 1996.** Elicitor-induced plant defence pathways. *Trends in PIant Science* **1,** 233-240.

**Charles, S. J., W. Jennifer and K. R. Barker 2005.** Plant parasitic nematodes of tobacco. In: *Plant-Parasitic Nematodes in Tropical and Subtropical Agriculture*, pp 675-708 (eds M. Luc, R. A. Sikora and J. Bridge). Wallingford, UK: CAB International.

**Dixon, R. A., M. J. Harrison and C. J. Lamb 1994.** Early events in the activation of plant defense responses. *Annual Review of Phytopathology* **32,** 479-501.

**Enyedi, A. J., N. Yalpani, P. Silverman and I. Raskin 1992.** Localization, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. *Proceedings of the National Academic of Science* **89,** 2480-2484.

**Ganguly, A. K., S. Anil, Pankaj and V. Singh 1998.** Salicylic acid induced resistance in tomato against *Meloidogyne incognita* Race-1. *Indian Journal of Nematology* **29,** 182-184.

**Garcia, P. C., R. M. Rivero, L. R. Lopez Lefebre, E. Sdnchez, J. M. Ruiz and L. Romero 2001.** Direct action of the biocide carbendazim on phenolic metabolism in tobacco plants. *Journal of Agricultural Food Chemistry* **49,** 131-137

**Hammond-Kosack, K. E. and J. G. Jones 1996.** Resistance gene-dependent plant defense responses. *Plant Cell* **8,** 1773-1791.

**Hartman, K. M. and J. N. Sasser 1985.** Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: *An Advanced Treatise on Meloidogyne. Vol II, Methodology*, pp. 69-77 (eds K.R. Barker, C.C. Carter and J.N. Sasser,). Raleigh, NC, USA: North Carolina State University Graphics.

**Khan, M. R. 2008.** *Plant Nematodes: Methodology, Morphology, Systematics, Biology and Ecology*. New Hampshire, USA: Science Publishers, pp.?.

**Khan, M. R. and M. W. Khan 1987.** Histophysiological alternations induced by *Meloidogyne incognita* in tomato. *International Nematological Network Newsletter* **4**, 10-12.

**Khan, M. R. and M. Akram 2000.** Effects of certain antagonistic fungi and rhizobacteria on wilt disease complex of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lycopersici*. *Nematologia Mediterranea* **28,** 139-144.

**Lucas, G. B. 1975.** *Diseases of Tobacco* 3rd ed. Raleigh, North Carolina, USA: Biological Consulting Associates, pp. 621.

**MacLachlan S. and S. Zalik 1963.** Plastid structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant of barley. *Canadian Journal of Botany* **41,** 1053-62.

**Malamy J., J. P. Carr, D. F. Klessig and L. Raskin 1990.** Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. *Science* **205,** 1002-1004.

**Nandi, B. N. C., Sukul and S. P. S. Babu 2000.** Exogenous application of salicylic acid reduces *Meloidogyne incognita* infestations of tomato. *Allelopathy Journal* **7,** 285-288.

**Nicholson, R. L. and R. Hammerschmidtt 1992.** Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology* **30,** 369-389.

**Opperman, C. H., C. G. Taylor and M. A. Conkling 1994.** Root-knot nematode directed expression of plant root specific gene. *Science* **23,** 221-223.

**Pankaj, G. Chawla, N. A. Shakil, V. Kishor and D. Rohatgi 2005.** Estimation of salicylic acid and its role in resistance mechanism in chickpea against *Meloidogyne incognita. Indian Journal of Nematology* **35,** 160-162.

**Ryals, J. K., U. H. Neuenschwander, M. G. Willits, A. Molina, H. Steiner and M. D. Hunt 1996.** Systemic

acquired resistance. *Plant Cell.* **8,** 809-1819

**Shane, N. and M. Kowblansky 1968.** Determination of acetyl salicylic acid, salicyclamide, acetominophone and caffeine in tablet or powder by independent methods. *Journal of Pharmaceutical Sciences* **57,** 1218-1223.

**Sharma, P. and S. K. Sain 2005.** Use of biotic compounds against damping off of cauliflower by *Pythium aphanidermatum. Indian Phytopathology* **58,** 395-401

**Shepherd, J. A., and K. R. Barker 1990.** Plant parasitic nematodes of tobacco. In: *Plant-Parasitic Nematodes in Tropical and Subtropical Agriculture*, pp 493-517 (eds M. Luc, R. A. Sikora and J. Bridge). Wallingford, UK: CAB International.

**Southey, J. F. 1986.** *Plant Nematology*. London, UK: Her Majesty's Stationary Office.

**Staswick, P. E., W. Su and S. H. Howell 1992.** Methyl jasmonate inhibition of root growth and induction of a leaf protein in an *Arabidopsis thaliana* mutant. *Proceeding of National Academy of Science* **89,** 6837-6840

**Summermatter, K., L. Sticher and J. E. Matraux 1995.** Systemic response in *Arabidopsis thaliana*-infected and challenged with *Pseudomonas syringae* pv. *syringae*. *Plant Physiology* **108,** 1379-1385

**Takahama, U. and T. Oniki 1992.** Regulation of peroxidasedependent oxidation of phenols in the apoplast of spinach leaves by ascorbate. *Plant Cell Physiology* **33,** 379-387

**Taylor, A. L. and J. N. Sasser 1978.** Biology, Identification and Control of Root-Knot Nematode (*Meloidogyne* spp.). Raleigh, North Carolina, USA: North Carolina State University Graphics.

**Wallace, H. R. 1987.** Effect of nematode parasites on photosynthesis. In: *Vistas on Nematotogy*, pp 253-259 (eds J. A. Veech and D. W. Dickson). Maryland. USA: SON Publication.

**Wobbe, K. K. and D. F. Klessig 1996.** Salicylic acid: an important signal in plants. In: *Plant Gene Research Series, Signal Transduction in Plant Growth and Development,* pp167-196 (ed D.P.S. Verma). Vienna, Austria: Springer-Verlag.

**Zieslin, N. and R. Ben Zaken 1993.** Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. *Plant Physiology and Biochemistry* **31,** 333-339.