Soil application of *Pseudomonas fluorescens* and *Trichoderma harzianum* reduces root-knot nematode, *Meloidogyne incognita,* on tobacco

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Summary. The effects of *Pseudomonas fluorescens* and *Trichoderma harzianum*, on the control of the root-knot nematode, *Meloidogyne incognita*, were investigated in three tobacco cultivars (RK-18 P8, RK-26 P3 and RK-12 P3). Soil application of the biocontrol agents was compared with two nematicides, Phorate and Furadan. Greatest reduction in the numbers of second-stage juveniles in soil, host root galls and egg mass indices were recorded with Furadan and *P. fluorescens* compared with experimental controls. The greatest increase in the plant growth and biomass of tobacco was obtained in cv. RK-18 P8 with *P. fluorescens* (16-32%) followed by Furadan (15-30 %) compared with experimental controls. Increases in total phenol (TP) and salicylic acid (SA) were negatively correlated with numbers of root galls and egg masses/root system. Greatest increases in the leaf contents of SA (20%) and TP (31%) were recorded in cv. RK-12 P3, whereas least increases were detected in cv. RK-18 P8 (17% TP and 10% SA). The study has revealed that cv. RK-12 P3 may be exploited commercially for nematode resistance to *M. incognita,* and *P. fluorescens* may be used as an alternative to nematicides in nematode infested field, to obtain higher productivity of tobacco.

Key words: salicylic acid, total phenols, chlorophylls, carotenoids.

Introduction

Tobacco is an important industrial crop and gives income worth billions of dollars annually to the world tobacco farmers. One of the major constraints in the production of this crop is regular occurrence of diseases. Root-knot nematodes, *Meloidogyne* spp., are a major problem in tobacco throughout the world and may result in up to 60% yield loss (Shepherd and Barker, 1990). Tobacco is highly susceptible to *M. incognita* and *M. javanica* (Charles *et al.,* 2005), which are widely distributed and possess high reproduction capacity and damage potential (Barker and Lucas, 1984; Johnson, 1998).

Plants have a variety of active and passive defense mechanisms against pathogen attack (Garcia *et al.*, 2001). The defense mechanisms, generally, include both chemical and physical barriers such as reinforced cell walls or synthesis of antimicrobial molecules (Hammond-Kosack and Jones, 1996). Phenolic compounds and salicylic acid (SA) are among the most influential and widely distributed secondary defense products in plants, and lay a completely different action in the host defense (Nicholson and Hammerschmidt, 1992; Takahama and Oniki, 1992). In incompatible interactions, the accumulation of these compounds is restricted to a few cells in close vicinity to the invading pathogen, accompanied by hypersensitive reaction/response (HR) which results in necrosis both of plant cells and pathogen, thus preventing further proliferation of the pathogens (Staskawicz, *et al.,* 1992). After

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infection, the systemic acquired resistance (SAR) pathway is activated leading to a drastic increase in SA and phenol concentrations in the infected cells and/or tissue (Neuenschwander *et al.*, 1996). Concentrations of these compounds also increases considerably in the rest of the plant, which results in the development of broad spectrum systemic resistance (Hunt and Ryals, 1996). Previous research has revealed that SA and phenol concentrations increase in plant tissues remote to site of infection (Vernooij *et al.*, 1994; Sharma and Sain, 2005).

In view of the enormity of the yield losses caused by root-knot nematodes to tobacco, it is necessary to minimize crop damage by adopting appropriate management methods available. Application of chemical nematicides does not always prove effective and economic (Pakeerathan *et al.*, 2009). In addition, poor target specificity of chemicals pose environmental and human toxicity hazards (Barker and Koenning, 1998). Therefore, environmentally friendly alternatives are required for nematode control.

Biological control is one possible safe alternative to pesticides for disease management, and is likely to be free from toxic residual effects. There are numerous microbial antagonists of root-knot nematodes and their application results in significant decrease in the nematode populations (Khan, 2007). *Pseudomonas fluorescens* and *Trichoderma* spp. are among the most commonly used biocontrol agents (BCAs) against plant nematodes (Sikora, 1992; Khan *et al.,* 2009). In addition to the suppressive action against target pathogens, the application of these BCAs triggers or activates latent defense mechanisms in plants (Ryals *et al.,* 1996).

The present study aimed to investigate the effect of *P. fluorescens* and *T. harzianum* on the control of *M. incognita* on three tobacco cultivars. Effectiveness of these BCAs was compared with two nematicides, Phorate and Furadan. These chemicals are widely used by the Indian farmers because they are highly effective and easily available. However, these nematicides have been banned for indiscriminate use in other countries, but not ian India. Number of galls, egg mass indices, plant growth, as well as biochemical characters such as total phenol (TP), SA, and leaf pigments of tobacco cultivars were assayed to un-

derstand the response of tobacco cultivars to the treatments of nematode inoculation, BCAs and nematicides.

Materials and methods

Germplasm of tobacco

Three cultivars of tobacco (*Nicotiana tabacum* L.), RK-12 P3, RK-18 P8 and RK-26 P3, were obtained from the Central Tobacco Research Institute, Rajamundri, Andhra Pradesh, India.

Nematode inoculum

Infected root samples of eggplant were collected from pure cultures of *M. incognita*. Roots were rinsed with distilled water, thereafter females and egg masses from the galled tissue were excised. The identification of the species was confirmed by the morphology of the perineal patterns of ten females (Hartman and Sasser, 1985). Egg masses were incubated on a coarse sieve lined with two layers of tissue paper placed in a Baermann funnel filled with water, at $25\pm2°C$ for a week. The hatched second-stage juveniles (J_2) were collected every 24 h. Nematodes in the suspension were counted and standardized to 2000 J_2/10 mL suspension.

Plant cultures

Clay pots (15 cm diam.) filled with 1 kg soil and compost (3:1) were autoclaved at 15 kg/cm² for 15–20 min. Tobacco plants of each cultivar were grown in clay pots (25 cm diam.) filled with autoclaved soil. Before transplanting of tobacco seedlings, 10 mL nematode suspension containing 2000 J_2 was added to the top soil of each pot. One day later, 4-week-oldseedlings (three to four leaves) of the tobacco cultivars were planted in the pots (one seedling/pot). Ten replicates were maintained for each treatment. Plants from five pots were harvested 15 days after inoculation for biochemical analysis, and then at 4 months, plants were harvested for measurement of plant growth parameters. Two control sets, uninoculated or inoculated with nematodes, were included. The uninoculated control did not receive nematodes or nematicide or BCA and the inoculated control received only nematodes. Pots were arranged in a completely randomized design and irrigated with tap water. Plants were regularly observed for any

visible symptom attributable to the nematode. At harvest, pots were flooded with water to recover the roots, and galling, egg mass production, and soil populations of root-knot nematodes, as well as length, fresh and dry weight of plants, were determined.

Gall and egg mass indices and estimation of nematode population densities in soil

Roots were gently rinsed under a slow stream of water and gall (GI) and egg mass (EMI) indices were measured on 0–5 scales: 0, no galls/egg masses; 1, one–two; 2, three–ten; 3, 11–30; 4, 31–100; and 5, >100 galls or egg masses per root system (Taylor and Sasser, 1978).

Meloigogyne incognita final soil population was determined at harvest using Cobb's decanting and sieving method (modified) followed by Baermann's funnel technique (Khan, 2008). The nematode suspensions were examined in a counting dish with a stereomicroscope to quantify the numbers of J_2 and the population was determined per kg soil.

Biochemical parameters

Total phenol and salicylic acid content

Contents of TP and SA in a 1 g leaf sample from each of the five tobacco plants in trial pots were assayed 15 days after nematode inoculation. The samples for TP assay were homogenized in 10 mL of 80% methanol and agitated for 15 min. at 70°C (Zieslin and Ben Zaken, 1993). The leaf samples from the five plants of a treatment were processed separately in three replicates. One milliliter of methanol extract was added to 5 mL of distilled water and 250 μL of Folin-Ciocalteau reagent (1N), and the solution kept at room temperature. Absorbance 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^{-1} fresh leaf (Sharma and Sain, 2005).

For SA assay, each 1 g leaf sample was cut into small pieces of 0.5–1.0 cm and was soaked in water overnight. The 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 water. 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 data were 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 y = mx ± c (Lowry *et al.*, 1951).

Leaf pigments

To estimate chlorophyll a, chlorophyll b and total chlorophyll and carotenoids in plant leaves, 1 g of fresh leaf tissue from interveinal areas was collected from the leaves of tobacco plants 15 days after nematode inoculation. The leaf tissue from each of three plants was ground separately in 40 mL of 80% acetone using a mortar and pestle. The suspension was decanted in a Buchner funnel and passed through two Whatman No. 1 filter papers, using a suction pump. Residues were ground twice by adding acetone and the suspension was decanted and filtered twice. The filtrate was transferred to a 100 mL volumetric flask and the volume was made up to the capacity by adding acetone. Optical density (OD) of the filtrate was read in a spectrophotometer (UV 2450, Shimadzu Japan) at 470 nm for carotenoid and 645 for chlorophyll a and 663 nm for chlorophyll b. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents were calculated (Arnon, 1949; Maclachlan and Zalik, 1963).

Biocontrol agents

Pseudomonas fluorescens and *T. harzianum* were obtained from the Institute of Microbial Technology, Chandigarh, India. The bacterium was inoculated by streaking on solidified nutrient agar medium in Petri plates. Plates were incubated at 37.8±2°C for 24 h. Mass culture of *P. fluorescens* was prepared in conical flasks containing King's B medium. The flasks were each inoculated with a similar colony of the bacteria from Petri plate whose gram-negative response had been tested. Bacteria were applied to soil at 2 mL pure culture/pot (1×10^{12} cfu mL^{-1}). For soil inoculation, *T. harzianum* was mass cultured on sorghum grains (Khan *et al.*, 2010). A known weight of fungus colonized sorghum grains were

ground with distilled water in an electric grinder and standardized to 2 g seeds/10 mL (1×10^6 cfu g^{-1}).

Nematicides

Furadan (Tata Holset, India) (10 G) and Phorate (Ambuja Agrochem, India) (10G) were applied to soil at 1.6 mg a.i. pot^{-1} before the transplanting of seedlings but after nematode inoculation. The dose of nematicides was determined on the basis of commercial nematicide doses (8 kg a.i. ha^{-1}).

Statistical analysis

The experiment was conducted during two consecutive years. Data obtained during the two years were not statistically different; hence results are based on the experiment conducted during the second year. All data were subjected to analysis of variance (ANOVA) using SPSS 11.0 for Windows-XP. Least Significant Differences (LSD) and F-values were calculated at three probability levels, $P \leq 0.05$, 0.01 and 0.001. Numbers of galls and egg masses were regressed against data obtained for SA, TP, dry weight of shoots and total chlorophyll to determine any correlations between these variables.

Results

Nematode control

Tobacco cultivars inoculated with 2000 J$_2$ of *M. incognita* exhibited stunted growth with mild chlorosis of the foliage. The GI and EMI were 3.0 and 2.66 (RK-18 P8), 2.33 and 1.66 (RK-26 P3) 2.0 and 1.33 and 1.0 (RK-12 P3), respectively (Table 1). Soil application with BCA or nematicides significantly ($P \leq 0.001$) reduced galling and egg mass production compared to the inoculated control. Maximum decreases in GI and EMI were recorded with the treatment of *P. fluorescens* or Furadan in comparison to the inoculated control. Application of *T. harzianum* was also found to be effective against the nematode but less so than *P. fluorescens* or Furadan. F-values of treatment effects on GI were significant at $P \leq 0.001$ and for EMI at $P \leq 0.01$ (Table 1).

Final population density in soil

The numbers of *M. incognita* J$_2$ in soil increased in comparison to the initial population (2000 J$_2$),

in inoculated control samples. Greatest numbers of J$_2$/kg soil were recorded in control pots of tobacco cv. RK-18 P8, followed by cvs. RK-26 P3 and RK-12 P3. Treatments with BCA or nematicides suppressed the soil population of J$_2$ over the initial population ($P \leq 0.001$, Table 1). In tobacco cultivar RK-18 P8, greatest decrease in the nematode population was recorded with Furadan (350%), followed by *P. fluorescens* (103%), *T. harzianum* (99.4%) and Phorate (40.4%) in comparison to the initial population. The F-value of treatment effects on soil population was highly significant at ($P \leq 0.001$, Table 1).

Plant growth and biomass

Inoculation with the root-knot nematode *M. incognita* caused significant reductions ($P \leq 0.001$) in plant length (24–30%), dry weight of shoots (28%) and roots (24%) of tobacco cv. RK-18 P8 ($P \leq 0.001$) in comparison to the uninoculated control (Table 1). The nematode infection caused 10-36% reduction in shoot dry weight of cvs. RK-18 P8 and RK-26 P3. The cv. RK-12 P3 did not exhibit significant decreases in any of the plant parameters (length, fresh or dry weights of shoots or roots at $P \leq 0.05$). Soil treatments with Furadan or *P. fluorescens* gave the greatest recovery of the plant growth parameters from 15–33% on cv. RK-18 P8 compared with the inoculated controls (Table 1). Application of *T. harzianum* significantly increased plant length, fresh and dry weights of inoculated plants, but to a lesser extent than the *P. fluorescens* or Furadan treatments. On cultivar RK-12 P3, no effect of BCA or pesticides was recorded as the cultivar was found to be tolerant to the nematode (Table 1). The ANOVA revealed significant F-values of treatment effects on root length at $P \leq 0.05$ (Table 1).

Total phenol and salicylic acid contents of leaf

Content of TP and SA of leaves of *M. incognita* inoculated control plants increased by 17 and 9.6% (cv. RK-18 P8, $P \leq 0.01$), 17 and 15% (cv. RK-26 P3, $P \leq 0.01$), and 20 and 31% (cv. RK-12 P3, $P \leq 0.001$), in comparison to the uninoculated control (Table 2). Treatments with BCA and pesticides induced much greater increases in the TP contents in the tobacco cultivars RK-18 P8, 20–34%, RK-26 P3, 48–71% and 6–12% and RK-12 P3, 57–78% in comparison to the inoculated control (Table 2). Treat-

Table 1. Effects of biocontrol agents (*Trichoderma harzianum* and *Pseudomonas flourescens*) and nematicides (Furadan and Phorate) on gall (GI) and egg mass (EMI) indices , final soil population and plant growth parameters of three tobacco cultivars inoculated with *Meloidogyne incognita* (2000 J_2 kg^{-1} soil). Each value is the mean of three replicates.

Tobacco cultivar	Treatment	GI (0–5)	EMI (0–5)	Soil population (J_2 kg^{-1})	Plant length (cm)[a]		Fresh weight (g) [a]		Dry weight (g) [a]	
					Shoot	Root	Shoot	Root	Shoot	Root
RK-18 P8	Uninoculated control	0	0	0	60.83	20.02	57.53	16.61	11.11	3.42
	Inoculated control	3	2.66	5814	45.7[***]	14.3 [***]	36.5 [***]	12.8 [***]	8.02[***]	2.61[***]
	T. harzianum	2	1.66	1003	53.9 [a]	18.59 [c]	42.79 [a]	15.95 [c]	10.12 [b]	3.01 [b]
	P. fluorescens	1.33	1	0985	54.12 [a]	18.92 [c]	43.23 [a]	16.17 [c]	10.23 [b]	3.03 [b]
	Furadan	1	1	445	55.55 [b]	19.8 [c]	46.09 [b]	15.73 [c]	10.56 [b]	2.97 [b]
	Phorate	2.33	2	1425	52.58 [a]	17.82 [c]	41.36 [a]	15.52 [c]	9.57 [a]	2.94 [b]
RK-26 P3	Uninoculated control	0	0	0	32.1	18.1	30.41	15.3	5.5	2.2
	Inoculated control	2.33	1.66	4354	26.6 [*]	15.62[***]	25.8 [*]	13.2 [***]	4.31[*]	1.91[*]
	T. harzianum	1.66	1.33	927	33.42	17.41[c]	29.41	14.72[b]	5.22	2.1
	P. fluorescens	1.33	1	875	34.38 [a]	17.8 [b]	29.89	14.9 [a]	5.8	2.01
	Furadan	1	0.66	346	31.32	17.29 [b]	29.1	15.1 [b]	5.33	2.02
	Phorate	2	1.66	716	30.25	16.9 [b]	28.21	14.61 [a]	5.1	1.95
RK-12 P3	Uninoculated control	0	0	0	48.3	16.4	49.8	13.2	10.3	2.8
	Inoculated control	1.33	1	2213	45.3	15.9	48	12.8	10	2.6
	T. harzianum	1	0.33	562	48.62	17	50	12.95	10.08	2.7
	P. fluorescens	0.66	0	540	48.66	17.1	50.26	13	10.09	2.72
	Furadan	0.66	0	207	47.52	16.35	49.3	12.88	10.1	2.68
	Phorate	1	0.66	479	45.58	16.30	48.4	13.11	10.05	2.48
LSD	$P \leq 0.05$	0.258	0.292	320.8	5.49	0.638	4.58	0.658	1.15	0.21
	$P \leq 0.01$	0.405	0.458	501.8	8.61	1	7.18	1.03	1.8	0.33
	$P \leq 0.001$	0.689	0.78	854.2	14.65	1.71	12.23	1.76	3.06	0.56
F-value	Treatment	18.058 [z]	9.785 [y]	43.362 [z]	NS	5.641[x]	NS	NS	NS	NS

[*] Values followed by the superscripts [*] ($P \leq 0.05$), [**] ($P \leq 0.01$) and [***] ($P \leq 0.001$) are significantly different from the control.
[a] Significantly different from the respective inoculated control ($P \leq 0.05$), [b] ($P \leq 0.01$) and [c] ($P \leq 0.001$).
[x] F-values followed by [x] are significant for ($P \leq 0.05$), [y] ($P \leq 0.01$) and [z] ($P \leq 0.001$), otherwise not significant (NS) at $P \leq 0.05$.

ments with *T. harzianum*, *P. fluorescens*, Furadan and Phorate resulted to 4.4, 4.9, 9.2 and 9% increases in SA contents of tobacco cv. RK-18 P8; 10.7, 11.6, 10 and 6% in cv. RK-26 P3, and 20, 20.3, 11 and 10% in cv. RK-12 P3 over control, respectively. F-values for treatment effects on SA content were significant at $P \leq 0.001$. A similar trend was

observed for TP content (Table 2). A negative correlation was found between galls or egg masses and TP or SA contents (Figure 1), indicating that increases in TP and SA was low in the cultivars that developed greater numbers of galls and egg masses.

Table 2. Effects of biocontrol agents and nematicides on leaf total phenol (TP), salicylic acid (SA), chlorophyll and carotenoids contents of tobacco cultivars inoculated with *Meloidogyne incognita* (2000 J_2/kg soil). Each value is the mean of three replicates.

Tobacco cultivar	Treatment	TP (µg catechol g^{-1} FW)	SA (ppm g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
RK-18 P8	Uninoculated control	86.5	13.55	1.028	0.999	2.027	0.134
	Inoculated control	101.5[**]	14.86[*]	0.897[**]	0.894	1.791[**]	0.125
	T. harzianum	121.5[c]	15.52	0.978[a]	0.958	1.936[a]	0.131
	P. fluorescens	136.5[c]	15.58	0.998[a]	0.899	1.897	0.132
	Furadan	131.5[c]	16.24[a]	0.972	0.953	1.925	0.129
	Phorate	126.5[c]	16.00[a]	0.957	0.941	1.898	0.128
RK-26 P3	Uninoculated control	91.5	14.20	1.002	0.889	1.891	0.128
	Inoculated control	106.5[**]	16.33[**]	0.894[**]	0.810	1.704[*]	0.118
	T. harzianum	131.5[c]	18.08[b]	0.957	0.871	1.828	0.123
	P. fluorescens	146.5[c]	18.24[b]	0.986[a]	0.874	1.860[a]	0.125
	Furadan	156.5[c]	17.93[a]	0.954	0.858	1.812	0.121
	Phorate	151.5[c]	17.31[a]	0.943	0.845	1.788	0.119
RK-12 P3	Uninoculated control	96.5	14.04	0.888	0.822	1.710	0.123
	Inoculated control	126.5[***]	16.84[***]	0.851	0.802	1.653	0.119
	T. harzianum	166.5[c]	20.21[c]	0.904	0.845	1.749[a]	0.122
	P. fluorescens	171.5[c]	20.27[c]	0.889	0.856	1.745[a]	0.123
	Furadan	161.5[c]	18.84[c]	0.879	0.812	1.691	0.121
	Phorate	156.5[c]	18.51[c]	0.876	0.808	1.684	0.120
LSD	$P \leq 0.05$	9.535	1.09	0.079	0.144	0.141	0.0153
	$P \leq 0.01$	13.071	1.66	0.109	0.198	0.193	0.0210
	$P \leq 0.001$	17.791	2.30	0.148	0.270	0.263	0.0286
F-value	Treatment	39.95[z]	36.81[z]	5.80[x]	NS	7.99[y]	NS

*Values followed by the superscripts [*] ($P \leq 0.05$), [**] ($P \leq 0.01$) and [***] ($P \leq 0.001$) are significantly different from the control.

[a] Significantly different from the respective inoculated control ($P \leq 0.05$), [b] ($P \leq 0.01$) and [c] ($P \leq 0.001$).

[x] F-values followed by [x] are significant for ($P \leq 0.05$), [y] ($P \leq 0.01$) and [z] ($P \leq 0.001$), otherwise not significant (NS) at $P \leq 0.05$.

Chlorophyll and carotenoid contents of leaves

Total chlorophyll content of leaves decreased in tobacco cvs. RK-18 P8 by 11.6% ($P \leq 0.01$), RK-26 P3 by 10% ($P \leq 0.05$) and in cv. RK-12 P3 by 3% (Table 2). Reduction in chlorophylls a and b in tobacco cultivars varied from 2.4–12.4%. Decreases in the carotenoid contents were sig-nificant ($P \leq 0.05$) for cv. RK-26 P3 (7.8%) over uninoculated controls (Table 2). Application of BCA and nematicides suppressed the negative effect of the nematode on leaf pigments result-ing in increased pigment contents. Among the two BCA, greater increases in the chlorophyll (6–9%) and caroteniods (4–5%, $P \leq 0.05$) were
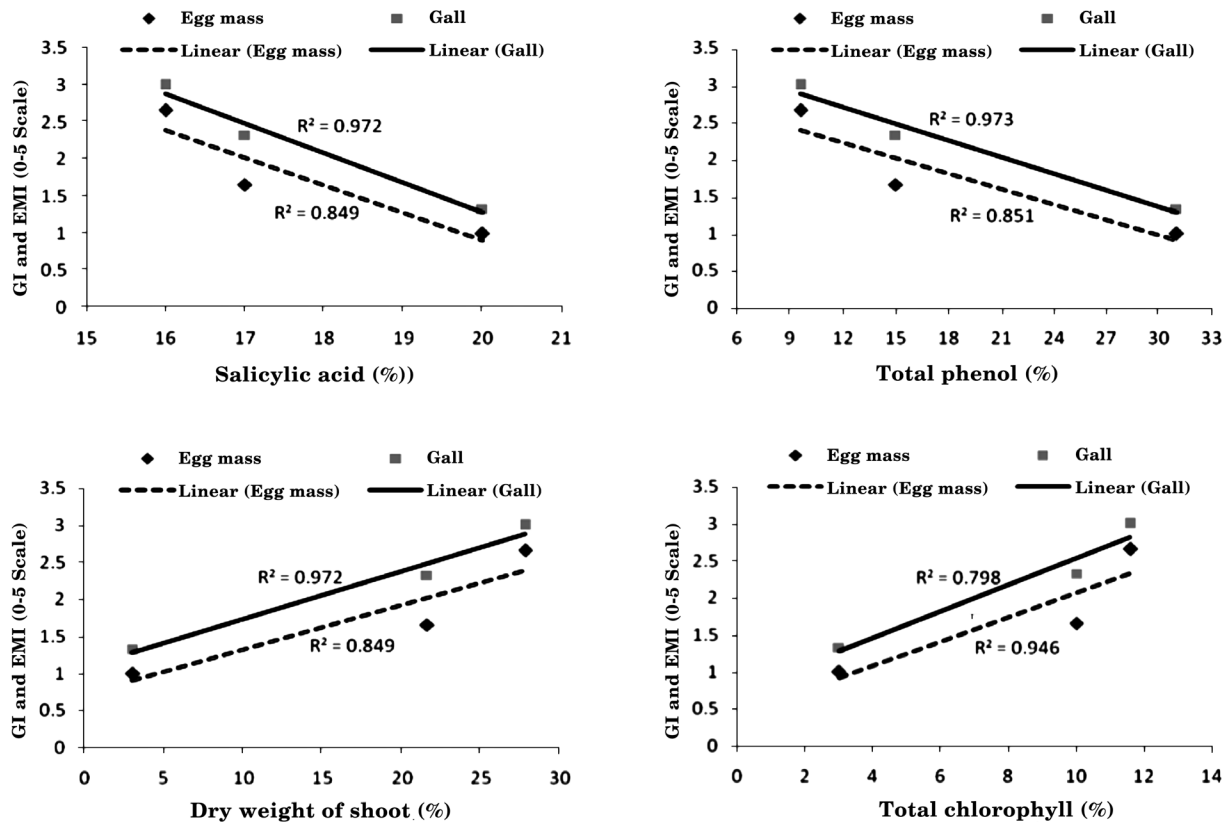
Figure 1. Correlations between gall (GI) and egg mass (EMI) indices with phenol, salicylic acid, total chlorophyll contents and dry weights of shoots of tobacco cultivars inoculated with *Meloidogyne incognita* (2000 J_2 pot^{-1}).

recorded in cvs. RK-18 P8 and RK-26 P3 compared with the controls. An increase of 4–12% (Furadan, $P \leq 0.01$) and 4–10% (Phorate, $P \leq 0.05$) in the leaf pigments was recorded with nematicide treatments. Overall effects of the various treatments were significant for total chlorophyll ($P \leq 0.01$) and chlorophyll a ($P \leq 0.05$), but not for chlorophyll b or carotenoids (Table 2).

Discussion and conclusions

Root-knot nematodes are important pests of solanaceous crops, reducing plant growth and yield, as observed in the present study and previously (Sasser, 1989). Shepherd and Barker (1990) have reported 50–60% decrease in the leaf yield of tobacco due to root-knot nematode infestation.

Gall formation and egg mass production had

a direct impact on plant growth of tobacco (Figure 1). Cultivars which developed greater galls and egg masses exhibited greater reduction in the dry weight of shoots. Development of numerous discrete galls on cvs. RK-18 P8 and RK-26 P3 confirm previous reports (Charles *et al.*, 2005). However, few galls developed on the cv. RK-12 P3. Varied reactions (highly resistant, resistant, moderately susceptible and susceptible) of tobacco germplasm to root-knot nematodes has been evaluated previously (Nian *et al.*, 2000).

The results of the present study showed that application of BCA or nematicides significantly controlled the root-knot nematode *M. incognita*. Application of *P. fluorescens* decreased GI from 3.0 to 1.33 and improved plant growth of two susceptible tobacco cultivars by up to 32%. *Trichoderma harzianum* suppressed GI from 3 to 2. Similar effects of *P. fluorescens* and *Tricho-*

derma spp. on different crops against *Meloidogyne* spp. have been reported (Hamid *et al*., 2003; Khan *et al*., 2005; Khan, 2007; Khan *et al*., 2007). The bacterium is a phosphate solubilizer (Khan *et al*., 2009) but may also suppress pathogens through antibiosis (Nielson *et al*., 1998), siedorphore production (Glick, 1995), induced systemic resistance (Kloepper *et al*., 1992), production of phytohormones (Garcia de Salamone *et al*., 2001) or other compounds (Marek-kazaczuk and Skorupska, 2001). Application *T. harzianum, T. hamatum* or *T. virens* has also demonstrated potential to suppress root-knot nematodes (Siddiqui and Shaukat, 2004). Our study suggests that there is considerable potential for exploiting *P. fluorescens* and *T. harzianum* for the management of root-knot nematodes in tobacco. However, the greatest suppression of galling and nematode soil population was recorded with the application of Furadan, and yield enhancement was greater with *P. fluorescens* than Furadan. This nematicide is reported to be highly effective against endoparasitic nematodes as the chemical is systemic in action (Hague and Gowen, 1987). However, use of carbofuran has been banned in many countries, because it possesses very high residual toxic effects to humans and other vertebrate, especially birds (Wobeser *et al*., 2004)

Chlorophylls are basic requirements for plant photosynthesis as these pigments absorb light for CO_2 fixation (Wallace, 1987). In the present study mild yellowing of leaves was observed on susceptible cultivars and this became gradually pronounced. At 3–4 month age, entire foliage of susceptible cultivars, especially RK18-P8, became discernibly yellow, whereas the foliage of the tolerant cultivar, RK-12 P3, remained normal. Our results revealed that the chlorophyll pigments are highly sensitive to alternation in host physiology induced by *M. incognita*. Infected plants develop water stress due to damage to roots and development of galls (Willcox-Lee and Lorea, 1987). Chlorophyll and carotenoid molecules are highly sensitive and become denatured in water stress conditions (Khan and Khan, 1987). Consequently contents of pigments are reduced, and subsequently photosynthetic activity of tobacco plants decreased, which was reflected in lower biomass production.

The content of TP in leaves in nematode inoculated plants of all three cultivars increased due to inoculation, but varied greatly. The least increase in TP was recorded in cv. RK-18 P8 which was found highly susceptible to the nematode, whereas TP contents werehigher in cv. RK-12 P3, which expressed tolerance against *M. incognita*. This indicates that the phenolic compounds contributed to the chemical defense of plants against nematode attack (Nicholson and Hammerschmidt, 1992; Hammond-Kosack and Jones, 1996). Salicylic acid 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; Moret and Munaz, 2007). In the present study, greatest concentration of SA was recorded in the cv. RK-12 P3 which did not show galling and plant growth reduction, whereas the smallest SA increase occurred in cvs. RK-18 P8 and RK-26 P3 which showed susceptibility to *M. incognita*. Ganguly *et al*. (1998) and Nandi *et al*. (2000) have demonstrated the activation of the SAR by SA and its negative correlation with galling caused by the *M. incognita*. Moreover, higher concentration of SA and phenol in BCA treated tobacco plants irrespective of nematode inoculation indicates that the BCA induced or enhanced systemic resistance in the plants through greater synthesis of SA and/or TP. It has been reported elsewhere that application of *P. fluorescens* (Zhou and Paulitz, 1994; Zehnder *et al*., 2001; Pieterse *et al*., 2002) and *T. harzianum* (Sharma and Sain, 2005) triggered and activated latent defense mechanisms in plants in response to infection by pathogens. However, a limited number of investigations have been conducted on the activation of TP and SA in nematode infected plants due to BCA.

Our study has demonstrated that the galls caused by *M. incognita* in tobacco can be effectively controlled by the application of *P. fluorescens* or Furadan. The bacterium also acted as a plant growth promoter and induced resistance by increasing plant phenol and SA contents. The cv. RK-12 P3 which showed highest increase of phenol and SA and considerable degree of tolerance to *M. incognita*. Our study suggests that this cultivar has considerable potential to combat nematode problems. Furthermore, the use of P. fluore-

scens has considerable potential for biocontrol of *M. incognita in tobacco*.

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