This article was downloaded by: [Aligarh Muslim University]
On: 01 November 2011, At: 03:53
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Archives Of Phytopathology And Plant Protection
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gapp20

Morphological and biochemical responses of selected germplasm of tobacco to soil inoculation with Pythium aphanidermatum

Mujeebur Rahman Khan [a], Ziaul Haque [a], M. Arshad Anwer [a] & M. Mahmud Khan [a]

[a] Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, 202002, India

Available online: 31 Oct 2011

To cite this article: Mujeebur Rahman Khan, Ziaul Haque, M. Arshad Anwer & M. Mahmud Khan (2011): Morphological and biochemical responses of selected germplasm of tobacco to soil inoculation with Pythium aphanidermatum , Archives Of Phytopathology And Plant Protection, DOI:10.1080/03235408.2010.490403

To link to this article: http://dx.doi.org/10.1080/03235408.2010.490403

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings,

demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

Archives of Phytopathology and Plant Protection
i*First* article 2011, 1–11

Taylor & Francis
Taylor & Francis Group

Morphological and biochemical responses of selected germplasm of tobacco to soil inoculation with *Pythium aphanidermatum*

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

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

(*Received 5 January 2010; final version received 10 April 2010*)

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

The effect of inoculation with *Pythium aphanidermatum* was studied on total phenol (TP), salicylic acid (SA), chlorophylls and carotenoid contents of leaves and plant growth characteristics of five tobacco cultivars, namely RK-10 P3, RK-12 P3, RK-13 P4, RK-18 P8 and RK-26 P3, to assess cultivar response at biochemical and morphological levels. Root rot measured at 0–5 scale was 2.66 on cv. RK-10 P3, followed by 2. 33 on cv. RK-18 P8, 1.33 on cv. RK-26 P3 and 1.0 on cv. RK-13 P4. The cv. RK-12 P3 did not develop measurable root rot. The rhizosphere population of root rot fungus increased over time, being highest on the cv. RK-10 P3 ($P \leq 0.001$), followed by cvs. RK-18 P8, RK-26 P3, RK-13 P4 and RK-12 P3. Inoculation with the fungus resulted in 5% (cv. RK-10 P3), 10.3% (cv. RK-18 P8, $P \leq 0.05$), 10.9% (cv. RK-26 P3, $P \leq 0.05$), 16.4% (cv. RK-13 P4, $P \leq 0.01$) and 41.5% (cv. RK-12 P3, $P \leq 0.001$) increase in the TP content of leaves. SA concentration in tobacco leaves increased marginally (0.8%–3%) in cvs. RK-10 P3, RK-18 P8 and RK-26 P3, but considerably (16%–17%, $P \leq 0.01$) in cv. RK-13 and RK-12 P3 in comparison to uninoculated plants. Total chlorophyll content of leaves in response to inoculation with *P. aphanidermatum* decreased by 27% and 23% in tobacco cvs. RK-10 P3 and RK-18 P8 ($P \leq 0.001$) and 17.6 ($P \leq 0.01$) and 10.6% ($P \leq 0.05$) in cv. RK-26 P3 and RK-13 P4, respectively. Reduction in chlorophylls a and b was 20% and 15% in cv. RK-10 P3 and 20% and 11% in cv. RK-18 P8. Total carotenoid contents of tobacco leaves decreased significantly in cvs. RK-10 P3 and RK-18 P8 ($P \leq 0.05$). 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. This study has revealed that greater synthesis of TP and SA may provide resistance in tobacco plants against *P. aphanidermatum*. The cv. RK-12 P3, in which greatest increase in the SA (17%) and TP (41.5%) was recorded, did not exhibit a significant decrease in plant growth variables and leaf pigments ($P \leq 0.05$).

Keywords: root rot; salicylic acid; total phenol; chlorophylls; carotenoids; tobacco; plant dry weight

Introduction

Tobacco is a high-value nonfood cash crop and is widely grown throughout the world including India (Shepherd and Barker 1990). The crop is regularly attacked by pathogens from seed sowing until the time it is fashioned in to tobacco products.

*Corresponding author. Email: mrkhan777in@yahoo.co.in

ISSN 0323-5408 print/ISSN 1477-2906 online
© 2011 Taylor & Francis
http://dx.doi.org/10.1080/03235408.2010.490403
http://www.tandfonline.com

Pythium aphanidermatum is an important pathogen of tobacco throughout the world and causes considerable damage to the crop (Martin and Loper 1999). The *Pythium* infection takes on many different forms in tobacco. The fungus causes damping off in nursery beds and root rot in the fields, especially during prolonged wet conditions and in poorly aerated soils (Chase 1999). The infected plants stop growing, and the leaves fade to a pale yellow (Lucos 1975; William 2003). 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 micro-organism, one or more defence mechanisms can be triggered to restrict the growth of the pathogen, and ultimately, it is destroyed (Dixon et al. 1994). Phenolic compounds and salicylic acid (SA) are among the most influential and widely distributed secondary products in the plant body and lay a completely different action in the defence of plants against pathogen attack (Nicholson and Hammerschmidt 1992; Takahama and Oniki 1992). In general, these compounds, namely phenolic acids and SA, 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 hypersensitive response, which results in necrosis of both plant cell and pathogen, thus preventing further proliferation of the pathogens (Staskawicz et al. 1992). However, the severity of infection and the defence mechanism vary with the cultivar. In view of the essential roles of phenolic compounds and SA in the development of pathogen resistance in plants, the present study was undertaken to evaluate the response of some tobacco cultivars to *P. aphanidermatum* with regard to biochemical parameters, namely total phenol (TP), SA, chlorophylls and carotenoid content, and morphological characteristics, namely root rot index, soil population, plant growth and biomass.

Materials and methods

Germplasm of tobacco

The germplasm of tobacco *(Nicotianum tobaccum* L.) consisting of five cultivars, namely RK-10 P3, RK-12 P3, RK-13 P4, RK-18 P8 and RK-26 P3, was procured from the Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh, India.

Preparation of fungal inoculum

Pure culture of *P. aphanidermatum* (Edson) Fitzg. was procured from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi. Inoculum of the fungus was prepared on sorghum grains. For inoculation, a known weight of fungus-colonised sorghum seeds were grinded with distilled water in an electric grinder and standardised to 2 g seeds/10 ml.

Plant culture

Clay pots (15 cm in diameter) filled with 1 kg of soil and compost (in the ratio 3:1) were autoclaved at 15 kg/cm^2 for 15–20 min. Before planting of tobacco seedling, 10 ml of fungal suspension (containing 2 g of colonised seeds/pot) was added to the top soil of the pot. A day later, three to four leaved seedlings (4-week old) of tobacco cultivars were transplanted in pots (1 seedling/pot). Ten replicates were maintained

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

for each treatment; plants from five pots were used for biochemical analysis and remaining five for plant growth variables. Plants were arranged in a completely randomised design on a roof receiving uniform sunlight. Plants were grown for 4 months. During this period, they were regularly observed for any visible symptom attributable to the pathogen. At harvest, four months after inoculation, plants were flooded with water to facilitate root recovery, and root rot (0–5 scale), rhizosphere population of the fungus, length and fresh and dry weight of plants (excluding root) were determined. The dry weights were determined by drying the plants in a hot air oven at 60°C for 2 days. Estimation of leaf pigments, TP contents and SA was done after 15 days of inoculation from another set of pots.

Root rot symptoms

At harvest, the roots of tobacco cultivars inoculated with *P. aphanidermatum* or the plants not inoculated with the fungus were also examined for root rot symptoms (brown to black rotting and decaying of root), which were measured on 0–5 scale: 0 = no rotting, 1 = 1–10% rotting, 2 = 11–25% rotting, 3 = 26–60% rotting, 4 = 61–80% rotting and 5 = 80–100% rotting.

Soil population of P. aphanidermatum

Soil population, in terms of colony forming units (CFU), of *P. aphanidermatum*/g soil was determined at harvest by dilution plate method. The suspension from 10^{-4} dilution was spread over potato dextrose agar in a Petri plate under laminar flow. Three plates were maintained for each treatment. The plates were incubated at 25°C $\pm$ 2°C for 72 hours in an incubator, and thereafter were examined under a colony counter to count the colonies of the fungus.

Estimation of TP

Fifteen days after inoculation, leaf samples (1 g) were collected from tobacco plants. The samples were homogenised in 10 ml of 80% methanol and agitated for 15 min at 70°C (Zieslin and Ben Zaken 1993). Leaf samples from the five plants were processed separately and served as five replicates. The methanol extract (1 ml) was added to 5 ml of distilled water, and 250 μl of Folin–Ciocalteau's reagent (1N) and solution was kept at 25°C. The absorbance of the blue colour developed was measured at 725 nm in a spectrophotometer (Spectronic 20, USA). TP was expressed as μg catechol/g fresh leaf (FL) (Sharma and Sain 2005).

Estimation of SA

SA present in tobacco leaves was estimated 15 days after inoculation. The leaves (1 g) were collected from the plants 15 days after inoculation and were cut into small pieces of 0.5–1.0 cm. The pieces were soaked in water overnight, filtered through the Whatman No. 1 filter paper and extracted in ethyl acetate. The ethyl acetate fraction was taken, and sodium sulphate was added to remove the moisture; the obtained filtrate was evaporated to dryness in a water bath. Methanol (10 ml) was added to the dried sample. Thereafter, the solution was used for recording the absorbance at 306 nm in a spectrophotometer (Shimadzu, 2450 PC; Japan) (Shane and

Kowblansky 1968). Standard curve of SA was prepared by making SA solution of different concentrations in methanol (0, 10, 20, 30, 40, 50 and 100 ppm). Absorbance at 306 nm of different concentrations of SA (ppm) was read in the spectrophotometer. The standard curve was prepared by plotting the absorbance data, 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 $y = mx \pm c$ (Lowery et al. 1951).

Estimation of leaf pigments

Chlorophyll a, chlorophyll b and total chlorophyll were estimated by grinding 1 g of fresh leaves from interveinal areas of 15-day 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 Whatman No.1 filter papers. The filtration was done with the help of suction pump. The residue was ground thrice by adding acetone. The suspension was then decanted in the Buchner funnel and filtered by a vacuum pump. At last, mortar and pestle were rinsed with acetone, and the solution was transferred in a Buchner funnel and filtered. The filtrate was transferred to a 100 ml volumetric flask, and the volume was made up to the capacity by adding acetone. The optical density of the filtrate was read using a spectrophotometer (Spectronic 20, USA) at 470 nm for carotenoid and 645 and 663 nm for chlorophylls. Chlorophyll a, chlorophyll b, total chlorophyll (Arnon 1949) and carotenoid contents (Maclachlan and Zalik 1963) were calculated using the following formula Chlorophyll a (μg/ml) = 12.7 (A663) – 2.69 (A645) Chlorophyll b (μg/ml) = 22.9 (A645) – 4.68 (A663) Total chl (μg/ml) = 17.76 (A645) + 7.34 (A663) Carotenoids (μg/ml) = 7.6 (A480) – 1.49 (A510)/ 227 where A is absorbance.

Statistical analysis

The experiment was conducted during two consecutive years. The data obtained during the two years were statistically identical; hence, the results are based on the experiment conducted during the second year because during second year, experiments were performed with greater preciseness and perfection gained due to the experience of the first year. All data were subjected to analysis of variance (ANOVA) using MINITAB 7.0 software for Windows XP. 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. Root rot indices were regressed over selected morphological and biochemical characteristics of tobacco cultivars, and correlation coefficients were calculated.

Results

Symptoms of root rot

Tobacco plants inoculated with 2 g culture of *P. aphanidermatum* exhibited stunted growth and mild yellowing of foliage. Characteristic root rots developed, and the affected root or part turned dark brown to black. The root rot measured on 0–5 scale was 2.66 on cv. RK-10 P3, followed by 2. 33 on cv. RK-18 P8, 1.33 on cv. RK-26 P3 and 1.0 on cv. RK-13 P4. The cv. RK-12 P3 did not develop measurable root rot (Table 1). F-value for root rot index was significant at $P \leq 0.01$ (Table 1).

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

Table 1. Effects of inoculation with *P. aphanidermatum* on length, fresh weight, dry weight of shoot and root of tobacco cultivars and on root-rot and soil population of the fungus.

Tobacco cultivars	Inoculation level (g/pot)	Root rot index (0–5 scale)	Soil population (10^3CFU/kg)	Plant length (cm)		Fresh weight (g)		Dry weight (g)	
				Shoot	Root	Shoot	Root	Shoot	Root
RK-10 P3	0.0	0.00	0.00	55.3	18.1	58.5	15.0	10.0	3.15
	2.0	2.66	5600	50.6**	16.4***	49.0**	14.1**	8.9**	2.50***
RK-18 P8	0.0	0.00	0.00	60.8	23.9	52.3	16.1	10.7	2.24
	2.0	2.33	5400	56.5*	21.6**	44.7**	15.4*	9.9*	2.05*
RK-26 P3	0.0	0.00	0.00	32.1	18.1	32.4	20.9	5.5	3.45
	2.0	1.33	2264	29.5*	16.5*	27.8**	19.7	5.1	3.25
RK-13 P4	0.0	0.00	0.00	41.4	20.0	43.9	15.3	8.2	2.60
	2.0	1.00	2018	38.9	17.9*	39.4*	14.8	7.9	2.45
RK-12 P3	0.0	0.00	0.00	46.8	16.2	49.4	13.0	10.3	2.80
	2.0	0.33	63	46.3	16.1	49.0	13.0	10.2	2.75
LSD	$P \leq 0.05$	0.876	0.595	6.700	2.829	4.599	1.282	0.505	0.348
	$P \leq 0.01$	1.278	0.868	9.189	3.879	6.307	1.758	0.693	0.477
	$P \leq 0.001$	1.916	1.302	12.508	5.281	5.585	2.394	0.943	0.649
F-value	cvs. ($df = 4$)	12.77^{b}	181.31^{c}	46.77^{c}	18.62^{c}	70.20^{c}	82.07^{c}	54.4^{c}	27.69^{c}
	Fungus ($df = 1$)	–	–	NS	6.31^{a}	NS	30.44^{b}	4.74^{a}	12.09^{b}
	cvs. × fungus ($df = 4$)	–	–	NS	NS	NS	2.97^{a}	NS	NS

Note: Each value is the mean of three replicates. Values followed by * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) are significantly different from the control (uninoculated), otherwise not significant (NS) at $P \leq 0.05$. *F*-values followed by ^{a}($P \leq 0.05$), ^{b}($P \leq 0.01$) and ^{c}($P \leq 0.001$) are significant, otherwise NS at $P \leq 0.05$.

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

Rhizosphere population

Rhizosphere population of *P. aphanidermatum* was monitored at harvest, four months after inoculation. The population of root rot fungus increased over time, highest population was recorded in the root zone of tobacco cv. RK-10 P3, followed by cvs. RK-18 P8, RK-26 P3, RK-13 P4 and RK-12 P3 (Table 1). *F*-values for rhizosphere population was highly significant at $P \leq 0.001$ (Table 1).

Plant growth and biomass

Inoculation with the root rot fungus *P. aphanidermatum* resulted in significant suppression in the length, fresh weight and dry weight of root and shoot of tobacco cvs. RK-10 P3 ($P \leq 0.01$ and $P \leq 0.001$) and RK-18 P8 ($P \leq 0.05$ and $P \leq 0.01$) in comparison to controls (Table 1). The per cent decrease in the variables considered was 11–20% and 7–15% in cvs. RK-10 P3 and RK-18 P8, respectively. Significant reduction in shoot and root length and fresh weight of shoot in the cv. RK-26 P3 was recorded at $P \leq 0.05$ over the control. The root length and fresh weight of shoot of the cv. RK-13 P4 also decreased ($P \leq 0.05$). The cv. RK-12 P3 did not exhibit significant decrease in any plant growth and biomass variable at $P \leq 0.05$. ANOVA has revealed significant *F*-values of length, fresh and dry weight of shoot and root for cultivars at $P \leq 0.001$, where *F*-value of fungus was significant for root variables ($P \leq 0.05$ or 0.01) and shoot dry weight ($P \leq 0.05$). *F*-value for interactive effects was significant only for fresh root weight ($P \leq 0.05$, Table 1).

TP and SA contents of leaf

TP content of leaves of *P. aphanidermatum*-inoculated plants increased by 5% (cv. RK-10 P3), 10.3% (cv. RK-18 P8, $P \leq 0.05$), 10.9% (cv. RK-26 P3, $P \leq 0.05$), 16.4% (cv. RK-13 P4, $P \leq 0.01$) and 41.5% (cv. RK-12 P3, $P \leq 0.001$) in comparison to controls (Table 2). Due to inoculation with *P. aphanidermatum*, SA content in tobacco leaves increased marginally (0.8%–3%) in cvs. RK-10 P3, RK-18 P8 and RK-26 P3, but considerably (16%–17%, $P \leq 0.01$) in cvs. RK-13 and RK-12 P3 over uninoculated plants (controls) ($P \leq 0.01$, Table 2). *F*-values of all three sources for SA were significant at $P \leq 0.001$, but the value was highest for fungus, followed by cultivars, and lowest for the interaction (Table 2). Same trend was observed for TP, with significant *F*-value of interaction at $P \leq 0.05$.

Chlorophylls and carotenoid contents of leaves

Total chlorophyll content of leaves in response to inoculation with *P. aphanidermatum* decreased by 27% and 23% in tobacco cvs. RK-10 P3 and RK-18 P8 over uninoculated plants, respectively ($P \leq 0.001$). Reduction in chlorophylls a and b was 20% and 15% for cv. RK-10 P3 and 20% and 11% for cv. RK-18 P8 (Table 2). In cvs. RK-26 P3 and RK-13 P4, the total chlorophyll reduced by 17.6% ($P \leq 0.01$) and 10.6% ($P \leq 0.05$), respectively, whereas in cv. RK-12 P3, a significant reduction was not recorded at $P \leq 0.05$ (Table 2). The carotenoid contents of tobacco leaves of all five cultivars decreased in a way similar to total chlorophyll (Table 2). Highest decrease in carotenoids was observed in cv. RK-10 P3 (9.7%, $P \leq 0.05$), followed by cvs. RK-18 P8 (8.6%, $P \leq 0.05$), RK-26 P3 (6.2%), RK-13 P4 (4.5%) and RK-12 P3

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

Table 2. Effects of inoculation with *P. aphanidermatum* on the leaf pigments, SA, TP per g FL of tobacco cultivars.

Tobacco cultivars	Inoculation level (g/pot)	TP (μg catechol g^{-1} FL)	SA (ppm/g FL)	Chlorophyll a (mg/g FL)	Chlorophyll b (mg/g FL)	Total chlorophyll (mg/g FL)	Carotenoids (mg/g FL)
RK-10 P3	0.0	76.5	13.46	1.002	0.989	1.991	0.134
	2.0	81.5	13.57	0.798***	0.840***	1.461***	0.121*
RK-18 P8	0.0	96.5	13.55	1.041	0.554	1.594	0.139
	2.0	106.5*	13.74	0.834***	0.493*	1.227***	0.127*
RK-26 P3	0.0	91.5	14.20	1.028	0.999	2.027	0.128
	2.0	101.5*	14.94	0.869*	0.802**	1.669*	0.120
RK-13 P4	0.0	91.5	13.79	0.799	0.731	1.530	0.131
	2.0	106.5**	15.90**	0.717*	0.658*	1.376	0.125
RK-12 P3	0.0	96.5	14.44	0.895	0.802	1.710	0.123
	2.0	136.5***	16.93**	0.874	0.794	1.640	0.122
LSD	$P \leq 0.05$	9.0869	1.0893	0.1067	0.1446	0.1549	0.0153
	$P \leq 0.01$	12.4620	1.6683	0.1464	0.1983	0.1225	0.0210
	$P \leq 0.001$	16.9622	2.3068	0.1993	0.2700	0.2892	0.0286
F-value	cvs. ($df = 4$)	22.18^{c}	227.23^{c}	5.27^{b}	NS	28.09^{c}	NS
	Fungus ($df = 1$)	42.70^{c}	419.56^{c}	15.43^{b}	NS	103.24^{c}	6.00^{a}
	cvs. × fungus ($df = 4$)	5.94^{b}	79.58^{c}	NS	NS	3.42^{a}	NS

Note: Each value is the mean of three replicates. Values followed by * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) are significantly different from the control (uninoculated), otherwise not significant at $P \leq 0.05$. *F*-values followed by ^{a} ($P \leq 0.05$), ^{b} ($P \leq 0.01$) and ^{c} ($P \leq 0.001$) are significant, otherwise not significant (NS) at $P \leq 0.05$.

(0.8%). Overall effect of the fungus inoculation and cultivars was significant for total chlorophyll ($P \leq 0.001$) and chlorophyll a ($P \leq 0.01$); effect of fungus was also significant for carotenoids ($P \leq 0.05$). Interactive effect was significant for only total chlorophyll ($P \leq 0.05$, Table 2).

Discussions

The tobacco cultivars inoculated with 2 g culture of *P. aphanidermatum* exhibited stunted growth, mild yellowing of leaves and rotting of roots. The blackened discolouration of roots observed in tobacco cvs. RK-10 P3, RK-18 P8, RK-26 P3 and RK-13 P4, in particular, due to infection with *P. aphanidermatum* has also been observed on other tobacco cultivars (Ahmed et al. 2008). Roots of susceptible cultivars turn dark brown (Oluma and Oladiran 1993; William 2003). Prominent root rot developed on the cvs. RK-10 P3 and RK-18 P8 has indicated their susceptibility to the pathogen. *Pythium* species are soil inhabitants, where they subsist as saprophyte or facultative parasite on roots or decaying organic matters (Lucos 1975). *P. aphanidermatum* multiplies rapidly in soil, especially in the root zone of susceptible plants. Root exudates of actively growing susceptible plants stimulate germination of oospores and sporangia (Stanghellini and Hancock 1971). Highest soil population of the fungus was recorded on the cv. RK-10 P3, which exhibited that greatest root rot was apparently due to the stimulatory effects of the root exudates of the cultivars.

The fungus infection resulted in significant suppression of the plant growth and dry matter production of tobacco cvs. RK-10 P3 and RK-18 P8. Within a few days of inoculation, the affected roots, parts, tissues or all become water soaked, lose cohesion and frequently develop a wet rot due to the action of pectolytic and cellulolytic enzymes secreted by the fungus (Chase 1999). The rotting disintegrates the internal tissues of the main root and impairs the ability of roots to absorb water and minerals, as a result, progressive decline of the aerial growth occurs (Oluma and Oladiran 1993), and ultimately the growth and biomass of the plants are reduced as observed in the present investigation. Correlation analysis has also revealed positive linear relationship between root rot index and per cent decrease, indicating that an increase in the root rot directly impaired vital physiological functions such as absorption of water, minerals, etc. and CO_2 assimilation, which led to subsequent decrease in the dry matter production as occurred in tobacco cultivars (Figure 1).

Chlorophylls are considered the basic unit for photosynthesis as these pigments absorb light and transfer energy to cell organelles for CO_2 fixation (Wallace 1987). On susceptible cultivars, mild yellowing of leaves was observed, which pronounced gradually with the advancement of plants' age. At 3–4 month of age, entire foliage of susceptible cultivars, as in RK-10 P3, became discernibly yellowish, whereas the foliage of resistant cultivars, especially RK-13 P4, remained normal and healthy. The chlorophyll pigments were found to be highly sensitive to alternation in host physiology induced by *P. aphanidermatum*. The water stress condition in the plants due to decay and loss of roots caused by the pathogen, chlorophyll and carotenoids molecules might have got denatured (Khan and Khan 1987). Correlation analysis has shown a linear negative relationship between leaf pigments and disease severity, where increase in the root rot index resulted to a corresponding decrease in the chlorophyll and carotenoid contents of the leaves (Figure 1). Decrease in the

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

chlorophylls and carotenoids resulted in a lower assimilation of carbohydrates, leading to lesser dry matter production as evident in the correlation analysis done between leaf pigments and dry weight of shoots (Figure 2).

TP content of leaves of all five cultivars increased in the fungus-inoculated plants, but varied greatly. The cv. RK-10 P3, which was found highly susceptible to the fungus, showed lowest increase in the TP, whereas the greatest increase of TP was in cv. RK-13 P4, which expressed resistant to *P. aphanidermatum*. This indicates that the phenolic compounds contribute in the defence of plants against pathogen attack (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 fungi, their

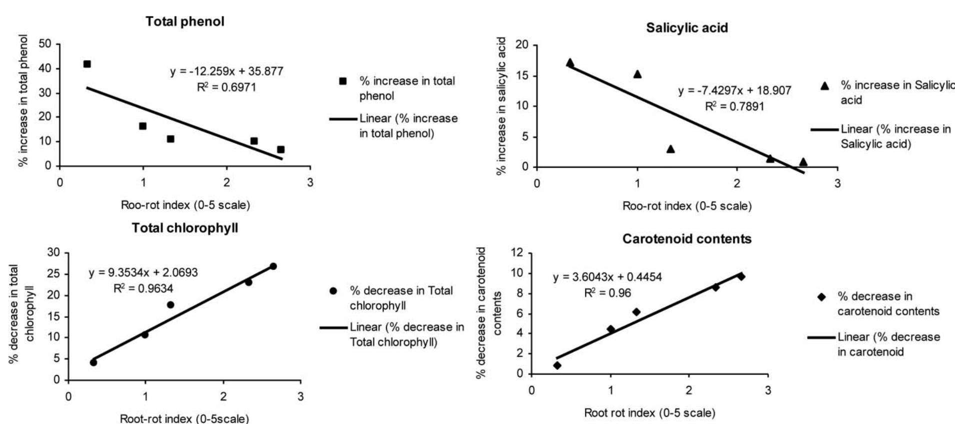

Figure 1. Correlation between disease (root rot index) and per cent change in TP, salicylic acid, total chlorophyll and carotenoid of tobacco cultivars subjected to inoculation of *Pythium aphanidermatum* (2 g).

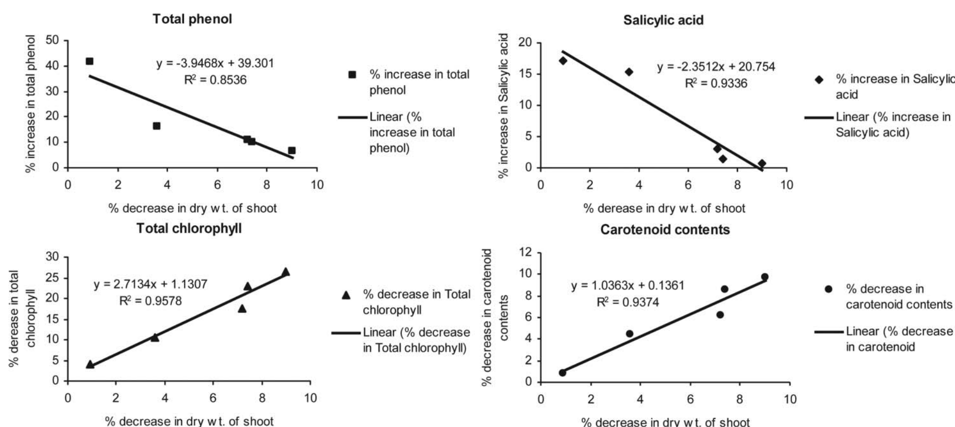

Figure 2. Correlation between per cent decrease in dry weight and per cent change in TP, salicylic acid, total chlorophyll and carotenoid of tobacco cultivars subjected to inoculation of *Pythium aphanidermatum* (2 g).

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

concentrations increase considerably, particularly in resistant plants, tolerant plants, or cultivars. The correlation analysis has also shown that greater concentration of phenols in the cultivars exhibited a lower root rot index, and the concentration decreased linearly with increase in root rot index as found in the present study (Figure 1). SA has also been implicated as one of the key components in the signal transduction pathway leading to plant resistance to various pathogens, including *P. aphanidermatum* (Ryals et al. 1996; Wobbe and Klessig 1996; Radhakrishnan and Balasubramanian 2009). Highest concentration of SA was recorded in the cv. RK-12 P3, which did not exhibit root rot and plant growth reduction, whereas lowest increase in SA occurred in cvs. RK-10 P3, RK-18 P8 and RK-26 P3, which showed susceptibility to *P. aphanidermatum*. Increase in tolerance or resistance of the cv. RK-12 P3 against the fungus was apparently due to the synthesis of SA in a high concentration in addition to other factors that were not determined. It has been reported that greater accumulation of SA in tobacco leaves results in a significant reduction of disease symptoms caused by the fungi *Phytophthora parasitica, Cercospora nicotianae* and *Peronospora tabacina* (Ryals et al. 1996). This has also become evident with the correlation analysis between increase in SA and decrease in root rot index (Figure 1). Correlation analysis between TP or SA and dry matter production of infected plants has shown a positive linear relationship (Figure 2). Phenol or SA do not have any significant role in plant growth promotion. The decrease in the shoot dry weight with the decrease in TP and SA was apparently due to the strong negative linear relationship between TP or SA and disease severity (Figure 2). In the cultivars, where concentration of TP or SA was greater, root rot was lower; as a result, decrease in dry matter production was also lower. *F*-values were much greater for SA than TP, indicating greater role of the former in the disease tolerance.

The study has revealed that among the five cultivars of tobacco screened, the cv. RK-12 P3 showed considerable degree of tolerance against *P. aphanidermatum*, which can be exploited commercially. The expressed tolerance can be considered as a reliable characteristic as it was based on the biochemical parameters, i.e. greater synthesis of TP and SA in response to the fungus infection.

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011

References

Ahmed HI, Labuschagne N, Korsten L. 2008. Supression of *Pythium ultimum* root rot sorghum by rhizobacterial isolates from Ethiopia and South Africa. Biol Control. 45(1):72–84.

Arnon D. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1–15.

Chase AR. 1999. Pythium root rot on ornamentals. Western Connection: Turf and Ornamentals. 8(1):1–4.

Dixon RA, Harrison MJ, Lambmb CJ. 1994. Early events in the activation of plant defense responses. Annu Rev Phytopathol. 32:479–501.

Garcia PC, Rivero RM, Lopez Lefebre LR, Sdnchez E, Ruiz JM, Romero L. 2001. Direct action of the biocide carbendazim on phenolic metabolism in tobacco plants. J Agric Food Chem. 49:131–137.

Hammond-Kosack KE, Jones JG. 1996. Resistance gene-dependent plant defense responses. Plant Cell. 8:1773–1791.

Khan MR, Khan MW. 1987. Histo-physiological alternations induced by *Meloidogyne incognita* in tomato. Int Nematol Network Newsl. 4:10–12.

Lowery OH, Roseenbrough NJ, Farr AL, Randall RH. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem. 193:265–275.

Lucos GB. 1975. Diseases of tobacco. North Carolina: Biological Consulting Associates.
Maclachlan S, Zalik S. 1963. Plastid structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant of barley. Can J Bot. 41:1053–1062.
Martin FN, Loper JE, 1999. Soilborne plant diseases caused by *Pythium* spp: ecology, epidemiology, and prospects for biological control. Crit Rev Plant Sci. 18:111–181.
Nicholson RL, Hammersehmid R. 1992. Phenolic compounds and their role in disease resistance. Annu Rev Phytopathol. 30:369–389.
Oluma HOA, Oladiran AO. 1993. *Pythium aphanidermatum* root rot of pawpaw (*Carica papaya* L.) in Nigeria. Mycopathologia. 123:111–115.
Radhakrishnan N, Balasubramanian R. 2009. Salicylic acid induced defence responses in *Curcuma longa* (L.) against *Pythium aphanidermatum* infection. Crop Prot. 28:974–979.
Ryals JK, Neuenschwander UH, Willits MG, Molina A, Steiner H, Hunt MD. 1996. Systemic acquired resistance. Plant Cell. 8:1809–1819.
Shane N, Kowblansky M. 1968. Determination of acetyl salicylic acid, salicyclamide, acetominophone and caffeine in tablet or powder by independent methods. J Pharm Sci. 57(7):1218–1223.
Sharma P, Sain SK. 2005. Use of biotic compounds against damping off of cauliflower by *Pythium aphanidermatum*. Indian Phytopathol. 58(4):395–401.
Shepherd JA, Barker KR. 1990. Plantparasitic nematodes of tobacco. In: Luc M, Sikora RS, Bridge J, editors. Plant-parasitic nematodes in tropical and subtropical agriculture. St. Albans, UK: Commonwealth Agricultural Bureau International. p. 493–517.
Stanghellini ME, Hancock JG. 1971. The sporangium of. *Pythium ultimum* as a survival structure in soil. Phytopathology. 61:157–164.
Staskawicz PE, Su W, Howell SH. 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein in an *Arabidopsis thaliana* mutant. Proc Natl Acad Sci USA. 89:6837–6840.
Takahama U, Oniki T. 1992. Regulation of peroxidase-dependent oxidation of phenols in the apoplast of spinach leaves by ascorbate. Plant Cell Physiol. 33:379–387.
Wallace HR. 1987. Effect of nematode parasites on photosynthesis. In: Veech JA, Dickson DW, editors. *Vistas on Nematology*. Maryland (USA): SON Publication. p. 253–259.
William N. 2003. Pythium diseases in the tobacco float system understanding the problem. Kentucky Pest News. Issue no. 976.
Wobbe KK, Klessig DF. 1996. Salicylic acid: an important signal in plants. In: D.P.S. Verma, editor. Plant gene research series, signal transduction in plant growth and development. Vienna: Springer-Verlag. p. 167–196.
Zieslin N, Ben Zaken R. 1993. Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiol Biochem. 31:333–339.

Downloaded by [Aligarh Muslim University] at 03:53 01 November 2011