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Full Length Research Paper

Effect of rhizobacterium on the rhizosphere and endophytic densities of M. javanica in tomato varieties

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The biological control potential of 0.2 cfu/ml of the bacterium; *Psuedomonas flourescens* was assessed by inundating two varieties of tomato; Roma VF and UC83B infected with 500; 1000; 1500 and 2000 eggs/(J^2) juveniles of the nematode *Meloidogyne javanica.* The control sets were only infected with *M. javanica* and the randomised block designs with five replicates were adopted. Plants were grown in 30cm mouth-wide black polyethene bags containing steam sterilized soil mixture of loam and sand in the ratio of 1:1. Endophytic and rhizosphere populations of *M. javanica* were assessed at 30, 60 and 90 days after inoculation (DAI). Result of the study revealed that *P. flourescens* did not inhibit *M. javanica* egg hatch and root penetration 30 DAI in both tomato cultivars. However, the endophytic population density of *M. javanica* varied greatly within the 60 and 90 DAI, in both tomato cultivars, which was attributed to host specific factors. There was no significant difference ($p>0.05$) between the height and the girth of the controls and that of the *P. flourescens* inundated replicates. There were significant difference s ($p<0.05$) in the root weight between the control and the *P. flourescens* induced replicates.

Key words: Endophytic and rhizosphere population, egg hatch, nematode penetration, feeding sites and plant growth stimulatory factors.

INTRODUCTION

Under natural field conditions, plant roots are constantly exposed to many soil micro-organisms (Onyenobi, 1986; Wang and McSorley, 2003) in the rhizosphere. Amongst the array of organisms in the soil a few such as plant parasitic nematodes are capable of parasitizing plants (Mai and Abawi, 1987, Imafidor and Nzeako, 2007). Plants infected with nematodes exhibit multiple symptoms such as stunting, wilting, chlorosis, and untriftly appearance which ultimately result to scarcity of food especially in sub-Saharan Africa (Imafidor and Nzeako, 2007, Abuzor and Haseeb, 2010). Annually plant parasitic nematodes alone result to 10.7% damage to the twenty life sustaining crops (LSC's) globally (Fawole et al., 1992 and Agrios, 1997).

Effective control against this menace had greatly relied on the application of inorganic substances (Muller and Gooch, 1982; Akhtar, 1999 and Siddiqui, 2004) which are both ecologically insensitive due to its effect on non target organisms and the high cost of application. Bashan (1998) and Siddiqui and Mahmood (1999) reported that rhizobacteria can promote growth in plants by enhancing nutrient uptake by roots while having antagonistic effect against plant pathogens. This possibility could be exploited in controlling the menace of *Meloidogyne javanica* infestion in economically important vegetables in Nigeria. On this premise; this study is aimed at determining the effect of the rhizobacterium; *Pseudomonas flourescens* on the rhizosphere and

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endophytic densities of *M. javanica* in two tomato varieties; Roma VF and UC83B.

MATERIALS AND METHODS

Pre-inoculation procedures

Sterilization of soil: A mixture of forest loam and sand (1:1) were steam sterilized for a period of 30 min and stored in a cool dry place for a period of 90 days to ensure disintegration of any surviving nematodes.

Isolation of bacterium: Twenty grams (20 g) of garden soil was obtained from the rhizosphere of okro plants at randomly selected sites, filled in dark polyethylene bags and taken to the laboratory for the isolation of the bio-control agents. Five gram of each of the garden soil sample was suspended in 50 ml of sterilized water in a 250 ml, Erlenmeyer flask. Standard Microbiological procedures were followed in the isolation of the bacterium according to Chessbrough (2003).

Purification of isolates: Each of the sub-cultured bacteria isolates were further sub cultured aseptically from the plates by streaking onto freshly prepared nutrient agar plates and incubated at 37°C for 24 h. After incubation, discrete colonies were picked and gram stained to confirm their purity. Each pure colony was inoculated onto sterile nutrient agar slants in screw-capped bottles incubated at 37°C for 24 h and stored in the refrigerator at 4°C as stock sample for the inoculum preparation (Chesbrough, 2003). *P. flourescens* sensitivity tests were carried out following the method by Blazevic et al. (1973).

Extraction of *M. javanica* from the root of tobacco plants for inoculum

Galled roots of tobacco plants were washed thoroughly in tap water to remove soil particles, cut into 1 to 2 cm length and immersed in Erlenmeyer flask containing 200 ml of 0.5% sodium hypochlorite (NaOCl) and vigorously shaken for three minutes. This was quickly passed through a 75 µm sieve (200 mesh) nested over a 26 µm sieve (500 mesh). The egg masses and juveniles of *M. javanica* trapped on the 500 mesh were back-washed with a gentle stream of tap water to remove residual NaOCl. The remaining root pieces in the 75 µm sieve were rinsed with tap water to recover additional eggmasses/juveniles. *M. javanica* eggs/juveniles were standardised following the procedure described by Hartman and Sasser (1985).

Planting: The seeds for the research were sourced from the National institute for horticultural research, Nigeria. The plants were raised in steam sterilized soil and were left for 14 days under light-shade conditions to ensure proper development of seedlings before transplanting.

Inoculation of plants with P. *flourescens*: 75 plants each were planted for the tomato cultivars; Roma VF and UC83B making a total of 150 plant each having four replicates with the fifth plant serving as the control. These were inundated with; 20 ml (0.2 cfu × 10^2/ ml) of *P. flourescens* except the fifth group serving as the control. Destructive bioassay was conducted on 30 days intervals to evaluate the response of the crops to the presence of *P. flourescens*.

Inoculation of plant with nematodes: Plants were inoculated inundatively with; 5, 10, 15, and 20 ml; at the base of the bagged plants representing; 500 eggmass/juveniles, (Group 2); 1000 eggmass/juvenile (Group 3); 1500 eggmass/juveniles (Group 4) and 2000 eggmass/juveniles (Group 5) respectively. The fifth plant in each group served as the control for the two tomato cultivars. The randomized block design was adopted for the research.

Post inoculation procedures

Determination of the population dynamics of M. javanica in the soil and root

Rhizosphere and endophytic populations of *M. javanica* were determined on 30 days intervals using the Bearmans funnel technique and the Hypochlorite sieving technique. Egg and *M. javanica* juveniles were counted using the binocular microscope.

Determination of gall index and eggmass index: Gall index and eggmass index was determined using the Hartman and Sasser (1985) scale.

Analysis of data

Data was analyzed using ANOVA.

RESULTS

Rhizosphere and endophytic densities of *M. javanica*: The rhizosphere densities of *M. javanica* increased slightly as plants aged in the *P. flouresens* induced plants, a pattern which varied as inoculums levels increased. The controls recorded slightly higher densities of *M. javanica* than the *P. flouresens* induced plants ($P>0.05$) (Table, 1). On individual cultivar basis UC83B had a slight increase in rhizosphere density of *M. javanica* than Roma VF at the various inoculums levels except at the 500 inoculum level ($P>0.05$). Endophytic populations of *M. javanica* varied amongst the *P. flouresens* induced cultivars and the controls as the plant aged (Table 1). However, *M. javanica* initial density did not significantly ($P>0.05$) influence the final density in the

Table 1. Rhizosphere and endophytic densities of *M. javanica* in tomato cultivars; Roma VF and UC83B at 30, 60 and 90 days after inoculation.

Rhizosphere density																
Inoculum level	**500**				**1000**				**1500**				**2000**			
Tomato cutivar	**R**	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**
Intervals in days																
30	3	0	1	1	3	0	8	3	1	0	6	1	1	3	1	2
60	2	3	14	1	6	1	11	7	3	4	9	1	2	2	1002	2
90	5	5	9	15	21	2	28	6	363	6	68	16	452	6	233	44

Endophytic density																
Inoculum level	**500**				**1000**				**1500**				**2000**			
Tomato cultivar	**R**	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**
Intervals in days																
30	3	0	2	6	2	0	3	29	4	0	1	12	1	2	2	2
60	22	34	85	31	24	0	53	31	19	46	22	200	9	25	41	17
90	314	64	4	68	300	34	29	52	825	5	16	280	177	75	175	120

R means Roma VF; U means UC83B; RC means Roma VF control; UC means UC83B control, Control; (tomato-*P. flouresens*), Each value in the table represents the mean of five replicates.

Table 2. Effect of *P flouresens* on Gall indexes and Reproductive factors (R) of *M. javanica* infected tomato cultivars and the control groups at 60 and 90 days intervals.

Interval in days	Tomato cultivars	Inoculum levels															
		500				1000				1500				2000			
		R	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**	**R**	**RC**	**U**	**UC**
60	Gall index	4	1	3	2	4	2	3	2	3	2	2	2	4	2	1	2
	Reproductive factor (R)	0.75	0.02	0.06	0.03	0.04	0.02	0.06	0.02	0.016	0.01	0.002	0.01	0.002	0.01	0.001	0.01
90	Gall index	4	1	4	4	4	1	4	4	4	2	4	5	4	2	4	5
	Reproductive factor (R)	0.15	0.03	0.08	0.12	0.07	0.01	0.07	0.04	0.07	0.01	0.07	0.06	0.07	0.01	0.07	0.05

(R)= Reproductive factor (Pf/Pi), R means Roma VF; U means UC83B; RC means Roma VF control; UC means UC83B control, Control (tomato-*P.flouresens*) Gall index according to Hartman and Sasser (1985) scale.

root as plant aged. The high gall index recorded in both cultivars at 60 and 90 days after inoculation indicated the suitability of the tomato cultivars to *M. javanica* (Table 2). We observed a variation in endophytic densities of *M. javanica* amongst the two cultivars and the controls. However, the treatments harbor higher endophytic population of nematodes than the control sets 30 days after inoculation with *M. javanica* (Table 1).

Effect of *P. flouresens* on tomato cultivars growth parameters: The height of *P. flouresens* induced plants were relatively shorter than those of the control sets ($P>0.05$). Data showed that inoculum size had no influence on the height of the tomato cultivars (Table 2). Wet root weight of *P. flouresens* induced plants were relatively higher than those of the control sets, a pattern which was consistent as inoculum level increased and plants aged ($p<0.05$). Girth of treatments showed slight variability amongst the tomato cultivars but varied consistently in comparison with the control sets (Tables 2 and 3).

DISCUSSION

M. javanica and *P. flourescens* inoculum treatment

The rhizosphere densities of *M. javanica* in the tomato cultivars; Roma VF and UC83B were below the initial population densities in all the inoculum levels at the 30 days interval (Table 1). This observation is consistent with studies by Khan et al. (1987) and Nzeako and Imafidor (2010) who attributed the decline in initial density

Table 3. Effects of *P. flourescens* on the growth parameters of *M. javanica* inoculated tomato cultivars in 30, 60 and 90 days intervals.

Inoculum levels	500				1000				1500				2000			
Tomato Cultivars	R	RC	U	UC	R	RC	U	UC	R	RC	U	UC	R	RC	U	UC
Height (cm)	9.6	18.1	8.5	12	10.5	20	8.3	19	12	18	20	14.5	9.4	22.2	9.5	19
Root weight (g)	0.5	1.4	0.5	0.4	0.45	0.55	0.3	1.4	0.45	0.15	0.3	0.6	0.2	1.35	0.3	0.5
Girth (cm)	0.2	0.45	1.1	0.2	0.15	0.4	0.1	0.8	0.2	0.35	0.2	0.25	0.1	0.4	0.2	0.2
Inoculum levels			500				1000				1500				2000	
Tomato Cultivars	R	RC	U	UC	R	RC	U		R	RC	U	UC	R	RC	U	UC
Height (cm)	42	75	42	81	46.5	74	46.5	63	48.5	68.5	50.5	63	34	60	34	67
Root weight (g)	14.5	7.5	17.5	5	19.4	7.8	19.4	8	14.6	6.4	8	5.2	9	4.1	5	12
Girth (cm)	6.5	6.5	3	0.6	4.5	0.6	4.5	0.5	2.5	0.7	3	0.4	35	0.8	2	0.7
Num. of fruits	0	1	0	0	0	0	0	0	0	0	8	0	0	0	0	1
Fruit weight (g)	0	1.6	0	8.1	0	0	0	0	0	0	30.2	0	0	0	0	2.2
Inoculum levels			500				1000				1500				2000	
Tomato Cultivars	R	RC	U	UC	R	RC	U	UC	R	RC	U	UC	R	RC	U	UC
Height (cm)	76	84	75	84.5	72	82	69.5	93	76	62	70	84.5	75	80	62	94.5
Root weight (g)	20	10	18.7	8.5	5	9.2	14.7	11.5	18.9	6.5	18.9	21	37.5	90	46.4	34
Girth (cm)	4.8	0.45	3.7	0.15	3	0.7	3.2	0.15	2.6	0.7	3	0.15	3	0.65	3.65	0.2
Num. of fruits	0	1	3	3	0	0	3	1	1	0	0	4	0	0	5	5
Fruit weight (g)	0	3.2	14.3	4.5	0	0	10.4	3.4	3.6	0	0	8.3	0	0	19.2	12.1

R means Roma VF; U means UC83B; RC means Roma VF control; UC means UC83B control, Control (tomato-*P. flouresens*), Each value in the table represents the mean of five replicates.

of rhizosphere nematodes to effective penetration and secure of feeding sites by the nematodes. However, the difference recorded in the rhizosphere densities of *M. javanica* between the treatments and the control sets suggests that nematode egg hatch or active penetration of the root tissues by *M. javanica* juveniles may have been inhibited by the presence of *P. flourescens* (Siddiqi and Mahmood, 1999; Kerry, 2000; Moussa and Hanna, 2010; Abduzor and Haseeb, 2010). The observed increase in endophytic population of *M. javanica* in *P. flourescens* inoculated plants contradicts Compant et al. (2005).

The high gall indexes did not record a corresponding reproductive factors (R) which contradicts studies by Khan (1987); Babalola (1990) and Imafidor and Nzeako (2008) where high gall indexes corresponded with high reproductive factors (Table 2). However, the study is in line with studies by Khan et al. (1987); Knox et al. (2004) and Nzeako and Imafidor (2010) who suggested that *M. javanica* infection can stimulate tumour stimulatory factors in plants even at very low densities.

P. flourescens induced growth stimulatory effects were observed in the height and wet root weight of the cultivars which was not observed in their girths of the cultivars.

Fruition in the tomato cultivars were variable suggesting host specific characteristics and harsh environmental factors.

Conclusively, the biological control agent; *P. flourescens* was observed not to have imposed an inhibitory effect on *M. javanica* egg hatch and secure of feeding sites in the rhizosphere. However, *P. flourescens* stimulated plant growth characteristics as observed in the height, and root weight of experimental replicates. The study also revealed that the growth stimulatory effect of *P. flourescens* also influences the formation of tumour cells in the treatments.

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