Comparative pathogenicity of reniform nematode on root-knot resistant and susceptible bidi tobacco

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Abstract Comparative pathogenicity of reniform nematode on root-knot resistant ABT 10 and susceptible bidi tobacco A119 revealed that ABT 10 was found significantly superior to A119 with respect to plant growth characters and as good as A119 with respect to multiplication of reniform nematode. Initial inoculum of 1,000 J₄ of the nematode found damaging to both ABT 10 and A119 varieties of bidi tobacco.

Keywords Pathogenicity · Reniform nematode · *Rotylenchulus reniformis* · Bidi tobacco

Introduction

Tobacco (*Nicotiana tabacum* L.), an important non-food narcotic cash crop, occupies less than 0.27 per cent of the net cultivated area and earns sizable amount of foreign exchange (Rs. 4,210 crores), central excise (Rs. 14,000 crores) to the national exchequer and VAT (Rs. 5,000 crores) besides providing direct and indirect employment to 36 million people including 6 million farmers and workers (Krishnamurthy 2011). Plant parasitic nematodes also known as eel worms or thread worms cause severe damage to the crop. Root-knot (*Meloidogyne incognita*; *M. javanica*), reniform (*Rotylenchulus reniformis*) and stunt

(*Tylenchorhynchus vulgaris*) nematodes are found predominantly attacking bidi tobacco in Gujarat. Research on root-knot nematodes in tobacco has been carried out in depth by many scientists. Very little information pertaining to reniform (Patel 1986; Patel et al. 1986a, b, c) and stunt nematodes (Patel and Patel 1991a, b, 1995, 1998; Patel et al. 1962) in bidi tobacco is available in literature. Similarly, a root-knot resistant bidi tobacco cultivar Anand Bidi Tobacco (ABT 10) was released in 2008 from Bidi Tobacco Research Station, AAU, Anand and no information is available about its reaction to reniform nematode. Therefore, present investigation was carried out.

Materials and methods

An experiment was conducted, during the year 2011 and 2012, to study comparative pathogenicity of reniform nematode on root-knot resistant and susceptible bidi tobacco cv. ABT 10 and A119, respectively. There were ten treatments comprising of two varieties viz. ABT 10 (V₁), and A119 (V₂) and five inoculum levels viz. 0 (Control, I₁), 10(I₂), 100(I₃), 1,000(I₄) and 10,000(I₅) J₄ per pot. Each treatment was repeated four times in completely randomized design (CRD). Thus forty disinfested pots of 15 cm diameter were filled with 500 cc sterilized soil and FYM. Each pot was transplanted with healthy seedling of bidi tobacco according to treatments keeping one seedling per pot. Second stage juveniles (J₂) of reniform nematode were extracted from well mixed soil collected from the pure culture microplot using petridish assembly method (Chawla and Prasad 1974). After 24 h, nematode suspension was carefully collected, concentrated and kept for 15 days at room temperature till juveniles convert into pre-adult stages (J₄). The nematode count (pre-adult female)

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Table 1 Comparative pathogenicity of reniform nematode on root-knot resistant and susceptible bidi tobacco: effect on plant height (cm) and shoot weight (g)

Treatment	Plant height (cm)			Shoot weight (g)					
				Fresh			Dry		
	2011	2012	Pooled	2011	2012	Pooled	2011	2012	Pooled
Variety (V)									
ABT10 (V ₁)	22.7	21.3	22.0	36.28	31.61	33.94	3.26	2.34	2.80
A119 (V ₂)	19.6	15.8	17.7	25.38	22.28	23.83	2.86	1.73	2.29
S.Em. $\pm$	0.7	0.5	0.4	0.55	0.91	0.53	0.09	0.07	0.06
CD 0.05	1.9	1.5	1.2	1.62	2.65	1.51	0.27	0.21	0.17
S.Em. $\pm$ (Y $\times$ V)	0.6			0.75			0.08		
CD 0.05 (Y $\times$ V)	NS			NS			NS		
Inoculum level J₄ (I)									
0 (I ₁)	25.5	23.6	24.5	37.39	35.20	36.30	3.48	2.51	3.00
10 (I ₂)	23.6	22.4	23.0	36.63	34.19	35.41	3.34	2.38	2.86
100 (I ₃)	23.4	21.8	22.6	35.48	32.80	34.14	3.31	2.19	2.75
1000 (I ₄)	19.1	14.6	16.9	26.98	20.11	23.55	2.71	1.90	2.31
10,000 (I ₅)	14.0	10.4	12.2	17.67	12.42	15.04	2.45	1.21	1.83
S.Em. $\pm$	1.0	0.8	0.7	0.88	1.44	0.84	0.14	0.12	0.09
CD 0.05	3.0	2.4	1.9	2.56	4.19	2.38	0.40	0.34	0.25
S.Em. $\pm$ (V $\times$ I)	1.5	1.2	0.9	1.24	2.03	1.19	0.19	0.16	0.13
CD 0.05 (V $\times$ I)	NS	NS	NS	3.62	5.93	NS	NS	NS	NS
Year									
S.Em. $\pm$ (Y $\times$ I)	0.9			1.19			0.13		
CD 0.05 (Y $\times$ I)	NS			NS			NS		
S.Em. $\pm$ (Y $\times$ V $\times$ I)	1.3			1.68			0.18		
CD 0.05 (Y $\times$ V $\times$ I)	NS			4.76			NS		
CV%	13.8	12.5	13.3	8.0	15.1	11.6	12.6	16.1	14.6

per ml in water suspension was estimated using stereoscopic binocular microscope for inoculation purpose. Required quantity (20 ml) of nematode suspension was inoculated per pot as per the treatment in the respective pot, while in control only 20 ml water without nematode was poured. The pots were kept on the bench in net house. The seedlings were watered regularly and protected from the insect damage using appropriate management measures. All agronomic practices in vogue were followed. Observations on plant growth, nematode penetration in stained roots (Franklin 1949) and multiplication were recorded 60 days after inoculation (60 DAI) and analyzed.

Results and discussion

Perusal of data revealed significant differences for plant growth characters (Tables 1, 2, 3, 4) in variety (V) i.e. plant height, shoot weight, number of leaves (Plates 1 & 2), root weight, root growth (Plates 3 & 4); inoculum level

Table 2 Interaction (V $\times$ I, Y $\times$ V $\times$ I) for fresh shoot weight (g) of bidi tobacco due to reniform nematode

Year/Variety/Inoculum level	Y ₁ (2011)	Y ₂ (2012)
V ₁ I ₁	41.45	41.58
V ₁ I ₂	40.94	40.07
V ₁ I ₃	40.28	39.43
V ₁ I ₄	35.23	23.48
V ₁ I ₅	23.48	13.48
V ₂ I ₁	33.33	28.83
V ₂ I ₂	32.31	28.31
V ₂ I ₃	30.67	26.17
V ₂ I ₄	18.74	16.74
V ₂ I ₅	11.85	11.35
Mean	30.83	26.94
S. Em. $\pm$ (V $\times$ I)	1.24	2.03
CD 0.05 (V $\times$ I)	3.62	5.93
S. Em. $\pm$ (Y $\times$ V $\times$ I)	1.68	
CD 0.05 (Y $\times$ V $\times$ I)	4.76	

Table 3 Comparative pathogenicity of reniform nematode on root-knot resistant and susceptible bidi tobacco: effect on number of leaves/plant, fresh root weight (g) and root growth index

Treatment	No. of leaves/plant			Fresh root weight (g)			Root growth index (0–5)*		
	2011	2012	Pooled	2011	2012	Pooled	2011	2012	Pooled
Variety (V)									
ABT10(V ₁)	9	9	9	2.26	2.16	2.21	4.15	4.15	4.15
A119 (V ₂)	8	8	8	1.66	1.53	1.59	3.90	3.90	3.90
S.Em. $\pm$	0.14	0.13	0.10	0.05	0.05	0.04	0.07	0.07	0.05
CD 0.05	0.41	0.38	0.27	0.16	0.14	0.10	0.21	0.20	0.14
S.Em. $\pm$ (Y $\times$ V)	0.14			0.05			0.07		
CD 0.05 (Y $\times$ V)	NS			NS			NS		
Inoculum level J₄ (I)									
0 (I ₁)	10	10	10	2.45	2.29	2.37	5.00	5.00	5.00
10 (I ₂)	10	10	10	2.37	2.19	2.28	5.00	5.00	5.00
100 (I ₃)	10	10	10	2.31	2.12	2.21	4.88	4.75	4.81
1,000 (I ₄)	8	8	8	1.45	1.39	1.42	3.00	3.13	3.06
10,000 (I ₅)	8	7	7	1.22	1.22	1.22	2.25	2.25	2.25
S.Em. $\pm$	0.22	0.20	0.15	0.09	0.07	0.06	0.12	0.11	0.08
CD 0.05	0.65	0.60	0.43	0.25	0.22	0.16	0.34	0.31	0.22
S.Em. $\pm$ (V $\times$ I)	0.32	0.29	0.21	0.12	0.11	0.08	0.17	0.15	0.11
CD 0.05 (V $\times$ I)	NS	NS	0.60	NS	NS	NS	NS	NS	NS
Year	NS			Sign.			NS		
S.Em. $\pm$ (Y $\times$ I)	0.21			0.08			0.11		
CD 0.05 (Y $\times$ I)	NS			NS			NS		
S.Em. $\pm$ (Y $\times$ V $\times$ I)	0.30			0.11			0.16		
CD 0.05 (Y $\times$ V $\times$ I)	NS			NS			NS		
CV %	7.1	6.5	6.8	12.2	11.3	11.8	8.2	7.5	7.9

* 0 = Minimum root growth; 5 = Maximum root growth

Table 4 Interaction (V $\times$ I) for number of leaves/plant of bidi tobacco due to reniform nematode

Variety /Inoculum	0 (I ₁)	10 (I ₂)	100 (I ₃)	1,000(I ₄)	10,000 (I ₅)
ABT10(V ₁)	10	10	10	8	8
A119(V ₂)	10	10	9	7	7
S.Em. $\pm$	0.21				
CD 0.05	0.60				

(I) i.e. fresh shoot weight in individual years and number of leaves in pooled as well as nematode multiplication (Tables 5, 6, 7, 8) i.e. number of eggs per egg mass (Plate 5), number of egg mass per plant and final population in pooled analysis for variety(V); number of eggs per egg mass, number of females (Plate 6) and egg mass per plant, soil and final population in individual years and in pooled for inoculum levels (I). Most of the interactions were not-significant. However, year effect was significant for plant height, shoot and root weight; V $\times$ I and Y $\times$ V $\times$ I for

Plate 1 Effect of reniform nematode on plant growth of ABT10 **Plate 2** Effect of reniform nematode on plant growth of A119

Plate 3 Reniform nematode on bidi tobacco root

Plate 4 Eggs of reniform nematode developed on bidi tobacco root

fresh shoot weight, while, $V \times I$ for number of leaves and number of egg mass per plant.

Between two varieties, root-knot resistant ABT 10 (V_1) recorded significantly higher plant growth than A119 (V_2) (Fig. 1) in individual years and in pooled results with reduced number of eggs per egg mass, egg mass per plant and final population of reniform nematode in pooled analysis.

Significant reduction in plant growth characters was observed at an initial inoculum of 1,000 J_4 (I_4) per plant compared to the lower inoculum levels of 100 (I_3) and 10 J_4 (I_2) per plant and no inoculation (I_1) during both the years and in pooled analysis (Fig. 2). Increase in inoculum level further reduced the plant growth characters with maximum reduction in 10,000 J_4 (I_5) per plant. Similarly, Increase in inoculum level significantly increased nematode multiplication i.e. number of egg mass and females per plant, soil and final population of reniform nematode with maximum in highest inoculum level (Fig. 3). However, initial

Table 5 Comparative pathogenicity of reniform nematode on root-knot resistant and susceptible bidi tobacco: Effect on number of eggs/egg mass and number of egg mass/plant

Treatment	No. of eggs/egg mass ($\sqrt{x+1}$ trans.)			No. of egg mass/plant ($\sqrt{x+1}$ trans.)		
	2011	2012	Pooled	2011	2012	Pooled
Variety (V)						
ABT10 (V_1)	5.36 (33)	5.16 (31)	5.26 (32)	5.36 (43)	5.20 (41)	5.28 (42)
A119 (V_2)	5.67 (37)	5.56 (36)	5.62 (36)	5.70 (51)	5.75 (54)	5.72 (52)
S.Em. $\pm$	0.16	0.16	0.11	0.18	0.22	0.14
CD 0.05	NS	NS	0.323	NS	NS	0.40
S.Em. $\pm$ ($Y \times V$)	0.17			0.20		
CD 0.05 ($Y \times V$)	NS			NS		
Inoculum level J_4 (I)						
0 (I_1)	1.00 (0)	1.00 (0)	1.00 (0)	1.00 (0)	1.00 (0)	1.00 (0)
10 (I_2)	6.69 (45)	6.19 (38)	6.44 (41)	2.02 (3)	1.79 (2)	1.91 (3)
100 (I_3)	6.51 (42)	6.26 (39)	6.39 (40)	3.79 (14)	3.74 (13)	3.76 (13)
1,000 (I_4)	6.56 (43)	6.56 (43)	6.56 (43)	10.04 (101)	9.92 (100)	9.98 (100)
10,000 (I_5)	6.82 (46)	6.81 (46)	6.81 (46)	10.83 (117)	10.91 (120)	10.87 (119)
S.Em. $\pm$	0.25	0.26	0.18	0.29	0.35	0.23
CD 0.05	0.73	0.76	0.51	0.83	1.02	0.64
S.Em. $\pm$ ($V \times I$)	0.37	0.39	0.27	0.40	0.49	0.32
CD 0.05 ($V \times I$)	NS	NS	NS	NS	NS	0.86
Year	NS			NS		
S.Em. $\pm$ ($Y \times I$)	0.27			0.32		
CD 0.05 ($Y \times I$)	NS			NS		
S.Em. $\pm$ ($Y \times V \times I$)	0.38			0.45		
CD 0.05 ($Y \times V \times I$)	NS			NS		
CV %	12.8	13.7	13.2	14.5	18.0	16.3

Figures in parentheses are original value

Table 6 Interaction ($V \times I$) for number of egg mass/plant of reniform nematode in bidi tobacco

Variety /Inoculum	0 (I_1)	10 (I_2)	100 (I_3)	1,000 (I_4)	10,000 (I_5)
ABT10 (V_1)	1.00 (0)	1.94 (3)	3.84 (14)	9.58 (91)	10.05 (101)
A119 (V_2)	1.00 (0)	1.88 (3)	3.68 (13)	10.38 (110)	11.68 (137)
S.Em. $\pm$	0.32				
CD 0.05	0.86				

Figures in parentheses are original value of $\sqrt{x+1}$ trans

inoculum of 1,000 J_4 per plant was at par with the highest inoculum level of 10,000 J_4 (I_5) per plant with respects to number of egg mass and females per plant in individual years and soil and final population in 2012 while number of females per plant and soil population in pooled analysis.

Table 7 Comparative pathogenicity of reniform nematode on root-knot resistant and susceptible bidi tobacco: effect on number of females/plant and soil population

Treatment	No. of females/plant ($\sqrt{x+1}$ trans.)			Soil population (500 CC) (Log x + 1 trans.)		
	2011	2012	Pooled	2011	2012	Pooled
Variety (V)						
ABT10 (V ₁)	6.23 (55)	6.05 (52)	6.14 (54)	2.56 (2,389)	2.56 (2,202)	2.56 (2,295)
A119 (V ₂)	6.51 (63)	6.46 (64)	6.48 (64)	2.63 (3,015)	2.62 (2,968)	2.63 (2,992)
S.Em. $\pm$	0.20	0.26	0.16	0.03	0.04	0.03
CD 0.05	NS	NS	NS	NS	NS	NS
S.Em. $\pm$ (Y $\times$ V)	0.23			0.04		
CD 0.05 (Y $\times$ V)	NS			NS		
Inoculum level J₄ (I)						
0 (I ₁)	1.00 (0)	1.00 (0)	1.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
10 (I ₂)	3.00 (8)	2.84 (7)	2.92 (8)	2.55 (370)	2.55 (370)	2.55 (370)
100 (I ₃)	4.86 (23)	4.58 (20)	4.72 (22)	2.88 (787)	2.92 (724)	2.90 (756)
1,000 (I ₄)	11.30 (129)	11.11 (125)	11.20 (127)	3.70 (5,395)	3.70 (5,389)	3.70 (5,392)
10,000 (I ₅)	11.68 (137)	11.73 (139)	11.70 (138)	3.84 (6,959)	3.78 (6,441)	3.81 (6,700)
S.Em. $\pm$	0.32	0.41	0.26	0.04	0.07	0.04
CD 0.05	0.94	1.18	0.73	0.13	0.19	0.11
S.Em. $\pm$ (V $\times$ I)	0.46	0.57	0.37	0.06	0.09	0.06
CD 0.05 (V $\times$ I)	NS	NS	NS	NS	NS	NS
Year	NS			NS		
S.Em. $\pm$ (Y $\times$ I)	0.37			0.06		
CD 0.05 (Y $\times$ I)	NS			NS		
S.Em. $\pm$ (Y $\times$ V $\times$ I)	0.52			0.08		
CD0.05 (Y $\times$ V $\times$ I)	NS			NS		
CV %	14.4	18.4	16.4	4.7	7.2	6.1

Figures in parentheses are original value

Table 8 Comparative pathogenicity of reniform nematode on root-knot resistant and susceptible bidi tobacco: Effect on final population

Treatment	Final population (Log x + 1 trans.)			Rr = Pf/Pi
	2011	2012	Pooled	
Variety (V)				
ABT10 (V ₁)	2.57 (2,444)	2.52 (2,254)	2.54 (2,349)	1.1
A119 (V ₂)	2.64 (3,079)	2.63 (3,032)	2.63 (3,055)	1.4
S.Em. ±	0.03	0.04	0.02	
CD 0.05	NS	0.11	0.06	
S.Em. ± (Y × V)	0.03			
CD 0.05 (Y × V)	NS			
Inoculum level J ₄ (I)				
0 (I ₁)	0.00 (0)	0.00 (0)	0.00 (0)	
10 (I ₂)	2.56 (378)	2.55 (377)	2.56 (377)	37.7
100 (I ₃)	2.89 (810)	2.81 (745)	2.85 (777)	7.8
1000 (I ₄)	3.71 (5,523)	3.71 (5,514)	3.71 (5,518)	5.5
10,000 (I ₅)	3.84 (7,097)	3.80 (6,580)	3.82 (6,838)	0.7
S.Em. ±	0.04	0.06	0.04	
CD 0.05	0.12	0.17	0.10	
S.Em. ± (V × I)	0.06	0.08	0.05	
CD 0.05 (V × I)	NS	NS	NS	
Year	NS			
S.Em. ± (Y × I)	0.05			
CD 0.05 (Y × I)	NS			
S.Em. ± (Y × V × I)	0.07			
CD 0.05 (Y × V × I)	NS			
CV %	4.6	6.5	5.6	

Figures in parentheses are original value

Plate 5 Effect of reniform nematode on root growth of ABT10

Plate 6 Effect of reniform nematode on root growth of A119

Significant interaction $V \times I$ for plant height and number of egg mass per plant also indicated similar trend indicating 1,000 J_4 per plant as damaging to A119 and ABT 10. Number of leaves per plant was significantly reduced at initial inoculum of 100 J_4 per plant in A119 while at 1,000 J_4 per plant in ABT 10.

Thus, the above results revealed that between two varieties, root-knot resistant ABT 10 was significantly superior to root-knot susceptible A119 with respect to plant growth characters like plant height, fresh and dry shoot weight, number of leaves per plant, fresh root weight and growth index and as good as A119 with respect to number of females per plant and soil population during both the years and in pooled analysis. Both the varieties were identical to each other with respect to

number of eggs per egg mass, number of egg mass per plant during 2011 and 2012 as well as for final population during 2011 but differ significantly from each other in pooled analysis for these characters. Both the varieties were uniform with respect to infection and multiplication of reniform nematode but they differ in plant growth characters. This may be due to profuse and fibrous root system and inherent capacity of ABT 10 compared to tap root system in A119. Under field conditions, ABT 10 has achieved 17 per cent higher yield over A119 in root-knot sick field (Patel and Patel 2010).

Among inoculum levels, initial inoculum of 1,000 J_4 (I_4) of reniform nematode found damaging to plant height, fresh and dry shoot weight, number of leaves per plant, fresh root weight and root growth index with good nematode multiplication. Interaction ($V \times I$ and $Y \times V \times I$) for fresh shoot weight also revealed that initial inoculum of 1,000 J_4 (I_4) of reniform nematode significantly reduced the fresh shoot weight in both ABT 10 and A119 varieties. Reduction in plant growth of bidi tobacco viz., seedling fresh and dry weight, root weight and plant height due to reniform nematode was reported by Patel (1981) and Patel et al. (1986a, b). Initial inoculum of 1000 reniform nematode per plant was found damaging to brinjal (Jiji and Venkitesan 1989), pea (Vats and Dalal 1998) and cotton (Patel et al. 2004).

Increase in initial inoculum level finally increased the population in each level with maximum in highest initial inoculum. However, maximum rate of reproduction was observed in lowest inoculum level of reniform nematode and there after decreased correspondingly with increase in initial inoculum level. This showed negative effects of high inoculum density on reproduction of reniform nematode. Similar observations were also made by Khan and Khan (1973) and Patel and Patel (1990).

Thus, it is concluded that root-knot resistant ABT 10 was found significantly superior to root-knot susceptible A119 with respect to plant growth characters and as good as A119 with respect to multiplication of reniform nematode. Initial inoculum of 1000 J_4 of the nematode found damaging to both, ABT 10 and A119 varieties of bidi tobacco.

Fig. 1 Effect of reniform nematode on Bidi tobacco: Shoot growth

Fig. 2 Effect of reniform nematode on Bidi tobacco: Number of leaves & root growth

Fig. 3 Effect of reniform nematode on Bidi tobacco: Nematode multiplication

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