INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19F–201/2020/24–6–1401–1408
DOI: 10.17957/IJAB/15.1576
http://www.fspublishers.org

Full Length Article

Synergistic Effect of *Meloidogyne incognita* and *Rhizoctonia bataticola* Causing Root Rot Diseases of Cotton

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Received 18 December 2019; Accepted 06 July 2020; Published 10 October 2020

Abstract

Root rot disease complex attributed to *Meloidogyne incognita* and *Rhizoctonia bataticola* is a serious threat to cotton in areas having warm climate. In this study, the impact of *M. incognita* and *R. bataticola* interaction was assessed at different levels of resistance and susceptibility in cotton. *M. incognita* and *R. bataticola* were inoculated on various cotton varieties, alone and in combination. Variety CRS-134 showed high resistant against *R. bataticola* after individual inoculation with 1.7 disease severity ratings (5–10% infection) whereas varieties FH-4243, MNH-554 and FH-183 were moderately resistant with 2.2, 2.1 and 2.4 disease severity ratings, respectively. Inoculation of *M. incognita* and *R. bataticola* in combination significantly enhanced the root rot severity in cotton. Results showed that in the presence of both *M. incognita* and *R. bataticola*, the disease severity increased to 6.2 in CRS-134, 4.9 in FH-4243, 5.9 in MNH-554 and 3.6 in FH-183. Hence, the synergistic effect of *M. incognita* and *R. bataticola* has drastic impact on cotton and could be minimized using resistant varieties. © 2020 Friends Science Publishers

Keywords: Cotton; *Meloidogyne incognita*; *Rhizoctonia bataticola*; Root rot; Synergistic effect

Introduction

Cotton is cultivated as an annual crop and shares a great part in the world's economy. Pakistan is the fourth cotton producing country in the world; however, it stands at 10th position in terms of yield (Shuli *et al*. 2018). It is the backbone of Pakistan economy; its contribution in GDP (Gross Domestic Product) is 0.8% and 4.5% in agriculture value addition (Economic Survey of Pakistan 2018–2019).

Cotton grows well in areas having 50 mm rainfall annually with heavy showers at the time of boll formation (Nazir 2007). Maximum yield in cotton depends on unfavorable temperature conducive for disease development and minimum insect pest attacks throughout the growing season. Among all factors responsible for low yield, plant parasitic nematodes such as *Meloidogyne incognita* and root rot fungi such as *Rhizoctonia bataticola* are considered key pests producing galls and rotting on cotton roots (Agrios 2005; Anwar and Mckerny 2007). Many studies on interactions between fungi and endoparasitic nematodes have been well documented (Powell 1971; Tu and Cheng 1971; Kellam and Schenck 1980; Atilano *et al*. 1981; Edin *et al*. 2019). *Meloidogyne* spp. not only causes malfunctioning of roots but also facilitates penetration of fungal pathogens (Singh 1975).

Pakistan lies between 24^{o} 00' N and 79^{o} 00' E, with subtropical climate and is vulnerable to climate change. The favorable conditions for the optimal growth of *Meloidogyne* spp. are short winter, high temperature, sandy loam soil and hot climate (Maqbool 1987). Srinivas *et al*. (2017) tested the

To cite this paper: Khan MA, SA Khan, H Riaz, N Ahmad, R Binyamin, W Ashraf, M Ishtiaq, M Ali, MA Zeshan, Q Shakeel, RM Ikram, U Waheed, A Mahmood, MY Wong (2020). Synergistic effect of *Meloidogyne incognita* and *Rhizoctonia bataticola* causing root rot diseases of cotton. *Intl J Agric Biol* 24:1401–1408

effect of seven temperature regimes on growth of *R. bataticola* and observed maximum mycelial growth at 35°C followed by 30°C and 25°C. Anwar and Mckerny (2007) reported that environmental changes particularly favor root rot fungi and root-knot nematodes, thus, their interaction leads to the crop failure.

M. incognita and *R. bataticola* are more prevailing pathogens in cotton growing areas of Sindh and Punjab (provinces of Pakistan) and responsible for high yield losses in cotton (Iqbal *et al*. 2012; Khan *et al*. 2017). The modifications induced by root-knot nematodes, either local or systemic, increase the susceptibility of host plants to other soil-borne fungi (Siddique *et al.* 2004). Cotton varieties cultivated in Pakistan are unable to reach their genetic potential because of biotic (root rot fungi; root knot nematodes) and abiotic (temperature) factors. The data presented in literature indicated that there are few resistant varieties of cotton against root-knot nematodes (Cook 1997; Robinson 1997; Kirkpatrick 1989; Anwar and Mckenry 2007; Khan *et al.* 2017). Using resistant varieties is a cheaper, more effective and eco-friendly approach for the management of *Meloidogyne* spp. (Sultana *et al.* 2013; Becker *et al*. 2003). This study was planned to identify resistant varieties of cotton against these potential pathogens and to evaluate the synergistic effect of *M. incognita* and *R. bataticola* on cotton.

Materials and Methods

Collection of cotton varieties

Thirty cotton varieties were collected from different research stations and institutes (*i.e*. Cotton Research Station Multan, Vehari, Bahawalpur, Faisalabad and Cotton Research Institute Multan). The experiments were done in research area at Department of Plant Pathology, University of Agriculture Faisalabad, under greenhouse trial following three sets using three replicates per experiment under completely randomized design. Firstly, the screening of cotton varieties was done to assess their responses against *M. incognita* and *R. bataticola* whereas their interaction was studied in next experiment. All the experiments were repeated twice.

Screening against *M. incognita*

The earthen pots having diameter of 20 cm were sterilized with 4% formaldehyde solution. The soil having 6% clay, 70% sand, 3% organic matter and 21% silt used in experiments was thoroughly mixed, air dried and sieved (3.5 mm pore size sieve) to remove debris and stones. The soil was also sterilized at 120°C for 20 min in an oven and then stored for two weeks at 25°C (Talavera and Mizukubo 2003). After germination, one plant per pot was maintained. The irrigation of plants was done carefully. The excessive irrigation or overhead watering was avoided to eliminate the risk of nematode drying or leaching out of the soil, especially for the first few days after nematode inoculation. *M. incognita* (isolated from cotton and identified based on morphological characteristics) was mass cultured on the roots of the susceptible tomato variety viz. Money Maker by single egg mass culture for regular supply. Second stage juveniles (J_{2s}) were extracted according to procedure described by Hussy and Barker (1973). Nematode suspension was prepared by pouring culture into a measuring cylinder and mixed vigorously by stirring and blowing. The counting of nematodes was done by taking 1 mL aliquots in a counting dish, repeated thrice and total population was estimated by multiplying the mean of three aliquots with total volume. Approximately 1000 nematodes were inoculated per pot after 60 days of planting. Root-knot galling index rated 0 to 5 was used in experiments to study the response of cultivars against *M. incognita* (Quesenberry *et al*. 1989; Anwar and Mckenry 2007) (Table 1).

Screening against *R. bataticola*

Resistance of cotton varieties was also evaluated against *R. bataticola*, a fungus causing root rot. *R. bataticola* (isolated from infected cotton roots and identified based on morphological characteristics) was cultured on PDA, 39 g per 1000 mL of water, in a 9 cm Petri plate. After pouring and inoculation, plates were kept at 28±2°C in an incubator (Sharma *et al*. 2012). The inoculation of *R. bataticola* was done on sixty days old cotton plants by picking the fungal colony along with PDA with spatula at the rate of 2 g mycelial mat/plant. The disease severity was calculated using appropriate disease rating scale (Ruppel *et al*. 1979) (Table 2).

Interaction of *M. incognita* and *R. bataticola*

A total of ten varieties were chosen, five varieties; CM-482, FH-169, MNH-554, FH-183, BT-8 were selected on the basis of resistant/susceptible response against *M. incognita* and five varieties; FH-177, P-5, CRS-2007, FH-4243, CRIS-134 on the basis of resistant/susceptible response against *R. bataticola* to assess the synergistic effect of both pathogens. *M. incognita* were applied by making holes around each plant at rate of 1 J_2 /g soil. *R. bataticola* was inoculated by picking the fungal colony along with PDA with spatula at the rate of 2 g mycelial mat/plant. The experiment was conducted in three sets and the data was collected after 7, 15 and 30 days. The parameters calculated were J_3 stage, J_4 stage, J_2 second stage, root rot severity, dry shoot weight, fresh shoot weight, dry root weight and fresh root weight. Data was managed by calculating means of repeated experiments and data presented in tables are from all replicated experiments. Standard errors of mean were calculated in Microsoft Excel 2010 and were statistically analyzed using Statistics 8.1 and SAS 9.3 software at 5% significant level (Steel *et al*. 1997).

Table 1: Root-knot galling index (Quesenberry *et al*. 1989; Anwar *et al*. 2007)

Ratings	Number of galls	Response
0	No gall	HR
1	1-2	R
2	3-10	MR
3	11-30	MS
4	31-100	S
5	> 100 galls per root system	HS

Table 2: Disease rating scale of root rot of cotton (Ruppel *et al*. 1979)

Scale	Status	Root severity
0	HR	No visible lesions on roots and yellowing of leaves.
1-2	R	Superficial, arrested dry lesions, at the point of inoculation, non-active lesions on tap root, no rooting. Total infected area <5%(1)or 5-10%(2)
2.1-4	MR	Deep dry rot at point of inoculation total infected area 10-25% (2.1-3) or 25-50% (3.1-4).
4.1-6	MS	Extensive rot of upper half of tap root. Total infected area 50-75%(4.1-5)or >75%(5.1-6)
6.1-8	S	More than 75% of tap root blackened, with rot extended well into the interior (6.1-7), roots usually misshapen most of the foliage yellowed and wilted (7.1-8).
8.1-9	HS	Plant dead 100% rotted, plants can be easily pulled from ground.

Table 3: Screening of cotton cultivars against *M. incognita*

S. No.	Varieties	No. of Galls	Galling Index	Response	S. No.	Varieties	No. of Galls	Galling Index	Response
1	BS-252	461.00a	5a	HS	16	CM-482	171.33fghij	5a	HS
2	S-one 886	127.67jkl	5a	HS	17	NIBGE-2	187.67efghi	5a	HS
3	MNH-554	36.67nop	3.6d	MS	18	A-501	204.67efg	5a	HS
4	FH-183	15.67op	2.6e	MR	19	BH-186	351.00c	5a	HS
5	PB-896	45.33nop	4cd	S	20	VH-329	155.67ghijk	5a	HS
6	FH-177	239.00de	5a	HS	21	CRS-2007	146.67hijk	5a	HS
7	FH-169	111.00klm	4.6ab	S	22	S-3	395.00bc	5a	HS
8	K-2129	267.67d	5a	HS	23	CIM-573	439.33ab	5a	HS
9	Akbar 802	67.67mno	4.3bc	S	24	FH182	386.33bc	5a	HS
10	MNH 886	193.33efgh	5a	HS	25	BT-12	218.33def	5a	HS
11	FH-142	407.00abc	5a	HS	26	BT-8	2.67p	1.6f	R
12	CM-615	81.33lmn	4.3bc	S	27	P-5	132.67ijkl	5a	HS
13	Red acala	219.67def	5a	HS	28	BH-172	386.33bc	5a	HS
14	CRIS-134	111.00klm	4.7ab	S	29	BT-10	144.00hijk	5a	HS
15	FH-4243	168.33fghij	5a	HS	30	P-11	5.33p	2f	MR

Values sharing common letters in each column do not differ significantly at $P \leq 0.05$ according to least significant difference test.

Results

Screening of cotton varieties against *M. incognita*

In this experiment number of galls was calculated and results showed that the number of galls varied significantly among all varieties. The varieties BS-252 (461) and CIM-573 (439.3) showed maximum number of galls. The smaller number of nematode galls was counted in variety FH-183 (15.6), P-11 (5.3) and BT-8 (2.6). Overall, twenty-one varieties showed highly susceptible response while only four varieties showed susceptible response to *M. incognita*. MNH-554 was moderately susceptible variety whereas two varieties (P-11 and FH-183) were moderately resistant. Only single variety BT-8 showed resistant response (Table 3).

Screening of cotton cultivars against *R. bataticola*

Only one variety (CRIS-134) showed resistant response against *R. bataticola*. Overall, nine varieties were moderately resistant; eleven varieties were rated moderately susceptible whereas eight varieties were susceptible to *R. bataticola*. Maximum disease severity (8.1) was calculated in variety FH-177 (Table 4).

Screening of cotton cultivars infected with *Meloidogyne incognita* and *Rhizoctonia bataticola*

Results showed that presence of *M. incognita* significantly induced severe root rot in those varieties that were resistant against *R. bataticola*. CRS-2007, FH-4243 and CRIS-134 were moderately susceptible, moderately resistant and resistant against *R. bataticola* but they were highly susceptible and susceptible against *M. incognita*, respectively (Table 3, 4). According to results taken after 7 days of data collection shown positive increase in disease severity as 1.4% root rot severity was noted in CRS-2007, 1.3% severity in FH-4243 and 2.1% severity in CRIS-134 with 1.33 g, 1.5 g and 1.5 g fresh root weight whereas 2.4 g, 2.3 g and 2.7 g fresh shoot weight, respectively. Number of juveniles (J_2) isolated from infected roots of varieties CRS-2007, FH-4243 and CRIS-134 were 82.2, 64.3 and 130.8, respectively (Table 5). Correlation analysis (0.976^{**} =Pearson's correlation coefficient) and regression equation

Table 4: Screening of cotton cultivars against *R. bataticola*

S. No.	Varieties	Severity	Status	S. No.	Varieties	Severity	Status
1	BS-252	6.13±0.14 d	S	16	CM-482	7.50±0.20b	S
2	S-one 886	6.97±0.08c	S	17	NIBGE-2	6.40±0.20d	S
3	MNH-554	2.13±0.14k	MR	18	A-501	5.43±0.24e	MS
4	FH-183	2.47±0.18jk	MR	19	BH-186	4.60±0.17f	MS
5	PB-896	3.07±0.08hi	MR	20	VH-329	4.43±0.20f	MS
6	FH-177	8.10±0.11a	HS	21	CRS-2007	5.57±0.17e	MS
7	FH-169	5.57±0.24e	MS	22	S-3	4.50±0.11f	MS
8	K-2129	3.57±0.08g	MR	23	CIM-573	5.23±0.20e	MS
9	Akbar 802	2.67±0.14ij	MR	24	FH182	6.10±0.11d	S
10	MNH 886	6.40±0.20d	S	25	BT-12	4.63±0.17f	MS
11	FH-142	3.43±0.20gh	MR	26	BT-8	5.43±0.6e	MS
12	CM-615	6.40±0.17d	S	27	P-5	7.37±0.12bc	S
13	Red acala	3.57±0.08g	MR	28	BH-172	4.33±0.12f	MS
14	CRIS-134	1.70±0.11l	R	29	BT-10	5.57±0.21e	MS
15	FH-4243	2.20±0.15k	MR	30	P-11	3.57±0.12g	MR

Values sharing common letters in each column do not differ significantly at P ≤0.05 according to least significant difference test. [R= resistant, MR= moderately resistant, S= susceptible, MS= moderately susceptible, HS= highly susceptible]

Table 5: Screening of cotton cultivars infected with *M. incognita* and *R. bataticola*

After 7 days								
Varieties	J2 second stage	J2developing stage	J4	Root rot	FRW	DRW	FSW	DSW
FH-177	138.83±2a	0.83±.16h	0.00	2.6±0.05ab	1.1±0.05g	0.50±0.05f	2.9±0.11e	1.4±0.05d
P-5	119.4±2d	4.93±0.59g	0.00	1.9±0.05cd	1.63±0.03e	0.83±0.03de	2.6±0.05efg	1.27±0.03de
CRS2007	82.2±1.5f	11.8±0.55e	0.00	1.4±0.29de	1.33±0.03f	0.46±0.13f	2.4±0.05fgh	1.17±0.03de
FH-4243	64.3±2g	19.56±0.52c	0.00	1.3±0.08e	1.5±0.05e	0.73±0.03e	2.3±0.05gh	1.13±0.03e
CRIS-134	130.8±2.8b	0.66±0.16h	0.00	2.1±0.05bc	1.5±0.05e	0.73±0.03e	2.7±0.05ef	1.33±0.03de
CM-482	132.4±1.2b	14.43±0.29d	0.00	2.7±0.05a	1.1±0.05g	0.53±0.03f	2.1±0.05hi	1.17±0.16de
FH-169	125.3±0.92c	7.47±0.29f	0.00	2.3±0.05abc	1.8±0.03d	0.9±0.05cd	1.93±0.03i	0.80±0.05f
MNH554	98.7±0.89e	53.16±1.52a	0.00	1.9±0.05cd	2.1±0.05c	1b±0.05c	3.53±0.12d	1.8±0.1c
FH-183	55.4±2.4h	13.37±0.96de	0.00	0.7±0.37f	2.3±0.05b	1.06±0.03b	4.4±0.05c	2±0.05c
BT-8	27.9±1.3i	21.93±0.74b	0.00	0.3±0.33fg	2.5±0.05a	1.1±0.0ab	5.06±0.08b	2.37±0.03b
After 15 days								
Varieties	J2 second stage	J2developing stage	J4	Root rot	FRW	DRW	FSW	DSW
FH-177	20.1±2.8a	4.83±0.44g	7.40±0.20h	4.6±0.05b	2.9±0.08h	1.3±0.1f	5.5±0.05hi	2.7±0.05f
P-5	13.5±0.31c	17.63±0.37e	30±0.28e	3.3±0.05e	3.3±0.05f	1.53±0.03e	5.8±0.05g	2.83±0.03ef
CRS2007	7±0.31e	29.53±0.29c	80.70±0.62b	3.8±0.05d	3.8±0.05e	1.83±0.03d	6.1±0.05f	2.9±0.05def
FH-4243	17.5±0.45ab	21.83±0.76d	44.93±0.38d	2.8±0.05f	3.3±0.05f	1.6±0.05e	6.4±0.05e	3.1±0.05cde
CRIS-134	15±0.50bc	93.23±1.51a	98.47±1.46a	4.4±0.05c	3g±0.05h	1.47±0.03e	5.4±0.05i	2.67±0.03f
CM-482	17.3±0.55b	8.7±0.43f	8.87±0.40h	4.8±0.06a	3.1±0.05g	1.53±0.03e	5.6±0.05h	2.7±0.05f
FH-169	16.7±0.72b	16.2±0.41e	21.23±0.46f	4.8±0.05a	4±0.05d	1.97±0.03cd	6.3±0.05e	3.07±0.08cde
MNH554	10.5±0.37d	33.90±0.45b	57.50±0.62c	4.2±0.03c	4.23±0.03c	2.03±0.03bc	7±0.05d	3.37±0.03c
FH-183	6±0.28e	22.5±0.45d	20.3±0.33f	1.9±0.03g	4.46±0.03b	2.17±0.06b	7.4±0.05c	3.2±0.35cd
BT-8	2.9±0.24f	3.90±0.20g	16.00±0.37g	1.8±0.05h	4.67±0.03a	2.16±0.03b	7.8±0.05b	3.8±0.05b
After 30 days								
Varieties	J2 second stage	J2developing stage	J4	Root rot	FRW	DRW	FSW	DSW
FH-177	0.00	44.53±1.25b	28.47±0.46a	6.3±0.05c	4.5±0.05g	2.07±0.03efg	6.93±0.08gh	3.2±0.05ef
P-5	0.00	33.0±1.4d	25.47±0.29c	5.3±0.05f	4.1±0.05i	1.9±0.11g	7.5±0.05ef	3.47±0.03de
CRS2007	0.00	22.5±0.45f	14.50±0.36e	5.6±0.05e	4.3±0.05h	2±0.05fg	7.9±0.05de	3.67±0.12cd
FH-4243	0.00	18.3±0.47g	22.43±0.29d	4.9±0.05g	4.8±0.05f	2.1±0.03ef	7.2±0.05fg	3.09±0.06efg
CRIS-134	0.00	54.47±0.55a	29.17±0.61a	6.2±0.05c	4.3±0.05h	2.03±0.03efg	6.7±0.15h	3±0.05fg
CM-482	0.00	36.2±0.66c	26.83±0.21b	6.9±0.03a	4.2±0.05hi	1.97±0.03fg	6.1±0.15i	2.8±0.05g
FH-169	0.00	13.27±0.13h	12.07±0.52f	6.7±0.05b	5±0.05e	2.2±0.05de	8.3±0.15d	3.6±0.10d
MNH554	0.00	30.23±0.52e	7.06±0.06g	5.9±0.03d	5.4±0.05d	2.33±0.05d	9.6±0.05c	4±0.15c
FH-183	0.00	3.1±0.36i	5.23±0.12h	3.6±0.05h	5.7±0.05c	2.6±0.05c	10.07±0.08c	4.6±0.11b
BT-8	0.00	3.10±0.05i	7.37±0.18g	3.3±0.05i	6.2±0.05b	2.83±0.03b	11.5±0.15b	4.9±0.05b

Values sharing common letters in each column do not differ significantly at P ≤0.05 according to least significant difference test.
J= juvenile, FRW= fresh root weight, DRW= dry root weight, FSW= fresh shoot weight, DSW= dry shoot weight

(y=0.0194x-0.1553) of root-knot nematode (*M. incognita*) with root rot fungus (*R. bataticola*) showed highly significant relationship (R^2=0.9314) between *M. incognita* (J_2 second stage) and *R. bataticola* after 7 days at P<0.01 (Fig. 1; Table 6). Data collected after 15 days shown 3.8% disease severity in variety CRS-2007, 2.8% in FH-4243 and 4.4% in CRIS-134. Variety FH-177 and CM-482 was highly susceptible and susceptible to *M. incognita* and *R. bataticola* with maximum disease severity, 4.6% and 4.8%, respectively. Increase in disease severity in cultivars resistant to *R. bataticola* represents the direct involvement of nematodes as the number of J_2 developing stage (J_3)

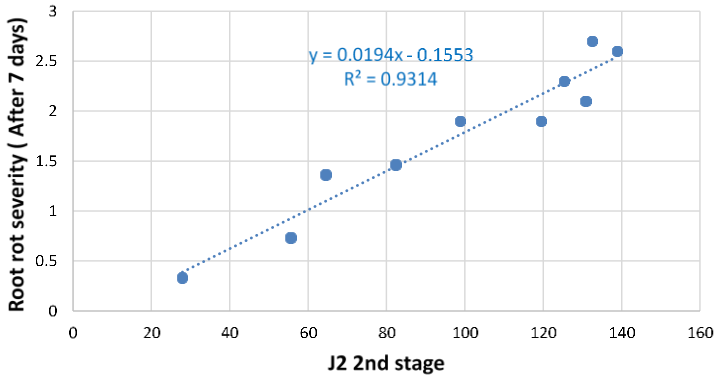

Fig. 1: Regression equation showing the effect of *M. incognita* and *R. bataticola* on root rot disease severity

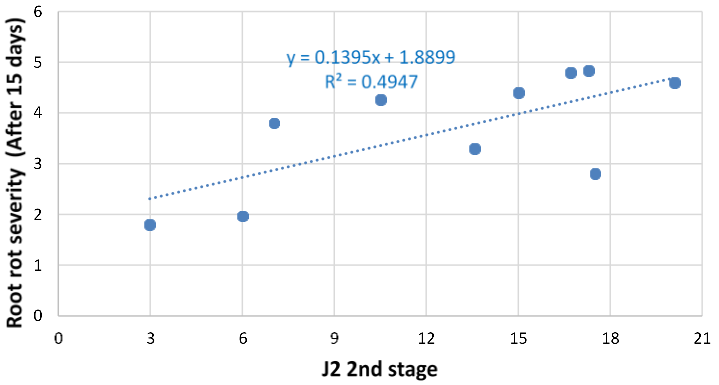

Fig. 2: Regression equation showing the effect of *M. incognita* and *R. bataticola* on root rot disease severity

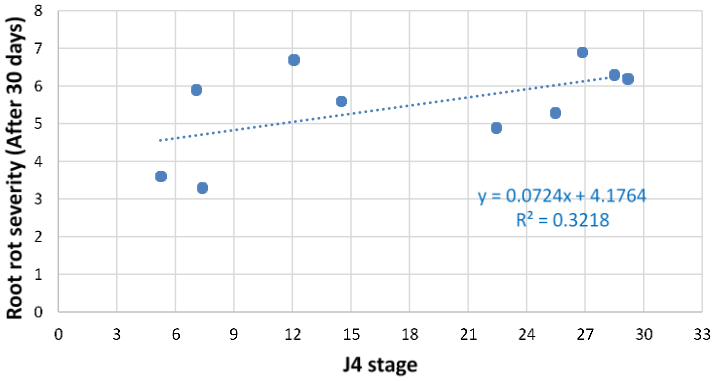

Fig. 3: Regression equation showing the effect of *M. incognita* and *R. bataticola* on root rot disease severity

counted in CRS-2007, FH-4243 and CRIS-134 was 29.53, 21.83 and 93.23 with 3.8 g, 3.3 g and 3 g fresh root weight and 6.1 g, 6.4 g and 5.4 g fresh shoot weight, respectively showing highly significant correlation (0.813^{**} =Pearson's correlation coefficient: R^2=0.4947) between *M. incognita* and *R. bataticola* at P<0.01 (Table 5 and 6: Fig. 2). After 30 days no J_{2s} were isolated from the samples whereas number of J_4 counted in varieties, FH-4243, CRIS-134, MNH-554, resistant to *R. bataticola* were 22.43, 29.17 and 7.06, respectively. A significant relationship (0.694^{*} =Pearson's correlation coefficient: R^2=0.3218) was observed between nematodes (J_4) and root rot severity at P<0.05 (Table 6: Fig. 3). Varieties that were moderately resistant (FH-183) and resistant (BT-8) against nematode showed minimum disease severity with maximum fresh shoot weight and fresh root weight in all experiments after 7, 15 and 30 days (Table 5).

Discussion

M. incognita is a very devastating and wide spread plant

Table 6: Correlation of *M. incognita* with *R. bataticola*

Stage	After 7 days
J_2 Second stage	Root rot severity
	0.976** 0.000
	After 15 days
J_2 Second stage	Root rot severity
	0.813** 0.002
	After 30 days
J_4 stage	Root rot severity
	0.694* 0.018

Upper values indicated Pearson's correlation coefficient;
Lower values indicated level of significance at 5% probability.
* = Significant (P<0.05); ** = Highly significant (P<0.01)

parasitic nematode. It not only causes damage to the roots but also provide space for entry to other soil-borne microorganisms. Cultivation of resistant varieties is a cheaper, more eco-friendly and effective method to reduce the population of *M. incognita*. Zhan *et al.* (2018) reported that cultivars breed with high level of resistance could reduce *Meloidogyne* population below economic damage. Mohanta and Mohanty (2012) conducted experiment to screen fifty-six okra cultivars/germplasm for their resistance to *M. incognita*. Present results are in line with these findings as the thirty cotton varieties were evaluated against root-knot nematode. Three varieties showed moderately resistant and resistant responses with the lowest nematode population whereas all other varieties showed susceptible responses with poor vigor and growth. Limited work has been done and reported on the screening of cotton varieties against *M. incognita*. This study is also supported by Anwar and Mckenry (2007) that there are few investigations on screening of cotton varieties against *M. incognita*. However, different researchers have reported varying levels of resistance and susceptibility on okra varieties against *M. incognita* (Sheela *et al*. 2006; Vinícius-Marin *et al.* 2017; Silva *et al*. 2019). Results in this study showed that susceptible varieties had more number of females and number of galls as compared to resistant cultivars. The findings in this study are in line with findings reported by Hussain *et al*. (2014). They found higher number of eggs, galls and females per plant in susceptible cultivars. After the entrance in roots, various compatible and incompatible reactions occur because of resistance (R) genes that lead to the visible reactions observed in the plant cells (Davis *et al.* 2000). The study concurs with the findings of Klink and Matthews (2009) and Ali and Abbas (2016) where they concluded that root-knot nematode infected all genotypes with different level of pathogenicity, which might be due to R genes. Mechanism of *M. incognita* infection and response of hosts had been elaborated by many researchers (Bendezu and Starr 2003; Williamson and Kumar 2006; Gheysen and Vanholme 2007; Ali *et al*. 2018). In this study, one variety was resistant and nine varieties were moderately resistant. Pande *et al.* (2004) supported the present evidences by conducting a trial on forty-seven chickpea germplasm against *R. bataticola* and among them 3 germplasm were resistant, 22 moderately resistant, 19 susceptible and 3 highly susceptible. Similar study was conducted by Khan *et al*. (2013) for sixty chickpea germplasm evaluation against *R. bataticola*, out of which 9 were resistant, 10 moderately resistant, 7 moderately susceptible, 17 susceptible and 17 highly susceptible.

Results from this study revealed that the presence of *M. incognita* significantly induced root rot severity in cotton varieties that were resistant against *R. bataticola*. This study concurred with Wheeler *et al*. (2019) that demonstrated the presence of *M. incognita* was favorable for the development of wilt symptom. Giant cells caused by nematodes produce metabolites that are significant source of food for *R. bataticola*. These swellings in roots increase fungal activity within root tissues and after colonization, the fungus moved into xylem tissues and caused wilting symptom (Hua *et al*. 2019). In this study, maximum disease severity was noted at second stage (J_2) of *M. incognita*. Correlation and regression equations for *M. incognita* and *R. bataticola* proved the significance of their interaction statistically. Interaction between nematode and fungi was first reported on cotton by Atkinson (1982). Al-Hazmi and Al-Nadary (2015) reported that in the presence of *M. incognita*, the maximum severity caused by *R. solani* was observed in *Phaseolus vulgaris*. Various studies has been conducted by several scientists on nematode and fungus interaction in various crops (Back *et al*. 2002; Back *et al*. 2006; Abuzar 2013; Safiuddin *et al*. 2014). Al-Hazmi and Al-Nadary (2015) reported the similar results that synchronized inoculation of fungus and nematode increased the disease index of fungus and root gall caused by nematodes. The cotton varieties resistant against *M. incognita* showed minimum disease severity with maximum fresh shoot weight and fresh root weight whereas there were variations in shoot-root weight in susceptible and resistant cultivars. Zwart *et al*. (2019) elaborated that affected plants produce more roots to overcome the limitations caused by nematodes and root efficiency reduced in the damage caused by root-knot nematode resulted in poor root-shoot ratio, the developing females withdraw the nutrients causing further damage, between the inoculum level and root weight a significant direct relationship was found, as the inoculum density increased, the root weight also enhanced. Setty and Wheeler (1968) and Afshar *et al*. (2014) explained that the higher root weight in affected plants might be due to amino acids, more tryptophan and larger amount of growth substance. It had inverse impact on shoot length. In this study inverse relationship was shown between root and shoot weight. The findings are contradictory to the hypothesis of Wareing (1970), that shoot and root are dependent on each other for carbohydrates, growth substances and nutrients. However, any reduction in root growth limit the shoot growth or vice versa.

Conclusion

This study concluded that interaction of *M. incognita* and *R. bataticola* disturbed the coordination between roots and

shoots leading to poor plant growth. The disease severity caused by *R. bataticola* with the presence of *M. incognita* increased to hundred percent. Thus, the cultivation of resistant and moderately resistant cotton cultivars in the field would help in reducing disease severity. Further studies are needed to investigate the interaction and resistant mechanism(s) as indicated in this study.

Author Contributions

MAK carried out research work. SAK and MYW provided technical support. HR, NA, RB, WA, MI, MA, MAZ, QS, RMI, UW and AM helped in writing the manuscript.

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