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Studies on interaction of nematode, *Pratylenchus delattrei* and fungal pathogen, *Fusarium incarnatum* associated with crossandra wilt in Tamil Nadu, India

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ABSTRACT

Investigations were under taken in pot culture experiment to assess a possible interaction between fungal pathogen of crossandra, *Fusarium incarnatum* and lesion nematode *Pratylenchus delattrei,* at various population density combinations, time of inoculation and their effect on plant growth and wilt incidence of crossandra. Among varies pathogen and nematode population densities tested, the inoculation of pathogen load @3% w/v and nematode @ 1/g soil resulted in maximum reduction of plant growth parameters *viz*., shoot length (49.8%), shoot dry weight (52.6%), root length (47.0%), root dry weight (47.4%), and flower yield (82.6%). More over maximum root lesion index of 3.9/1-5 scale, wilt disease incidence of (50%) and nematode population up to 365 per 200 cc soil, were also recorded. Other experimental results revealed that the treatment involving inoculation of nematode prior to fungal pathogen recorded more disease incidence (58.3%), nematode (381/200cc) and pathogen (12.1×10^3), populations and there was significant reduction in root length, root weight, shoot height and shoot weight when compared with inoculation of pathogen and nematode simultaneously or inoculation of pathogen prior to nematode.

KEY WORDS: Crossandra, *Fusarium incarnatum*, nematode, *Pratylenchus delattrei*, wilt

INTRODUCTION

Crossandra (Fire cracker) is an important commercial flower, mainly grown in India, Tropical Africa and Madagascar. Crossandra (*Crossandra infundibuliformis*) is affected by various fungal, bacterial, nematode and viral diseases. Among the various fungal diseases, wilt disease caused by *Fusarium* spp. is one of the major problem in Crossandra production and limits the crop cultivation. Some reports are also available stating that there is a consistent association of *Fuarium solani* and nematode complex in crossandra wilt that are causing major crop yield losses (Srinivasan, and Muthukrishnan,1975). The nematode *Pratylenchus delattrei* causes a serious damage to crossandra crop (Jonathan *et al* 2001). Nematode fungal interactions are important biological phenomena and are great significance in agriculture. Much experimental evidences indicated a biological interaction between nematodes and certain soil-born fungi (Botseas and Rowe,1994; Jonathan *et al* 1996, Bhagawathi *et al*., 2000). In some interactions the nematodes are not essential for the establishment and development of fungal pathogens. However, the nematodes usually assist and enhance the pathogencity mechanism of the fungus towards modifications in the host plant (Jordan,1987, Mauza and Webster,1992, Bowers *et al*., 1996). But in case of crossandra wilt till

date systemic work was not carried to find the extend of losses they cause, role of each pathogen and their interactive effect on disease incidence, pathogen population, plant growth and yield etc. Hence, keeping all this in view, the present study was carried out at Department of Plant Pathology, Agricultural College and Research Institute, TNAU, Madurai, to evaluate the interaction effect of these two organisms on the crossandra growth, yield and wilt incidence in two experiments. The first experiment was conducted to assess the population combination of nematode and fungal pathogen and other experiment included time of inoculation of nematode and fungal pathogen, one prior to the other.

MATERIALS AND METHODS

An intensive systemic survey was conducted to assess the wilt incidence in different crossandra growing areas of Tamil Nadu *viz.,* Madurai, Dindigul, Trichy, Karur and Thiruvellur etc during the crop season, August 2013 to June 2014. Ten Soil samples were collected from each farm from root zone of wilt affected crossandra plants in polythene bags using soil auger after removing top 2-3 inches of surface soil up to 15 inches. The samples were mixed homogeneously to constitute a composite sample and from the composite sample one kg of soil sealed and transported in cool boxes to the laboratory for nematode extraction. The wilt disease incidence was expressed using the formula

$$\text{Per cent disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

The experiments were carried out in glass house at Department of Plant Pathology, Agricultural College and Research Institute, TNAU, Madurai, from August 2013 to August 2014. The *Fusarium* was isolated from the diseased tissues of crossandra by tissue segment method following Rangaswami (1958). Nematode was isolated from soil by following Cobb"s sieving method and counted by suspending in known volume of water in a Doncaster counting dish and mean of the counts was taken. Similarly, the culture of lesion nematode *P. delattrei* collected from roots and soil by Cobb"s sieving method were maintained on crossandra growing pots after confirmation of the species.

The pathogen *F. incarnatum* was multiplied on sand-maize medium (Riker and Riker, 1936), and used as inoculums source. Earthen pots of five kg were filled with sterilized pot mixture containing red soil, sand and farm yard manure in equal proportions and two seedlings of crossandra were planted in each pot. The inoculation of nematode and fungus were carried as per treatment proposed in two experiments, by imposing of treatments from 10^{th} day of planting with a purpose to study the population density of nematode and fungal pathogen required to cause severe disease and their interactive effects on crossandra in first experiment and to know the time of occurrence of nematode and fungus one prior to other that lead to sever disease of crossandra in second experiment.

The treatments imposed in the first experiment includes T_1) Inoculation of

nematode alone (1/g soil), T_2) Inoculation of pathogen alone(3%)w/v, T_3) Inoculation of pathogen (3%) + inoculation of nematode (1/g), T_4) Inoculation of pathogen (3%) + inoculation of nematode (1/2g), T_5) Inoculation of pathogen (3%) + inoculation of nematode (1/4g), T_6) Inoculation of pathogen (2%) + inoculation nematode (1/g), T_7) Inoculation of pathogen (1%) + inoculation of nematode (1/g) , T_8) Control (No pathogen/No Nematode.

The treatments imposed in second experiment includes : T_1) Inoculation of nematode (1/g) + pathogen (3%), simultaneously T_2) Inoculation of nematode (1/g) seven days earlier followed by pathogen (3%), T_3) Inoculation of pathogen (3%) seven days earlier followed by nematode (1/g) T_4) Control check (No pathogen/No Nematode).

In the above two experiments each treatment was replicated six times in pots and the experiments were laid out in a completely randomized design (CRD) under glass house conditions. The experiments were terminated at 150 days after planting. Observations on plant growth parameters (shoot length (cm), shoot weight (g), root length (cm), root weight (g) and flower yield (g)) were recorded. The root lesion index was recorded following 1-5 scale (Ponochet, 1988). Data collected was statistically analyzed after making necessary transformations using the software OPISTAT.

RESULTS AND DISCUSSION

The associated fungal and nematode pathogens with crossandra wilt was isolated and identified based on both morphological and molecular characters as *F.incarnatum* by National Facility (NFCCI & FIS), Mycology and Plant Pathology Group, Agarkar Research Institute, Pune and the nematode by local nematologist at Agricultural College, Madurai. The interaction effects of *F.incornatum* and *P.delattrei* alone and in different population combinations were carried out on crossandra. The results of the interaction effects of these pathogens are presented in Table 1 and 2.

In general all the plant growth parameters viz.. shoot and root length, shoot and root dry weight were decreased significantly in all the treatments when compared to uninoculated check. The maximum percent reduction of shoot length and shoot dry weight was recorded in T_3 (49.8 and 52.6%) that is inoculation of pathogen @ 3% + nematode (1/g soil) followed by T_6 which included, inoculation of pathogen (2%) + inoculation of nematode (1/g) by recording 25.8 and 39.7 percent respectively.

The root length and root dry weight also decreased significantly in all the treatments as compared to uninoculated check. The maximum per cent reduction was observed in T_3 (47.0 and 47.4%) followed by T4 (38.8 and 35.8%) respectively, where as T_4 and T_6 are statistically on par with each other. Highest reduction in flower yield was observed in T_3 (82.6%) followed by T_6 (51.8%),when compared with uninoculated check. Overall the combination of fungus and nematode resulted in more reduction of plant growth and yield than by either of them alone. It was also observed that the population of nematode was more in combined application than each one alone, indicating the synergistic effect of fungus and nematode. Similar results were also already observed by Hosieni *et al* (2010).

As represented in Table 2, maximum population of nematode 365 per 200 cc soil and root lesion index of 3.9 was recorded in T_3 followed by T_6 with nematode population of 353 and root lesion index of 3.7, where as maximum number of pathogen colony

forming units were recorded in T_3 followed by T_4. Wilt incidence was also maximum in T_3 (50%) followed by T_6 with wilt incidence of 25.2 percent. Similar trend was observed by Pablo Castillo *et al* (1998) between *Pratylenchus thornei* and *F.oxysporium f.sp.ciceri* on chickpeas ,and Vidyasagar *et al* (2012) in tomato between *M.incognata* and *Rhizoctonia solani*. Khan,1993 also observed the same trend between *Pratylenchus loosi* and *Rhizoctonia solani* and stated that there is a synergistic effect that the role of nematode as a facilitator on fungus penetration into root by influence on host physic and physiology.

The experimental results represented in table 3 revealed that all plant growth parameters such as root length, shoot length, shoot dry weigh root dry weight and flower yield are decreased and pathogen population (nematode + fungi), root lesion index and wilt incidence are heigher in T_2 which comprising of inoculation of nematode (1/g) seven days earlier followed by pathogen (3%), when compared with T_1 (Inoculation of nematode (1/g) + pathogen (3%) simultaneously), or T_3 (inoculation of pathogen (3%) seven days earlier fallowed by nematode (1/g)). The similar results were also observed by Swaransingh *et al* (2010), between *M.incognita* and *Macrophomina phaseolina* on Lentil. Inoculation of nematode 7days early may prepare the roots for fungus invasion and synergistic effect resulting in increased penetration ability of the fungus or nematode influence the physiological changes in crossandra root and there by the root was more susceptible. It was also observed that combined interaction involving fungus application one week earlier to nematode decreased nematode reproduction and this might be due to production of adverse effect of fungus mass on the nematode penetration or fungal invasion of nematode feeding site. This supports the results recorded by Hoseini *et al* (2010) on tea plants between *P.loose* and *F. proliferatum* etc. Swaransingh *et al*. (2010) also recorded same results in lentil. Over all the experimental results supported the hypothesis that feeding injury by root lesion nematodes provides a direct avenue of entry of root infecting fungi into the root system and physical and physiological activity of nematode feeding on plant roots was also related to entry of root fungi into roots. An alternative explanation is that the nematode enhanced host susceptibility to mycelial growth of the fungus. Increased susceptibility of the plant might allow root infecting fungi to move quickly into roots by enhancing their ability to colonize the plant roots. Nematode feeding causes vast changes on hormonal balance and biological changes on host that make host susceptible to fungi.

CONCLUSION

These investigations provide baseline data for understanding the relationship of nematode and fungus and their role in causing wilt in crossandra. It was observed that nematode *P.delattrei* acts as predisposing factor for *F.incarnatum*. Hence, it is important that while designing management strategies both the pathogens should be taken in to consideration.

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Table 1: Studies on interaction between nematode *Pratylenchus delattrei* and fungus *F. incarnatum* population densities and their effect on the plant growth parameters of crossandra

Sl. No.	Treatments	Shoot				Root				Flower yield (g)	% decrease over control
		Height (cm)	Percent decrease over control	Dry weight (g)	Percent decrease over control	Height (cm)	Percent decrease over control	Dry weight (g)	% decrease over control		
1	Inoculation of nematode alone (1 nem/g soil)	50.4	2.6	20.9	31.3	19.2	31.7	32.0	27.4	8.0 (16.47)*	20.4
2	Inoculation of Pathogen alone(3%)	58.2	10.7	24.3	20.1	20.5	27.0	36.2	17.9	7.1 (15.45)	29.7
3	Inoculation of pathogen (3%) + inoculation of nematode (1/g)	32.7	49.8	14.4	52.6	14.9	47.0	23.2	47.4	1.8 (7.62)	82.6
4	Inoculation of pathogen (3%) + inoculation of nematode (1/2g)	50.3	22.8	19.5	35.9	17.2	38.8	28.3	35.8	5.7 (13.86)	43.2
5	Inoculation of pathogen (3%) + inoculation of nematode (1/4g)	52.3	19.7	20.1	33.9	20.1	28.5	33.0	25.2	6.6 (14.90)	34.5
6	Inoculation of pathogen (2%) + inoculation of nematode (1/g)	48.4	25.8	18.3	39.7	17.3	38.4	28.4	35.6	4.9 (12.76)	51.8
7	Inoculation of pathogen (1%) + inoculation of nematode (1/g)	49.2	24.5	21.2	30.3	18.0	35.9	30.2	31.5	7.3 (15.67)	27.7
8	Control (No pathogen/No Nematode)	65.2	-	30.4	-	28.1	-	44.1	-	10.1 (18.54)	
	CD (P=0.05)	0.23	-	0.26	-	0.22	-	0.46	-	0.09 (0.101)	
	SE(m)	0.08	-	0.09	-	0.08	-	0.16		0.03 (0.035)	

Each value is mean of six replicates.

*Figure in the parentheses are arc sine transformed values

Table 2: Studies on interaction between nematode *Pratylenchus delattrei* and fungus *Fusarium incarnatum* population densities and their effect on the occurrence of wilt of crossandra

T.No.	Treatments	Nematode population			Root lesion index	Fungal cfu/g soil 10^{-3} at 150 DAP	Wilt incidence (%)
		Initial population/200cc	population/200cc soil at 150 DAP	Percent increase over control			
1	Inoculation of nematode alone (1 nem/g soil)	200	350 (18.74)*	75.0	3.8 (2.19)*	0.0 (1.00)*	0.0 (1.00)*
2	Inoculation of Pathogen alone(3%)	0	0 (1.00)	0.0	0.0 (1.00)	9.8 (3.29)	8.3 (3.05)
3	Inoculation of pathogen (3%) + inoculation of nematode (1/g)	200	365 (19.13)	82.5	3.9 (2.21)	11.2 (3.49)	50.0 (7.14)
4	Inoculation of pathogen (3%) + inoculation of nematode (1/2g)	100	218 (14.80)	118.0	2.4 (1.84)	10.3 (3.36)	33.3 (5.86)
5	Inoculation of pathogen (3%) + inoculation of nematode (1/4g)	50	126 (11.27)	152.0	1.1 (1/45)	10.0 (3.32)	16.6 (4.20)
6	Inoculation of pathogen (2%) + inoculation of nematode (1/g)	200	362 (19.05	81.0	3.7 (2.17)	7.6 (2.93)	25.2 (5.12)
7	Inoculation of pathogen (1%) + inoculation of nematode (1/g)	200	353 (18.82)	76.5	3.6 (2.14)	8.7 (3.11)	8.3 (3.05)
8	Control (No pathogen/No Nematode)	-	0.00 (1.00)	-	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)
	CD (P=0.05)	-	4.27	-	0.16 (0.038)	0.13 (0.019)	0.45 (0.042)
	SE(m)	-	0.16	-	1.49 (0.013)	0.06 (0.007)	0.12 (0.015)

Each value is mean of six replicates.

*Figure in the parentheses are arc square root transformed values.

Table 3: Studies on interaction between nematode, *Pratylenchus delattrei* and fungus *Fusarium incarnatum* in *vivo* on the occurrence of wilt of crossandra and plant growth parameters

T.No.	Treatments	Shoot		Root		Nematode population/200cc soil		Percent increase in nematode population	Root lesion index	Fungal cfu/g soil 10^{-3} at 150 DAP	Wilt incidence (%)	Flower yield (g)
		Height (cm)	Dry weight (g)	Height (cm)	Dry weight (g)	Initial population	150 DAP					
1	Inoculation of nematode (1/g) + Pathogen (3%) Simultaneously	31.7	13.4	14.9	23.2	200	365 (19.13)**	82.5	3.90 (2.22)**	11.2 (3.49)**	41.7 (6.54)**	1.90 (8.07)*
2	Inoculation nematode (1/g) seven days earlier followed by pathogen (3%)	30.2	13.1	15.2	21.2	200	381 (19.54)	90.5	4.20 (2.28)	12.1 (3.62)	58.3 (7.70)	1.10 (6.07)
3	Inoculation of pathogen (3%) seven days earlier followed by nematode (1/g)	34.3	15.5	16.4	27.4	200	344 (18.57)	72.0	3.40 (2.09)	9.60 (3.26)	25.0 (5.10)	2.10 (8.33)
4	Control (No pathogen/No Nematode	63.2	29.4	27.1	42.1	-	0.00 (1.00)	0.0	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	9.33 (17.78)
	CD (P=0.05)	0.02	0.25	0.16	0.16	-	6.02 (0.16)	-	0.10 (0.022)	0.13 (0.020)	0.58 (0.055)	0.06 (0.1180
	SE(m)	0.07	0.09	0.05	0.05	-	2.03 (0.053)	-	0.03 (0.007)	0.05 (0.007)	0.19 (0.018)	0.02 (0.040)

Each value is mean of six replicates

*Figure in the parentheses are arc sine transformed values

** Figure in the parentheses are arc square root transformed values