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Studies on Interaction Between *Ceratocystis fimbriata* and *Meloidogyne incognita* on Pomegranate Wilt Complex

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Disease complexes involving nematode and fungi have gained momentum in the recent years. There was no conspicuous gall production in nematode alone and in combination with fungi. The affected roots showing brownish discolouration were observed in fungal spore suspension, fungal giant culture and fungal giant culture + *M. incognita* and *M. incognita* alone treatments. In uninoculated treatment there was no brownish discolouration. The two pathogens (*M. incognita*, *C. fimbriata*) adversely affected plant growth parameters like shoot and root length, fresh and dry weight of shoot, fresh and dry weight of root per plant. Among the two pathogens inoculated individually, maximum reduction in plant growth parameters noticed in fungal giant culture (*C. fimbriata*) treatment over control compared to fungal spore suspension, or *M. incognita*. Reduction in plant growth parameters caused by fungal spore suspension were noticed which is on par with *M. incognita*. With respect to of combined inoculations with nematode and fungi, greatest reduction in growth parameters was noticed in *M. incognita* + *C. fimbriata*. However, the treatment receiving the inoculum both pathogens, showed a highest reduction in plant growth parameters in comparison with other treatments.

Key words:*Ceratocystis fimbriata, Meloidogyne incognita*, Interaction, Wilt.

Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family punicaceae and pomegranate is a native of Iran. It is commercially an important fruit crop of both tropical and subtropical regions. In India, it is regarded as a "vital cash crop", grown in an area of 1, 16,000 ha with a production of 89,000 MT with an average productivity of 7.3 MT (Anon., 2012). Karnataka state has the distribution of cultivating pomegranate under tropical condition in an area of 12,042 ha with a production of 1, 29, 547 tonnes (Anon., 2012). Wilt caused by *Ceratocystis fimbriata* is the most severe disease in Karnataka which causes yellowing, drooping and death of pomegranate plant leading to loss to the farmers. There is no more information available on mode of spread of pathogen, predisposing factor for fungal pathogen, Root knot nematode infestation, their reproduction, symptom and interaction between *Ceratocystis fimbriata* and *Meloidogyne incognita*, hence the study was conducted.

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MATERIAL AND METHODS

Survey and collection of soil samples

A nematode random survey of the major pomegranate growing areas of the north Karnataka

was carried out by collecting soil and root samples, for the different plant parasitic nematodes associated with pomegranate. 28 samples were collected from rhizoshere of pomegranate crop during 2008-2009.Composite soil samples were collected with the help of a scoop from a depth of 15-20 cm from the soil at random. Each sample consisted of 10-15 soil cores. After 48 hrs the nematode suspension was collected and examined under a stereoscopic binocular microscope. The different plant parasitic nematodes present in the suspension were identified to the level of genus.

Pomegranate (cv Kesar) is used as the test plant to study the pathogenecity. The seedlings obtained from farmer Shanker Reddy of Koppal district. In general 20-30 days old seedlings of uniform size and growth and free from wilt were used for transplanting.

Soil sterilization

Sandy loam soil, from the field of Krishi Vigyan Kendra, Saidapur free from stones and hard lumps was obtained and mixed thoroughly with compost in 6:1 proportion. Steamed sterilized soil was used for culturing nematodes and also for root invasion studies by autoclaving the compost mixture at 120°C under 1.04 kg/cm^2 pressure for two hours then once again autoclaved after 48 hours. The autoclaved soil was allowed to cool to room temperature and later used for experimental work.

Pure culture of *M. incognita*

Pure culture of the root-knot nematode was obtained from Coleus *M. incognita* Horticultural College, Arabhavi, UHS, Bagalkot.

After picking up single egg mass, the perennial pattern of the adult female was verified before inoculating to the culture pots. Egg masses were transferred to the Petri dish partially filled with tap water and then it was incubated at room temperature. The larvae emerging from egg masses were pipetted into a small quantity of fresh water, stirred well and the suspension poured into the three holes made around base of the pomegranate plants.

Hatching of larvae and inoculation

The egg masses from stock cultures were isolated carefully to a wire gauze sieve containing two layers of facial tissue paper trimmed down to edge of the gauze and kept in a Petri dish holding sufficient water to remain in contact with the bottom of the wire gauze and wet the egg masses. The hatched larvae passed through the tissue paper and sank to the bottom of the petridish. After 24 hours the contents of the Petri dish were emitted into a beaker, diluted to a suitable volume and population counts made with the help of a Fenwick's multichamber counting slide.

Based on the requirement the suspension was diluted with water. Twelve days old transplanting the *pomegranate* seedlings in test pots, three holes of 2-2.5 cm deep were made on at 1.25 cm from the base of the plant suspension containing a pre-determined number of larvae was pipetted equally into all the holes. The holes were then filled by gently pressing the soil around the plants. The plants were watered to keep the soil moist.

Interaction studies with *M. incognita*

To establish interaction of *M. incognita* on pomegranate, a pot culture experiment was designed under glass house conditions. The treatment included inoculation of the nematode larvae of *M. incognita* @ 100 per pot alone and in combination with *C. fimbriata*. Four replication were maintained for each treatment. The pots were kept in the glass house for observation.

***C. fimbriata* culture**

Spore suspension

C. fimbriata isolated from naturally infected pomegranate root, was cultured on PDA, at 20±2°C for fifteen days. The spores were isolated by flooding the Petri plates with sterile distilled water and gently agitating the cultures with an artist's brush. Spores were filtered through four layers of cheese cloth, and spore was adjusted to required concentration by using haemocytometer.

Number of spore in the diluted suspension/millilitre = Average number of spore above

one large squareX1ml/0.004mm^3(or)

Number of spore in the diluted suspension/millilitre = No of spore counted x 250,000

This number has to be multiplied by the original dilution of the suspension to ascertain the density of original suspension in number of cells/ml (Karuna Vishunavat and Kolte, 2005).

Apparently healthy seedlings were soaked in spore suspension of *C. fimbriata* under vacuum for 30 minutes.

Giant culture

Sand corn meal medium was prepared in the proportion of 90:10 in order to get maximum inoculum of fungus. Four hundred gram of sand corn meal medium was taken in 1000 ml flasks and watered to 20 per cent of its weight and sterilized at 1.04kg /cm^2 pressure for one hour. The pure culture of C. *fimbriata* was inoculated flasks under aseptic condition and incubated at 25±1°C for 20 days. Four per cent of giant cultures was used for further studies.

T_1- Spore suspension alone
T_2-Giant culture alone
T_3-*M. incognita*alone
T_4- Giant culture+ *M. incognita*
T_5- Control
The data was statistically analysed.

RESULTS

Disease complexes involving nematode and fungi have gained momentum in the recent years. The data on interaction (Plate-1) between *M. incognita* and wilt inducing fungi are presented in Table 1.There was no conspicuous gall production in nematode alone and in combination with fungi. The affected roots showing brownish discolouration were observed in fungal spore suspension, fungal giant culture and fungal giant culture + *M. incognita* and *M. incognita* alone treatments. In uninoculated treatment there was no brownish discolouration.Significant reduction in shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight was noticed in all the treatments in comparison to uninoculated control.

Table 1. Influence of single or combined inoculations *Ceratocystis fimbriata, Meloidogyne incognita* on plant growth parameters, in pomegranate

Treatments	Length (cm)		Shoot weight (g)		Root weight (g)	
	Shoot	Root	Fresh	Dry	Fresh	Dry
F.S	76.50	10.75	15.75	6.62	4.88	2.12
F.G	43.50	8.25	9.75	4.00	2.88	1.37
M.i	56.50	10.00	11.25	4.75	3.13	1.25
FG+*M.i*	39.00	7.50	7.88	3.50	1.63	0.62
U. C	99.00	17.00	22.88	8.87	6.75	2.75
SEm±	0.43	0.43	0.96	0.43	0.02	0.43
CD @ 1%	1.15	1.15	2.52	1.15	0.05	1.15

F.S – Fungal spore suspension (*Ceratocystis fimbriata*)
F.G – Fungal giant culture (*Ceratocystis fimbriata*)
M.i- Meloidogyne incognita
F.G+ *M.i - Ceratocystis fimbriata + Meloidogyne incognita*
U.C – Uninoculated control

The two pathogens (*M. incognita*, *C. fimbriata*) adversely affected plant growth parameters like shoot and root length, fresh and dry weight of shoot, fresh and dry weight of root per plant. Among the two pathogens inoculated individually, maximum reduction in plant growth parameters noticed in fungal giant culture (*C. fimbriata*) treatment over control compared to fungal spore suspension, or *M. incognita*. Reduction in plant growth parameters caused by fungal spore suspension were noticed which is on par with *M. incognita*. With respect to of combined inoculations with nematode and fungi, greatest reduction in growth parameters was noticed in *M. incognita* + *C. fimbriata*. However, the treatment receiving the inoculum both pathogens, showed a highest reduction in plant growth parameters in comparison with other treatments.

In these interactions, it was observed that the effect of the combined inocula (*M. incognita* + *C. fimbriata*) on plant growth parameters was additive in nature, where inoculations were simultaneous where in the resultant effect on growth parameters was almost equal to sum total of individual effects. However in treatment receiving a combined inocula of all the two

organisms (*M. incognita* + *C. fimbriata*), the resultant effect was more than the simple additive effect.

T_1 - Fungal spore suspension (*C. fimbriata*)
T_2 -Fungal giant culture (*C. fimbriata*)
T_3 - *Meloldogyne incognita*
T_4 - *C. fimbriata*+*Meloidogyne incognita*
T_5 - Uninoculated control

Plate 1: Influence of single or combined inoculation of *Ceratocystis fimbriata* and *Meloidogyne incognita* on plant growth parameters in pomegranate

DISCUSION

Nematode and fungi cause severe damage to pomegranate. However, the effect on plantgrowth and wilt incidence increased when both pathogens were present together, increased incidence in the presence of nematode on Coleus was also reported by Senthamarai*et al.* (2006), Krishna Rao and Krishnappa (1994) on chickpea.

Plants receiving simultaneous inoculations of *M. incognita* and *Ceratocystis fimbriata* giant cultureshowed the greatest reductions in plant growth parameters as compared to other treatments. These findings are in conformity with those recorded by Bhabesh Bhagawati *et al.* (2007), Aberdeen and Patil Kulkarni (1969) who suggested that the greater damage in simultaneous inoculation of fungus giant culture and nematode might be attributed to the prior invasion of nematodes into the roots, thereby making the host more favourable for fungal infection by offering a metabolically rich substrate and/or nematode might also modify the rhizosphere, thus favouring the fungal growth.

Number of galls per plant, juvenile population in soil and root were maximum in nematode alone treatment, but were significantly less in other treatments receiving fungi. However, the treatment receiving concomitant inoculation of *M. incognita* and *Ceratocystis fimbriata giant culture*, giant culture alone and spore suspension alone were recorded significantly lowest number of galls and nematode population over other treatments. The reduction in galling and nematode population could be possibly attributed to deleterious effects of metabolites of *Ceratocystis fimbriata*on the juveniles of root-knot nematode. This is further supported by greater reductions when fungi and nematodes were inoculated simultaneously.These results are in conformity withthe results recorded by Akhtar Haseeb *et al.* (2007) and Hung *et al.* (2003), who reported that reduction of nematode population build up, number of galls might be due to reduced root system. Thus, the nematode faced competition for food. In addition to fungal disruption of nematode feeding sites, plants affected by disease complex may be more prone to early senescence and death which in turn might prevent nematodes from completing life cycles leading to reduced reproduction.

Summary

There was no conspicuous gall production in nematode alone and in combination with fungi. The affected roots showing brownish discolouration in fungal spore suspension, fungal giant culture and fungal giant culture + *M. incognita* and *M. incognita* alone. In uninoculated treatment there was no brownish discolouration. The two organisms (*M. incognita* and *C. fimbriata*) adversely affected plant growth parameters like shoot and root length, fresh and dry weight of shoot, fresh and dry weight of root per plant. Of the two organisms inoculated individually, fungal giant culture caused greater reduction in plant growth parameters over control than either by fungal spore suspension, or *M. incognita*. While reduction in plant growth parameters caused by

fungal spore suspension. Greatest reduction in growth parameters was caused by *M. incognita* + *C. fimbriata*.

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