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CONTROL OF ROOT ROT-ROOT KNOT DISEASE COMPLEX IN OKRA BY SOME INDIGENOUS PLANTS OF KARACHI

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ABSTRACT

This study has been made out to check the efficiency of leaves of our wild plants (*Melia azedarach, Withania somnifera, Thespesia populnea* and *Terminalia catappa*) as organic amendment for the control of soil borne/root rot fungi (*Fusarium oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina*) and root knot nematode (*Meloidogyne javanica*) in okra. Amended leaves of all plant species suppressed the rate of fungi as well as knot formation caused by root knot nematode. Growth of okra plant was also improved after soil amendment. Dry powder of all four tested plants @ 1 and 2 % were found to be more effective than 0.5% concentration.

Key-words: Wild plants, root rot fungi, soil amendment, root knot nematode, okra.

INTRODUCTION

Chemical pesticides are generally used to control plant pathogens but these are expensive and hazardous to our live-stock. As a result researchers go for bio-remedial against plant pathogens (Abbasi *et al*., 2014). Biological control of soil pathogenic microbes is a method in contrast of chemical pesticides which is cheap and harmless to environment (Weller, 1988; O'Sullivan and O'Gara, 1992). In Pakistan *Meloidogyne* spp. contaminated nearly all the agriculture crops whereas *M. incognita* and *M. javanica* are the most familiar (Zarina and Shahina, 2010) Root knot nematode (*Meloidogyne javanica*) is a parasite and cause root knot diseases in several crops. Parasitism disrupts the root system so the uptake of water and nutrients has become reduced (Abad *et al*., 2003).

Fungi which stick with the soil surface and in the soil matrix are called soil borne or root rot fungi. These fungi cause diseases in a huge variety of crops which are known as soil borne diseases. *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* (Kühn) and *Fusarium* spp. are common soil borne pathogenic fungi due to which heavy losses occurs. About 500 plant species around the world are destroying by *Macrophomina phaseolina* (Mihail and Taylor, 1995). Genus *Fusarium* possess the species that are widely dispersed and most diverse plant infecting fungi, produced wilts and blights diseases (Agrios, 2005). *F. oxysporum* Snyd & Hans., caused reduction in yield of pea about 2.2 ha (Tu, 1987). Another soil borne fungus *R. solani* is distributed worldwide and is amongst the world's highly destructive plant pathogens, it survive as an active mycelium for a long time in soil and attacks more than 2000 species of crop plants (Parameter, 1970).

The soil borne plant pathogens particularly above described fungi and root knot nematodes cause root rot and root knot disease complex in plants. Many reports have shown that infection with the root knot nematodes and some root pathogenic fungi in combination, resulting huge losses in crop plants than either pathogen acting alone (Shahda and EL-Saedy, 1990; Mahgoub, 1996). When root knot nematode *Meloidogyne* spp., associate with root infecting fungi it produces greater losses than either pathogen alone (Starr *et al*., 1989).

There are various alternative methods have been reported for managing soil-borne pathogens, such as application of soil organic amendments, soil solarization, fumigation, crop rotation etc. (Oka *et al*., 2007). Soil organic amendment has been explored as a method of suppressing nematodes and soil-borne root infecting fungi (Akhtar and Malik, 2000; Ikram and Dawar, 2013). Application of green manure in soil also improved soil fertility as well as plant growth and its productivity (Pakeerathan *et al*., 2009). Therefore, this study has been carried out to evaluate fungicidal and nematicidal activity of four plant species that are commonly found in Karachi as soil organic amendment for management of soil borne microorganisms (fungi and nematode).

MATERIALS & METHODS

Collection of plant species

Thespesia populnea (L) Sol. Ex Corr, *Withania somnifera* (L.) Dunal, *Terminalia* catappa L. and *Melia azedarach* L. were collected from different localities of Karachi, washed with tap water, shade dried and their leaves were separated. Leaves were ground in powder form.

Isolation of nematode juveniles

Roots of brinjal (*Solanum melongena*) were cut into pieces and transfer in a bottle containing calcium hypochlorite solution (1%), shake it and pass through sieves. After washing with tap water, eggs were collected by the suspension passed from 100-mesh sieve first then pass through 400-mesh now transferred into a beaker. After eggs hatching, the second stage juveniles (J_2) were counted with the help of counting chamber in per ml (Hussey & Barker, 1973).

Organic amendment

Garden loam soil was used for present work and artificially infested by *R. solani, F. oxysporum and M. phaseolina* (1000 spores/pot). Before infestation it was autoclaved then ground powders of plants were mixed with 300g soil in plastic pots @ 0.5, 1 and 2% w/w concentrations. Pots were watered daily (3 weeks) for decomposition of organic matter. After three weeks 5 seeds of okra were sown in pots and after germination of seeds two seedlings kept in each pot. Then soil was artificially infested by *M. javanica* @2000 juveniles (J_2) per pot. There were three replicates for each pot. After 45 days plants were uprooted and estimation of different growth parameters was carried out. For each plant species pots were set in following manure:

a) Pots amended with plant powder + Nematode (Control**:** Pots without plant powder + Nematode).
b) Pots amended with plant powder + Fungi (Control**:** Pots without plant powder + Fungi).
c) Pots amended with plant powder + Nematode & Fungi (Control**:** Pots without plant powder + Nematode & Fungi).

Data record

After 45 days plants of okra were uprooted. Shoot and root length and fresh weight were measured. All treatments have three replicates so mean and standard error was taken. Analysis of variance (ANOVA) was applied to data.

Colonization of pathogenic fungi

To check the occurrence of fungi, 1 cm long root pieces were cut, surface sterilized by $Ca(OCl)_2$ (1%) for 1 minute then transferred on to Petri dishes (5 pieces per dish) containing Potato Dextrose agar (PDA) medium amended with appropriate amount of antibiotics (Penicillin and Streptomycin). Dishes were incubated for 3-6 days at 28°C. After that infection % age was calculated by following formula.

$$\textbf{Colonization \%} = \frac{\text{No. of root pieces colonized by fungus}}{\text{Total number of root pieces}} \times 100$$

Colonization percentages were converted into roots colonization index (RCI) according to 0-5 scale where 0 = no, l = l-10%, 2 = 11-25%, 3 = 26- 50%, 4 = 51-75% and 5 = 75-100% root pieces infected by pathogen (Shahzad & Ghaffar, 1992).

Infection by root knot nematode

Roots infected by root knot nematodes were examined by RKI scale Sasser *et al*. (1984). The number of knots were counted by root knot index (RKI) where, 0 = no, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = more than 100 galls present on per root system.

RESULTS

All amended plant leaves not only suppressed infection of fungi and nematode but also enhanced growth of okra plant. Leaves powder at 1% and 2% are more effective than 0.5% concentration. It was also noticed that after 45 days the pods of okra were also growing. Shoot length and weight were increased $F = (776.87, P<0.001)$ and $(F = 140.79, P<0.001)$ respectively in all treatment. Maximum increased in length (33.93 cm) and weight (2.96 gm) was observed when soil was amended with *T. populnea* leaves at 1% concentration, followed by *W. somnifera* @ 2% concentration. Soil amended with 1% *T. populnea* powder shows maximum increased in length and weight of root as compare to control and other treatments (Table 1).

ANOVA results showed that the three factors (treatments, concentrations and inoculations) were highly significant ($P<0.001$) in all variables, while interaction among these factors were also high (Table 2).

Soil amended with *W. somnifera* leaves @ 2% with inoculum (Nematode + Fungi) & (Fungi) showed minimum RCI (0.6%) and (0.7%) respectively on the other hand *T. catappa* leaves @ 1% with inoculums of (Nematode +

Fungi) showed 0.9% RCI as compared to control. Minimum (1 RKI) was recorded in *M. azedarach* leaves @ 1% with inoculum (Nematode), *W. somnifera* leaves @ 2% with inoculum (Nematode) & (Nematode + Fungi), *T. populnea* leaves @ 2% with inoculum (Nematode) and *T. catappa* leaves @ 1% with inoculum (Nematode) (Table 1).

Table 1. Mean ± standard error of growth parameters of okra plant with root knot index and root colonization percentage.

Treatments	Conc.	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	RKI	RCI
INOCULATION: NEMATODE							
Control		17.06±0.06	14.2±0.2	0.52±0	0.31±0.01	5	-
M. azedarach	0.5%	19.33±0.23	12.53±0.29	0.41±0.01	0.26±0.01	3	-
	1%	29.4±0.70	13.63±1.83	2.42±0.29	0.52±0.20	1	-
	2%	29.13±0.13	15.5±1.89	1.60±0.003	0.25±0.04	1.3	-
W. somnifera	0.5%	18.4±0.26	12.4±0.23	0.35±0.02	0.23±0.03	3.3	-
	1%	27.96±0.50	16.53±0.86	1.75±0.12	0.91±0.02	1.3	-
	2%	29±0	19±0	1.66±0.06	0.22±0	1	-
T. populnea	0.5%	20±0	15.7±0.06	0.71±0.003	0.44±0	2.1	-
	1%	29.86±0.46	17.16±0.38	1.9±0.11	0.50±0.008	1.4	-
	2%	30.16±0.44	19.16±0.16	1.88±0.16	0.50±0.04	1	-
T. catappa	0.5%	18.03±0.03	13.73±0.37	0.54±0.003	0.3±0.02	3.6	-
	1%	24±0	15.5±0	0.81±0	0.21±0	1	-
	2%	25.2±0.11	15.8±0.43	1.84±0.006	0.54±0.07	2	-
INOCULATION: FUNGI							
Control		19.33±0.23	11.93±0.87	0.44±0.01	0.2±0.005	-	5
M. azedarach	0.5%	20.13±0.06	9.4±0.4	0.8±0.01	0.13±0.01	-	4
	1%	30.13±0.08	17.3±0.35	2.16±0.03	0.39±0.28	-	2
	2%	30.7±0.35	19±0	2.26±0.26	0.93±0.03	-	1
W. somnifera	0.5%	20±0	10.5±0.28	0.81±0.01	0.14±0.01	-	3
	1%	28.03±0.03	15.03±0.26	1.84±0.006	0.85±0.06	-	1.6
	2%	32.63±0.34	18.5±0.43	2.52±0.08	0.91±0.01	-	0.7
T. populnea	0.5%	22.26±0.23	15.1±0.208	0.92±0.02	0.41±0.02	-	3
	1%	33.13±0.13	19.46±0.74	2.83±0.03	1±0.23	-	2
	2%	32.83±0.12	19.7±0.36	2.4±0.23	0.97±0.11	-	2.1
T. catappa	0.5%	21.16±0.12	13.5±0.28	0.52±0.01	0.25±0.02	-	4
	1%	23.93±0.17	14.8±0.49	0.82±0.01	0.26±0.06	-	1.3
	2%	24.26±0.17	14.3±0.47	0.82±0.02	0.3±0.02	-	1.1
INOCULATION: NEMATODE + FUNGI							
Control		16.76±0.14	8.6±0.305	0.48±0.008	0.21±0.01	4.6	5
M. azedarach	0.5%	17.9±0.05	15.96±0.26	0.62±0.017	0.37±0.03	3.2	3.6
	1%	27.06±0.06	18.03±0.31	1.54±0.002	0.86±0.06	1.1	1.6
	2%	32.16±0.16	18.4±0	2.4±0.1	0.92±0.01	1.1	1.3
W. somnifera	0.5%	17.13±0.13	15.66±0.24	0.52±0.01	0.25±0.02	4	3.2
	1%	28.33±0.33	13.4±0.305	1.85±0.04	0.61±0.04	1.6	1
	2%	31.43±0.72	19.5±0.26	2.16±0.27	0.76±0.12	1	0.6
T. populnea	0.5%	19±0	10.43±0.53	0.61±0.01	0.27±0.01	2.4	4
	1%	33.93±0.48	20.4±0.702	2.96±0.14	1.41±0.29	1.3	2.3
	2%	33.3±0.15	19.8±0.34	2.7±0.11	1±0.15	1.1	2
T. catappa	0.5%	17.33±0.24	8.96±0.32	0.52±0.01	0.18±0.04	3.8	4
	1%	19.33±0.24	11.4±1.02	0.56±0.03	0.24±0.04	2.1	0.9
	2%	22.26±0.17	15.03±0.08	1.47±0.01	0.34±0.02	2.3	1.2

Conc. =Concentrations, RKI=Root knot index, RCI=Root colonization percentage.

Table 2. Three way ANOVA of growth parameters in okra.

SOURCE	Variables											
	Shoot Length (cm)			Root Length (cm)			Shoot Wt (g)			Root Wt (g)		
Main Effect	**F**	**P**	**LSD 0.05**	**F**	**P**	**LSD 0.05**	**F**	**P**	**LSD 0.05**	**F**	**P**	**LSD 0.05**
Treatment	776.87	0.0000***	0.20	47.03	0.0000***	0.47	140.79	0.0000***	0.07	29.19	0.0000***	0.06
Concentration	6493.53	0.0000***	0.20	290.87	0.0000***	0.47	855.56	0.0000***	0.07	82.87	0.0000***	0.06
Inoculation	269.86	0.0000***	0.17	22.78	0.0000***	0.40	12.99	0.0000***	0.06	8.19	0.0005***	0.05
Interactions												
Trt. x Conc.	220.31	0.0000***		11.99	0.0000***		44.74	0.0000***		9.08	0.0000***	
Trt. x Inoc.	36.66	0.0000***		15.61	0.0000***		13.92	0.0000***		5.43	0.0001***	
Conc. x Inoc.	29.95	0.0000***		27.44	0.0000***		8.25	0.0000***		10.06	0.0000***	
Trt. x Conc. x Inoc.	21.45	0.0000***		10.92	0.0000***		10.80	0.0000***		4.76	0.0000***	

***= P<0.001, **= P<0.01, *= P<0.05, ns= non significant

DISCUSSION

Present investigation proved that amendment suppressed the growth of *R. solani, F. oxysporum and M. phaseolina* as well as root knot nematode, not only fungi and nematode attack alone but also when these pathogens combined together. Growth of okra (*Abelmoschus esculentus*) plant was also good due to amendment. The tested species of plant have several pharmacological properties but in present work emphasized to root rot fungi and root knot nematode. These indigenous plants commonly found in Karachi and get attention to researchers for their phytochemicals which possessed antimicrobial and phrmacological activities. In *vivo* and in *vitro* antifungal and nematicidal properties of *Thespesia populnea*, *Withania somnifera*, *Terminalia catappa* and *Melia azedarach* have been reported by many workers (Abid *et al*., 1997; Babayi *et al*., 2004; Carpinella *et al*., 2005; Wani, 2006; Radwan *et al*., 2007; Khan *et al*., 2008; Mahesh & Satish, 2008; Bobbarala *et al*., 2009; Hemaiswarya *et al*., 2009; Pakeerathan *et al*., 2009; Ntalli *et al*., 2010; Cavoski *et al*., 2012).

Pesticides cause adverse effect on human health as well as our environment because these are carcinogenic and mutagenic (Ikram & Dawar, 2013). Whereas, synthetic nematicides are extremely toxic not only for plant parasitic nematodes but also the beneficial fungi and bacteria present in soil (Agrios, 2005). Therefore plant diseases manage by the use of plant extract and their dry powder is a cheap and effective to control plant and human diseases.

Amendment provides energy and nutrients to soil which are good for its beneficial microorganisms, it also help in the survival of crops (Drinkwater *et al*., 1995). Several reports have been shown that soil organic amendment reduced pathogenic microorganisms. Khan *et al*., (2008) stated that *W. somnifera* leaves extract amended in soil control nematode population and increased papaya yield as compare to carbofuran (nematicide). Pakeerathan *et al*., (2009) used *T. populnea* leaves as green manure which improved plant growth and reduced nematode infestation in tomato field. Dry powder of different parts of *Eucalyptus* sp., were effective against *R. solani, Fusarium* sp. *and M. phaseolina* (Dawar *et al*., 2007). Ehteshamul-Haque *et al*., (1996) used *Datura fastuosa, Stoechospermum marginatum* and neem cake for control of root rot-root knot disease complex caused by soil borne fungi and nematode in okra. Significant reduction in *M. incognita* population in tomato, okra and lentil was noticed when soil amended with *M. azedarach* leaves (Wani, 2006; Radwan *et al*., 2007).

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