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ROOT-ROT ROOT-KNOT DISEASE COMPLEX OF VEGETABLES IN KARACHI REGION AND THEIR NON-CHEMICAL CONTROL

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ABSTRACT

Root-rot root-knot complex disease is aggressively infecting the vegetable crops all over the world. During our survey, conducted during September 2013 to October 2014, it was observed that this disease in the vegetable crops of different regions of Karachi including Malir, Landhi, Murad Memon Goth, Gadap Town and Sharafi Goth causes heavy losses. During present study, twelve different genera of fungi were isolated from different vegetable crops (cabbage, brinjal, tomato, radish and spinach). The fungi included *Alternaria solani, Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, A. terreus, Acremonium fusidioides, Cladosporium* sp., *Drechslera hawaiiensis, Eurotium amstelodami, Fusarium oxysporum, Macrophomina phaseolina, Penicillium commune, Rhizoctonia solani, Trichoderma harzianum* and *Ulocladium* sp. Besides these fungi, root-knot nematodes (*Meloidogyne* spp.) were also isolated from the infected roots of vegetable crops. It was also observed in the present study that the infection rapidly increased due to many factors such as, the presence of excess moisture, winds, dust storms and as well as by mechanical vectors. The extracts of different parts of three medicinal plants *Annona squamosa, Ocimum basilicum* and *Cassia fistula* were used for the control of root-rot root-knot disease complex. Aqueous extract of *A. squamosa* not only proved to be most effective in the control of soil-borne plant pathogenic fungi and root-knot nematodes but also promoted the plant growth.

Keywords: Vegetable, Root-rot, Root Knot, Disease complex, Karachi, Nematode, Fungi, organic amendment

INTRODUCTION

The nature has endowed Pakistan with diverse types of climatic conditions. Therefore, large varieties of vegetables are cultivated in Pakistan throughout the year as summer and winter vegetables particularly in Sindh province (Athar and Bokhari, 2006). The yield of vegetables is reducing gradually every year due to different biotic and abiotic factors including soil-borne fungi and nematodes. Soil-borne plant pathogens cause significant losses in almost all crops particularly to the vegetables (Usman *et al.,* 2013; 2014). Usman *et al.,* (2014) reported the different fungi from different areas of Sindh as well as Karachi and identified *Alternaria solani, Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, A. terreus, Acremonium fusidioides, Cladosporium* sp., *Drechslera hawaiiensis, Eurotium amstelodami, Fusarium oxysporum, Macrophomina phaseolina, Penicillium commune, Rhizoctonia solani, Trichoderma harzianum* and *Ulocladium* sp., from the soil of vegetable fields.

Root-knot nematodes *(Meloidogyne* spp.) are aggressive pest of vegetables and widely infect plants (Khan *et al.,* 2005). *Meloidogyne* spp. are particularly damaging to vegetable crops in warmer climates. Usually root system deformed and underground organs including tap roots of carrot and potato tubers aggressively damaged and become valueless in market (Sikora and Fernandez, 2005). Vegetable production in tropical and sub-tropical areas is highly dependent on suitable plant parasitic nematode control (Sikora and Fernàndez, 2005), particularly against *Meloidogyne* spp., that are usually the most damaging. Root-knot nematodes are difficult to control and manage. These are deep-rooted in soil. So there are very limited options to control them (Radwan *et al.,* 2012). Several researchers are engaged in standardizing the root-rot and root-knot disease management strategies by nonchemical and eco-friendly strategies including organic amendment, natural fertilization, biological control, heat-based method, soil management and sanitation to improve the productivity of vegetables (Collange *et al.,* 2011). Some studies have also been done on antimicrobial and antifungal activity of *Annona squamosa*, *Ocimum basilicum* and *Cassia fistula* flowers and seed along with some other medicinal plants (Phongpaichit *et al.,* 2004; Kumar *et al.,* 2006; Duraipandiyan and Ignacimuthu, 2007; Sangetha *et al.,* 2008). Several principles examined in the extracts of *Cassia fistula* include protein, saponins, glycosides, triterpenoids, flavonoids, amino acids, carbohydrates and alkaloids (Panda *et al.,* 2010). *A. squamosa* is widely distributed in tropical and subtropical regions. It is used as antifungal and nematicidal. It suppresses the mycelia growth of fungi (Bermejo *et al.,* 2005).

The main objectives of the present studies were; 1) to survey and collect infected (root-rot root-knot) vegetable plants along with respective soil and roots samples from vegetable growing areas of Karachi, 2) to compare the

fungi and nematode composition of assemblages in root-rot root-knot diseases in various vegetables, and 3) to apply different plants extracts as a organic amendment for the reduction of root-rot root-knot complex disease.

METHODS AND MATERIALS

Collection and isolation of fungi

There are fifteen samples of root-rot fungi of each vegetable including cabbage (*Brassica oleracea* L.), brinjal (*Solanum melongena* L.), tomato (*Lycopersicon esculentum* Mill.), radish (*Raphanus sativus* L.) and spinach (*Spinacia oleracea* L.) showing wilting, stunted growth, chlorosis and blotches were collected from different areas of Karachi (Malir, Landhi, Murad Memon Goth, Gadap Town and Sharafi Goth) from September 2013 to October 2014. The infected root samples were cut into small pieces (2 cm) and surfaces sterilized by 1% Ca $(OCl)_2$ for 1 min. These pieces were transferred on potato dextrose agar (PDA) medium containing antibiotic (Penicillin and Streptomycin) drops. The Petri dishes were incubated for 5 days at 28 ± 2 °C (Usman *et al.*, 2014). Infection percentage was calculated with the help of following formula:

$$\text{Infection } (\%) = \frac{\text{Number of plants infected by a pathogen}}{\text{Total number of plants}} \times 100$$

Soil dilution plate technique

One gram of soil was suspended in 9 mL of sterilized distilled water and dilution of 1:10, from which the dilutions of 1:100, 1:000 and 1:0000 were made. One mL aliquot sample was poured in sterilized Petri plates containing Potato Dextrose Agar (PDA). There were three replicates in per sample. The Petri dishes were kept in incubator at 28 ± 2 °C (Waksman and Fred, 1922). The fungal colonies developing on plates were counted.

Identification of fungi

Isolated fungi were identified after Ellis (1971; 1976), Barnett and Hunter (1972), Sutton (1980), Nelson *et al.* (1983), Domsch *et al.* (1980) and Singh *et al*. (1991).

Isolation of root-knot nematodes

The root-knot nematode species were identified with the help of perineal pattern as described by Taylor and Netscher (1974).

Organic amendment for control of root-rot root-knot disease

The experiment was conducted in screen house in pots of 35 cm diam. Clay soil mixed with dry leaf powder *Annona squamosa*, *Ocimum basilicum* and *Cassia fistula* at concentration of 0.5, 1.0 w/w was shifted in 8 cm diam., plastic pots with 300 g soil in per pot. The soil was maintained at 50% maximum water holding capacity. After 15 days, 3 weeks old okra seedlings were transplanted singly in every pot. A day after transplantation, seedlings were artificially infested with *M. javanica* root-knot nematodes at 10-egg mass per plant and selected highly destructive soil-borne pathogens such as fungi *R. solani, M. phaseolina* and *F. oxysporum* were artificially infested in soil at 100 spores/pot. After inoculation of pathogen, the different parameters of plants were studied. There were 3 replicates of each treatment. The pots were randomized on benches of screen house. Plants were uprooted after 45 days of inoculation and different growth parameters and colonization of roots was recorded using following formula.

$$\text{Colonization } \% = \frac{\text{Number of root pieces colonized by the pathogen}}{\text{Total number of root pieces}} \times 100$$

Root colonization data were converted into roots colonization index (RCI) according to a 0-5 scale of Shahzad and Ghaffar (1992) where 0=0, l=l-10, 2=11-25, 3=26- 50, 4=51-75 and 5=75-100% root pieces colonized by the pathogen. However, Root knot index (RKI) was determined using Sasser *et al.* (1984) scale.

Analysis of data:

Data were analyzed and subjected to Analysis of variance (ANOVA). The follow up of ANOVA included least significant difference (LSD), Duncan's multiple range test was used to compare the treatment means.

RESULTS AND DISCUSSION

Twelve genera of fungi were isolated from roots of the studied vegetable crops (cabbage, brinjal, tomato, radish and spinach) from different areas of Karachi. Fungi were identified as *Alternaria solani, Aspergillus flavus, A.*

fumigatus, A. niger, A. oryzae, A. terreus, Acremonium fusidioides, Cladosporium sp., *Drechslera hawaiiensis, Eurotium amstelodami, Fusarium oxysporum, Macrophomina phaseolina, Penicillium commune, Rhizoctonia solani, Trichoderma harzianum* and *Ulocladium* sp., (Table. 1). The studied vegetables were in the following order of infectibility.

Cabbage > Brinjal > Tomato ≈ Radish ≈ Spinach

Table 1. Occurrence of fungi isolated from roots and soil samples of vegetable plants.

Fungi isolated from root samples					
Name of fungi	Cabbage	Brinjal	Tomato	Radish	Spinach
Acremonium fusidioides	+	--	--	--	--
Alternaria solani	+	+	--	--	--
Aspergillus oryzae	+	--	--	--	--
Cladosporium sp.	+	--	--	--	--
Eurotium amstelodami	+	--	--	--	--
Fusarium oxysporum	+	+	+	+	+
Macrophomina phaseolina	+	+	+	--	+
Penicillium commune	--	+	--	+	--
Rhizoctonia solani	+	+	+	+	+
Trichoderma harzianum	--	+	--	--	--
Ulocladium sp.	+	--	--	--	--
Unidentified black mycelium	+	+	--	--	--
Fungi isolated from soil samples					
Alternaria solani	--	--	+	--	--
Aspergillus flavus	+	+	+	--	+
A. fumigatus	+	--	--	--	+
A. niger	+	+	+	+	--
A. terreus	--	+	--	--	--
Drechslera hawaiiensis	--	--	+	--	+
Fusarium oxysporum	+	+	+	+	+
Macrophomina phaseolina	+	+	+	+	+
Penicillium commune	+	+	--	--	--
Rhizoctonia solani	+	+	+	+	--
Trichoderma harzianum	--	+	--	--	--

Present (+), Absent (--)

Ten different fungi were isolated from roots of cabbage. Among these *Fusarium oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* were dominant with mean values of 56, 46 and 35%, respectively. The infection result of brinjal roots showed that *Fusarium oxysporum* and *Macrophomina phaseolina* were dominant with mean values of 59 and 39%, respectively (Fig. 1). At times, multiple fungi infection was observed in tomato, radish and spinach roots. In such situations, *F. oxysporum* and *R. solani* were pre-dominant as compared to other species. On overall basis, the occurrence of *Fusarium oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina* was pre-dominant in all samples of vegetables collected (Fig. 1).

ANOVA result of infection (%) of fungi isolated from roots of vegetable plant samples collected from Karachi. All vegetables showed highly significant differences among fungi species. All species are aggressively infecting all vegetable particularly cabbage ($F=209.377$, $P<0.001$), brinjal ($F=173.33$, $P<0.001$), radish ($F=110.040$, $P<0.001$), tomato ($F=88.056$, $P<0.001$) and spinach ($F=37.372$, $P<0.001$) crops.

Root-knot nematodes were isolated from root samples of vegetable plants. Total 55 soils samples were collected from the different regions of Karachi to calculate the population density and prevalence of root-knot nematode. Out of 55 samples, 35 were infected with root-knot nematodes. The population of root-knot nematode (*Meloidogyne* spp.) in brinjal and cabbage was significantly high as compared to other vegetables. Population of root-knot nematode was 63 numbers of knots in brinjal and 43knots in cabbage while the prevalence of root-knot nematode in

brinjal was 95% and in cabbage75% were recorded. The prevalence of root-knot nematode was 71% in tomato as shown is Table 2.

Table 2. Root-knot nematodes (*Meloidogyne* spp.) associated with different vegetable crops.

Name of vegetable	Number of knots/plants	J2/ 40 g of root	Population density (%)	Prevalence (%)
Cabbage	43	97	26	75
Brinjal	63	129	35	95
Tomato	47	33	9	71
Radish	35	68	18	61
Spinach	29	47	13	67

Eleven fungi were isolated from soil samples collected from the rhizosphere of different vegetable crops. Maximum eight different fungi were isolated from the rhizosphere of brinjal plants. Among these *Aspergillus flavus, A. niger* and *F. oxysporum* were dominant with mean values of 56, 41 and 37% respectively, as compared to other fungal species. However, the roots of cabbage showed that *F. oxysporum* and *Rhizoctonia solani* were found dominant with mean values of 56 and 46 37%, respectively as compared to other fungal species. Whenever, the multiple combined occurrencesof fungi was present in case of result tomato, radish and spinach roots, *F. oxysporum* and *M. phaseolina* were dominant with average mean value of 57 and 37%, respectively as compared to other fungal species. When compared on the basis of all vegetable, the occurrence of *Fusarium oxysporum* and *Aspergillus flavus* was maximum with 53 and 45% occurrence in the samples collected Karachi as shown in Fig. 2.

The results of ANOVA for occurrence (%) of different fungi in soil samples were collected from Karachi. All vegetable showed highly significant differences among fungi species. Almost all species of fungi are infecting vegetable particularly cabbage ($F=25.780$, $P<0.001$), brinjal ($F=46.635$, $P<0.001$), radish ($F=63.719$, $P<0.001$), tomato ($F=47.638$, $P<0.001$) and spinach ($F=75.796$, $P<0.001$) crops.

Effect of organic amendment to control the control of root-rot root-knot

For organic amendment, the pot experiment were carried out in screen house and regularly irrigated with tap water. The forty five days data on different growth parameters of Okra were recorded (Table 4).

Number of leaves was increased and significantly high ($F=11.57$, $P<0.001$) during 45 days of treatment as compared to control. The maximum results were recorded in the treatments of *A. squamosa* stem extract and *O. basilicum* leaf extract. Results of stem and root lengths were significantly high ($P<0.001$, $F=105.7$ and $F=69.26$) during the 45 days. Shoot and root lengths were increased in all treatments but maximum increases in length were recorded in *A. squamosa* leaf extract treatment with mean of 46.27 cm in stem length and 14.67 cm mean were recorded in *O. basilicum* stem extract treatment in root length. However, significantly high ($P<0.001$) results were observed in fresh root weight ($F=25.62$ and $F=14.19$). However, non-significant ($P<0.06$) results were recorded in fresh stem weight. *A. squamosa* leaf extract treatments increased maximum fresh stem weight with mean of 5.76 g as compared to other treatments and control. Maximum fresh root weight was observed in *C. fistula* fruit extract with mean of 1.73 g were noted in okra plant as compared to control. Minimum Root-Knot Index (1.0 RKI) was observed in both concentrations of (0.5 and 1.0%) leaves and stem extract of *A. squamosa* recorded in okra plant. In Root Colonization Index (RCI)**,** *A. squamosa* leaf extract showed minimum 0.7% RCI in both at 0.5 and 1% concentrations as compares to control (Table 3).

The results of analysis of variance (ANOVA) of different parts of plant are shown in Table 3. Treatment shows highly significant ($P<0.001$) effects on all variables including number of leaves, stem and root lengths, fresh stem and root weights. Concentration factor was found to be highly significant ($P<0.001$) with respect to all variables, similarly. Inoculums level did not show significantly ($P<0.06$) influence on fresh stem weight but showed significant effect on all other variables including number of leaves, stem and root lengths and fresh root weight. Interaction of treatment×concentrations was highly significant ($P<0.001$) for all variables. The interaction of treatment×inoculums did not show significant ($P<0.06$) effects on all variables including stem length and fresh stem weight. Interaction of concentration×inoculums also did not show significant ($P<0.06$) influence on stem length only. The interaction of all three factors (treatments× concentrations× inoculums) was found significant ($P<0.01$) with respect to stem and root length and fresh root weight.

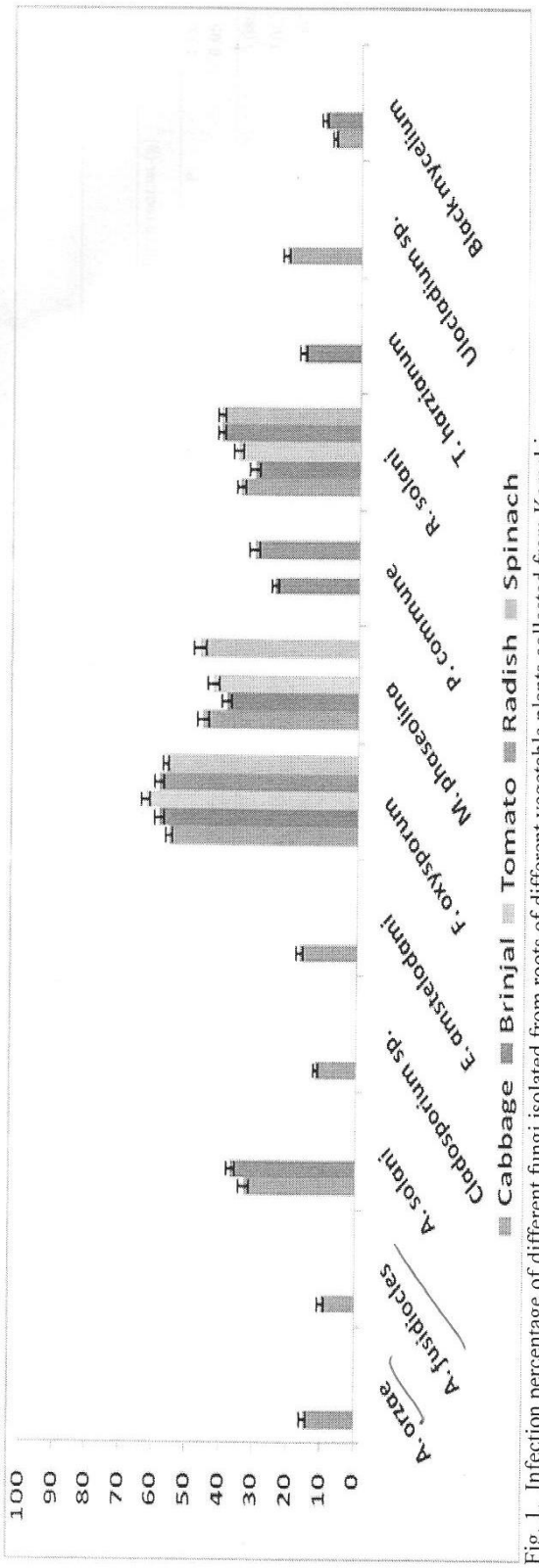

Fig. 1. Infection percentage of different fungi isolated from roots of different vegetable plants collected from Karachi.

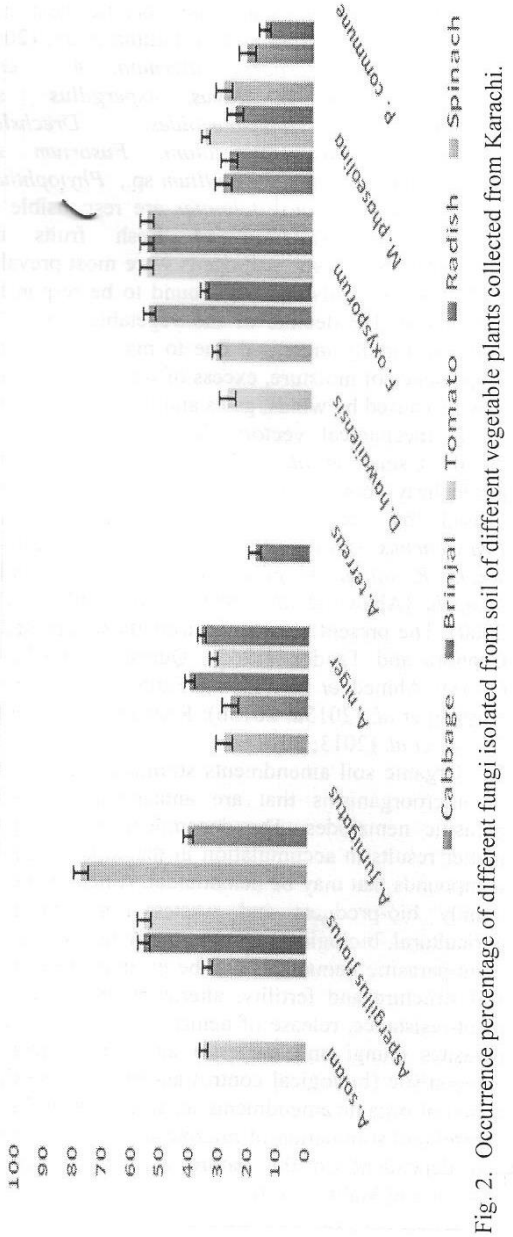

Fig. 2. Occurrence percentage of different fungi isolated from soil of different vegetable plants collected from Karachi.

Lichtenzveig *et al.* (2006) reported that the pathogenic micro-fungal floras of field soils cause root-rots, vascular wilts and seedling damping-off diseases in plants. Abawi and Widmer (2000) reported that production of food crops and vegetables are generally affected by different soil-borne pathogens. The severity and incidence soil and root diseases are indirect appraisal of soil fertility or soil use. Plant pathogens have caused almost 20% reductions in the principal food and cash crops worldwide. Since pathogenic fungi alone caused 10-30% reduction in the yield of major food and cash crops (Agrios, 2005). Ahmed and Raza (1991) reported that the soil-borne fungi perpetuate seasonally on specific host crop debris which are buried in soil. Fatima *et al.,* (2009) indicated that *Alternaria alternata*, *A. citri*, *Aspergillus niger*, *A. flavus*, *Aspergillus* sp., *Cladosporium cladosporioides*, *Drechslera australeinsis*, *Fusarium solani*, *Fusarium* sp., *Geotrichum candidum*, *Penicillium* sp., *Phytophthora capsici* and *Rhizopus stolonifer* are responsible for postharvest deterioration of fresh fruits and vegetables. Soil-borne pathogens were most prevalent in the soil of fields and also found to be responsible for most of the decline of the vegetable crops. The infection rapidly increased due to many factors such as, presence of moisture, excess of water and infection may be caused by winds, gales and dust storms as well as by mechanical vectors (Hussain *et al.,* 2013a; 2013b; Usman *et al.,* 2014). Soil-borne pathogens particularly root-rot root-knot is destructive disease caused by nematodes (*Meloidogyne* spp. and *Pratylenchus* spp.) and several fungi including *F. solani*, *R. solani*, *M. phaseolina*, *P. ultimum* and *T. basicola* (Abawi *et al.,* 1985; Abawi and Widmer, 2000). The present results confirms those reported by Chandra and Tandon (1965); Qureshi and Ghafoor (1966); Ahmed *et al.* (1991); Fatima *et al.* (2009); Hussain *et al.* (2013a; 2013b); Raza *et al.* (2013) and Usman *et al.* (2013; 2014).

Organic soil amendments stimulate the activities of microorganisms that are antagonistic to plant-parasitic nematodes. The decomposition of organic matter results in accumulation in the soils of specific compounds that may be nematicidal. Amendments are mainly bio-products and wastes from industrial, agricultural, biological and other activities. Control of plant-parasitic nematodes can be by improvements of soil structure and fertility, alteration of the level of plant-resistance, release of nemato-toxic compounds), parasites (fungi and bacteria) and other nematode antagonistic (biological control agents). The mode of action of organic amendments leading to plant disease control and stimulation of microorganisms is complex and dependent on the nature of the amendments (Akhtar and Malik, 2000).

Table 6. ANOVA results of different growth parameters of okra plants observed after45 days of treatments.

SOURCE	After 45 Days															
	No. of Leaves			Stem Length (cm)			Root Length (cm)			Fresh stem wt (g)			Fresh 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**	**F**	**P**	**LSD 0.05**
Treatment	11.57	0.0000***	0.33	105.07	0.0000***	1.24	69.26	0.0000***	0.53	25.62	0.000***	0.44	14.19	0.0000***	0.080
Concentration	228.22	0.0000***	0.26	447.75	0.0000***	0.96	446.22	0.0000***	0.41	52.37	0.0000***	0.34	113.52	0.0000***	0.062
Inoculation	13 81	0.0000***	0.26	10.86	0.0001***	0.96	6.74	0.0019**	0.41	1.44	0.2412ns	0.34	22.40	0.0000***	0.062
Interactions															
Trt. x Conc.	6.48	0.0000***		34.14	0.0000***		20.58	0.0000***		7.66	0.0000***		9.96	0.0000***	
Trt. x Inoc.	3.41	0.0018***		1.49	0.1687ns		2.38	0.0222*		1.15	0.3366ns		13 78	0.0000***	
Conc. x Inoc.	9.22	0.0000***		2.38	0.0568ns		9.06	0.0000***		5.07	0.0010***		7.48	0.0000***	
Trt x Conc. x Inoc	1.37	0.1736ns		2.05	0.0174*		2.68	0.0016**		1.03	0.4334ns		3.94	0.0000***	

F= F-ratio, P= P- value (P<0.001 is showing highly significant, P< 0.01 is showing significant, P< 0.05 is showing least significant, P< 0.06 or up is a non significant, ns=Non significant), LSD= Least significant difference

Table 4. Effect of different plant extracts (**A**= *Annona squamosa*, OB= *Ocimum basilicum* and CF= *Cassia fistula*) on different growth parameters of okra plant during 45 days with Mean and Standard error.

Treatments	Dose	No. of leaves	Stem length (cm)	Root length (cm)	Fresh stem wt (g)	Fresh root wt (g)	RKI	RCI
Control		3±0	22.67±0.33	3.83±0.44	1.17±0.09	0.35±0.01	4.1	3.9
Annona Leaf								
Fungi	0.5%	6.67±0.33	41.37±0.73	6.73±0.20	3.58±0.52	0.72±0.13	1.9	1.5
	1%	7±0.58	38.87±0.70	7.97±0.20	4.73±0.64	0.74±0.30	1.5	1.4
Nematode	0.5%	5.67±0.33	44.27±1.24	8.27±0.24	2.71±0.10	1.48±0.25	1.4	1.7
	1%	5.67±0.33	46.27±0.32	6.83±0.57	5.76±1.06	1.20±0.12	1.1	0.7
Fungi+Nematode	0.5%	5±0	46.13±0.23	5.73±0.03	3.89±0.39	1.10±0.06	1.1	0.9
	1%	4.67±0.33	44.33±0.63	7.17±0.15	4.53±0.78	1.07±0.03	1	1
Annona Stem								
Fungi	0.5%	5.67±0.33	27±1.15	9.67±0.33	2±0.32	0.50±0.04	1.7	1.7
	1%	7.33±0.33	27.67±1.20	14.33±0.88	1.87±0.09	0.86±0.07	1.5	1.4
Nematode	0.5%	5.33±0.33	29±0.58	12.33±0.33	1.71±0.08	0.53±0.03	1.5	1.3
	1%	6.33±0.33	30.67±2.19	12.13±0.47	2.36±0.32	1±0.11	1.7	1.2
Fungi+Nematode	0.5%	5.33±0.33	25.67±2.19	12±0.58	1.92±0.14	0.76±0.05	1.1	1.1
	1%	6.67±0.67	34.33±0.33	13.67±1.45	2.08±0.28	1.27±0.15	1.1	1.3
OB Leaf								
Fungi	0.5%	6.67±0.33	41.17±0.73	7.37±0.69	3.63±0.48	0.72±0.13	1.9	1.7
	1%	7.33±0.67	42.50±1.89	7.57±0.74	4.57±0.93	1±0.08	2	2.1
Nematode	0.5%	5.67±0.33	43.63±1.23	8.57±0.33	4.40±0.74	1.15±0.11	2.1	1.9
	1%	5.67±0.33	38.83±3.93	6.67±0.69	5.56±1.15	1.13±0.07	1.7	2
Fungi+Nematode	0.5%	4.67±0.33	44.50±2.29	7.03±0.29	3.79±0.46	1.03±0.03	1.8	2.1
	1%	4.33±0.33	40±1.73	7.53±0.09	4.11±1.25	1±0	1.8	1.7
OB Stem								
Fungi	0.5%	5.67±0.33	27±1.15	9.67±0.33	1.75±0.43	0.51±0.03	2.1	2.3
	1%	7±0.58	29±0.58	14.67±0.88	1.97±0.03	0.94±0.05	2.2	2.1
Nematode	0.5%	4.67±0.33	29±0.58	12±0.58	1.66±0.06	0.49±0.03	1.4	2.2
	1%	6±0.58	31.67±1.76	11.47±0.29	2.52±0.31	0.98±0.13	1.3	1.9
Fungi+Nematode	0.5%	5.33±0.67	16.33±6.69	10.67±1.20	1.41±0.41	0.78±0.05	1.7	2
	1%	7±0.58	34.33±0.33	11.33±1.33	2.08±0.28	1.27±0.15	1.5	2.1
CF Fruit								
Fungi	0.5%	5±0	33.50±0.30	8.60±0.76	1.96±0.37	1.57±0.03	1.4	2.4
	1%	5±0	43.40±0.47	10.87±0.28	4.97±0.09	1.73±0.04	1.7	2.1
Nematode	0.5%	3.67±0.33	36.27±1.08	8.73±0.44	1.91±0.19	0.99±0	2.4	2.4
	1%	4.33±0.33	42.23±1.03	12.50±1.18	3.33±0.49	1.03±0.03	2.1	2.3
Fungi+Nematode	0.5%	5.33±0.33	32.80±2.61	6.80±0.40	1.63±0.27	0.97±0.01	1.9	2.1
	1%	4±0	41.33±1.60	8.10±0.83	1.91±0.33	0.95±0.05	1.9	2

Use of organic amendment treatment play effective role to control of chemical, cultural and physical soil-borne pathogens on plants. Several compounds of medicinal plants such as toxic, terpenids and phenolic compounds are reported as an effective and helpful in the reduction of nematodes and have a potential nematicidal properties in

their tissues (Akhtar and Mahmood, 1994; Shaukat and Siddiqui, 2001; Siddiqui and Shaukat, 2002; Shaukat *et al*., 2004). Annon aceous acetogenins (AAs), a common secondary metabolite of *A. squamosa*, is characterized by branched C_{32} and C_{34} fatty acids ending in a γ-lactone and exhibits a broad range of biological properties such as cytotoxic, antitumor, antiparasitic, pesticidal, antimicrobial, and immune suppressive activities (He *et al.,* 1997; Bermejo *et al.*, 2005). In addition, it is also progressive results in soil amendment against the activity of plant nematodes (Singh and Sitaramaiah, 1970; Muller and Gooch, 1982; Akhtar, 1993; Akhtar and Alam 1993; Akhtar and Mahmood, 1994; Akhtar, 1997; Akhtar and Malik, 2000).

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