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EFFECT OF *ANNONA SQUAMOSA*, *OCIMUM BASILICUM* AND *CASSIA FISTULA* USED AS ORGANIC AMENDMENTS AGAINST THE SOIL-BORNE PATHOGENS IN OKRA PLANT

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ABSTRACT

The present study was carried out for the management of soil-borne diseases caused by *Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani* and root-knot nematodes (*Meloidogyne javanica*) in Okra-by using plant parts of *Annona squamosa*, *Ocimum basilicum* and *Cassia fistula* as organic amendments. Soil treatment with different parts of plants including leaves and stem powder at the concentration of 0.5 and 1% w/w were employed for the reducing of soil-borne pathogens. Results indicated that the soil-borne pathogens were suppressed significantly the growth parameters of test plant (Number of leaves, stem and root length and root and stem weight) of okra improved. *Annona squamosa*, *Ocimum basilicum* and *Cassia fistula* leaves, stem and fruit powder were effective at the concentration of 1% for the enhancement of growth of okra. These plants may hopefully be used as organic amendments for minimizing the soil-borne diseases in okra.

Key-words: Okra, Soil-borne fungi, root-knot nematode, Organic amendment

INTRODUCTION

Okra (*Abelmoschus esculentus* L. (Moench.) is a popular vegetable crop and many of its varieties are grown worldwide. The production of okra is negatively affected by many insects and diseases caused by fungi, viruses, bacteria, mycoplasmas and nematodes (Hussain *et al*., 2011a; Ahmad *et al*., 2012; Arain *et al*., 2012; Iqbal *et al*., 2012; Srivastava *et al*., 2012). In addition to other pathogens, the soil-borne diseases caused by fungi *Fusarium* spp., *M. phaseolina* and *R. solani* results in severe damage to crop plants leading to annual loss in yields (Sultana *et al.*, 2005; Mithal, 2006; Abbasi *et al.*, 2008; Joseph *et al.*, 2008; Usman *et al.*, 2013). The association of root-knot with root-rot fungi such as *Macrophomina phaseolina*, *Fuasrium* sp., and *Rhizoctonia solani* cause diseases in different vegetable crops particularly chilli, brinjal, okra, tomato and spinach (Maqbool *et al.*, 1988; Usman *et al.*, 2013; Hussain *et al.*, 2013b).

Several studies have been made on antimicrobial and antifungal activity of *Annona squamosa*, *Ocimum basilicum* and *Cassia fistula* along with some other medicinal plants including Phongpaichit *et al.* (2004); Kumar *et al.* (2006); Duraipandiyan and Ignacimuthu (2007) and Sangetha *et al.*, (2008).

Annona squamosa is distributed in tropical as well as subtropical regions of the world. It is used as antifungal and nematicidal (Bermejo *et al.*, 2005; Farzana *et al.*, 2016). Although synthetic chemicals do control plant diseases and are highly effective against plant pathogens the chemical residues have been found in underground water (Nega, 2014) making water unsuitable for human consumption. Therefore there is a need to develop alternative strategies to control diseases caused by plant pathogens. These strategies like crop rotation, organic amendments and use of microorganism as biocontrol are important tools against plant pathogens (Abid *et al.*, 1997; Siddiqui and Shaukat, 2002). The extracts of medicinal plants *Annona squamosa, Cassia siamea* and *Momordica charantia* have been shown to inhibit the growth of *Aspergillus, Fusarium, Cladosporium* and *Alternaria* species (Ghangaonkar, 2007).

In the present studies, three plant species (*Annona squamosa*, *Ocimum basilicum* and *Cassia fistula*) are tested to determine their fungicidal potential against soil-borne pathogens causing root-rot/ root-knot disease complexes.

MATERIALS AND METHODS

Inoculation of Plant extracts of different plants and soil-borne pathogens

Experiment was conducted in a screen house. The size of pot was 8 cm diam. Clay soil was mixed with dry leaves powder of *Annona squamosa*, *Ocimum basilicum* and *Cassia fistula* @ 0.5, 1.0 w/w was transferred in 8 cm

diam., plastic pots @ 300 g per pot (All 3 plant powders in the same pot). The soil was maintained at 50% maximum water holding capacity. After 15 days, 3 weeks old okra seedlings were transplanted singly in each pot. A day after transplantation, seedlings were inoculated with *M. javanica* root-knot nematodes @ 10 egg masses per plant and fungal species *R. solani, M. phaseolina* and *F. oxysporum* were inoculated together in the same pot soil at 100 spore/pot. After inoculation, all treatments (Fungi, Nematodes and Nematode + fungi) with extracts of plants were applied to okra plants. There were 3 replicates of each treatment. Un-amended inoculated and without inoculated plants placed as controls. The all pots were kept on ranks of green house. The seedlings of plant were uprooted after inoculation of 45 days and different parameters of growth with colonization of roots were 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 Index (RCI) and Root-Knot Index (RKI):

The data of root colonization were changed into Roots Colonization Index (RCI) on (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% of the root pieces were observed harbouring colonies of pathogen. However, Root knot index (RKI) was followed by method of Sasser *et al.* (1984) scale.

Identification of Fungi and Nematodes:

Isolated fungi were examined by using 10 × and 40 × magnifications on the microscope to identify hyphae, sporangia, sporangiophores, conidia, conidiophores and some other morphological characters including growth pattern, colony texture and growth rate of the colonies. The standard manuals or refernces including Ellis (1971); Barnett and Hunter (1972); Sutton (1980); Nelson *et al.* (1983) and Singh *et al.* (1991) were also used for the confirmation of different species. The different species of root-knot nematodes were detected and identified with the reference of perennial pattern (Taylor and Netscher, 1974).

RESULTS AND DISCUSSION

Data is presented in Fig. 1. An increase in number of leaves was observed in all treatments ($F=11.57$, $P < 0.001$). However, highly significant increase was noted in okra test plant treated by either *A. squamosa* stem powder or *O. basilicum* leaf powder.

Stem and root lengths were significantly high ($P < 0.001$, $F = 105.7$ and $F = 69.26$). We found significant increase in both shoot and root lengths of the treated plants however, the maximum increase in plant length was recorded in plant treated by *A. squamosa* leaf powder with an average of 46.27 cm stem length and mean were recorded in Plants treated by *O. basilicum* stem powder resulted in 14.67 cm root length. The significantly high ($P<0.001$) numbers was noted in fresh root weight ($F=25.62$ and $F=14.19$). We found that *A. squamosa* leaf powder increased fresh stem weight with an average of 5.76 g than other treatments and untreated control. Maximum fresh root weight of 1.73 g recorded in okra plant treated with *C. fistula* fruit extracts. Minimum root-knot index (1.0 RKI) was recorded on okra plant treated with either 0.5 or 1.0% *A. squamosa* leaves or stem powder (Fig. 2).

All 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. We did not find any significant effect of pathogens inoculums level ($P < 0.06$) on fresh stem weight but we did find significant inoculum effects on all other variables - number of leaves, stem and root lengths and fresh weight of root. 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. 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.

Phytopathogens including bacteria, nematodes, virus and fungi cause significant damage to plants resulting in yield reductions in infested corps (Oka *et al.*, 2000; Vidhyasekaran, 2004). Plant extracts of plant parts are beneficial for the soil because these organic materials ultimately provide nutrients to plants and improve crop yields (Akhtar and Malik, 2000). Additional use of microorganisms in treated soil improves the activities of enzymes (Rodriguez-Kabana *et al.* 1983). Considerable development has been made in the use of organic matters as soil management against soil-borne plant pathogens. Organic amendments have produced positive effects on crop viability, biological activities, soil nutrients and physical conditions (Kang *et al.*, 1981; Hungalle *et al.*, 1986). The additional use of organic amendment usually promote the soil and increase the ability of soil to exchange ions and hold water due to release of nutrients by organic material, the developed soil improved growth of plants root (Akhtar and Malik,

2000). Our study confirms the results of Akhtar and Malik (2000) that 0.5 and 1% or additional concentration of *A. squamosa*, *O. basilicum* and *C. fistula* found effective to the control of soil-borne pathogens particularly root-root root-knot disease complex.

Table 1. The effect of different concentrations (0.5 and 1%) of different plant powder on the mycelia development of fungi and population of nematodes and growth parameters of okra plants with Mean and Standard error.

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

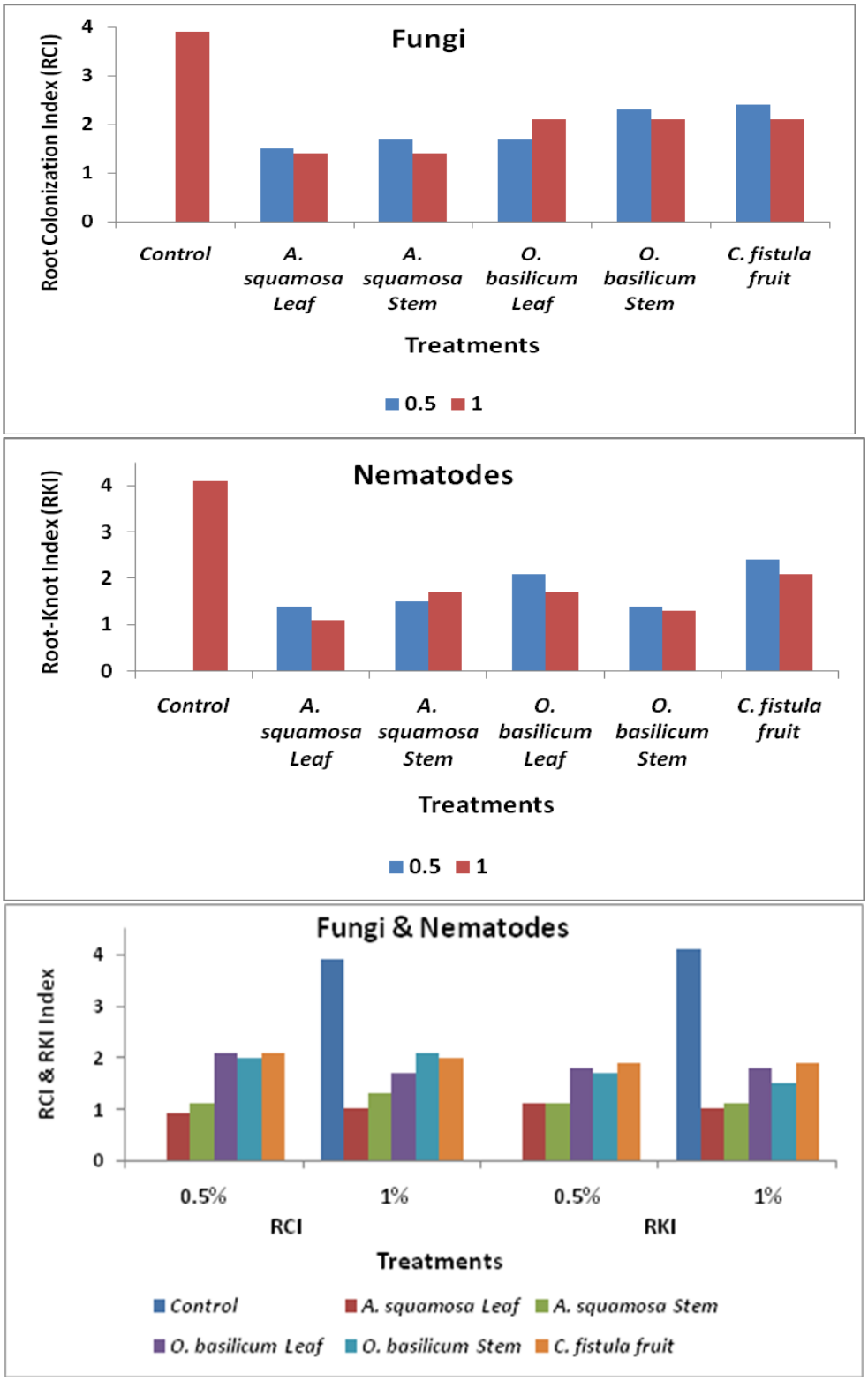

Fig 2. The alone and combined effect of different concentration (0.5 and 1%) of plant powder on the Root Colonization Index (RCI) of fungi and Root-Knot Index (RKI) of nematodes.

Dang *et al.* (2011) described that the methanol extract of *A. squamosa* is significantly active for the control of several fungi and phyto-nematodes. *A. squamosa* has Annonaceous acetogenins (AAs) secondary metabolites and fatty acids which shows a vast range of bio properties including antiparasitic, immune-suppresive, cytotoxic, antimicrobial, antitumor and pesticidal activities (He *et al.,* 1997; Bermejo *et al.,* 2005). Our present study confirms the results of these above mentioned references that *A. squamosa* was not only inhibiting the soil-borne plant pathogen but also helpful for the improving of okra plant growth.

Green manure amendment has been shown to inhibit population of parasitic phytonematodes (Dickson and Hewlett, 1989). The products of plants may be released through decomposition of residues, leaching from residues, exudation from roots and volatilization (Halbrendt, 1996). The present organic amendment results confirm and closely related to the findings of Shahzad (1994); Zaki (1988); Phongpaichit *et al.* (2004); Bermejo *et al.* (2005); Duraipandiyan and Ignacimuthu (2007); Sangetha *et al.* (2008) Dang *et al.* (2011) and Farzana *et al.* (2016). Findings of our studies to confirms the above mentioned results and indicated that the soil-borne pathogens were suppressed by the extract (Leaves, stem and roots) of plants *Annona squamosa*, *Ocimum basilicum* and *Cassia fistula* at the concentration of 1% were found effective for growth okra test plant.

CONCLUSION

The leaves and stem of *A. squamosa* have the ability to suppress the root-root and root-knot disease complex and also improve the plant growth. Further experiments using larger plots or small field trails should be conducted to see if we can achieve similar results what we have seen in screen house experiments. In addition, it is also proved that these plants may be utilized the minimizing of soil-borne diseases as an organic amendment.

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