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EFFICACY EVALUATION OF *AZADIRACHTA INDICA*, *CALOTROPIS PROCERA*, *DATURA STRAMONIUM* AND *TAGETES ERECTA* AGAINST ROOT-KNOT NEMATODES *MELOIDOGYNE INCOGNITA*

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Abstract

Different management strategies are being adopted to control root-knot nematode, *Meloidogyne incognita*, one of the most detrimental pests of agricultural crops. Although application of nematicides is the most commonly used practice, they cause pollution of ground water, so safe and efficient alternatives are needed. The use of antagonistic plants for the control of nematodes is a very attractive alternative. In the present study, nematicidal efficacy of four medicinal plants viz. *Azadirachta indica* A.Juss., *Calotropis procera* (Ait.) R.Br., *Datura stramonium* L., and *Tagetes erecta* L., was ascertained for the control of *M. incognita*. All leaf amendments at different dosages significantly improved the plant growth characteristics of okra and reduced root-knot infections compared with the untreated control. *Azadirachta indica* and *C. procera* caused the maximum reductions in number of galls, egg masses and reproduction factor (Rf) of the nematode.

Introduction

Root-knot nematodes are cosmopolitan in distribution, occur in soil and are rarely seen. Control of these nematodes is not well developed. Many noxious chemicals have been tried over the last few decades but only a few have stood the test of time. Most of these compounds are expensive and out of the reach of many farmers. Among various control strategies, the use of antagonistic plants as organic amendments has gained the interest of scientists because they pose few to no environmental hazards. The incorporation of organic material into the soil reduces root-knot nematodes densities, resulting in an increase in yield (Muller & Gooch, 1982). In addition to their suppressive effects on nematode density, organic amendments with antagonistic plants improve soil texture, increase water-holding capacity, supply nutrients to deficient soil and stimulate microbial population of actinomycetes, bacteria, fungi and other elements which might be antagonistic to nematodes (Badra *et al*., 1979; Godoy *et al*., 1983; Rodríguez-Kábana, 1986). Medicinal and antagonistic plants have some advantages over synthetic nematicides. They may contain novel compounds that pests are not yet resistant to, they are potentially less toxic than pure compounds, they biodegrade rapidly, may possess multiple modes of action and are derived from renewable resources (Quarles, 1992). Linford *et al*., (1938) were the first to study the nematicidal effect of chopped pineapple (*Ananas comosus*) leaves used as an organic amendment against *Meloidogyne* spp. Some of the plant species and parts antagonistic to *Meloidogyne* spp. are leaves and flowers of marigold (*Tagetes* sp.), leaves, roots and seeds of neem (*Azadirachta indica*), and leaves and seeds of chinaberry (*Melia azadirach*) (Rather *et al*., 2007). Neem (*A. indica* A.Juss.) is a member of the mahogany family, Meliaceae. It is native from the Indo-Pakistan subcontinent and is known to many people as a "wonder tree" due to its many uses in medicine, agriculture, industry, etc. It has been found, mainly in the last decade or so, that neem materials can affect more than 200 insect species as well as mites, nematodes, fungi, bacteria, and even a few viruses. *Calotropis procera* (Asclepiadaceae), *Datura stramonium* (Solanaceae) and *Tagetes erecta* (Asteraceae) are commonly found in various parts of the country and have been reported to possess nematicidal properties (Ahmad *et al*., 1991, 1996; Walia & Gupta, 1995). The present study was carried out to evaluate the nematicidal efficacy of leaves of these antagonistic plants as soil amendments against root-knot nematode (*Meloidogyne incognita*) at various dosages.

Materials and Methods

Collection of plant material: *Calotropis procera* (ak) and *Datura stramonium* (jimson weed) were collected from the Cholistan desert of Bahawalpur, while *Tagetes erecta* (marigold) and *Azadirachta indica* (neem) were collected from the Botanical Garden of Agriculture Extension Department, Bahawalpur.

Multiplication *Meloidogyne incognita*: *Meloidogyne incognita*, raised from a single egg mass, was mass multiplied on the most susceptible variety of tomato (Money maker). The juveniles were extracted using the method described by Hussey & Barker (1973) and by the Whitehead and Hemming Tray method (Whitehead & Hemming, 1965).

Soil used for pot experiment: The soil (sand 55.6%, silt 19.4%, clay 25%, pH 7.6 and organic matter 0.98%) used in the pot experiment was sterilized with formalin. The soil was then sieved through a 3.5 mm sieve to remove large stones and plant residues.

Bioassay for the assessment of nematicidal efficacy of the test plants: The leaves of *A. indica*, *C. procera*, *D. stramonium* and *T. erecta* were washed and dried under shade. The dried leaves were then mixed thoroughly with formalin-sterilized soil at 25, 50 and 75 g/kg of soil. Five kg of amended soils were transferred to 20 cm diameter pots along with 1 g of nitrogen in the form of urea. The pots without added organic matter served as controls. The pots were watered daily to facilitate decomposition of organic matter. Two weeks after amendment, three seeds of okra (*Abelmoschus esculentus* cv. 'Punjab selection') were sown and after germination one healthy seedling was maintained. The plants were inoculated with 5000 freshly

hatched second stage juveniles of *M. incognita* 10 days after germination. Each treatment was replicated five times. The pots were arranged in a completely randomized design in the greenhouse at 25 ± 2°C for six weeks. The pots were watered when needed.

Data collection: The okra plants were gently removed from the pots after six weeks. The shoots were excised from the roots. The lengths of shoots and roots were measured with a ruler. The shoots and roots of individual plants were weighed with an electric balance. The galls and egg masses on the whole root systems were counted under a stereoscope at a magnification of 40×. For estimations of total nematode populations, eggs were extracted from the okra roots of individual plants by using the method described by Hussey & Barker (1973). The juveniles were extracted from the soil of each pot following the Whitehead and Hemming Tray Method (Whitehead & Hemming, 1965). The total number of eggs and nematodes in soil constituted the total population. The reproduction factor (Rf) was calculated by dividing the final population (Pf) by the initial population (Pi). The percent increase and reduction in these parameters over the control was calculated as follows:

$$\% \text{ Reduction/ increase} = \frac{A - B}{A} \times 100$$

where, A = Value of the control plants
B = Value of the inoculated plants

Statistical analysis: A completely randomized design was used in the experiment. The experiment was repeated twice. As there were no differences in the mean values of all the corresponding treatments of the repeated experiments, the data of both trials were averaged before statistical analysis. All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278. The means were compared by Duncan's Multiple Range Test (DMRT) at 5%. Standard errors of means, trend lines, regression equations and R^2 values were calculated in Microsoft Excel 2003.

Results

Effect of antagonistic plants on root and shoot lengths of okra: The analyses of variance regarding root and shoot lengths of okra plants showed significant effects of the addition of organic amendments ($F = 5.70$; $df = 3$; $P = 0.002$ and $F = 301.91$; $df = 3$; $p<0.001$ respectively), the concentrations of the amendments ($F = 151.95$; $df = 2$; $P < 0.001$ and $F = 837.25$; $df = 2$; $p<0.001$ respectively), and the interaction between amendments and their concentrations ($F = 5.74$; $df = 6$; $p<0.001$ and $F = 44.31$; $df = 6$; $p<0.001$ respectively). Roots and shoots were longest in treatments with *A. indica*, followed by those with *C. procera*, at the concentration of 75 g/kg of soil. The amendments at 25 g/kg of soil were the least effective. It was observed that with the increase in concentration, there was a corresponding increase in root and shoot length over the control. The individual root and shoot lengths and relationships between these parameters and concentrations of individual amendments are given in Figs. 1 & 2.

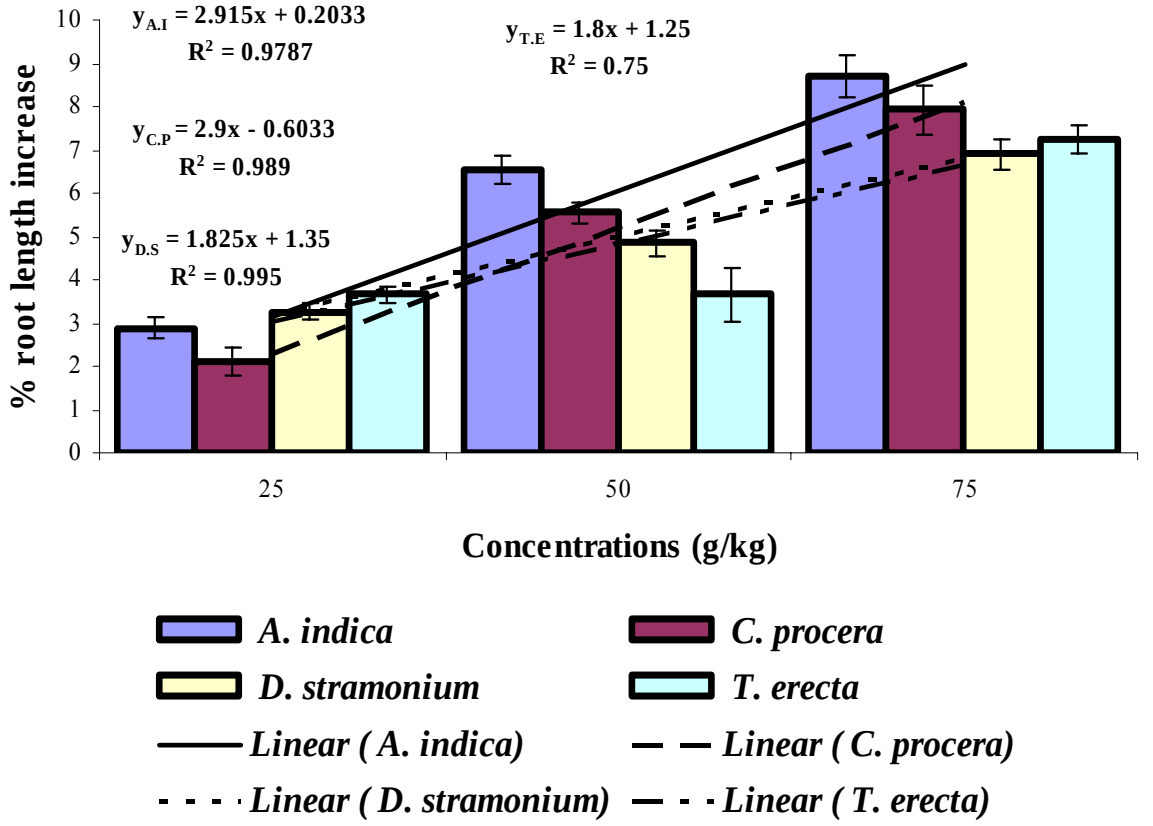

Fig. 1. Effect of organic amendments at various concentrations on % increase in root length of okra. A.I (*Azadirachta indica*), C.P (*Calotropis procera*), D.S (*Datura stramonium*) andT.E (*Tagetes erecta*).

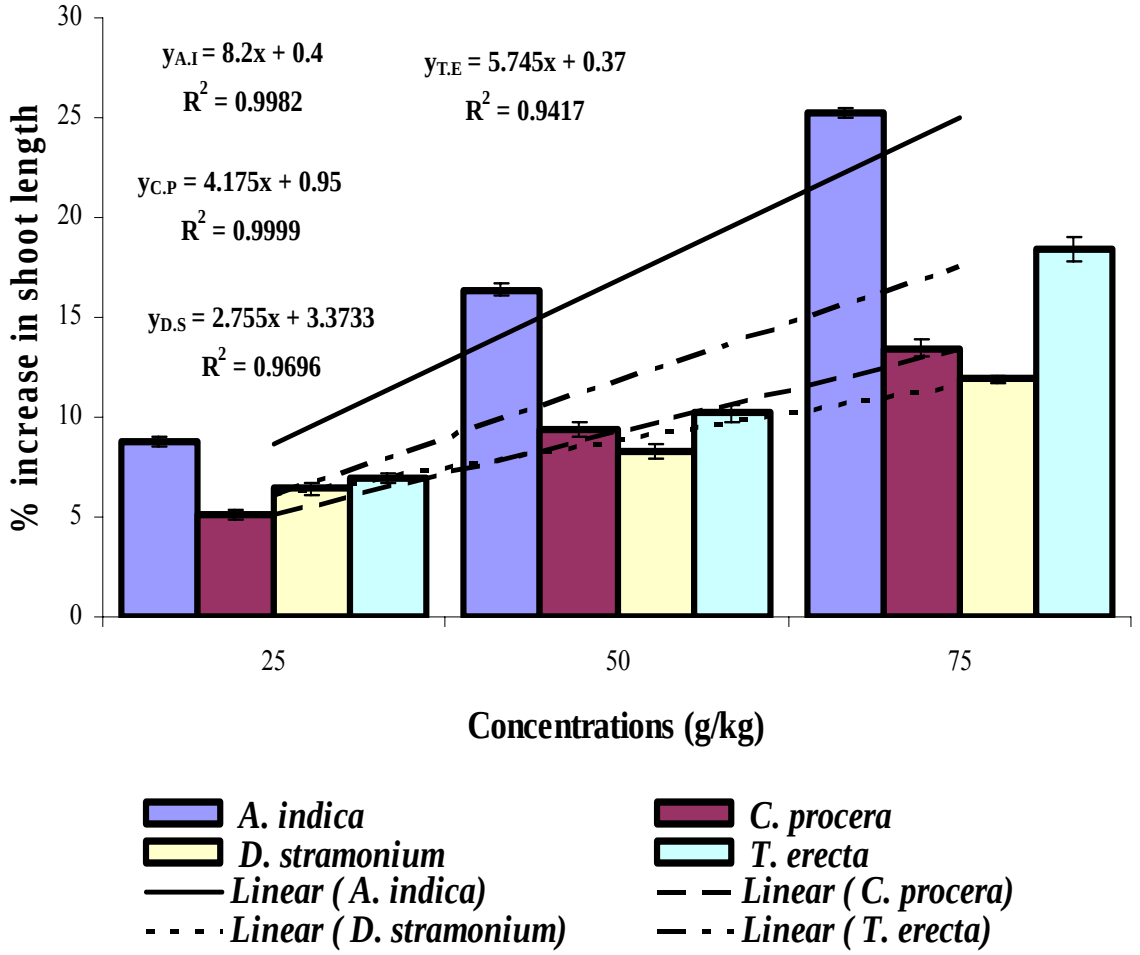

Fig. 2. Effect of organic amendments at various concentrations on % increase in shoot length of okra. A.I (*Azadirachta indica*), C.P (*Calotropis procera*), D.S (*Datura stramonium*) and T.E (*Tagetes erecta*).

Effect of antagonistic plants on shoot and root weights of okra plants: The analyses of variance regarding fresh root and shoot weights and dry shoot weight of okra plants showed significant effects of the amendments ($F = 2.00$; $df = 3$; $P = 0.128$, $F = 80.34$; $df = 3$; $p<0.001$ and $F = 24.70$; $df = 3$; $p<0.001$ respectively), their doses ($F = 189.71$; $df = 2$; $p<0.001$, $F = 1106.76$; $df = 2$; $p<0.001$ and $F = 487.03$; $df = 2$; $p<0.001$ respectively) and the interaction between them ($F = 2.77$; $df = 6$; $P = 0.023$, $F = 33.12$; $df = 6$; $p<0.001$ and $F = 6.02$; $df = 6$; $p<0.001$ respectively). All the amendments significantly increased percent fresh and dry weights over their controls, with the maximum increase seen from *A. indica*, followed by *C. procera*. Similarly, increases in these parameters were higher at higher concentrations of the amendments. A direct relationship was observed between the concentrations of the amendments and these parameters, which are shown with trend lines and equations in Figs. 3 & 4.

On the other hand, a decrease in root weight was observed by the applications of amendments. The reductions in root weights were the maximum in the case of *C. procera* and *D. stramonium*. Similarly, the magnitude of reduction increased with an increase in the concentration of amendments and this increase in reduction of root weight was found directly proportional to the dosage of the amendments. The relationships between doses of individual amendments and reduction in root weight are shown with trend lines and regression equation in Fig. 5.

Effect of antagonistic plants on number of galls, egg masses and reproduction factor: The analyses of variance regarding number of galls, egg masses and reproduction factor showed significant effects of amendments ($F = 2.91$; $df = 3$; $P = 0.045$, $F = 6.30$; $df = 3$; $p<0.001$ and $F = 8.29$; $df = 3$; $p<0.001$ respectively), their concentrations ($F = 53.53$; $df = 2$; $p<0.001$, $F = 93.09$; $df = 2$; $p<0.001$ and $F = 101.56$; $df = 2$; $p<0.001$ respectively) and the interaction between amendments and their concentrations ($F = 0.72$; $df = 6$; $P = 0.636$, $F = 1.60$; $df = 6$; $P = 0.171$ and $F = 1.29$; $df = 6$; $P = 0.281$ respectively). All the amendments caused significant reductions in the number of galls, egg masses, and reproduction factor. The most significant reductions were observed with *A. indica* and *D. stramonium*. Higher concentrations of amendments caused higher reductions. The reductions in these parameters were found to be directly proportional to the concentrations. These relationships are shown by trend lines and regression equations in Figs. 6, 7 and 8, respectively.

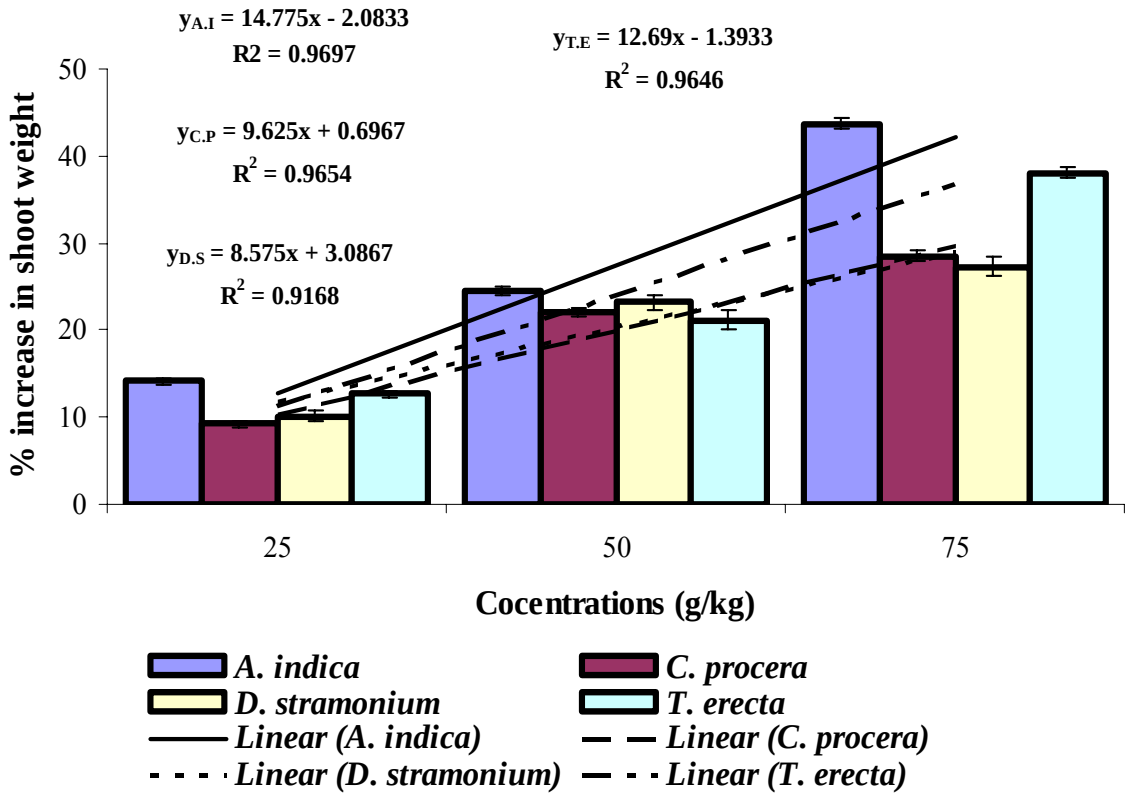

Fig. 3. Effect of organic amendments at various concentrations on % increase in shoot weight of okra. A.I (*Azadirachta indica*), C.P (*Calotropis procera*), D.S (*Datura stramonium*) and T.E (*Tagetes erecta*).

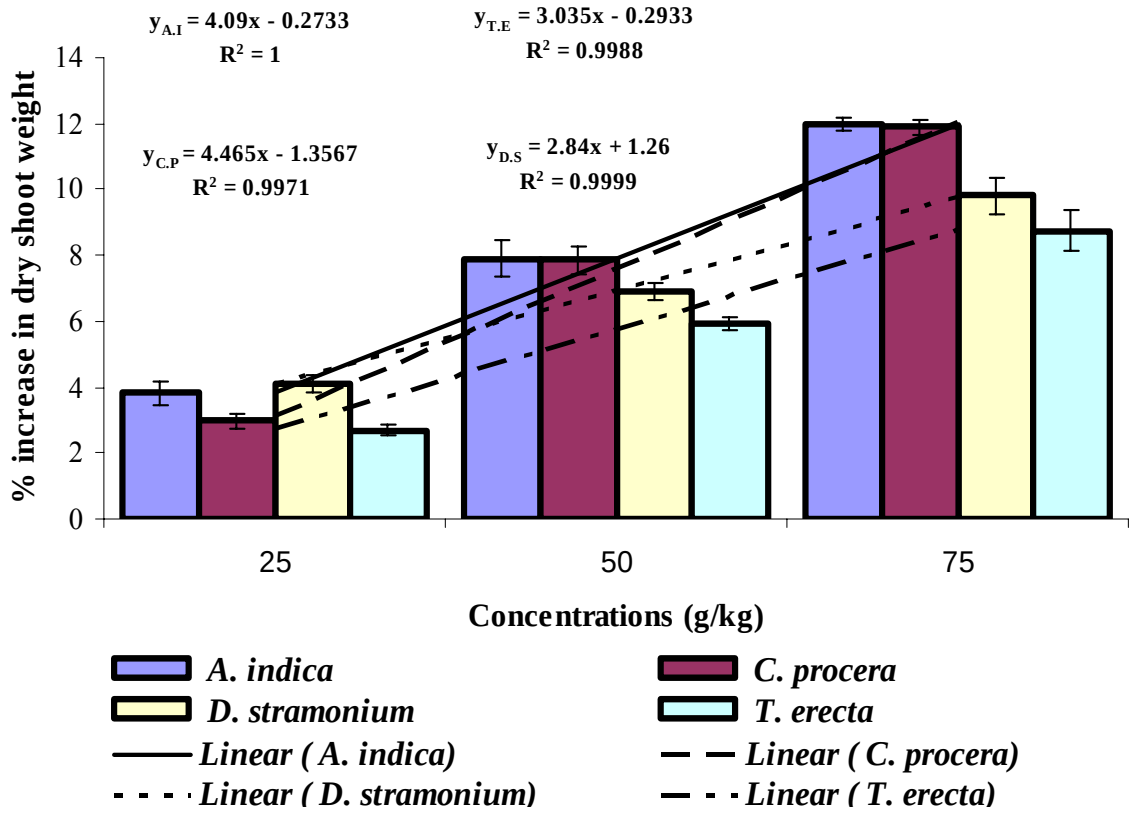

Fig. 4. Effect of organic amendments at various concentrations on % increase in dry shoot weight of okra. A.I (*Azadirachta indica*), C.P (*Calotropis procera*), D.S (*Datura stramonium*) and T.E (*Tagetes erecta*).

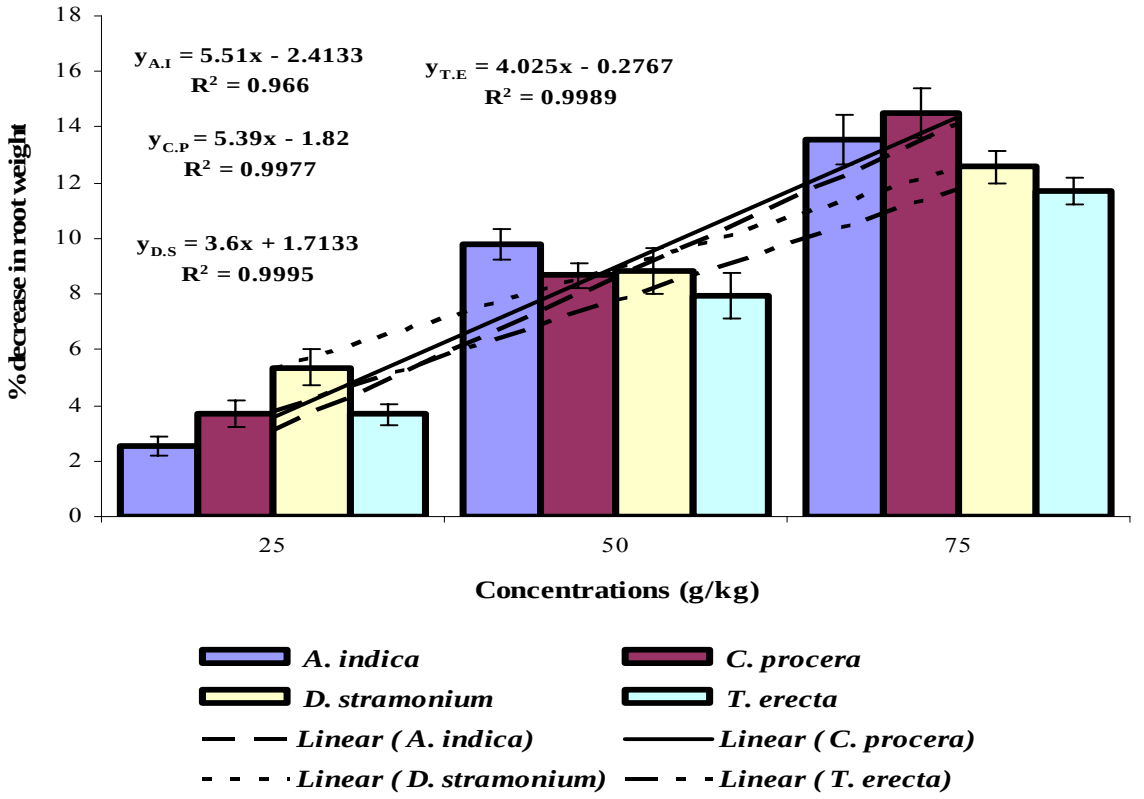

Fig. 5. Effect of organic amendments at various concentrations on % reduction in root weight of okra. A.I (*Azadirachta indica*), C.P (*Calotropis procera*), D.S (*Datura stramonium*) and T.E (*Tagetes erecta*).

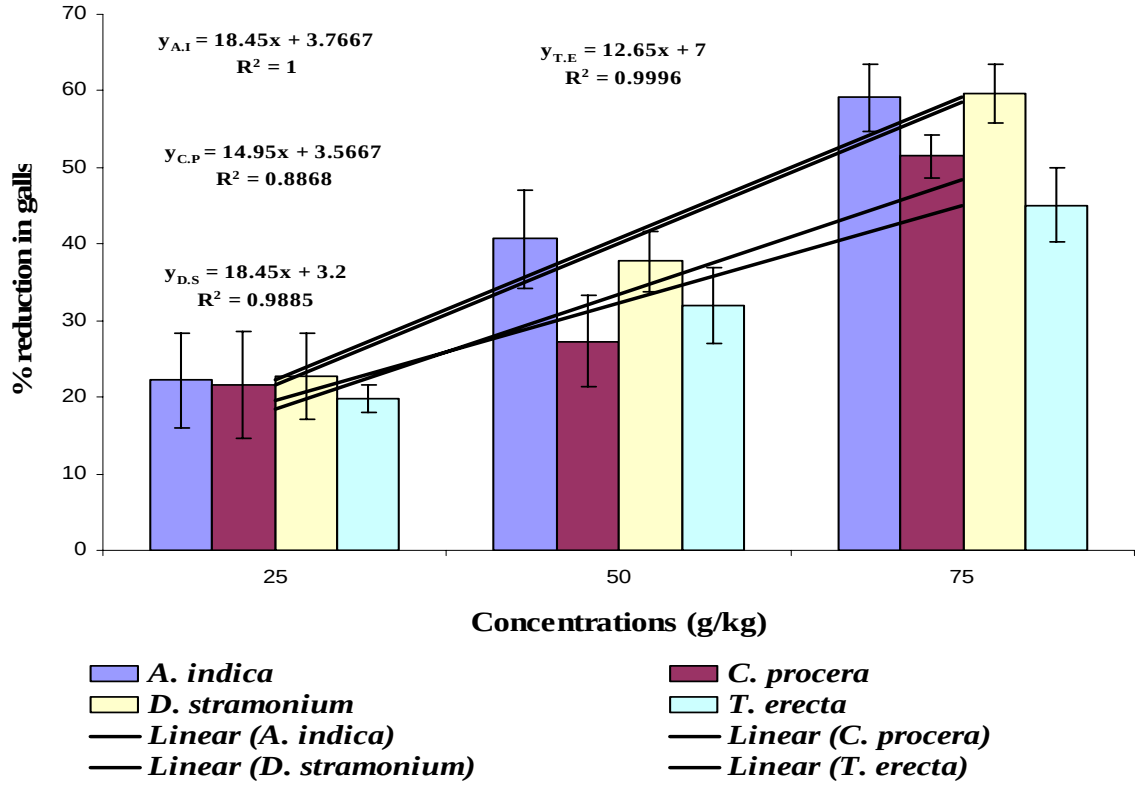

Fig. 6. Effect of organic amendments at various concentrations on % reductions in galls by *M. incognita* on okra. A.I (*Azadirachta indica*), C.P (*Calotropis procera*), D.S (*Datura stramonium*) and T.E (*Tagetes erecta*).

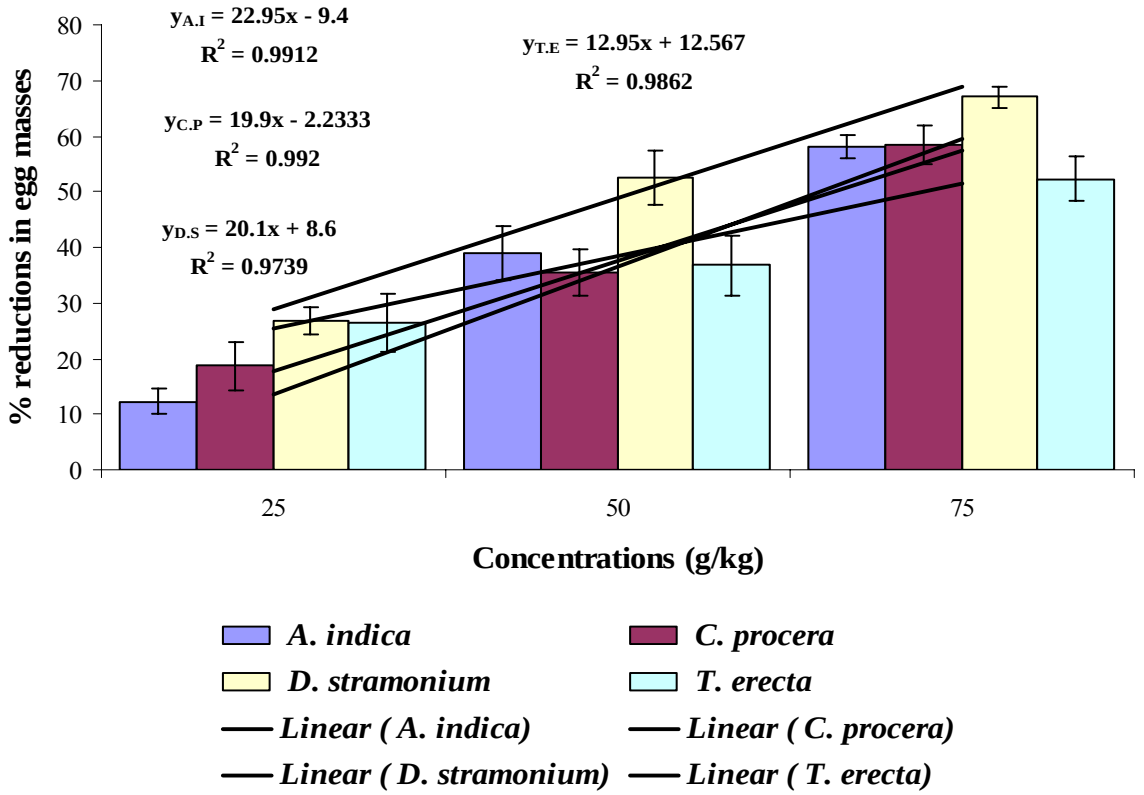

Fig. 7. Effect of organic amendments at various concentrations on % reductions in egg masses by *M. incognita* on okra. A.I (*Azadirachta indica*), C.P (*Calotropis procera*), D.S (*Datura stramonium*) and T.E (*Tagetes erecta*).

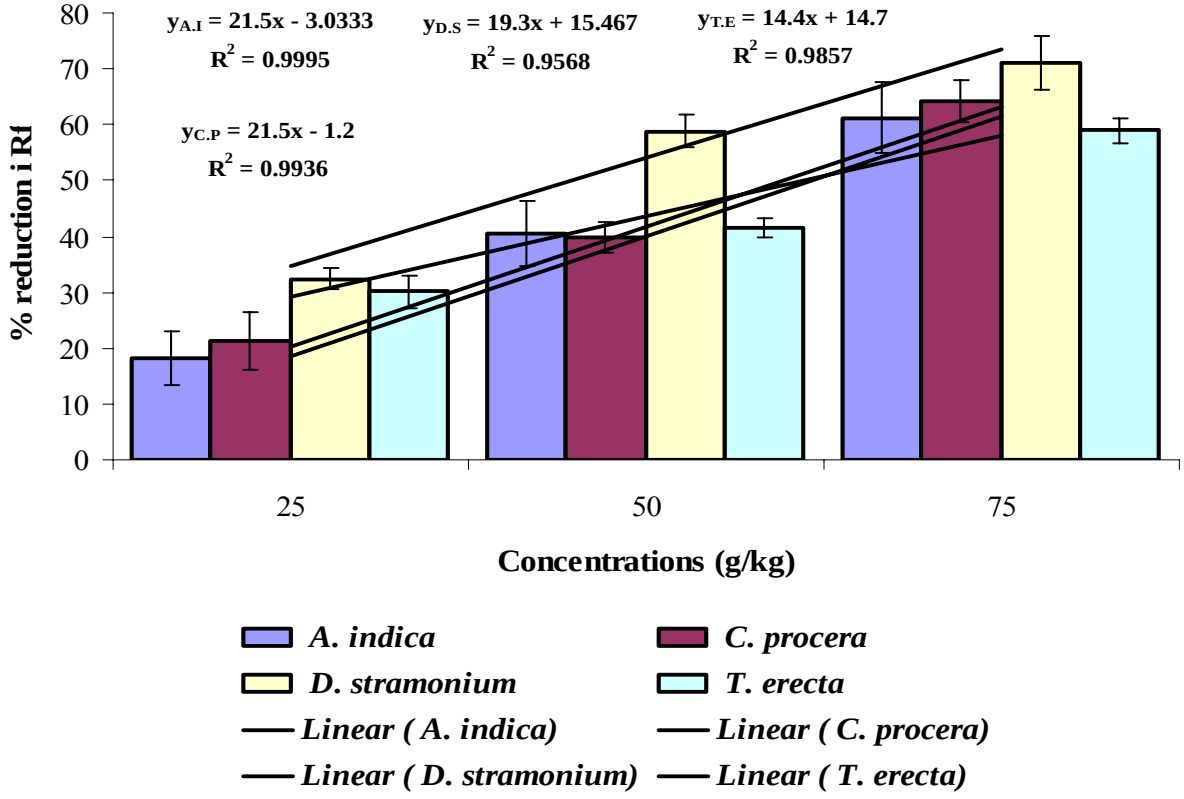

Fig. 8. Effect of organic amendments at various concentrations on % reductions in reproduction factor (Rf) of *M. incognita* on okra. A.I (*Azadirachta indica*), C.P (*Calotropis procera*), D.S (*Datura stramonium*) and T.E (*Tagetes erecta*).

Discussion

All the plants used as amendments caused significant reductions in *M. incognita* infections, resulting in increases of all growth parameters of okra except root weight. Higher concentrations of amendments were more effective than lower ones. The results agree with those of Muller & Gooch (1982), Ali (1990) and Akhtar & Alam (1990). The use of organic soil amendments is the cheapest and most effective way to control plant diseases caused by nematodes. Amendments not only change physical and chemical properties of soil but also support a wide variety of antagoniststic microorganisms like fungi, bacteria, etc. (Jaffee *et al*., 1998; Timm *et al*., 2001), that through competion, antibiosis or parasitism can retard the populations of the plant disease-inciting agents like fungi, bacteria and nematodes. The addition of soil amendments results in a considerable increase in the liberation of CO_2 through the saprophitic activities of soil saprophytes, which can suppress the activities of disease-causing agents (Papavizas & Davey, 1992). Due to rapid multiplication of micro-organisms within the soil, soil nitrogen, which is often scarce, is quickly consumed, resulting in nitrogen deficiency. The nitrogen deficiency greatly reduces the growth of pathogens. It has also been reported that nematode populations may be reduced due to the accumulation of toxic substances that are produced by the decomposition of organic amendments in soil (Akhtar *et al.,* 1982; Khan *et al.,* 1966). Sayre (1980) postulated two hypotheses, which explain the effectiveness of soil amendments in two ways. The decomposition products from soil amendments are directly toxic to plant nematodes, and manipulation of soil microbial populations by the addition of amendments initiates a cascade of events favoring the build-up of bacteria, microbivorers, nematode-trapping fungi and other soil antagonists that destroy parasitic nematodes. The breakdown of organic matter releases compounds into soil that may be toxic to nematodes. Uhlenbroek & Bijloo (1958, 1959) found the highly nematicidal compounds α-terthienyl and 5-(3-buten-1- yny1)-2,2'-bithienyl in *Tagetes* sp. Debprasad *et al.*, (2000) reported that *T. erecta* contains dodecanoic acid, myristic acid, palmitic acid, steric acid, octaeicosane-8-one, triacontane-1-o1, α-sesquiphellandrene, β-sesquiphellendrene, 2-methyl-6-(4-methyl cyclohexadienyl), hept4-en-2-o1, myristoleic acid and trieicosane. These compounds showed nematicidal activity against *M. incognita*. The nematicidal effect of neem is attributed to naturally occurring chemicals, e.g. azadirachtin, nimbin, salannin, nimbidin, kaempferol, thionemone, quercetin, and others. Devakumar *et al*., (1985) identified limonoids in neem that were highly active against nematodes. The limonoids are compounds belonging to the beta-furanotriterpenoid group. So far, at least nine neem limonoids have demonstrated an ability to control a range of insects (Anon., 1992). New limonoids are still being discovered in neem, but azadirachtin, salannin, meliantrol, and nimbin are the best-known ones. The nematicidal activity of *D. stramonium* is due to alkaloids and hyoscine, which have been assayed for nematicidal action on *Hoplolaimus indicus*, *Helicotylenchus multicinctus*, and *M. incognita*. The alkaloids killed 90 to 100% of all nematode species, whereas hyoscine was effective only against *H. indicus* with 90% mortality (Qamar *et al*., 1995). In summary, the results obtained in this study suggest that application of organic amendments of the test plants to soil is effective in the control of root-knot nematodes.

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