African Journal of Agricultural Research Vol. 8(1), pp. 93-101, 8 January, 2013
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR12.500
ISSN 1991-637X ©2013 Academic Journals

Full Length Research Paper

Characterization of nematicidal activity of plant residues and their application with moisture approach against *Meloidogyne incognita* in tomato

Faheem Ahmad[1]*, M. A. Siddiqui[2] and Olubukola O. Babalola[1]

[1]Department of Biological Sciences, Faculty of Agriculture, Science and Technology, North-West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa.
[2]Section of Plant Pathology and Plant Nematology, Department of Botany, Aligarh Muslim University, Aligarh-202002, U.P, India.

Accepted 24 August, 2012

Eco-friendly methods and strategies for nematode management have been recommended due to their non hazardous impact on environment. In this current study, experiment was conducted to control *Meloidogyne incognita* in tomato *cv*. 'K25' by using organic amendment as dip or soil-additive. The root-dip treatment of *Justicia adhatoda* L. inhibited larval penetration in roots of tomato more than the root-dip of *Lantana camara* L. The standard concentration (s) proved to be more effective as compared to diluted concentrations. In another treatment, mixing organic residue of both test plants with soil at 0.5, 1.0 and 3.0% (w / w) 5 days prior to tomato transplanting, improved plant growth response and reduced root-knot development in roots at a 6 and 12% moisture levels. The 3% (w / w) of residue mix of *J. adhatoda* L. had phytotoxic effects on tomato. The 3% (w / w) of organic residue dosage of *J. adhatoda* L. at 12% moisture level caused minimum root-knot development in the roots of tomato. Therefore, the availability of these plant materials in tropical and subtropical regions of the world, especially developing countries will provide an economic and feasible means of controlling nematodes in vegetable crops.

Key words: Organic amendments, nematicidal properties, root-knot nematode, management.

INTRODUCTION

Root knot nematodes (RKN), *Meloidogyne* spp., are recognized in all temperate and tropical areas, and are among the most damaging agricultural pathogens in the world (Trifonova et al., 2009). It has been estimated that the global losses to *Meloidogyne incognita* (Kofoid and White) Chitwood amounts to $ 78 billion (Chen et al., 2004). Yield losses in tomato due to RKN, *Meloidogyne* spp., in India were 15 to 60% (Krishnappa et al., 1992). In the more developed agricultural system, synthetic nematicides are one of the primary means of control but are mostly inappropriate for subsistence farmers in developing countries. The disturbance of soil ecosystems, resurgent outbreaks, and the toxicity of fumigants to humans and animals have become serious concerns. Due to negative impact of chemical pesticides, the eco-friendly approach should be developed to reduce environmental burden. Instead of positive benefits to agricultural and environmental sector, eco-friendly organic manures also reduced the input cost as compared to synthetic chemical pesticides. The above facts motivated to the scientific communities that such type of eco-friendly effective approach would be highly benefited to reduce the incidence of pest diseases in economic crops throughout the world.

*Corresponding author. E-mail: 23849843@nwu.ac.za or ahmad_nematol@yahoo.com. Tel: +27183892627. Fax: +27183892134.

The proper decomposition of organic residue in soil plays a key role in changing the soil properties and develops unfavorable condition for nematodes activities against host plants. The organic soil amendments also improved soil structure and promotes root growth of the host plant (Craswell and Lefroy, 2001; Young and Crawford, 2004). Various chemical compounds released during decomposition may induce disease resistance in the roots against pests. The organic manure suppresses RKN through the production of toxic root exudates and by being incorporated into the soil where it increases organic matter, thereby providing a food source for antagonistic organisms (Valenzuela and Smith, 2002). Therefore, decomposed materials ultimately serve as nutrients for plants and enhance crop yields (Akhtar and Alam, 1993). There are several examples of plant species which upon further investigation against plant-parasitic nematode (PPN) have been found to contain nematicidal compounds that can be used to control RKN (Taba et al., 2008; Ahmad, 2009; Ahmad et al., 2010; Douda et al., 2010). Plant parts and their products used as organic amendments against nematodes, especially those with high nitrogen /carbon ratios have been reported to exhibit nematicidal activity, mainly through the release of ammonia from the plant residue during their decomposition in soil or through increased population of antagonistic micro-organisms (Oka et al., 2006). The nemato-toxic compounds produce during decomposition of plant residue have been reported to reduce roots infection caused by PPN, while several secondary metabolites such as terpenoids, alkaloids and phenolic compounds also showed nematicidal activity (Thoden et al., 2009).

The objectives of this study were to (1) evaluate comparative inhibiting potential of different dilutions of dried leaf powder extract of *Lantana camara* L. and *J. adhatoda* L. against penetration of second stage juveniles (J_2) of *Meloidogyne incognita* into the roots of tomato (*Lycopersicon esculentum* Mill. *cv.* 'K25') seedlings, (2) evaluate nematicidal efficacy of various doses of dried leaf powder of *L. camara* L. and *J. adhatoda* L. against root-knot development caused by *M.incognita,* and also its role on plant growth parameters of tomato.

MATERIALS AND METHODS

Root-knot nematode reproduction and inoculation

Second stage juveniles (J_2) of *M. incognita* were obtained from a pure culture that was previously initiated by egg masses and propagated on eggplant (*Solanum melongena* L.) in the glasshouse. Egg masses were hand picked using sterilized forceps from heavily infected roots. These egg masses were washed in distilled water and then placed in 15 mesh mm sieves (8 cm in diameter) containing crossed layers of tissue paper. Infective J_2 of *M. incognita* in water suspension was adjusted to approximately 1000 freshly hatched juveniles and added in 10 ml of water suspension. These J_2 were used for inoculation in the pots of root-dipping and soil-amendment-study.

Root-dipping treatment

Dried leaf powder of two test plants viz., *L. camara* L. and *J. adhatoda* L. were mixed 15 g in 45 ml distilled water separately and extract were centrifuged at 2000 rpm for 15 min and after centrifuge, the extract were filtered through Whatman No.1 filter paper (Whatman International Ltd., Maidstone, UK). The obtained extracts arbitrarily termed as standard concentration (S), were stored at 4°C. The different concentrations were also prepared as S / 2 (2 fold) and S / 10 (10 fold) from standard concentration (S) with adding the requisite amount of sterilized distilled water (DW) at the time of experiment. Freshly washed roots of three weeks old seedlings of tomato cv. 'K25', were dipped in the various concentration (S, S / 2 and S / 10) of dried leaf powder of both selected plants separately for 45 min and 90 min incubation period. Plants without dip treatment served as untreated control. Each treatment was replicated four times. After dip treatment, roots were again washed and tomato seedlings transplanted singly into plastic pot (5 cm in diameter) containing thoroughly washed sand. The tomato plants were immediately inoculated with 1000 freshly hatched second stage juveniles (J_2) of *M. incognita*. The inoculated tomato seedlings were uprooted from the pots after 72 h and roots were washed with tap water and juveniles (J_2) were isolated from sand by using Cobb's sieving and decanting method along with Baermann's funnel techniques (Southey, 1986) and their number counted. The numbers of larvae that have penetrated into the roots were determined by deducting the larvae present in sand from the initial inoculum levels.

Soil-amendment-study

Leaf powder was separately prepared from air dried leaf of *L. camara* and *J. adhatoda*. Dried leaf powders of both test plants were separately passed through Glen Creston grinder (Dalton Garden, Stanmore, UK) and filtered with 2 mm mesh sieve. The filtered powder was mixed separately with soil at doses of 0.5, 1.0 and 3.0% (w / w) and filled into 1 kg clay pots (15 cm in diameter). Pots containing soil without amendments served as untreated control. Soil moisture level of amended and unamended pots expressed as percentage of water, was estimated as:

Moisture weight of amended soil at 25 ± 4°C temperature = Xg
Dry weight of amended soil = Yg
Weight differences = Xg - Yg = Zg
Percent level of moisture = Zg × 100 / Xg

The moisture of amended and unamended soil was adjusted at 6 and 12% level by adding known amount of water to soil. These moisture levels were maintained throughout the experiment.

Seeds of tomato cv. 'K25' were surface sterilized in 0.1% sodium hypochlorite (NaOCl) for two minutes and then rinsed three times with sterilized distilled water. The tomato seeds were sown in clay pots (25 cm in diameter) filled with autoclaved soil. One week after germination, tomato seedlings were transplanted individually into 1 kg clay pots filled with amended soil including untreated uninoculated (UU) and untreated inoculated (UI) control pots. After five days of tomato seedlings transplantation, approximately 1000 freshly hatched second stage juveniles were inoculated in each pot. The experiments were set up in a randomized complete block design with four replications of each treatment in glasshouse at 25 ± 4°C. Plants were harvested at 50 days after inoculation of nematodes. Data on both shoot and root regarding length, fresh and dry weight were recorded. Infected root system of tomato were washed with tap water, fixed in 4% formalin for 24 h and stained in

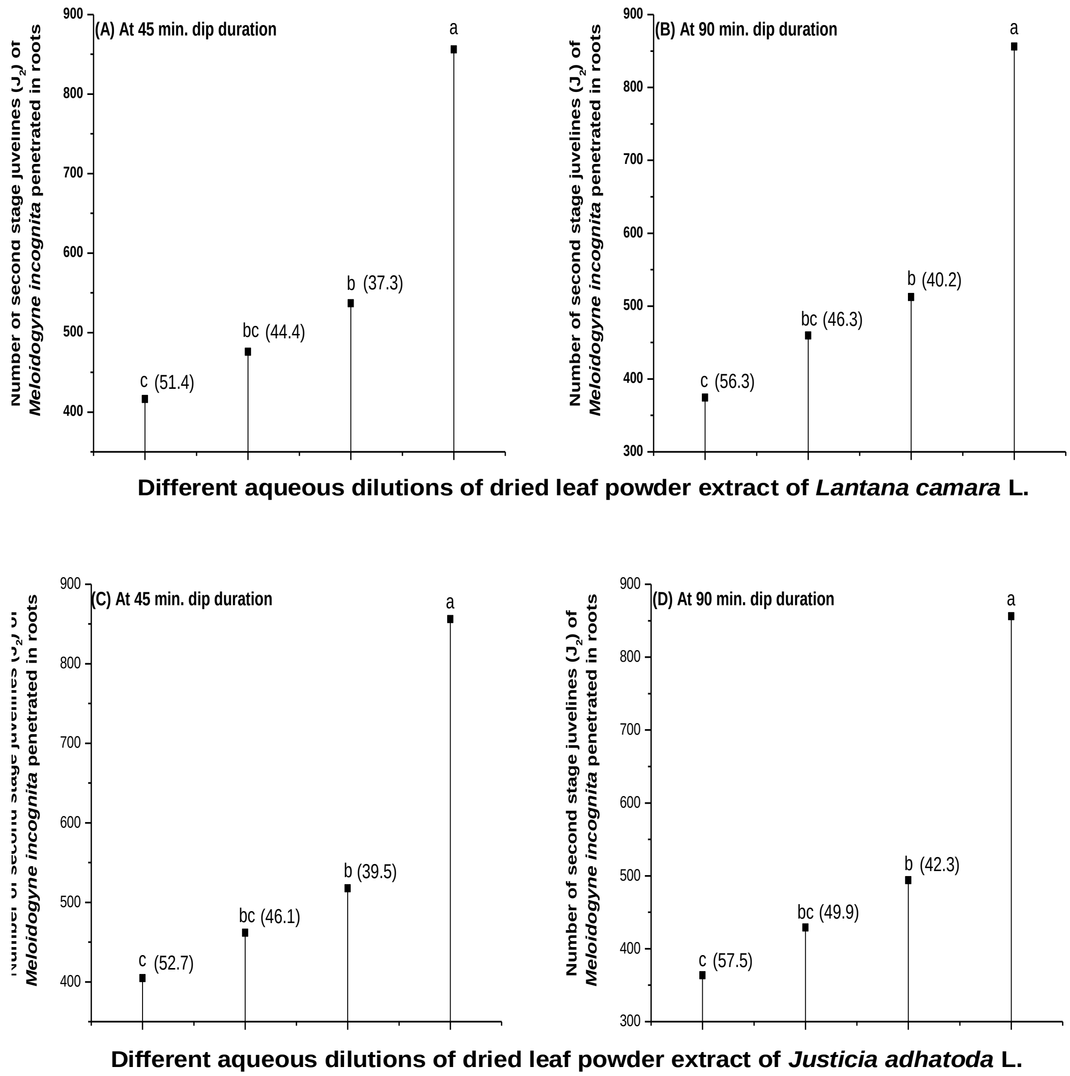

Figure 1. Effect of root dip in different aqueous dilutions of dried leaf powder extract of *Lantana camara* L. and *Justicia adhatoda* L. on the penetration of second stage juveniles (J_2) of *Meloidogyne incognita* in roots of tomato cv. 'K 25' seedlings at different dip duration. Each mean consists of four replications. Means followed by the same letters do not differ significantly at p = 0.05 according to Duncan's multiple range test (DMRT). Figures within bracket are percent inhibition in penetration over distilled water control (856.0) (DW = Distilled water control).

0.01 lactic acid fuchsine (Byrd et al., 1983) and examined number of galls per root system. Development of galls on per root system was rated on scale of 0 - 5: 0 = 0, 1 = 1 - 2, 2 = 3 - 10, 3 = 11 - 30, 4 = 31 - 100 and 5 = greater than 100 galls or egg masses per root system (Taylor and Sasser, 1978). The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 12.0 software (SPSS Inc., Chicago, IL, USA). Least significant differences (L.S.D) were calculated at p = 0.05 and Duncan's Multiple Range Test (DMRT) was employed to test for significant differences between treatments.

RESULTS

Root dipping for 45 and 90 min and dip treatment dilutions of S, S / 2 and S / 10 of dried leaf powder of *L. camara* and *J. adhatoda* showed significant control of juvenile's penetration into the roots of tomato compared to untreated control (Figure 1). The root dipping responses were directly related to the aqueous concentrations and dip duration. The standard concentration (S) of *L. camara* and *J. adhatoda* were found more effective against J_2 penetration, and penetrations were inhibited as 51.4 and 52.7% at 45 min dip duration, respectively (Figures 1a and c). Whereas the 56.3 and 57.5% inhibition were observed during dip duration in the same aqueous concentration of extract for 90 min, respectively (Figures 1b and d). Other concentration doses (S / 2 and S / 10) of *L. camara* and *J. adhatoda* at 45 min dip duration showed 44.4, 37.30 and 46.1, 39.5% inhibition

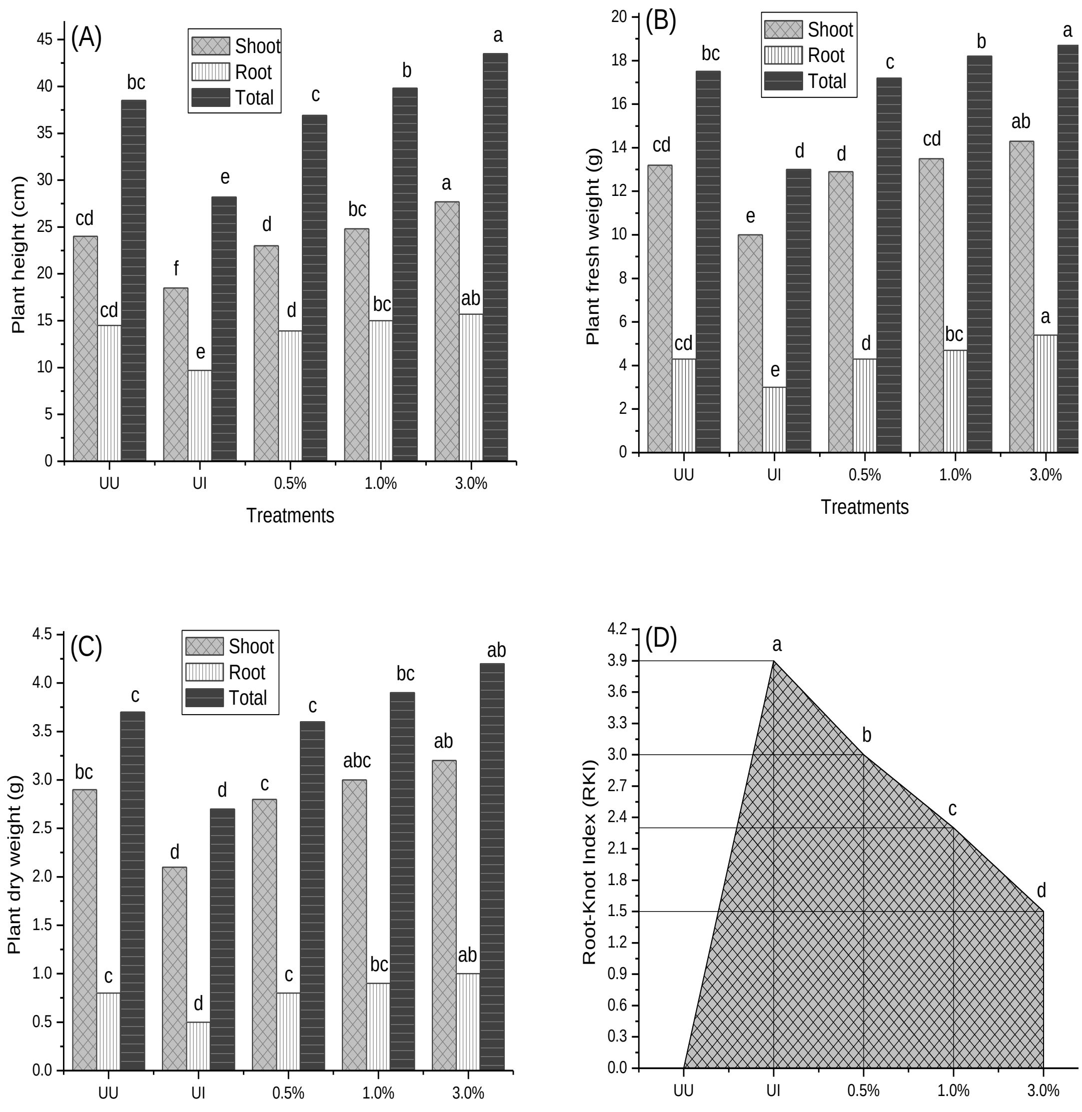

Figure 2. Effect of various doses (w / w) of dried leaf powder of *L. camara* L. at 6% moisture level on tomato cv.'K 25' inoculated with *M. incognita* (1000 J_2 / pot). Each mean consists of four replications. Means followed by the same letters do not differ significantly at p = 0.05 according to Duncan's multiple range test (DMRT). (A) Effect on the plant height (cm) of tomato. (B) Effect on the plant fresh weight (g) of tomato. (C) Effect on the plant dry weight (g) of tomato. (D) Effect on the root-knot development in roots of tomato caused by *M. incognita*. UU = Untreated uninoculated; UI = Untreated inoculated.

against J_2 penetration (Figures 1a and c) and in same extract concentrations, Inhibition were recorded as 46.3, 40.2 and 49.9, 42.3% at 90 min dip duration, respectively (Figures 1b and d). However, juvenile's inhibition against penetration was not more influenced by increasing concentrations strength of dried leaf powder of both test plants (Figures 1a, b, c, d)) from (S / 10 to S / 2) and (S / 2 to S).

The obtained results from the pot experiment indicated that the potted soil mixed with different applied doses 0.5, 1.0, and 3.0% (w / w) of *L. camara* and doses 0.5, 1.0 % of *J. adhatoda* at both moisture level (6 and 12%) were found to increase growth of tomato (Figures 2 to 5). The dose 3% (w / w) of *J. adhatoda* was showed to inhibit the plant growth, while root-knot development was found minimum in roots (Figures 3 and 5). The amended soil which was maintained at 12% moisture level showed slightly better performance in increasing growth parameters in comparison to same amended soil at 6% moisture level. Best improvement in growth parameters of tomato was observed in pots treated with dose 3% (w / w) of *L. camara* at 12% moisture level. However, minimum tomato growth was recorded in pots treated with dose 3% (w / w) of *J. adhatoda* at 6% moisture level

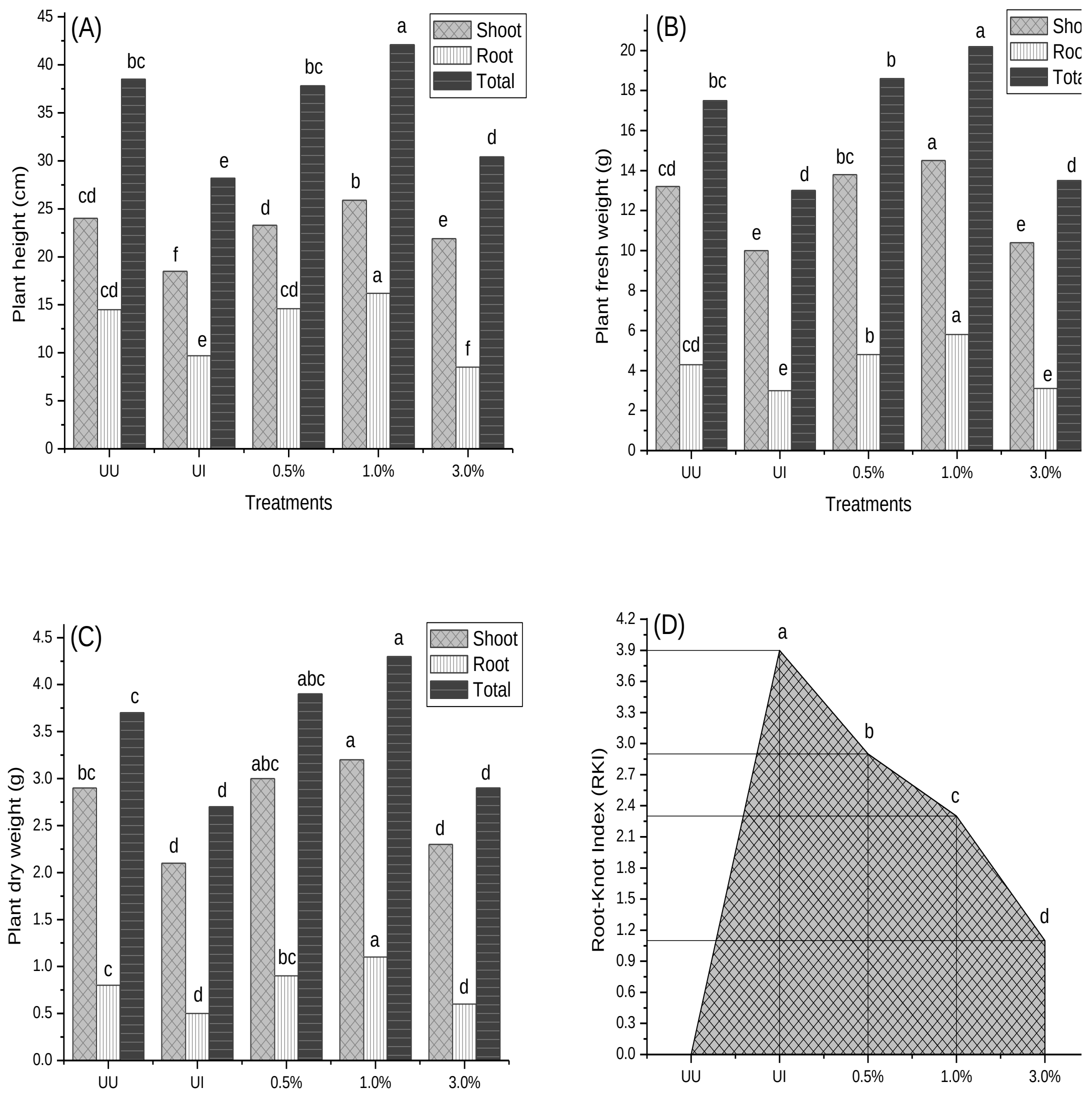

Figure 3. Effect of various doses (w / w) of dried leaf powder of *J. adhatoda* L. at 6% moisture level on tomato cv. 'K 25' inoculated with *M. incognita* (1000 J_2 / pot). Each mean consists of four replications. Means followed by the same letters do not differ significantly at p = 0.05 according to Duncan's multiple range test (DMRT). (A) Effect on the plant height (cm) of tomato. (B) Effect on the plant fresh weight (g) of tomato. (C) Effect on the plant dry weight (g) of tomato. (D) Effect on the root-knot development in roots of tomato caused by *M. incognita*. UU = Untreated uninoculated; UI = Untreated inoculated.

(Figures 2 to 5).

Data represented in Figures 2 to 5 also showed a considerable decrease in galls per root system with increasing rate of applied doses 0.5, 1.0, and 3.0% (w / w) at 6 and 12% moisture level as compared with value recorded in control. Higher applied doses of organic additives resulted in greater effects in reducing number of galls per root system as compared to target lower doses. In respect to higher dose at 12% moisture level, applied dose 3% (w / w) of both test plants drastically reduced galling in roots of tomato when compared either with untreated control or other tested doses (Figures 4 and 5). However, lower dose 0.5% (w / w) of *L. camara* was the least effective at 6% moisture level while same dose at 12% moisture level exhibited slightly better performance in reducing root-knot galling (RKI = 2.7) (Figure 4).

DISCUSSION

Aqueous concentrations of dried leaf powder of *L. camara* and *J. adhatoda* were recorded effective against J_2 penetration in roots of tomato, as previously reported as nematicidal activity against PPN (Anwar et al., 1985;

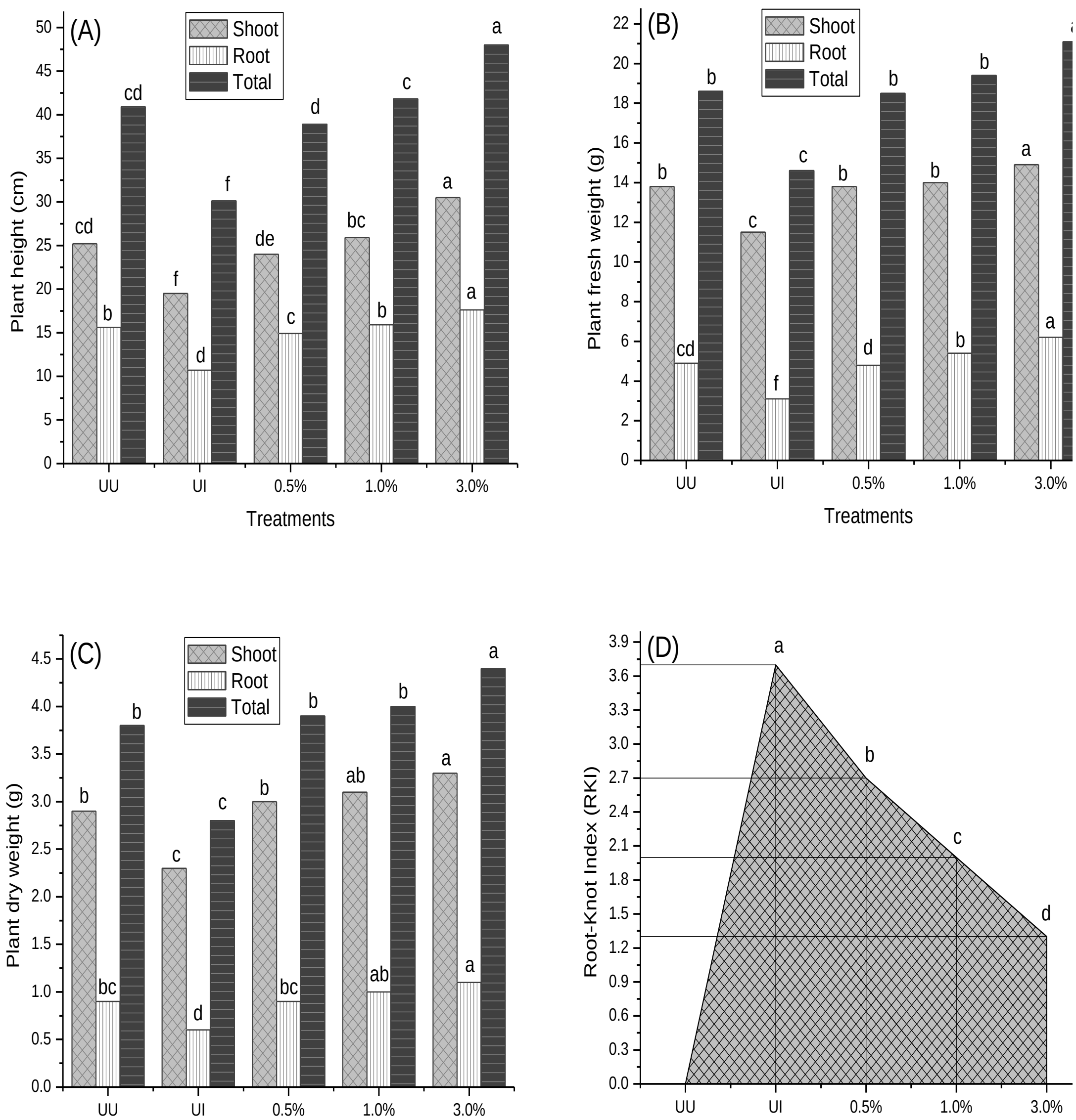

Figure 4. Effect of various doses (w / w) of dried leaf powder of *L. camara* L. at12 % moisture level on tomato cv. 'K 25' inoculated with *M. incognita* (1000 J_2 / pot). Each mean consists of four replications. Means followed by the same letters do not differ significantly at *p* = 0.05 according to Duncan's multiple range test (DMRT). (A) Effect on the plant height (cm) of tomato. (B) Effect on the plant fresh weight (g) of tomato. (C) Effect on the plant dry weight (g) of tomato. (D) Effect on the root-knot development in roots of tomato caused by *M. incognita*. UU = Untreated uninoculated; UI = Untreated inoculated.

Qamar et al., 2005). However, this present results revealed that aqueous concentrations were effective even at concentrations that were too low to inhibit J_2 against penetration in roots. Therefore, the treatments of aqueous concentrations might have repellent action or may have played a direct role in plant defense mechanisms. The protection of root-knot disease of tomato using the aqueous extract of organic residue could be due to either (1) loss of mobility due to paralysis as revealed here by *in vitro* experiment, or (2) disturbed orientation and host findings (linked to disturbance of chemoreception or injuries of sensory organs) disrupting the nematodes co-ordinance in stimuli gradients and affecting the response to root's attractants Such type of consequences of behavioral disturbance have also been suggested by Green (1971). The aqueous dilutions may also have acted against nematodes by changing root conditions inimical to them or dry means of some chemicals absorbed by the roots, or by way of some chemical reactions triggered by these chemicals, causing resistance against invasion and development of plant pathogens (Bell, 1981).

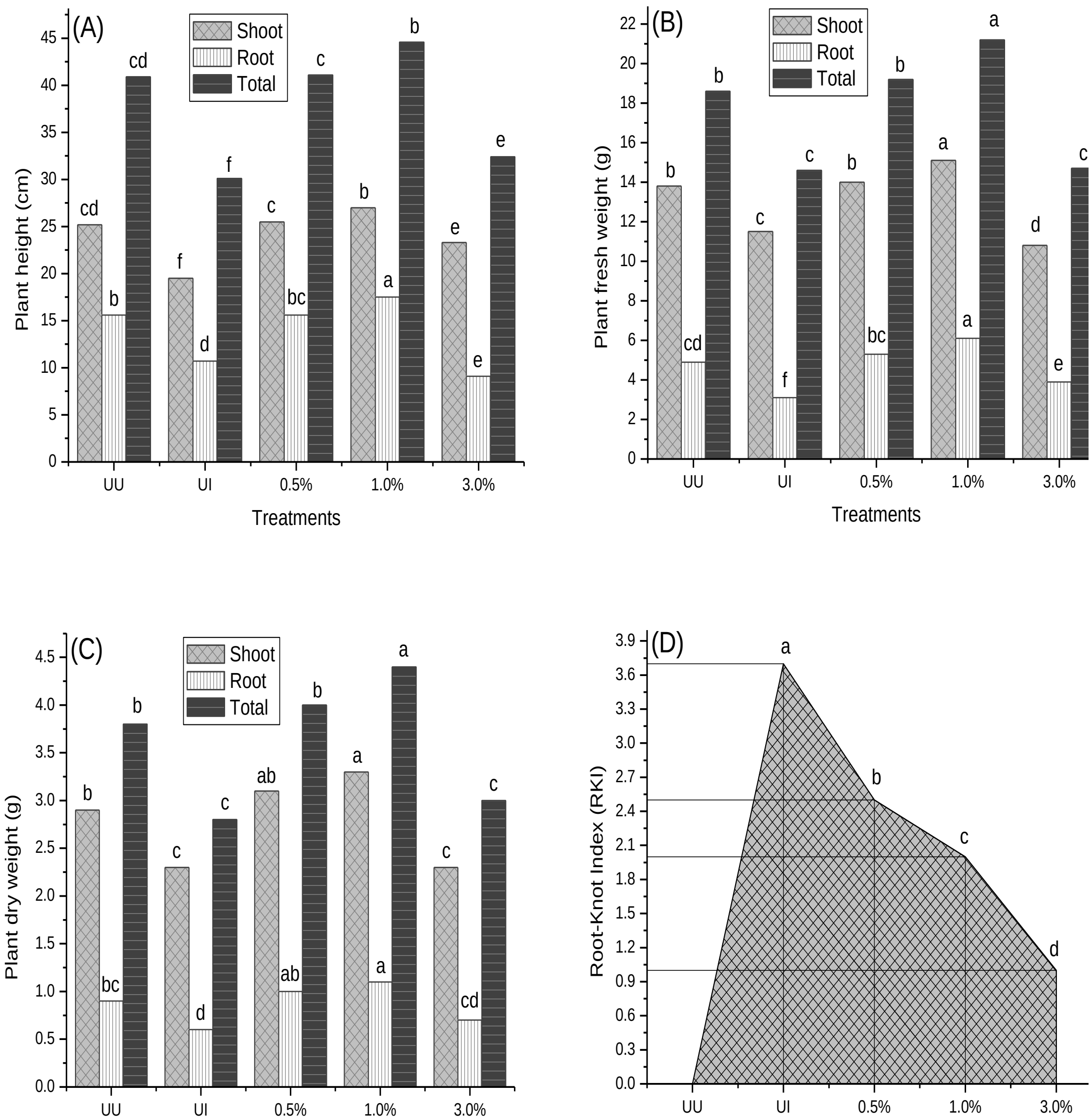

Figure 5. Effect of various doses (w / w) of dried leaf powder of *J. adhatoda* L. at 12% moisture level on tomato cv. 'K 25' inoculated with *M. incognita* (1000 J_2 / pot). Each mean consists of four replications. Means followed by the same letters do not differ significantly at p = 0.05 according to Duncan's multiple range test (DMRT). (A) Effect on the plant height (cm) of tomato. (B) Effect on the plant fresh weight (g) of tomato. (C) Effect on the plant dry weight (g) of tomato. (D) Effect on the root-knot development in roots of tomato caused by *M. incognita*. UU = Untreated uninoculated; UI = Untreated inoculated.

The root-knot developments were found more common in roots grown in unamended soil as compared to those roots which were grown in soil amended with plant residues. Since roots growing in unamended soil supported more root-knot developments causing severe wound to the roots system resulted weak growth of tomato plants. It has been known that application of organic residue could enhance plant growth and increase plant tolerance to damage caused by nematodes (El-Hamawi et al., 2004; Ferraz and de Freitas, 2004). The effects of dried leaf powder used as organic soil amendments at higher moisture level were slightly better to those obtained when it was applied with same organic soil amendments at low moisture level. Because, appropriate moisture level contributed well to decomposition of plants residue and active nematicidal compounds which are released in soil to inhibit the activity of nematodes (Ahmad, 2009). The results presented in this paper show that soil treated with following doses 0.5, 1.0, and 3.0% (w / w) of target plant residues suppressed root-knot development in root system of tomato. Although at the both moisture levels, all amended soil were performed improvement in plant growth parameters including length, fresh and dry weight,and reduction in root-knot development except applied dose 3% (w / w) of *J. adhatoda* exhibited only reduction in growth parameters indicating phytotoxic effects The 3% (w / w) dose of *J. adhatoda* have negative mode of action for plant growth parameters that may either kill the non-target organisms in the rhizospheres of plant roots or change the ions ratio in soil causing deleterious impact on length, fresh and dry weight of tomato plant. Stowe (1979) and Melkania (1983) have also been reported phytotoxic effect on plants growth and indicated in their report that vary in the amount of indigenous secondary metabolites may be possible to release different amount of phytotoxins in soil causing deleterious impact on growth characteristics. The secondary metabolites produced by plants, and are by-products of primary metabolic processes (Levin, 1976).

The chemical constituents of organic soil amendments stimulate the development of populations of microorganisms (Riegel et al., 1996) which may include nematode antagonists or cause the proliferation of microbial species capable of producing substances toxic to nematodes. Suggested mechanisms responsible for nematode suppressing effect included the release of nematicidal compounds from the organic amendments and the stimulation of nematode antagonistic organisms (Odour-Owino, 2003; Kimenju et al., 2004). It must be kept in mind that application of dried leaf powder used as organic soil amendments at moisture level performs better results due to release of nematicidal compounds in soil during decomposition of plants residue. Begum et al. (2000) and Qamar et al. (2005) observed that isolated chemical constituents such as lantanoside, lantanone, camaric acid and oleanolic acid from aerial parts of *L. camara*, possessing nematicidal activity agains RKN, *M. incognita*. Ahmad et al. (2010) also noted that various concentrations of leaf extract of *L. camara* were deleterious to RKN, *M. incognita*. Similarly, *J. adhatoda* has also shown nematicidal activity against *M. incognita* (Anwar et al., 1985) as also found in this present study. Thus, the protective and nutritional properties of dried leaf powder of *L. camara* and *J. adhatoda* make it an attractive alternative to chemicals especially in an intensive farming system, where plant nutrition and disease control including nematodes, are the main limiting factors. For this reason it is suggested that the use of plants residue too would be more efficient against nematodes when used in combination with other management practices that are currently available.

ACKNOWLEDGEMENTS

The support of the fellowship of North-West University is highly acknowledged. The authors express their profound gratitude to Prof Irshad Mahmood (AMU, India) for critically reading and his helpful suggestions that enhanced the value of the manuscript.

REFERENCES

Ahmad F, Rather MA, Siddiqui MA (2010). Nematicidal Activity of Leaf Extracts from *Lantana camara* L. against *Meloidogyne incognita* (Kofoid and White) Chitwood and its use to Manage Roots Infection of *Solanum melongena* L. Braz Arch Biol Technol. 53:543-548.

Ahmad F (2009). Studies on the management of root-knot nematode (*Meloidogyne incognita*) with organic soil amendments. Ph.D. Thesis, Aligarh Muslim University, Aligarh. p. 262.

Akhtar M, Alam MM (1993). Utilization of waste materials in nematode control: A review. Biores. Technol. 45:1-7.

Anwar M, Sultanul-Haq, Saxena SK (1985). Effect of some plant extracts on the larval hatching of *Meloidogyne incognita* (Kofoid and White) chitwood. 1949-III. Ind. Forest. 111:841-845.

Begum S, Wahab A, Siddiqui BS, Qamar F (2000). Nematicidal constituents of the aerial parts of *Lantana camara*. Nat. Prod. Res. 63:765-767.

Bell AA (1981). Biochemical mechanisms of disease resistance. Annu. Rev. Plant. Physiol. 32:21-81.

Byrd DW, Kirkpatrick T, Barker K (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. J .Nematol .15:142 -143.

Chen ZX, Chen SY, Dickson DW (2004). Nematology advance and perspectives. Vol. II. CAB International, UK. p. 608.

Craswell ET, Lefroy RDB (2001). The role and function of organic matter in tropical soils. Nutrient Cycl. Agroecosys. 61:7-18.

Douda O, Zouhar M, Maza-kova J, Nova-kova E, Pavela R (2010). Using plant essences as alternative mean for northern root-knot nematode (*Meloidogyne hapla*) management. J. Pest. Sci. 83:217-221.

El-Hamawi MH, Youssef MMA, Zawam HS (2004). Management of *Meloidogyne incognita*, the root-knot nematode, on soybean as affected by marigold and sea ambrosia (damsisa) plants. J. Pest. Sci. 77:95-98.

Ferraz S, De freitas LG (2004). Use of antagonistic plants and natural products. In: Nematology-Advances and Prospectives, Volume II: Nematode Management and Utilization (Eds. ZX Chen, SY Chen, DW Dickson). CABI Publishing, Cambridge, MA. pp. 931-977.

Green CD (1971). Mating and host finding behavior of plant nematodes. In: *Plant Parasitic Nematodes*, ed. Zuckerman, B.M., Mai, W.F. and Rohde, R.A. Academic Press, New York. pp. 247-266.

Kimenju JW, Muiru DM, Karanja NK, Nyongesa WM, Miano DW, Mutua GK (2004). Assessing the role of organic soil amendments in management of root-knot nematodes on common bean, *Phaseolus vulgaris* L. J. Trop. Microbiol. 3:14-23.

Krishnappa K, Ravichandra NG, Reddy BMR (1992) Nematode pests of national importance and their management. In: Pest management and pesticides: Indian scenario (Eds. BV David). Namrutha, Madras, India. pp. 57-66.

Levin DA (1976). The Chemical Defenses of Plants to Pathogens and Herbivores. Annu. Rev. Ecol. Syst .7: 121-159.

Melkania NP (1983) Influence of certain selected tree species on ground flora. Ph.D. Thesis, Kumaun University, Nainital. p. 442.

Odour-Owino P (2003). Integrated management of root-knot nematodes using agrochemicals, organic matter and the antagonistic fungus, *Paecilomyces lilacinus* in natural field soil. Nematol. Mediterr. 31:121-123.

Oka Y, Tkachi N, Shuker S, Rosenberg R, Suriano S, Fine P (2006). Laboratory studies on the enhancement of nematicidal activity of ammonia releasing fertilizers by alkaline amendments. Nematology 8:335-346.

Qamar F, Begum S, Raza SM, Wahab A, Siddiqui BS (2005). Nematicidal natural products from the aerial parts of *Lantana camara* Linn. Nat. Prod. Res. 19:609-613.

Riegel C, Fernandez FA, Noe JP (1976) *Meloidogyne incognita* infested soil amended with chicken litter. J. Nematol. 28:369-378.

Southey JF (1986). Laboratory methods for work with plant and soil nematodes. (London: Ministry of Agriculture, Fisheries and Food, HMSO). p. 202.

Stowe LG (1979) Allelopathy and its influence on the distribution of plants in an Illinois old field. J. Ecol. 67:1065-1085.

Taba S, Sawada J, Moromizato Z (2008). Nematicidal activity of Okinawa

Island plants on the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. Plant. Soil 303:207-216.

Taylor AL, Sasser JN (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* spp.). NCSU, Graphics, Raleigh, NC, USA. p. 111.

Thoden TC, Hallmann J, Boppré M (2009). Effects of plants containing pyrrolizidine alkaloids on the northern root-knot nematode *Meloidogyne hapla*. Eur. J. Plant. Pathol. 123:27-36.

Trifonova Z, Karadjova J, Georgieva T (2009). Fungal parasites of the root-knot nematodes, Meloidogyne spp. in Southern Bulgaria, Estonia. J. Ecol. 58:47-52.

Valenzuela H, Smith J (2002). "*Tropic sun", sunnhemp*. Hawaii, USA, University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources. (www.ctahr.hawaii.edu/oc/freepubs/pdf/GreenManureCrops/sunnhemp.pdf).

Young IM, Crawford JW (2004). Interactions and self-organisation in the soil-microbe complex. Science 304:1634-1637.