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USE OF *RHIZOPHORA MUCRONATA* IN THE CONTROL OF *MELOIDOGYNE JAVANICA* ROOT KNOT NEMATODE ON OKRA AND MASH BEAN

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Abstract

Aqueous and ethanol extracts of leaves and stem of *Rhizophora mucronata* plant were tested for their nematicidal activity against *Meloidogyne javanica* root knot nematode. Results showed that *R. mucronata* exerted more lethal effect in mortality of juveniles as compared to hatching of juveniles. Of the different plant parts used, stem showed more nematicidal effect as compared to leaves in aqueous extract as well as in ethanol extract except that in mortality leaves showed more nematicidal effect in ethanol extract. Soil amendment @ 0.1, 1 and 5 % w/w was carried out with dried powder of leaves and stem of *R. mucronata* in the control of root knot nematode on mash bean and okra plants. Germination of seeds, shoot length, root length, shoot weight and root weight significantly increased in both mash bean and okra where leaves and stem powder of *R. mucronata* plant was used @ 5% w/w. Maximum inhibition in root knots were observed when *R. mucronata* plant parts powder was used @ 5% w/w followed by 1% w/w. Stem powder were found to be more effective as compared to leaves in the control of root knots.

Introduction

Plant parasitic nematodes, often referred to as "hidden enemies" are among the most wide spread and important pathogens causing serious losses to crop plants. Root-knot nematode *Meloidogyne* spp., are world wide in distribution and are known to attack a wide variety of crops (Goodey *et al*., 1965). Of a total 70 *Meloidogyne* spp., identified so far (Luc *et al*., 1988), only 4 species viz., *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood and *M. hapla* Chitwood are of major economic importance. *M. incognita* root knot nematode is the most abundant and damaging nematode in Pakistan infecting about 102 plant species (Maqbool & Shahina, 2001). *Meloidogyne* species induce major morphological and physiological changes within roots, attack nearly every crop sown where yields and quality are reduced (Sasser, 1980). Damage caused by root-knot nematode is much higher in tropical and sub tropical countries (Taylor & Sasser, 1978). In Pakistan, *Meloidogyne* root knot nematodes are recognized as important parasites of vegetable crops, where more than 100 plants have been found infested with root knot nematode from different cultivated zones of the country (Maqbool, 1988; Zaki, 2000). Although the application of chemical nematicides have been found as an effective measure for the control of nematode but due to high toxic residual effect of these chemicals (Akhtar, 1991), there is therefore need to develop alternative nematode control strategies (Siddiqui & Alam, 1985). Organic amendments are generally used for the improvement of crop plants and increasing agricultural productivity. Stone *et al*., (2000) showed that addition of organic matter amendments (organic wastes and plant residues) to field soils suppress a variety of soil borne diseases. Various organic amendments have a suppressive effect on plant parasitic nematodes

(Alam, 1976, 1990). Mehdi *et al*., (1999) observed that use of *Avicennia marina* as organic amendment showed promising results in the control of soil borne root infecting fungi like *Macrophomina phaseolina, Fusarium solani*, *Rhizoctonia solani* and *M. javanica* root knot nematode in tomato. Mangroves have been reported to contain compounds like tannins, alkaloids, polyphenols (Combs & Anderson, 1949) which have antimicrobial activity (Jamale & Joshi, 1978; Nishiyama, 1978; Ross *et al*., 1980). The aim of the present study was to use *R. mucronata* as a biocontrol agent in suppression of root knot nematode and increase the productivity of mash bean and okra.

Materials and Methods

R. mucronata plant parts viz., leaves and stem were used for the control of root knot of crop plants. *R. mucronata* parts were collected from coastal areas. Dried powder was used as such for soil amendment and for *in vitro* experiment. Mangrove plant parts was soaked in distilled water and after 24 hrs the extract was filtered with Whatman's filter paper.

***In vitro* experiments:** Egg masses of *Meloidogyne javanica* obtained from the roots of egg plant (*Solanum melongena* L.) were collected by the method of Hussey & Barker (1973). Suspension of eggs in distilled water was prepared 30-40 eggs/ml containing 0.1 ml egg suspension and 2ml of aqueous and ethanol extract was transferred in glass cavity block of 2.5 cm diam., and was kept at room temperature, this was 50% extract. Similarly 100% test extract was made. Cavity block containing 0.1 ml egg suspension in 2 ml distilled water served as control. Each treatment was replicated three times. After 24, 48 and 72 hrs exposure, the numbers of hatched larvae were counted under a low power stereoscopic microscope (Cayrol *et al*., 1989).

In case of mortality, suspension of eggs in distilled water was prepared and poured on a plastic sieve with tissue paper on a funnel and incubated at room temperature for 24 hrs. After hatching juveniles were collected and suspension of juveniles in distilled water was prepared. 0.1 ml of freshly hatched juveniles suspension (30-40 juveniles/ml) and 2 ml of test extract was transferred in a cavity block, 2.5 cm diam., and kept at room temperature. Cavity block with distilled water served as control. Each treatment was replicated three times. At 24, 48 and 72 hrs exposure the mortality % was calculated (Cayrol *et al*., 1989).

***In vivo* experiments:** Roots of egg plant (*Solanum melongena* L.) infested with *Meloidogyne javanica* root knot nematode were collected from the experimental plot of the Department of Botany, University of Karachi. They were washed under running tap water and cut into small pieces then dipped in 100ml of 1% Na $(OCl)_2$ in a bottle and mouth was tightly closed. After shaking vigorously by hand for 5 min., the content was poured onto a 100 mesh sieve fitted over a 400 mesh sieve, followed by washing under running tap water for 1 min. The residues from 400 mesh sieve were transferred into 250ml beaker. Number of eggs and larvae/ml of suspension were determined with the help of counting dish (Hussey & Barker, 1973). Soil obtained from the experimental plots of the Department of Botany, University of Karachi was sieved through 2mm sieve to discard non soil particles and transferred in 8cm diam., plastic pots @ 300gm/pot. The soil used was sandy loam (Sand, Silt, Clay; 70, 19, 11%), pH range from 7. 5 - 8.1 with moisture holding capacity (MHC) of 24.04 % (Keen & Raczkowski, 1922), total nitrogen

1.5 % (Mackenzie & Wallace, 1954), total organic matter 2.4 %. Soil after amendment with *R. mucronata* plant part viz., leaves and stem @ 0.1, 1 and 5 % w/w was kept in 8cm diam, plastic pot @ 300 gm/pot. Soil moisture was adjusted at 40% M.H.C. (Keen & Raczkowski, 1922). Non-amended soil served as control. There were three replicates of each treatment. After 1 week of amendment, 5 seeds of mash bean and okra were sown in each pot. The pots were arranged in randomized complete block design, then 2 week old plants were infested @ 2000 eggs/ pot (Hussey & Barker, 1973). After 45 days of inoculation, data on germination, plant height, shoot weight, root weight and root knots were recorded. Infection of roots by root knot nematodes was estimated by using the 0-5 scale (Taylor & Sasser, 1978). Data were analyzed and subjected to analysis of variance (ANOVA) following the procedure as given by Gomez & Gomez (1984).

Results

***In vitro*:** The present study showed the efficacy of mangrove plant parts viz., leaves and stem aqueous and ethanol extracts in the control of root knot nematode. All parts of *R. mucronata* showed nematicidal effect by reducing the hatching of eggs to varying degree as the exposure period increased. Aqueous extract used @ 100 % showed greater reduction in hatching of eggs as compared to 50 % (Table 1). Similarly ethanol extract used at 1000 ppm showed reduction in eggs hatching followed by 500 and 250 ppm. After 24 hrs no eggs hatched in ethanol extract of leaves of *R. mucronata* (Table 1). Of the different plant parts used, stem showed more nematicidal effect in aqueous extract as compared to leaves whereas leaves showed more significant result as compared to stem in ethanol extract. Aqueous and ethanol extract of *R. mucronata* plant parts showed significant mortality of *M. javanica* larvae. The mortality increased with the increase in extract concentration and exposure time. Siddiqui *et al*., (2000) obtained same result by using ethylacetate and hexane fraction at different concentration in the mortality of *M. javanica*. In aqueous extract killing of juveniles of *M. javanica* increased as the exposure period increased exerting maximum lethal effect @ 100 % whereas 50 % treatment with different mangrove plant part showed less lethal effect as compared to 100 % (Table 1). Mehdi *et al*., (2001) observed the same results by using aqueous, methanolic and chloroform extracts of *A. marina* which caused significant mortality of *M. javanica* juvenile. Ethanol extract used @1000 ppm showed highest mortality % of juvenile as compared to 500 and 250 ppm (Table 1). In the present study, ethanol extract used @ 1000 ppm showed more nematicidal effect as compared to aqueous extract. Mehdi *et al*., (2001) reported that aqueous extract of *A. marina* and *R. mucronata* caused significant mortality of *M. javanica* juvenile.

***In vivo* effect:** *R. mucronata* plant parts powder viz., leaves and stem used @ 5% showed an increase in plant height as compared to control. Germination of seeds showed significant increase where *R. mucronata* plant parts powder was used @ 5% w/w in mash bean and okra (Table 2). Greater plant height was observed in okra and mash bean when *R. mucronata* plant parts powder was used @ 5% w/w. Maximum shoot and root weight were observed in okra where *R. mucronata* dried leaves and stem powder was used @ 5% w/w ($p<0.001$). Shoot length, shoot weight, root length and root weight of okra plant were significantly increased ($p<0.001$) (Table 2). Present results showed that *R. mucronata* plant parts viz., leaves and stem were more effective in the control of *M. javanica* infection in mash bean and okra. Maximum inhibition in root knots of okra and mash bean were recorded where *R. mucronata* plant parts was used @ 5% w/w followed

by 1% w/w. Maximum knots were observed in okra ($p<0.001$) as compared to mash bean ($p<0.01$). All plant parts powder of *R. mucronata* were equally effective in control of *M. javanica* (Table 2). Similarly Mehdi *et al*., (2001) reported that *A. marina* and *R. mucronata* with or without *Pseudomonas aeruginosa* significantly reduced the root knot infection in tomato. Results from the present study suggested that *R. mucronata* plant parts viz., leaves and stem as an organic amendment could therefore be used as a potential approach for the improvement of plant growth and in the control of root knot nematodes.

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References

Akhtar, M. 1991. *Studies on the management of plant parasitic nematodes with organic soil amendments.* Ph.D. Thesis. Aligarh Muslim University, Aligarh-202002, India, pp 195.

Alam, M.M. 1976. *Organic amendments in relation to nematodes*. Ph.D. Thesis, Aligarh Muslim University. Aligarh ,pp150.

Alam, M.M. 1990. Neem in nematode control,. In: *Nematode Biocontrol (Aspects and Prospects*). M.S. Jairajpuri, M.M Alam and I. Ahmed (Eds.) BS Publishers and Distributors (Pvt.) Ltd. Delhi-110032, India pp 51-55.

Cayrol, J.C., C. Djian and I. Pijarowski. 1989. Studies on the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus. Rev*. *Nematol*., 12:331-336.

Combs, C.A and H. Anderson. 1949. Use of mangrove bark. *Australian leather trade Rev*. 43:270-274.

Gomez, K.A. and A.A. Gomez. 1984. *Statistical procedures for Agricultural Research.* 2nd ed. Willey, New York, pp 680.

Goodey, J.B., M.T. Franklin and D.J. Hooper. 1965. *Goodey's the nematode parasites of plant Catalogued under their hosts.* Farnham Royal, Commonwealth Agriculture Bureax. 3rd edition, pp 214.

Hussey, R.S. and K.R. Barker. 1973. *A comparison of nematodes of collecting inocula for Meloidogyne* spp, including a new technique. *Pl. Dis. Report*., 61: 328-331.

Jamale, B.B. and G.V. Joshi. 1998. Effect on age of mineral constituents. Polyphenoloxides and peroxides in mangrove leaves. *J. Exp. Biol*., 16(1): 117-120.

Keen, B.A. and H. Raczkowski. 1922. Clay contents and certain physical properties of soil. *J.Agric. Sci*., 11:441-449.

Luc, M., A.R. Magneetic and R. Fortuner. 1988. A reappraisal of Tylenchina (Nematol) 9. The family Heteroidae Filip've and Schuurmans Stekhovem, 1941. *Revue Nematol*., 11: 159-176.

Mackenzie, H.A. and H.S. Wallace. 1954. The kjeldahl determination of nitrogen: A critical study of digestion conditions, temperature, Catalyst and oxidizing agents. *Aust.J.Chem*., 7:55-70.

Maqbool, M.A. 1988. An overview of nematode problem and research in Pakistan. In: *Advances in Plant Nematology* US-Pak Int. workshop on Plant Nematology, (1986). NNRC, University of Karachi, Karachi Pakistan, pp 23-46.

Maqbool, M.A. and F.Shahina. 2001. *Biodiversity of Nematode Fauna in Pakistan.* NNRC, University of Karachi, Karachi- 75270, Pakistan, pp 180.

Mehdi, F.S., I.A. Siddiqui, S. Erum and R. Ali. 1999. Effect of *Avicennia marina* and *Paecilomyces lilacinus* on root rot-root knot diseases of tomato. *Pakistan Journal of Biological Sciences*, 2(4): 1462-1466.

Mehdi, F.S., I.A. Siddiqui, T. Zia and N.I. Ali. 2001. Use of mangrove for the control of *Meloidogyne javanica* in tomato. *Nematol. Medit.*, 29: 127-129.

Nishiyama**,** Y., P.C. Sanchez and M. Kozaki. 1978. Inhibitory functions of Mangrove bark towards cell growth of microorganisms. Hakko, Kogaku, Kaishi, 56: 712-717.

Ross, S.A., S.E. Megalla, D.W. Bisby and A.H. Awad. 1980. Studies for determining some antibiotic substance in some Egyptian plants. Screening of some Antimicrobial activity. *Fitoterpia*, 51: 303-308.

Sasser, J.N. 1980. Root knot nematode. A global menace to crop production, *Plant Disease*, 104: 36-41.

Siddiqui, I.A., A.Q. Shamin., V. Sultana, S. Ehteshamul-Haque and A. Ghaffar. 2000. Biological control of root rot and root knot disease complex of tomato. *Plant and Soil* 227: 163-169.

Siddiqui, M.A. and M.M. Alam. 1985. Evaluation of nematicidal properties of different parts of margosa and Persian Lilac. *Neem Newsletter*, 2:1-4.

Stone, A.G., G.E. Vallad, L.R. Cooperband, W.R. Stevenson and R.M. Goodman. 2000. Impact of animal organic amendment on disease incidence in a three year vegetable rotation. *Soil Science Abstracts*, pp 85.

Taylor, A.I. and J.N. Sasser. 1978. *Biological identification and control of root knot nematodes* (*Meloidogyne* species). North Carolina State University Raleigh Graphics, USA. pp.111.

Zaki, M.J. 2000. Biomanagement of root- knot nematodes problem of vegetables. DFID, UK Research Project Report. Department of Botany. University of Karachi, Karachi, 75270.

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