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Nematicidal Activity of *Avicennia Marina* in Different Solvents Fraction against *Meloidogyne Javanica*

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Abstract

In vitro Avicennia marina powdered parts (leaves, stem and pneumatophore) were extracted in aqueous extract (100, 50% w/v) and solvents (ethanol, ethyl acetate, hexane and chloroform) with different concentrations (1000, 500, 250ppm) were used for the assessment of nematicidal activity. All parts of *A. marina* significantly reduced the hatching of *Meloidogyne javanica* eggs. However, of the different parts used, pneumatophore extract in all solvents proved to be much efficient in reduction of eggs whilst leaves extract showed maximum mortality of *M. javanica* larvae. Of the different solvents used, maximum reduction in eggs-hatching and increment in larval mortality was attained by ethanol and ethyl acetate with 1000ppm concentration. 250ppm fraction of solvents and 25% of aqueous extract had little effect on hatching and mortality of *M. javanica* larvae.

Keywords: *A. marina* parts, Aqueous and solvent extracts, *Meloidogyne javanica*, Hatching test, Mortality test.

Introduction

Avicennia marina (Forssk) Vierh (Avicennaceae) is a dominant species in mangrove forests and can tolerate approximately 90% of salt concentration (Saifullah et al., 1994; Macnae, 1966; Burchett et al., 1984; Burchett et al., 1989). High concentration of salt is present in the xylem sap and excess amount of salt is excreted from plant through leaf surfaces (Scholander et al., 1962). An ultrafiltration system is present in roots and leaves for exclusion of salt which depend upon the integrity of semi-permeable membrane (Scholander, 1968).

Meloidogyne javanica caused highly destructive losses in crop production all over the world, whereas, in Pakistan, about 100 plants have been found to be infected with root knot nematodes from different cultivated zones of Pakistan and these *Meloidogyne* spp root knot nematodes, are identified as important parasites of vegetable crops (Maqbool, 1988; Zaki, 2000; Webster, 1969). These root knot nematodes substantially reduced the nutrient and water uptake because of damaged root system, as a result of which plants become weak and low yielding with the characteristics of typical root gall formation (Abad et al., 2003). Infective stage of nematode is the second-stage juvenile (J_2) which penetrates the roots, goes through three successive moults and forms adult females or males. The oesophageal glands of the nematode initiate secretions forming binucleate cell. Rapid divisions of the nuclei results in several large multinucleate cells whilst surrounding cells divide and produce characteristic galls called root knots (Gheysen and Fenoll, 2002; Davis et al., 2000; Williamson and Kumar, 2006). These root knot nematodes produce measurable changes in physiology and morphology of the host (Williamson and Gleason, 2003).

Many researchers reported the chemical constituents of mangrove forest which possesses biologically active antibiotic, antiviral, antibacterial and antifungal compounds. These compounds include tannins, alkaloids, polyphenols, salts, organic acids, carbohydrates, hydrocarbons, benzoquinone, naphthofurans, napthoquinone, sesquiterpenes, triterpenes, flavonoids, polymers and sulfur derivatives (Combs & Anderson, 1949; Jamale and Joshi, 1978; Nishiyama et al., 1978; Ross et al., 1980; Han et al., 2007). Many active compounds like, alkaloids, diterpenes, phenols, polyacetylenes, sesquiterpenes, thyenil derivatives are reported to be used in management of plant parasitic nematodes (Chitwood, 2002).

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Present study has been carried out for assessment of nematicidal activity of *A. marina* in different solvents.

Materials and Methods

Plant samples

Avicennia marina parts (leaves, stem and pneumatophore) were collected from coastal areas of Karachi. These plant samples were washed properly by sterilised distilled water to remove dirt and air dried under shade and powdered individually using an electric grinder. The powder obtained was stored in an air tight container for further studies.

Extract preparation

For the aqueous extraction of plant material, 20g powdered *A. marina* parts were soaked in 200ml deionised water for 48 hours at room temperature (30±1ºC). The extract was filtered through two layers of Whatman No.1 filter paper and centrifuged at 5000g for 15 minutes. The supernatant was collected and stored at 6˚C prior to work. This gives stock solution (S or 100%), whereas, S/2 (50%) w/v concentrations were prepared by dissolving 50ml stock solution (S or 100%) in 50ml deionised water. Appropriate quantities of antibiotics (0.5ml/l Benzyl Penicillin Potassium Salt and 0.5ml/l Streptomycin Sulphate) were added to avoid bacterial contamination (Harbone, 1984).

For solvent extraction, 200g of powdered *A. marina* parts was extracted with 500ml of ethanol, ethyl acetate, chloroform and hexane separately, placed on rotary shaker at 190-220rpm for 24 hours. It was filtered twice, using two layered Whatman No.1 filter paper and centrifuged at 5000g for 15 minutes. The supernatant was collected and solvent was evaporated under rotary vacuum evaporator. The residue, after weighing, was dissolved in appropriate amount of respective solvents to make ppm solutions (1000, 500, 250) (Harbone, 1984).

a. Hatching test: *M. javanica* eggs were collected from roots of egg plant (*Solanum melongenosa* L.) in the field of Department of Botany, University of Karachi, according to Hussey and Barker (1973). To assess the influence of *A. marina* extracts on egg hatching of *M. javanica*, a modified method of Meyer et al. (1982), was used where 1000, 500 and 250ppm concentrations of *A. marina* parts were prepared in ethanol, ethyl acetate, chloroform and hexane whilst aqueous extract was prepared at 50 and 100% concentration. 1ml of egg suspension (25-40 eggs/ml) and 1ml of different concentrations of *A. marina* parts were transferred in glass cavity blocks of diameter 2.5cm. There were triplicates of each treatment. Hatching of *M. javanica* were taken after 24, 48 and 72 hours.

b. Juvenile mortality test: Mortality test was conducted in triplicate where eggs and egg masses of *M. javanica* were hand-pricked by using a fine needle, placed in sterile distilled water and incubated in incubator (30±1ºC). Emerged juveniles after 72 hrs were collected and suspended in distilled water. 1 ml of freshly emerged juveniles suspension containing 40-50 juveniles/ml was mixed with 1 ml of each of different concentrations of aqueous and solvent extracts of plant were transferred separately to glass cavity blocks having a diameter of 2.5cm) and kept at room temperature. Glass cavity blocks containing only the juveniles' suspension were regarded as control and each treatment was replicated thrice. The number of dead juveniles was recorded at 24, 48 and 72 hours exposure using low power stereomicroscope. Nematodes were counted dead if no movement was recorded during two seconds even through mechanical prodding. The nematode mortality was expressed as percentage of the total nematode incubated (Cayrol et al., 1989).

Statistical Analysis

Data were subjected to three way analysis of variance (FANOVA) and significance at 5% level was tested using Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984).

Results and Discussion

Hatching test: Aqueous extract of powdered *A. marina* leaves at 100% revealed significant ($P<0.001$) reduction in hatching of juveniles after 72 hrs in contrast to control (Fig. 1). *A. marina* pneumatophore at 50% showed less effect on hatching of J_2. On the other hand, ethyl acetate, chloroform and ethanol extracts showed greater reduction of eggs hatching at higher (1000 ppm) concentration of *A. marina* pneumatophore after 72 hrs ($P<0.001$) (Fig. 2). Increase in hatching of *M. javanica*second stage juveniles was observed with respect to time. However, after 72 hrs, maximum reduction of hatching was

accompanied. All *A. marina* parts gave significant results for inhibition of hatching. However, *A. marina* pneumatophore and leaves were found best amongst them. Of the different solvents used, maximum reduction in eggs hatching was attained by *A. marina* ethyl acetate extract (Fig. 2).

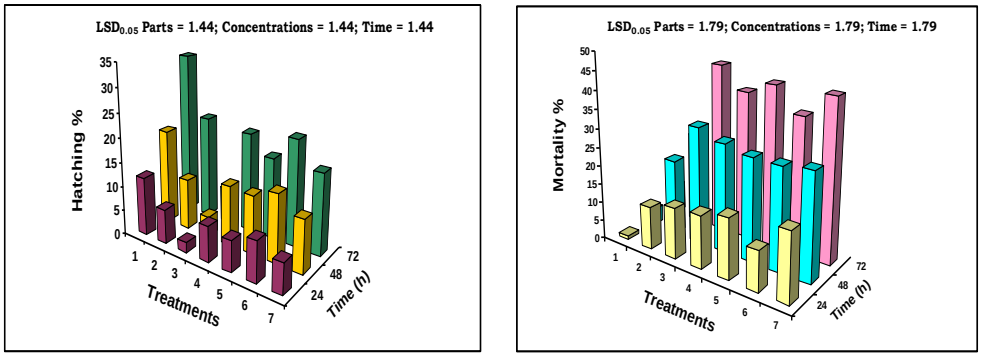
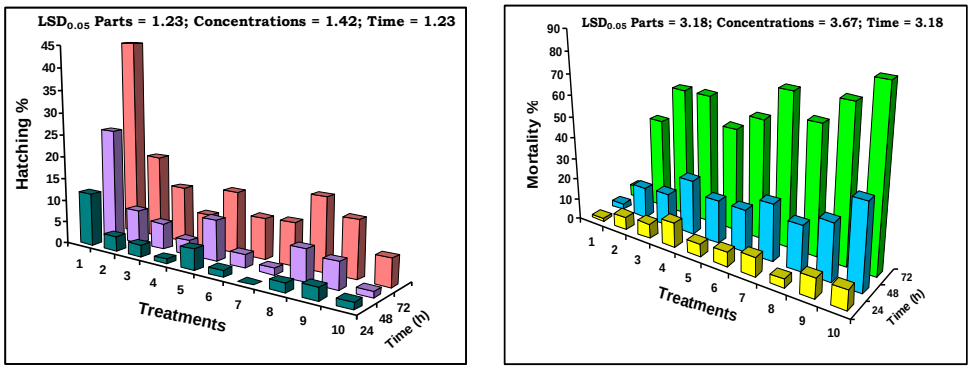


Fig 1: Nematicidal activity of aqueous extract of *A. marina* parts. (1 = Control; 2 = 50% leaves; 3 = 100% leaves, 4 = 50% stem; 5 = 100% stem; 6 = 50% pneumatophore; 7 = 100% pneumatophore)

Chloroform extract



Ethanol extract

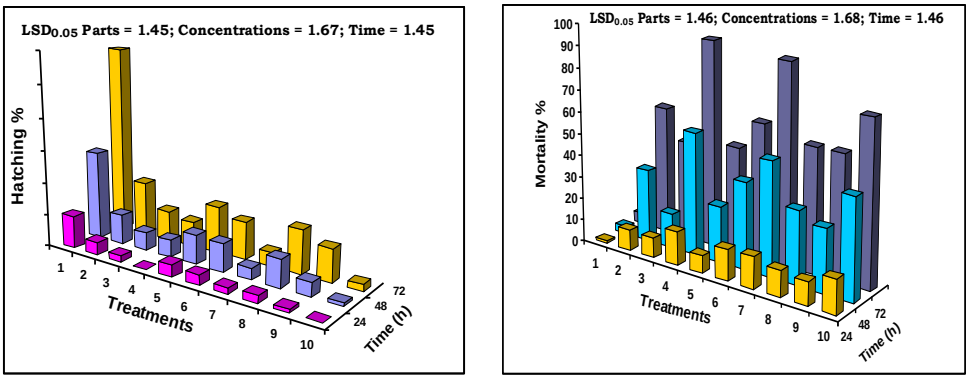


Fig 2: Nematicidal activity of solvent extracts of *A. marina* parts at different days.

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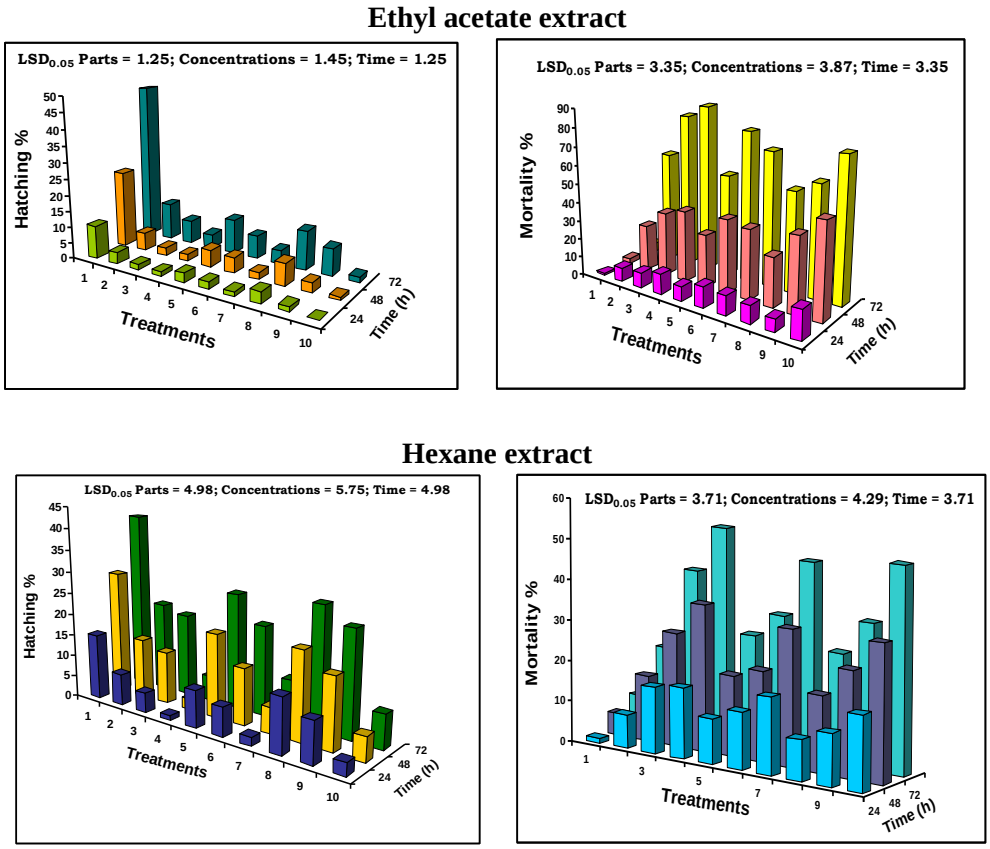

Fig 2: Nematicidal activity of solvent extracts of *A. marina* parts at different days.

(1 = Control; 2 = 250 ppm leaves; 3 = 500 ppm leaves; 4 = 1000 ppm leaves; 5 = 250 ppm stem; 6 = 500 ppm stem; 7 = 1000 ppm stem; 8 = 250 ppm pneumatophore; 9 = 500 ppm pneumatophore; 10 = 1000 ppm pneumatophore).

Mortality test: All parts of *A. marina* at different concentrations showed significant ($P<0.001$) mortality of *M. javanica* larvae. However, 100% aqueous extract of *A. marina* leaves resulted in the greatest juveniles mortality followed by 50% mortality. Similarly, ethyl acetate and hexane extract of *A. marina* leaves and ethanol, chloroform extract of *A. marina* pneumatophore at 1000 ppm caused significant ($P<0.001$) mortality of larvae (Fig. 2). It was noteworthy that extract concentrations and mortality of juveniles were in proportion to each other. As the extract concentration increased, mortality of larvae also increased with respect to time. However, there was an increase in J_2 mortality as the exposure time extended to 48 and 72 hrs. Maximum mortality of larvae was observed by *A. marina* extracted in 1000ppm fraction of ethanol. 250ppm fraction of organic solvents had little effect on mortality of *M. javanica* larvae (Fig. 2). Our result was contrary to that of Mehdi et al. (2001), where aqueous, methanol and chloroform extracts of *A. marina* and *R. mucronata* attributed maximum mortality of *M. javanica* juveniles.

Some chemical compounds act as nematicides, plants secretes salt mostly NaCl through leaf glands located in the leaves to maintain favourable K^+/Na^+ ratio (Luttge, 1971; Sobrado, 2001; Waisel et al.,1986; Sobrado and Greaves, 2000). Some species of *Avicennia* have active secreting glands to excrete excess salts. This excretion is governed by various factors (Liphschitz and Waisel, 1982). Besides NaCl being secreted with the nutrient solution, some other macronutrients like K^+, Mg^2, Ca^{2+} and SO_4^{2-} and micronutrients like Zn are also present (Sobrado and Greaves, 2000; Scholander et al., 1962; Atkinson et al., 1967; Boon and Allaway, 1986) which may be a cause of nematicidal activity. There are many active compounds in mangrove parts which are insoluble in water. Thus, they could be resuspended in water. If these

compounds are exposed to organic solvents, they become easily soluble and may serve as evidence for the killing of nematodes at high concentration.

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