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EFFECT OF *BARLERIA ACANTHOIDES* VAHL. ON ROOT-KNOT NEMATODE INFECTION AND GROWTH OF INFECTED OKRA AND BRINJAL PLANTS

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Abstract

Barleria acanthoides Vahl. is a xerophytic herb found in Karachi. In the present study *B. acanthoides* was used against root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood, *In vitro* and greenhouse experiments. Its effects on root-knot infection, growth, chlorophylls and protein contents in leaves of okra (*Abelmoschus esculentus* (L.) Moench. var. Arka anamika) and brinjal (*Solanum melongena* L. var. Black beauty) plants were observed. Aqueous extracts of *B. acanthoides* significantly inhibited egg hatching of root-knot nematode and caused appreciable mortality of second stage juveniles of *M. javanica In vitro*. Soil amendment with shoot material of *B. acanthoides* at 1% and 2% w/w significantly suppressed nematode galling in okra and brinjal roots. *B. acanthoides* amendment resulted in enhanced growth, chlorophyll and total protein contents in okra and brinjal compared to unamended *M. javanica* inoculated plants.

Introduction

Among the plant parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.) are capable of reproducing on over 2,000 species of plants (Sasser & Freckman, 1987) and are responsible for approximately 50% of overall nematode damage. The various species of *Meloidogyne* induce major morphological and physiological changes within roots, attack nearly every crop sown where not only yields are greatly affected but quality is also reduced (Sasser, 1980). After hatching from eggs, second-stage juveniles invade roots of host plants and migrate intercellularly to differentiating vascular regions.

The symptoms of nematode infection are the formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting, mineral deficiency, weak and poor yielding plants (Abad *et al.*, 2003).

Although the application of chemical nematicides have been found as an effective measure for the control of nematodes but due to high toxic residual effect of chemicals on the environment and particularly on non-target organisms (Akhtar & Malik, 2000; Anastasiadis *et al*., 2008), there is an urgent need to develop alternative strategies for the control of nematodes. Crops and weeds may exhibit biochemical mechanisms to counteract the activity of nematodes. Numerous plant species, representing 57 families, have been shown to contain nematicidal compounds (Sukul, 1992).

Barleria species exhibit several medicinal properties. For instance, leaves of *B. prionitis* L., are chewed to relieve toothache (Chopra *et al*., 1956) and juice of the leaves is used in ulcer and fever (Ambasta, 1986). *B. lupulina* Lindl., has strong inhibitory effect against acne-inducing bacteria (Chomnawang *et al*., 2005). Root decoction or infusions of pounded leaves of *B. eranthemoides* R. Br., is drunk for treatment of dysentery and against infectious diseases (Maregesi *et al*., 2007).

In view of the therapeutic properties of *Barlaria* species, present research was undertaken (1) to test the activity of *Barleria acanthoides* Vahl., against root-knot nematode *Meloidogyne javanica* (Treub) Chitwood and (2) to examine the effect of *B. acanthoides* on physio-chemical response of root-knot infected plants of okra and brinjal.

Materials and Methods

Extract preparation: *Barleria aconthoides* Vahl., that generally grow on rocky soil, was collected from Karachi University campus. Plants were washed with tap water and dried under sunlight. Dried stem and leaves were powdered in an electric blender. Aqueous extract (10% w/v) was prepared by soaking the powder for 48 hours in sterilized distilled water. After straining through muslin cloth it was filtered through Whatman No.1 filter paper.

Culture preparation of root-knot nematodes: Roots of plants infested with root-knot nematodes were collected from Karachi University garden. The root-knot nematodes species were identified with the help of perennial pattern as described by Taylor and Netscher (1974). The root-knot nematode *Meloidogyne javanica* (Treub) Chitwood, was cultured on brinjal seedlings in a greenhouse from a single egg mass. Nematode (*M. javanica*) eggs were extracted from infected roots using a 2% NaOCl solution and the eggs released from the roots were collected using the modified technique described by McClure *et al.* (1973). The egg suspension was poured on a cotton-wool filter paper and incubated at 28±2ºC to obtain freshly hatched juveniles (J_2). Juveniles were collected within 48 h were used.

Egg hatching test: Eggs of *M. javanica* were collected by the method of Hussey and Barker (1973). A suspension of eggs in distilled water was prepared. One ml of egg suspension (30–45 eggs/ml) and one ml of plant extract was transferred in glass cavity block (diameter 2.5 cm) and kept at room temperature. Each treatment was replicated thrice. The glass cavity block containing one ml egg suspension and one ml distilled water served as control. After 96 h exposure, the number of hatched eggs was counted under a low power (6X) stereomicroscope. The toxicity of plant extract was assessed as the mean percentage of the hatched eggs.

Mortality test of nematode larvae: Eggs/eggs masses of *M. javanica* were placed in distilled water and incubated at 28±2°C. After hatching, the juveniles were collected and a suspension of juveniles in distilled water was prepared. One ml of freshly hatched juveniles suspension (40–50 juveniles/ml) and one ml of plant extract was transferred in glass cavity block and kept at room temperature. Each treatment was replicated thrice. The glass cavity block containing one ml nematode suspension and one ml distilled water served as control. After 72 h exposure, the number of killed juveniles was counted under a low power stereomicroscope. The toxicity of plant extract was assessed as the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol *et al.*, 1989).

Greenhouse experiments: Soil was obtained from experimental field of Department of Botany, University of Karachi containing sandy loam soil, pH 8.1 and amended with dried powder of *B. aconthoides* @ 1% and 2% w/w in 8.1 cm diam., plastic pots. Two week after amendment, 5 okra (*Abelmoschus esculentus* (L.) Moench. var. Arka anamika) seeds or one seedling of brinjal (*Solanum melongena* L. var. Black beauty) was transplanted in each pot separately. After 4–5 days of brinjal seedlings transplantation or after 10 days of okra seed germination approximately 2000 freshly hatched second stage juveniles were introduced in holes made around the roots of each plant. Unamended and uninoculated pots served as positive controls, while unamended and nematode inoculated

pots as negative controls. Treatments and controls were replicated thrice. The pots were placed in a completely randomized design in a green house and watered daily. After 8 weeks of nematode inoculation, plants were gently removed from pots and the roots were carefully washed in running water.

Estimation of chlorophyll contents: One g fresh leaf material was extracted in 80% acetone. The extract was centrifuged at 1000 *g* three times for about 5 min., and the supernatant was collected. The absorbance of supernatant was recorded at 645 and 663 nm and chlorophyll a and b contents were estimated in accordance with Maclachlan and Zalik (1963). The amount of chlorophylls was expressed as mg/g fresh weight.

Estimation of total protein: Half g fresh leaves were plunged in hot 80% ethanol to kill the tissue quickly. After keeping for 5 min, ethanol was decanted and the tissue was then crushed in a mortar with 10 ml of 5% Trichloroacetic acid (TCA) and centrifuged at 1000 *g* for 5 min. The tissue was then washed separately with 5 ml each of absolute ethanol, ethanol-chloroform mixture (3:1 v/v) and finally with ethanol-ether mixture (3:1 v/v). The washed residue was then incubated in 5 ml of 0.5 N NaOH for 16 h at 37˚C. The sediment was removed by centrifugation at 4000 *g* for 20 min., and washed once with 5 ml of 0.5 N NaOH. The extract and washing were combined and made upto 10 ml with 0.5 N NaOH. The extract was then used for protein determination. The total protein contents were estimated by using the method of Bradford (1976), and expressed in mg/g fresh weight.

Statistical analysis of data: Data sets were subjected to analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) depending upon the experimental design. The follow up of FANOVA included Least Significant Difference (LSD). Duncan's Multiple Range Test (DMRT) was also used to compare the treatment means (Sokal & Rohlf, 1995).

Results and Discussion

The results showed that the aqueous extract of powdered shoot of *B. acanthoides* plant species inhibited egg hatching and is capable of causing appreciable mortality of *M. javanica* juveniles *In vitro* (Table 1). These results are in large part due to the fact that plant extract possess compounds toxic to root-knot nematode. Expression of egg hatching and larval mortality by *Argemone mexicana* plant extract has also been shown by Shuakat *et al.,* (2002, 2004). Presumably, egg and larval stage (J_2) are vulnerable to the nematicidal potential of *B. acanthoides* extracts.

Soil application with dried powder of *B. acanthoides* significantly ($p<0.001$) suppressed the root knot development in brinjal and okra plants. Maximum inhibition in gall formation was observed after amendment with 2% as compared to untreated controls where maximum number of knots was observed (Tables 2 & 3). Increasing dosages of the amendments were found to be effective in reducing plant-parasitic nematodes in soil (Akhtar & Mahmood, 1996). Nematicidal activity of organic amendments in soil can be attributed to chemical mineralization with the ultimate release of ammonia, increased nitrogen and carbon dioxide levels (Akhtar, 1998). Release of toxic compounds from plant tissues are also reported to reduce plant parasitic nematode infection, several plant terpenoids and phenolic compounds are known to have nematicidal properties (Akhtar & Mahmood, 1994, Shaukat & Siddiqui, 2001, Siddiqui & Shaukat, 2002, Shaukat *et al*., 2004).

Table 1. Effect of *B. acanthoides* on hatching and mortality of root knot nematodes.

Treatments	Hatching%	Mortality%
Barleria acanthoides	10 b	66 a
Control	60 a	7 b
LSD $_{0.05}$	5.4	4.9

DMR test (0.05): Means followed by the same letters are not significantly different from each other.

Table 2. Effect of *B. acanthoides* on nematode infection and growth of okra plants.

Treatments	Shoot length (cm)	Shoot weight (cm)	Root weight (g)	Root-knot/ Root system
Control (uninfected)	23.2 a	2.02 a	1.46 bc	0 d
Control (infected)	18.2 c	1.35 d	1.71 a	86 a
B. acanthoides 1%	22.2 a	1.59 c	1.48 b	48 b
B. acanthoides 2%	20.5 b	1.82 b	1.38 c	30 c
LSD $_{0.05}$	1.6	0.18	0.09	12

DMR test (0.05): Means followed by the same letters are not significantly different from each other.

Table 3. Effect of *B. acanthoides* on nematode infection and growth of brinjal plants.

Treatments	Shoot length (cm)	Shoot weight (cm)	Root weight (g)	Root-knot/ Root system
Control (uninfected)	15.3 a	1.22 a	0.57 b	0 d
Control (infected)	9.3 c	0.63 c	0.8 a	58 a
B. acanthoides 1%	12.2 b	0.87 b	0.66 b	41 b
B. acanthoides 2%	14.3 a	1.11 a	0.6 b	29 c
LSD $_{0.05}$	1.6	0.17	0.09	12

DMR test (0.05): Means followed by the same letters are not significantly different from each other.

Table 4. Effect of *B. acanthoides* on physiology of okra plant infected by root knot nematodes

Treatments	Chl "a+b" mg/g	Chl "a/b" mg/g	Total proteins mg/g
Control (uninfected)	0.417 a	1.45 c	2.82 a
Control (infected)	0.291 c	2.25 a	2.36 c
B. acanthoides 1%	0.340 b	1.79 b	2.42 c
B. acanthoides 2%	0.394 a	1.6 c	2.53 b
LSD $_{0.05}$	0.027	0.16	0.06

DMR test (0.05): Means followed by the same letters are not significantly different from each other.

Table 5. Effect of *B. acanthoides* on physiology of brinjal plant infected by root knot nematodes.

Treatments	Chl "a+b" mg/g	Chl "a/b" mg/g	Total proteins mg/g
Control (uninfected)	0.418 a	1.41 c	3.05 a
Control (infected)	0.298 c	1.83 a	2.41 c
B. acanthoides 1%	0.346 b	1.67 b	2.54 b
B. acanthoides 2%	0.389 b	1.59 b	2.5 b
LSD $_{0.05}$	0.017	0.14	0.08

DMR test (0.05): Means followed by the same letters are not significantly different from each other.

Soil treated with dried powder of *B. acanthoides* also improved plant growth. Plant height and fresh shoot weight were significantly ($p<0.001$) increased compared to untreated controls (Tables 2 & 3). Only root weight showed increase in untreated infected plants which was due to the formation of galls or giant cells (Postuka *et al*., 1986). The galls/giant cells are adapted to provide nutrient sink from which the nematode is able to feed. Organic matter amendment to soil have been shown to have beneficial effects on soil nutrients, soil physical conditions, soil biological activity and crop viability (Kang *et al*., 1981; Hungalle *et al*., 1986; Addabdo, 1995).

Organic amendment with *B. acanthoides* also altered the chlorophyll and total protein contents of brinjal and okra plants. Infected plants showed reduction in total chlorophylls compared to untreated infected plants. However, they increased significantly ($p<0.001$) in organic amended infected plants. Chlorophyll a/b ratio was highest in infected plants compared to untreated controls. The chlorophyll a/b ratio decreased in organic amended infected plants (Tables 4 & 5). The results showed that in infected plants chlorophyll b content was more affected than chlorophyll a. The degradation of chlorophyll b is different from that of chlorophyll a. Chlorophyll b is degraded by first being converted to chlorophyll a (Ito *et al*., 1993; Scheumann *et al*., 1996). Due to this reason, greater reduction of chlorophyll b content was observed compared to chlorophyll a. Protein content also exhibited a significant ($p<0.001$) reduction in infected plants compared to untreated controls. However, proteins level was increased in leaves of amended plants (Tables 4 & 5). Decreased level of proteins can be attributed to root-knot nematode induced gall formation and the development of giant cells that represent major sink for amino acids, which were imported into the roots *via* the vascular system as reported by Hoth *et al.*, (2005). The amino acid utilization by galls or giant cells reduces their availability for protein synthesis. In addition, amino acids were also probably formed due to the proteolysis of existing tissue proteins that essentially decreases the overall protein level. The results suggest that *B. acanthoides* has a potential to control root-knot nematode infection thereby improves the growth of the okra and brinjal infected plants.

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