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Some Studies on the Control of Citrus Nematode (*Tylenchulus semipenetrans*) by Leaf Extracts of Three Plants and Their Effects on Plant Growth Variables

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Abstract: The effects of water extracts of neem (*Azadirachta indica*), Ak (*Calotropis procera*) and datura (*Datura alba*) were seen on the larval mortality of citrus nematode (*Tylenchulus semipenetrans*). The results were highly significant and revealed that of the three test plants; neem gave the maximum larval mortality followed by Ak and datura. The standard extracts were more toxic to *Tylenchulus semipenetrans* larvae than other concentrations. Similarly, maximum mortality was recorded after 48 h of exposure followed by 24 and 12 h. When the standard extracts of leaves of the test plants were applied in pots on *Citrus jambhiri*, growth variables were enhanced to the maximum by neem followed by Ak and datura. On the other hand significant reductions were recorded in nematode populations and rate of multiplication in treatments where extracts were applied as compared to control.

Key words: Citrus nematode, *Tylenchulus semipenetrans*, larval mortality, *Azadirachta indica, Calotropis procera, Datura alba*

INTRODUCTION

Citrus is the world's recognized second commercial fruit. High vitamin 'C' contents, abundance of essential mineral elements and other dietary values make its esteem more prominent. Pakistan is among the leading citrus growing countries and it is the source of foreign exchange earnings. In Pakistan, citrus is the largest group of fruits produced over an area of 194.2 thousand ha with an annual production of 1830.3 thousand tons[1]. Amongst different microorganisms like fungi, bacteria, viruses and nematodes which are the important agents affecting the quality and quantity of citrus, nematodes play a key role.

Plant parasitic nematodes present some of the most difficult pest problems encountered in our agricultural economy. Each year these minute organisms exact an ever increasing toll from almost every cultivated area of the world.

In agriculturally advanced countries, it has been noted that among nematodes, citrus nematode *Tylenchulus semipenetrans* is causing immense damage to citrus trees[2]. Eighty species and varieties of the genus citrus were found to be susceptible to *Tylenchulus semipenetrans*[3]. This nematode occurs throughout the world citrus growing areas and causes a serious disease known as slow decline. Affected trees exhibit reduced vigor, chlorosis, leaf fall, die back and reduced production and quality of fruit[4]. The nematode (*Tylenchulus semipenetrans*) is the primary cause of citrus decline. The nematode never kills trees but growth and yield of infected trees slowly decline. Severity of the disease can vary from minor to severe decline of infected trees. Symptom expression is influenced by several physical and chemical factors as shown by several investigations[5].

Taking into account the world wide distribution of citrus nematode, it is necessary to find out the most effective and feasible control measure. The use of chemicals for nematode control on large scale is an expensive and impracticable operation. In Pakistan, the prices of nematicides are very high and only small number of farmers can afford the use of pesticides. This situation demands the search for cheaper alternative control measure which can be made available to small growers.

There are reports that certain plant parts and extracts possess nematicidal properties[6-8]. Application of the plant parts or extracts to nematode infested soil affects nematode directly and stimulates soil microbes that reduce nematode populations[9,10].

Under this context, the use of plant extracts with nematicidal properties can prove to be effective, cheaper and safer control measure.

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MATERIALS AND METHODS

Leaves of datura (*Datura alba*), neem (*Azadirachta indica*) and Ak (*Calotropis procera*) were washed under running water and chopped. Twenty five grams of chopped leaves of each test plant were then macerated in an electric blender and soaked separately in 100 mL of distilled water. After 24 h, samples were filtered through Whatman filter paper No.1. The extracts were arbitrarily termed as standard "S" and subsequent dilutions viz., S/2 and S/4 were prepared by the addition of requisite quantities of distilled water.

In order to evaluate the effect of different water extracts on the larval mortality of *Tylenchulus semipenetrans*, 1 mL of nematode suspension containing about 30 juveniles was poured into the petri dish with the help of pipette and 5 mL of the extract was added in the petri dish with the help of pipette. The same procedure was repeated for all the extracts. Petri dishes containing distilled water served as control. Each treatment was quadruplicated. The petri dishes were placed at room temperature which ranged from 25 to 30°C. Dead and surviving nematodes were counted after 12, 24 and 48 h. Mortality was assessed by touching the nematodes with fine needle and percentage of larval mortality was calculated by the following formula:

$$\% \text{ mortality} = \frac{\text{Number of larvae killed}}{\text{Total No. of larvae}} \times 100$$

The effect of the standard extracts of the leaves of the test plants was then studied in pots. For this purpose three months old seedlings of highly susceptible citrus cultivar Jatti khatti (*Citrus jambhiri*) were transplanted singly in pots (15x12 cm) containing formalin sterilized sandy loam soil. After one week of transplanting, the plants were inoculated with approximately 5000 juveniles/pot of *T. semipenetrans*. Standard extracts of leaves of each of datura, neem and Ak @ 100 mL/pot were applied after five days of inoculation. Each treatment was replicated five times. After three months, plants with soil were gently removed from pots and their roots were carefully washed under running tap water. Data were recorded on plant height, fresh and dry weights of shoot and root, final population of nematodes in soil, number of females per gram of root and rate of multiplication. All the data recorded were analyzed statistically by using analysis of variance and means were compared by using least significant difference test at 5% level of probability[11].

RESULTS

Analysis of variance regarding plant extracts, time of exposure, concentrations and their interaction was highly significant. Comparison of treatment means of leaf extracts of test plants indicated that neem gave the maximum mortality followed by datura and Ak as shown in Table 1. Similarly comparison of treatment means regarding time of exposure revealed that the maximum mortality was observed after 48 h of exposure followed by 24 and 12 h (Table 2). Further, it is evident from the comparison of means (Table 3), that standard concentrations of the leaves extracts of test plants showed maximum mortality as compared to other concentrations. Mortality in distilled water was negligible. Mean individual percent mortalities in leaf extracts of test plants at different concentrations and exposure times are given in Table 4.

When standard leaf extracts of test plants were applied in pots, plant height and fresh and dry weights of shoots and roots were greater. Comparison of treatment means indicated that plant height was the maximum in Ak and neem extracts followed by datura extract showing 98.86, 96.15 and 49.91% increases over control (Fig. 1).

Comparison of treatment means revealed that fresh and dry shoot weights were maximum in neem extract which showed 200.14 and 223.63% increases over control, followed by Ak and datura extracts showing 187.85 and 196.63, 112.29 and 126.97% increases over control, respectively (Fig. 2 and 3).

Similarly, it is obvious from the comparison of treatment means that fresh and dry root weights were maximum in neem extract giving 205.71 and 122.52% increases over control and increases in these parameters in case of Ak and datura extracts were 166.03 and 65.76%, 62.85 and 18.91% over control, respectively (Figs. 4 and 5).

On the other hand significant reductions were recorded in nematode populations and rate of multiplication in treatments where extracts were applied as compared to control. Final population of nematodes in soil, number of females per gram of root and rate of

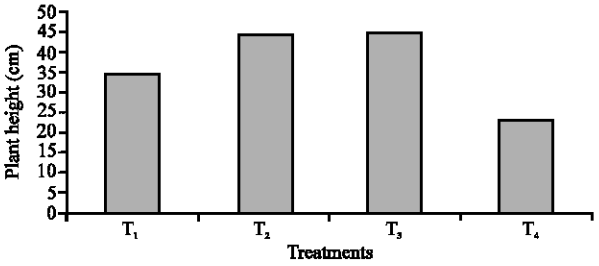

Fig. 1: Effect of plant extracts on plant height (cm)

Table 1: Effect of plant extracts on larval mortality of *Tylenchulus semipenetrans*

Plant extracts	% mortality
Neem	19.44A
Ak	14.78B
Datura	17.23B
LSD value	0.3796

Separation of means by LSD test at 5 % level

Table 2: Effect of different exposure times on larval mortality of *Tylenchulus semipenetrans*

Exposure time	% mortality
48 h	19.02A
24 h	17.09B
12 h	15.25C
LSD Value	0.3796

Separation of means by LSD test at 5 % level

Table 3: Effect of different concentrations on larval mortality of *Tylenchulus semipenetrans*

Concentrations	% mortality
S	28.06A
S/2	22.30B
S/4	18.00C
Distilled water	0.25D
LSD value	0.4383

Separation of means by LSD test at 5 % level.

Table 4: Effect of leaf extracts of test plants on larval mortality of *Tylenchulus semipenetrans*

		Percent mortality after		
Plants	Concentrations	12 h	24 h	48 h
Datura	S	100.00	100.00	100.00
	S/2	75.55	81.10	96.66
	S/4	54.44	81.10	86.66
	D.W	1.11	1.11	2.22
Neem	S	100.00	100.00	100.00
	S/2	100.00	100.00	100.00
	S/4	69.44	84.44	89.99
	D.W	0.00	0.00	1.11
Ak	S	100.00	100.00	100.00
	S/2	57.77	71.10	87.8
	S/4	45.55	58.88	69.99
	D.W	0.00	1.11	1.11

Data are means of four replicates

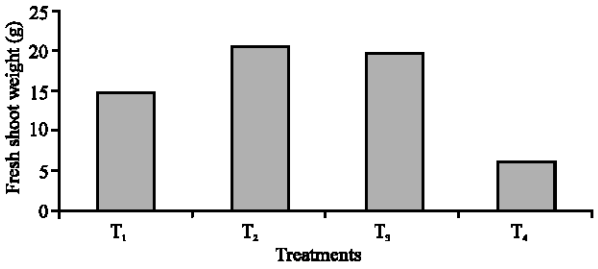

Fig. 2: Effect of plant extracts on fresh shoot weight (g)

multiplication was minimum in case of neem extract showing 78.72, 70.24 and 78.68% reductions over control, respectively. Ak extract was equally effective as neem extract giving 71.74, 60.57 and 71.82% decreases over control. Datura extract was significantly different from neem and Ak extracts in case of final population of

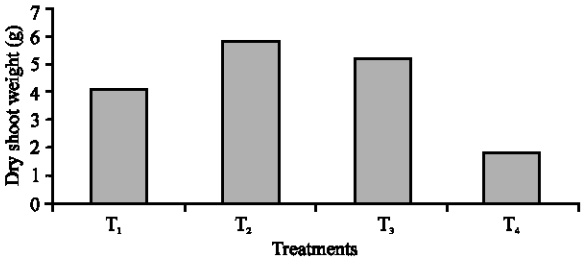

Fig. 3: Effect of plant extracts on dry shoot weight (g)

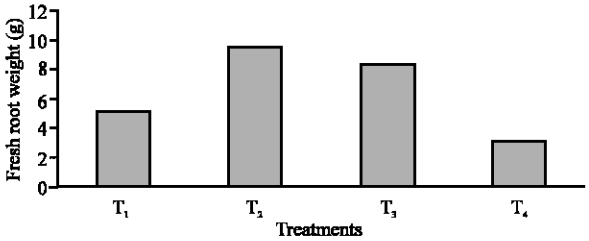

Fig. 4: Effect of plant extracts on fresh root weight (g)

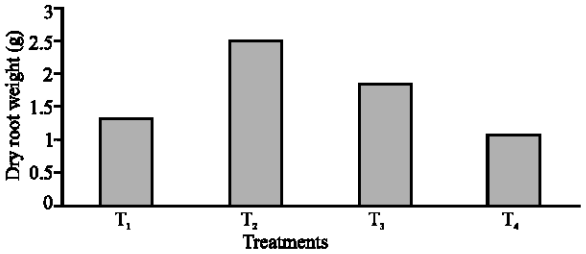

Fig. 5: Effect of plant extracts on dry root weight (g)

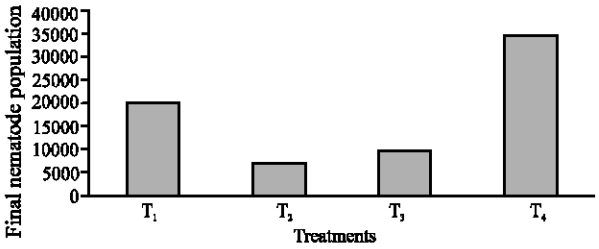

Fig. 6: Effect of plant extracts on final nematode population

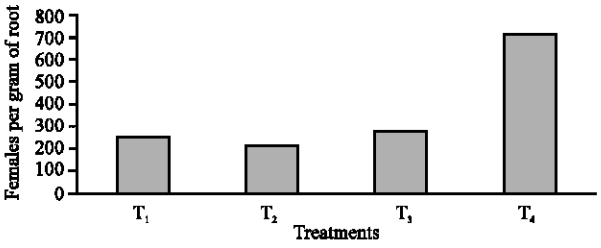

Fig. 7: Effect of plant extracts on females per gram of root

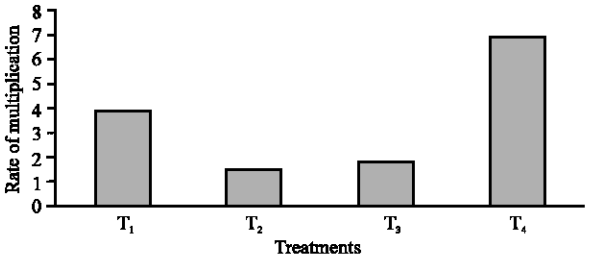

Fig. 8: Effect of plant extracts on rate of multiplication

T_1 = Datura extract T_2 = Neem extract
T_3 = Ak extract T_4 = Control (Nematode alone)

nematodes in soil and rate of multiplication while it was equally effective as neem and Ak extracts in case of number of females/g of root showing 44.50, 64.22 and 44.49% reductions in these parameters over control (Figs. 6-8).

DISCUSSION

When the water extracts of neem, Ak and datura and their subsequent dilutions were studies on the larval mortality of citrus nematode in laboratory, significant results were achieved. The nematicidal properties of these plants were tested in laboratory[6,7,12,13]. The mortality of the larvae might be attributed to the chemicals contained in the extracts. These chemicals might have penetrated directly and inhibited acetyl cholinesterase and other esterases like cholinesterase enzyme. Hydrolysis of acetyl choline by acetyl cholinesterase is a vital part of neurotransmission in the nervous system. Cholinesterase and esterases also function in various metabolic systems. The existence of acetyl cholinesterase in plant parasitic nematodes was first demonstrated by using a site specific acetyl thiocholine substrate reaction that was inactivated by known cholinesterase inhibitors[14].

It was suggested that the inhibition of body activity may result from acetyl cholinesterase inhibition that was irreversible at high concentrations due to continued binding of the enzymes[15]. Various other effects observed include the prevention of or decreased mobility and delay in the molting processes. Many of these behavioral changes may relate to inhibition of various esterases enzymes.

When the standard extracts of leaves of the test plants were applied, plant height, fresh and dry weights of shoot and root were significantly more as compared to control. The reproduction factor was significantly low in pots where neem extract was applied. The findings are in line with those of other workers[7,16-19].

The protective action of the leaf extracts of these plants against citrus nematodes appears to be due to the presence of chemicals and growth stimulators in them. Possibly these chemicals are either absorbed by the root or an elicitor/activator reaction was initiated by some factor present in the extracts[20,21]. According to Kast[22] an induced defense mechanism may have some practical utility in integrated biological nematode management strategies. The root improvement could be attributed to poor penetration and later retardation in different activities of 2nd stage juveniles such as feeding and/or reproduction as suggested by Bunt[23].

From the present as well as from the earlier study[24,25], it would appear that leaves of neem possess some broad spectrum factor which has the potential against nematodes with different modes of feeding. Systemic action of azadirachtin, a triterpenoid from neem, against citrus nematode has been reported[26]. The effective nematicidal activity of neem leaf extract might have been due to the action of its active principles viz., Nimbine, Nimbinine, Nimbidine, Thionemone and Margosine-o[27].

These results indicate that extracts of these medicinal plants can prove helpful in the control of plant parasitic nematodes. Application and use of plant extracts will probably be easy and economical as compared to chemical treatment. The active principles of these medicinal and poisonous plants, if extracted in pure form in large quantities, would probably form the base for the development of indigenous nematicides.

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