J. Sci. Technol. (Dinajpur) Vol. 6:97-102 (2008)
ISSN 1994-0386

EFFICACY ON ROOT-KNOT (*Meloidogyne javanica*) OF TOMATO OF TWO ALCOHOLIC PLANT EXTRACTS WITH EMPHASIS ON CHEMICAL INVESTIGATIONS

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ABSTRACT

An experiment was conducted to find out the components present in the neem (*Azadirachta indica*) and biskatali (*Polygonum hydropiper)* alcoholic leaf extracts and also evaluate their efficacy in controlling the root-knot disease of tomato caused by *Meloidogyne javanica* along with Curaterr (Carbofuran). Thin Layer Chromatography showed four and three distinct compounds were present in neem and biskatali leaf extracts, respectively. Laboratory experiment showed significant effect of both leaf extracts on mortality of juvenile where neem gave higher percent of mortality than biskatali leaf extracts. In field experiment, all the treatment gave higher plant growth and yield with lower development of gall, egmasses, J2, J3, J4 and adult. Chemical Curaterr and both the alcoholic leaf extracts were used as side dressing after 7 days of inoculation. Curaterr showed superior results over neem and biskatali leaf extracts in case of all plant growth characters; yield parameters along with the number of galls, adult females and different juvenile's stages of the nematode. Neem leaf extract gave better effect than biskatali in all cases of plant growth characters, yield, gall numbers and nematode development.

Key words: *Neem and Biskatali leaf extracts, Thin Layer Chromatography, Mortality, Root-Knot and Tomato*

INTRODUCTION

Tomato (*Lycopersicon esculentum L.*) is one of the most popular and nutritious vegetable crop in Bangladesh and is very often threatened by many diseases. Among the diseases root- knot is a serious disease caused by *Meloidogyne* spp. Moreover hot and humid climate makes it a suitable bed for the predominantly occurring *Meloidogyne javanica* and *M. incognita* along with other 14 genera of plant parasitic nematodes (Chowdhury, 1976). Use of chemical is very expensive and it is a difficult task for the common farmers to determine the precise dose of the chemical for its proper application in the field. It also provokes environment pollution and health hazards. Recently, various plant extracts and plant parts have been reported to have nematicidal properties (Mahmood *et al*., 1984). Chemical analyses have indicated that neem (*Azadirachta indica*) and Biskatali (*Polygonum hydropiper*) are the sources of many compounds with medicinal properties which are found to be toxic in nature and to be lethal to many insects and nemic pathogens including root-knot nematode *Meloidogyne javanica* (Kundu *et.al.*2007 and Javed *et.al.* 2007). Before going for formulation of a plant product, the nature of action including its active principle should be known. Still now this information is not available. To know the mode of action and its active principle, chemical analysis of the materials is necessary. With this view in mind, the present research work was undertaken with the following objectives: to determine the effect of plant extracts of neem and biskatali on the incidence of root-knot disease of tomato, to study the comparative effect of those extracts and chemical (Curaterr) in controlling root-knot of tomato and to find out the components present in neem and biskatali leaf extracts.

MATERIALS AND METHODS

Experiment 1: Chemical investigation on the leaf extracts of neem and biskatali: Neem and biskatali leaves were oven dried overnight at temperature of 85°C. Then the dried leaves were powdered in blender. After powdering, 100 g of each neem and biskatali leaves were kept in cheese

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cloth and placed in Sox let's apparatus for extraction. Ethyl alcohol (95%) was used for extraction.This extraction was done by Sox let's apparatus in a water bath keeping the temperature not more than 85 °C. The alcoholic extracts of both neem and biskatali leaves were then concentrated by a vacuum rotary evaporator.

Thin Layer Chromatographic (TLC) technique was employed for the identification of a number of compounds present in these extracts and R_f values were determined for every spot produced by the compounds. From the TLC it is found that four (4) components are present in neem leaf extract and three (3) components in biskatali leaf extract (Table 1). Further study should be taken to find out the chemical structures of components are present both in neem and biskatali leaf extracts. The R_f (Ratio of flow) values between solvent front and components were calculated as follows:

$$R_f = \frac{\text{Distance traveled by the component}}{\text{Distance travelled by solvent front}}$$

Experiment 2: Laboratory study of juvenile mortality with alcoholic extracts of neem and biskatali: Eggmasses of *Meloidogyne javanica* were collected from the roots of brinjal plants which were previously inoculated by a single eggmass of *M javanica*. Surface sterilization of the collected eggmasses was done with 0.001% mercuric chloride solution for about 1 minute then the sterilized eggmasses were placed on small nylon sieves and kept about 24 hours for hatching. The freshly hatched juveniles were collected and the nematode suspension was diluted in such a way that each ml suspension contained approximately 100 juveniles which were counted with the help of a stereo-binocular microscope.

Alcoholic extracts of neem and biskatali were diluted separately in 100 ml water and marked it as standard solutions (S). Subsequent dilutions S/2, S/10, S/50 and S/100 were prepared by the addition of required quantity of distilled water. One hundred freshly hatched J_2 juveniles were transferred with steripack disposable syringe to petridishes containing 1 ml of various dilutions (S, S/2, S/10, S/100) of plant extracts and petridishes with only distilled water were designated as control. After 12, 24 and 48 hours, the number of dead and living nematodes were counted by observing under stereoscopic microscope and mean percentages were calculated. Based on the mortality test two effective doses ($S/_2$ for neem and S for biskatali) were selected for the pot experiments in order to evaluate their effect on the root-knot of tomato plants inoculated with *Meloidogyne javanica*.

Experiment 3: Net house experiment

Healthy, mature and disease free seeds of tomato (*Lycopersicon esculentum L.*) cv. Ratan were collected and surface sterilization was done with mercuric chloride solution (0.001%). Then seed were sown in two pot containing 4 kg sterilized and dried soil containing Sandy loam soil, sand and well decomposed cowdung at the ratio of 2:2:1. One month aged seedlings were then transplanted in pot. Eight surface sterilized eggmasses were placed in each pot in two holes (2.5 cm deep), four on each side of the plant after 10 days of transplanting. 10 ml of each extracts and 1 g of chemical (curaterr) were applied as side drench just after 7 days of inoculation. The experiment was laid out in a Randomized Complete Block Design with four treatments and three replications. Four treatments were as follows: T_0 = Control, T_1 = Neem leaf extract (S/2), T_2 = Biskatali leaf extract (S), T_3 = Chemical (cureterr). All data were analyzed following standard procedures for analysis of variance. All the experiments were conducted in the lab of Chemistry, Seed Pathology Center and Net house of Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh.

RESULTS AND DISCUSSION

The spotted TLC plates of neem and biskatali leaf extracts were eluted by mixed solvent of ethyl acetate and benzene at the ratio of 4:1. The R_f values of different spots observed on eluted and developed plates and have been presented in Table 1 and Plate 1.

The standard (S=100% conc.) alcoholic extract of neem was found highly toxic to the juveniles. The standard concentration caused 100% mortality of juveniles after 12 hours of exposure (Table 2).

Incase of S/2 (50% conc.) extract of neem there observed 64.28%, 65% and 67% mortality after 12,

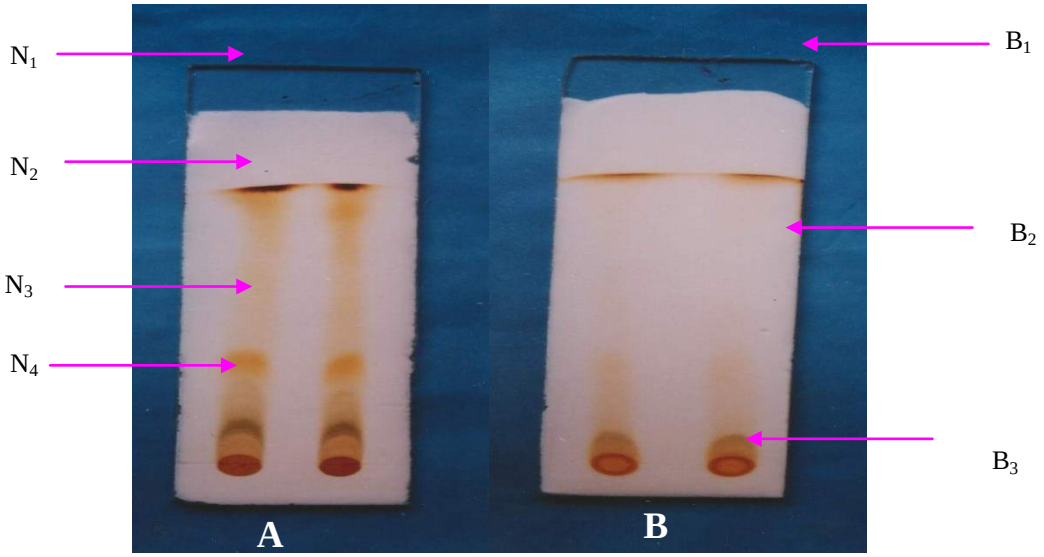

Plate 1. The neem and biskatali extracts showed different spots on TLC plates under iodine bath

Table 1. R_f values of different compounds in refluxing neem and biskatali leaf extracts in mixed solvent

Leaf extracts	Solvent of extraction	Eluting solvents	Ratio	Number of components present	R_f values
Neem	Ethyl alcohol	Ethyl acetate : benzene	4 :1	4	0.099 0.22 0.32 0.78
Biskatali	Ethyl alcohol	Ethyl acetate : benzene	4 :1	3	0.092 0.29 0.76

24 and 48 hours, respectively. The standard alcoholic extract of biskatali showed moderate level of toxicity against the survival of *M. javanica* juveniles. The concentration of bisktali leaf extract appeared 20%, 21% and 21% mortality of juveniles after 12, 24 and 48 hours, respectively with S/2. In both cases, juvenile mortality was found to increase with the increase of concentrations and exposure periods (Table 2). S/2 (half standard) concentration of neem leaf extract and S (standard) concentration of biskatali leaf extract were selected for testing against root- knot of tomato in pot experiment due to their high toxic effect on juvenile mortality as observed in the laboratory test (Table 2).

Table 2. Effect of plant extracts of neem and biskatali on juvenile mortality of root-knot nematode *Meloidogyne javanica*

Plant extract	**Exposure time (hr)**	**Percent mortality at different concentration**				
		Control	**S**	**S/2**	**S/10**	**S/100**
Neem	12	0	100	64.28	7.14	7.14
	24	0	100	65.0	7.14	7.14
	48	0	100	67.0	7.14	7.14
Biskatali	12	0	50	20.0	5.0	5.0
	24	0	52	21.0	5.0	5.0
	48	0	52	21.0	5.0	5.0

S=Standard solution (prepared by alcoholic extraction from 100 g oven dried leaf powder dissolving in 100 ml distilled water)

Mayabini-Jene (2000) observed 90 to 100% mortality of larvae and adult of *Nilaparvata lugens* within 24 hours exposure period working with the Benzene extract of leaves of biskatali (*P. hydropiper*). Ahmed *et al.* (1991) reported that leaf extracts of neem, gave maximum 100% inhibition of egg hatching and larval mortality which are significantly affected by the concentration of the leaf extracts and exposure time. The effect of alcoholic extracts of neem and biskatali in alone and chemical nematicide Curaterr on different growth parameters were found statistically significant compared to control treatment. The length of shoot (80.50 cm) and root (29.38 cm) was found highest in Curaterr followed by higher significant shoot length (72.75 cm) with neem leaf extract and (69.03 cm) with biskatali leaf extract. The application of leaf extracts also showed significant increase in shoots and root weight. The highest shoot (130.90 g) and root (18.75 g) weight was recorded also in Curaterr. Application of neem leaf extracts showed the second heights shoot and root weight but biskatali leaf extract showed statistically similar result with neem leaf extract (Table 3). Curaterr and biskatali leaf extract showed statistically similar effects on the number of fruits per plant followed by neem leaf extracts but Curaterr gave the highest (8.25). In case of fruits weight Curaterr showed the best result (103.00 g) followed by neem leaf extract (71.00 g) and biskatali leaf extract (60.38g) (Table 3). Effect of the treatments on different stages of nematode development and diseases parameters were statistically significant. Number of nematodes at every developmental stage was found lowest in Curaterr. The lowest number (10.13) of galls was found with the chemical treatment followed by significant and identical numbers 17.25 and 18.25 galls in the treatments neem and biskatali leaf extract, respectively. The lowest number (6.38) of eggmasses was obtained with Curaterr followed by lower number (8.38) with neem and (11.03) with biskatali leaf extracts (Table 3).Like that of galling incidence the lowest number of adult, J_4, J_3 and J_2 was found with Curaterr followed by neem leaf extract and biskatali leaf extract, respectively. In case of J_4 and J_3 both the effect of neem leaf extract and biskatali leaf extract was statistically similar (Table 4).

Chemical nematicide Curaterr was found to influence the plant growth characters corresponding lower galling incidence, eggmass, development of adult females, J_2, J_3, and J_4, juveniles in the *M. javanica* inoculated plants. It is no doubt to say that nematicidal properties

Table 3. Effect of different treatments on the growth and galling incidence of tomato plants infected with *Meloidogyne javanica*

Treatments	Length of shoot (cm).	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	No. of fruits/ plant	fruit weight /plant	No. of galls/g of root	No. of eggmasses /g of root
To (Control)	58.63 d	16.38 c	38.88 d	5.00 c	3.05 c	10.63 d	21.50 a	14.38 a
T_1 (Neem leaf extract)	72.75 b	20.25 b	104.8 b	7.75 b	5.16 b	71.00 b	17.25 b	8.38 c
T_2 (Biskatali leaf extract)	69.13 c	19.63 b	98.13 c	8.63 b	7.13 a	60.38 c	18.25 b	11.13 b
T_3 (Curaterr)	80.50 a	29.38 a	130.90 a	18.75 a	8.25 a	103.00 a	10.13 c	6.38 d
LSD (p=0.05)	3.317	2.274	3.390	1.716	2.747	2.877	1.980	1.960

Each value is an average of eight replications
Values in the column having common letter (s) do not differ significantly at P=0.05 level by DMRT

Table 4. Effect of different treatments on the number of adult females, J_2, J_3 and J_4 stages of *Meloidogyne javanica*

Treatments	No. of adult females/ 10 galls	No. of J_2 stages/ 10 galls	No. of J_3 stages/ 10 galls	No. of J_4 stages/ 10 galls
To (Control)	27.00 a	10.1 a	9.0 a	14.2 a
T_1 (Neem leaf extract)	10.1 c	6.1 c	7.9 bc	8.2 bc
T_2 (Biskatali leaf extract)	13.2 b	7.4 b	8.2 b	8.9 b
T_3 (Curaterr)	5.4 d	2.7 d	2.2 d	2.9 d
LSD (p=0.05)	3.8	1.9	2.1	3.2

Each value is an average of eight replications, Values in the column having common letter (s) do not differ significantly at p=0.05 level by DMRT

of the chemical suppressed the growth stages of the nematode as evident with the highest plant growth characters already stated. Among the two alcoholic plant extracts, neem leaf extract hindered the development of adult females, J_2, J_3 and J_4 at the higher significant level which intern might have helped in increasing the plant growth characters as found in the experiment. In respect of leaf extract of biskatali more or less lower suppressing effect was observed on adult females which are the source of new generations of nematodes. As a result comparatively lower plant growth characters with higher number of eggmasses were observed with this extract which reflects the results of the mortality test. Hassan (1995) stated that Furadan 5 G and Miral 3G (Carbofuran) gave superior response in plant growth characters corresponding lower number of galls, adult females and egg masses in brinjal infected with *M. javanica*. Nanjegowada *et al.* (1998) similarly observed that Carbofuran was more effective in reducing root galling and increasing plant growth of tomato plants infected with *M. javanica*. Research findings of Hasan *et al.*(2005) also showed that Curaterr (Carbofuran) improve the plant growth characters including yield of mungbean by suppression of gall, and different stages of nematode development. Guzzman and Saxsena (1998) reported that neem oil, neem extracts and neem cake showed high nematicidal activity against root-knot nematode *M. incognita* in both laboratory and green house test. Like the finding of the present study there *in vitro* test showed a progressive significant increase in mortality of J_2 juveniles and inhibitory effects on production of galls in tomato plants corresponding with higher root weight. Siddique and Alam (1987) observed significantly reduced galling and population build up in tomato with water soluble extract of neem. Nanjegowada *et al.* (1998) used various neem products against *M. incognita* in a tomato nursery and found that, all the neem products and Carbofuran significantly reduced the nematodes population as well as increases the plant growth compared to control. In this study Carbofuran was to be more effective in reducing root galling as similarly observed in the present study. Recent reports made by Siddique and Alam (2001) revealed that root-knot development on tomato was significantly inhibited and growth of tomato was significantly improved by using neem based commercial products and aqueous extracts of different neem plant parts. All these reports on chemical nematicide Curbofuran and plant extracts are in agreement with the findings of the present study. From the over all study it has been summarized that chemical Curaterr was superior to plant extract in increasing the plant growth characters with yield by suppressing the galling incidence, eggmass production and nematode development. But, considering the environmental pollution and health hazards alcoholic leaf extract of neem could be used as substitute to chemical nematicide to control root - knot disease of tomato. However, further study is required to establish the action of neem extract on nematode under field conditions and also the chemical investigation on the neem estracts.

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