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Evaluation of some plant extracts for nemato-toxic potential against juveniles of *Meloidogyne incognita in vitro*

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ABSTRACT

Aqueous leaf extracts were utilized to assess the nematicidal or nematostatic property on second stage juvenile of *Meloidogyne incognita*. The juvenile were incubate at various concentration of leaf extract viz., 250, 500, 1000 and 2000 ppm. Corrected mortality using Abbot's formula was recorded after 12, 24 and 48 hours respectively. Correlation coefficient (Pearson) was checked to explain the association between percentages mortality of juvenile with extract concentrations. Linear regression was used to denote concentration and rank dependent outcome of four aqueous plant leaves extracts on the second stage juvenile (J_2) mortality. All leaf extracts were found to be nematicidal or nematostatic in property. Maximum juvenile mortality rate was recorded in *Xanthium strumarium* throughout the incubation period as followed by *Acalypha indica*, *Argemone mexicana* and *Colocasia gigantean*. Concentration depended effect of *X. strumarium* and *C. gigantean* proved maximum and minimum level when analyzed by values of regression and correlation. Aqueous leaves extracts of these aforementioned weeds give us an idea about nematicidal properties and therefore may be used as biopesticide in future.

Keywords: Nemato-toxic, Nematicidal, Correlation, Liners Regression, Meloidogyne incognita, Mortality.

INTRODUCTION

Population in the world is currently growing at the rate of 1.09% per annum [1] Due to this alarming figure, sufficient nourishment for the whole population of the world is one of the most important tasks of the present era. Moreover, agricultural productivity is hampered due to various pests. Among the major pest groups, plant parasitic nematodes assort huge economic losses to important cropping system. The annual yield loss by the plant parasitic nematodes in various countries around the world is estimated to be 12.30% ($157 billion) [2], out of which $40.3 million is reported from India [3].

Nematodes are difficult to control due to microscopic size and hence they are more often overlooked [4]. The phytoparasitic nematodes can be managed by two ways: Chemical and Non-chemical. The chemical nematicides used to control nematodes are costly, environmentally hazardous, toxic in nature, contaminate ground water, adversely affect water quality, aquatic life and human health. Therefore, to sustain agricultural production there is a need to develop cheap and eco-friendly non chemical methods to overcome the nematode infestation. Hence, the use of plant extracts is gaining attention due to their easy availability, cost effectiveness, pollution-free and eco-friendly nature. Therefore, the plant-based nematicides are a substitute in present times. The nematicidal properties in plant leaf extracts have been reported by various researchers *viz.*, [5, 6, 7]. In view of the aforementioned, the objective of this study is to evaluate the nematicidal potentials of aqueous leaves extracts of four commonly growing plant *viz.*, *Xanthium strumarium* L. (Cocklebur), *Acalypha indica* (Indian Mercury), *Colocasia gigantean (*Indian taro) *Argemone mexicana* (Mexican poppy).

MATERIAL AND METHODS

Preparation of aqueous leaf extract

Fresh leaves of four plants *viz.*, Cocklebur, Indian Mercury, Indian taro and Mexican poppy were washed thoroughly with tap water; 25 g fresh leaves of each weed were air dried and grinded in mortar and pestle to make the powder. Then 2g of dried powder was mixed with 1000 ml DDW. After mixing thoroughly, the material was centrifuged for 10 minutes at 10,000 rotations per minute (rpm). This extract was filtered through Whatman filter paper no. 1 and this filtered extract was called as standard 2000 parts per million (ppm). By using this standard filtrate1000, 500 and 250 ppm concentrations

solutions were prepared by adding required amount of DDW.

Root-Knot culture and inoculum

The root-knot nematode (RKN) was sampled from the brinjal plant root at Jaspura village of Banda district of Uttar Pradesh. The further identification and confirmation of the species was made by comparing the characteristic features of second stage juvenile and perineal pattern with that of description given by [8]. The identified species was maintained as pure culture by using single egg on brinjal plant. After 60 days, egg-masses were hand-picked from the infected roots of brinjal and kept in 5 ml of 0.5% sodium hypochlorite (NaOCl) solution for 5 minutes [9]. To collect hatched second-stage juveniles (J_2), sterilized eggs were placed into a hatching chamber comprising of a Mesh Nylon Filter (25 µm in diameter) in an autoclaved dish (modified Baermann). Second-stage juveniles were allowed to pass through the filter. The juveniles collected within 48 h were used.

Mortality assay on second stage juvenile (J2) of *M. incognita*

The nematicidal or nematostatic activity of different aqueous concentration of leaf extracts of different plants was determined by keeping approximately 100 Second stage juvenile in 2000, 1000, 500, 250 ppm. The mortality was observed after 12, 24 and 48 hours of treatment with aqueous extract of leaves. Petridish containing DDW was served as control. Every treatment was reproduced five times. The Petridishes were held at 28 ±°C. Approximately, 100 freshly hatched 2nd stage juveniles (J_2) of *M. incognita* race 3 were shifted separately in to Petridishes containing 10 ml of different concentrations (2000 ppm, 1000 ppm, 500 ppm, 250 ppm) of aqueous extract of leaves. The mortality of nematodes was counted after exposure time of 12, 24 and 48 hours. The juvenile mortality in the exposed concentration was corrected for death in the control (DDW) using Abbott's formula.

Statistical analysis

The study was made using SPSS version 22.0 software to analyze the sampled data. In the beginning, the means were compared with ANOVA to assess the statistical significance of recorded data. Pearson rank Correlation test was conducted to ascertain the association among the percentages of larval mortality and extract concentrations. Linear regression was conducted to show ranking and dose dependent effect of four extracts of plant leaves on mortality of 2nd stage juvenile (J_2) of *M. incognita* race 3. The Present study is based on the two variables namely mortality and concentration. Relationship between these variable has been estimated through the "best fit" regression line. Further correlation is also examined between mortality and concentration for the evaluation of degree of association. The size of the correlation coefficient (r) indicates how well the linear regression model explains the relationship between the two variables, which ranges between 0 and 1, with higher values of r demonstrating a stronger association between the two variables. The coefficient of determination (R^2) describes how independent variable (X) clarifies the deviation in the dependent variable (Y). In this study, concentration is taken as independent variable while mortality is taken as dependent variable. Generalized form of regression and correlation equation 1 and 2, respectively:

$$Y = a + bX \dots\dots\dots\dots (1)$$

$$\text{where}, a = \frac{(\sum y)(\sum x^2)-(\sum x)(\sum xy)}{n(\sum x^2)-(\sum x)^2}$$

$$b = \frac{n(\sum xy)-(\sum x)(\sum y)}{n(\sum x^2)-(\sum x)^2}$$

$$r = \frac{n(\sum xy)-(\sum x)(\sum y)}{\sqrt{[n\sum x^2-(\sum x)^2][n\sum y^2-(\sum y^2)]}} \dots\dots\dots (2)$$

RESULT

Interpretation of results of the present study clearly demonstrates the effect of concentration and exposure time of leaf extract on juvenile mortality (Table 1 and Figure 1). The mortality rate of juvenile incubated for 12 h in aqueous leaf extract of *Xanthium strumarium were found to be* 25%, 35%, 48% and 51% when the juvenile were kept in 250, 500, 1000, 2000 ppm aqueous concentrations, respectively. The mortality increased after 24 h and reached up to 28% 39%, 61% and 79%. Following the same trend, another high was observed after 48h of incubation as the levels soared up to 32%, 49%, 67% and 83% at 250, 500, 1000 and 2000 ppm concentration.

Table 1: Effect of aqueous leaf extract of Plant on the survival of second-stage juveniles of *Meloidogyne incognita* with respect to five different concentrations

Treatment	Concentration ppm.	Mortality of second stage juvenile (J_2) of *M. incognita* (Mean S.E.)		
		12 Hours	24 Hours	48 Hours
Xanthium strumarium	0	0.63±0.632 (1%)	1.40±0.872(1.4%)	1.80±0.917 (2%)
	250	24.60±2.68 (25%)	27.60±1.03 (28%)	30.82±1.22 (32%)
	500	35.00±2.67 (35%)	38.80±1.16 (39%)	47.62±1.94 (49%)
	1000	47.40±2.04 (48%)	60.0±1.30 (61%)	64.45±2.57 (67%)
	2000	50.80±3.97 (51%)	78±1.14 (79%)	80.07±1.93 (83%)
L.S.D.(P=0.05)		7.812	3.297	5.382
Acalypha indica	250	28.20±3.338 (28%)	34.60±1.536 (35%)	38.10±2.451 (40%)
	500	30.80±3.499 (31%)	38.80±3.826 (39%)	56.83±4.391 (59%)
	1000	37.20±1.855 (38%)	49.60±4.686 (50%)	58.40±2.482 (61%)
	2000	43.80±0.97 (44%)	61.80±2.223 (63%)	72.60±2.786 (75%)
L.S.D. (P=0.05)		7.052	4.564	8.408

Argemone mexicana	250	21.80±3.2 (22%)	20.4±1.208 (21%)	30.82±1.22 (32%)
	500	27.40±3.415 (28%)	28.60±2.6 (29%)	47.62±1.942 (49%)
	1000	33.80±3.121 (34%)	39.20±1.934 (39%)	51.00±1.975 (53%)
	2000	40.40±1.208 (41%)	46.40±1.806 (47%)	60.20±2.653 (62%)
L.S.D. (P=0.05)		7.693	5.311	5.485
Colocasia gigantean	250	17.60±1.249 (18%)	20±1.871 (21%)	26.60±1.03 (28%)
	500	21.20±1.158 (21%)	25.60±2.441 (26%	35.80±3.023 (37%)
	1000	29.60±1.6 (30%)	34.20±1.655 (35%)	39.40±3.341 (41%)
	2000	31.20±2.311 (32%)	43.40±1.806 (44%)	52.80±2.518 (55%)
L.S.D. (P=0.05)		4.446	5.351	7.098

Note: Each value is an average of five replicates, DW=Distilled Water (Control). The corrected value of mortality using Abbott's (1925) are given in parentheses. (Mean ±S.E.), ppm= parts per million

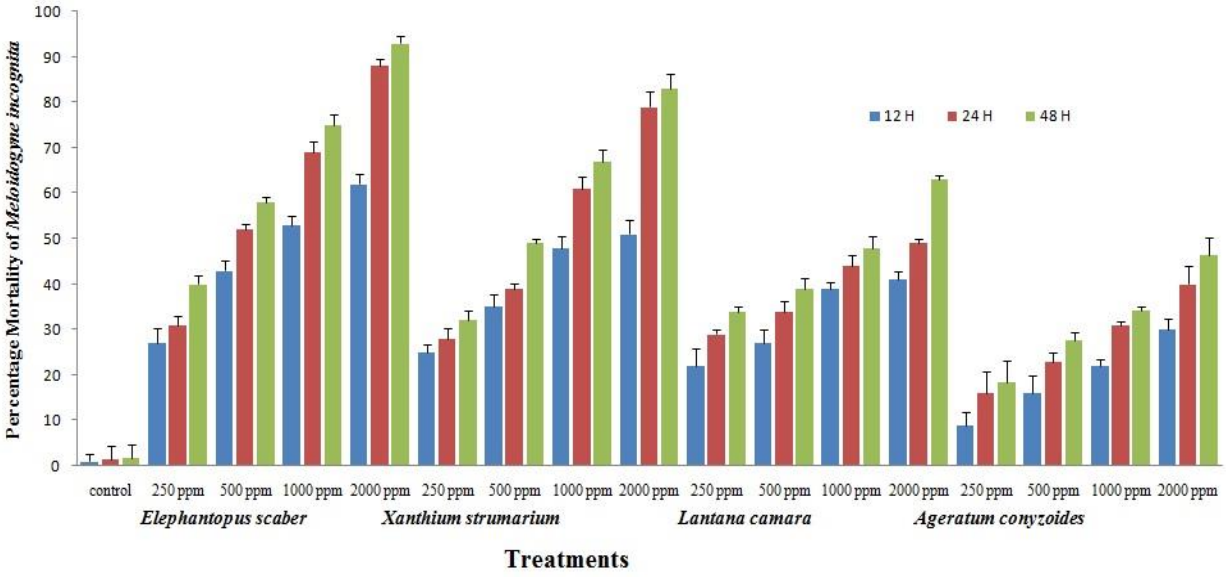

Figure 1: Percentage mortality of Juvenile of *Meloidogyne incognita* on different concentration.

Acalypha indica leaf extract showed 28%, 31%, 38% and 44% mortality when incubated for 12h at 250, 500, 1000, 2000 ppm aqueous concentration, respectively. Similarly, 35%, 39%, 50% and 63% mortality was observed when incubated for 24h at 250, 500, 1000, 2000 ppm aqueous concentration, respectively. The mortality increased to 40%, 59%, 61% and 75% when incubated for 24 h at 250, 500, 1000, 2000 ppm aqueous concentration.

The nematicidal activity of aqueous leaf extracts of *Argemone mexicana* showed 22%, 28%, 34% and 41% mortality at 250, 500, 1000, 2000 ppm aqueous concentrations respectively after 12 h of incubation. The rate of mortality aggravated to 21%, 29%, 39% and 47%when incubated for 24 h. Mortality increased up to 32%, 49%, 53% and 62% when incubated for 48 h at 250, 500, 1000, 2000 ppm aqueous concentration.

The minimum rate of mortality was observed in liquid leaf extract of *Colocasia gigantean*. The mortality of second 2ndjuvenile was found as follows: 18, 21, 30 and 32% at 12 h; 21, 26, 35 and 44% at 24 h and 28, 37, 41 and (55) % at 48 h respectively.

The outcomes reveal that the nematicidal activity of leaf extract was increased with an increase in concentration and exposure time. A significant relationship was observed between extract concentration of plant leaves and mortality of juveniles at P=0.05 (Table 2). The data showed in table 3, regression and correlation of regression revealed the best concentration dependent effect of aqueous leaf extracts on nematode mortality *Xanthium strumarium (*$R^{2=}$.749) follow by Acalypha indica ($R^{2=}$.650), *Argemone mexicana*(R^2=.556) and *Colocasia gigantean* (R^2=.453).

Table 2: Correlation coefficient (Pearson) describing the relation between two continuous variables

Relationship between *Xanthium strumarium aqueous* extract and % of mortality of juvenile (J_2)		
	***Xanthium strumarium extract* concentration (ppm.)**	**J_2**
Pearson correlation (XS.)	1	.865
P-value (1-tailed)	-	.05
N	15	15
% of mortality of juvenile (J_2)	.865	1
P-value (1-tailed)	.05	-
N	15	15

Relationship between *Acalypha indica aqueous* extract and % of mortality of juvenile (J_2)		
	***Acalypha indica* extract concentration (ppm.)**	**J_2**
Pearson correlation (AI.)	1	.806
P-value (1-tailed)*	-	.05
N	15	15
% of mortality of juvenile (J_2)	.806	1
P-value (1-tailed)*	.05	-
N	15	15
Relationship between *Argemone mexicana aqueous* extract and % of mortality of juvenile (J_2)		
	***Argemone mexicana* extract concentration (ppm)**	**J_2**
Pearson correlation (AM)	1	.745
P-value (1-tailed)*	-	.05
N	15	15
% of mortality of juvenile (J_2)	.745	1
P-value (1-tailed)*	.05	-
N	15	15
Relationship between *Colocasia gigantean aqueous* extract and % of mortality of juvenile (J_2)		
	***Colocasia gigantean* extract concentration (ppm)**	**J_2**
Pearson correlation (CG)	1	.673
P-value (1-tailed)*)	-	.05
N	15	15
% of mortality of juvenile (J_2)	.673	
P-value (1-tailed)*	.05	-
N	15	15

* Correlation is significant at 0.05 level (1- tailed) test, %=Percentage

Table 3: Regression values and correlation of regression of the effect of different aqueous leaves extract of plant on mortality of second stage juvenile (J2) of *Meloidogyne incognita*.

Treatment	Regression equation	R^2 value	Rank
Xanthium strumarium	y = 0.031x + 16.77	0.749	1
Acalypha indica	y = 0.024x + 18.68	0.650	2
Argemone mexicana	y = 0.020x + 15.82	0.556	3
Colocasia gigantean	y = 0.017x + 12.85	0.453	4

DISCUSSIONS

Many secondary products of plants such as theinyls, alkaloids, phenols, sesquiterpines, diterpenes and polyacetylene possess nematicidal or nematostatic activity against phytoparasitic nematodes [10, 11, 12]. Nemato-toxic potential of liquid extract plant depends on concentration and the duration of incubation in extract [13, 14].

Similar results have been found by earlier worker such as [14, 15]. The nematostatic effect of leaf extracts may be the result of lipophilic properties that dissolve the cytoplasmic membrane of nematode cells and destroy the enzyme protein structure [17, 18]. The mechanism of plant leaf extract action may incorporate denaturation, degradation of proteins and obstruction of the electron transport framework (ETS) or ADP phosphorylation [18].

From the results, it is evident that the presence of water soluble phytochemicals and toxicants is lethal for nematodes. The results suggest that aqueous leaf extracts have significant potential at different concentrations as Biopesticide against phytoparasitic nematode. Practically, the use of aqueous leaf extracts of plants is more important and easily available resource to the poor farmers. From this study, it can be concluded that the use of botanical means may serve as an alternative to the chemicals in order to bring sustainability to agriculture.

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