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Screening of *Derris indica* Bennet. for cytotoxicity against *Artemia salina* and phytotoxicity on mustard seeds

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Abstract: Chloroform extracts of the fruit shell, leaves, root bark, root wood, seeds, stem bark and stem wood of *Derris indica* Bennet. were tested against the brine shrimp, A*rtemia salina* nauplii. All the test extracts of *D. indica* were found to be effective. The LC_{50} values of the extracts were 15312.37, 92.074 and 29.661 ppm for the fruit shell; 60922.83, 61.522 and 23.777 ppm for the leaf; 15312.37, 51.477 and 19.169 ppm for the root bark; 2598.584, 30.480 and 8.260 ppm for the root wood; 545.025,26.730 and 7.719 ppm for the seed; 60922.83, 114.549 and 29.572 ppm for the stem bark and 7734.618, 58.501 and 23.694 ppm for the stem wood at 30 minute, 24 hours and 48 hours post exposures respectively at doses 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ppm against *A. salina* . The toxicity of the extracts could be arranged in the order: seed > root wood > root bark> stem wood > leaf > fruit shell > stem bark extract. However, the extracts did not significantly inhibit the germination of mustard oil seeds, and thus its application to crops or to the crop field may not cause any harm to crop plants.

Key words: *Derris indica*, cytotoxicity, A*rtemia salina* nauplii, phytotoxicity, mustard seeds.
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Introduction

Natural products derived from plants, as an alternative to conventional insecticides for insect control, is now-a-days very popular among the IPM practitioners. Plant-derived pesticides are more readily biodegradable; therefore they are less likely to contaminate the environment. Moreover, the botanical pesticides break down readily in soil and are not stored in plant or animal tissues.

Being a medicinal plant, *Derris indica* Bennet. might contain some antipathogenic properties. Powdered seeds of this plant are valued as febrifuge and tonic, and are used in bronchitis and whooping cough. The seeds are also reported to be used as a fish poison (Kirtikar and Basu, 1935), and the seed oil is used as a soap liniment to treat scabies, herpes and rheumatism (Burkill, 1966). The leaf extract is active against *Micrococcus pyogenes* var. *aureus* (Anon, 1969). The leaf juice is prescribed in flatulence dyspepsia, diarrhoea and cough and also considered as a remedy for leprosy and gonorrhoea. The root juice is used for cleansing foul ulcers and sores, and to treat gonorrhoea. Roots are used as fish-poison by the aborigins of Australia (Kirtikar and Basu, 1935). The dried flowers are used as decoction to quench thirst in diabetes. The fresh bark juice is given internally in bleeding piles (haemorrhoids) and a decoction of bark is used against beri-beri (Anon, 1969). Cheema *et al.* (2003) have advocated the commercial utilization of sorghum water extracts for weed management in wheat. Ethanol extracts of *D. trifoliata* showed different mortality rates of brine shrimp which increased proportionally with the increasing concentrations of the extracts (Saifullah and Azam, 2011). Daruliza et al. (2012) traced the anti-Candida activity and brine shrimp toxicity assay of *Ganoderma boninense.* The insecticidal activity of this plant against *Callosobruchus maculatus* has been determined (Mondal and Islam, 2008), and antibacterial and larvicidal potentials have also been worked out (Mondal *et al.,* 2010). Germination characteristics of Maize seeds under different ageing treatment have been done by Siadat *et al*., (2012). Another seed germination test was conducted by Geetha *et al*., (2011). Seed priming is known as technique of seed enhancement that improves germination or seedling growth in many crops such as Dry bean (*Phaseolus vulgaris* L.), cordia (*Cordia millennii*) (Adebisi, 2011), coffee (*Coffea arabica* L.) (Gebreselassie *et al*., 2010), capsicum (*Capsicum annum*) and *Agropyron elongatum* (Tavili *et al.*, 2010)

Various workers investigated *D. indica* giving emphasis mostly on the chemical constituents and their medicinal profile but very few works have been done on its pesticidal importance. In this investigation, cytotoxic and phytotoxic activity tests of *D. indica* were carried out on the brine shrimp, *Artemia salina* nauplii and the musatard seeds respectively to evaluate the efficacy of the plant parts as a possible source of potential secondary metabolites to be used as environment friendly pest control agents.

Materials and Methods

Preparation of plant materials for extraction: The fresh leaves, fruit shell, root bark, root wood, seeds, stem bark, and stem wood of *D. indica* were collected from the campus of the University of Rajshahi, Bangladesh. After drying under shade the plant materials were powdered in a grinder machine.

Chemical extraction of the collected materials: Chloroform was selected as a solvent to extract seven different parts of *D. indica* separately. The ground dried materials, *viz*. leaves, fruit shell, root bark, root wood, seeds, stem bark, and stem-wood were extracted with sufficient amounts of chloroform (500g × 1500ml × 3 times) for each of the items. Separate extracts were collected by the cool method after 72 hours of plunging for each of the plant parts. Extracts were subjected to

filtration and evaporation of the solvent. The residues were kept in a refrigerator after proper labeling.

Since the lethality test involves the culture of brine shrimp nauplii, i.e., the nauplii should be grown in water with salinity similar to that of sea water, while the seawater contains 3.8% sodium chloride. Accordingly, a 3.8% sodium chloride solution was prepared by dissolving 38 gm sodium chloride in 1000 ml distilled water. The P^H of the brine water thus prepared was maintained between 8 and 9 using $NaHCO_3$.

Brine water was taken in a small tank and shrimp eggs (1.5 gm/L) were added to one side of the perforated tank with a constant oxygen supply. A constant temperature (37ºC) and sufficient light were maintained. After 48 hours, shrimp nauplii were collected and used for the experiment. For the seed germination test, fresh and healthy mustard seeds were collected from the market.

Cytotoxicity test:

Preparation and application of doses on *A. salina*

Chloroform extracts of the *D. indica* samples were applied against the brine shrimp nauplii. For the fruit shell, leaves, root bark, root wood, stem bark and stem wood samples 4mg were initially dissolved in 200µl of pure dimethylsulfoxide (DMSO) to make them hydrophilic before adding 19.98 ml of water to get a concentration of 200 ppm for each of the samples separately which were used as stock solutions for all the extracts and from these concentrations other successive doses were prepared separately for each of the extracts through the serial dilution method. A series of concentrations, e.g. 200, 100, 50, 25, 12.5 and 6.25 ppm were prepared for the extracts separately. However, for the seed extract 2 mg was initially dissolved in 100 µl of DMSO to make it hydrophilic before adding 19.98ml of water to get a concentration of 100 ppm which was used as the stock solution for the seed extract. The following concentrations were made from the stock solution: 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ppm.

Brine shrimp eggs were hatched in simulated seawater to get nauplii. Test samples were prepared by the addition of the requisite amounts of DMSO for obtaining desired concentrations of the test sample. The nauplii were counted by visual inspection and were taken in vials containing 5ml of brine water. Then samples of different concentrations were added to the pre-marked vials with the help of a micropipette. The vials were left for 24 hours and then the nauplii were counted again to find out the cytotoxicity of the test agents and compared to the results with positive control.

Preparation and application of doses on mustard seeds:

In this experiment 4 doses from the fruit shell, leaves, root bark, root wood, seed, stem bark and stem wood extracts of *D. indica* were made as 1 mg, 0.75 mg, 0.50 mg and 0.25 mg/ml freshwater. Because of insolubility of the extract in water it was needed to add 100µl DMSO with the weighed extract before mixing with water.

For application of doses a number of petridishes 60mm diam. were used. Filter papers were placed inside the petridishes and doses were applied separately. Five mustard seeds were put in every petridish and three replications were set for each concentration and a control with three replications was also maintained. All the petridishes were kept covered to avoid drying. The humid condition inside the petridishes helped the seeds to germinate. Then the petridishes were placed in a safe place with plenty of light and air. Germination (%) was carefully recorded at various concentrations of different extracts of *D. indica*.

Collection and analysis of data for cytotoxicity

The test tubes containing the nauplii with the treated brine water were kept on a rack near the window in the laboratory. The recorded mortality was corrected by the Abbott's (1925) formula:

$$P_r = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where,
P_r = Corrected mortality (%),
P_o = Observed mortality (%), and
P_c = Control mortality (%).

Mortality data were subjected to statistical analysis according to Finney (1947) and Busvine (1971) by using software developed at the Department of Agricultural and Environmental Science, University of Newcastle-upon -Tyne, U.K. The dose-mortality relationship was expressed as median lethal concentrations (LC_{50}).

Results and discussions

The results of dose-mortality assays of *D. indica* extracts against *A. salina* nauplii are presented in table 1 and illustrated in Fig. 1. Most of the test extracts showed remarkable dose-mortality effects against the 1 day old nauplii of *A. salina.* The degrees of activity of the extracts against the brine shrimp nauplii could be arranged in the order: seed > root wood > root bark> stem wood > leaf > fruit shell > stem bark extract.

In general, the application of the chloroform extracts of different parts of *D. indica* on the germination of mustard seeds produced significant effects (Table 2).

The ethanolic extracts of *D. scandens* (Roxb.) Benth, along with other test extracts showed cytotoxicity (LC_{50}<30 µg/ml) against lung and prostate cancer cell lines (Acharya and Thomas, 2007). LC_{50} values of petroleum ether, chloroform and methanol extracts on *A. salina* Leach were recorded as 1.14, 1.1, and 54.9mg/l respectively. Chemical analysis revealed the presence of fatty acids, steroids, triterpenoids, alkaloids, phenols, and phenyl propanoids, tannin, and mucilage in the extracts (Uyub *et al.,* 2010).

The present results are more or less similar to those of Mondal and Islam (2008). However, the fruit shell and the leaf extracts did not offer any mortality of the test insect (*Callosobruchus maculatus*) and the intensity of activity could be arranged in a descending order as seed > root wood > root bark >stem wood > stem bark.

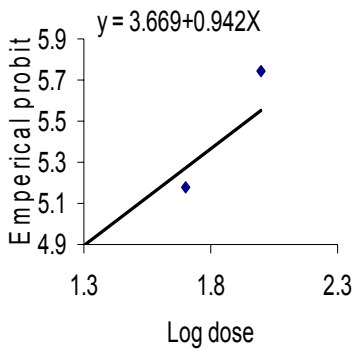
Table1: Cytotoxicity of *D. indica* extracts against *A. salina* nauplii.

Test extract	Time exposed	LC_{50} value (ppm)	95% Confidence limits		Regression equation	χ^2 Value(df)
			Lower limit	Upper limit		
Fruit shell	30 min	15312.7	34.995	6700098	Y=2.791+0.528X	0.546 (4)
	24 h	92.074	50.777	166.959	Y=3.182+0.925X	0.319 (4)
	48 h	29.661	20.117	43.734	Y=3.284 + 1.166X	0.994 (4)
Leaf	30 min	60922.83	5.791	6.409e+08	Y=2.721+0.476X	0.550 (4)
	24 h	61.522	37.700	100.397	Y=3.259+0.973X	0.278 (4)
	48 h	23.777	16.546	34.168	Y=3.194+1.312X	0.911 (4)
Root bark	30 min	15312.37	34.995	6700098	Y=2.791+0.528X	0.546 (4)
	24 h	51.477	27.505	96.342	Y=3.766+0.721X	0.279 (4)
	48 h	19.169	11.440	32.122	Y=3.788+0.945X	1.225 (4)
Root wood	30 min	2598.584	110.001	61387.03	Y=2.737+0.663X	0.204 (4)
	24 h	30.480	16.123	57.620	Y=3.989+0.682X	5.686e-02(4)
	48 h	8.260	4.036	16.905	Y=4.125+0.954X	2.352 (4)
Stem bark	30 min	60922.83	5.791	6.409e+08	Y=2.721+0.476 X	0.550 (4)
	24 h	114.549	47.100	278.582	Y=3.612 + 0.674 X	0.374 (4)
	48 h	29.572	17.862	48.960	Y =3.712 + 0.876 X	8.718e-02(4)
Stem wood	30 min	7734.618	68.723	870519.9	Y =2.530 + 0.635 X	0.389 (4)
	24 h	58.501	28.500	120.083	Y =3.859 + 0.646 X	0.356 (4)
	48 h	23.694	13.599	41.286	Y = 3.867+0.824 X	0.597 (4)
Seed	30 min	545.025	81.267	3655.26	Y= 2.452 + 0.931X	0.799 (4)
	24 h	26.730	16.271	43.912	Y 3.678 + 0.926 X	1.335 (4)
	48 h	7.719	4.673	12.750	Y =4.063 + 1.056 X	1.568 (4)

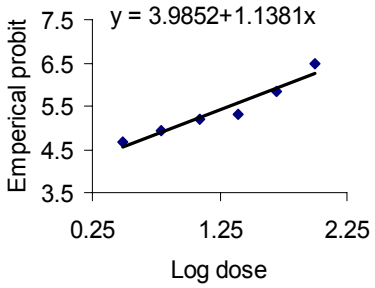
Table2. Germination (%) of Mustard seeds by extracts of different parts of *D. indica*

Treatment with following extratives	Germination %age
	Mustard seed
Fruit shell	90
Leaf	92
Root bark	85
Root wood	90
Stem bark	80
Stem wood	75
Seed	85
Control	95

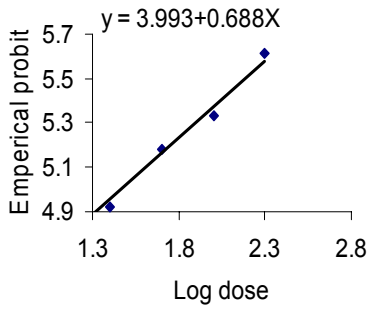
It is important to evaluate the newly found bioactive agents to try against some test crop plants to see whether or not they cause any detrimental effects on target crop(s). Thus, phytotoxicity tests are necessary. Ndakidemi and Dakora (2003) employed legume seed flavonoids and nitrogenous metabolites for an improvement in their understanding of seed chemistry whether they would permit manipulation of these molecules for effective control of pathogens, insect pests, *Striga* and destructive weeds, as well as for enhanced acquisition of N and P via symbioses with soil rhizobia and AM fungi.


y = 3.669+0.942X
Emperical probit
5.9
5.7
5.5
5.3
5.1
4.9
1.3
1.8
2.3
Log dose

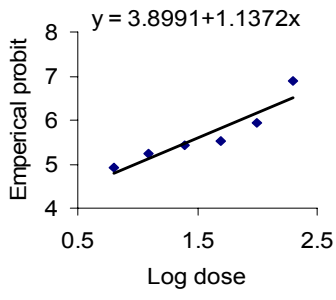
A


y = 3.9852+1.1381x
Emperical probit
7.5
6.5
5.5
4.5
3.5
0.25
1.25
2.25
Log dose

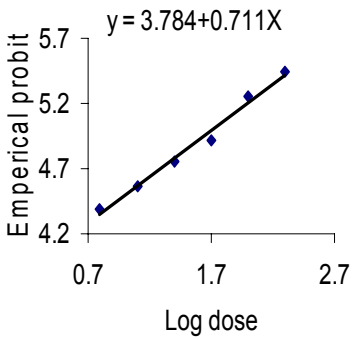
B


y = 3.993+0.688X
Emperical probit
5.7
5.5
5.3
5.1
4.9
1.3
1.8
2.3
2.8
Log dose

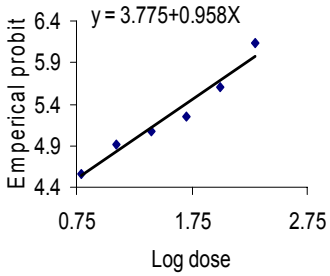
C


y = 3.8991+1.1372x
Emperical probit
8
7
6
5
4
0.5
1.5
2.5
Log dose

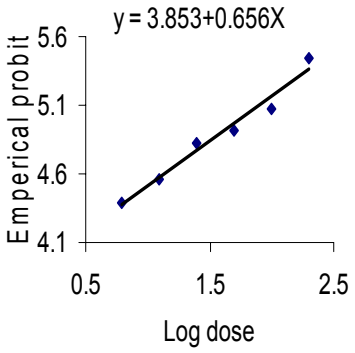
D


y = 3.784+0.711X
Emperical probit
5.7
5.2
4.7
4.2
0.7
1.7
2.7
Log dose

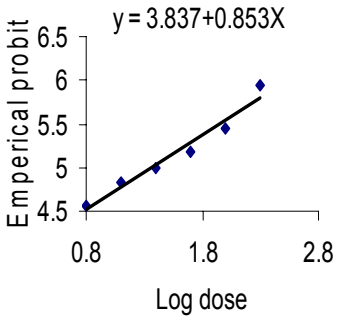
E


y = 3.775+0.958X
Emperical probit
6.4
5.9
5.4
4.9
4.4
0.75
1.75
2.75
Log dose

F


y = 3.853+0.656X
Emperical probit
5.6
5.1
4.6
4.1
0.5
1.5
2.5
Log dose

G


y = 3.837+0.853X
Emperical probit
6.5
6
5.5
5
4.5
0.8
1.8
2.8
Log dose

H

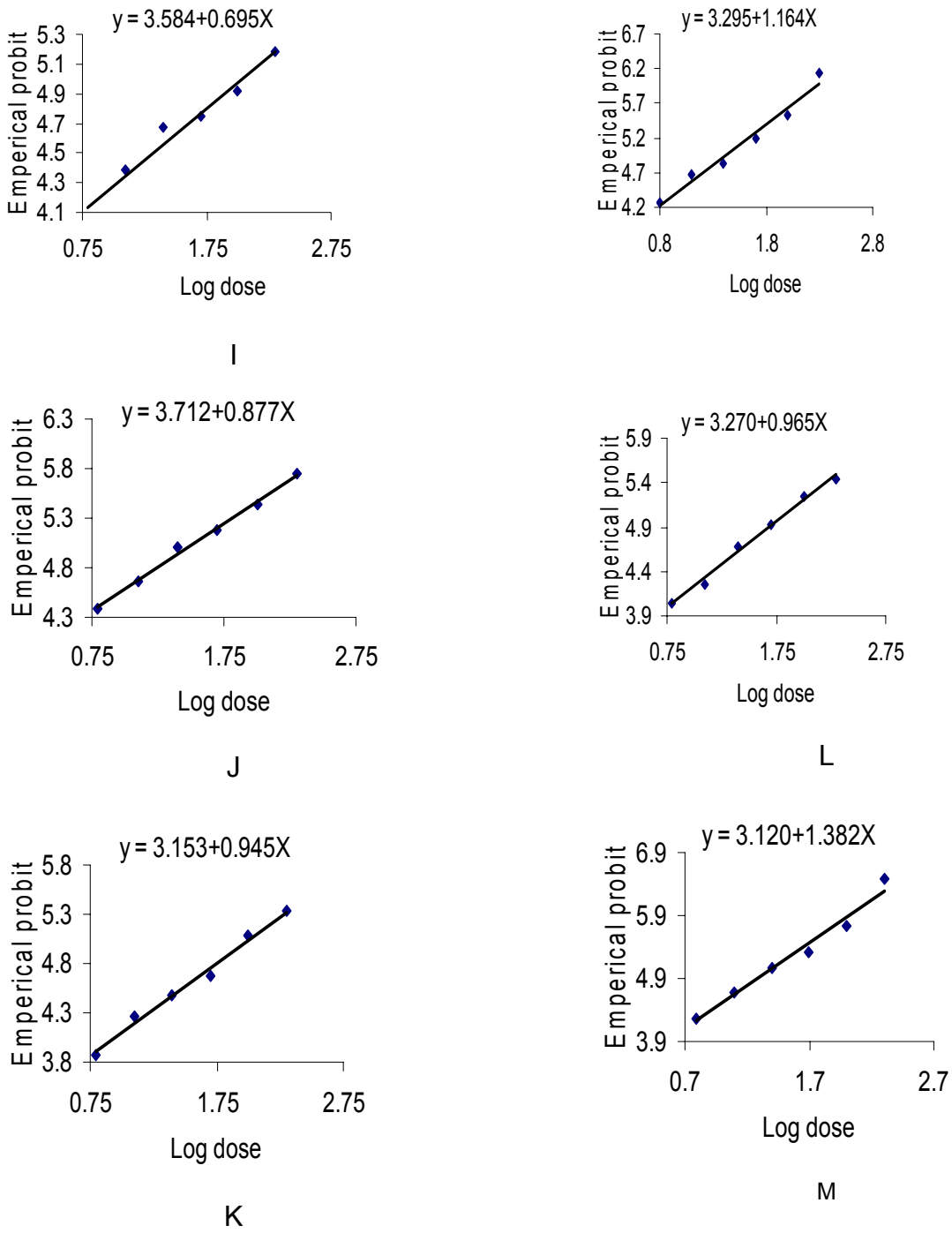


Fig. 1. Probit mortality regression lines of the chloroform extracts of *D. indica*: A- Seed/ 24 h; B- Seed/ 48 h; C- root wood/ 24 h; D- root wood/ 48 h ; E- root bark/ 24 h and F- root bark/ 48h;.G- stem wood/ 24 h; H- stem wood/ 48 h; I- Stem bark/ 24 h; J- Stem bark / 48 h; K- fruit shell/ 24 h and L- fruit shell / 48 h; M- leaf/ 24and N- leaf / 48 h of exposure against *A. salina*

However, it is also important to see whether or not the plant secondary metabolites having insecticidal or biological activity cause any barrier to the sprouting and growth of seedlings. Phlomina and Srivasuki (1996) reported that leaf leachates of 5 multipurpose tree species (*Eucalyptus camaldulensis, Acacia nilotica, Derris indica*, *Cassia siamea* and *Sesbania grandiflora)* had varying degrees of inhibitory and stimulatory effects on germination percentage. Velu *et al.*(1996) reported that *Acacia* sp. retard the plant growth and development. Thakur and Bhardwaj (1992) reported that when wheat seeds were exposed to leachates from leaf extracts of *Eucalyptus globulus, Populus ciliata, Juglans regia* and *Robinia pseudoacacia* germination was not affected.

A perusal of the data shows that *D. indica* extracts produced significant mortalities against *A salina* nauplii. But the extracts had, in general, no significant phytotoxicity against mustard seeds. However, more comprehensive studies are needed in this line.

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