

PISCICIDAL EFFECTS OF EXTRACTS OF KARENJA PLANT *PONGAMIA PINNATA* (L.) PIERRE AND VAT PLANT *CLERODENDRUM VISCOSUM* (VENT.) ON SINGHI FISH *HETEROPNEUSTES FOSSILIS* (BLOCH)

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ABSTRACT

The piscicidal properties of the seed, leaf and bark of the indigenous plants, karenja *Pongamia pinnata* (L.) Pierre and vat *Clerodendrum viscosum* (Vent.) were studied on the predatory fish Singhi, *Heteropneustes fossilis* (Bloch) under laboratory conditions. Distilled water, 50% ethyl alcohol and absolute ethyl alcohol extracts of the plant parts of the plants were tested in this experiment. The altered behaviour due to the effects of the extracts was noted, initially hyperactivity, losing stamina and in the later period, after a few hours, the affected fishes becoming sluggish and inactive. Mortality data varied depending upon the toxicity of the plant parts, extracts and concentrations. On the basis of concentrations, the relative toxicity of the piscicides on *H. fossilis* for *P. pinnata* was found to be in the order of seed > bark > leaf and for *C. viscosum*, seed > leaf > bark. The toxicity of seed and leaf extracts of *C. viscosum* was greater than *P. pinnata* but opposite for bark extract for the fish. On the basis of LC₅₀ values the toxicity of both the plant parts were similar as to seed > bark > leaf.

Key words: Toxicity, Plant toxins, Behaviour, Mortality, *H. fossilis*.

INTRODUCTION

Eradication of predatory and undesirable fishes from fish farms and ponds is an important task in any culture practice. Predatory and undesirable fishes prey on the fish fry of economic variety and directly affect the production of cultured fish. As to control and eradication of unwanted fishes from the ponds, require effective piscicides, which are usually not easily accessible, farmers use synthetic compounds and even pesticides. However, none of these compounds in culture ponds seldom are appreciated, especially due to their long term persistence in the ecosystem as well as in the cultured fisheries. Indiscriminate use of pesticides poses a great risk to aquatic organisms, especially fishes and consequently to human. Therefore, a good control measure is one that will be effective in killing

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the target organism, which is not injurious to people and animal but easily available, economical, disappear in aquatic environment without any cumulative adverse effect and have traditionally been used to harvest fish in almost all parts of the world. Botanicals, which are biologically degradable, and having piscicidal activities with shorter residual effects are being appreciated. Such piscicidal plants contain different active ingredients known as alkaloids such as nicotine, pyrethrum, resin, tannin, saponin and diosgrin (Wang and Huffman 1991).

Plant preparations satisfy most of the requirements of an ideal piscicide. Floral species of the families Meliaceae, Rutaceae, Leguminosae, Asteraceae, Annonaceae and Canellaceae are the most promising for use in pond ecosystem (Jacobson 1989). Among the plant products Rotenone, Nicotine, Saponin, Sabadilla, Quassia, Ryania and Pyrethrum are the chief products, which have piscicidal properties. Works on the potentiality of plant species as piscicides are considerable (e.g., Nayar 1955, Chopra *et al.* 1958, Shirgur 1972, 1974, 1975, Weiss 1973, Chaiyvareesajja *et al.* 1987, Van Andel 2000, Singh and Singh 2002, Neuwinger 2004, Sudhansu and Singh 2004).

There are literatures reporting on the piscicidal properties of some indigenous plants in Bangladesh (e.g., Haque and Tilton 1970, Chowdhury *et al.* 1981, Ameen and Shahjahan 1987, Ameen *et al.* 1987, Latifa *et al.* 1987, 1988, 1992, 1997, 2002, 2004, Ahmed 1992, Latifa and Begum 1993, Nasiruddin *et al.* 1997, 1998, 2006, 2009, Nasiruddin and Sultana 2007). The present study on the dry seed, leaf and bark extracts of *Pongamia pinnata* (L.) Pierre (Bengali-Karenja) and *Clerodendrum viscosum* (Vent.) (Bengali- Vat) as piscicides is concerned with assaying of the extracts of two indigenous plants' seed, leaf and bark i.e., *C. viscosum* and *P. pinnata* on the fresh water predatory catfish, *Heteropneustes fossilis* (Bloch) under laboratory conditions. These plant preparations might help in controlling the undesirable fish species in commercial culture ponds.

MATERIALS AND METHODS

Toxic effects of the seed, leaf and bark of the indigenous plants *Pongamia pinnata* (Bengali-Karenja) and *Clerodendrum viscosum* (Bengali-Vat) were used to determine the behaviour, and toxicity of a predatory fish *Heteropneustes fossilis*. Healthy and live specimens of *H. fossilis* were purchased from fish markets of Chittagong city on the day of the experiment, transported immediately to the laboratory in aerated plastic containers filled in with water. Then the fishes were kept in a big glass aquarium (60 cm x 30 cm x 30 cm) containing tap water

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and acclimatized for 3-4 hours in laboratory condition. Only the healthy and active fishes were used for the experiments. The average length of the fish was 15.0 ± 8.0 cm and weight was 14 ± 3 g.

At the prior to the experiments the plant parts were grinded using metallic mortar and pestle, then crushed into fine powder with an electric grinder and sieved through 0.0025 sq cm mesh size sieve. Required amount of plant powder was weighed in an electronic pan balance (SHINKO DENSHI Co. LTD. Model: KS- 300A, made in China) to mix with the solvents. To 10 g of finely ground dry powder taken in a flat bottom flask, 100 ml of either distilled water or 50% ethyl alcohol or absolute ethyl alcohol (MERCK) solvent was added and soaked for 24 hours. The flasks were stirred vigorously with a magnetic stirrer to ensure maximum extraction of the toxic components. After 24 hours, the solution was filtered through fine cloth, and was kept as “stock liquid extract” (Latifa *et al.* 1992). The toxicity test doses were calculated from this “stock solution” by appropriate dilution (APHA 1976).

Concentrations of the doses were prepared in triplets after range finding tests had been conducted which determined 1-99% mortality. The ranges of concentrations were chosen to affect quick kill of the fishes. The desired concentrations of different test solutions were obtained by appropriate dilution of the stock solution (APHA 1976). For each set of experiments the calculated volume of stock solution was added to the calculated volume of water so that the final volume of the dose in the aquarium was always five litre in each of the replicates. The dose concentrations were recorded in terms of parts per million (ppm).

Five concentrations of each extract after preliminary screenings were used in the final experiment. In each test, a set of five healthy, active test fishes were released randomly. Each concentration of the relevant toxicant was replicated three times and the test fish were kept for relevant test exposure period i.e., 24 hours. For each set of experiment, a control set was run with equal number of test fishes released in same volume of deep tube well water and replicated. The behaviour of the fishes was noted at the start and at the end of the experiments in the highest dose of each experiment and control. Mortality was counted only for those fishes which were killed within 24 hours after exposure to the various concentrations of the toxicants.

Mortality data of the fishes was subjected to probit analysis (Finney 1971). The regression equation was calculated to see the slope functions of concentration

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and mortality data of an extract. LC₅₀ values with 95% confidence limit were calculated using a computer based probit analysis programme. Chi-square and F-tests were done to determine the result of toxicity on the test fish for each type of extracts. The values of χ^2 was determined and compared with tables of the statistics for (n-1) degree of freedom at 0.05 level of significance level (Fisher and Yates 1963). Analysis of Variance of mortality of fishes against the doses was made to estimate variation among treatments (n-1) and replication (r-1) at 0.01 level of significance level (Chowdhury 1969). Relative potency or toxicity values were calculated on the basis of potency, which is reciprocal of the equitoxic doses. The relative potency of equitoxic toxicants was obtained by taking the highest LC₅₀ value of toxicant as unit and comparing with the respective LC₅₀ values of other toxicants. The comparative analysis amongst the different plant extracts were made in terms of LC₅₀ values of each toxicant.

RESULTS AND DISCUSSION

Behaviour of fish

In control set, fishes exhibited normal behaviour throughout the experiments. They moved freely and swiftly round the aquaria with no abnormal activities. No slime was secreted. There was no change in body colour. Fins and barbels were normal. They showed no signs of respiratory distress. They were morphologically and physically well balanced. There was no mortality in the control experiments.

After exposure to *P. pinnata* seed extracts, the fishes showed vigorous movement within 15 minutes. The fishes became restless, distressed and moved up and down with a few minute intervals. Such movement continued for a while. Later the fishes remained motionless. Their movement slowed down within an hour and gradually relaxed and moved to the bottom. They became motionless and died at different time intervals. Barbels and fins were paralysed. Profuse slime secretion was seen to occur. Dead fishes became stiff and sticky. Most of the dead fishes were seen to float parallelly to the surface water. Some were seen floating at 90° angle.

With *P. pinnata* leaf extracts, most of the cases fish tended to remain aggregated in the corner of the aquaria. They also showed tendency to surfacing and swam actively near the surface. The fish moved up and down to gulp air and became motionless and gradually settled to the bottom of the aquaria. Opercular movement decreased, as a result gulping of air increased. The fish lost balance and barbels straight. Gradually barbels and fins were paralyzed. Exposed fish died

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at different time intervals and were seen floating at various angles to the water surface. The fish that died later settled at the bottom and those that died earlier were seen to be floating. Slime secretion was seen to be occurred.

When the fish were exposed to *P. pinnata* bark extracts, they showed jerking and abnormal movement within 20 minutes. They occasionally moved upward and tried to escape out from the aquaria. They also occasionally moved up towards the water surface for respiring. Gradually the exposed fish became physically imbalanced. After 24 hour of exposure, their skin and eye colour changed and faded. Barbels were more or less straight and paralyzed. Slime secretion was also observed. Some dead fishes were seen floating at 45° angle at the bottom of the aquaria. Haemorrhagic blood was seen around the mouth opening.

After exposing the fish to the seed extracts of *C. viscosum*, at the very beginning they reacted abnormally by exhibiting signs of distressed movement. The first visible reaction was seen within 10 minutes of exposure. They showed surfacing and swam excitedly. At that time the fish moved towards the surface for few minutes intervals. Such rapid movement continued for a while and then they became immobilized at the bottom, became gradually motionless and balanceless. Barbels and fish body straightened. Fins were stupefied, followed by death. Some of the dead fishes floated at the surface with their head up and body down. The fish that died later settled at the bottom and that died earlier floated parallelly to the water surface or at an angle of 90°. Slime secretion was observed to large extent. The eyes of the dead fish were whitish and bulging. The colour of the body was faded. After death, the mouth of the fish was found to be widely open.

On exposure to *C. viscosum* leaf extracts, the fish moved up and down to gulp air but most of the time, they remained immutable and motionless in their position. The first visible reaction started within 20 minutes of exposure. The fish tended to jump out of the aquaria. Opercular movement was rapid. They showed jerking movement. They gradually became inactive and became balanceless. They died at different time intervals. Yellowish slime covered the body. Blood was observed from mouth of some fishes. Mouth remained wide even after death. Dead fishes were found floating at an angle of 45° or 90°.

The first visible reaction of test fish started within 30 minutes of exposure to the bark extracts of *C. viscosum*. Slowly they became motionless and gradually settled to the bottom of the aquaria. The fish became balanceless. Fins and barbels straightened and gradually were paralyzed. This was followed by gradual loss of equilibrium to complete paralysis. Moderate slime secretion was seen to occur on

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the body surface. As time passed fish exhibited sluggish movement. Finally, they were non-reactive to mechanical touch. Dead fish floated at various angles to the water surface or remained at the bottom of the experimental aquaria.

During the experiment on exposure to the extracts containing toxicants the fish showed various abnormalities and stressful behaviour. These behavioural responses before death showed similarities with the findings of Latifa *et al.* (1992, 1997, 2002, 2004) and Nasiruddin *et al.* (1997, 1998, 2006, 2009). The response of the test fish was quick and abrupt immediately after their release into the highest concentration of the extracts. Immediately after their release, fishes showed vigorous movement, tried to escape by jumping out. Different workers have reported their observations about the physical, altered and behavioural changes under toxicant exposure of the fishes in the respective media and have reported that an increase in the dose level caused a decrease in the induction time of total loss of equilibrium in the species (Sylvester 1975, Latifa *et al.* 1987, 1988, 1992, 1997, 2002, Nasiruddin *et al.* 1997, 1998, 2006, 2009, Singh and Singh 2002). Loss of balance and occasional jumping out of the experimental aquaria exhibited due to the nervous reaction of the fish towards the toxicant has also been reported by Adeogun (1994). Increase in slime secretion may reduce the oxygen uptake and cause suffocation in fish (Olaifa *et al.* 2008).

Toxicological effects of the plant parts extracts on mortality of the fishes

The effect of different concentrations of the two plant extracts on mortality of *H. fossilis* was determined during the exposure period of 24 hours (Table 1). It was observed that the toxicants of the different extracts of the two plants at different concentrations caused different percentage of mortality of the test fish. Probit mortality lines for the species with the two plant part extracts of the two experimental plants are shown in Figures- 1A-F. LC₅₀ values and confidence limits of each toxicant for the test fish were determined.

The relative toxicities of the plant part extracts

The LC₅₀ and relative potency values of the distilled water, 50% ethyl alcohol and absolute ethyl alcohol extracts of *P. pinnata* and *C. viscosum* for *H. fossilis* were calculated. The LC₅₀ and relative potency values and range of toxicity of all the extracts of the two plant parts are given in Table 1.

Absolute ethyl alcohol extract of *P. pinnata* seeds was the most toxic extract having a low LC₅₀ value (62.797 ppm) and high relative potency value (18.407). The least toxic extract was the distilled water extract of the *P. pinnata* leaf having a high LC₅₀ value (1155.925 ppm) and low relative potency value

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(1.000). The relative position of the experimental extracts on the basis of their LC₅₀ values were: absolute ethyl alcohol extract of seed > absolute ethyl alcohol extract of bark > 50% ethyl alcohol extract of seed > 50% ethyl alcohol extract of bark > distilled water extract of seed > distilled water extract of bark > absolute ethyl alcohol extract of leaf > 50% ethyl alcohol extract of leaf > distilled water extract of leaf. The order of effectivity of the extracts was: seed > bark > leaf.

In case of *C. viscosum*, amongst the different extracts, distilled water, 50% ethyl alcohol and absolute ethyl alcohol extracts of seeds, and absolute ethyl alcohol extract of bark were toxic having LC₅₀ values of 209.973, 195.204, 114.178 and 283.306 ppm respectively. Distilled water extract of leaf was the less toxic extract (LC₅₀ = 779.042 ppm). The relative position of the extracts on the basis of their LC₅₀ values i.e., toxicities were: absolute ethyl alcohol extract of seed > 50% ethyl alcohol extract of seed > distilled water extract of seed > absolute ethyl alcohol extract of bark > 50% ethyl alcohol extract of bark > absolute ethyl alcohol extract of leaf > 50% ethyl alcohol extract of leaf > distilled water extract of bark > distilled water extract of leaf. The order of effectivity of toxicity of the extracts was; seed > bark > leaf.

TABLE 1. TOXICITY PARAMETERS OF THE DEFERENT EXTRACTS OF *PONGAMIA PINNATA* AND *CLERODENDRUM VISCOSUM* SEED, LEAF AND BARK ON *HETEROPNEUSTES FOSSILIS* AFTER 24 HOURS EXPOSURE.

Plant Parts	Solvent	Dose Range (ppm)	Mortality range	χ^2 Value	P Value (χ^2 at 5%)	F Value	P Value (F-test) at 1%	Estimated regression equation	LC ₅₀ (ppm)	Confidence limit		Relative potency	Range of toxicity*
										Lower	Upper		
<i>P. pinnata</i> (Seed)	Distilled water	100-1000	20.00-93.33	19.574	p<0.05	35.50	p<0.01	-0.4927+2.1873x	326.956	209.841	462.047	18.407	Most toxic
	50% ethyl alcohol	50-750	13.33-93.33	45.043	p<0.05	37.23	p<0.01	3.0307+0.8742x	202.622	132.614	299.860	5.704	Toxic
	Absolute ethyl alcohol	25-500	26.67-93.33	1.189	p>0.05	22.43	p<0.01	2.2896+1.5101x	62.797	31.518	101.287	3.535	Moderately toxic
<i>P. pinnata</i> (Leaf)	Distilled water	750-1750	13.33-86.67	0.953	p>0.05	19.47	p<0.01	-12.608+5.7501x	1155.925	992.214	1319.671	1.977	Fairly toxic
	50% ethyl alcohol	500-1500	20.00-93.33	9.558	p<0.05	71.00	p<0.01	-8.6291+4.6483x	852.809	699.189	999.826	1.355	Less toxic
	Absolute ethyl alcohol	250-1250	13.33-93.33	9.270	p>0.05	76.00	p<0.01	-4.5160+3.4376x	584.648	443.278	727.125	1.000	Least toxic
<i>P. pinnata</i> (Bark)	Distilled water	200-1000	26.67-86.67	7.061	p>0.05	49.00	p<0.01	-1.6029+2.5211x	415.974	269.142	552.325	6.881	Toxic
	50% ethyl alcohol	100-800	20.00-93.33	9.864	p<0.05	30.50	p<0.01	-0.4675+2.2492x	273.202	176.973	383.837	4.231	Toxic
	Absolute ethyl alcohol	50-600	13.33-86.67	7.707	p>0.05	23.12	p<0.01	0.7680+1.90215x	167.979	106.118	252.453	2.778	Moderately toxic
<i>C. viscosum</i> (Seed)	Distilled water	100-500	26.67-93.33	19.214	p<0.05	31.27	p<0.01	-1.3404+2.7337x	209.973	141.698	274.104	6.823	Toxic
	50% ethyl alcohol	50-400	13.33-86.67	16.020	p<0.05	26.72	p<0.01	-0.0226+2.1947x	195.204	136.678	289.649	3.990	Toxic
	Absolute ethyl alcohol	25-300	13.33-86.67	8.398	p>0.05	34.00	p<0.01	1.1701+1.8640x	114.178	75.510	182.045	3.710	Toxic

TABLE 1 (Contd.)
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C. <i>viscosu</i> <i>m</i> (Leaf)	Distilled water	250-1250	20.00-80.00	7.187	p>0.05	34.85	p<0.01	-1.3363+2.2288x	699.141	484.248	1032.930	1.717	Moderately toxic
	50% ethyl alcohol	100-1000	13.33-86.67	5.923	p>0.05	30.5	p<0.01	-0.3644+2.0669x	396.347	260.365	579.630	1.484	Fairly toxic
	Absolute ethyl alcohol	100-800	20.00-86.67	6.076	p>0.05	39.25	p<0.01	-0.5237+2.2509x	283.306	177.382	411.034	1.000	Less toxic
C. <i>viscosu</i> <i>m</i> (Bark)	Distilled water	500-1500	26.67-93.33	6.344	p>0.05	26.36	p<0.01	-6.0101+3.8114x	779.042	581.781	940.920	2.749	Toxic
	50% ethyl alcohol	250-1250	26.67-93.33	40.386	p<0.05	31.27	p<0.01	-2.2647+2.6766x	524.934	354.246	685.260	1.965	Moderately toxic
	Absolute ethyl alcohol	200-1000	20.00-86.67	3.327	p>0.05	31.59	p<0.01	-2.3541+2.7724x	453.613	314.964	594.467	1.114	Fairly toxic

*Range of toxicity: On the basis of LC₅₀ values

1. Most toxic 1 < 100 ppm
2. Toxic 100 < 300 ppm
3. Moderately toxic 300 < 500 ppm
4. Fairly toxic 500 < 700 ppm
5. Less toxic 700 < 900 ppm
6. Least toxic 900 < 1200 ppm

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All the extracts were more or less toxic with variations in concentrations. Due to the effects of different concentrations mortality rate of the fishes varied. In the present study in terms of concentration, the toxicities of the plant parts of *P. pinnata* followed the pattern: seed > bark > leaf and that for *C. viscosum* was: seed > leaf > bark. But in terms of LC₅₀ values the order of toxicity for both the plant parts was: seed > bark > leaf. As to concentration ranges the effects of seed and leaf extracts was greater with *C. viscosum* extracts than *P. pinnata* extracts but opposite for bark extracts. Chi-square values showing insignificant values indicated a good relationship between observed and expected mortalities. Analysis of data made between mortality and concentrations showed that most of the analyses were significant at 0.01 level which again indicated a good relationship between the concentrations used and mortalities obtained.

Along with the many medicinal applications of these plants such as remedy for skin diseases, useful in diseases of eye, good for tumours, piles, wounds, ulcers, urinary discharges, remedy of diabetes, increases appetite, lessens expectoration, useful in inflammations, bronchitis, asthma, etc. (Joshi 2000), these plant part extracts can be used as fish poison and might be helpful as controlling agent of undesirable fish species in the nursery, rearing and stocking ponds of a fish culture farm. From the present study it has appeared that the effectiveness of *P. pinnata* and *C. viscosum* plant parts (dry seed, leaf and bark) extracts seemed to be promising in different concentration levels. The present study was mainly directed at determining the ichthyotoxicity of *P. pinnata* and *C. viscosum* plant part extracts on *H. fossilis* and their prospect as fish toxicant. The toxicity may vary with other fish species.

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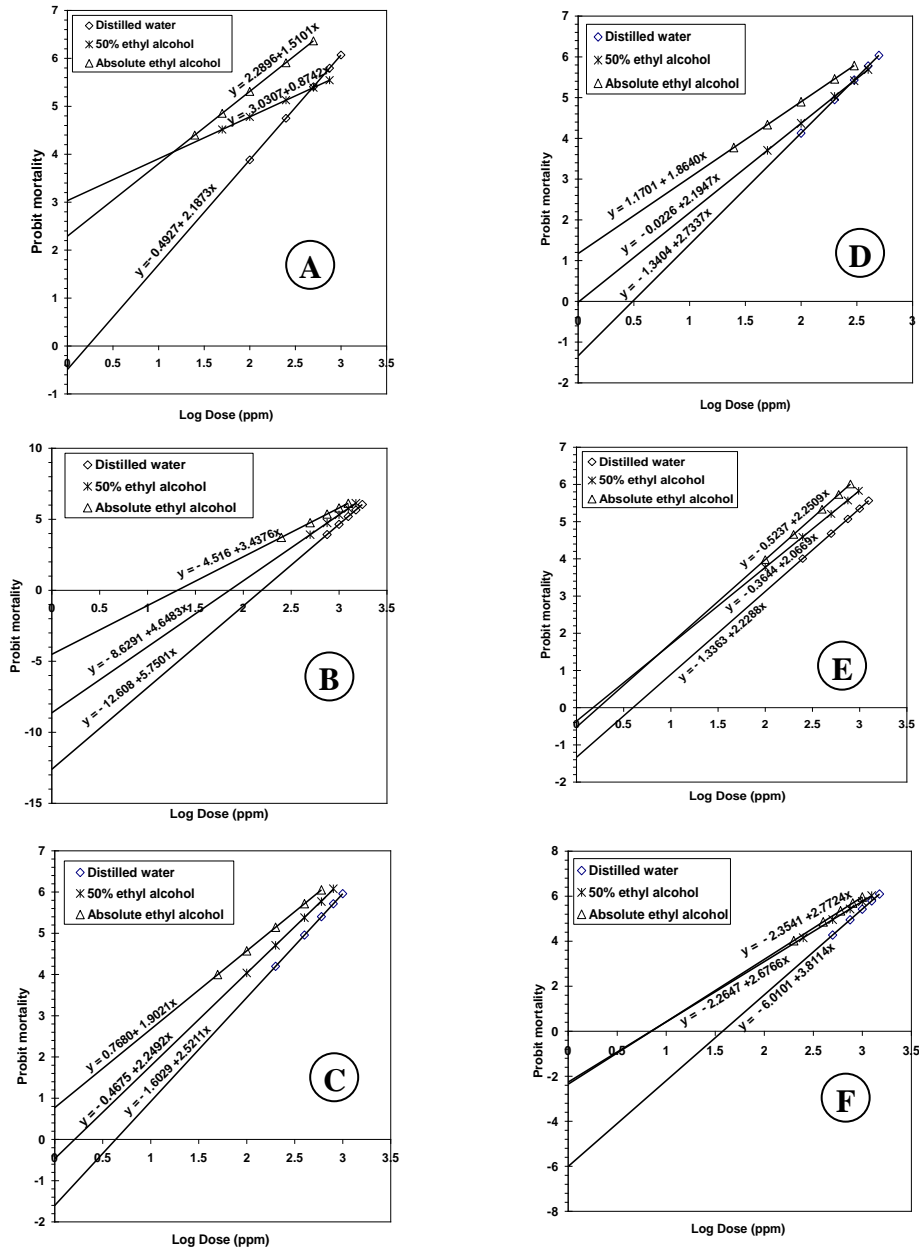


FIGURE 1. REGRESSION LINES FOR DETERMINING THE LC₅₀ VALUES OF DISTILLED WATER, 50% ETHYL ALCOHOL AND ABSOLUTE ETHYL ALCOHOL EXTRACTS OF *PONGAMIA PINNATA* SEED (A), LEAF (B), BARK (C) AND *CLERODENDRUM VISCOSUM* SEED (D), LEAF (E) AND BARK (F) TO *HETEROPNEUSTES FOSSILIS* AFTER 24 HOURS OF EXPOSURE.

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