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Toxic effects of *Jatropha gossypifolia* and its binary and tertiary combinations with other plant molluscicides in natural ponds

Efectos tóxicos de *Jatropha gossypifolia* y de sus combinaciones binarias y terciarias con otros molusquicidas vegetales en estanques naturales

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ABSTRACT

The toxic effect of leaf, stem-bark and latex of *Jatropha gossypifolia* against *Lymnaea acuminata* and *Indoplanorbis exustus* was studied in natural ponds. Toxicity of binary (1:1) and tertiary (1:1:1) combinations of the rutin (*Croton tiglium*), ellagic acid (*Euphorbia hirta*), betulin (*Euphorbia lathyrus*) and taraxerol extracted from stem bark (*Codiaeum variegatum*) with *Jatropha gossypifolia* latex powder were studied against freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus* in ponds. Combinations of *Jatropha gossypifolia* latex powder with Rutin of *Croton tiglium*, Ellagic acids of *Euphorbia hirta* and Betulin of *Euphorbia lathyrus* were more toxic than their individual components and other combinations.

RESUMEN

Se estudió el efecto tóxico de la hoja, de la corteza del tallo y del látex de *Jatropha gossypifolia* sobre *Lymnaea acuminata* y *Indoplanorbis exustus* en estanques naturales. Se estudió la toxicidad de combinaciones binarias (1:1) y terciarias (1:1:1) de la rutina (*Croton tiglium*), del ácido elágico (*Euphorbia hirta*), de la betulina (*Euphorbia lathyrus*) y del taraxerol extraido de la corteza del tallo (*Codiaeum variegatum*) con látex en polvo de *Jatropha gossypifolia* sobre los caracoles de agua dulce *Lymnaea acuminata* y *Indoplanorbis exustus* en estanques. Combinaciones de polvo de látex de *Jatropha gossypifolia* con rutina de *Croton tiglium,* con ácido elágico de *Euphorbia hirta* y con betulina de *Euphorbia lathyrus* resultaron más tóxicas que sus componentes aislados o que otras combinaciones.

KEY WORDS: Toxicity, snail, Compounds and Fascioliasis.
PALABRAS CLAVE: Toxicidad, caracoles, compuestos, Fascioliasis.

INTRODUCTION

Snails are well known carriers of diseases and vectors of pests. A large number of snails inhabits freshwater, where the larvae of parasitic trematodes also pass part of their life. Many aquatic snails act as vectors for the larvae of trematodes and thereby cause a number of diseases. Two diseases carried by

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aquatic snails, fascioliasis and schistosomiasis cause immense harm to man and his domestic animals.

Fasciola hepatica and *Fasciola gigantica* are the causative agents of endemic fascioliasis in eastern Uttar Pradesh. Both flukes are transmitted by the vector snails *Lymnaea acuminata* and *Indoplanorbis exustus* (HYMAN, 1970; SINGH AND AGARWAL, 1981; AGARWAL AND SINGH, 1988; SINGH, SINGH, MISHRA AND AGARWAL, 1996). The problem is particularly severe in northern parts of Uttar Pradesh. One of the obvious solutions is to destroy the vector snails and remove a link in their life-cycle (GODAN, 1983; SINGH *ET AL.* 1996). Synthetic molluscicides, though extensively used for control of pests, are a serious environmental hazard (REIDINGER, 1976 SINGH AND AGARWAL, 1981; TRIPATHI AND SINGH, 2004). Molluscicides of plant origin are gaining greater acceptance amongst users as they have greater biodegradability and cost less than synthetic molluscicides (MARSTON AND HOSTETTMANN, 1985; SINGH *ET AL.* 1996).

The molluscicidal activity of *Croton tiglium, Codiaeum variegatum, Jatropha gossypifolia* and *Euphorbia hirta* in the laboratory has been established (YADAV AND SINGH, 2001; SINGH AND AGARWAL, 1988; SINGH, YADAV, TIWARI AND SINGH, 2005).

The present study deals with the use of *Jatropha gossypifolia* as a molluscicidal agent, isolated and in binary and tertiary combinations with different active compound such as rutin of *Croton tiglium,* ellagic acids of *Euphorbia hirta,* taraxerol of *Codiaeum variegatum* and betulin of *Euphorbia lathyrus* against freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus* in ponds.

MATERIALS AND METHODS

Leaf, stem bark and latex of *Jatropha gossypifolia* were collected locally from their natural habitat and identified by the Botany Department, University of Gorakhpur (U.P) India. Leaf and stem bark of *Jatropha gossypifolia* were kept in an incubator at 37° C for 48h. Dried pieces of leaf and stem bark, were pulverized in a grinder. The latex of *Jatropha gossypifolia* was drained into glass tubes by cutting their stem apices. This latex was lyophilized at -40° C and the lyophilized powder was stored for further use. The freeze-dried powder was mixed with an appropriate volume of distilled water to obtain the desired concentration. The crude powder so obtained was used for the toxicity testing of doses in different concentrations used in natural ponds (Table I).

Rutin ($C_{27}H_{30}O_{16}$) (EC NO-205-814-1), ellagic acid ($C_{14}O_6O_8$) (4,4,5,5,6,6-hexahydroxydiphenic acid, 2,6,2,6-dilactone) (EC NO-207-508-3), and betulin ($C_{30}H_{50}O_2$) (Lup-20(2a)-ene-3β28-diol) (EC NO-207-475-6) were supplied by Sigma Chemical Co. P.O. Box 14508 St. Louis. Mo.63178 USA. Rutin is obtained from leaves of *Croton tiglium* (ICMR, 1990a), ellagic acid is found in flowers of *Euphorbia hirta* (GUPTA AND GARG, 1966) and betulin is found in the stem bark of *Euphorbia lathyrus* (ICMR, 1990b). Taraxerol is extracted from the stem-bark of *Codiaeum variegatum* (YADAV, TIWARI AND SINGH, 2005) and latex of *Jatropha gossypifolia* was prepared using the method of SINGH, SINGH AND SINGH, (1995). Different binary (1:1) and tertiary (1:1:1) combinations were prepared using lyophilized powder of *Jatropha gossypifolia* with rutin, ellagic acid, taraxerol and betulin.

Adult *Lymnaea acuminata* (2.6±0.3 cm in shell height) and *Indoplanorbis exustus* (0.87±0.035 cm in shell height) were collected from Ramgarh Lake of Gorakhpur district and acclimatized to laboratory conditions for 72h. Toxicity experiments were performed using the method of SINGH AND AGARWAL, (1988).

The experiment was conducted in freshwater ponds, 29.28 m^2 in area and 9.19 m^3 in water volume. Each pond was stocked with 100 snails with a size difference not greater than 1.5 times (APHA, 1992). The experimental animals were exposed continuously for 96h to four different concentrations and each set of experiments was replicated six times. Control groups were kept under similar conditions without any treatment. Water

Table I. Concentrations used for the toxicity testing of different parts of *Jatropha gossypifolia* and different combinations (1:1) of *Jatropha gossypifolia* latex with rutin, ellagic acids and taraxerol against *L. acuminata* and *I. exustus* and their tertiary combinations (1:1:1) with betulin against *L. acuminata*.
Tabla I. Concentraciones usadas para el ensayo de toxicidad de distintas partes de Jatropha gossypifolia, *de distintas combinaciones (1:1) del látex de* Jatropha gossypifolia *con rutina, ácido elágico y taraxerol sobre* L. acuminata *y* I. exustus, *así como de sus combimaciones terciarias (1:1:1) con betulina sobre* L. acuminata.

Treatments	Concentration used (mg/L)	
	Lymnaea acuminata	*Indoplanorbis exustus*
J. gossypifolia latex	5.00, 7.00, 9.00, 9.50	7.00, 9.00, 11.00, 13.00
J. gossypifolia Stem-bark	7.00, 8.00, 9.00, 10.50	8.00, 10.00, 12.00, 14.00
J. gossypifolia leaf	9.0, 10.50, 12.00, 13.00	12.00, 14.00, 16.00, 18.00
J. gossypifolia latex + rutin	0.5, 0.8, 1.5, 2.0	1.5, 3.5, 5.5, 7.5
J. gossypifolia latex + ellagic acids	2.00, 3.00, 4.00, 5.00	3.00, 4.00, 5.00, 7.00
J. gossypifolia latex + taraxerol	5.00, 7.00, 9.00, 11.00	9.00, 11.00, 13.00, 15.00
J. gossypifolia latex + rutin + betulin	0.5, 0.9, 1.3, 1.8	
J. gossypifolia latex + ellagic acids + betulin	0.9, 1.2, 1.5, 1.7	
J. gossypifolia latex + taraxerol + betulin	2.00, 2.50, 3.00, 3.50	

analysis for various physico-chemical parameters, viz. temperature, pH, dissolved O_2, total ammonia, free CO_2 and total alkalinity were carried out every week. Water temperature ranged from 27.4-28.6° C. The other parameters were within the following ranges: total alkalinity 43-62 ppm, pH 6.8-7.7, dissolved oxygen 7.8-10.3 mg/L, total ammonia 0.29-1.59 µg at N/I.

Mortality was recorded at 24h intervals up to 96h. Lethal concentrations (LC_{50}) values, upper and lower confidence limits (UCL, LCL) and slope values were calculated by the probit log method using POLO computer programme of RUSSELL, ROBERTSON AND SEVIN, (1977). The regression coefficient was determined between exposure time and different values of LC_{50} (SOKAL AND ROHLF, 1973).

RESULTS

The toxicity of the LC_{50} values of extracts of latex, stem bark and leaf of *Jatropha gossypifolia* for periods ranging from 24h to 96h for freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus* are given in (Table II). Toxicity against both freshwater snail species was time as well as dose dependent. There was a significant negative correlation between LC_{50} values and exposure time (II-IV). Thus with an increase in exposure time, the LC_{50} of *Jatropha gossypifolia* latex decreased from 10.32 mg/L (24h);> 8.61 mg/L (48h);> 6.67 mg/L (72h);> to 5.66 mg/L (96h) against *Lymnaea acuminata* and 10.69 mg/L (24h);> 9.60 mg/L (48h);> 8.73 mg/L (72h);> to 8.31 mg/L (96h) in the case of *Indoplanorbis exustus*. The same trend of toxicity was observed in the case of stem bark and leaf of *Jatropha gossypifolia* against both the snails for all the exposure periods (Table II).

Binary combinations (1:1) of *Jatropha gossypifolia* latex with Rutin of *Croton tiglium*, Ellagic acid of *Euphorbia hirta* and taraxerol of *Codiaeum variegatum* were more toxic for *L. acuminata* and *I. exustus* than single treatment for all the exposure periods (Table III.) Toxicity (24h) of *J. gossypifolia* latex + rutin against *L. acuminata* (LC_{50}=1.36 mg/L) and *I. exustus* (LC_{50}=4.57 mg/L) was highest (Table III) and *J. gossypifolia* latex + ellagic acid against *L. acuminata* (LC_{50}=5.05 mg/L) and *I. exustus* is

Table II. Toxicity of latex, stem-bark and leaf of *Jatropha gossypifolia* against *Lymnaea acuminata* and *Indoplanorbis exustus* at different time exposure periods.
Concentrations given are the final concentrations (mg/L) in natural ponds. t-ratio was more than 1.96. The heterogeneity factor was less than 1.0. The g-values were less than 0.5. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments. ts, testing significance of the regression coefficient-latex, stem bark and leaf extracts -0.9926**; -0.97214*; -0.87392* in *L. acuminata* and -0.98311*; -0.99969**; -0.99889* in *I. exustus* (*, Linear regression between x and y. **, Non-linear regression between log x and log y).
Tabla II. Toxicidad del látex, de la corteza del tallo y de la hoja de Jatropha gossypifolia *sobre* Lymnaea acuminata *y* Indoplanorbis exustus *con distintos tiempos de exposición.*
Las concentraciones indicadas son las concentraciones finales (mg/L) en estanques naturales. La t*-ratio fue superior a 1,96. El factor de heterogeneidad fue inferior a 1,0. Los valores de* g *fueron menos de 0,5. Una regresión negativa significativa (P<0.05) se observó entre el tiempo de exposición y el* LC_{50} *de los tratamientos. ts, test de significación del coeficiente de regresión - látex, corteza del tallo y extractos de hojas -0.9926**; -0.97214*; -0.87392* en* L. acuminata *y -0.98311*; -0.99969**; -0.99889* en* I. exustus (*, *Regresión linear entre* x *e* y. **, *Regresión no-linear entre* log x *y* log y).

Hours	Plant extracts	*Lymnaea acuminata* LC_{50} (95% confidence limits)	*Lymnaea acuminata* Slope value	*Indoplanorbis exustus* LC_{50} (95% confidence limits)	*Indoplanorbis exustus* Slope value
24h	Latex	LC_{50}=10.32 (14.53-23.17)	2.11±0.277	LC_{50}=10.69(10.318-11.135)	6.375±0.298
	Stem bark	LC_{50}=12.66 (11.80-14.03)	4.224±0.445	LC_{50}=13.73 (13.066-14.707)	7.207±0.380
	Leaf	LC_{50}=24.60 (19.73-39.95)	2.629±0.508	LC_{50}=17.32 (16.112-17.866)	10.99±0.532
48h	Latex	LC_{50}=8.61 (8.263-9.043)	4.458±0.261	LC_{50}=9.60 (9.286-9.932)	6.999±0.289
	Stem bark	LC_{50}=10.39 (10.10-10.78)	6.11±0.434	LC_{50}=12.31 (11.707-13.118)	5.927-0.320
	Leaf	LC_{50}=13.35 (12.91-13.94)	6.800±0.484	LC_{50}=16.40 (16.290-16.758)	10.46±0.481
72h	Latex	LC_{50}=6.67 (6.298-7.026)	5.347±0.693	LC_{50}=8.73 (8.328-9.115)	8.354±0.324
	Stem bark	LC_{50}=8.78 (8.634-8.951)	6.817±0.419	LC_{50}=11.01 (10.356-11.765)	4.759±0.299
	Leaf	LC_{50}=11.67 (11.47-11.90)	6.859±0.450	LC_{50}=15.22 (14.938-15.535)	9.174±0.437
96h	Latex	LC_{50}=5.66 (5.350-5.936)	5.412±0.262	LC_{50}=8.31 (7.773-8.776)	7.110±0.310
	Stem bark	LC_{50}=8.14 (7.997-8.279)	9.058±0.442	LC_{50}=9.52 (8.981-9.984)	5.210±0.304
	Leaf	LC_{50}=10.55 (10.29-10.79)	11.79±0.489	LC_{50}=14.26 (14.049-14.478)	8.378±0.427

LC_{50}=6.19 mg/L). The same trend was also observed in *J. gossypifolia* latex+taraxerol against freshwater snails *L. acuminata* and *I. exustus*. The order of toxicity of binary combinations against both the snails were *J. gossypifolia* + rutin > *J. gossypifolia* latex + ellagic acid > *J. gossypifolia* latex + taraxerol (Table III).

Regarding tertiary combinations (1:1:1) of *J. gossypifolia* latex powder with taraxerol, rutin, ellagic acid and betulin against *L. acuminata*, the toxicity (24h) of *J. gossypifolia* latex + ellagic acid + betulin (LC_{50}=1.70 mg/L) was highest, as shown in Table IV. The order of toxicity of various tertiary combinations against *L. acuminata* was *J. gossypifolia* latex + ellagic acid + betulin> *J. gossypifolia* latex + taraxerol + betulin> *J. gossypifolia* latex + rutin + betulin (Table IV).

The slope values given in the toxicity tables were steep and the heterogeneity factor was less than 1.0, which indicates that the results found fall within the 95% confidence limits of LC values. The regression test ('t' ratio) was greater than 1.96 and the potency estimation test ('g' value) was less than 0.5 at all probability levels.

DISCUSSION

It is evident from the results that *Jatropha gossypifolia* leaf, stem bark and latex are toxic against both freshwater

Table III. Toxicity of binary combinations (1:1) of *Jatropha gossypifolia* latex with rutin, ellagic acid and taraxerol against *Lymnaea acuminata* and *Indoplanorbis exustus* at different time exposure periods.
Concentrations given are the final concentrations (mg/L) in natural ponds. t-ratio was more than 1.96. The heterogeneity factor was less than 1.0. The g-values were less than 0.5. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments. ts, testing significance of the regression coefficient - of extracts *J. gossypifolia* latex + rutin; *J. gossypifolia* latex + ellagic acid; *J. gossypifolia* latex + taraxerol in *L. acuminata* -0.97986*; -0.99964*; -0.99715* and -0.98576**; -0.99952*; -0.99967* in *I. exustus* (*, Linear regression between x and y. **, Non-linear regression between log x and log y).
Tabla III. Toxicidad de combinaciones binarias (1:1) de látex de Jatropha gossypifolia *con rutina, ácido elágico y taraxerol sobre* Lymnaea acuminata *y* Indoplanorbis exustus *con distintos tiempos de exposición.*
Las concentraciones indicadas son las concentraciones finales (mg/L) en estanques naturales. La t-ratio *fue superior a 1,96. El factor de heterogeneidad fue inferior a 1,0. Los valores de* g *fueron menos de 0,5. Una regresión negativa significativa (P<0.05) se observó entre el tiempo de exposición y el* LC_{50} *de los tratamientos. ts, test de significación del coeficiente de regresión - de extractos de látex de* J. gossypifolia + *rutina; látex de* J. gossypifolia + *ácido elágico; látex de* J. gossypifolia + *taraxerol en* L. acuminata -0.97986*; -0.99964*; -0.99715* and -0.98576**; -0.99952*; -0.99967* *en* I. exustus (*, *Regresión linear entre* x *e* y. **, *Regresión no-linear entre* log x *y* log y).

Hours	Plant extract + Compounds	*Lymnaea acuminata*		*Indoplanorbis exustus*	
		LC_{50} (95% confidence limits)	Slope value	LC_{50} (95% confidence limits)	Slope value
24h	*J.gossypifolia* latex+rutin	1.36(1.248-1.520)	2.512±0.124	4.57(4.068-5.203)	2.456±0.115
	J.gossypifolia latex+ellagic acid	5.05(4.627-5.732)	4.331±0.240	6.19(5.708-6.936)	3.398±0.211
	J.gossypifolia latex+taraxerol	10.02(9.584-10.57)	5.984±0.283	14.18(13.841-14.600)	6.849±0.369
48h	*J.gossypifolia* latex+rutin	1.05(0.985-1.151)	2.659±0.122	3.47(3.138-3.813)	2.622±0.113
	J.gossypifolia latex+ellagic acid	4.26(3.935-4.732)	3.500±0.190	5.26(4.857-5.821)	3.718±0.208
	J.gossypifolia latex + taraxerol	9.17(8.843-9.562)	5.529±0.252	13.00(12.753-13.289)	7.403±0.358
72h	*J.gossypifolia* latex + rutin	0.85(0.762-0.938)	2.899±0.125	2.76(2.487-3.079)	2.762±0.113
	J.gossypifolia latex + ellagic acid	3.55(3.232-3.938)	2.980±0.184	4.39(3.996-4.809)	3.495±0.204
	J.gossypifolia latex + taraxerol	7.90(7.666-8.157)	5.109±0.230	11.82(11.556-12.103)	7.447±0.347
96h	*J.gossypifolia* latex + rutin	0.73(0.618-0.837)	2.816±0.127	2.24(1.821-2.636)	2.475±0.113
	J.gossypifolia latex + ellagic acid	2.84(2.644-3.030)	3.342±0.185	3.58(3.253-3.871)	3.962±0.216
	J.gossypifolia latex + taraxerol	6.98(6.797-7.175)	4.772±0.224	10.76(10.474-11.041)	7.631±0.352

snails *Lymnaea acuminata* and *Indoplanorbis exustus*. Toxicity of *J. gossypifolia* latex is highest in comparison to leaf and stem bark (Table II).

The latex of *Jatropha gossypifolia* can be used as a potential source of molluscicides as the crude preparation of the latex has sufficient time dependent molluscicidal activity. Molluscicidal activity can be increased several times when mixed with other plant-derived molluscicides such as rutin from *Croton tiglium*, ellagic acid from *Euphorbia hirta* and taraxerol from *Codiaeum variegatum* (ICMR, 1990a; GUPTA AND GARG, 1966; ICMR, 1990b; YADAV ET AL. 2005). The highest increase in the toxicity was observed when *J. gossypifolia* latex + Rutin (1:1) were tested against both the freshwater snail *Lymnaea acuminata* and *Indoplanorbis exustus*. There was an increase of 7.6 times and 2.34 times in 24h toxicity against *L. acuminata* and *I. exustus*, respectively, compared to the single treatment with *J. gossypifolia* latex preparation alone. The tertiary combina-

Table IV. Toxicity of tertiary combinations (1:1:1) of *Jatropha gossypifolia* latex with rutin, taraxerol, ellagic acid and betulin against *Lymnaea acuminata* at different time exposure periods. Concentrations given are the final concentrations (mg/L) in natural ponds. t-ratio was more than 1.96. The heterogeneity factor was less than 1.0. The g-values were less than 0.5. Significant negative regression (P<0.05) was observed between exposure time and LC_{50} of treatments. ts, testing significance of the regression coefficient- of extracts *J. gossypifolia* latex+rutin+betulin -0.98631*; *J. gossypifolia* latex+ellagic acid+betulin -0.99945**; *J. gossypifolia* latex+taraxerol+betulin -0.99927*. (*, Linear regression between x and y. **, Non-linear regression between log x and log y).
Tabla IV. Toxicidad de combinaciones terciarias (1:1:1) de látex de Jatropha gossypifolia *con rutina, taraxerol, ácido elágico y betulina sobre* Lymnaea acuminata *con distintos tiempos de exposición. Las concentraciones indicadas son las concentraciones finales (mg/L) en estanques naturales. La* t-ratio *fue superior a 1,96. El factor de heterogeneidad fue inferior a 1,0. Los valores de* g *fueron menos de 0,5. Se observó una regresión negativa significativa (P<0,05) entre el tiempo de exposición y el* LC_{50} *de los tratamientos. ts, test de significación del coeficiente de regresión - de extractos de látex de* J. gossypifolia*+rutina+betulina -0,98631*; látex de* J. gossypifolia*+ácido elágico+betulina -0.99945**; látex de* J. gossypifolia*+taraxerol+betulina -0,99927** (*, Regresión linear entre* x *e* y. ***, Regresión no-linear entre* log x *y* log y).

Hours	Plant extract + Compounds	*Lymnaea acuminata* LC_{50} (95%confidence limits)	Slope value
24h	*J.gossypifolia* latex + rutin + betulin	6.12 (5.948-6.310)	7.452±0.351
	J.gossypifolia latex + ellagic acid + betulin	1.70 (1.599-1.855)	4.788±0.293
	J.gossypifolia latex + taraxerol + betulin	3.24 (3.150-3.364)	6.250±0.334
48h	*J. gossypifolia* latex + rutin + betulin	5.59 (5.434-5.740)	8.135±0.356
	J. gossypifolia latex + ellagic acid + betulin	1.46 (1.389-1.556)	5.430±0.282
	J.gossypifolia latex + taraxerol + betulin	2.99 (2.922-3.074)	6.691±0.327
72h	*J. gossypifolia* latex + rutin + betulin	5.28 (5.092-5.462)	10.26±0.401
	J. gossypifolia latex + ellagic acid + betulin	1.26 (1.169-1.352)	4.345±0.259
	J.gossypifolia latex + taraxerol + betulin	2.69 (2.621-2.761)	6.696±0.315
96h	*J. gossypifolia* latex + rutin + betulin	5.01 (4.785-5.212)	9.097±0.395
	J. gossypifolia latex + ellagic acid + betulin	1.03 (0.943-1.14)	4.263±0.261
	J.gossypifolia latex + taraxerol + betulin	2.40 (2.312-2.498)	8.461±0.346

tions (1:1:1) of *J. gossypifolia* latex + ellagic acid + betulin showed a potent effect compared to other combinations (Table IV).

The increase in mortality with increased exposure periods could be affected by several factors, which may be acting separately or jointly. For example, uptake of active moiety is time dependent, which leads to a progressive increase in the entrance of the drug and its effects on the snail body (SINGH AND AGARWAL, 1988; 1993a; 1993b). Stability (life span) of active moiety of pesticides in the environment and the rate of their detoxification in animal bodies also alter the relationship of exposure periods to mortality (MITRA, SUD AND MITRA, 1978; KOUNDINYA AND RAMAMMURTHY, 1979; MATSUMURA, 1985).

Statistical analysis of the data on toxicity brings out several important points. The χ^2 test for goodness of fit (Heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance etc. do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep slope values. The steepness of the slope line indicates that there is a large increase in the mortality of snails with a relatively small increase in the concentration of the toxicant. The slope is, thus, an index of the susceptibility of the target animal to the pesticides used. A steep slope is also indicative of rapid absorption and onset of effects. Even though the slope alone is not a very reliable indicator of the toxicological mechanism, yet it is a useful parameter for such a study. Since the LC_{50} of the latices of euphorbial lay within the 95% confidence limits, it is obvious that in replicate tests of random samples, the concentration response lines would fall within the same range (RAND AND PETROCELLI, 1988).

In conclusion, it may be stated that binary combinations of *Jatropha gossypifolia* with other common plant products can be used in natural ponds to control the population of vector snails. These binary combinations can enhance the efficiency and reduce the doses of plant derived molluscicides, so that the areas of treated water will be environmentally safe. For proper utilization of plant product combinations as molluscicides further studies are however, necessary, to elucidate the mechanism of action in the snail body.

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