

EVALUATION OF PISCICIDAL ACTIVITY OF PLANT *ASCLEPIAS CURASSAVICA* LINN (FAM. ASCLEPIADACEAE).

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ABSTRACT

Milkweed, *Asclepias curassavica* belong to family Asclepiadaceae is evaluated for its piscicidal activity. Aqueous and methenolic extract of leaves were tested on fish *Poicellia reticulata*. They showed promising toxic activity. LC₅₀ obtained at different time intervals are 7.42 mg/lit (24hr.), 9.31 mg/lit (48hr.) and 11.22 mg/lit (72hr.) for aqueous extract. Where as for methanolic extract, it is 4.32 mg/lit (24hr.), 6.14 mg/lit (48hr.) and 8.42 mg/lit (72hr.). This plant has a great potential to be evaluated for its toxicity on various pest. Further studies will reveal the toxic principles and their biological activities.

KEY WORDS:

Introduction:

Asclepias curassavica is one of Various plants from Asclepiadaceae family passes very high medicinal properties. The plant is used medicinally by various tribes in the tropics for the insipidus properties of its roots. *Asclepias curassavica* is known as milk weed. Taxonomical information of this plant is mentioned in below Table 1 (Stone, 1970).

Table 1: Taxonomical classification of *Asclepias curassavica*

Kingdom	Plantae
Division	Angiosperms
Order	Gentianales
Family	Asclepiadaceae
Genus	<i>Asclepias</i>
Species	<i>curassavica</i>

The milkweed produce white poisonous sap from which it is named. it grow to be 2 to 3 ½ feet herb. Leaves opposite, oblong-lanceolate, acute at both ends, 6-15 cm long, 1-3 cm wide; cymes on peduncles 3-6 cm long, 4-15-flowered; flowers bright red-and yellow; calyx-lobes to 4mm long; corolla to 9 mm long; corona-scales to 4mm long; stamens tube to 3 mm long; follicles ovoid-oblong, 5-7.5 cm long; (Stone, 1970). Various literatures have demonstrated numerous ethnomedical uses of *Asclepias curassavica* in different countries. As per the published reports, different parts of the plant have got different ethno-medicinal indications worldwide (Raja S. and R. Koduru 2014) In India, the hot water extract of entire plant is used as a fish poison, cathartic (humans), and emetic (Gupta *et al.*, 1971). The fresh petals are used as eye drops for chronic eye diseases (Jhon 1984). The root was used as an emetic (Duke 1994). and anti diarrhoeal agent. The plant is also reported to be used as a poultice for ringworm Oakes and Morris 1958). The hot water extract of dried leaves are used as an anthelmintic (Mitra *et al.*, 1974). The plants that shows fish poisonous property are called as piscicidal plants. They stun fish when it passes through the gills or in some cases ingested. The fish then floats to the surface for easy capture. This is a very common practice all over the world hence variety of indigenous plants are used by local tribes for this purpose.

The presence of trash and weed fishes in cultured pond is a serious problem in freshwater aquaculture. These fishes compete for the food and habitat with major cultivated carps. *P. reticulata* is the common weed fish which have low food value. They quickly escape in netting so, difficult to get rid of in persistent water reservoirs which cannot be dried. they engulf the fry and food of cultured carp at several stages of their rearing (Jhingran, 1975), thus create nuisance in cultured carp production and put a great loss to the fisherman. For eliminating unwanted population of such weed fishes from cultured ponds, fish farmers made several efforts, by use of synthetic chemical Agro-pesticides (Marking, 1992). Due to their broad spectrum action and bioaccumulation and persistence nature, they adversely affect both the quality of fish and their consumer (Cullen and Connell, 1992). A Safe alternative for these harmful synthetic agro-biocides is environmentally safe, plant originated piscicides which are less expensive, biodegradable, readily available, easy to handle and safe to mankind and environment (Marston and Hostettmann, 1985; Singh *et al.*, 2010). A large diversity of

biocidal plants belonging to different families (Kulakkattolickal, 1989) and their products (Bhatia, 1970) have been reported as piscicidal in India (Mohaptra and Nayak 1998; Jawale and Dama 2012; Jawale and Dama, 2010). The toxicity of Biocidal plant extract to the freshwater fish has been studied by number of investigators (Bhatta *et al.*, 1987; Bhatta and Farswam, 1992; Jawale *et al.*, 2012). But the piscicidal activity of *Asclepias curassavica* on the freshwater fish was many time reported in ethno botanical reports (Chopra 1933; Trinorgau, 2016; Primitivewayscom, 2016; Rajashekharan and Shivanand 2001), thus initiated the necessity to evaluate and confirm piscicidal property in the laboratory. Hence, the present paper deals with the evaluation of piscicidal property of leaves extract of *A. curassavica* leaves on the guppy fish *P. reticulatus*.

MATERIALS AND METHODS

Green tendered and well developed plant leaves were collected from nearby forest area of Nashik (Maharashtra) and were air dried in a shady place to retain their active ingredients intact. Dried leaves were powdered in a table model grinder for extraction. Powdered leaves (500 gm) were soaked in distilled water and methanol in airtight wide mouth bottle separately and kept for 7 days. After that, the cold extract from the bottles along with respective solvents were filtered. Around 6.3 gm dried hydroscopic methanolic extract and 5.1 gm of dried aqueous extract was obtained using rota-evaporator. dried extracts were kept in the desiccators until assayed. Dried extracts were used for toxicological testing. Stock solution was prepared by dissolving plant extract (1gm) in (1lit) distilled water to make its strength 1000 ppm. Different concentrations were prepared by adding a required dose of stock solution in water. Fish *Poicilia reticulata* of 2.4 ± 0.2 cm length and 215.6 ± 20.2 mg body weight were collected from the river. They were brought to laboratory and maintained separately in glass aquariums under controlled laboratory conditions (27.2°C and 75.5% RH; 16 h day light) for 7 days with optimum aeration. Piscicidal activity was carried out by acute static bioassay of various ppm concentration of alcoholic extract as per the protocol given in OCED (2000). All experiments were repeated three times and performed in triplicate. The fishes were exposed for 24, 48, 72 hours at different concentration of extract. Mortality was recorded at every 24 hr. up to 72 hr. exposure period. Fishes were considered dead if they failed to respond to a stimulus provided with glass rod. The recorded mortality data was used to calculate the LC_{50} values, upper and lower confidence limits, slope function and regression results using SPSS statistical analysis software (Version 10.0) using Probit Analysis Statistical Method (Finney, 1971). The LC_{50} values (with 95% confidence limits) were calculated. Differences among the results were considered to be statistically significant when P value was < 0.05 . Also, the MS Excel 2007 was used to find regression equation ($Y = \text{mortality}$; $X = \text{concentrations}$), the LC_{50} was derived from the best-fit line obtained.

RESULTS

The acute toxicity studies of leaves extract of *A. curassavica* were determined at different time intervals and presented in table 2. There is a significant negative correlation between LC_{50} values and exposure periods i.e. LC_{50} values decreased from 24 to 72 hr. If exposure periods increase the LC_{50} values decreases 7.42 ppm (24 hr) $>$ 9.31 ppm (48 hr) $>$ 11.22 (72 hr). for methanolic extract and 4.32 ppm (24 hr) $>$ 6.14 ppm (48 hr) $>$ 8.42 (72 hr). for aqueous extract. The results of regression analysis indicated that the mortality rate (Y) is positively correlated the concentration (X) having a regression coefficient (R) close to one in each case. The toxicity study shows the overall picture of test progress and indicates that, the rate of mortality increased with increasing concentration of plant extracts in a linear fashion.

Table 2. LC_{50} values and regression equation results for *P. reticulatus* treated with *A. curassavica* extracts.

Plant extract	Exposure period	LC_{50} in ppm	Regression equation
Aqueous dried extract	24 h.	7.42	$Y = 3.94x + 0.89$
	48 h.	9.31	$Y = 2.00x + 0.55$
	72 h.	11.22	$Y = 1.00x + 2.88$
Methanolic dried extract	24 h.	4.32	$Y = 4.64x + 0.63$
	48 h.	6.14	$Y = 3.94x + 0.22$
	72 h.	8.42	$Y = 1.66x + 0.88$

Y mortality rate, x concentrations

During toxicity studies of exposures to plant extracts of *A. curassavica* leaves caused significant behavioural changes in the guppy fish *P. reticulata*. When fishes were kept in toxic medium, They shows first symptoms of loss of sensitivity (response), characterizes by raising of the fins, subsequently a rigorous, spasmic and mostly superficial movements of

fins and operculum was observed. Often the mouth was partially closed and slight twitching of the jaws was discernable. After initial 10-15 hrs, there is a heavy discharge of mucus through the gills and a mucus layer was formed on eyes and all over the body. Fish shows stupefaction. when touched with glass rod, did not respond to external stimulus and shows knockdown effect. In the last hours of exposure movements were staggered, mouth is somewhat opened, rigidity in fins and tail, eyes are dull and body colour become totally lifeless. Finally fish died with rigor mortis in curved formed.

DISCUSSION

Piscicidal evaluation of *A. curassavica* plant in present study indicated the significant positive correlation between dose and mortality. It could be due to increased concentration of extract in aquarium water and resulted in more intake or entry of active piscicide in the fish body. There is no significant different between observed and expected mortality. Since calculated chi-square values are less than the table chi-square value. Thus, it is likely that extracts of *A. curassavica* plant will be a useful plant product for killing weed and trash fishes in aquaculture. Toxicity experiments showed that extract of *A. curassavica* leaves, caused significant behavioral changes in fish *P. reticulatus*. In the present study the abnormal behavior of the fish may be due to suffocation, leading into forceful respiration efforts. Several chemical constituents including cardenolides, flavanols, phenols, aliphatic, alicyclic compounds and alkaloids were present in *A. curassavica* plant. (Kupchan *et al.*, 1964; James *et al.*, 1982). Various photochemist have reported presence of Conduritol F, Conduritol F 3-O-beta-D-glucoside, Ascurogenin, Calotoxin, Calotropagenin, 16-alpha-Hydroxy, calotropin, Clepogenin, 4''-O -β D-Glucopyranosylgofruside, 4'' O-β- Cellobiosylgofruside, Curassavogenin, Uscharidin, Uscharin, Voruscharin, Hyperoside, soquercetrin, Alkaloids Cardenolides/bufadienolid in the leaves of this plant (Abe *et al.*, 1991; James *et al.*, 1982; Groeneveld *et al.*, 1994; Duffey and Scudder, 1972; Tschesche *et al.*, 1958; Haribal and Renwick 1998; Abe *et al.*, 2000). Behavioural responses of fish after exposure to the plant extract indicate suffocation stress. also increase secretion of mucus indicate the local absorption of the toxicant, and to minimize its effect more profuse mucus secretion is reported. (Gill *et al.*, 1991; Goodmann *et al.*, 1985; Shaikh *et al.*, 2012; Tiwari and Singh 2005). This study indicates the piscicidal activity of *A. curassavica* extracts which can be used in aquaculture and as biocide in other agricultural practices. Although long term toxic effect has to be studied further to establish its commercial use. Present experiments shows that locally available plants can be used as piscicides as an alternative to harmful Agro-pesticides that are in use today to eradicate unwanted fishes in the ponds. The use of plant poisons to catch fish is still used in many tribes in the world today. Their experience of using various plants selected for their soap like properties, led to the universal finding that phytochemicals from these plants would also stun fish when used in a specific manner. Most important chemicals that occur in nearly all plants used for stunning fish are saponin and rotenone (Primitivewayscom. 2016). Chopra (1933) reported the possibility of piscicidal plant to have insecticidal property. Recently many authors have reported piscicidal and also insecticidal activity of *Cestrum nocturnum* leaves. (Jawale and Dama 2010; Jawale *et al* 2012; Jawale, 2014, Jawale, 2015). More laboratory investigation of various parts of this plant extracts on various organism could lead into development of eco-friendly biocides. Hence Further studies are undertaken to evaluate the toxicity of this extract on the various pest species.

REFERENCE

- Abe F., Yamauchi T., Honda K. and Hayashi N. (2000).** Conduritol F glucosides and terpenoid glucosides from *Cynanchum liukiense* and distribution of conduritol F glucosides in several *Asclepiadaceae* plants. *Chemical and Pharmaceutical Bulletin*; 48(7): 1090-1092.
- Bhatia H. L. (1970).** Use of Mahu oil cake in fishery management. *Indian Farming*. **20**: 39-40.
- Bhatt J. P., and Farswam V. S. (1992).** Haemolytic activity of piscicidal compounds of some plants to a fresh *Barilius bendalensis* (Ham). *J Environ Biol*. **13**(4): 333-342.
- Bhatt J. P., Dobryal A. K. and Farswam Y. S. (1987).** Growth response in the fry of *Schizothrorax richardsonii* (Gray) to the plant toxins. *J Environ Biol*, 8(2): 207-215.
- Chopra, R. N. (1933).** Indigenous drugs of India. The Art Press, Calcutta.
- Cullen M. C. and Connell D. W., (1992).** Bioaccumulation of chlorohydrocarbon pesticides by fish in the natural environment. *Chemosphere*. **25**(11): 1579-1587.
- Duffey S. S and Scudder G. G. E.(1972).** Cardiac glycosides in North American *Asclepiadaceae*, a basis for unpalatability in brightly coloured hemiptera and coleoptera. *Journal of Insect Physiology*; 18: 63.
- Duke J. A. (1994).** Amazonian ethnobotanical dictionary, Boca Raton, FL: "CRC Press" Pp 181.
- Finney D. J. (1971).** *Probit analysis*. S. Chand and Company Ltd. Ram Nagar, New Delhi, Pp 333.



- Gill T. S., Pandey J. and Tewari H. (1991). Individual and combined toxicity of common pesticides to teleost, *Puntius conchonioides*, Hamilton. *Indian J. Exp. Biol.* **29**(2): 145-148.
- Goodmann L. S., Gillman A.G., Rall T. W. and Murad F. (1985). *The pharmacological basis of therapeutics*. Macmillan Publishing Company, New York.
- Groeneveld H. W, Van berkel Y. E. M, Binnekamp A, Seykens D. (1994). Some quantitative aspects of cardenolide synthesis from malonate in *Asclepias curassavica*. *Phytochemistry*; **37**(6): 1605-1610.
- Gupta M. L, Gupta T. K, Bhargava K. P. A. (1971). Study of antifertility effects of some indigenous drugs. *Journal of Research in Indian Medicine*; **6**: 112-116.
- Haribal M, Renwick J. A. A. (1998). Identification and distribution of oviposition stimulants for monarch butterflies in host's and non hosts. *Journal of Chemical Ecology*; **24**: 891-904.
- James N. S., Carolyn J. N. and Mark L. S. (1982). Cardenolides in the latex and leaves of seven *Asclepias* species and *Calotropis procera*. *Phytochemistry*; **21**(9): 2343-2348.
- Jawale C. S. and Dama L. B. (2010). Hematological changes in the fresh water fish, *Cyprinus carpio* exposed to sub-lethal concentration of piscicidal compounds from *Cestrum* species (Family : *Solanaceae*). *Nat. J. Life Sci.* **7**(1): 81-84.
- Jawale C. S. and Dama L. B. (2010). Insecticidal potential of *Cestrum* sp. (*Solanaceae*: *Solanales*) against *Tribolium castaneum* and *Tribolium confusum* (Herbst) (*Coleoptera*- *Tenebrionidae*). *Deccan Curr. Sci.* **3**(2): 155-161.
- Jawale C. S., Vinchurkar A. S., Dama L. B., Pawar Kishor, Dama S. B. and Shaikh Yasmeen. (2012). *Cestrum nocturnum* (L) a prospective piscicide for control of predatory fish *Channa punctatus* (bloch.). *Trends in fishery research* Vol. 1 , 1: 14-17
- Jawale C. S and Dama L. B. (2012). Biological Activities of *Cestrum* Species (family : *Solanaceae*): Biocidal Properties of *Cestrum nocturnum* . Germany: LAP Lambert Academic Publishing.
- Jawale C. S. (2014). Larvicidal activity of some saponin containing plants against the dengue vector *Aedes aegypti*. *Trends in Biotechnology research*, **3**(1), 1-11.
- Jawale C. S. (2015). Potential mosquito larvicide of *cestrum nocturnum* and *Sapindus mukorossi*. *Trends in Life sciences* , **4**(4), 174-178.
- Jhingran V. G. (1975). *Fish and fisheries of India*. Hindustan Publishing Corporation (India), New Delhi. Pp.954.
- John D. (1984). One hundred useful raw drugs of the kani tribes of Trivandrum forest division, Kerala, India. *International Journal of Crude Drug and Research*; **22**(1): 17-39.
- Kulakkattolickal A. T. (1989). Piscicidal plants of Nepal: ripe fruit of *Catunaregam spinosa* (Thunb.) (*Rubiaceae*) and leaves of *Polygonum hydropiper* L (*Polygonaceae*) as fish poisons. *Aquaculture*. **78**: 293-301.
- Kupchan S. M., Knox J. R., Kelsey J. E., Saenzrenauld J. A. (1964). *Calotropin* A cytotoxic principle isolated from *Asclepias curassavica*. *Life Science*; **25**(146): 1685-1686.
- Marking L. L. (1992). Evaluation of toxicants for the control of carp and other nuisance fishes. *Fisheries*. **17**:6-12.
- Marston A. and Hostettmann K, (1985). Plant molluscicides. *Phytochem.* **24**: 639-652.
- Mitra R, Mehrotra S, Mehrotra B. N., Kapoor L. D. (1974). Pharmacognostic study of *Asclepias curassavica* Linn. *Bulletin Botanical Survey of India*; **16** (1-4): 82-88.
- Mohapatra, B. C. and G. B. Nayak (1998). Assessment of toxicity of ripe fruit pulp of Hingan, *B. roxburghii* on different fishes. *J. Aquacult. Trop.*, **6**: 19-21.
- (2000). Series on testing and assessment number 23 guidance document on aquatic toxicity testing of difficult substances and mixtures (September 2000), OECD, Paris.
- Raja Sundararajan and Ravindranadh Koduru (2014). *Asclepias curassavica*: a review of ethnomedical, phytochemical and pharmacological information. *Iajpr*, **4** (4), 1739-1755.
- Rajasekharan, P. E. and Shivanand, T. N., (2001) Blood Flower : The Hindu Newspaper section: features 2-18-02 <http://hinduonnet.com/thehindu/2001/02/18/stories/1318045e.htm> *Asclepias curassavica*
- Shaikh Yasmeen, Suryawanshi G. D., Dama L. B. and Mane U.H. (2012). Behavioural changes of fresh water bivalve molluscs *Lamellidens marginalis* due to acute toxicity of cadmium. *DAV Int. J. Sci.* **1**(2): 103-106.
- Singh S., Yadav R. and Singh A. (2010) Molluscicides from some common medicinal plants of eastern Uttar Pradesh, India. *J. Appl. Toxicol.* **30**(1):1-7.
- Stone B. C.(1970). The flora of Guam. *Micronesica* 1970; **6**:487
- Tiwari S. and Singh A. (2005). Possibility of using latex extracts of *Nerium indicum* plant for control of predatory fish *Channa punctatus*. *Asian Fish Sci.* **18**:161-173.
- Trinorgau. (2016). *Trinorgau*. Retrieved 19 May, 2016, from http://keys.trin.org.au/key-server/data/0e0f0504-0103-430d-8004-060d07080d04/media/Html/taxon/Asclepias_curassavica.htm Tschesche R, Forstmann D, Rao Varanasi