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EXPLOITING THE LARVICIDAL PROPERTIES OF *Parthenium hysterophorus* L. FOR CONTROL OF DENGUE VECTOR, *Aedes albopictus*

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ABSTRACT

The larvicidal properties of invasive weed Parthenium hysterophorus L. extract was compared with that of other natural plant extracts; Stevia, Chrysanthemum and Neem extract & oil. The extracts obtained from these respective plants under test were dissolved independently to get 20% stock solutions, which was diluted to lower concentrations with tap water. Various doses (0.5, 1.0, 1.5, 2.0, 2.5 and 3%) were tested as per standard dose-response larvicidal bioassays for determining the toxicity in term of LC_{50}/LC_{90}. The results revealed highest LC_{50} value (2.086 to 2.815 %) and LC_{90} value (5.836 to 8.533 %) for Stevia extract and the least LC_{50} (0.849 to 1.455%) and LC_{90} (1.875 to 2.638 %) for Parthenium extract against Aedes albopictus respectively. Parthenium extract proved superior to the other plants extracts against 3rd and 4th instars larvae of Ae. albopictus in both the exposure periods. The overall, high values of LC_{50} & LC_{90} for Stevia extract indicating its low toxicity while the minimum LC_{50} & LC_{90} values by Parthenium extract exhibit its high toxicity against the 3rd and 4th instars larvae of Ae. albopictus in both the 24 & 48 hours exposure periods. The five tested plants, in terms of efficiency against 3-4 instars mosquito larvae were ranked as Parthenium > Chrysanthemum > Neem extract > neem oil > Stevia. These results are helpful in exploiting the use of crude extract obtained from invasive weed (Parthenium) in dengue vector control strategies as effective and safe larvicides in the public health program of dengue vector control. This will not only help in control of the deadly disease but will also be utilized in mechanical control of the invasive weed i.e. P. hysterophorus.

Key words: *Aedes albopictus*, larvicides, parthenium, phyto-chemicals.

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INTRODUCTION

Many plants including weeds have developed protection mechanisms, such as repellents and even insecticidal effects, to defend themselves against insect pest attacks. Thus extracts obtained from their different botanical parts having sap can be wisely used in suppressing insect pests (Ahmad *et al*., 2012). We therefore, exploited the insecticidal properties due the allopathic nature of the *Parthenium hysterophorous* in the control of mosquito vectors of deadly diseases as potential natural alternative to the use of synthetic insecticides for environment friendly control of dengue vector mosquito (Khan and Khan, 2014). Dengue is a serious health problem in many areas of Pakistan and having potential of becoming established in other areas of Pakistan (WHO, 2012) and therefore, need proper attention by controlling its potential vectors through environment friendly techniques. The use of plant extracts (Chowdhury *et al*., 2008) for insect control has a number of useful qualities as these are ecologically safe, no risk to public health and environment and rich stock of chemicals of different biological actions as well. Previous researchers from different parts of the world had also reported potential of some plant extracts in mosquito control program. Weed plants due to its inbuilt toxic nature have pesticidal and even medicinal properties also (Chansang *et al*., 2005).

The leaf extract of *Centella asiatica* has larvicidal properties and is an inhibitor for adult emergence against *Culex quinquefasciatus* (Rajkumar and Jebanesan, 2005). Similarly, the extract of *Solanum xanthocarpum* was found to be toxic against the larvae of *Anopheles stephensi* (Chowdhury *et al*., 2008). According to Indian reports (Mandal, 2011)**,** 100% protection from the bite of *Culex quinquefasciatus* mosquito was observed due to the repellant action of *Eucalyptus* and *A. indica* seed oil. Another Indian (Nathan *et al*., 2007) reported that the leaf extracts of *Eucalyptus tereticornis* showed 99% mortality at 160 ppm against the larvae of *An. stephensi*. Petroleum ether extract of *Chrysanthemum colocynthis* was extremely vigorous against fourth-instars larvae of *Aedes aegypti* in Pakistan (Rahuman *et al*., 2008).

High toxicity of similar nature of plant extract against *An. stephensi* and similar high larvicidal properties of hexane extracts from *Chrysanthemum sinensis* leaves against *Aedes* spp. after 24 h of exposure were also noted in Indian studies (Mohan *et al*., 2008;

Radhika *et al*., 2012). Kumar *et al*., 2012 confirmed the potential of celery seed oil as the prospective larvicidal, repellent and irritancy agent for the control and management of *Aedes aegypti* population. Choochote *et al*., 2004 reported that the ethanol extracted *A. graveolens* possessed excellent larvicidal activity against fourth instars exhibiting LD_{50} and LD_{95} values of 81.0 and 176.8 mg/L, respectively. Ahmad *et al*., 2011 reported high toxicity of Parthenium and Stevia extracts against the larvae of *Anopheles* species from Pakistan. In previous studies different researcher reported different plant extracts as an effective botanical against Red pumpkin beetle and fruit flies. (Misbahullah *et al.*, 2012; Khan *et al*., 2012).

Keeping in view the recent trend of mosquito control through various plant extracts in many regions of the world, we assessed the efficacy of crude extracts of some plants with main emphasis on invasive weed, *Parthenium hysterophorus* against mosquito species, *Aedes albopictus* by estimating the differences in doses of extracts and exposure period against third and fourth instars larvae after 24 and 48 hours. This study was of dual nature i.e. exploiting the toxic effect of the weed against the deadliest mosquito and ultimately the mechanical control of this noxious weed.

MATERIALS AND METHODS

The comparative larvicidal potential of *Stevia rebaudiana*, *Parthenium hysterophrus*, *Chrysanthemum morifolium*, Neem (*Azadirachta indica*) extract and oil were tested against the recently established dengue vector (Khan and Khan, 2014), *Aedes albopictus* larvae under laboratory conditions. There were four replications per treatment.

Preparation of plant crude extracts

The plant species under investigation were collected from field or the local market. Parthenium weed was collected in bulk from the nearby Experimental farm of Nuclear Institute for Food and Agriculture (NIFA), Peshawar. The sweet plant (*Stevia* spp.) was obtained from the Biotechnology group, NIFA. The leaves of the plants were dried at ($28 \pm 2°C$) under shade condition and crushed by using electrical stainless steel blender and sieved to get fine powder from which the extract was prepared following the method of Khan *et al*., 2012. The weight (250 g) of crude extract of the different tested plants species was taken of the shade dried leaves powder in separate containers. Distilled water (1250 ml) was added and kept for 16 hours with shaking periodically, and the filtrate of extract was collected. The pooled extracts were concentrated separately by rotary vacuum evaporator at 40°C under condensed pressure of 22-26 mm of Hg and evaporated to desiccation and stored at 4°C in air tight vials. The extract obtained from each plant was dissolved independently and 20% stock solutions were prepared separately for each plant extracts. The stock solution was diluted to lower desired concentrations of 0.5, 1, 1.5, 2.0, 2.5 and 3% with tap water by using Abid unit calculator (Farid, 2006).

Dose-Response Larvicidal Bioassays

Mosquito's larvicidal trials were carried out as per World Health Organization standard procedure (WHO, 2012) with slight modifications following the method by indigenous Indian techniques (Rahuman *et al*., 2008b). From the stock solution different concentrations (0.5, 1.5, 2, 2.5, and 3 %) were prepared for testing their larvicidal efficiency. Twenty five third instar larvae of *Aedes albopictus* were selected and transferred into cups of prepared test solution. Mosquito larval diet was added to the larvae. The numbers of dead larvae were counted after 24 and 48 hours of exposure. Percent mortality was recorded from the average of four replications. However, at the end of 24 and 48 hours the selected test samples that were turned out in their toxic potential to equal to temephos (standard check) were selected. Comparative toxicity of plant crude extracts against 3rd-4th instars *Aedes albopictus* and for 24 and 48 hrs exposure periods was calculated by using Polo plus software, Leora Berkeley, CA using range of LC50/LC90 as standard.

RESULTS AND DISCUSSION

Table-1 shows the results on LC_{50}, LC_{90}, lower and upper confidence limits, slope and chi square (χ^2) values of five different botanical extracts for 24 hr exposure of 3^{rd} instar larvae of *Aedes albopictus*. The results revealed highest LC_{50} value (2.815 %) and LC_{90} value (8.533 %) for Stevia extract and the least (1.024%) and (2.452 %) for Parthenium extract against *Aedes albopictus*. Overall, LC_{50} & LC_{90} values were found maximum for Stevia extract indicating its low toxicity while Parthenium extract gave minimum LC_{50} & LC_{90} values that exhibited high toxicity against the mosquito species.

Table-2 indicates LC_{50}, LC_{90}, lower and upper confidence limits, slope and chi square (χ^2) values of five different botanical extracts for 48 hr exposure of 3^{rd} instar larvae of *Aedes albopictus*. The results showed highest LC_{50} value (2.086%) and LC_{90} value (5.836%) for Stevia extract and the least (0.849 %) and (1.875 %) for Parthenium extract against *Aedes spp*. Overall, LC_{50} & LC_{90} values were maximum for Stevia extract indicating its low toxicity while Parthenium extract gave minimum LC_{50} & LC_{90} values that exhibited high toxicity against the mosquito species.

Table-3 indicates LC_{50}, LC_{90}, lower and upper confidence limits, slope and chi square (χ^2) values of five different botanical extracts for 24 hr exposure of 4^{th} instar larvae of *Aedes albopictus*. The results showed highest LC_{50} value (2.544 %) and LC_{90} value (6.452 %) for Stevia extract and the least (1.455 %) and (2.638 %) for Parthenium extract against

Aedes spp. Overall, LC_{50} & LC_{90} values were found maximum for Stevia extract indicating its low toxicity while Parthenium extract gave minimum LC_{50} & LC_{90} values that exhibited high toxicity against the mosquito species.

Table-4 indicates LC_{50}, LC_{90}, lower and upper confidence limits, slope and chi square (χ^2) values of five different botanical extracts for 48 hr exposure of 4th instar larvae of *Aedes albopictus*. The results showed highest LC_{50} value (2.254%) and LC_{90} value (6.527%) for Stevia extract and the least (1.287%) and (2.126%) for Parthenium extract against *Aedes* spp. Overall, LC_{50} & LC_{90} values were found maximum for Stevia extract indicating its low toxicity while Parthenium extract gave minimum LC_{50} & LC_{90} values that exhibited high toxicity against the mosquito species.

In our study the different botanical extracts, in various concentrations, yielded significant variable mortalities in 3rd and 4th instars larvae of *Aedes* spp. after 24-48 hours period, where Parthenium extract gave highest while Stevia extract lowest toxicity. The application of Parthenium extract was highly toxic that resulted in maximum larval mortalities. Parthenium extract proved superior to the other plant extracts against 3rd and 4th instars larvae of *Aedes albopictus* after 24-48 hours exposure periods. The five tested plants, in terms of efficiency against 3-4 instars mosquito larvae can be ranked as Parthenium > Chrysanthemum > Neem extract > neem oil > Stevia. The present results are in conformity to that reported by some earlier researchers. Tatiana *et al*. (2005) found Parthenium extract effective against *Leishmania amazonensis.*

According to Sathish (2008), the leaf and flower extracts of the weed *Lantana camara* and *Parthenium hysterophorous* exhibited larvicidal activity against third instar larvae of *Culex quinquefasciatus* with maximum mortality. Previous Pakistani reports by Ahmad *et al*., 2011 also highlighted that among the three plants extracts tested in two media, *Parthenium hysterophorous* had the powerful anti-oxidative enzymes activity. In our trials the LC_{50} & LC_{90} values were found maximum for Stevia extract indicating its low toxicity while Parthenium extract gave minimum LC_{50} & LC_{90} values that exhibited high toxicity against the two mosquito species. According to Indian reports by Kumar *et al.* (2012) hexane and petroleum ether extracts prepared from the stem of *P. hysterophorus* were found effective exhibiting LC_{50} values of 379.76 and 438.57 mg/L, respectively.

Thus *P. hysterophorous*, after testing its efficiency under field conditions, might be recommended in the Integrated Vector Management (IVM) program of mosquito in the province. Thus during the vector control process, the first priority should be given in exploiting the toxic potential of the invasive weed (*P. hysterophorus*) by attacking the mosquito of deadly diseases in their larval habitats. After testing its efficacy under field condition, this will lead to an environment friendly tactics for reducing risk of vector infection. Its insecticidal effects can provide potential natural alternative to the use of synthetic insecticides. Thus botanical extracts will continue to play an important role in suppressing vectors of deadly diseases including dengue epidemics.

CONCLUSION

Our results can be successfully utilized in developing safe plant based bio-pesticide in public health sector on one hand and the mechanical control of the invasive weed as additional outcome for the farming community of the country.

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Table-1. Comparative toxicity of different plant crude extracts against 3rd instar larvae of *Aedes albopictus* (24 hrs exposure).

Treatment	% Lethal concentration				Slope	χ^2
	LC_{50}	95% CL	LC_{90}	95% CL		
Chrysanthemum	1.50	0.95-1.80	2.62	2.17-4.26	5.30±0.62	12.54
Parthenium	1.02	0.50-1.309	2.45	1.78-5.36	3.38±0.30	21.26
Neem extract	1.48	0.80-1.78	2.82	2.31-5.45	4.57±0.64	10.49
Neem oil	1.86	1.46-2.16	3.70	2.97-6.62	4.31±0.58	7.254
Stevia extract	2.81	2.48-3.35	8.53	6.14-15.35	2.66±0.39	0.770

Table-2. Comparative toxicity of plant crude extracts against 3rd instar larvae of *Aedes albopictus* (48 hrs exposure).

Treatment	% Lethal concentration				Slope	χ^2
	LC_{50}	95% CL	LC_{90}	95% CL		
Chrysanthemum	1.34	0.97-1.58	2.32	2.00-3.03	5.42±0.65	7.24
Parthenium	0.84	0.45-1.13	1.87	1.42-3.09	3.72± 0.34	16.16
Neem extract	1.37	1.21-1.50	2.48	2.27-2.80	4.97± 0.56	1.28
Neem oil	1.42	0.94-1.69	2.25	1.90-3.21	6.46± 0.73	12.88
Stevia extract	2.08	1.83-2.35	5.83	4.57-8.93	2.86± 0.42	0.39

Table-3. Comparative toxicity of plant crude extracts against 4th instar larvae of *Aedes albopictus* (24 hrs exposure).

Treatment	% Lethal concentration				Slope	χ^2
	LC_{50}	95% CL	LC_{90}	95% CL		
Chrysanthemum	1.54	1.19-1.77	3.06	2.61-4.26	4.28±0.57	4.97
Parthenium	1.45	0.97-1.73	2.63	2.19-4.17	4.96±0.57	11.63
Neem extract	1.65	1.50-1.79	3.07	2.78 -3.54	4.75±0.52	2.24
Neem oil	1.61	1.17-1.99	4.60	3.36-9.81	2.82±0.34	7.17
Stevia extract	2.54	2.28-2.89	6.45	4.97-10.33	3.17±0.49	1.09

Table-4. Comparative toxicity of plant crude extracts against 4th instar larvae of *Aedes albopictus* (48 hrs exposure).

Treatment	% Lethal concentration				Slope	χ^2
	LC_{50}	95% CL	LC_{90}	95% CL		
Chrysanthemum	1.42	1.25-1.56	2.61	2.38-2.97	4.85±0.58	2.58
Parthenium	1.28	0.90-1.54	2.12	1.81-2.91	6.08±0.68	10.94
Neem extract	1.53	1.36 -1.66	2.67	2.44-3.01	5.29±0.61	1.93
Neem oil	1.30	0.48-1.72	3.22	2.38-9.45	3.20±0.41	13.62
Stevia extract	2.25	1.98-2.56	6.52	4.96-0.66	2.77±0.42	0.84

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