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LARVICIDAL EFFICACY OF AQUA NANOEMULSION OF *ALOE VERA* OIL AGAINST *AEDES AEGYPTI* MOSQUITO

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ABSTRACT

Essential oils act as advantageous alternative to insecticides for the control of mosquitoes. But their improper dispersion in water is a problem and to overcome this, present study was conducted by preparing *Aloe vera* oil nanoemulsions in water using sonication technique. Thermodynamic stability tests revealed that aqueous nanoemulsion having *A. vera* oil and Tween-20 in 1:3 ratio was found to be maximally stable (out of the tested three nanoemulsions prepared @ 1:1, 1:2 and 1:3). Size of spherical droplets of the stable aquananoemulsion recorded through transmission electron microscopy (TEM) was found to range from 20 to 50 nm(38.33±1.67). Different concentrations of the stable nanoemulsion of *A. vera* oil @750, 650, 550, 450, 350, 250 ppm (in triplicate) were tested for their larvicidal potential against *Aedes aegypti*. Amongst these 650 ppm was found to be the most effective as 100% larval mortality was observed within 24 hr.

Key words: *Aedes aegypti*, *Aloe vera* oil, aqua nanoemulsion, larvicidal efficacy, size ofdroplets, sonication technique, TEM

Aedes aegypti mosquito acts as a vector for the transmission of many deadly diseases like dengue fever, chikunguniya, yellow fever and zika virus fever. This mosquito well establishes its population generally in most households at almost every tropical urban conditions and also in some subtropical areas (Phillipis, 2008) and typically bites during the day time (Becker et al., 2010). Vector control is the most successful and excellent method to decrease incidence of mosquito borne diseases. The control of mosquitoes worldwide is dependent on continued applications of chemical insecticides (Yang et al., 2002). Most of these synthetic chemicals are expensive and destructive to the environment, toxic to humans, animals and other non target organisms and also results in creating resistance among mosquitoes (Wattanachai and Titanon, 1999; Amer and Melhorn, 2006). Such associated problems prompted the researchers to explore alternative, simple, environmentally safe and sustainable methods of mosquito control.

Essential oils extracted from plants provide an efficient and eco-friendly alternate to manage this menace and most important that the chances of developing mosquito resistance to such bioinsecticides are unlikely (Adeyemi, 2010). There has been a concerted effort worldwide to screen traditional plants for their insecticidal property (Asfaw et al., 2007). Larvicidal potential of indigenous plants and their products including essential oils have been reported in many parts of India (Singhand Bansal, 2003; Kocher and Riat, 2017; Riatand Kocher, 2019). Among these *Aloe vera* is one of the few plants popular for its medicinal value and larvicidal efficiency because of the presence of active ingredients namely chromones and anthraquinones (Pitasawat et al., 2007; Raitand Kocher, 2017). But usage of essential oils poses a problem of their improper dispersion in larval aquatic habitat. To overcome this, present interest has been devoted to the development of nano sized oil emulsions in water by downsizing the oil droplets so as to form effective and homogenously dispersed larvicidal formulation (Anjali et al., 2012; Montefuscoli et al., 2014; Sugumar et al., 2014; Kaur et al., 2019). Keeping in view the importance of nanoemulsions in mosquito larval control, the present study was planned to explore the larvicidal efficacy of aqua nanoemulsion of *A. veera* oil against *Ae. aegpyti*.

MATERIALS AND METHODS

Pure form of *A. vera* oil was obtained from Katyani Exports, New Delhi. The aqua nanoemulsionswere formulated using *A. vera* oil, non-ionic surfactant (Tween-20) and distilled water. The concentrations of these components that could form a stable nanoemulsion were standardized using different concentrations of Tween-20 and water. However, the concentration of *A. vera* oil was fixed (6%) for all the formulations

following the procedure given by Sugumar et al. (2014). Firstly, coarse emulsion was prepared by adding water to organic phase which contains oil and surfactant in different ratios 1:1, 1:2, 1:3 (v/v) using a magnetic stirrer, which was later subjected to ultrasonication using 40 KHzsonicator.

For screening of the stable nanoemulsion, these were visually observed for optical transparency, appearance and phase separation and then were analysed for thermodynamic stability stress tests (Shafiq et al., 2007). For this, the prepared nanoemulsions were centrifuged at 3000 rpm for 30 min and observed for phase separation (if any). The nanoemulsion which was found to be the stable one during this process was further analyzed for heating-cooling cycle.This was performed by keeping the stable nanoemulsion at 40^{o}C (maintained in hot air oven) and 4^{o}C (maintained in refrigerator), alternatively for 48hr at each temperature. The cycle was repeated three times and evaluated for any physical change like phase separation and appearance. Then morphology of most stable nanoemulsion was observed via transmission electron microscopy (TEM) by negatively staining one drop of oil emulsion with phosphor tungstic acid and positioning this on a copper grid. TEM micrographs were acquired using a transmission electron microscope (Hitachi H7650), with a tungsten source and operating at 120 Kv.

For the collection of mosquito larvae water samples were taken from different peridomestic water collections like desert coolers, earthen pots and road side ditches etc. of urban zones of Ludhiana district using plastic dippers from June to September, 2017. From the various types of mosquito larvae present in the collected water samples, *Ae. aegypti* larvae were recognized from the other types of mosquito larvae (if present) on the basis of their morphological features by following the standard keys given by Becker et al. (2010) and Bar and Andrew (2013). Different concentrations of *A. vera* oil in water @ 750, 650, 550, 450, 350 and 250 ppm were prepared by diluting the most stable nanoemulsion using dechorinated water. In the treatment trial twenty 4th instar *Ae. aegypti* larvae were exposed to these concentrations in triplicate. A vehicle-control set (having Tween-20 in de-chlorinated water in same ratio as treatment set) and a control set (having 250ml de-chlorinated water only) containing twenty 4th instar larvae each in beakers were also run simultaneously (in triplicate).

All mosquito larvae were adequately fed with mixture of dog biscuits and yeast ground in ratio 3:1 (2mg/100ml).The experimental sets were kept in B.O.D. incubator at 26±2°C. Mortality of larvae after 3, 6, 12, 24 and 48hr of treatment were recorded in nanoemulsion treated, vehicle-control and control sets. Data was statistically analyzed by comparing the mortality record from eucalyptusoil nanoemulsion treated sets with control and vehicle-control sets by using ANOVA (Duncan multiple range test). LC_{50} and LC_{90} values after 12 hr of post-exposure, were worked out by log probit technique (Finney, 1971) employing the computer programme POLO (Robertson et al., 1980).

RESULTS AND DISCUSSION

Screening and characterization of aqua nanoemulsion: Among the prepared nanoemulsions having *A. vera* oil (6%) and Tween-20 in different ratios i.e. 1:1, 1:2 and 1:3, the nanoemulsion with 1:3 ratio was found to be the most stable one after observing through thermodynamic stabilization testsand this emulsion showed no phase separation and highly transparent after heating and cooling cycle. The nanoemulsions having oil and Tween-20 in other ratios (1:1 and 1:2) were turbid and milky in appearance and showed phase separation after heating and cooling cycle. The most stable aqua nanoemulsion (having *A. vera* oil and Tween-20 in 1:3 ratio) analysed by transmission electron microscopy (TEM) showed spherical nature of droplets with their droplet size ranging from 20-50 nm (38.33±1.67).As the other two nanoemulsions (1:1 and 1:2) showed phase separation, therefore those were not considered for TEM study.

Larvicidal efficiency: Exposure of 4th instar *Ae. aegypti* larvae to 250 ppm of *A. vera* oil based aqua nanoemulsion (1:3) showed 3.33±1.69% mortality after 3hr and this mortality rate was found to increase with increase in the exposure time as 11.67±3.37, 21.67±3.37, 36.67±4.41 and 48.33±3.37% mortality after 6, 12, 24 and 48hr, respectively. With the increase in concentration of *A. vera* oil based aqua nanoemulsion to 350ppm, the percent mortality got increased from 5.00±0.00 (after 3hr) to 13.33±1.67, 23.33±6.67, 41.67±4.41 and 58.33±3.33 after 6, 12, 24 and 48hr respectively. The exposure of 450ppm nanoemulsion showed an increasing trend in larval mortality i.e. from 25.00±2.35 to 81.67±1.66 within 3 to 48hr. Treatment with 550ppm concentration of nanoemulsion resulted in 48.33±1.73, 65.00±3.34, 75.00±2.89 and 90.00±1.66% larval mortality after

3, 6, 12 and 24 hr respectively and there was 100% mortality of larvae after 48hr. Similarly, with the exposure of 650ppm of nanoemulsion for 3, 6 and 12 hr, the mortality rate recoded was 75.00±2.89, 85.00±2.89, 96.67±1.66% respectively and further increase of exposure to 24 hr, there was 100% mortality of larvae (Table 1). When the larvae were exposed to 750 ppm concentration of nanoemulsion, 100% mortality was observed even within 12 hr of exposure (Table 1).

During the present study, 650 ppm of *A. veera* oil based aqua nanoemulsion was found to be the most effective concentration out of the tested six concentrations, as it resulted in 100% larval mortality within 24 hr or before their conversion to next developmental stage i.e. pupae. No mortality was recorded in control and vehicle-control sets. The values for LC_{50} and LC_{90} of *A. veera* oil aqua nanoemulsion computed after 12 hrs against 4th instar larvae of *Ae. aegypti* were worked out to be 267.12 and 480.64 ppm, respectively.

The mucilagenous pulp of *A. vera* contains 93-96% water and rest are solid active compounds for its biological activity. There are about 75 different compounds which include anthraquinones, chromones or phenolic compounds, lignin tannic acids, polysaccharides, flavonoids, saponins, sterols, amino acids and salicylic and enzymes (Reynolds and Dweek,1999). The main reason for mortality of larvae in present study after their exposure to *A. vera* oil may be due to the presence of flavonoids as one of the component of this essential oils (Pitasawat et al., 2007; Kocher and Riat, 2017; Kaur et al., 2019; Riat and Kocher, 2019). In general the essential oils enter the larval body either through larval surface or tracheal systems.The body wall of mosquito larvae can easily absorb large quantities of the toxic substances present in their aquatic habitat (Matsumura, 1985; Lu and Kacew, 2002). As these larvae are generally so small that their body surface area is readily exposed to relatively higher concentrations than that of mammals (Martinez et al., 2013). These oils cause lysis of cells present in the epithelial tissue of larval midgut and in turn undergo necrosis.

Similar degenerative changes and necrosis have also been observed in *Anophelesstephensi* larvae after exposure to crude *A. veera* oil (Riatand Kocher, 2017). Duarte et al. (2015) have shown the larvicidal ability of *Rosmarinus officinalis* and *Pterodonemarginatus* Vogel oil based nanoemulsions against different species of mosquito. Neem oil nanoemulsions have also been found to be very effective against *Culex quinauefasciatus* compared to synthetic insecticides/ larvicides (Anjali et al., 2012). Scientists have reported larvicidal potential of nanoemulsions of basil and eucalyptus oils against *Ae. aegypti* and *Cx. Quinquefasciatus* mosquitoes, respectively (Ghosh et al., 2013; Sugumar et al., 2014). In our laboratory also, a recent study has shown a good larvicidal potential of eucalyptus oil based aqua ananoemulsion at its 70 ppm concentration against *Ae. aegypti* (Kaur et al., 2019). Thus, essential oil based nanoemulsions may serve as one of the significant novel strategies for management of mosquito vector resulting in reduction of mosquito larval population, ensuring biosafety and decreasing the magnitude of epidemiology of mosquito borne diseases.

Table 1. Larvicidal effect of aqua nanoemulsion- *A. vera* oil on 4th instar larvae of *Ae. aegypti*

Oil concentration (ppm)	% larval mortality (Mean±S.D) (n=20)				
	3 hr	6 hr	12 hr	24 hr	48 hr
250	3.33±1.69^{a}	11.67±3.37^{b}	21.67±3.37^{b}	36.67±4.41^{b}	48.33±3.37^{b}
350	5.00±00^{a}	13.33±1.67^{b}	23.33±6.67^{b}	41.67±4.41^{b}	58.33±3.33^{c}
450	25.00±2.35^{b}	36.67±4.41^{c}	53.33±4.41^{c}	68.33±3.33^{c}	81.67±1.66^{d}
550	48.33±1.73^{b}	65.00±3.34^{b}	75.00±2.89^{b}	90.00±1.66^{a}	100.00±0.00^{c}
650	75.00±2.89^{c}	85.00±2.89^{c}	96.67±1.66^{c}	100.00±0.00^{c}	-
750	91.67±1.66^{c}	96.67±1.66^{c}	100.00±0.00^{c}	-	-
0 (Control)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0 (Vehicle-control)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

N= No. of larvae taken; Figures followed with different superscripts indicate significant difference ($p<0.05$ DMRT)

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REFERENCES

Adeyemi M M. 2010. The potential of secondary metabolites in plant material as deterrents against insect pests. African Journal of Pure and Applied Chemistry 4:243-246.

Ame A, Mehlhorn H. 2006. Larvicidal effects of various essential oils against *Aedes*, *Anopheles* and *Culex* larvae (Dipetra, Culicidae). Parasitology Research 99: 466-472.

Anjali C H, Sharma Y, Mukherjee A, Chandrasekaran N. 2012. Neem oil (*Azadirachta indica*) nanoemulsion- a potent larvicidal agent against *Culex quinquefasciatus*. Pest Management Science 68:158-163.

Asfaw D, Aseyed T, Abebe D, Kissi M, Daniel M, Girum T. 2007. Screening of some Ethopian medicinal plants for mosquito larvicidal effects and phytochemical constituents. Journal of Pharmacology 3: 231-243.

Bar A and Andrew J. 2013. Morphology and morphometry of *Aedes aegypti* adult mosquito. Annual Review and Research in Biology 3(1):52-69.

Becker N, Petric D, Zgomba M, Boase C, Dahl C, Madon M, Kaiser A. 2010. Mosquitoes and their Control. (2nd ed.) Springer Publication. pp. 9-40.

Duarte J L, Jesus R R A, Anna E M F M O, Rodrigo A S C, Adriana M F, Raimundo N P S, Deborah Q F, Jose C T C, Caio P F. 2015. Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. Revista Brasileira de Farmacognosia 25:189-192.

Finney D J. 1971. ProbitAnalysis.(3rd ed.) Cambridge: Cambridge University Press. pp. 68-72.

Ghosh V, Mukherjee A, Chandrasekaran N. 2013. Formulation and characterization of plant essential oil based nanoemulsions: Evaluation of its larvicidal potential against *Aedes aegypti.* Asian Journal of Chemistry 25: 321-323.

KaurN, Kocher D K, Sidhu A. 2019. Synthesis and testing of *Eucalyptus globulosa* oil based nanoemulsion for its larvicidal potential against *Aedes aegypti.* African Entomology 27(2) in press.

Kocher D K, Riat A K. 2017. Larvicidal potential of *Eucalyptus globulus* oil against *Aedes* and *Culex* mosquitoes. Journal of Insect Science 30(1): 5-9.

Lu F C, Kacew S. 2002. Lu's Basic Toxicology: Fundamentals, Targets Organ and Risk Assessment. (4th ed.). Taylor and Francis Inc., New York, ISBN: 9781420055986. pp: 386-392.

Martinez M L, Poser G V, Henriques A, Gattuso M, Rossini C. 2013. Simaroubaceae and picramniaceae as potential sources of botanical pesticides. Industrial Crops and Products 44: 600-602.

Matsumura F. 1985. Toxicology of Insecticides. (2nd ed.) Plenum Press, New York, USA, ISBN-13: 978-1-4612-9508-2.pp. 112-120.

Montefuscoli A R, Gonzalez J, Palma S D, Ferrero A A, Band B F.2014. Design and development of aqueous nanoformulations for mosquito control. Parasitology Research 113: 793-800.

Phillips M. 2008. Dengue reborn: Widespread resurgence of a resilient vector. *Environmental* Health Perspectives 116(9):A382-A388.

Reynolds T, Dweck A C. 1999. *Aloe vera* gel: a review update. Journal of Ethnopharmacology 68: 3-37.

Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chithong U, Kanajanapothi D, Rattanachanpichai E, Tippawankosal P, Riyong D, Tuetun B, Chaiyasit D. 2007. Aromatic plant-derived essential oil: an alternative larvicide for mosquito control. Journal of Fitoterapia 78: 205-210.

Riat A K, Kocher D K. 2017. Study of histoarchitectural changes in *Anopheles stephensi*larvae following exposure to *Eucalyptus globulus* and *Aloe vera*oils. Turkish Journal of Zoology 41: 763-773.

Riat A, Kocher D K. 2019.Larvicidal potential of *Eucalyptus globulus* oil against *Anopheles stephensi.* International Journal of Mosquito Research 6 (2): 24-26.

Robertson J L, Russel R M, Savin N E. 1980. POLO: A User's Guide to Probit or Logit Analysis. Pacific South-West Forest and Range Experiment Station, Berkeley, U.S.A.

Shafiq S, Shakeel F, Talegoankar S, Ahmad F J, Khar R K, Ali M. 2007. Development and bioavailabilty assessment of ramipril nanoemulsion formulation. European Journal of Pharmaceutics and Biopharmaceutics 66:227-243.

Singh K V, Bansal S K. 2003. Larvicidal properties of a perennial herb *Solanum xanthocarpum* against vectors of malaria and dengue/ DHF. Current Science 84:749-751.

Sugumar S, Clarke S K, Nirmala M J, Tyagi B K, Mukherjee A, Chandrasekaran N. 2014. Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus.* Bulletin of Entomological Research 104: 393-402.

Wattanachai P, Tintanon B. 1999. Resistance of *Aedes aegypti* to chemical compounds in aerosol insecticideproducts in different areas of Bangkok, Thailand. Journal of Communicable Diseases 25: 188-191.

Yang Y C, Lee S S, Lee H K, Kim M K, Lee H S. 2002. A piperidine amide extracted from Piper longumlarvae fruit shows activity against *Aedes aegypti* mosquito larvae. Journal of Agricultural and Food Chemistry 50: 3765-3767.

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