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Comparative efficacy of conventional insecticide and eco-friendly biopesticide in the management of *Anopheles stephensi* mosquito (Vector of *Plasmodium* parasite): A synthesis of child outcomes.

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ABSTRACT

Of the total deaths annually due to malaria, most of which are children under the age of 5 years worldwide (WHO 2015). Malaria is caused by parasites from the genus *Plasmodium*. For the control of malaria parasite *Plasmodium* spp., the vector mosquito *Anopheles* has to be controlled. As the mosquitoes complete their development in water, their control in aquatic habitat is easier. The synthetic insecticides, due to their quick action, are considered as the first line of action, but their large scale use has resulted in environmental hazards and development of resistance. Therefore, environmentally safe plant products having insecticidal properties are considered to be a potential alternative tool in mosquito control programs in the recent time. The present study was specifically designed to evaluate the comparative larvicidal activity of conventionally used synthetic insecticide cypermethrin and a leaf extract of naturally occurring *Parthenium hysterophorus* against the 4th instar larvae of *Anopheles stephensi*. The static exposure of five different concentration viz., 10, 20, 40, 60 and 80 mg/L of cypermethrin to the 24 hrs old 4th instar larvae of *A. stephensi* caused 39.5%, 46.75%, 60.25%, 71.25% and 83.25% mortality respectively as compared to control (2.5%), however, the same concentrations of leaf extract of *Parthenium hysterophorus* resulted in 14.25%, 26.50%, 38.75%, 45.5% and 59.25% mortality as compared to control (3.75%). These results suggest that the leaf extracts of *Parthenium hysterophorus* could be useful as an alternative for synthetic insecticides for the control of the *A. stephensi*. Further detailed research is needed to identify the active ingredient in the extracts that can be use in mosquito management program.

Key words: *Anopheles stephensi, Parthenium hysterophorus, Cypermethrin*

Introduction

Arthropods are awfully perilous vectors of pathogens and parasites that may hit as epidemics or pandemics in the increasing world population of humans and animals (Bonizzoni et al., 2013; Mehlhorn et al., 2012; Benelli et al., 2016). Among them, mosquitoes (Diptera: Culicidae) act as vectors, transmitting important pathogens and parasites of so

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many noxious diseases such as malaria, dengue, chikungunya, Japanese encephalitis, Zika virus and lymphatic filariasis (Jensen and Mehlhorn, 2009; Benelli and Mehlhorn, 2016; Pastula et al., 2016; Saxena et al., 2016). Each year, mosquito-borne diseases infect nearly 700 million people, resulting more than one million deaths (Amerasan et al 2016). Unfortunately, no treatment is on hand for most of the arboviruses transmitted by mosquitoes, with special reference to malaria which is an important cause of global morbidity and mortality (Smith et al., 2005) that affects 350–500 million people each year (Murugan et al., 2015) living in many parts of the world and kills over 500,000 people annually, most of which are children under the age of 5 (WHO, 2015). Malaria is caused by *Plasmodium* parasites through the bites of infected *Anopheles* mosquitoes. Mosquito are usually targeted with synthetic insecticides, insect growth regulators and microbial control agents, however, there indiscriminate use cause development of resistance, biological magnification through food chain thereby resulting strong adverse effects on the human health and environment.

To overcome these problems, major emphasis has been made on the use of natural products which can provide an alternate to synthetic insecticides (Junwei *et al*., 2006). Many plants have been found to contain toxic chemicals which are helpful for the control of insects (Robert, 2001) and are useful for field applications in mosquito control programmes (Sethuraman *et al*., 2010; Yankanchi and Patil, 2010; Arivoli *et al*., 2011; Palanisamy *et al*., 2012; Muthu *et al*., 2012; Patil *et al*., 2014). The pharmacological and insecticidal properties of plants have been recognized in many parts of the world, especially in India, where plant materials are easily available and their use in health practices is a tradition.

Therefore, this study was carried out with the aim to investigate the comparative efficacy of plant *Parthenium hysterophorus* and conventionally used insecticide cypermethrin against the larvae of *Anopheles* mosquito.

Parthenium hysterophorus is a belligerent ever-present annual herbaceous weed belonging to Asteraceae family, with no economic importance. It is commonly called as Santa maria or famine weed. In India, it is locally known as carrot grass, congress grass or *Gajar Ghas*. Chemical analysis of *P. hysterophorus* (Seema, 2011) has indicated that all its parts including trichomes and pollen contain toxins called sesquiterpene lactones (SQL). Maishi et al.1998 reported that *P. hysterophorus* contains a bitter glycoside parthenin. Cypermethrin is a type II pyrethroid insecticide which is widely used throughout the world in agriculture, forestry, horticulture, public health and animal husbandry.

Materials and Methods

The mosquito larvae of *Anopheles stephensi* were collected from various parts of AMU campus and were reared to adults in the laboratory at 75±5% relative humidity, 27± 2°C temperature and 14L:10D. The larvae were fed on a powdered mixture of dog biscuits and yeast tablets in the ratio of 3:1. The emerged adults were fed with rat blood and with 10% glucose solution (Patil *et al*., 2014). The plant leaves of *Parthenium hysterophorus* were collected from the Aligarh Muslim University Campus, shade dried at room temperature, powdered coarsely and extracted with petroleum ether (BP 60-80°C) in the soxhlet apparatus for 8-10 hrs (500 ml of petroleum ether for 100 g) according to Karmegam *et al*. (1997) method. The weighed quantity of the plant material was reduced to a viscous dark green residue and the crude extracts were further concentrated to paste. Five different concentrations of *Parthenium hysterophorus* and cypermethrin insecticide *viz*. 10mg, 20mg, 40mg, 60mg and 80mg/L aqueous solutions were prepared in ten separate jars. In each jar, 100 4^{th} instar larvae (24h old) of *A. stephensi* were introduced for static larvicidal essay. Each experiment was conducted with four replicates and control was also run simultaneously (WHO, 2005). Dead larvae were counted after 06 hrs, 12 hrs, 18 hrs and 24 hrs.

Results and Discussion

Mosquitoes are the principal vectors of many diseases that contribute to major disease burden in India. The disease transmission can be interrupted by controlling the vectors using various methods. The larval stages of the mosquitoes are attractive targets for pesticides exposure because they breed in water and are easy to deal them in this habitat. The indiscriminate application of conventionally used synthetic pesticides in the water body has built up resistance in vector mosquitoes necessitating exploring of some better alternative means (Hag et al 1999). The biopesticides, especially those derived from plants, have the capability to replace these conventionally used insecticides (Amer and Mehlhorn, 2006). A considerable number of plant materials have been tested against the three medically important mosquito genera *Aedes*, *Anopheles* and *Culex* showing susceptibility to such biopesticides (Shaalan, et al 2005). Sukhthankar et al (2014) carried out larvicidal bioassays with methanolic extract of leaves of *Chromolaena odorata* (family Asteraceae) against late instar larvae of disease vectors *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* and recorded highest mortality (90%) in *Cx. quinquefasciatus* at 110 ppm followed by *Ae. aegypti* at 138 ppm and *An. stephensi* at 8306 ppm. Rajan and Varghese (2017) studied the comparative larvicidal activity of aqueous extracts of dried leaf powder of *Lantana camara* and *Catharanthus roseus* against the larvae of mosquito and reported that *Lantana camara* was an ideal candidate as a larvicide than *Catharanthus roseus* and required 80mg/100ml concentration to kill 100% larvae in six hours.

The present results also showed the larvicidal activity of different concentrations of conventionally used cypermethrin and leaf extract of *Parthenium hysterophorus* against the 4th instar larvae of *Anopheles stephensi* which have been shown in figure 01and 02. The static exposure of five different concentration viz., 10, 20, 40, 60 and 80 mg/L of cypermethrin to the 24 hrs old 4th instar larvae of *A. stephensi* for different durations caused varied mortality. The exposure of 10, 20, 40, 60 and 80 mg/L of cypermethrin for 6 hours duration caused 10.25, 13.25 20.25, 22.75 and 26.25% death of the 4th instar larvae respectively (Fig. 01) as compared to control (1-1%), however, the exposure of the same concentration of leaf extract of *Parthenium hysterophorus* for similar duration showed 3.5, 5.75, 6.25, 8.75 and 11.25% larval loss respectively (Fig.02) as compared to control (1.25). Similarly the exposure of same concentrations of cypermethrin for 24 hrs duration caused 39.5%, 46.75%, 60.25%, 71.25% and 83.25% mortality respectively as compared to control (2.5%), however, the same concentrations of leaf extract of *Parthenium hysterophorus* resulted in 14.25%, 26.50%, 38.75%, 45.5% and 59.25% mortality as compared to control (3.75%).

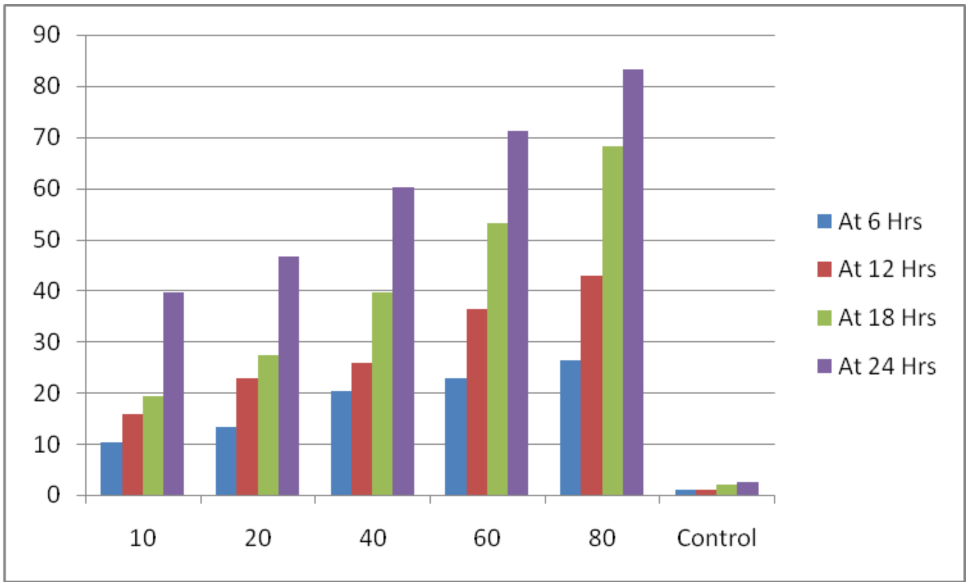

Fig.01. Mean mortality of 4th instar larvae of *Anopheles stephensi* following static exposure of different concentrations of cypermethrin for different durations.

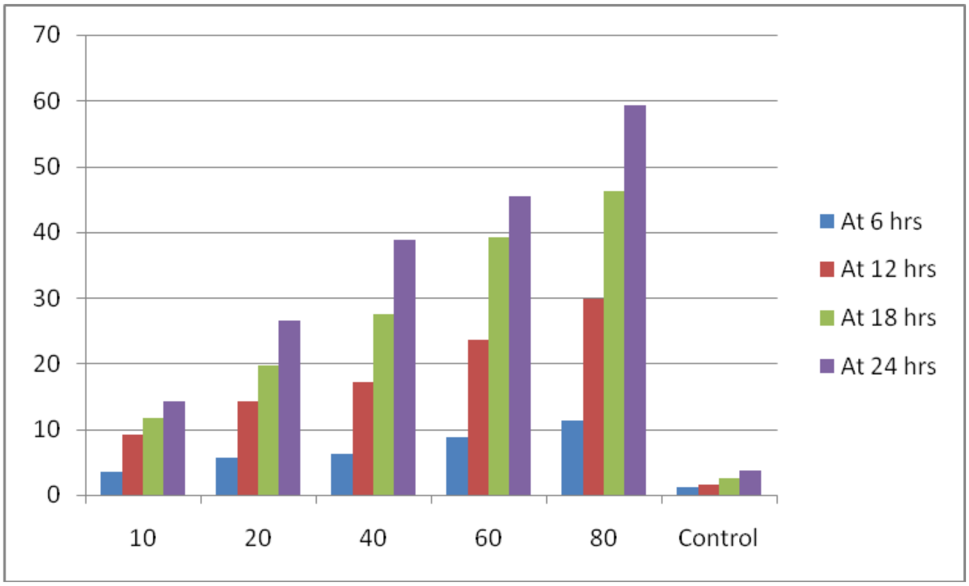

Fig.02. Mean mortality of 4th instar larvae of *Anopheles stephensi* following static exposure of different concentrations of *Parthenium hysterophorus* for different durations.

Mohan et al (2007) studied the comparative toxicity of cypermethrin alone and in combination with the root extract of *Solanum xanthocarpum* against anopheline larvae and recorded that petroleum ether extract was most toxic, with LC50 of 1.41 and 0.93 ppm and LC90 of 16.94 and 8.48 ppm at 24 and 48 hours after application, respectively, followed by carbon tetrachloride and methanol extracts. The values for cypermethrin were an LC50 of

0.0369 ppm after 24 hours and 0.0096 ppm after 48 hours and LC90 of 0.0142 and 0.0091 ppm after 24 and 48 hours, respectively. The ratios of cypermethrin and petroleum ether extracts tested were 1:1, 1:2 and 1:4. Of the various ratios tested, the cypermethrin and petroleum ether extract ratio of 1:1 was observed to be more efficient than the other combinations.

The larvicidal, pupaecidal, repellent, ovicidal and oviposition-deterrent activity of various plants against the *Anopheles stephensi* have also been studied by Ramkumar et al (2015), Dinesh, et al (2015), Hadjiakhoondi (2006), Roni et al (2013), Elango et al (2010). Maurya et al (2007) also studied the larvicidal activity of leaf extracts of *Aloe barbadensis* (Liliaceae) and *Cannabis sativa* (Moraceae) against the *Anopheles stephensi* under laboratory conditions and reported that the carbon tetrachloride extract of *A. barbadensis* was the most effective as compared to the other extracts tested. Yadav et al. (2013) evaluated the larvicidal properties of plant extract of *Thevetia peruviana* in different solvents against the larvae of malaria and dengue vectors and observed that the leaf extracts in acetone solvent was most effective (LC50 0.0268% against *An. stephensi* and 0.0219% against *Aedes aegypti*), while chloroform extract was least effective (LC50>0.05%). Raghavendra (2009) studied the comparative efficacy of the aqueous and hexane extracts of dried fruit of *Solanum nigrum* against *Anopheles culicifacies*, *An. stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* and showed that the hexane extract posses good mosquito larvicidal efficacy killing 100% larvae at 100 ppm as compared to aqueous extract which registered the same mortality at 1000 ppm. Shamia et al (2006) evaluated the larvicidal efficacy of *Ageratum conyzoides, Argemone mexicana* and *Azadirachta indica* against *Anopheles stephensi* to develop an effective ecofriendly insecticide of plant origin and recorded that methanol extract of *A. indica* was remarkably more effective as compared to petroleum ether extracts of *Ag. conyzoides* , carbon terra chloride extract of *Ar. Mexicana* and petroleum ether extract of *Az. Indica.* The impact of Az. *Indica* methanol extract on the life cycle of malaria vector was further recorded and observed that methanol extract of Az. *Indica* causes deformities in different developmental stages, extending the developmental period, reducing the hatching rate, influencing adult emergence and prolonging the larval-pupal periods of the malaria vector.

Meenakshi and Jayaprakash (2014) tested different dilutions (100, 200, 300, 400, 500 ppm) of the crude extract of mangrove plant *Rhizophora mucronata* for larvicidal property against *Anopheles stephensi* and *Aedes aegypti* and reported that the larva of *A. stephensi* was more susceptible than *Aedes aegypti.* Varun et al (2013) studied the activity of leaf extracts of three invasive weeds- *Vernonia cinerea, Prosopis juliflora* and *Cassia tora* against 3rd instar larvae of *Anopheles stephensi* and showed that the leaf extracts of these plants can be use as ecofriendly larvicide. The exposure of 2% dilution of leaf extract of *Agave sisalana* to the 3rd instar larvae of *An. stephensi* produced 100 mortality, whereas, same mortality is achieved by the application of 1% dilution to the larvae of *Cx. quinquefasciatus* and *Ae. Aegypti* (Singh et al., 2014)

Batabyal et al (2007) tested the toxicity of seed extracts of three Indian medicinal plants, *Azadirachta indica, Momordica charantia* and *Ricinus communis against the larvae of Anopheles stephensi* and reported that *A. indica* methanol extract can be use as alternate to synthetic insecticides. Similarly the ethanolic leaf extract of *C. obtusifolia* against *A. stephensi* can also be use as an alternative to synthetic insecticide in mosquito control programs (*Rajkumar and Jebanesan, 2009)*.

Nathan et al (2005) studied the larvicidal, pupicidal, adulticidal and antiovipositional activity of neem limonoids against *Anopheles stephensi* Liston (Diptera: Culicidae) and observed that Azadirachtin, salannin and deacetylgedunin posses high bioactivity at all doses as compared to the rest of the neem limonoids. The study of Panneerselvam et al (2012) to evaluate the larvicidal, pupicidal, repellent, and adulticidal activities of methanol crude extract of *Artemisia nilagirica* against two important vector mosquitoes, viz., *Anopheles* *stephensi* and *Aedes aegypti* (Diptera: Culicidae) suggests that the leaf extract have the potential to be used as an ideal eco-friendly approach for the control of vector mosquito as target species. Kamaraj et al (2010) studied effect of various parts (viz., leaf, flower, bark and root) of ten medicinal plants viz., *A. vasica, A. squamosa, C. auriculata, H. indicus, H. javanica, P. somniferum, P. zeylanica, S. indicum, S. torvum,* and *V. negundo* in different solvents viz., hexane, chloroform, ethyl acetate, acetone and methanol and reported that highest larval mortality was found in leaf acetone extract of *Adhatoda vasica*, bark ethyl acetate extract of *Annona squamosa*, methanol leaf and flower extract of *Cassia auriculata*, leaf ethyl acetate extract of *Hydrocotyle javanica*, methanol leaf and seed extract of *Solanum torvum* and leaf hexane extracts of *Vitex negundo* against the fourth instar larvae of *An. stephensi* and *Cx. quinquefasciatus.* Further they suggest that the leaf methanol extract of *S. torvum* and bark ethyl acetate extract of *A. squamosa* from Southern India have the potential for use to control mosquitoes. Patil et al (2010) studied the comparative larvicidal activity of crude chloroform, dichloromethane and methanol extracts of the leaves and roots of six Indian plants, *Aegle marmelos* L., *Balanites aegyptica* L., *Calotropis gigantica* L., *Murraya koenigii* L., *Nyctanthes arbor-tristis* L. and *Plumbago zeylanica* L., against the early fourth instar larvae of *Aedes aegypti* L. and *Anopheles stephensi* and reported that the methanol extracts of plants were more effective than the other extracts. Varied larvicidal and ovicidal activity of leaf extract of *Acalypha indica* in different solvents viz, benzene, chloroform, ethyl acetate and methanol against *Anopheles stephensi* was also reported by Govindarajan et al (2008).

Govindarajan in 2011 further studied the larvicidal and ovicidal activity of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of the leaf of three plants, *Eclipta alba*, *Cardiospermum halicacabum*, and *Andrographis paniculata*, against the early third-instar larvae of *Anopheles stephensi* (Liston) (Diptera: Culicidae) and reported that all extracts showed moderate larvicidal effects; however, the highest larval mortality (100%) was found with methanol extract of *E. alba* at 200 ppm. The methanol extracts of *Pelargonium citrosa* leaf for their biological, larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency and biting deterrency was also tested against *Anopheles stephensi*. The larval mortality was dose dependent. The extracts significantly decreased fecundity, egg hatchability and longevity. The larval, pupal and adult growth and development was completely inhibited by the treatment. The leaf extract treatment also significantly enhanced biting deterrency. Therefore, these plant derived materials could be useful as an alternative for synthetic insecticides controlling field populations of mosquitoes (Jeyabalan et al 2003). The adulticidal, repellent, and ovicidal potential of the crude hexane, ethyl acetate, benzene, aqueous, and methanol solvent extracts of some medicinal plants viz., *Andrographis paniculata*, *Cassia occidentalis*, and *Euphorbia hirta* against the mosquito vector, *Anopheles stephensi* (Diptera: Culicidae) was also studied by Panneerselvam and Murugan (2013) who observed that all extracts showed moderate adulticide effects; however, the highest adult mortality was found in methanol extract of *A. paniculata* followed by *C. occidentalis* and *E. hirta.* On the basis of the results they suggest that the leaf extracts of *A. paniculata*, *C. occidentalis*, and *E. hirta* have the potential to be used as an ideal eco-friendly approach for the control of the *A. stephensi.*

To conclude, the results of the present study showed that the leaf extracts from *Parthenium hysterophorus* can be used in the control of mosquito vector *Anopheles stephensi* in malaria management programme. Further study is required to purify the active compounds from this plant for developing larvicide.

Child outcome projections:

As the application of pesticides has turned out to be an obligatory input to agriculture and public health, their versatile use had resulted in contamination of all basic necessities of life, i.e. air, water and food. The possible hazards from pesticide residues in food have been much discussed and hotly debated in the scientific literature, the popular press and the political arena. However, the indiscriminate use of these pesticides, due to their high killing efficiency, in mosquito management developed resistance in the target insect on one hand and contaminate the environment reaching the human body through food on other hand resulting in human health problems especially in children. Earlier studies also show that the main route of pesticide exposure in urban areas is through dietary intake of fruits and vegetables (Tang et al., 2018). The presence of cypermethrin residues along with other pesticides in fruits and its health risks for the infants, toddlers and adults was studied by Lozowicka, et. al (2016). The study found that the fruit with the highest contribution to the ADI were found to be apples (316%, 58%), cherries (96%, 37%) and pears (129%, 33%) for infants and adults. Findings further indicated that dietary exposures to insecticide residues in fruit may be likely to pose certain health risks for the infants and toddlers. Likewise, Fluegge, Nishioka, & Wilkins (2016), while studying the effects of simultaneous prenatal exposures to organophosphate and synthetic pyrethroid insecticides on infant neurodevelopment at three months of age found that both insecticide exposures have been inconsistently linked with poorer neurodevelopmental outcomes. On the same lines, Sun, et. al (2011) conducted an estimation of dietary exposure to cypermethrin residues for the Chinese children. The study revealed that one vegetable and one fruit covered 30.7 and 22.5% of the total intake for children, respectively. Exposure to cypermethrin was found alarmingly high amongst rural children of the chosen population. Galea et al (2015) investigated exposure to cypermethrin in relation to spray events on children living near the agricultural lands. Although the study could not establish a link between high exposure risk and spraying event but the sample was nevertheless at high risk of exposure to the pesticide with bad health outcomes. In their monumental systematic review, Burns et al (2013) collated evidence of adverse neurodevelopmental outcomes in infants and children due to pre-partum exposure to pesticides that includes cypermethrin.

Since natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors, *Parthenium hysterophorus* is among one of them which is a well known herb in India. Bagchi, Raha and Mukherjee (2016) through an extensive review, studied the threats and benefits of this herb. While hedonising the effects, they found the herb as a multi-faceted indigenous medicine that has benign effects on children while used as a pesticide. Kalaiselvi et al (2013) tested the antioxidant potential and antimicrobial potential of the leaf extract *Parthenium hysterophorus*. The antioxidant property was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and hydrogen peroxidase assay. The anti-bacterial and antioxidant properties were found to be better for the aqueous leaf extract and the extract containing the silver nanoparticles.

To conclude, *P. hysterophorous* may be chosen as a preferred option as a pesticide as compared to cypermethrin while considering environmental safety and general wellbeing of human population especially children.

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