LARVICIDAL ACTIVITY OF THE COLD AND HOT WATER LEAF EXTRACTS OF *AZADIRACHTA INDICA* A. JUSS (MELIACEAE) AGAINST THE LARVAE OF *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

ADETIMEHIN, Adeyemi Daniel and UYI, Osariyekemwen Osa
Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria.

Corresponding Author: Uyi, O. O. Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. **Email:** osariyekemwen.uyi@uniben.edu **Phone:** +2348038013012

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ABSTRACT

This study investigated the potency of the cold and hot water leaf extracts of Azadirachta indica A. Juss (Meliaceae) against the third instar larvae of Culex quinquefasciatus (Say) (Diptera: Culicidae), the vector of lymphatic filariasis. Ten actively swimming third instar larvae of Cx. quinquefasciatus were exposed to different concentrations of 0.0, 1.0, 2.0 and 3.0 % (w/v) of cold and hot water leaf extracts of A. indica, after which percentage larval mortality was monitored for 24 and 48 hours. Results from this study revealed that both extract types (cold and hot water) of A. indica demonstrated excellent larvicidal activities against the larvae of Cx. quinquefasciatus at all tested concentrations (excluding the control – 0.0 % w/v). Percentage larval mortality was slightly a function of both concentration and exposure duration. Following a 48 hour exposure period of Cx. quinquefasciatus larvae to different concentrations of A. indica cold and hot water leaf extracts, the highest concentration (3.0 % w/v) of both extract types was observed to demonstrate a perfect larvicidal activity (100 %) against the test insect. In conclusion, this study recommends the application of A. indica cold and hot water leaf extracts in the control of Cx. quinquefasciatus specifically at their potential breeding sites and also in areas where lymphatic filariasis is endemic.

Keywords: *Azadirachta indica*, Leaf extracts, Larvicidal activity, *Culex quinquefasciatus*

INTRODUCTION

Mosquitoes are nuisance species found all over the world (with the exception of completely frozen areas) and are chiefly responsible for many of the vector-borne diseases affecting humans and other animals (Aigbodion and Uyi, 2013; Baskar *et al*., 2016). Several mosquito species including those belonging to the genera *Anopheles*, *Culex* and *Aedes*, have been reported to possess the capacity of transmitting dreadful, debilitating and life threatening diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya, yellow fever and zika virus to more than two-fifth of the world population (Mohan and Ramaswamy, 2007; Nour *et al*., 2012). *Culex quinquefasciatus* Say (Diptera: Culicidae), is a major vector of *Wuchereria bancrofti* which causes bancroftian filariasis. This mosquito species is commonly found within residential houses, with high prevalence in urban and/or semi-urban areas, particularly in environs surrounded by polluted stagnant water bodies (e.g. gutters), where it constitutes a biting nuisance (Aigbodion *et al*., 2011).

In Africa, the prevalence of lymphatic filariasis is quite alarming, with over 40 million people in the sub-Saharan region affected (Aigbodion *et al*., 2011). Nigeria is believed to bear the highest burden of lymphatic filariasis (elephantiasis), with an estimated 80 to 120 million people at risk (Okorie *et al*., 2013). This disease often triggers clinical manifestations

including but not limited to lymphoedema, lymphangitis, itching, creeping sensations, arthritis and chyluria (Abdullahi *et al*., 2015). In addition, infection with lymphatic filariasis invariably brings about a significant reduction in man hours, absence from school, reduced economic growth, unforeseen expenses on treatment and prevention amongst others (Abdullahi *et al*., 2015).

Despite the fact that several efforts and resources have been invested in the field of mosquito/vector control, the medical and economic burden caused by mosquito-borne diseases continues to grow due to the inefficiency of current control measures. For this reason, there is an urgent need to identify new techniques in tackling the menace caused by these insect pests. With respect to vector control, the use of broad spectrum synthetic insecticides is the most widely practiced technique, and its usage over the years has produced remarkable results (Baskar *et al*., 2016). However, following the indiscriminate use of these insecticides by man in recent times, a number of undesirable outcomes such as the evolution of resistance strains of insect pests, hazards to human health and non-targeted organisms, environmental pollution amongst others, have emanated (Remia and Logaswamy, 2010). These unexpected outcomes have stimulated renewed interests in the need to search for easily affordable, available, user- and eco-friendly alternatives in the management of insect pests.

In line with the search for sustainable alternative approaches to synthetic insecticides in mosquito control, one of the most widely applied methods is through the utilization of natural products from plant origin. In accordance to the use of plant products, one of the most extensively used plant species is *Azadirachta indica* (also known as neem), a medium-to-large, deep rooted, evergreen tree, native to dry areas of India, Afghanistan and Pakistan (Alouani *et al*., 2009). Following its widespread introduction and cultivation in the tropics and subtropics, several studies have empirically demonstrated its usefulness in insect pest control and ethno-medicine (Chakkaravarthy *et al*., 2011; Raja *et al*., 2013). Several authors have reported its anti-tumor, anti-ulcer, anti-microbial, anti-malaria, anti-fertility and anti-diabetic activities (Paul *et al*., 2011; Maragathavalli *et al*., 2012; Kavitha *et al*., 2017). With respect to pest control, there exist a plethora of publications on the efficacies of extracts, powders and/or oils from several parts of *A. indica* plant against several insect pests including mosquitoes (Baskar *et al*., 2016). For instance, Maragathavalli *et al*. (2012) reported that the methanolic and ethanolic leaf extracts of *A. indica* caused maximum mortality against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* after 24 hours. Similarly, Shankar *et al*. (2013), examining the repellent potentials of some local plants including *A. indica*, revealed that burning the leaves of *A. indica* using glowing charcoal repelled significant numbers of mosquitoes with a 6 hour protection time in all selected houses.

Although, studies focusing on the larvicidal activities of *A. indica* leaf extracts using expensive and volatile extracting solvents such as methanol, acetone, ethyl acetate, ethanol, petroleum ether and chloroform are not uncommon (Okigbo *et al*., 2010; Chakkaravarthy *et al*., 2011; Nour *et al*., 2012;Maragathavalli *et al*., 2012; Okbatinsae and Haile, 2017), but studies on the larvicidal activity of the aqueous leaf extracts of *A. indica* using only water as the extracting solvent are scanty (Rashid and Ahmad, 2013). This study seeks to present a more user-friendly and cost effective means of controlling mosquito species using the larvicidal activities of the hot and cold water leaf extracts of *A. indica* against the third instar larvae of *Cx*. *quinquefasciatus*.

MATERIALS AND METHODS

Collection and Preparation of Plant Powders: Fresh leaves of *A. indica* were collected from a tree located in an open field within the vicinity of the National Youth Service Corps (NYSC) School (12° 26′ 40.8″N, 04° 12′ 18.1″E) Birnin Kebbi, Kebbi State, Nigeria. Following collection, the leaves were washed with running distilled water, chopped and air-dried in the general laboratory of the school to a constant weight. The dried leaves were blended

into fine powder using an electric blender (Pyramid, PM – 444B3, People's Republic of China). The relatively homogenous powder was then preserved in an air-tight and water-proof container prior to the onset of the experimental trials.

Cold and Hot Water Extracts Preparation: Cold water extract of *A. indica* was prepared by weighing 2.0, 4.0, and 6.0 g of *A. indica* leaf powder in separate plastic containers with screw caps. Thereafter, 200 ml of distilled water was added into each container containing different concentrations (grams) of *A. indica* leaf powder. The resulting mixtures were kept for 48 hours with periodic shaking (Rashid and Ahmad, 2013), then filtered with the aid of a filter paper (Whatman No. 1) to obtain three different concentrations viz. 1.0, 2.0 and 3.0 % w/v of *A. indica* leaf extracts.

Hot water extract of *A. indica* was prepared using the same method stated above. However, 200 ml of freshly boiled distilled water was used to dissolve the plant powders (1.0 – 3.0 g) in their respective containers, to obtain three different concentrations (1.0, 2.0 and 3.0 % w/v) of *A. indica* leaf extracts.

Collection of Mosquito Species: Freshly laid eggs of *Cx. quinquefasciatus* in rafts were collected from an open stagnant water (gutter) within Birnin Kebbi metropolis (12° 26′ 53.1″N, 04° 12′ 0.4″E), Kebbi State, Nigeria. The collection site is characterized by the presence of different kinds of polyethylene bags, decomposing food substances amongst other dirt. After collection, the eggs alongside water samples from the collection site were transported to the general laboratory, National Youth Service Corps (NYSC) School, Birnin Kebbi, Kebbi State, Nigeria, after which they were transferred into a 5 liter transparent plastic bucket. Following a 6 day post collection period, the newly molted third instar larvae of about the same age were used for the experimental bioassay.

Larval Bioassay: The experiment was conducted in the general laboratory, National Youth Service Corps (NYSC) School, Birnin Kebbi, Kebbi State, Nigeria. To perform the larvicidal bioassay, 30ml each of different concentrations (1.0%, 2.0%, and 3.0% (w/v)) of *A. indica* cold and hot water leaf extracts was measured into labeled 100ml transparent plastic cups. Thereafter, 10 actively swimming third instar larvae of *Cx. quinquefasciatus* from the stock population were introduced into each of the plastic cups. A control treatment (0 % w/v) were distilled water (30 ml) was used was also setup for comparison. Each treatment (concentration) including the control was replicated four (4) times. The experiment was laid out in a completely randomized design (CRD). Larval mortality was monitored after 24 and 48 hours. Live and dead larvae were counted and percentage mortality was calculated using the equation: number of dead larvae/ total number of larvae x 100 %. Larvae were confirmed dead when there was no response to probing with the blunt end of a pin. Dead larvae were removed as soon as possible in order to prevent decomposition which may cause rapid death of the remaining larvae (Remia and Logaswamy, 2010).

Statistical Analysis: The effects of concentration of *A. indica* (hot and cold water extract) and exposure time on the percentage mortality of *Cx. quinquefasciatus* were analysed using a two way analysis of variance (ANOVA). When the overall results were significant, the differences among the treatments were compared using the Tukey's Honest Significant difference (HSD) test. The effect of hot and cold water leaf extracts on the percentage mortality of the mosquito larvae was analysed using student's *t* test. All analyses were performed using Genstat 12.0 (VSN International, Hemel Hempstead, UK).

RESULTS

***Azadirachta indica* Cold Water Leaf Extract:** The cold water leaf extract of *A. indica* exhibited excellent larvicidal activity against the larvae of *Cx. quinquefasciatus* at all tested concentrations compared to the control. Larval mortality significantly differed as a function of both concentration ($F_{3, 31} = 300.24$; $p<0.001$)

and exposure time ($F_{3, 31}$ = 29.04; p<0.001) (Figure 1). Following a 24 hour exposure period of *Cx. quinquefasciatus* larvae, the control (0.0 % w/v) exhibited the lowest larvicidal activity (0 % mortality), followed by the 1.0 % w/v treatment which caused 30 % mortality (Figure 1).

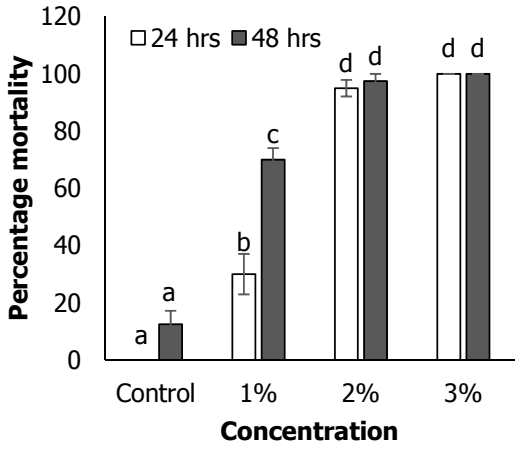

Figure 1: **Percentage mortality of *Culex quinquefasciatus* larvae caused by different concentrations of *Azadirachta indica* cold water leaf extract following a 24 and 48 hour exposure period**. *Means capped with different letters are significantly different [after Tukey's Honest Significant Difference (HSD) Test: P < 0.05)]*

There was no significant difference between the 2.0 and 3.0 % w/v treatments as they both accounted for 95 and 100 % larval mortalities respectively after a 24 hour exposure interval (Figure 1). At the end of 48 hour exposure duration, 2.0 and 3.0 % w/v treatments of *A. indica* cold water leaf extract exhibited significantly higher (p<0.001) mortalities of *Cx. quinquefasciatus* (97.5 and 100 % respectively) when compared to the control (12.5 %) and the 1.0 % w/v (70 %) treatments (Figure 1). Finally, a significant interaction ($F_{3, 31}$ = 12.88; p<0.001) was found to exist between the concentrations of *A. indica* cold water leaf extract and the duration of exposure.

***Azadirachta indica* Hot Water Leaf Extract:** The hot water leaf extract of *A. indica* demonstrated significant larvicidal activity against the larvae of *Cx. quinquefasciatus* at all concentrations excluding the control. Larval mortality significantly differed as a function of both concentration ($F_{3, 31}$ = 100.87; p<0.001) and exposure time ($F_{3, 31}$ = 76.53; p<0.001) (Figure 2). At the end of a 24 hour exposure period, percentage larval mortality was significantly higher across all concentrations with the control causing the least mortality (0 %) (Figure 2).

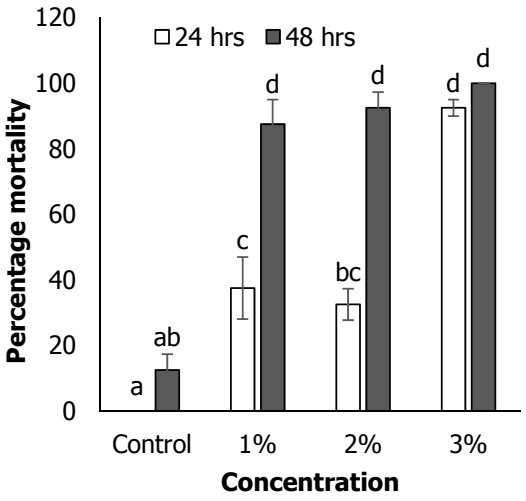

Figure 2: Percentage mortality of *Culex quinquefasciatus* larvae caused by different concentrations of *Azadirachta indica* hot water leaf extract following a 24 and 48 hour exposure period. *Means capped with different letters are significantly different [after Tukey's Honest Significant Difference (HSD) Test: P < 0.05]*

In contrast, no significant difference was observed among all the three concentrations (1.0, 2.0 and 3.0 % w/v) of *A. indica* hot water leaf extract, as they all accounted for 87.5, 92.5 and 100 % mortalities respectively of *Cx. quinquefasciatus* larvae following 48 hour exposure period (Figure 2). There was a significant interaction ($F_{3, 31}$ = 12.6; p<0.001) between the concentration and period of exposure. Finally, the percentage mortality cause by the cold and hot water leaf extracts did not differ ($t_{1,9}$ = 0.93; P = 0.390) (Figure 3).

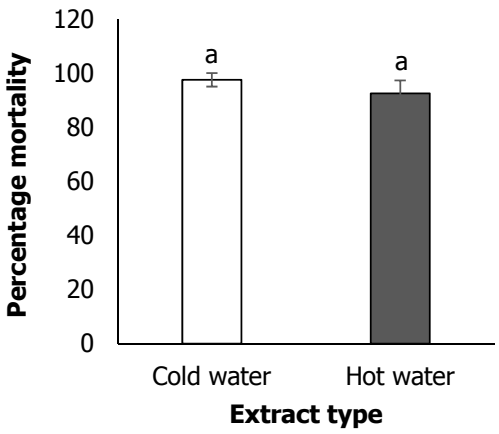

Figure 3: Comparative effects of the cold and hot water leaf extracts of *Azadirachta indica* against the larvae of *Culex quinquefasciatus* following a 48 hour exposure period. *Means capped with the same letters are not significantly different (after a Two Sample t test: P > 0.05)*

DISCUSSION

This study investigated the larvicidal activity of the hot and cold water leaf extracts of *A. indica* against the larvae of *Cx. quinquefasciatus*, the principal vector of lymphatic filariasis. Results from this study revealed that both extract types demonstrated excellent larvicidal activities against *Cx. quinquefasciatus* larvae. Admittedly, there exist a plethora of published articles and empirical evidences on the insecticidal activities of several parts (leaves, stems and roots) of *A. indica* against several insect pests including mosquitoes (Baskar *et al*., 2016). Similarly, a number of studies have empirically demonstrated the larvicidal activities of the leaf, stem and root extracts of *A. indica* using volatile and expensive extracting solvents such as acetone, methanol, ethanol, chloroform, petroleum ether and ethyl acetate against the larvae of several mosquito species including *Cx. quinquefasciatus* (Chakkaravarthy *et al*., 2011; Maragathavalli *et al*., 2012; Nour *et al*., 2012). However, studies focusing on the larvicidal activities of the aqueous leaf extracts of *A. indica* are scarce (Rashid and Ahmad, 2013). In this study, the cold water leaf extract of *A. indica* demonstrated excellent larvicidal activity against the larvae of *Cx. quinquefasciatus* with percentage larval mortality increasing with an increase in the concentration of the leaf extract. For instance, following a 24 hour exposure period, the highest concentration (3 % w/v) of *A. indica* cold water leaf extract exhibited the highest larvicidal activity (100 %) against the larvae of *Cx. quinquefasciatus*. This result was consistent with the findings of other authors (Okigbo *et al*., 2010; Maragathavalli *et al*., 2012; Nour *et al*., 2012; Rashid and Ahmad, 2013) who reported high mortalities in insect pests such as *A. aegypti and Cx. quinquefasciatus* with increasing concentrations of the leaf, seed, and root extracts of *A. indica*. As has been documented in previous studies (Tonk *et al*., 2006; Okigbo *et al*., 2010; Nour *et al*., 2012; Okbatinsae and Haile, 2017), percentage larval mortality also increased with an increase in the duration of exposure. For example, following 48 hour exposure period, the 1.0 % w/v treatment accounted for 70 % mortality when compared to the 24 hours exposure interval where it only accounted for 30 % mortality. Similarly, the 2.0 % w/v treatment accounted for 97.5 % mortality of *Cx. quinquefasciatus* larvae at the end of the 48 hours exposure period.

Comparatively, the hot water leaf extracts of *A. indica* demonstrated a remarkable larvicidal activity against the test insect. At the highest concentration (3.0% w/v), the hot water leaf extract of *A. indica* caused 92.5 % mortality in *Cx. quinquefasciatus* after 24 hour exposure period, and this concurred with the findings of Tonk *et al*. (2006), Nour *et al*. (2012) and Rashid and Ahmad (2013) where mortalities of insect pests such as *A. aegypti*, *Cx. quinquefasciatus* and *Culex pipiens fatigans* increased significantly with increasing concentrations of the plant extracts used. Furthermore, percentage larval mortality increased with an increase in the period of exposure. At the end of 48 hour exposure period, the hot water leaf extracts of *A. indica* at the lowest concentration (1.0 % w/v) accounted for 87.5 % mortality, followed by the 2.0 % w/v treatment which caused 92.5 % mortality and

then the 3.0 % w/v treatment which exhibited 100 % larvicidal activity against the larvae of *Cx. quinquefasciatus*.

Conclusion: Undoubtedly, the high larvicidal activities of the hot and cold water leaf extracts of *A. indica* can be attributed to the presence of certain phytochemicals present in the leaves of the plant. Our claim can be validated by the findings of Kavitha *et al*. (2017) who conducted phytochemical analysis on the leaves of *A. indica*. The authors reported the presence of certain phytochemicals such as alkaloids, saponins, phenols, flavonoids, glycosides, tannins amongst others in the leaves of *A. indica*. In summary, this study presents a user- and cost effective alternative approach to conventional insecticides in the management of several mosquito species within urban, semi-urban, and rural areas. In addition, this recommends the application of cold and hot water leaf extracts of *A. indica* in the control of *Cx. quinquefasciatus*, particularly in the regions where lymphatic filariasis is endemic.

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REFERENCES

ABDULLAHI, A. G., ALAKU, I. A. and HUDU, S. B. (2015). Epidemiology of malaria and lymphatic filariasis; prevention and control under single disease control program in rural Nasarawa State, Nigeria. *Journal of Biology, Agriculture and Healthcare,* 5(2): 55 – 57.

AIGBODION, F. I. and UYI, O. O. (2013). Temporal distribution of and habitat diversification by some mosquitoes (Diptera: Culicidae) species in Benin City, Nigeria. *Journal of Entomology,* 10(1): 13 – 23.

AIGBODION, F. I., UYI, O. O., AKINTELU, O. H. and SALAU, L. A. (2011). Studies on some aspects of the ecology of *Culex quinquefasciatus* (Diptera: Culicidae) in relation to filarial infection in Benin City, Nigeria. *European Journal of Experimental Biology,* 1(4): 173 – 180.

ALOUANI, A., REHIMI, N. and SOLTANI, N. (2009). Larvicidal activity of a neem tree extract (Azadirachtin) against mosquito larvae in the Republic of Algeria. *Jordan Journal of Biological Sciences,* 2(1): 15 – 22.

BASKAR, K., MOHANKUMAR, S., SUDHA, V., MAHESWARAN, R., VIJAYALAKSHMI, S. and JAYAKUMAR, M. (2016). Meliaceae plant extracts as potential mosquitocides – a review. *Entomology, Ornithology and Herpetology,* 5: 172. DOI: 10.4172/2161-0983.1000172.

CHAKKARAVARTHY, V. M., AMBROSE, T., VINCENT, S., ARUNACHALAM, R., PAULRAJ, M. G., IGNACIMUTHU, S. and ANNADURAI, G. (2011). Bioefficacy of *Azadirachta indica* (A. Juss) and *Datura metel* (Linn.) leaves extracts in controlling *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Entomology,* 8(2): 191 – 197.

KAVITHA, M., RAJA, M., KAMARAJ, C., KARTHIK, R. R., BALASUBRAMANIAM, V., BALASUBRAMANI, G. and PERUMAL, P. (2017). In vitro antimicrobial activity of *Azadirachta indica* (leaves) against fish pathogenic bacteria isolated from naturally infected *Dawkinsia filamentosa* (Blackspot barb). *Medicinal and Aromatic Plants*, *6*(294), pp.2167-0412.DOI: 10.4172/2167-0412.

MARAGATHAVALLI, S., BRINDHA, S., KAVIYARASI, N. S., ANNADURAI, B. and GANGWAR, S. K. (2012). Mosquitoes larvicidal activity of leaf extract of neem (*Azadirachta indica*). International *Journal of Advanced Biological Research,* 2(1): 138 – 142.

MOHAN, D. R. and RAMASWAMY, M. (2007). Evaluation of the larvicidal activity of the leaf extract of a weed plant, *Ageratina adenophora*, against two

important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. *African Journal of Biotechnology,* 6(5): 631 – 638.

NOUR, A. H., SANDANASAMY, J. D. and NOUR, A. H. (2012). Larvicidal activity of extracts from different parts of neem (*Azadirachta indica*) against *Aedes aegypti* mosquitoes' larvae. *Scientific Research and Essays,* 7(31): 2810 – 2815.

OKBATINSAE, G. and HAILE, A. (2017). In vitro studies of larvicidal effects of some plant extracts against *Anopheles gambiae* larvae (Diptera: Culicidae). *Journal of Medicinal Plants Research,* 11(4): 66 – 72.

OKIGBO, R. N., OKEKE, J. J. and MADU, N. C. (2010). Larvicidal effects of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens* against mosquito larvae. *Journal of Agricultural Technology,* 6(4): 703 – 719.

OKORIE, P. N., ADEMOWO, G. O., SAKA, Y., DAVIES, E., OKORONKWO, C., BOCKARIE, M. J., MOLYNEUX, D. H. and KELLY-HOPE, L. A. (2013). Lymphatic filariasis in Nigeria; micro-stratification overlap mapping (MOM) as a prerequisite for cost-effective resource utilization in control and surveillance. *PLoS Neglected Tropical Disease,* 7(9): e2416.

PAUL, R., PRASAD, M. and SAH, N. K. (2011). Anticancer biology of *Azadirachta indica* L (neem): a mini review. *Cancer Biology and Therapy*, 12(6): 467 – 476.

RAJA, R. A. Y., KRISHNA, K. C., LOKANATHA, O., MAMATHA, S. and DAMODAR, R. C. (2013). Antimicrobial activity of *Azadirachta indica* (neem) leaf, bark and seed extracts. *International Journal of Research in Phytochemistry and Pharmacology*, 3(1): 1 – 4.

RASHID, M. and AHMAD, A. (2013). The effect of neem (*Azadirachta indica*) leaves extract on the ecdysis and mortality of immature stages of common house mosquito *Culex pipiens fatigans*. *Biologia,* 59(2): 213 – 219.

REMIA, K. M. and LOGASWAMY, S. (2010). Larvicidal efficacy of the leaf extract of two botanicals against the mosquito vector *Aedes aegypti* (Diptera: Culicidae). *Indian Journal of Natural Products and Resources,* 1(2): 208 – 212.

SHANKAR, B. S., SARAVANAN, T., RAGAVI, M., KAVIYA, G., ANUSHREE, A., SAMRAJ, A. and TENNYSON, S. (2013). Screening of local plants for their repellent activity against mosquitoes (Diptera: Culicidae). *Journal of Mosquito Research,* 3(14): 97 – 104.

TONK, S., BARTARYA, R., KUMARI, M., BHATNAGAR, V. P. and SRIVASTAVA, S. S. (2006). Effective method for extraction of larvicidal component from leaves of *Azadirachta indica* and *Artemisia annua* Linn. *Journal of Environmental Biology,* 27(1): 103 – 106.