Biocontrol of Mosquito Larvae using different parts of Neem (*Azadirachta Indica* A.Juss) in Katsina Metropolis, Katsina State, Nigeria

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Abstract: The present study include the phytochemical composition of different parts of neem (Azadirachta indica A.Juss) and its biocontrol of mosquito larvae in Katsina metropolis where the leaves and stem bark of neem (Azadirachta indica) were collected from Umaru Musa Yar'adua University, Katsina while Anopheles mosquito larvae were collected from the stagnant water around households. The preliminary phytochemical screening of aqueous extract of stem bark, fresh and dried leaves revealed the presence of eight phytochemicals that include alkaloids, flavonoids, saponins, tannins, phytosterols, phenolic compounds, cardiac glycocides and terpanoids with different proportions. Different concentrations of all the botanicals (Stem bark, Fresh and Dried leaves) were made separately and twenty larvae of Anopheles were placed in different concentrations. Control was set up for each experiment. Observation of mortality was carried out after each 24 hours. Statistically, there was a significance difference ($p<0.05$) between the mortality of the mosquito larvae. The dried leaves extract caused the highest (x-y %) (13.33-36.67%) mortality rate with the least LC50 and LC90 values, followed by fresh leaves (8.33-26.67%) and the least (6.67-23.33%) mortality was found in stem bark at 24 hours after exposure, similar trend was observed at 48 hrs, 72 hrs and 96 hrs against Anopheles larvae. Conclusively, aqueous extracts of neem stem bark, fresh and dried leaves could have some biologically active components of larvicidal effect. The use of this plant parts is recommended for biological control of mosquito larvae and further research to isolate the active component is equally suggested.

Keywords: Phytochemical, LC_{50} and LC_{90}, Mortality, Azadirachta indica, mosquito larvae.

1. Introduction

Poorly drained storm water forms stagnant pools that provide breeding sites for disease vectors including mosquito, because of this, some diseases are more common in the wet season than the dry season. Mosquitoes act as a vector for most of the life-threatening diseases such as malaria, yellow fever, dengue fever etc in almost all tropical and subtropical countries and many other parts of the world (Qualls *et al.,* 2006). Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world (WHO, 2012). Mosquito borne diseases are prevalent in more than 100 countries across the world (Russell *et al* 2009). However, the mosquitoes are becoming resistant to a wide range of pesticides (Rathore *et al.,* 1986). This makes room to consider some other bioinsecticide which are cheap, appropriate and could safely be used for vector control. The widespread use of synthetic insecticides for the control of pest as well as human diseases vectors has led to concern about toxicity and environmental impact (Qualls *et al.,* 2006). Neem tree (*Azadirachta indica* A.Juss) is a deciduous tree that is native to northern western India and has long been recognized for its insecticidal properties. This tree typically grows in the tropical and subtropical part of Asia, but nowadays they are also cultivated in other warm regions of the world because of its considerable climatic tolerance (Sushree *et al.,* 2000). Neem has been used as insecticides even before the advent of synthetic organic insecticides (Tanzubil *et al*., 1996). Phytochemicals are substances produced mainly by plants, and these substances have biological activity. In the pharmaceutical industry, plants represent the main source to obtain various active ingredients. They exhibit pharmacological effects applicable to the treatment of bacterial and fungal infections and also chronic-degenerative diseases such as diabetes and cancer (Nadia *et al*., 2018). They are chemical compounds produced by plants, generally to help them thrive or prevent competitors, predators, or pathogens. Some phytochemicals have been used as poisons and others as traditional medicine (Nadia *et al*., 2018). Mosquitoes control reduces the number of mosquitoes and their effect to human health and other domestic animals. Biocontrol or biological control is a method of controlling pest such as insect, mite, weeds and plant diseases using other species of animal or plants (Venek *et al*., 2006). This research is aimed to

study the phytochemical composition of different parts of neem (*Azadirachta indica* A.Juss) and its biocontrol of mosquito larvae in Katsina metropolis.

2. Materials and Methods

2.1. Study Area

The research was carried out in Katsina metropolis. Katsina State covers an area of 23,938 km^2, and located between latitudes 11^o 08'N and 13^{o}22'N and longitudes 6^{0}52'E and 9^{0}20'E. The State is boarded by Niger Republic to the north, Jigawa and Kano States to the east, Kaduna State to the south and Zamfara State to the west. Katsina city is the centre of an agricultural region producing groundnuts, cotton, millet, maize, guinea corn, beans, cassava; rice etc. apart from agriculture, craft works is another traditional occupation of the people of Katsina State (Abba, 2017).

2.2. Collection and Preparation of Plant Materials

Leaves and stem bark of neem (*Azadirachta indica*) were collected from the school compound of Umaru Musa Yar'adua University and then identified at herbarium of the Department of Biology of the same institution. All the plant materials were placed in a ventilated area before grinding them into fine powders using clean pestle and mortar. The powder were placed in well labeled containers separately and kept in the laboratory. The powder was then weighed in 2, 4, 6 and 8 grams and used in this study. For fresh leaves, 6.50, 13, 19.54 and 26 grams of fresh leaves were weighed and crushed using a blinder and used in the study (Kabula *et al*., 2011).

2.3. Preparation of Samples for Phytochemical Screening of Botanicals

Extraction was carried out according to techniques of Sasidharan *et al.* (2011). Fifty grams of powdered dried leaves stem bark and fresh leaves paste were sucked in 200ml of distilled water respectively and kept for three days. The solutions were filtered using muslin cloth and concentrated under water bath till the extract were allowed to dry into semi-solid state, the extract were used for phytochemical screening to detect the presence of secondary metabolites.

2.4. Preliminary Phytochemical Screening of Neem Extracts

The aqueous extracts were subjected to preliminary phytochemical screening using standard procedure and methods described by Safowara (1993).

2.5. Selection and Collection of Mosquito Larvae

Anopheles mosquito larvae found in the water body of the stagnant water around households were collected. Method of Rathy, (2015) was adopted where genus of *Anopheles* larvae are collected for the study and the larvae were identified and differentiated using Kent *et al*., (2005) method where the larva morphology was used as the basis for selection and identification.

2.6. Larvicidal Bioassay

WHO (2005) procedure for bioassay of the larvicidal activity were adopted. A set of twenty larvae (*Anopheles*) were introduced each in to the treatment plastics containing 2 liters of the stagnant water collected separately, each larvae was placed into the water that it was collected from. To the treatments sets, for dried neem leaves and stem bark, respective grams (2, 4, 6, and 8) of the leaves and stem bark powder were diluted in 50ml of tap water and added respectively (Ohaga *et al*., 2007). For fresh neem leaves, it was calculated that 6.50 g of fresh leaves produced 2 g of dried leaves. Therefore, respective grams (6.50, 13, 19.54 and 26 g) of fresh leaves were crushed in 50 ml of tap water each using a blinder to correspond to the concentration of dried neem powder. A control set containing only larvae. The plastics were covered with mosquito net and maintained the room temperature and relative humidity. Larvae are considered dead if settled and remained motionless at the bottom of the buckets with no response to light or mechanical stimulus. The mortality of these was checked after each 24 hours and percent mortality was calculated using a formula of:

Mortality = Number of dead larvae x 100

Total number of larvae

The experiment was replicated three times (Ohaga *et al.*, 2007) and results were presented in chats.

2.7. Statistical analysis

All the analysis were carried out using SPSS 20.0 and charts were plotted in Microsoft excel 2010. The data obtained were subjected to one-way analysis of variance (ANOVA) and means between treatments were compared using Turkey's-b at the significance level $p<0.05$ confidence level. The results were presented in tables and charts. The LC_{50} and LC_{90} were also calculated by using probit analysis with SPSS (20.0) software package.

3. Result

3.1. Phytochemical Screening

Preliminary phytochemical screening of stem bark, fresh and dried neem leaves revealed the presence of 8 phytochemicals namely; alkaloid, flavonoids, saponins, tannins, phytosterols, phenolic compound, terpanoids and cardiac glycocides (Table 1).
The results shows that all the 8 phytochemicals mentioned above were presence in the aqueous leaves (fresh and dried) extracts in different proportions, phytosterols was absent in the aqueous extract of stem bark of neem.

Table1. Phytochemical Compositions of Aqueous Extract of Stem Bark, Fresh and Dried Leaves of Neem (*Azadirachta indica*) in Katsina Metropolis

Phytochemicals	Fresh Leaves	Dried Leaves	Stem Bark
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phytosterols	+	+	_
Phenolic compound	+	+	+
Terpanoids	+	+	+
Cardiac glycocides	+	+	+

Key: + = presence, - =absence of the secondary metabolites.

3.2 Larvicidal Effect of study botanicals against *Anopheles* Larvae

In this research the highest mortality (36.67%) was recorded in larvae treated with 8g/ml of dried neem leaves extract, while the least (6.67%) was recorded in the samples treated with 2 g/ml of stem bark neem extract within the first 24 h after treatment (Figure 1), at 48 h after treatment, the highest (75.00%) mortality was recorded in larvae treated with 8 g/ml of dried neem leaves extract, followed by (63.33%) in treatment of 6 g/ml of dried leaves extract. The least was found in stem bark extract (18.33%) at a concentration of 2 g/ml (Figure 2), at 72 h after treatment, total mortality (100%) of larvae was recorded in treatment with 6 and 8 g/ml of dried neem leaves extract and 8 g/ml of fresh neem leaves extract and the least mortality (31.67%) was recorded in treatment with 2 g/ml of stem bark of neem extract (Figure 3), at 96 h after treatment, highest (86.67%) mortality was recorded in treatment with 6 g/ml of fresh neem leaves extract and 8g/ml of stem bark extract, followed by (85.00%) in treatment with 4 g/ml of dried neem leaves extract, the least (55.00%) was found in treatment with 2 g/ml of stem bark (Figure 4) and no dead larvae were found in the untreated larvae. Therefore, Mortality of *Anopheles* mosquito larvae varied with different botanical extract at different time.

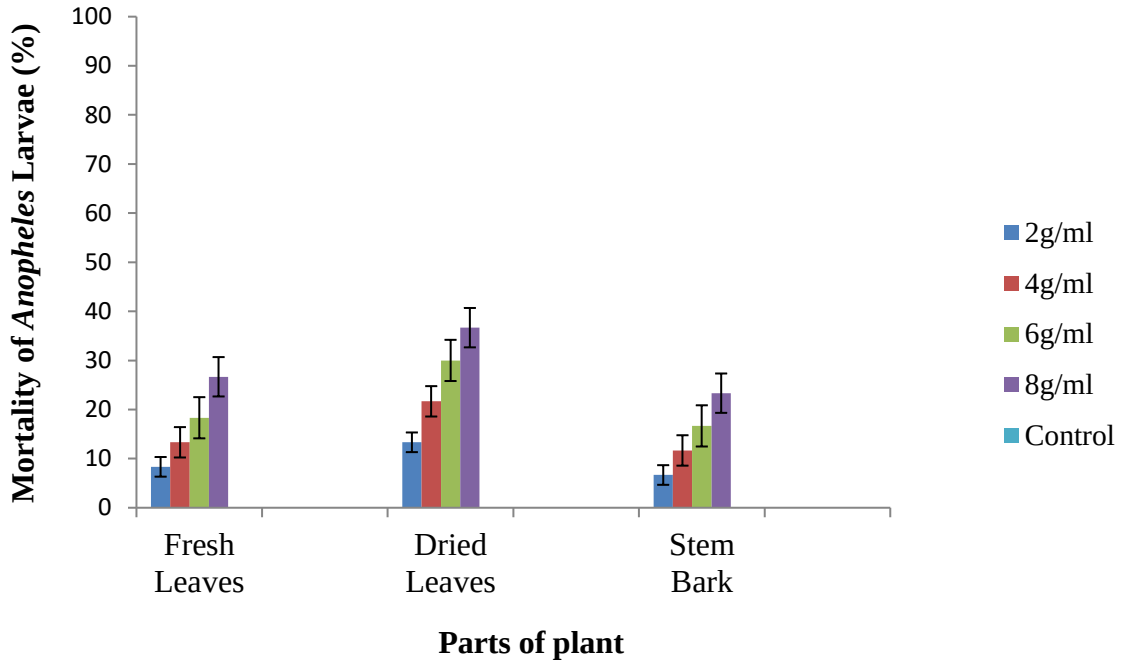

Figure 1: Mortality of *Anopheles* Larvae Exposed to Neem Stem Bark, Fresh and Dried Leaves Extracts at 24 h after Treatment

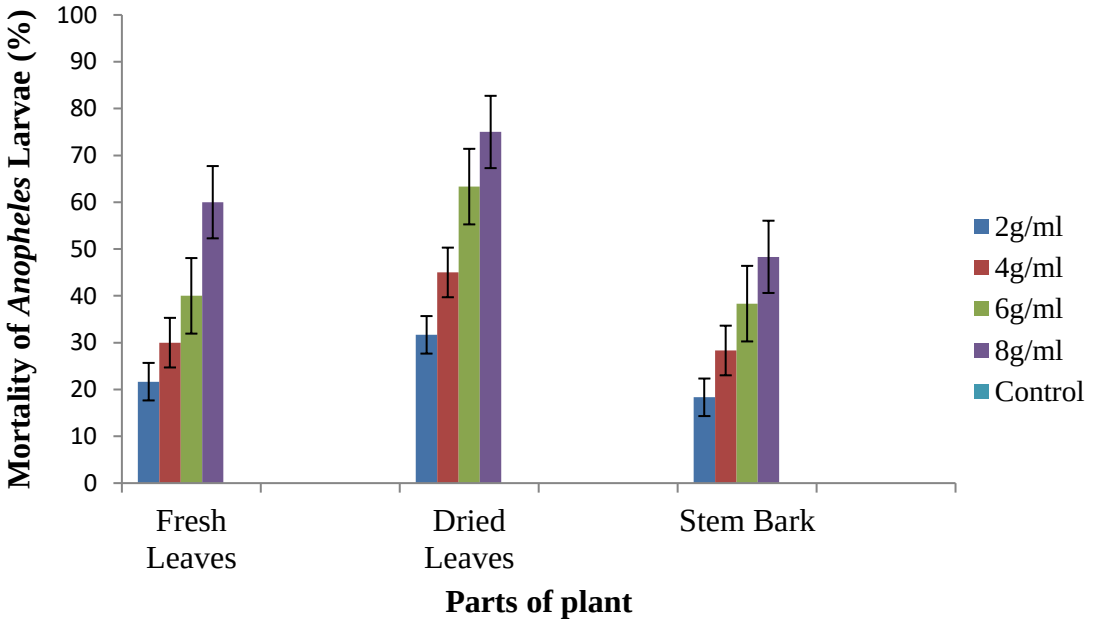

Figure 2. Mortality of *Anopheles* Larvae Exposed to Neem Stem Bark, Fresh and Dried Leaves Extracts at 48 h after Treatment

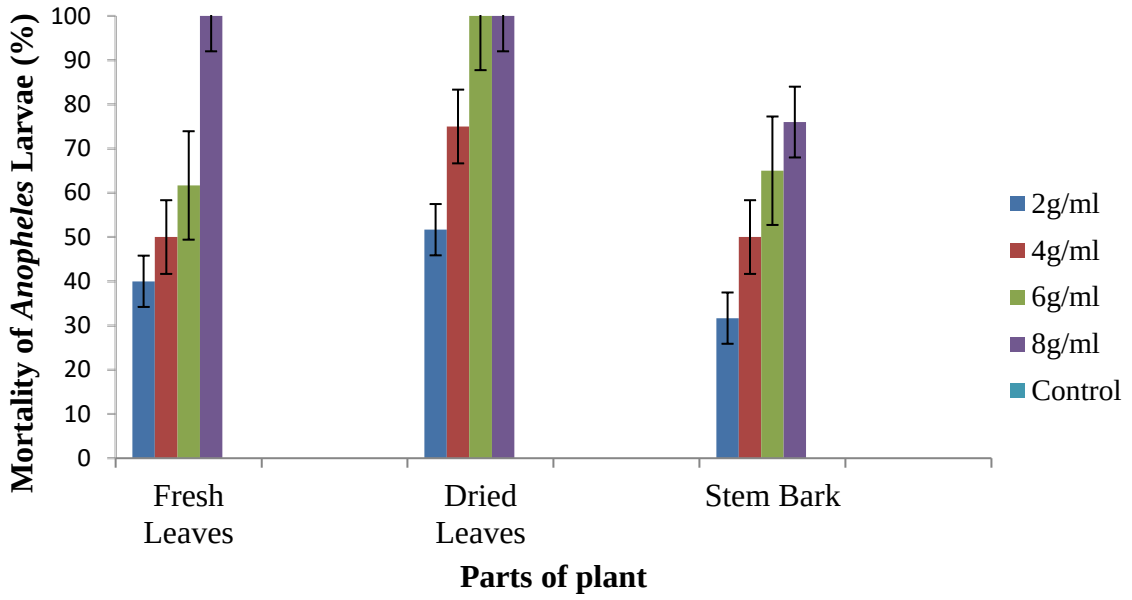

Figure3. Mortality of *Anopheles* Larvae Exposed to Neem Stem Bark, Fresh and Dried Leaves Extracts at 72 h after Treatment

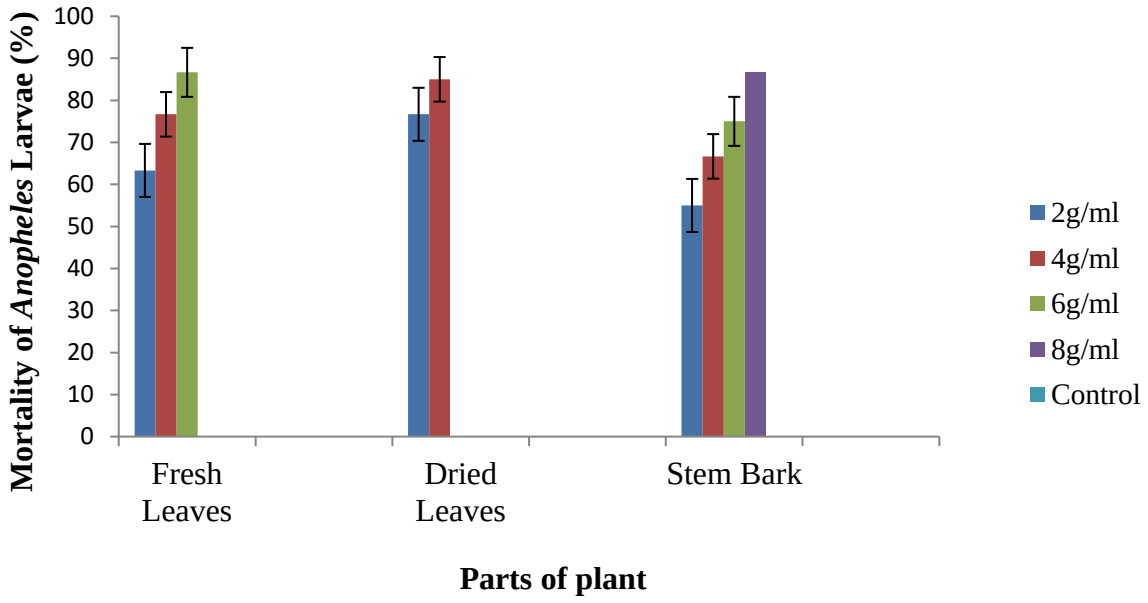

Figure4. Mortality of *Anopheles* Larvae Exposed to Neem Stem Bark, Fresh and Dried Leaves Extracts at 96 h after Treatment

3.3 Lethal Concentrations (LC_{50} and LC_{90}) of study botanicals against *Anopheles* Mosquito Larvae

Table 2 Presents the Lethal Concentrations (LC_{50} and LC_{90}) of different parts of neem at different concentrations in 24h after exposure against *Anopheles* larvae. The results show that stem bark had the highest values of lethal concentration LC_{50} and LC_{90} as 3.262 and 7.861 g/ml respectively, followed by fresh leaves with LC_{50}= 2.470g/ml and LC_{90}= 6.545 g/ml, the least LC_{50} and LC_{90} were found in dried leaves with 2.204 and 4.628 g/ml. chi-square test indicated that there was a significant differences ($p<0.05$) in LC_{50} and LC_{90} between all the test botanicals.

Table2. LC_{50} and LC_{90} of Stem Bark, Fresh and Dried Leaves against *Anopheles* Larvae at 24 hrs after Exposure

Time	Slope (SEM)		LC_{50} (g/ml)	LC_{90} (g/ml)	X^2	p (<0.05)
24 h	DL3.657(±1.088)	FL	2.204	4.628	1.986	0.000
	3.255(±0.930)		2.470	6.547	0.844	0.001
	SB 3.643(±0.921)		3.262	7.861	0.167	0.000

Key: DL-Dried leaves, FL-Fresh leaves and SB-Stem bark

4. Discussion

The result of preliminary phytochemical screening of the plants studied showed that the leaves of the plants (both fresh and dried) were rich in most of the secondary metabolites. Therefore, compounds detected here may be responsible for their insecticidal activities of the organisms. Plants are poor living organisms, without any mechanical way of escaping from insulting environment. They are naturally blessed with phytochemicals to take care of diseases, pest and herbivores. The most important of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins (Sukumar *et al.,* 1991). Phytochemical analysis of methanolic leaf extracts of *Azadirachta indica* has shown the presence of biological compounds like, alkaloids, flavonoids, saponins, reducing sugars, terpenoids and tannins (Anupam *et al.* 2013). The preliminary phytochemical screening of *A. Indica* on leaf, Stem-bark and Root, revealed that alkaloids, tannins, saponins, phenols, flavonoids and glycosides were adequately present (Nwokocha *et al*., 2011) . *A. indica* contains several active ingredients which act in different ways under different circumstances.

In the present study, dried leave was found to be most effective followed by fresh leaves and the least was the stem bark sample. At equal concentrations, dried neem leaves appears to act faster on the *Anopheles* mosquitoes larvae mortality than the fresh leaves and stem bark extract. However, the LC_{50} and LC_{90} for the aqueous samples of stem bark, fresh and dried leaves showed their efficacy in causing 50% and 90% mortality of *Anopheles* mosquito larvae within 24h after treatment. Recent findings reported LC_{50} of aqueous extract of the study botanicals against *Culex pipiens* (Abdelouaheb *et al*., 2009). Their findings revealed that all the botanicals were effective in causing 50% mortality at a very low concentration within short periods. Result from this study have shown that dried leaves had the lowest values of LC_{50} in *Anopheles* mosquito larvae, inferring it to be the most effective botanical extract against the study larvae. Although all the botanicals showed considerable effect in killing 50% and 90% of *Anopheles* mosquito larvae but the lethal concentration of dried leaves was found to be the least.

With regard to mosquitocidal plants, it has been known that at least 344 species of plants including the neem tree are known to contain bioactive materials that show some mosquitocidal activity (Shaalan *et al*., 2005).

Botanicals have been used in various forms to control mosquitoes, for example ancient peoples used smoke from burning cattle or goat dung to drive out mosquitoes from their caves or huts before sleeping (Kihamfa *et al*., 2011). Later on, certain herbs and barks of some trees were added to the smoldering fire to enhance the repellent action of smoke. Plant products can be used either for the type of activity they possess. A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors (Shaalan *et al*., 2005).

The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent (Shaalan *et al*., 2005). Another study by Waka *et al.* (2004) stated that local plants with repellent or insecticidal action may play an important role in regions where mosquitoes bite in the early evenings or in situations when there are not enough bed nets to cover all the beds in a dormitory.

Furthermore, the results of another study indicated that plant-based compounds such as Azadirachtin may be an effective alternative to conventional synthetic insecticides for the control of *Culex pipiens* (Aliero *et al*., 2003). However, this study explored the use of different local preparations made from different parts of neem tree on *Anopheles* mosquito larvae. The results of this study shows that aqueous extract from neem stem bark, fresh and

dried leaves have larvicidal effects on larval stages of the mosquitoes. Therefore, both types of the preparations can be made and used by local people to control the breeding of mosquitoes in anthropogenic habitats especially in rural areas.

5. Conclusion

Katsina state is located within the tropical region of the world with high prevalence of malaria throughout the year. Control programs using conventional insecticides to target anthropogenic mosquito habitat are very expensive because these habitats are widespread, particularly in cities of most African countries. Conclusively it can be said that neem has some biologically active components that show a significant larvicidal activity. So neem products may be used as mosquito population controlling agent, which is a vector of many malarial parasite. They are cheaper, biodegradable and can be used easily by an ordinary man without hazardous effects. It also shows that these anthropogenic breeding sites, both rural and urbanized areas can be controlled by local preparations of neem as aqueous extract.

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