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Efficacy of plant extracts against the tree hole breeding mosquitoes

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ABSTRACT

Mosquitoes are the vector of many serious diseases of human. They belong to order Diptera, super family is Culicidae and subfamilies are Culicinae, Anophilinae and Toxorhincitinae. Adult male feed on nectar while female on blood except the members of family Toxorhrincitinae. Females are in a habit of laying eggs near water, in water or onto the water. This water may be present in tires, ponds, polythene bags, tree holes, earthen pots and different catchment areas. Tree holes become filled with water, after it rains. Some of this vaporizes while the left over becomes a good place for mosquitoes to lay eggs. Mostly Aedes spp. lay their eggs in tree hole standing water. These tree holes are the habitat of many other immature of insects, which are later on consumed by the larvae of mosquitoes. The larvae were collected from here with pipette and transferred to the lab for rearing and experimentation. Different chemical insecticides are in use for controlling mosquitoes, but there are many side effects with chemical use like resurgence and resistance in pest, mammalian toxicity and non-specific killing action. Soft twigs, leaves, seeds and fruit peel were taken from different medicinal plants, including Citrus reticulate, Citrus maxima, Calotropis gigantis, Citrus limetta, Jasminum officinale, Gardania augusta, Nicotiana tabacum, Citrullus colocynthis, Mentha piperata for the preparation of extracts through soxhlet apparatus, which were then applied on 3rd and 4th larval instars of mosquitoes and efficiency of extracts was recorded in term of percentage mortality and LC 50. The highest mortality percentage mortality (100%) was given by Citrus limetta, Citrullus colocynthes and Citrus reticulate extracts. The lowest LC 50 (207.182 ppm) was shown by Mentha piperata extract.These results indicate that it is better and effective to control mosquitoes by using botanicals instead of chemicals.

Key Words: *Botanicals, Larvicide, Biopesticide, Mosquitoes, LC 50 and Percentage mortality*

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I. Introduction

Mosquitoes have been creating serious issues for many years in parts of the world, e.g. in America, Africa, Canada, Thailand, Srilanka and Asia. They are known to transfer diseases to human e.g. dengue, yellow fever, chickengunya, filariasis, encephalitis, malaria and cause deaths (Anon. 2016).

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Mosquitoes lay eggs in the stagnant water or near it. There larvae and pupae are aquatic, larvae feed over the other fauna present in the water. We can see stagnant water around us in many forms like in tires, polythene bags, earthen pods, holes dug by animal hoofs around and tree holes. Tree holes are excellent breeding places for mosquito. *Aedes* mosquitoes prefer to breed in tree holes (Yenoviak, 2001; Mehmood *et al*., 2016; Qasim *et al*., 2014). To control the mosquito, traditional chemical insecticides are in use, both larvicides and adulticides. There are some side effects of the use of chemical insecticides like resistance and resurgence in insects, mammalian toxicity, a nonspecific action which kills all the hazardous as well as beneficial insects. So, keeping in view these hazards, some alternate option should be used (Govindarajan *et al*., 2011). Plants possess different chemicals in them, which deter insect's feeding, oviposition, adult emergence capacities and can also kill the insects by feeding on limonoids present in citrus, azadirechtin in neem etc. (Akram et al., 2010). These chemicals can be extracted from plants through different ways. These chemicals show efficient result in controlling mosquitoes and also these are safe for environment with no health concern. The first ever extracted phytochemical was pyrethrin from chrysanthimum. After extraction these chemicals are used in different formulations and concentration against insects. In mosquito control program citrus extracts give good results as a larvicide. The milkweed can be used as fresh latex and there extract both have good larvicidal values, oviposition deterrent and female irritant (Singh *et al*., 2005). The acetone Nicotiana tabacum extract is efficient in controlling the immature of mosquitoes (Ileke *et al*., 2015). Citrullus colocynthis weed give excellent results against larvae (Rahuman *et al*., 2008). Mentha piperata has good larvicidal values in it against mosquitoes (Kumar *et al*., 2011). This study is helpful in finding the best alternate of chemical insecticides. These plant extracts are ecofriendly, cheaper and have less hazardous effects.

II. Materials and Methods

Old trees, having holes in them in University of Agriculture Faisalabad were selected, including *Mangifera indica*, *Ficus religiosa*, *Delbergia sissoo* and *Moringa oleifera*. These trees were tagged with the name and number over their bark. The immature of mosquitoes were collected from these holes with the help of pipette. The immatures after identification were transferred to separate trays in Biosystematics Laboratory in the Department of Agri-Entomolgy, University of Agriculture Faisalabad for further rearing. Here in the lab, the larvae were fed with fish diet and yeast, in the trays. When these larvae developed into pupae, these were transferred to beakers and placed in the rearing chamber. Adult emergence took place in the rearing chamber. For the adult males, foam soaked in 10% sucrose was placed in a vial and for the feeding of adult female,albino rats trapped in a trap was placed in the rearing chamber, where adult female fed over rat's blood (Arivoli *et al*., 2012). After mating, the female laid eggs on the wet filter paper placed in a petri dish, from where these were collected and frozen for off-season experimentations. For preparing extracts, plant parts were collected from different localities. The plants which were selected for the preparation of extracts were *Citrus reticulate*, *Citrus maxima*, *Calotropis gigantis*, *Citrus limetta*, *Jasminum officinale*, *Gardania augusta*, *Nicotiana tabacum*, *Citrullus colocynthis* and *Mentha piperata*. Soft twigs and leaves of *Calotropis gigantis* plant, *Jasminum officinale*, *Gardania, Mentha piperata, Citrullus colocynthis* and *Nicotiana tabacum* were taken cleaned and dried in oven for almost 48 hours at 50°C. These dried parts were then grounded in the grinder to form powder. The powder was then processed through soxhlet apparatus with acetone for the extraction of oil (Vogel, 1978). Peel of *Citrus reticulate, Citrus maxima* and *Citrus limetta* was taken and dried in oven for almost 60 hours at 50°C. The dried peel was then grounded into powder in the grinder. The powder was then run through soxhlet apparatus with acetone for extraction of oil. After the preparation of these extracts from different plant parts, stock solution with 1% concentration was prepared by dissolving 1ml of extract into 99ml of acetone. Further concentrations were made from this, which were 300,400,500,600,700 and 800ppm. In beaker of 250ml, 200ml of the particular concentration was used, in which 30 larvae of 3rd and 4th instar were released. Five replicates of each treatment were used. Control contained only acetone. The experiment was conducted in laboratory conditions at 30±2c^{o} and 70±5% relative humidity. The experiment was conducted according to CRD. Percentage mortality was calculated.

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III. Results and Discussion

Percentage mortality on *Citrus limetta* peel extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp.*

The highest mortality (100%) was given by 800 ppm concentration at 72 hours interval, while the lowest was 36%, given by 300 ppm concentration at 24 hours interval (Figure 01).

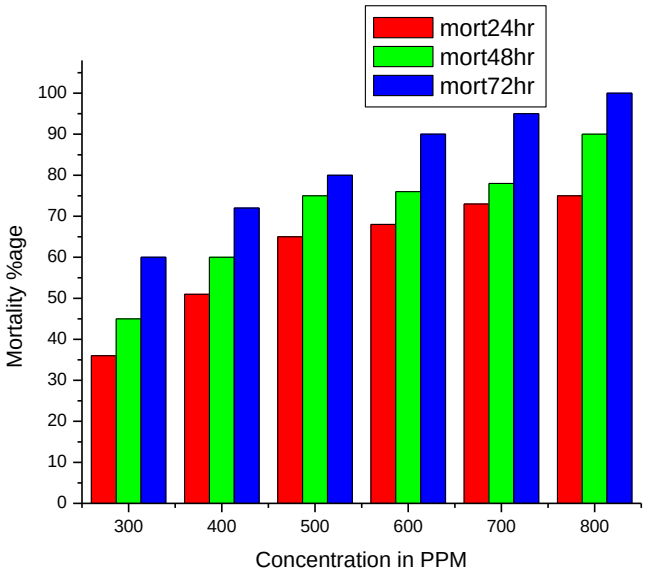

Figure 01. % age mortality given by *Citrus limetta* peel extract.

The LC 50 after 24 hours interval was 393.468 ppm, while after 48 hours LC 50 was 318.843 and after 72 hours LC 50 was 235.544 (Table 01).

Table 01. LC 50 and regression equation by *Citrus limetta*

Time	LC 50	Regression Equation
24 hrs	393.468	y = 12.054ln(x) - 57.023
48 hrs	318.843	y = 12.597ln(x) - 57.618
72 hrs	235.544	y = 12.386ln(x) - 52.652

The use of different concentrations (ppm) of *Citrus limetta* peel extracts on the 3rd and 4th larval instars of *Aedes* sp. showed 100% mortality after 72 hours at 300 ppm and 800 ppm. Lowest LC 50 (235.544 ppm) was observed after 72 hours. 50 % mortality was observed at 400 conentration after 24 hours in case of *Citrus reticulate* against *Aedes* sp. larvae. Similar experiment was conducted by Akram *et al*. (2010) on the 4th larval instars of *Aedes albopictus* by using the different concentrations of ten different varieties of citrus found that at higher concentration would be more effective and safe.

Percentage mortality on *Mentha piperata* leaf extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp.*

The highest mortality (87%) was given by 400 ppm concentration at an interval of 72 hours, while the lowest mortality (30%) was given by 300 ppm concentration at an interval of 24 hours (Figure 02). The LC 50 was 581.261 after 24 hours, while LC 50 was 409.28 after 48 hours, and 207.182 after 72 hours (Table 02). The extract of *Menthapiperata* was found effective in controlling *Aedes* larvae at 400ppm after 72hours. The lowest LC 50 observed was 235.544 ppm after 72 hours. Similarly Kumar *et al.* (2011), used *Menthapiperata* to control larvae of *Aedes aegypti.* LC_{90} values after 24 hours were 111.9 ppm and 295.8 ppm respectively.

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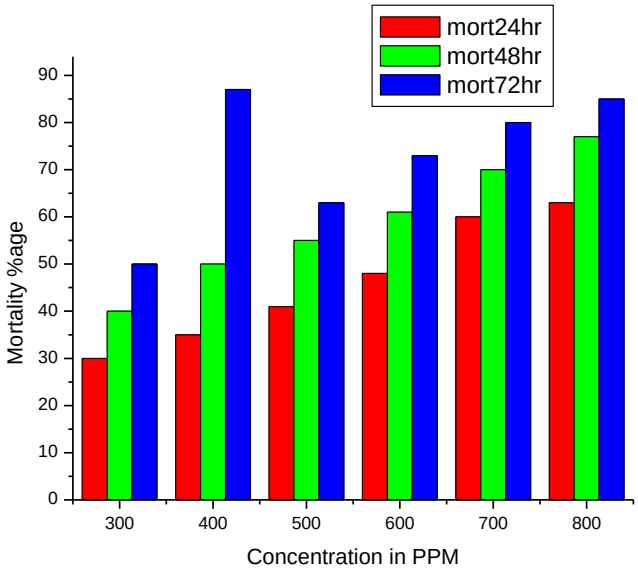

Figure 02. % age mortality given by *Mentha piperata.*

Table 02. LC 50 and regression equation by *Mentha piperata*

Time	LC 50	Regression Equation
24 hrs	581.261	y = 10.633ln(x) - 52.682
48 hrs	409.28	y = 10.917ln(x) - 50.66
72 hrs	207.182	y = 7.4724ln(x) - 24.855

Percentage mortality on *Citrus maxima* peel extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp.*

The highest mortality (91%) was given by 800 ppm concentration at an interval of 72 hours, while the lowest mortality (30%) was given by 300 ppm concentration at an interval of 24 hours (Figure 03).

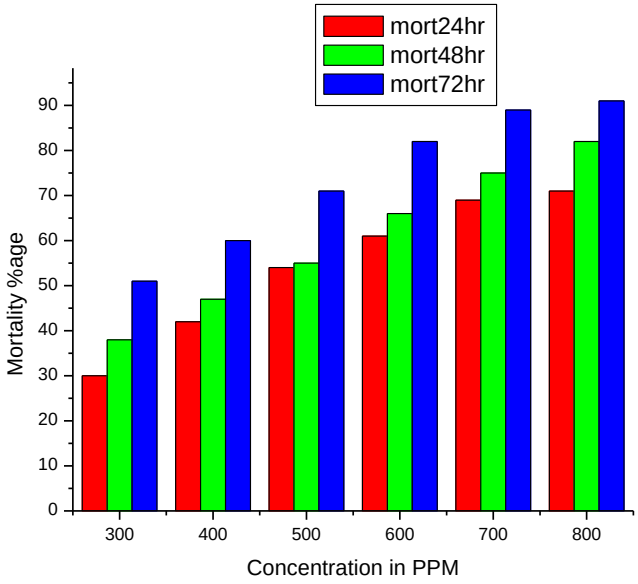

Figure 03. % age mortality given by *Citrus maxima.*

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LC 50 observed was 470.507 ppm after 24 hours, while LC 50 was 548.123 ppm after 48 hours and 302.384 ppm after 72 hours (Table 03).

Table 03. LC 50 and regression equation by *Citrus maxima*

Time	LC 50	Regression Equation
24 hrs	470.507	y = 13.094ln(x) - 65.578
48 hrs	548.123	y = 13.709ln(x) - 67.63
72 hrs	302.384	y = 13.204ln(x) - 60.418

Percentage mortality on calotropis leaf extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp.*

The highest mortality (83%) was given by 800 ppm concentration at an interval of 72 hours, while the lowest mortality (30%) was given by 300 ppm concentration at an interval of 24 hours (Figure 04). The LC 50 was 606.436 ppm after 24 hours, while LC 50 was 479.67 ppm after 48 hours, and 421.702 ppm after 72 hours (Table 04). The aqueous leaf extract of *Calotropus* is a good larvicide, oviposition deterrent and adult emergence inhibitor against *Anopheles* and*Culex*mosquitoes (Elimam *et al.*, 2009). The experiment found that the use of aqueous leaf extract of *Calotropus* resulted 50% mortality against the 3rd and 4th larval instars of *Aedes* sp. at 700 ppm after 24 hours, but did not showed 100% mortality even at 800 ppm. The lowest LC 50 observed was 421.702 ppm.

Table 04. LC 50 and regression equation by *Calotropis* leave extract

Time	LC 50	Regression Equation
24 hrs	606.436	y = 9.9666ln(x) - 48.862
48 hrs	479.67	y = 11.355ln(x) - 55.096
72 hrs	421.702	y = 13.63ln(x) - 67.385

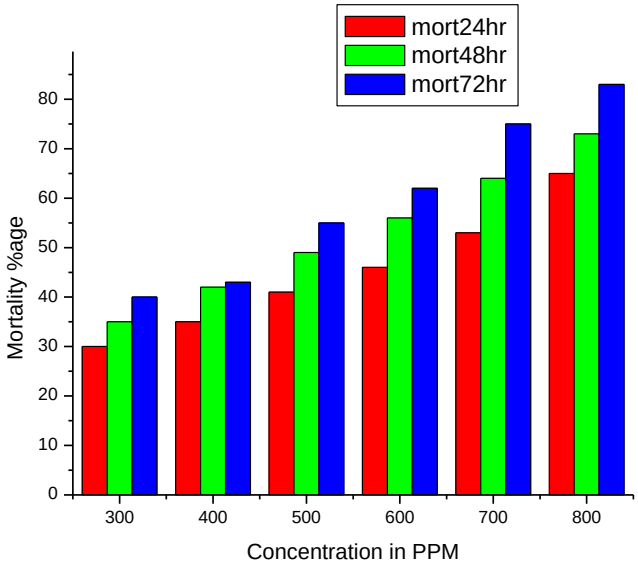

Figure 04. % age mortality given by *Calotropis* leave extract.

Percentage mortality on Citrus reticulate peel extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp.*

The highest mortality (100%) was given by 800 ppm concentration at an interval of 72 hours, while the lowest mortality (35%) was given by 300 ppm concentration at an interval of 24 hours (Figure 05). The LC 50 measured was 395.875 ppm after 24 hours, while 324.634 ppm after 48 hours, and 259.77 ppm after 72 hours (Table 05).

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Table 05. LC 50 and regression equation by *Citrus reticulata*

Time	LC 50	Regression Equation
24 hrs	395.875	y = 14.687ln(x) - 72.845
48 hrs	324.634	y = 15.073ln(x) - 72.163
72 hrs	259.77	y = 13.629ln(x) - 60.775

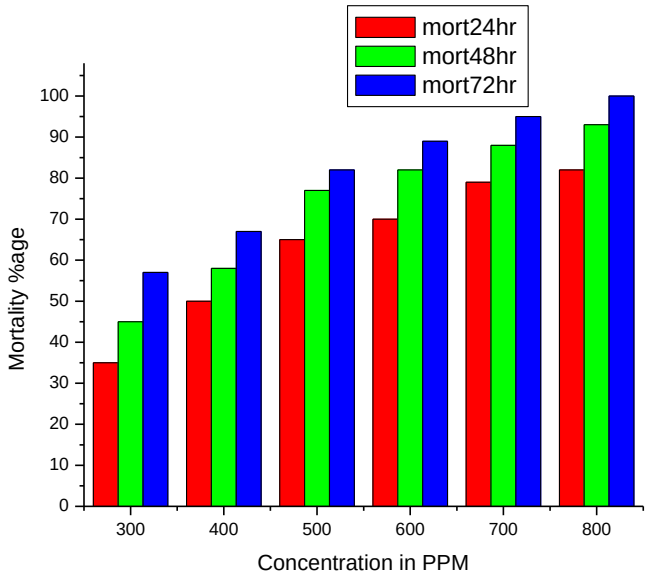

Figure 05. % age mortality given by *Citrus reticulate.*

Percentage mortality on *Citrullus colocynthis* fruit extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp.*

The highest mortality (100%) was given by 800 ppm concentrationat an interval of 72 hours, while the lowest mortality (38 %) was given by 300 ppm concentration (Figure 06).

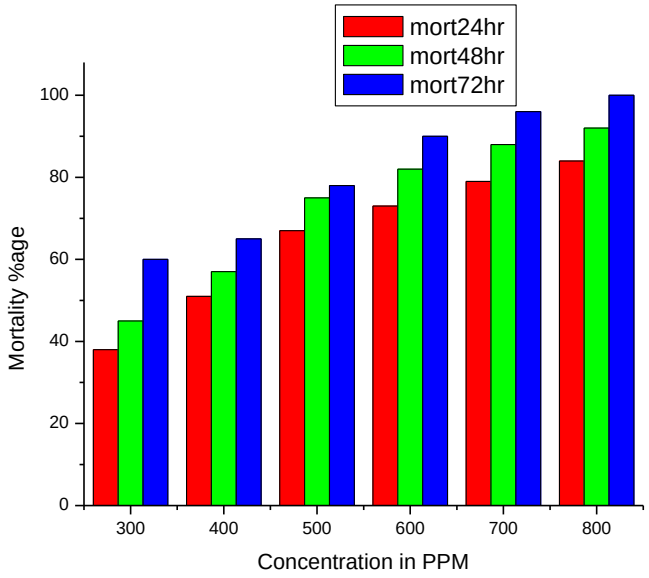

Figure 06. % age mortality given by *Citrullus colocynthis.*

In case of *Citrullus colocynthis,* the LC 50 was 378.607 ppm after 24hours, while LC 50 was 328.685 ppm after 48 hours, and 257.546 ppm after 72 hours (Table 06). The extract of *Citrullus colocynthis*

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was found effective. 50% mortality was observed at 400ppm after 24 hours and 100% mortality was observed at 800 ppm. Lowest LC 50 observed after 72 hours was 257.546 ppm. Arivoli *et al.* (2012) tested seven different plant extracts including *Citrullus colocynthis* against mosquito larvae at four different concentrations; 250, 500, 750 and 1000ppm and found the best results at 352.84 ppm.

Table 06. LC 50 and regression equation by *Citrullus colocynthis*

Time	LC 50	Regression Equation
24 hrs	378.607	y = 14.356ln(x) - 70.225
48 hrs	328.685	y = 15.038ln(x) - 72.147
72 hrs	257.546	y = 13.393ln(x) - 59.348

Percentage mortality on *Gardania augusta* leave extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp*.

The highest mortality (60%) was given by 800 ppm concentration at an interval of 72 hours, while the lowest (20%) was given by 300 ppm at an interval of 24 hours (Figure 07).

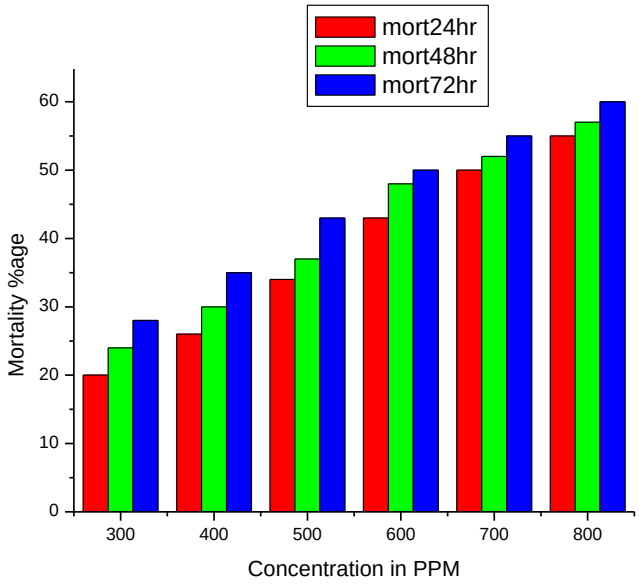

Figure 07. % age mortality given by *Gardenia augusta.*

The LC 50 measured was 720.467 ppm after 24 hours, while 603.23 ppm after 48 hours, and 667.073 ppm after 72 hours (Table 07). The *Gardania augusta* leave extracts found 50% mortality after 24 hours using 700 ppm. This experiment showed the similar results as, Ochieng *et al.* (2010) used the extract of *Gardania ternifolia* against the 2nd larval instars at different concentrations and found that this one is effective against the larvae of *Anopheles* and *Aedes*.

Table 07. LC 50 and regression equation by *Gardenia augusta*

Time	LC 50	Regression Equation
24 hrs	720.467	y = 11.155ln(x) - 58.399
48 hrs	603.23	y = 9.9807ln(x) - 48.9
72 hrs	667.073	y = 10.588ln(x) - 53.853

Percentage mortality on *Jasminum officinale* leave extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp.*

The highest mortality (60%) was given by 800 ppm concentration, while the lowest mortality (20%) was given by 300 ppm at an interval of 24 hours (Figure 08).

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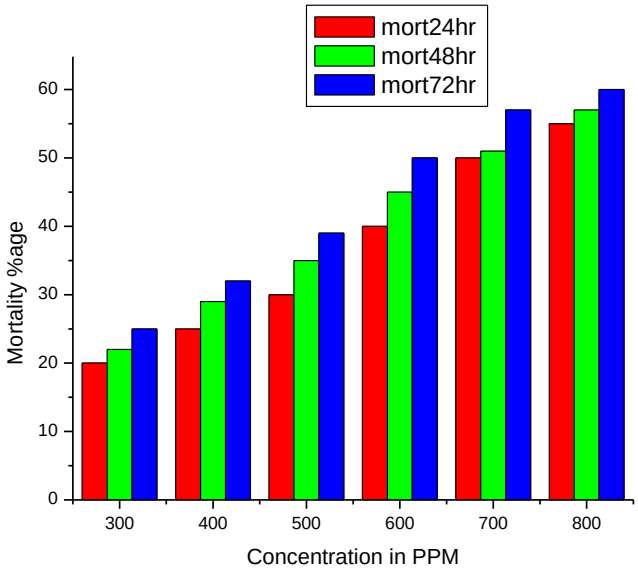

Figure 08. % age mortality given by *Jasmine officinale.*

The LC 50 measured was 746.652 ppm after 24 hours, while LC 50 was 689.591 ppm after 48 hours, and 613.328 ppm after 72 hours (Table 08).

Table 08. LC 50 and regression equation by *Jasmine officinale*

Time	LC 50	Regression Equation
24 hrs	746.652	y = 11.162ln(x) - 58.844
48 hrs	689.591	y = 10.923ln(x) - 56.394
72 hrs	613.328	y = 11.426ln(x) - 58.343

Percentage mortality on *Nicotiana tabacum* leaf extracts at different concentration (PPM)s against 3rd and 4th instar larvae of *Aedes spp.*

The highest mortality (79%) was given by 800 ppm concentration at an interval of 72 hours, while the lowest mortality (25%) was given by 300 ppm concentration at an interval of 24 hours (Figure 09).

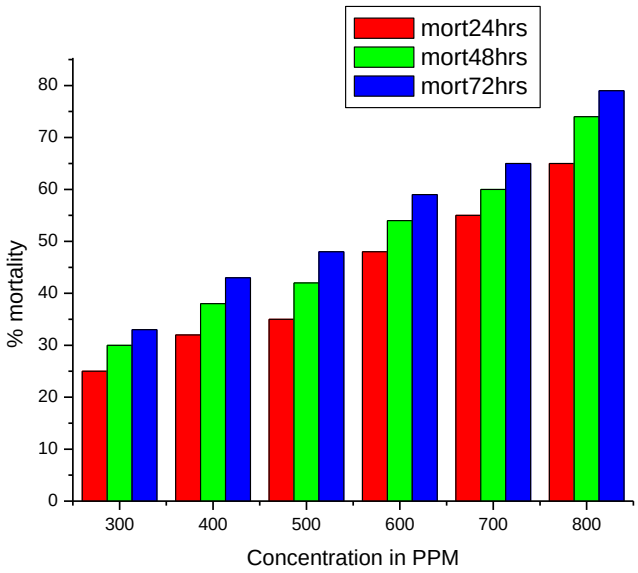

Figure 09. % age mortality given by *Nicotiana tabaccum* leave extract.

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The LC 50 measured was 615.786 ppm after 24 hours, while LC 50 was 525.89 ppm after 48 hours, and 471.019 ppm after 72 hours (Table 09). *Nicotiana tabacum* leaf extracts used against the 3rd and 4th larval instars of *Aedes* species found 50% mortality at 600ppm after 24 hours. The lowest LC 50 observed was 471.019, after 72 hours. Similarly Olofintoye *et al.* (2011) used the extracts of *Nicotianatabacum*on the larvae of subfamily *Culicinae* and *Anophilinae*.The extract was found efficient in controlling the mosquito larvae, 50% mortality of *Culicinae* larvae in first 24 hours.

Table 09. LC 50 and regression equation by *Nicotiana tabaccum*

Time	LC 50	Regression Equation
24 hrs	615.786	y = 12.073ln(x) - 62.544
48 hrs	525.89	y = 12.747ln(x) - 64.862
72 hrs	471.019	y = 13.226ln(x) - 66.405

IV. Conclusion

Citrus limetta, *Citrullus colocynthis* and *Citrus reticulate* gave 100% mortality at 800ppm and 50% at 300ppm after 72 hours and is excellent to control *Aedes* mosquitoes, after that *Mentha piperata* leaf extracts showed 50% mortality at 300ppm. *Calotropis gigantis* and *Citrus maxima* gave 50% mortality at 400ppm after 72 hours. *Nicotiana tabacum* and *Gardania augusta* showed 50% mortality at 600ppm after 24 hours. *Jasminum officinale* showed 50% mortality at 700 ppm after 24 hours. The lowest LC 50 was showed by 207.182 ppm concentration of *Mentha piperata* after 72 hours followed by *Citrus limetta,* which was 235.544 ppm, after 72 hours. These results indicate that it is better and effective to control mosquitoes by using botanicals instead of chemicals.

V. References

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APA (American Psychological Association)

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