Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 7266 - 7273
Received 15 December 2020; Accepted 05 January 2021.

# Control of Culex Mosquito Larvae by Extracts of *Mentha Spicata* L. (Mint) and *Citrus Limon* L. (Lemon)

**Faiza Asad Phulpoto[1], Safdar Ali Ujjan[1], Javed Ahmed Ujan[1], Kiran Naz Sahito[1], Sanaullah Abbasi[2], Abdul Majid Ansari[2], Sham Lal[3], Akhtar Hussain Lashari[4]**

[1]Department of Zoology Shah Abdul Latif University Khairpur, Sindh, Pakistan
[2]Department of Biochemistry Shah Abdul Latif University Khairpur, Sindh, Pakistan
[3]Institute of Microbiology Shah Abdul Latif University Khairpur, Sindh, Pakistan
[4]School of Environment, Beijing Normal University, Beijing 100875, China

## ABSTRACT

**Aim:** Mosquitoes represent the major arthropod vectors of human disease worldwide by transmitting various diseases. This study evaluated the control of Culex mosquito larvae by extracts of *Mentha Spicata* L. (Mint) and *Citrus Limon* L. (Lemon).

**Methodology:** The extracts of mint (*M. spicata*) and lemon (*C. limon*) were were prepared and the larvicidal activity of mint, lemon, and mix of mint and lemon was assessed in 4 different dilutions (2%, 4%, 6% and 8%) along with control group in glasses. The extracts were exposed to larvae for three days and data were noted after 24 h, 48 h and 72 h. The number of dead larvae was counted and noted. The experiment was performed in triplicates.

**Results:** The highest larval mortality was found with mint extract (1.91 mg/dL $LC_{50}$ value), followed by lemon peel extract (2.32 mg/dL $LC_{50}$ value) and mix extract (4.29 mg/dL $LC_{50}$ value) respectively. In terms of % mortality mint showed high mortality rate of 94% followed by lemon 88% and mix extract 70%. The % mortality was directly proportional to the concentration of the extract.

**Conclusion:** Mint and Lemon extracts can be considered as a powerful arsenal for the control of mosquito population if used separately.

**Keywords:** Mint, Lemon, Extract oil, Larvicidal activity, Mosquito larvae, Mortali

## 1. INTRODUCTION

Mosquito is a Spanish language word consists of two parts Mosca and Diminuto that stands for 'Tiny fly' because mosquito is basically a type of fly that belong to order Diptera (Culicidae: Diptera) (Brown, 1993). The upgraded recent classification fauna of Culicidae is divided in to two sub families' 135 genera 3500 species from which 100 species are said to be harmful and disease ausing insects (Clements, 1992; Reinert, 2000; Reinert, 2001). Like all insects osquito possesses 3 body parts upper part known as Head, middle part Thorax and lower part Abdomen. Mosquitoes have a thin and light body stature that helps them fly (Harbach, 2007). Typically, female and male both mosquitoes get nourishment from plant juices and honeydew derived from part of plants but in many species female mouth parts are adapted for piercing of skin to suck animal blood to consume an energy to mature eggs (Wahid et al., 2003).

Mosquitoes are said to be small flies because they belong to the order Diptera these insects are responsible for many deaths because they work as vector and spread many deadly ailments like yellow fever, dengue, Zika virus, malaria, jaundice, elephantiasis and many more. Around 782,000 people die because of mosquitoes borne diseases (WHO, 2010). Many types of

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 7266 - 7273
Received 15 December 2020; Accepted 05 January 2021.

mosquito control methods are in use but each day new techniques are introduced to find more effective and safe ways to kill this deadly creature that is harmful for mankind. Chemical control includes chemical sprays whereas the bi-control techniques include man-made insecticides derived from plant source that are quite ecofriendly. Many plants are used in these procedures example: Neem, Thyme, Citrus plants, Lemon grass, Marigold etc (Lees et al., 2015). Therefore, this study evaluated the control of Culex mosquito larvae by extracts of *Mentha Spicata* L. (Mint) and *Citrus Limon* L. (Lemon).

## 2. MATERIALS AND METHODS

### 2.1 Phyto-extraction

The leaves of *Mint spicata* (Family:Labiatae) and *Citrus Limon* were purchased from a local market of Khairpur city. The peel of lemon and leaves of mint were washed, dried, and pulverized in electrical grinding machine (Cambridge CG-501). The powder material was preserved in airtight jars. For extraction, powder material (8g/80ml) was added in Propanol-2 (analaR, BDH England) followed by filtration after 5-6 h. The filtrate was kept at 60°C in incubator for evaporation and the final extract was preserved in sterilized bottle sealed parafilm to prevent from contamination (Vogel, 1978).

### 2.2 Collection and rearing of mosquito larvae

Mosquito larvae were collected from stagnant water that was a breeding site of mosquitoes around Shah Abdul Latif University and Khairpur city into jars. Larvae were brought for identification and quantification at the laboratory, Department of Zoology, Shah Abdul University, Khairpur. They were kept at 75±5% humidity and at the temperature 28±2°C (Imam et al., 2014). Mosquito larvae were identified by following key previously reported for identifying larval species (Leopoldo, 2004).

### 2.3 Preparation of different concentrations from extracts

The larvicidal activity of crude extract was assessed in 4 different dilutions (2mg/dL, 4mg/dL, 6mg/dL and 8mg/dL) with total volume of 250 ml and prepared as followed.

- 2mg/dL= 5ml of extract with 245ml of water
- 4mg/dL = 10ml of extract with 240ml of water
- 6mg/dL = 15ml of extract with 235ml of water
- 8mg/dL = 20ml of extract with 230ml of water

Each of three extracts (Mint, Lemon, and Mix of them) had their own 4 concentrations separately in glasses.

### 2.4 Quantification of Mortality

In each glass (N=12) containing different concentration of either mint extract, lemon extract or mint and lemon extract, 15 mosquito larvae were placed carefully with the help of brush and the whole set up was covered with mosquito net (Fig. 1). The larvae were left exposed to each extract for duration of 24 h, 48 h and 72 h along with control (WHO, 2005). After exposing time, the mortality number of larvae was noted. During whole experiment pH and Temperature were also recorded (Bilal et al., 2013).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 7266 - 7273
Received 15 December 2020; Accepted 05 January 2021.

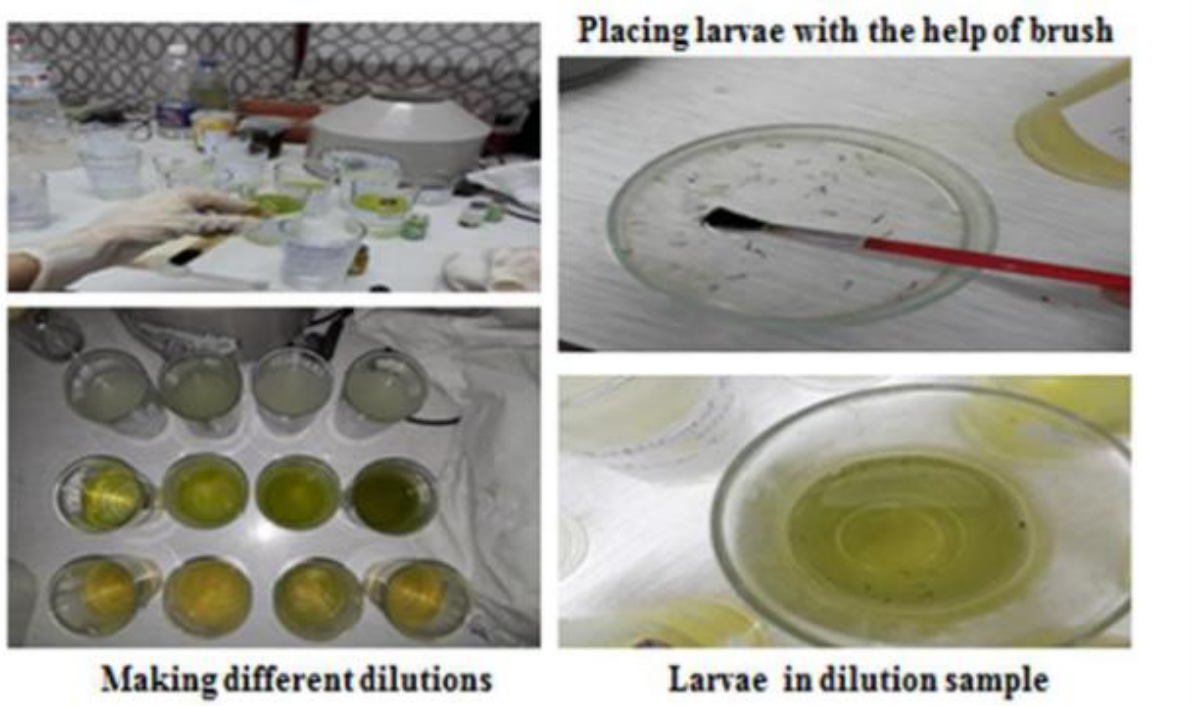



**Fig. 1.** Steps of making dilution of extracts and transferring larvae in extracts

### 2.5 Interpretation of data

The data was assessed through the Ldp Line version 1.0 (available at: ldp-line.software.informer.com/download) and the equations of probit analysis were used to find LC50 values (Where LC = Lethal Concentration).

### 2.6 Statistical analysis

The experiment was performed in three replicates and results were recorded in average ± standard deviation. Probit analysis was performed to analyze the relationship of dose with response by using IBM SPSS statistics 21.0 software.

## 3. RESULTS

The data for mint, lemon and mint lemon mixture experiments and number of dead mosquito larvae by the application of three different extracts are shown in Table 1. The LC50 values (Probit analysis) for each extract are shown in Fig. 2-4.The results obtained showed that when mosquito larvae were exposed to mint extract for 1$^{st}$ day, the LC50 (where LC= Lethal concentration) values attained were 14.64 mg/dL. The extract was evidenced to be more effective on the 2$^{nd}$ day showing the LC50 value of 4.26 mg/dL and on the day 3$^{rd}$, the LC50 of mint extract was found 1.91 mg/dL and that is the highest LC50 among all other extracts used. The Lethal Concentration value of Lemon extracts on mosquito larvae was recorded as 475.44 mg/dL on the 1$^{st}$ day, whereas 2nd day value was 5.67 mg/dL and LC50 value on 3$^{rd}$ day was 2.32 mg/dL. The mix extract showed moderate effect on mosquito larvae compared to other two extracts, but all the values were showing significant effect. The LC50 due to mix extract on 1$^{st}$ day was 47.63 mg/dL and on 2$^{nd}$ day it was 10.19 mg/dL and on the 3$^{rd}$ day 4.29 mg/dL. Overall, all the dilutions made from three extract showed effective mortality rate on mosquito larvae

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 7266 - 7273
Received 15 December 2020; Accepted 05 January 2021.

hence no pupal growth was seen and none of the larvae became an adult mosquito within the time duration of 24 to 72 h (Table 1). In control experiment both pupal and adult mosquito were observed. Overall, mint and lemon extracts showed high potency to act as a larvicide and has effects on physical growth and mortality of mosquito larvae. In terms of percentage mortality mint showed high mortality rate 94% whereas lemon showed 88% and Mix extract showed 70% (Fig. 5). The concentration of the extract is straight proportional to the percentage of larval mortality (Fig. 5).

**Table 1. No. of dead larvae recorded at 24 h, 48 h and 72 h in each extract**

| | **Mint leaves Extract** | | | **Lemon peels extract** | | | **Mint leaves + Lemon peels extract** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **No. of dead larvae** | | | **No. of dead larvae** | | | **No. of dead larvae** | | |
| **Conc.mg/dL** | **Day 1** | **Day 2** | **Day 3** | **Day 1** | **Day 2** | **Day 3** | **Day 1** | **Day 2** | **Day 3** |
| 2 | 3 | 5 | 8 | 2 | 6 | 7 | 1 | 4 | 5 |
| 4 | 3 | 4 | 10 | 2 | 6 | 11 | 0 | 2 | 4 |
| 6 | 5 | 8 | 12 | 2 | 7 | 11 | 2 | 5 | 7 |
| 8 | 7 | 12 | 15 | 3 | 8 | 12 | 4 | 6 | 9 |
| 2 | 2 | 4 | 8 | 3 | 5 | 8 | 3 | 3 | 7 |
| 4 | 3 | 6 | 10 | 2 | 6 | 8 | 2 | 5 | 7 |
| 6 | 5 | 8 | 11 | 4 | 9 | 12 | 5 | 7 | 9 |
| 8 | 5 | 10 | 14 | 4 | 10 | 13 | 4 | 8 | 10 |
| 2 | 2 | 6 | 9 | 3 | 5 | 7 | 2 | 4 | 6 |
| 4 | 3 | 8 | 12 | 3 | 5 | 8 | 4 | 7 | 8 |
| 6 | 5 | 9 | 12 | 2 | 4 | 11 | 3 | 5 | 8 |
| 8 | 5 | 12 | 15 | 5 | 11 | 14 | 4 | 8 | 11 |

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 7266 - 7273
Received 15 December 2020; Accepted 05 January 2021.

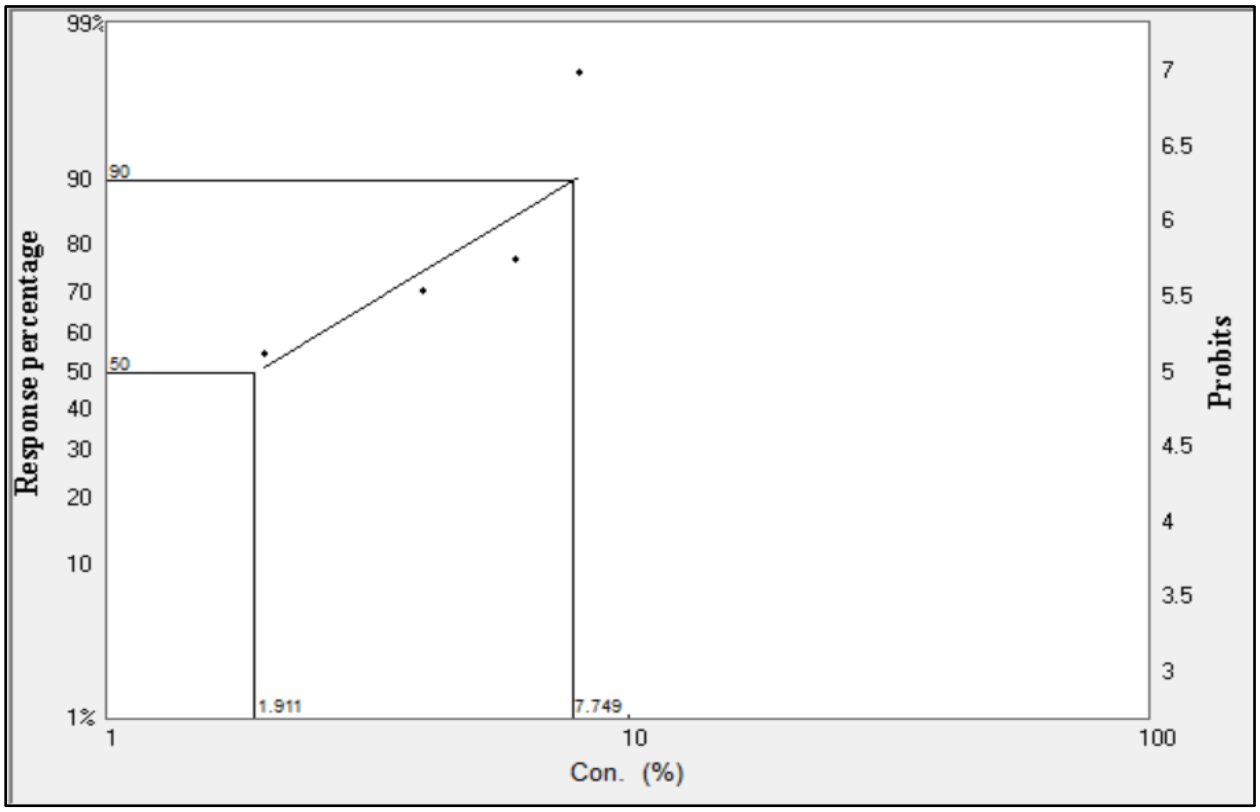



**Fig. 2.** LC50 value results for mint extract (Concentration vs Response)

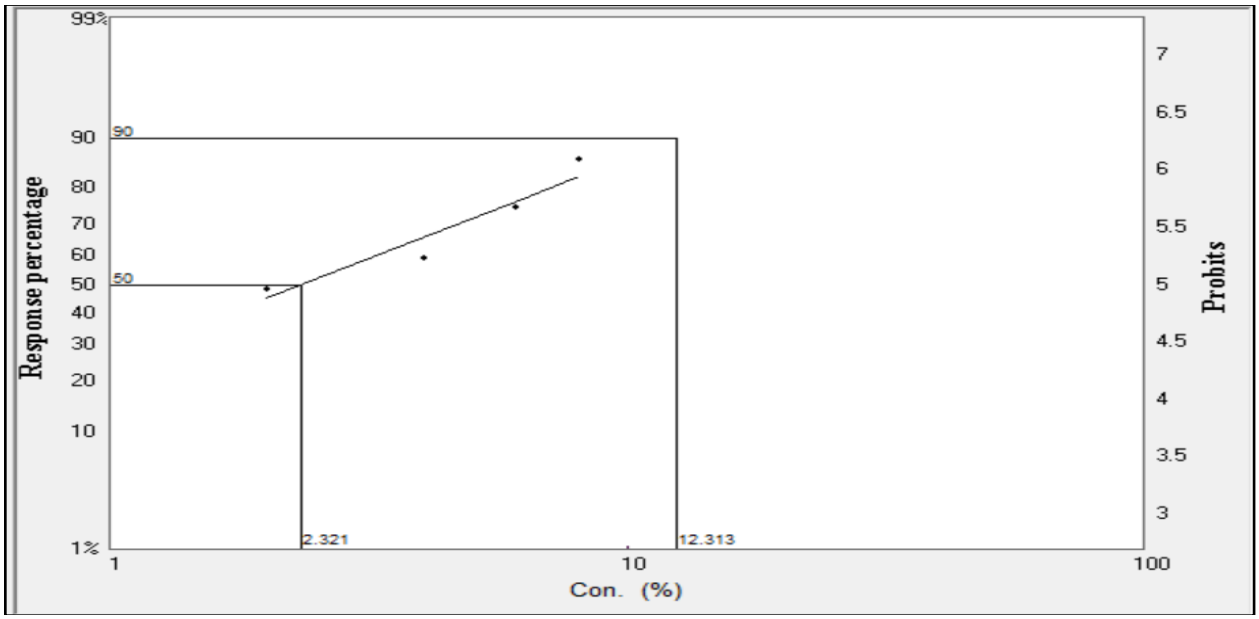



**Fig. 3.** LC50 value results for lemon extract (Concentration vs Response)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 7266 - 7273
Received 15 December 2020; Accepted 05 January 2021.

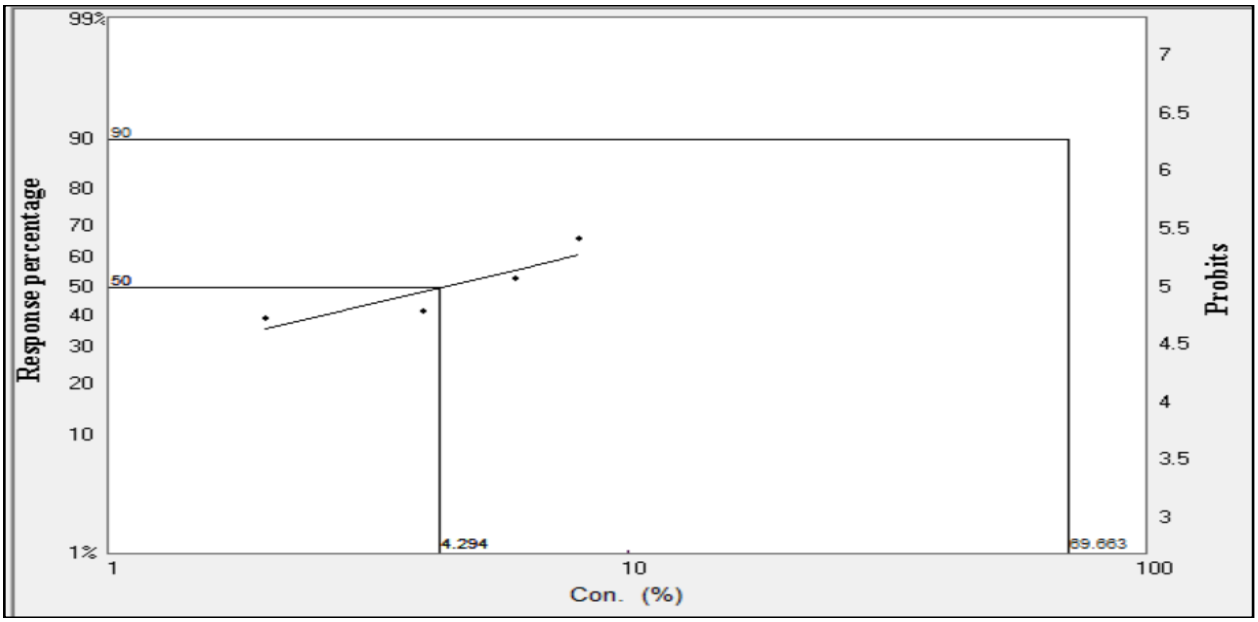
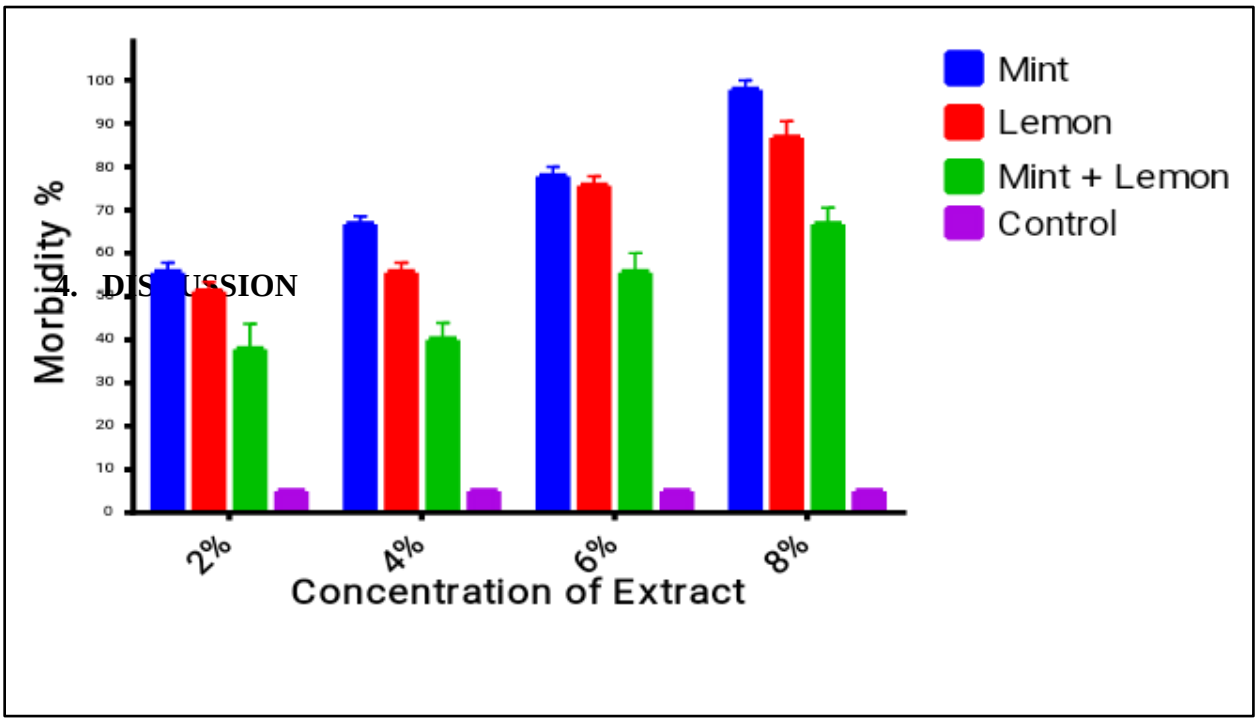



**Fig. 4.** LC50 value results for mint and lemon mix extract (Concentration vs Response)




## 4. DISCUSSION

**Fig. 5.** Morbidity rate of larvae in response to various concentration of extracts

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 7266 - 7273
Received 15 December 2020; Accepted 05 January 2021.

## 4. DISCUSSION

The plants have been reported to produce many chemical secondary compounds to safeguard against pathogens and some have been historically used in controlling pests (Miresmailli&Isman, 2014; Pavela, 2014). The purpose of this experiment was to calculate the efficacy of different plant extracts on the life span of mosquito larvae to prove the hypothesis. The research was assessed in the Department of Zoology's lab at Shah Abdul Latif Khairpur Mir's. In the initial step the extracts were prepared (Vogel, 1978) then mortality of mosquito larvae was checked by application of these extracts in different dilutions (WHO, 2005). The natural extracts obtained from *Mint spicata* leaves, *Citrus* limon peel, and mix extract of both indicated the larvicidal activity and thus possess the capacity to be used in insecticides. All three crude extracts showed moderate larvicidal effects however, the highest larval mortality was found with mint leaves powder extract with 1.91 mg mg/dL, LC50 value, along with lemon extract 2.32 mg/dL, while mix extract had the lowest LC50 value 4.29mg/dL after 24h 48h 72h of exposure, respectively. These results are in agreement with previous study (Bilal et al., 2013). Previously various studies have been conducted on plant extracts against various crops' pests. In our study lowest mortality was noted with lowest concentration of plant extract. This indicated that increase in concentration of extracts increased the larval mortality. Compared to control significant difference was observed for each of extract used in this study. Mint leaves extract showed highest larvicidal activity followed by lemon peels extract and mint and lemon mixture extract.

## 5. CONCLUSION

The current research shows the effect of all the three extracts of mint, lemon and mint-lemon mixed used in this study against Culex mosquito larvae with highest activity with mint extract. Based on mentioned findings this study suggests that the mint leaves and lemon peels can be used to control Culex mosquito larvae to prevent the spread of diseases.

**Acknowledgement**
The authors acknowledge Department of Zoology, Shah Abdul Latif University, Khairpur, Sindh Pakistan for providing laboratory facilities to perform experimental work.

## 6. References

1. Bilal H, Akram W, Hassan AS. Larvicidal activity of *Citrus limonoids* against Aedes albopictus larvae. J Arthropoda Borne Dis. 2012; 6(2):104–11.1.
2. Brown, L. The New Shorter Oxford English Dictionary on Historical Principles. Oxford [Eng.]: Clarendon. 1993. ISBN 978-0-19-861271-1.
3. Clements, A.N. (1992). The biology of mosquitoes, Vol.1. Development, nutrition and reproduction. Chapman and Hall, New York, NY.
4. Harbach RE. Mosquito Taxonomic Inventory. Available at: http://mosquito-taxonomic-inventory.info/ 2007. (accessed 20 Apr 2011).
5. Imam H, Zarnigar, Sofi G, Seikh A. The basic rules and methods of mosquito rearing (A. aegypti). Trop Parasitol. 2014; 4(1): 53–55.
6. Lees RS, Gilles JRL, Hendrichs J, Vreysen MJB, Bourtzis K. Back to the future: The sterile insect and other technique against mosquito disease vectors. Curr. Opin. Insect Sci. 2015; 10:156–162.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 7266 - 7273
Received 15 December 2020; Accepted 05 January 2021.

7. Leopoldo MR. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with the Dengue virus transmission. Zootaxa (589) Magnolia Press Auckland Newzeland. 2004.
8. Miresmailli S, Isman MB. Botanical insecticides inspired by plant-herbivore chemical interactions. Trends in Plant Science. 2014;19,29-35.
9. Pavela R. Insecticidal properties of Pimpinella anisum essential oils against the Culex quinquefasciatus and the nontarget organism Daphnia magna. Journal of Asia Pacific Entomol. 2014;17:287-293.
10. Reinert JF. New classification for the composite genus Aedes (Diptera:Culicidae:Aedinii), elevation of subgenus Ochlerotatus to generic rank, reclassification of the other subgenera and notes on certain subspecies. J. Am. Mosq. Contr. Assoc. 2000; 16:175-188.
11. Reinert JF. Revised list of abbreviations for genera and subgenera of Culicidae (Diptera) and notes on generic and subgeneric changes. J. Am. Mosq. Contr. Assoc. 2001;17: 51-55.
12. Vogel AI. Text Book of Practical organic Chemistry. The English language Book Society and Longman, London. 1978.
13. Wahid I, Sunahara T, Mogi M. "Maxillae and mandibles of male mosquitoes and female autogenous mosquitoes (Diptera: Culicidae)". Journal of Medical Entomology.2003;40 (2):150–8.
14. World Health Organization (WHO). Dengue transmission research in WHO bulletin. 2010.
15. World Health Organization (WHO). Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES. 2005.