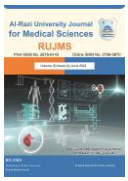
Original Research *Al-Razi Univ J Med Sci 2021; 5(1)*

RUJMS

Al-Razi University Journal of
Medical Sciences



https://doi.org/10.51610/rujms/3.5.1.2021

Morphological Effects of Some plant Extracts on Mosquito Larvae of Dengue Fever Vector *Aedes aegypti (Diptera)* from Al-Hodeidah Governorate- Yemen.

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Abstract

Background: Mosquito vectors have medical important as *Aedes spp*. It transports different pathogens which cause different diseases such as Yellow Fever, Chikungunya and Zika. The incidence of Dengue Fever has grown dramatically in new decades. Nowadays, using natural control as plant extracts has shown to be environmentally safe, degradable and have target-specific. ***Aims***: The present study was carried out to study the morphological changed of Aedes aegypti larvae as result of treatment with *Datura stramonium, Mentha piperita, Nicotiana glauca* and *Peganum harmala* in methanolic and aqueous extracts. ***Methods***: Tested Ae. aegypti larvae were examined by light microscope and anatomy microscope to record changes in their morphology affected by different plant extracts. ***Results***: The control group did not show any morphological damage. Larvae treated with D. stramonium exhibited black color and bend in methanolic extract also black color with spots in aqueous extract. *Mentha piperita* methanolic extract showed dark color body, cuticle pigmentation, abandoned and thin body but dark gut and the larvae in aqueous extract. Dark color and thick body appeared in *N. glauca and P. harmala* extract in addition the detachment between thorax and abdomen segments of treated larvae was clearly appeared in high aqueous extract of P. harmala. ***Conclusion***: The high concentration 800 ppm of extracts had the highest morphological effect on the *Ae. aegypti* larvae.

Keywords: *Aedes aegypti,* morphological, treatment, methanolic extract and aqueous extract.

Introduction

Aedes genus is a viral vector, this virus causes diseases such as Dengue fever (DF), Yellow fever (YF), Chikungunya (CHIK) and Zika (ZIK)[1,2,3,4]. The incidence of dengue has grown dramatically in new decades. About half of the world's population is now at

risk[5]. A seasonal spike in dengue has been reported in Yemen, with more cases in 2019. There have been 14.540 suspected cases of DF, with 79 associated deaths recorded during the 8th months of this year [6]. The treatment method for these diseases is to control mosquitoes borne diseases through insecticides. It was proved that insect species may develop resistance to their insecticide. Moreover, synthetic insecticides are obviously known to cause environmental pollution, bad side effects of the insecticides to human, domestic animals and wild life health have been also detected[7, 8, 9]. Until now there are no vaccines or drugs for this specific dengue fever causing it to spread_histrionically in the past few years[8, 10]. In Yemen, there are neither studies about the morphological effects of plant extracts on *Aedes aegypti* larvae. From this point, it is necessary to conduct intensive effort to use plant extracts for controlling this important vector and their morphological effects. There are few studies on morphological effects of plant extracts against *Ae. aegypti* larvae. In Saudi Arabia, two studies recorded some morphological changes in the larval siphon and pupa of *Ae. Aegypti*[11, 12]. Other study, evaluated ethanolic plants extract of *Thalassia hemprichii* and *Holothuvia atra* against *Ae. aegypti* larvae, they found *H. atra* extract exhibited the high morphological effect[13]. Sharma et al., (2015)[14], evaluated the effect of *Achyranthes aspera* leaf and stem extracts against *Ae. aegypti* larvae and recorded many morphological changes such as the aggressive anal gill biting behaviour forming ring-shaped structure. Sutiningsih et al., (2018)[15] noted that *Brucea javanica* extract had morphological effects on *Ae. aegypti* larvae such as visible damaged heads, cuticles, digestive and respiration tracts, respiratory siphons, and setae. The tested larvae were smaller than normal larvae. Literature review revealed that there is no studies in this field in Yemen, this study considered the first work which examined the morphological changes in *Ae. aegypti* larvae affected by plant extracts. Therefore, this study aimed to determine the changes of plant extracts and to identify its effects on the morphology of *Ae. aegypti* (L.) larvae.

Aim of the study

The present study was carried out to study the morphological changed of *Ae. aegypti* larvae as result of treatment with *D. stramonium, M. piperita, N. glauca* and *P. harmala* in methanolic and aqueous extracts.

Materials and Methods:

Plant Collection and extraction

Leaves of four plant species (*Datura stramonium, Mentha piperita, Nicotiana glauca* and *Peganum harmala)* were collected from different places in Sana'a Governorate, during 2017. The plant leaves were cleaned; air dried for one week at room temperature and powdered. Two hundred gram leaves powder were extracted with distilled water and absolute methanol for six hours using soxhlet apparatus (50gm/ 500ml). The obtained aqueous extract was concentrated by oven at 40 C^0. The yield extract was kept in plastic dark vials and stored in a cold temperature until used against *Ae. aegypti* larvae.

Aedes aegypti collection and strain rearing:

Mosquito strain of *Ae. aegypti* was used in this study. The parental strain larvae were collected from Al-Hodeidah-Yemen. Larvae were rearing and reproductive. To get the 3rd instar and 4th instar stages in these experiments, Twenty instar larvae were put in plastic enamel and exposed to high and low concentrations, the same procedure for rearing larvae was performed.

Morphological study:

Tested *Ae. aegypti* larvae were examined by light microscope and anatomy microscope to record changes in their morphology affected by different plant extracts. The morphological examinations were performed at the highest (800 ppm) and the lowest (200 ppm) concentrations of methanolic and aqueous extracts to clarify the differences between the samples.

Results:

Morphological effect of the four selected plants on Ae. aegypti larvae:

Observation of morphological vicissitudes in *Ae. aegypti* larvae was intended to decide damaged target body parts resulted after the treatment with low and high concentrations, 200 and 800 ppm comparing with control larvae. In *D. stramonium* and *N. glauca* aqueous extract, no mortality was recorded in 200 ppm after 24 hr, larvae examination observed after 48 hr. Moreover, *M. piperita* aqueous extract had no mortality in 200 ppm either after 24, 48 or 72 hr, for these reasons in 400 ppm after 24 hr. larvae were selected to study morphological changes.

The control group did not show any morphological abnormalities (Fig: 1, A).

Datura stramonium extract showed different larval morphological changes in law and high concentrations in methanolic and aqueous extracts. Treated larvae with 200 ppm *D. stramonium* aqueous extract after 48 hr as well as with 800 ppm after 24 hr. and 200 ppm methanolic extract, after 24 hr, exhibited different morphological changes. Morphological alterations in methanolic extract were mild in the lower concentration, while very devious and bend larvae with dark or black color appeared in the higher concentration (800 ppm), (Fig: 1 B and D).

Aqueous extract at lower concentration (200 ppm) after 48 hr. caused cuticle damage. In addition, larvae showed dark color with black spots, while larvae appeared black and fragile at higher concentrations, (Fig: 1 C and E). Larvae treated with *M. piperita* was 400 (there is no mortality in 200 ppm) and 800 ppm concentration of aqueous extracts and 200 ppm and 800 ppm concentration of methanolic extract, exhibited several morphological effects. Using of the low concentration methanolic extract (200 ppm), showed a dark color body and cuticle pigmentation in larvae, while in the high concentration (800 ppm), their bodies appeared abandoned, thin and black color. There was no mortality recorded on 200 ppm aqueous extract after 24, 48 and 72 hr. Therefore, the morphological effect was observed in 400 ppm after 48 hr. treated larvae exhibited dark gut. In high aqueous extract concentration (800 ppm), the larvae appeared dark (Fig: 2. B, C, D and E).

Larvae treated with the low concentration (200 ppm) of *N. glauca* methanolic extract effects in color and cuticles larvae. They become dark and thick bodies' larvae, while in high

concentration larvae appeared thick and black. Morphological effect in 200 ppm aqueous extract after 48 hr. to change in color of larvae, they become light dark larvae, while 800 ppm aqueous extract resulted in thin, black with dark alimentary canal larvae (Fig: 3. B, C, D and E).

Discussion:

The present results reveal that, methanolic and aqueous extracts of the four selected plants had high effect on larvae morphology as morphological distortions at 800 ppm and low effect at 200 ppm lead to death, this result is in agreement with previous studies carried by Mahyoub et al., (2016)[13] and Sutiningsih et al., (2018)[15]. *Aedes aegypti* larvae when treated with *D. stramonium* methanolic extract, showed morphological changes as bends, these results exhibited in the lower concentration (200 ppm) and increased in the higher concentration (800 ppm), dark color of larvae being more pronounced by four methanolic and aqueous plants extracts in 800 ppm, similar observation were reported by Sharma et al., (2015)[14] when the larvae of *Ae. aegypti* were exposed to *Achyranthes aspera* hexane extract.

The killer cause of *M. piperita* methanolic extract insect stages may be due to the effects on the nervous and digestive system of the insect by touching these extracts to the surface[16]. Darkening of larvae body was the major morphological changes exhibited by larvae treated with the four plant extracts. This darkening may be attributed to activation of phenoloxidase in tissues injured by extract component. Also, *M. piperita* extract caused the darkening of the gut which may be attributed to activation of phenoloxidese or to accumulation of leaf extract in the larval gut[17]. Most of the naturally occurring phenolic compounds have pigments and the role of phenolics have mechanisms of plants against phytophagous insects and some of phenolic sequestered in the cuticle, where it imparts a yellowish tinge to the exocuticle[18]. Methanolic extract of *D. stramonium* showed mild bent larvae at 200 ppm and high bent larvae at 800 ppm, which was due to the effect of tropane alkaloids (hyoscyamine and scopolamine) on nervous system of larvae[19]. Morphological effects of *P. harmala* showed detached larvae in aqueous extract of 800 ppm, this result agrees with that of Sutiningsih et al., (2018)[15]. There are poor references on the morphological effects of the four plants extracts.

Conclusion

With regard to the influence of the tested extract on the morphology of larvae, data showed many morphological effects. These effects were more pronounced in the higher concentration (800 ppm). In most cases methanolic extracts were more effective than aqueous extracts.

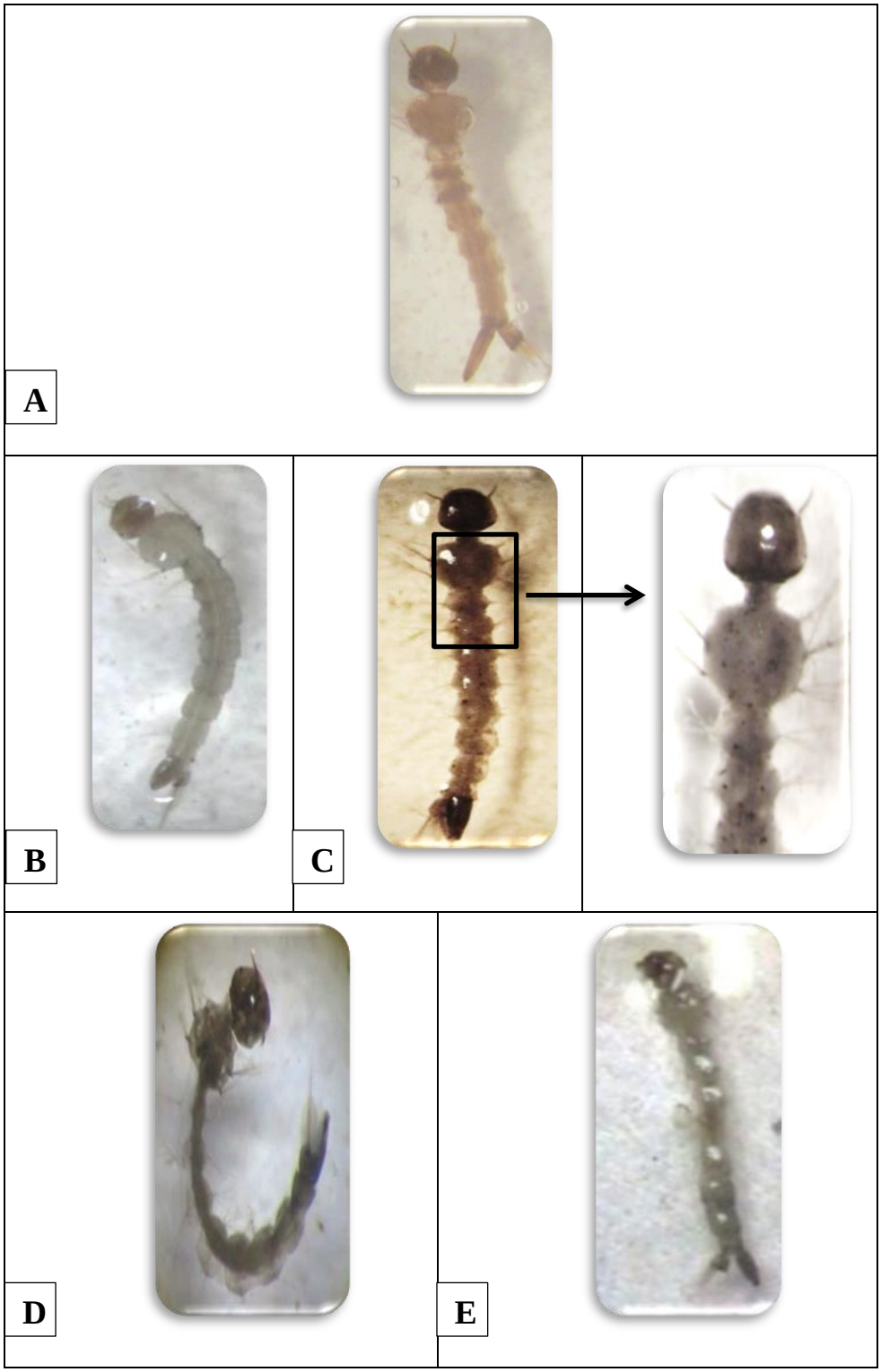

Fig. 1: Morphological abnormalities in *Ae. aegypti* larvae after treatment with ***D. stramonium***. A: Control larvae, larvae treated with B: 200 ppm methanolic extract, C: 200 ppm aqueous extract, D: 800 ppm methanolic extract and E: 800 ppm aqueous extract. after 24 hr.

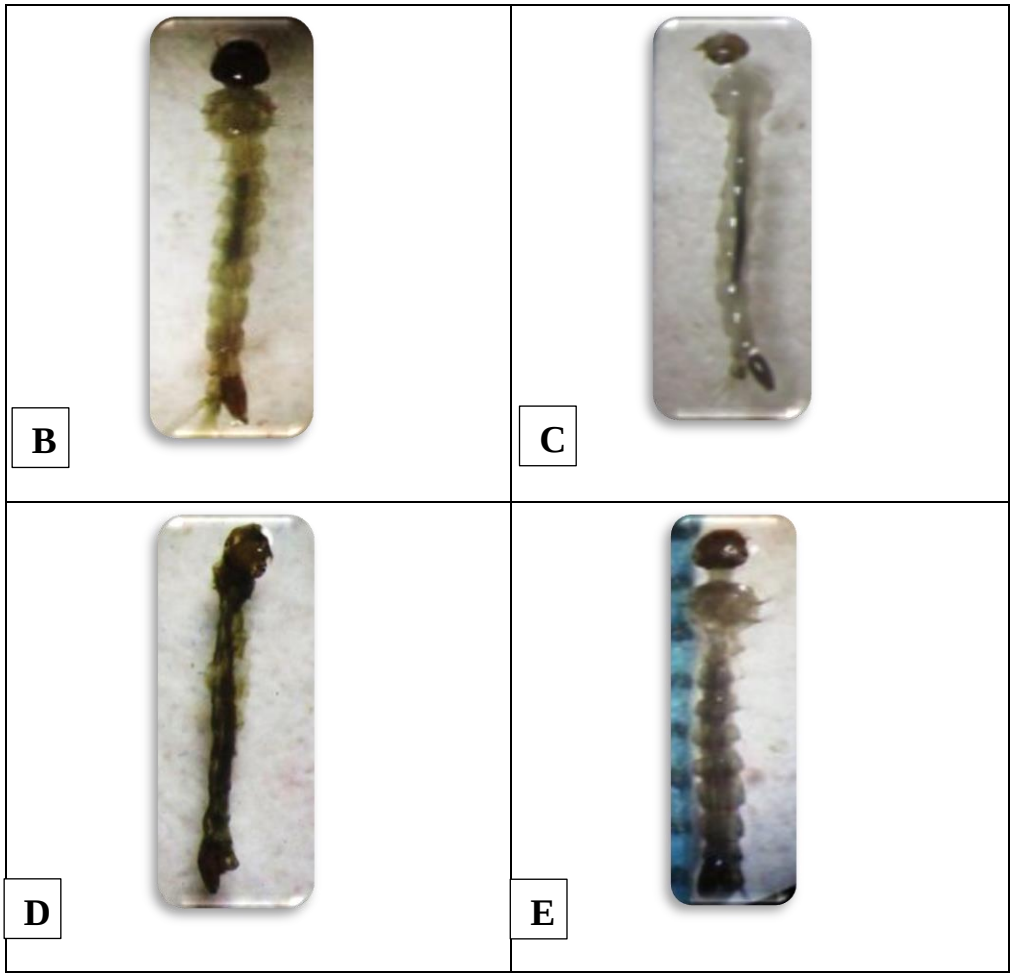

Fig. 2: Morphological abnormalities in *Ae. aegypti* larvae (third and fourth instar) after treatment with ***M. piperita***. Larvae treated with (B): 200 ppm methanolic extract after 24 hr., (C): 400 ppm aqueous extract after 48 hr. (D): 800 ppm methanolic extract and (E): 800 ppm aqueous extract. after 24 hr.

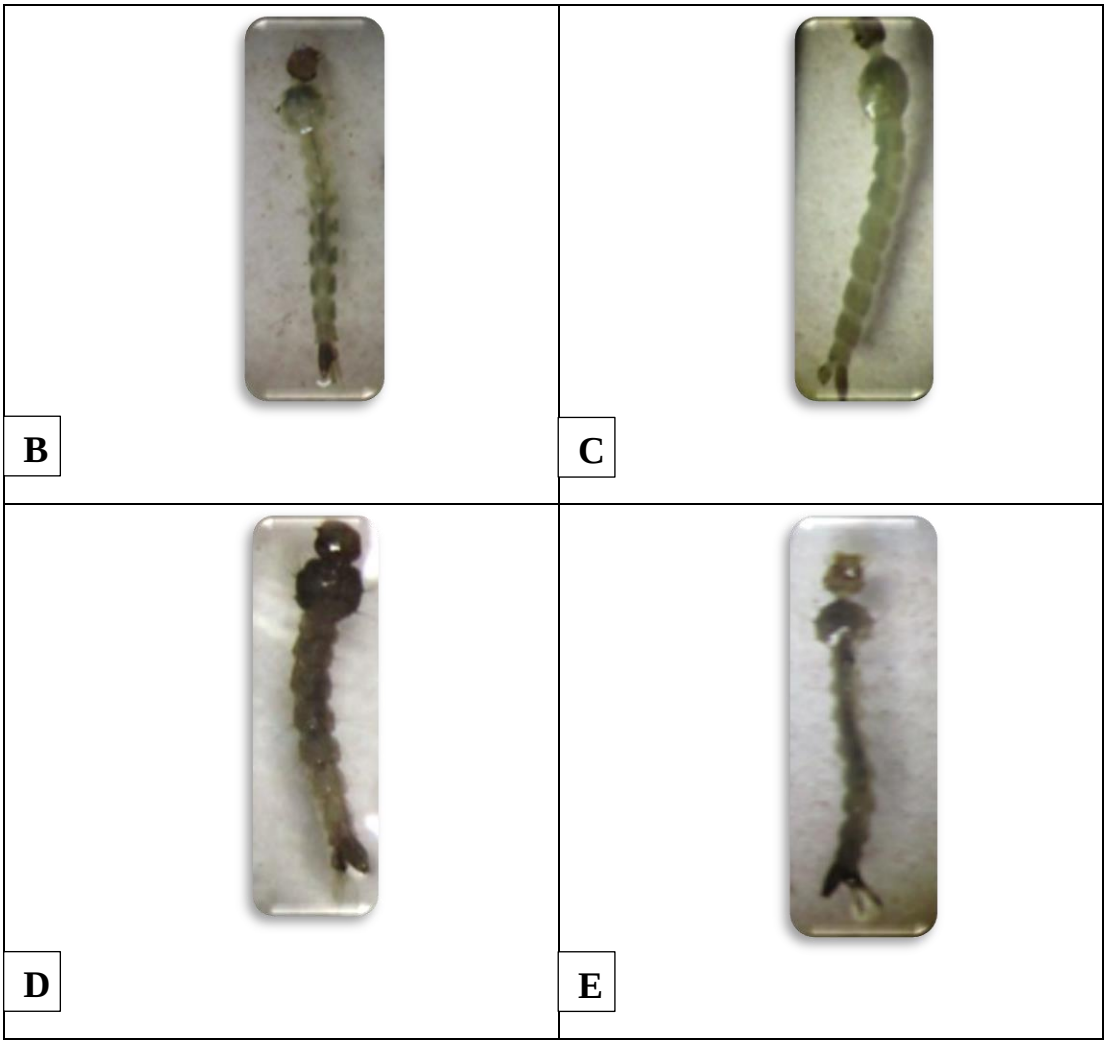

Fig. 3: Morphological abnormalities in *Ae. aegypti* larvae (third and fourth instar) after treatment with ***N. glauca***. Larvae treated with (B): 200 ppm methanolic extract after 24 hr., (C): 200 ppm aqueous extract after 48 hr. (D): 800 ppm methanolic extract and (E): 800 ppm aqueous extract. after 24 hr.

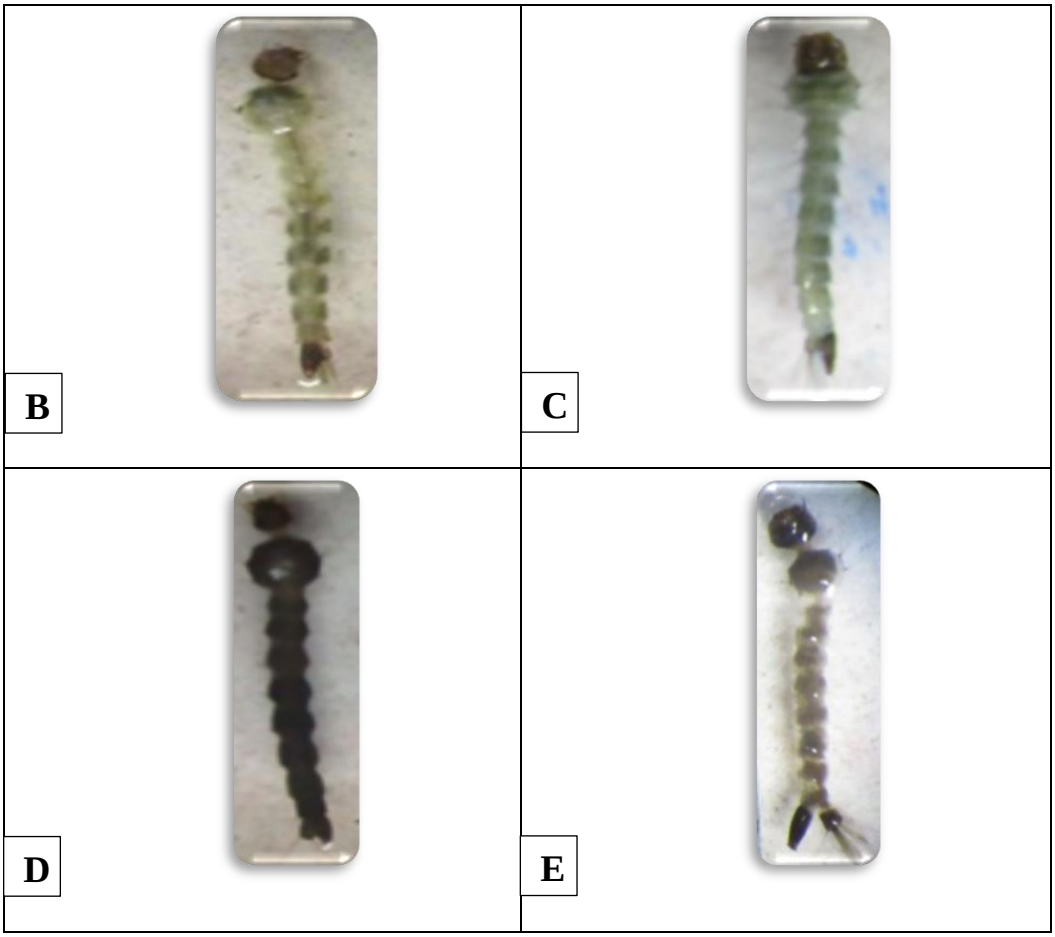

Fig. 4: Morphological abnormalities in *Ae. aegypti* larvae (third and fourth instar)after treatment with ***P. harmala*** after 24 hr. Larvae treated with (B): 200 ppm methanolic extract, (C): 200 ppm aqueous extract, (D): 800 ppm methanolic extract and (E): 800 ppm aqueous extract.

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