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Toxic effect of Crude Alkaloid extract of *Citrullus collocynthis* against *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

The present study was carried out to investigate the toxic effect of crude alkaloid extract of *Citrullus collocynthis* on the mortality rates of immature larval instars of *Culex pipiens* mosquito (Diptera: Culicidae). Results of the present study showed that crude alkaloid extract was more toxic to the first and second instars larvae and mortality rates were reached at concentration 20 mg/ml to (87.2 and 79.6 %) respectively compared to (4.6 and 3.8 %) respectively at control treatments. Whereas the 3rd and 4th instars larvae were less toxic and mortality rates were reached at concentration 20 mg/ml to (66.4 and 50.2 %) respectively compared to (1.4 and 1.2 %) respectively at control treatments. Egg mortality rates were also increased at higher concentrations 20 mg/ml to reach 50.2 % compared to 9.6 % at control treatments. Pupae stages were more resistant at higher concentrations 20 mg/ml reached to 41.7 % compared to 6.4 % at control treatments. The present study also revealed that crude alkaloid extract of *C. collocynthis* negatively impact on hatchability, immature stages development period and female productivity. Moreover the present study showed that cumulative mortalities rates were increased as the concentrations raised resulting in that crude alkaloid extract of C. *collocynthis* was more toxic against of immature stages of *Culex pipiens* mosquito.

Key words *: Mosquito Culex pipiens, Natural products, Citrullus collocynthis, Crude alkaloid extracts.*

Introduction

Mosquitoes are among the best known groups of insects, because of their importance to human as pests and vectors of some of the most serious human diseases (Snow *et al.,* 2005; Asiry *et al.,* 2017). Among these mosquito borne diseases dengue, yellow fever and West Nile Virus are well-known as endemic in Southeast Asian countries transmitted by *Culex pipiens* complex as well as lymphatic filariasis caused by worm bancrofti affected about 106 million people worldwide (Rajesh *et al.,* 2015). Some of these diseases such as Dengue fever and Dengue haemorrhagic fever are common vector borne diseases currently present and more than one hundred countries in the world are infected annually about 80 million people worldwide (Murray *et al.,* 2013). Mosquito as well as their carrying diseases they are the most important blood sucking groups of insects (Rai *et al.,* 2007). One of the control strategies of mosquito borne diseases is the stopping of disease transmission by ether killing ,preventing mosquitoes to bite human or by causing mortality against larvae . Natural products act as larvicidal or ovicidal brought attention for several of investigators because they have bioactive chemicals which act against certain groups of insects and are eco-friendly as well as some plant extracts may act as insecticides, repellents and toxic substances (Inocent *et al.,* 2008; Kamaraj *et al.,* 2010; Masotti *et al.,* 2012). Plants

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derived pesticides are less toxic for human and delay development of resistant by pests because they are easily biodegradable (Raghavendra *et al.,* 2011) plant extracts from several different families have been tested and have appeared promising as larvicides (Deepalakshmi and Jeyabalan, 2017; Grace *et al.,* 2020). Previous studies were done on *Citrullus collocynthis* plant extracts against the dengue fever vector *Aedes aegypti* of (Asiry *et al.,* 2017) indicated that ethanolic extract was more effective to reduce *A. aegypti* larvae under the lower concentrations after 72 h. compared to other tested plants. Several previous studies indicated that *C. collocynthis* could be used as potent larvicidal products against larval stages (Rahuman *et al.,* 2008; Gurudeeban *et al.,* 2010 and Arivoli and Tennyson, 2011).

The present study was carried out to evaluate toxic effect of crude alkaloid extract of *C. collocynthis* against *Cx.pipiens*.

Materials and Methods

Preparation of Crude Alkaloid extract

Fruits of *C. collocynthis* were collected during spring season and washed carefully, then dried in an air oven at 45 ˚C, plant fruits were ground by a blander grinding the dried fruits to obtain a plant powder. 20 g of plant powder was dissolved with 200 ml of absolute ethanol continued with soxhlet apparatus 24h and then the extracts were dried via rotary vacuum evaporator at 60 ˚C, following the procedure of Asiry *et al.,* (2017). Plant powders were kept in a container until used. Crude alkaloid extracts were prepared depending on the standard methods (Harborne, 1984). Stock solutions were prepared by dissolved of 2gm of dried solid Crude alkaloid extract with 5 ml of ethanol and accomplished with distilled water to obtain 20 mg/ml. Other concentration also were prepared by diluting and control treatments were prepared with distilled water mixed with 5 ml ethanol.

Mosquito rearing

Egg rafts of *Cx. pipiens* were obtained from Laboratory at college of Science, University of Kufa and maintained at 27 ±2 ˚C with 75-85 relative humidity. The immature larval stages were reread in plastic trays filled with tap water and were fed on bread extract with yeast extract. The Pupae were transferred daily from the trays to a closed cup containing tap water and were kept inside breeding cage. Adults were supplied with 10 % sugar solution for males nourishment. The adult females were provided a blood meal by placing a pigeon on top of the breeding cage overnight for oviposition.

Ovicidal and Larvicidal bioassay

Four different test concentrations 2.5, 5, 10, and 20 mg/ml were selected and the effects of the tested extracts were determined by following the standard procedure of (WHO, 2005). Each concentration was replicated five times. The larval mortality rates were observed and counted after 24 h of the exposure period. Mortality of eggs also was counted. Mortality of pupae also was determined. Cumulative mortalities of immature stages were observed and counted daily until emergence of adults and during experiment immure stages development period were recorded. The productivity of female also was recorded.

Statistical analysis

Before performing analysis tested concentrations were transferred to angular transformed. Data analyses were subjected to Factorial experiments with completed randomized design by using values with Least Significant Difference L.S.D. At Probability level of 0.05 for significantly exhibition (Al-Rawi and Khalafalla, 2000). Mortality rates were corrected depending on Abbott's formula (Abbott, 1925).

The Results

Results of the current study showed that crude alkaloid extract of *C. collocynthis* significantly effected the mortality rates of immature stages of *Cx. pipiens* (Table, 1). At high concentration of 20 mg/ml the percentage mortalities reached to 50.2, 87.2, 79.6, 66.4, 50.2 and 41.7 % for eggs, 1st instars larvae, 2nd instars larvae, 3rd instars larvae, 4th instars larvae and pupae respectively of *Cx. pipiens* after 24 h from exposure period compared to control treatments were 9.6, 4.6, 3.8, 1.4, 1.2 and 4.6 % for eggs, 1st instars larvae, 2nd instars larvae, 3rd instars larvae, 4th instars larvae and pupae respectively. The increased mortality rates was found to be concentration dependent. Among the immature stages of *Cx. pipiens* the 1st instars larvae were more vulnerable than other instars larvae, whereas eggs, 4th instars larvae and pupae were more resistant immature stages to crude alkaloid extract of *C. collocynthis*.

The present study also revealed that crude alka-

Table 1. Effect of different concentrations of crude alkaloid extract of*C. Collocynthis* against immature stages of *Cx.pipiens* after 24 h.

Conc. Mg/ml	Percentage Mortality rate (%)					
	Eggs	1st instars larvae	2nd instars larvae	3rd instars larvae	4th instars larva3	pupae
0	9.6	4.6	3.8	1.4	1.2	6.4
2.5	19.2	19.6	16.3	14.2	10.6	13.4
5	29.4	37.4	35	28.6	25.3	21
10	38.6	66.7	55.4	49.6	33.6	31.6
20	50.2	87.2	79.6	66.4	50.2	41.7
$L.S.D.P._{0.05}$	4.62	9.04	9.04	9.04	9.04	2.93

loid extract of *C. Collocynthis* negatively impact on hatchability, immature stages development period and female productivity (Table, 2). There is no significant effect of crude alkaloid extract of *C. Collocynthis* on hatchability, but crude alkaloid extract of *C. Collocynthis* significantly effected immature development period and female productivity. Immature development period was reached to 15.6 days at concentration 5 mg/ml compared to 13.8 days at control treatments. Immature development period was prolonged as concentrations of crude alkaloid extract raised.At control treatments Female productivity was 169 egg /female whereas at concentration 5mg/ml Female productivity were declined to 90.6 egg/female. At higher concentrations 20 and 10 mg/ml of crude alkaloid extract female productivity did not appeared because of maximum mortalities rates.

The data presented in Figure, 1 indicated that crude alkaloid extract of *C. collocynthis* was more toxic against the cumulative moralities rates of immature instars larvae and were reached the maximum at the 2nd stage larvae with concentration 20 mg/ml. At concentration 10 mg/ml the cumulative moralitiesrates were reached to the top curve at pupae stages. Other concentrations clearly appeared different values of the cumulative moralities rates ranged between 73.6 to 57.6 % at concentrations 5 and 2.5 mg/ml respectively compared to 30 % at control treatments.

Table 2. Effect of different concentrations of crude alkaloid extracts of *C. Collocynthis* on Hatchability, Immature development period and productivity of female of *Cx.pipiens*.

Conc. Mg/ml	Parameters		
	Hatchability (%)	Immature stages development period (Day)	Female productivity (Egg/female)
0	96	13.8	169
2.5	94	14.4	151.6
5	93.6	15.6	90.6
10	—-	——	——
20	——	——	——
$L.S.D._{P.0.05}$	——	1.04	17.6

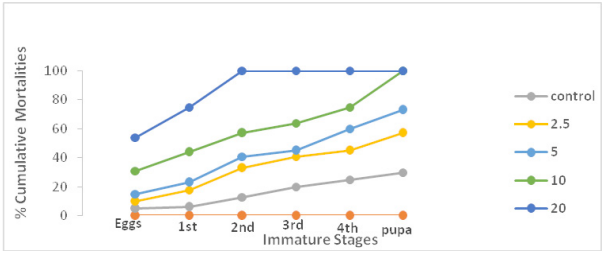

Fig. 1. Effect of crude alkaloid extract of *C. Collocynthis* on the cumulative mortalities rates of immature stages of *Cx. pipiens*.

Discussion

The results of present study demonstrated the toxic effect of crude alkaloid extract of *C. collocynthis* against immature instars larvae of *Cx. pipiens*. The 1st and 2nd instars larvae were more sensitive to toxic crude alkaloid extract than 3rd and 4th instars larvae, as well as eggs and pupae stages which were more resistant to the activity of crude alkaloid extract. The different instars larvae susceptibility result agree with several previous studies (Rahuman *et al.*, 2008; Arivoli *et al.*, 2015; Asiry *et al.*, 2017) who found that 2nd instars larvae was more susceptible than 3rd instars and the later was more susceptible than 4th instars larvae to the phytochemicals and this may support the ideas of previous investigations (Kumar and Dutta, 1987) who observed that insects age and physiological status of larvae plays an important role in influencing susceptibility. In earlier studies it

was found that the 1st and 2nd instar larvae were more effective to toxic phytochemicals due to their thin layer cuticles (Mordue and Blackwell, 1993). The findings of the present study revealed that crude alkaloid extract of *C. collocynthis* had larvicidal activity against larvae mosquito. Present findings with other earlier studies occurring on phytochemicals as insecticides may play a more chief role in mosquito control programs in the future, as well as will contribute to reduce in the application of synthetic insecticides. The biological activity of the crude alkaloid *C. collocynthis* extracts may be due to different phytochemicals, including alkaloids which possess deterrent, anti-feedant, growth regulating and fertility reducing properties in insects (Prabuseenivasan *et al.,* 2004). Phytochemical analysis of plant extracts demonstrated the presence of carbohydrates, proteins, separated amino acids, tannins, saponins, phenolic, flavonoids, alkaloids, anthranol, steroids, Cucurbitacin A, B, C, D, E, caffeic acid and cardic glycoloids (Al-Snafi, 2013). These compounds may jointly or independently contribute to produce toxic activity against *Cx. pipiens.* It was earlier reported that whole plant is effective against mosquito larvae (Rahuman *et al.,* 2008). Likewise, study of Rahuman and Venkatesan (2008) who investigated several extracts of *C. collocynthis* all tested extracts showed moderate larvicidal effects except petroleum ether extract was found a high larval mortality against the 4th instar larvae of *Cx. quinquefasciatus*. Whereas Study of Helfi *et al.,* (2016) have studied alcoholic extracts of *C. collocynthis* showed that were more toxic against the larvae of *Chrysomya bezzaina* fly showed 100% mortality in larvae in each instars. The present results with the earlier reports on same plant indicated that crude alkaloids may be more effective. This also agrees with the report of Arivoli and Tennyson (2011) who found whole plant crude extracts of *C. collocynthis* have larvicidal activity against *Cx. quinquefasciatus* larvae appeared to be more susceptible followed by *Aedes aegypti* and *An. stephensi*. Investigation of Hamid *et al.,* (2016) indicated that ethanol extracts of *C. collocynthis* fruit pulp and seeds demonstrated considerable mortality in *An. arabiensis* and *Cx. quinquefasciatus* the percentage mortality in *An. arabiensis* larvae treated with ethanol extracts of *C. collocynthis* fruit pulp showed relatively higher larvicidal potentiality ranged between 90 % to 45% and when they were treated with seed extract ,mortalities ranged between 85% and 35% whereas mortality ranged between 90% to 45% against *Cx. quinquefasciatus* and the results agreed with current study. These findings also agree with the report of Asiry *et al.,* (2017) who have tested *C. collocynthis* ethanolic extracts against the larval stages of *Aedes aegypti*. Present findings are in agreement with that of results obtained by Satti and Edriss, (2014) who revealed that ethanolic extracts 0.5% of *C. collocynthis* fruits lead to mortalities reached to 57.5% against 4th instars larvae of *An. arabiensis*. Ethanolic extract of *C. collocynthis* fruits also was tested by Torkey, *et al.* (2009) who investigated chloroform and ethanolic extracts against *Aphis craccivora* showed maximum insecticidal activity. Study of Al-Murmidhi and Al – Hasnawi, (2009) who investigated the activity of crude phenols extract of *C. collocynthis* against *Musca domsestica* flies at concentration 20 mg/ml, mortality rates of eggs and larvae were reached 85.44% and 90 % respectively compared to 18.43% and 16.6% at control treatments. The results of the present study conformed with several studies that proved other plant extracts have bio-active chemicals serve as potential, environment safe and alternative to synthetic insecticides may act as repellents, contact insecticides, fumigants, or could intervene with any of the vital functions of the insects (Rahuman and Venkatesan, 2008; Helfi *et al.,* 2016; Hamid *et al.,* 2016). Study of Raghavendra, *et al.* (2011) who demonstrated the toxicity of the *Eugenia jambolana* leaf extracts against three species of mosquitoes in the order of *Aedes aegypti*, *Cx. quinquefasciatus*, *An. stephensi* were found to be significantly different among all tested extracts.

The present study revealed that crude alkaloid extract of *C. collocynthis* cause increase of egg mortalities rates and pupae of *Cx. pipiens* in higher concentration but at the lower concentrations the mortalities rates were decreased. The effect of crude alkaloid extract of *C. collocynthis* on egg mortality and pupae stages may be added significance to larvicidal activity of crude alkaloid extract of *C. collocynthis*. Study of Rajkumar and Jebanesan, (2004) who demonstrated ovicidal activity of *Solanum trilobatum* leaf extract against egg rafts of *Cx. quinquefasciatus* and these findings agreement with results of present study. The lesser effect of the crude alkaloid extract of *C. collocynthis* on eggs may be contributed to egg shell is comprised of several layers to protect the embryo additionally chitin (Moreira *et al.,* 2007). The toxic effect of alkaloid substances against eggs may

be causing induration or interfering with the embryogenesis process (O'Kane and Baker, 1934). The present study showed that crude alkaloid extract of *C. collocynthis* had also a toxic effect on pupae of *Cx. pipiens* which were not much affected by that crude alkaloid extract of *C. collocynthis* and this may be contributed to the non-feeding behaviour of pupae stages wheras the phytochemicals enter the insect system through oral feeding and affect the gut and other organs (Deepalakshmi and Jeyabalan, 2017). Present study demonstrated that crude alkaloid extract of *C. collocynthis* leads to prolonged immature larval stages development and pupation. The cumulative moralities rates of immature instars larvae showed increased mortalities rates related with increase in concentration of the crude alkaloid extract. Present study also demonstrated that crude alkaloid extract of *C. collocynthis* negatively affected female productivity and this may be explained to either by sexual dysfunction that disruption of hormonal system responsible for reproduction or by decreasing metabolic levels which affect the maturation of eggs (Stockel *et al.,* 1994; Amira and Boudjelida, 2013). In the same line Investigation of Bramhim *et al.* (2016) who tested the activity of aqueous extract of *C. collocynthis* fruits on the 4th instar larvae against two species of mosquitoes *Cx. pipiens* and *Culiseta longiareolata* the results showed there was a reduction in female fertility of the emerged larvae for *Cx. pipiens* the number of eggs laid was 409, 261 and 123 egg/female with concentrations 10, 50 and 100 mg/ml respectively compared to 598 egg/female at control treatments. In similar study of Mansoor and Thameri (2010) who investigated toxic effect of phenols and alkaloids extracts of *C. collocynthis* against *Sarcophaga haemorrhoidalis* meat fly larvae the alkaloid extract at concentration 50% 1st ,2nd and 3rd instars larvae mortality rates reached 70,56.6 and 50% respectively also affect mortality rates of pupae. The results of the present study may be contributed to offer a background for further investigations to find out the active mosquito larvicidal compounds from crude alkaloid extract of *C. collocynthis*. However, further studies are needed to investigate the biochemical structure and mode of action of the active compounds which have ovicidal and larvicidal activity for crude alkaloid extract of *C. collocynthis*.

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