

Laboratory evaluation of the root exudates of the *Clerodendron inerme* in controlling on *Aphis craccivora* Koch

Maryem Akbal Hasoon* and Ahmed Saeed Mohamed

ABSTRACT

This study was conducted to check for the biological efficacy of root exudates of *Clerodendron inerme* on some biological aspects of *Aphis craccivora*. Also, the study included the detection of the compounds in root exudates of *C. inerme* by using the gas chromatography-mass spectrometry technique. Results showed that the high concentration of root exudates recorded high cumulative mortality of the adult insects after exposure time 1, 2, and 3 days from treatments 28.5, 33.8, and 40.3% respectively compared with 32.4, 39.3, and 48.5% respectively of nymphs insects. The developmental period of the immature stages has increased from 6.5days in the control treatment compared with 9.3days at high concentration while the fecundity of adult females reduced to reach 18.00nymph at high concentration compared with 26.66nymphs in the control treatment. GC-MS analysis of root exudates of *C. inerme* showed the presence of many compounds such as 2-Propenoic acid, ethenyl ester, 2-Phenanthrenol.

Keywords: *Aphis craccivora*, *Clerodendron inerme*, GC-MS Analysis

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INTRODUCTION

The cowpea aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) is one of the important pests and cause substantial damage in a good number of agricultural crops especially cowpea as sucking on vital plant sap and interfering with photosynthetic functions and it the transmission of some viruses diseases to the host plant such as cowpea mosaic and papaya mosaic that negatively affects the productivity or yield of the crop (Choudhary *et al.*, 2017). In order to protect the environment from the harmful effects of using chemical pesticides in addition to showed resistance of *A. craccivora* of pesticides (Kandil *et al.*, 2017). So, Researchers have resorted to using environmentally safe alternatives such as entomopathogenic fungi (Mohmed, 2019a), and plant extracts Because it contains many secondary metabolic compounds similar to those found in chemical pesticides (González Valdivia *et al.*, 2017). The presence of secondary metabolism compounds in plants is not limited to leaves, stems, and roots, as it was

diagnosed that the roots of plants release a wide range of compounds like allelic chemicals, polysaccharides, amino acids, aliphatic acids, aromatic acids, fatty acids, sterols, phenolics, enzymes, vitamins, and other secondary metabolites (Bertin, 2003). There are no studies that have systematically investigated root exudates collection and its effect in controlling the pest. So, the present study evaluates the novel method of the potential of root exudates of *C. inerme* as a deterrent against *A. craccivora* under laboratory conditions.

MATERIAL AND METHODS

Insect Collection and Rearing

Cowpea aphids were collected from the cowpea and broad bean crop fields of the College of Agriculture, Al Qasim Green University. *A. craccivora* was reared on healthy cowpea in greenhouses by planting potted which is the source of the insect for next experiments. Cowpea aphids were collected from the cowpea and broad bean crop fields of the College of Agriculture, Al Qasim Green University. *A. craccivora* was reared on healthy cowpea in

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Preparation of root exudates

A large 5-liter bottle was brought, a hole was made in the bottom and then covered the hole with silicone to easy withdraw the root exudates by a medical syringe, A *C. inerme* plant was uprooting the plant from the ground carefully to preserve its roots, which was washed with distilled water several times to remove the dust stuck in it, the plant was placed inside the bottle, Then the bottle is placed in a large pot containing soil to obtain darkness and conditions similar to the original medium of the plant. An amount of 100 mL was withdrawn from the bottle after different period time 7, 14, and 21 days which were considered as concentrations to be used in subsequent experiments. As for the control treatment, only distilled water was used.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To diagnose and identify compounds of the root exudates of *C. inerme*, this analysis was performed by Instrument of Gas Chromatography-Mass spectrometry Shimadzu's in laboratories of the Ministry of Science and Technology – Baghdad.

Bioassay Test

To perform biological tests for the effect of the root exudates of *C. inerme* 10 adults and nymphs of *A. craccivora* were placed separately on the filter paper into 9 cm diameter Petri dishes using a hairbrush. Aphids were sprayed directly using a 1cc insulin syringe with the root exudates of *C. inerme* concentrations. The same number was used for controls as well. Leaves of cowpea were placed for feeding the adults and nymphs. Petri dishes had a 1-cm hole covered with nylon mesh on the lid for ventilation. The dishes were sealed by adhesive tape to avoid the adults' and nymphs' escape. The treated plates were incubated at ($25 \pm 2^\circ\text{C}$ and $65 \pm 5\% \pm 10$ R. H.). Mortality was recorded after 1, 2, and 3 days post-treatment. To calculate the developmental period of the immature stages and fecundity of adult females, 20 days day old first-instar nymphs (neonate aphids) were collected and treated with the root exudates of *C. inerme* concentrations as in

previous experience. Neonate aphid's treatment has been pursued to reach the adult stage. The developmental period of the nymphal stage was recorded to reach the adult stage. To determine the fecundity of adult females emerged adults were selected (3 adults per replicate and three replicates per treatment) and placed onto leaves of cowpea. The number of nymphs produced by these adults was recorded (Mohmed, 2019b).

Statistical analysis

RESULTS

GC-MS analysis of root exudates of *C. inerme*

The results analyzing of the mass spectrum of GCMS of the root exudates of *C. inerme* in Fig.1 showed containing the root exudates on

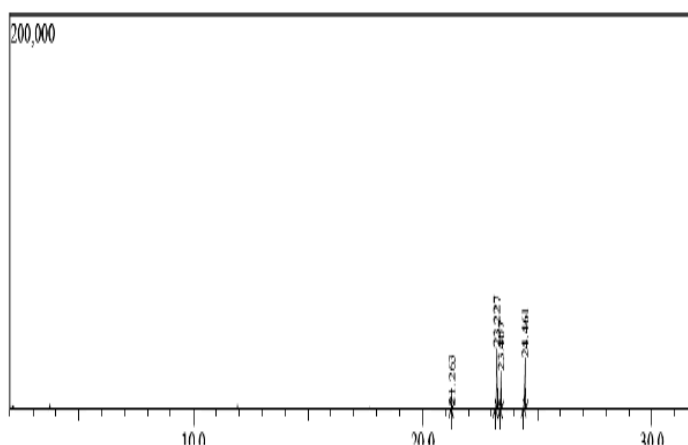


Figure 1. GC-MS Chromatogram of root exudates of *C. inerme*

Table 1. Compounds found in the root exudates of *C. inerme*

Name of compound	Retention time	Peak area	Peak Height
2-Propenoic acid, ethenyl ester	21.263	2898	1799
2-Phenanthrenol, 12-Hydroxyabieta-9	23.227	79389	31447
Diisooctyladipate, Hexanedioic acid	23.407	32390	18940
Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-6-methoxy-1	24.461	67329	25543

many important chemical compounds such as 2-Propenoic acid, ethenyl ester, 2-Phenanthrenol, 12-Hydroxyabieta-9, Diisooctyladipate, Hexanedioic acid, and Phenanthrene,

1,2,3,4,4a,9,10,10a-octahydro-6-methoxy-1, listed in Table. 1, according to Retention time. The results agreed with (Li *et al.*, 2012) which indicated that the root extract of *C. inerme* contained many compounds as 6-methylene-3,3-diphenyl-1,4-cyclohexadiene.

A. craccivora mortality

The results presented in Table 2 show that the root exudates of *C. inerme* concentrations showed effectiveness in controlling on adult and nymph of *A. craccivora* during different time periods. The highest concentration recorded high mortality in both adult and nymph stages with the increase in periods time to reach 48.5and 40.3% respectively, compared with 6.7and 4.1% in control treatment after 3 d of exposure. A significant difference was observed toward the decrease of mortality in adults compared to nymphs.

Table 2. Effects of root exudates of *C. inerme* concentrations on mortality of adult and nymph of *A. craccivora*at different time periods.

Conc. period time	Mortality(%)							
	Nymph				Adult			
	Time periods/days				Time periods/days			
	1	2	3	Mean	1	2	3	Mean
o	0.0	4.1	6.7	3.6	0.0	4.1	4.1	2.7
7	25.3	30.5	36.1	30.6	22.2	26.0	31.4	26.5
14	28.7	33.0	40.6	34.1	24.6	27.1	33.7	28.4
21	32.4	39.3	48.5	40.0	28.5	33.8	40.3	34.2
L.S.D ≤ 0.05	Con.=4.2 per.=3.3 con.xper.=7.1				Con.=4.1 per.=3.4 con.xper.=7.0			

Developmental period and fecundity of *A. craccivora*

The statistical analysis of the results in table 3.showed

Table 3. Effects of root exudates of *C. inerme* concentrations on developmental period and fecundity of adult of *A. craccivora*.

Concentration	Developmental period (days)	Productivity nymph / Female
0	6.5	26.66
7	7.6	22.00
14	8.0	20.33
21	9.3	18.00
L.S.D (P≤0.05)	0.3	0.5

The period of development increased with an increase in the concentration of root exudates of *C. inerme* compared with control treatment, it increased from 6.5days in control treatment to

about 9.3 days at high concentration. While Female productivity decreased as the concentration increased where the average number of births adult females of *A. craccivora* significantly reduced from 26.66nymphs in the control treatment to 18.00nymphs at high concentration.

DISCUSSION

Our hypothesis was that compounds of *C. inerme* root exudates had a positive effect on some biological aspects of *A. craccivora* under laboratory conditions. To validate this hypothesis, a newly developed procedure to collect root exudate can be performed. With further study, we found that all three concentrations affected aphid mortality, developmental period, and fecundity of adults. The possible reason might be the contents of root exudates of chemical compounds, which appeared clearly in an analysis gas chromatography-mass spectrometry. Recent studies have shown that the chemical constituents of the root exudates may act as a signalling chemical in plant defences against herbivores (Murata *et al.*, 2019) such as JA which is a common signalling chemical that elicits the production of defensive metabolites in plants against herbivores or plant competitors (Kong *et al.*, 2018). Many explanations for our results where most of the published research indicates the effectiveness of plant extracts from the leaves, stems, and fruits of plants in controlling pests because they contain many secondary metabolites such as phenols, terpenes etc. (Ibanez *et al.*, 2012) . The roots of a plant must have a role in the production, and it may be the main source of their production through the metabolic processes that conduct in the root. So, root exudates similar to extract of a plant in effected on pest as a toxic, repellent, antifeedant growth, impair their feeding and reduces the fertility of *A. craccivora*. The nymphs were more sensitive than adults to root exudates maybe back to cuticle at this stage of development which be thinner and more vulnerable to root exudates and therefore facilitates the penetration into the cuticle of the insect or structure may affect the amount of a chemical compound that enters its body (Benelli

et al., 2020). The results obtained from these studies have exposed the good potential for the use of root exudates of *C. inerme* for the control and can be considered as a promising effective pesticide against the cowpea aphid *A. craccivora* or it can be tested on other pests.

This study has presented new methods of research and selection and optimization of a new type of bio pesticide of pest represented by root exudates of plants Which aimed to open new horizons in the use of alternative methods for chemical pesticides, which be safe for the environment, cheap, and easy to obtain.

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