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Research article

Insecticidal effect of crude plant extract of *Adhatoda vasica* against *Brevicoryne brassicae*

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ABSTRACT

The present study evaluates the insecticidal activity of (acetone, methanol and water) crude leaf extract of *Adhatoda vasica* against *Brevicoryne brassicae*. The alcoholic extracts (5 %) that extracted by acetone and methanol showed 100 and 81.6% mortality of adult *B. brassicae,* respectively and 100 and 90% mortality of nymphs *B. brassicae*, respectively. Phytochemical screening revealed that the presence of flavones, volatiles oils, tannins, saponines, glycosides, alkaloids, resins and terpenoids in methanol (80%) crude extract. It can be concluded that the acetone and methanol crude leaf extract of *A. vasica* nees can produce high mortality percentage in (nymphs and adults) of *B. brassicae*, so it can be used as insecticides.

Keywords: *Adhatoda vasica, Brevicoryne brassicae,* Insecticidal, plant extract.

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INTRODUCTION

During the previous periods, the pesticides caused economic substantial losses in agricultural crops. For combating the agricultural insect pests, many countries and corporations were adopted on synthetic insecticides that begun in the 1940s [1]. There are many chemical synthetic insecticides were used and commercially. The accumulation of synthetic insecticides in the environment with toxic residues led to the many problems such as resistance of insects, resurgence of new insect pests, contamination of the agro ecosystem, contamination of air, water and soil, and other many problems.

Substances obtained from natural sources can be controlling insect pests (pyrethrum, rotenone and nicotine) and can be used as biological control agents [2]. The use of plant material or crude plant extracts (botanical insecticides) for the protection of crops and stored products from insect pests is probably as old as crop protection itself [3]. Plants have evolved a wide variety of chemical compounds which are known as secondary metabolites were flavonoids, volatiles oil, tannins, saponines, glycosides, alkaloids and resins [4] for protecting plants against pests.

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Therefore, these compounds were used in the production of pesticides. *Adhatoda vasica* (L.) (Acanthaceae) is an insecticidal plant [5]. Leaf extract has anti feeding activity against *Spodoptera littoralis* [6]. In present study, the possibility of using *A. vasica* extract against *B. brassicae*.

MATERIALS AND METHODS

Collection of plant material

The leaves of *A. vasica* (Acanthaceae) were collected from different places in the gardens of University of Baghdad during October 2014. The collected plants were washed with clean tap water and then left at room temperature at 22-25 °C for 2-3 weeks for drying. After that, the collected plants were grinded by electric blander to convert the dried plants to cross dried powder.

Crude extraction

Crude plant extract was prepared by using two solvents (acetone and methanol 80%), dried leaves powder (200 gm) was kept in a thimble and extracted by soxhlet apparatus, which contained 600 ml of the solvent, at 50°C for 36 h with an extraction ratio of (1:3) [7]. The extract was filtered and then concentrated under reduced pressure in a vacuum evaporator below 40 °C. Preparation of an aqueous extract of dried leaves powder (150 gm) was added to a flask contained 450 ml of distilled water and piu onto hot plate stirrer apparatus at 80°C for 24 h with an extraction ratio of (1:3 (extract : water) [8]. The extract was filtered and then concentrated under reduced pressure in a vacuum evaporator below 40°C.

Phytochemical screening

Phytochemical analysis were performed to assess the qualitative chemical composition of different crude extracts using commonly employed precipitation and coloration reactions to identify the major secondary metabolites like flavonoids, volatiles oil, tannins, saponines, glycosides, alkaloids and resins. The phytochemical analysis was carried out using different reagents [9].

Preparation of *B. brassicae* colonies

Colonies of *B. brassicae* pest were prepared in order to conduct the bio-evaluation of pests. The pest were obtained from *Ricinus Communis Linn* plants which infected with these pests. *B. brassicae* infected the lower and the upper surface leaves of the plant. Aluminum plate trays were prepared with dimensions (3.5 x 42 x 32) cm each was contained cotton wetted with distilled water and leaves of *Ricinus Communis Linn* plant infected *B. brassicae* pest. The trays were incubated in an incubator at temperature 27°C and relative humidity of 80-85 %. After that, clean and free infection of castor leaves were placed in the same trays in contact with infected plant leaves. The witting of cotton and the cultivation conditions were maintained for pest colonies growth. After that, different concentrations of crude extracts (20 %) as stock solution were prepared (0.25, 0.5, 1 g/L) to obtain different dilution of 1.25, 2.5 and 5 ml/L. Then, spray tower was used to spray 3 ml of each concentration on dishes that contained the pests [10].

Bio-evaluation of extract on *B. brassicae* pest (nymphs and adults)

Bio-evaluation of different plant extracts in terms of effect of them on *B. brassicae* (nymphs and adults) was performed. Six dishes as replicate were prepared for each concentration contained castor leaves with diameter of 2/ inch. A number of *Bemisia Tobaci* adults pest (male and female) were added to each dish. The dishes were then incubated in an incubator at temperature 27 °C and relative humidity (80-85 %). During 48 h, eggs were formed and ovulation has finished. After that, the adult pest and excess eggs were removed with leaving 10 eggs for each dish in order to get converged nymphs age. After process of hatching, all nymphs were transferred to new dishes. These dishes were splashed with 3 ml of each dilution of plant extract (1.25, 2.5, and 5) ml/L by spray tower. Then, the dishes were incubated for three days and the bio evaluation for crude plant extracts effects, was determined according to the killing percentage of pests. Thereafter, the plant crude extracts were compared with control sample that contained distilled water, dispersing and adhesive factors (citowett), as well as with chemical synthetic pesticides (1.8% Abamectin) at the same amount mentioned previously and according to the manufacturer's instructions. The relative efficiency of plant extracts was estimated according to the Henderson equation [11].

$$\left(1- \frac{\text{Insect population before treatment} * \text{Insect population after treatment}}{\text{Insect population after treatment} * \text{Insect population before treatment}}\right) \times 100 \text{ RE}$$

Statistically

Results were analyzed statistically by using CRD (Complete Randomize Design. The least significant differences (LSD) test was used to compare between means at 0.05 level of probability.

RESULTS AND DISCUSSION

Detection of active materials (secondary metabolites) in the crude plant extracts of *A. vasica* leaves

Investigation of active materials (secondary metabolites) in the methanolic extract of *A. vasica* leaves showed the presence of flavonoids, volatiles oil, tannins, saponines, glycosides, alkaloids and resins. While, coumarins and phenolic compounds were not found (**Table 1**). The methanolic extract effect on nymphs of *B. brassica* higher (little) than acetone extract, with an average of mortality reached to 62.6 and 62.2 %, respectively.

Table 1. Secondary metabolites composition in the crude extract of *A. vasica* leaves. Bio-evaluation of the crude plant extracts on the different phases of *B. brassicae pest.*

No.	Test	Reagent	Color reagent	Result
1	Coumarine	Toluene: acetone: formic acid	Nil	-
2	Flavonoids	Ethyle acetate + KOH	Yellow	+
3	Volatiles oil	UV. light	Pinkish	+
4	Tannins	Lead acetate	Gel precipitate	+
5	Saponines	Mercuric chloride	White precipitate	+
6	Glycosides	Keede reagent	Violet ring	+
7	Alkaloids	Dragendroffs reagents	Orange-red	+
8	Resins	HCL 4%	Turbidity	+
9	Phenolic compound	Potassium – ferric cyanide	Nil	-

The statistical analysis showed no clear statistical differences between them. On the other hand, the effect of water extract was zero (0%). It was found that, the best concentrations were (5, 2.5 and 1.25) ml/L, with an averages of killing reached to 63.3, 51.6 and 9.4 %, respectively. The statistical analysis showed a clear statistical difference among them (**table 2**).

In addition, the best killing effect on nymphs of *B. brassicae* was observed at the dilution of 5 ml/L for both acetone and methanolic extract (100 and 90 %, respectively). The statistical analysis showed an existence of statistical differences. In addition, the less effective interaction on nymphs of *B. brassicae* at the dilutions of 5, 2.5 and 1.25 ml/L for the water extract with zero (0 %) of relative efficiency for both of them.

Table 2. Effect (relative efficiency) of different dilutions (5, 2.5 and 1.25 ml/L) of acetone, methanolic and water extracts of *A. vasica* plant leaves on nymphs of *B. brassicae*. LSD between extracts = 0.56 at level 0.05. LSD between dilutions= 0.56 at level 0.05. LSD for interaction between extracts and dilutions = 0.98 at level 0.05.

Extracts	5	2.5	1.25	Average
Acetone	100	73.3	13.3	62.2
Methanol	90	81.6	1.5	62.6
Water	0	0	0	0
Average	63.3	51.6	9.4	
Control	0	0	0	
Abamectin 0.5ml/L	100	-	-	

In **table 3**, acetonic extracts effect on adults of *B. brassicae* pest higher than methanolic extract with an average of killing reached to 57.2 and 47.1%, respectively. The statistical analysis showed no clear statistical differences between them. In addition, an average of the killing of water extract was zero (0%). In addition, the best killing effect on adults of *B. brassicae* at dilution 5 ml/L for both acetone and methanolic extract with the values reached to 100 and 81.6%, respectively. The statistical analysis showed an existence of statistical differences, in addition to the less effective interaction on adults of *B. brassicae* at level (dilution) 5, 2.5 and 1.25 ml/L for the water extract with zero (0%) effective.

Table 3. Effect (relative efficiency) of acetone, methanolic and water extract of *A. vasica* plant leaves on adults of *B.brassicae.* LSD between extracts = 0.43 at level 0.05; LSD between dilutions = 0.43 at level 0.05. LSD for interaction between extracts and concentrations = 0.78 at level 0.05.

Extracts	5	2.5	1.25	Average
Acetone	100	70	1.6	57.2
Methanol	81.6	58.3	1.6	47.1
Water	0	0	0	0
Average	60.5	45.7	1.06	
Control	0	0	0	
Abamectin 0.5ml/L	100	-	-	

Several study focused on the extract the active materials from leave and seed of different plants [12,13] As mentioned previously, acetone and methanolic extracts were very poisonous for adults and nymphs of *B. brassicae* pest that were have convergent effect. This convergence may belong to the type of active materials (secondary metabolites) in organic crude extracts which were clarified previously. In addition, higher impact of acetone than methanolic extract as insecticides against adults of *B. brassicae* may relate to the ability of acetone to dissolve and extract the active materials more than methanol, which reflected in biological impact against pests. Moreover, the lack of effect for the water extract as insecticides probably is due to the absence of ability of water as a solvent for extract active materials [14]. Result of this study agrees with Shuaa *et al*, [15]. This referred that the alcoholic extract of plant gave a killing average reached to 45.6% versus aphid and house flies. This may relate to the difference in insect types and their physiological differences.

Results showed that the acetone and methanol 80% crude leaf extract of *A. vasica* nees can cause mortality in (nymphs and adults) of *B. brassicae*, so it can be used as insecticides.

Conflict of interest

The authors declare that they have no conflict of interests.

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