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The Toxic effects of *Achillea millefolium* and *Heliotropium* bacciferum on specific life performance aspects of the *Callosobruchus maculatus* in vitro

Wafaa Mashkoor Husein[1] and Abdulla Nezar Ali[2*]

ABSTRACT

Nowadays, one of the main pests affecting stored seeds is *Callosobruchus maculatus*, which drastically lowers their quality and marketability. Especially for grains intended for human or animal feed, the conventional chemical insecticides used to manage this pest can carry serious risks. Consequently, the quest for substitute pest management strategies, such as the application of plant extracts and bioactive substances, has received a lot of attention lately. Multiple insect pests can be successfully controlled by bioactive substances found in particular plant components, according to recent research. The purpose of this work was to assess how well-known insecticidal plants *Achillea millefolium* and *Heliotropium bacciferum* affected life of *C. maculatus* cycle and to extract their bioactive components. Part of the components taken from the leaves of *A. millefolium* and *H. bacciferum* were identified by GC-Mass spectrometry. Three replicates were examined using ethanol, ethyl acetate, hexane extracts, cold water, hot water, and ethanol at four concentrations: 10, 7.5, 5, and 2.5 mg.mL^{-1} over 24-, 48-, 72-, and 96-hour periods. The most notable effect on beetle life metrics was found to be shown by *H. bacciferum* extracts. Mortality rates from adult and egg extracts were especially high at 10% concentration over 96 hours. Egg mortality from a 10% ethanol extract of *H. bacciferum*, for example, was 74%; egg mortality from ethyl acetate and hexane extracts was 69% and 65.07%, respectively; egg mortality from hot and cold-water extracts was (51%) and (47%). Adult death rates were (84%) for the 10% ethanol extract, (79%), (75%), (59%), and (55%) for the ethyl acetate, hexane, hot water, and cold-water extracts, in that order.

Keywords: *A. millefolium*, *H. bacciferum*, *Callosobruchus maculatus*, plant extraction, phytochemical.

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INTRODUCTION

Among the primary insects impacting different stored grains is the *Callosobruchus maculatus*, which is a member of the order Coleoptera and the family Bruchidae (Mssillou, 2022). It causes significant losses by infesting legume family seeds, such as those of cowpeas, chickpeas, lentils and mung beans. The beetle finishes out its entire cycle in storage, where the infestation starts in the field. Through their development and feeding inside these seeds, the larvae increase seed damage, lower their nutritional value, and reduce their rate of germination. (Nisar *et al.,* 2022).

Because their edible seeds can be used as cereals, vegetables, or animal feed, legume crops like *Vigna unguiculata* are essential food sources. They contain high levels of proteins, including essential amino acids like lysine and tryptopha (Horn *et al.,* 2022; Konda and Kumar, 2022). Cowpeas offer various health benefits, such as preventing inflammation, oxidation, diabetes, atherosclerosis, and cardiovascular diseases (Apea-Bah *et al.,* 2017).

Researchers prioritize finding new, safe, environmentally friendly alternatives to combat pests. Plant-based insecticides or microbial products are potential replacements for traditional chemical pesticides (Aimad *et al.,* 2022). Most plants contain toxic, repellent, or attractive substances to insects (Mohammed Rahman, 2019). Natural products derived from plants or microorganisms have become popular alternatives to conventional chemical insecticides, with microbial agents showing potential in pest management (Ravindran *et al.,* 2018).

Due to the economic importance of the beetle and the significant damage it causes to legumes, and considering the health impacts of pesticides, researchers have explored plant-based alternatives. This study investigates the effects of organic solvent and aqueous extracts from medicinal plants such as *A. millefolium* and *H. bacciferum* on various life aspects of *C. maculatus*.

MATERIALS AND METHODS

Insect Rearing

Adult *C. maculatus* were obtained from local markets in Al-Diwaniyah Governorate. Ten pairs (10 males and 10 females) were isolated and reared in the laboratory on clean, uninfected cowpea seeds in 800 mL plastic bottles containing 250 g of seeds. The bottles were sealed with muslin cloth and secured with rubber bands, then placed in an incubator at 30±2°C and (70±5%) relative humidity (Al-Hadidi, 2018). The seeds were manually cleaned to remove any foreign materials and placed in a freezer at -20°C for two weeks as a precautionary measure to eliminate any potential pre-existing infestations (Al-Rubaie, 2014).

Collection of *A. millefolium* and *H. bacciferum* Plants

Leaves of *A. millefolium* and *H. bacciferum* (heliotrope) were collected from the Salhoubia area in the Al-Muthanna desert and Sawa Lake in March 2023 during the flowering period. The leaves were cleaned, washed to remove dust, and air-dried in a shaded room by spreading them on white paper and using airflow to prevent fungal growth. Once dried, the leaves were ground using an electric grinder (America type) and stored in airtight glass containers at room temperature until use.

Preparation of Alcoholic Extracts of plants

Extraction was carried out in the laboratory following a standard procedure. A 20 g of plant powder was added to 200 mL of each organic solvent like ethanol, hexane, and ethyl acetate in a Soxhlet extractor using filter paper for 24 h at a temperature of (40-45°C). The filtrate was concentrated using a rotary evaporator at 45°C until it became a gummy consistency, then dried in an electric oven.

Studying the Effect of Aqueous Extracts on Beetle Eggs

Eggs from newly emerged adults after mating were collected within 24 hours. Three replicates were used for each treatment at four concentrations (2.5, 5, 7.5, and 10 %), along with a control treatment (distilled water only). The eggs were sprayed with 1 mL of the extract from 15 cm, then incubated at 30±2°C and (70±5 %) relative humidity. The hatching rate was monitored over intervals of (24, 48, 72, and 96 h).

Studying the Effect of Aqueous Extracts on Adult Beetles

24 hours after their emergence, 10 adults, 5 males and 5 five females were separated and placed in dishes containing fifteen clean cowpea seeds apiece. At four concentrations 2.5, 5, 7.5, and 10% three replicates of each treatment were employed, in addition to a control treatment consisting solely of distilled water. To achieve complete exposure, 1 mL of the extract from 15 cm was sprayed on the adults. They were then allowed to dry and incubated at 30±2°C and 70±5% relative humidity.

The rates of adult death were observed every 24, 48, 72, and 96 hours.

Studying the Effect of Alcoholic, Ethyl Acetate, and Hexane Extracts on Beetle Eggs

Eggs from just emerging adults were gathered and treated within a day. For each extract alcoholic, ethyl acetate, and hexane three replicates were employed at four concentrations (2.5%, 5%, 7.5%, and 10%), plus a control treatment. After applying 1 mL of the extract from a 15 cm distance, the eggs were left to incubate at 30±2°C and (70±5%) relative humidity. Over periods of 24, 48, 72, and 96 hours, the hatching rate was tracked.

Studying the Effect of Alcoholic, Ethyl Acetate, and Hexane Extracts on Adult Beetles

Separating newly developed individuals (24 hours old), 10 adults 5 males and 5 females were placed in each dish with 15 clean cowpea seeds. Every treatment (alcoholic, ethyl acetate, and hexane) had three repetitions at four concentrations (2.5, 5, 7.5, and 10%), plus a control treatment (distilled water only). After thoroughly exposing the adults to 1 mL of the extract sprayed from 15 cm, they were allowed to dry and maintained at 30±2°C and (70±5%) relative humidity. Over intervals of 24, 48, 72, and 96 hours, adult mortality rates were tracked.

Statistical Analysis

The factorial experiment was designed according to a Completely Randomized Design (CRD). Data were analyzed using Analysis of Variance (ANOVA) with the statistical software Statistic version 10. Mortality percentages were adjusted using the Abbott equation (Abbott, 1925), and the statistical analysis of mortality was performed using the angular transformation. The means were tested with the Least Significant Difference (LSD) test at a probability level of $P \leq 0.05$.

Results and Discussion

Effect of aqueous extracts (cold water - hot water) and organic extracts of ethyl alcohol, ethyl acetate and hexane of *A. millefolium and H. bacciferum* on the mortality rate of the southern cowpea beetle *C. maculatus eggs.*

Table (1) shows the significant effect of aqueous extracts (hot and cold) of *the plant H. bacciferum* on the mortality rates of eggs of the southern bean beetle *C. maculatus.* It was noted from the table (1) that there are significant differences between the concentrations of the different treatments of the extract of the *plant H. bacciferum* aquatic (cold-hot) (0, 2.5, 5 and 7.5, 10 mg/mL) on the mortality rate of the egg. The results indicated that the concentration of 10 mg/mL led to the highest percentage of mortality, as the percentage of egg mortality was (63.03% and 63.82%), respectively compared to the control treatment (0%). The current results showed a significant effect of *H. bacciferum extract.* There are also substantial differences between the treatments used for the effect of different exposure times on eggs of the southern cowpea beetle C. *maculatus.* 96-hour exposure time gave the highest egg mortality rate (43.29%) compared to other exposure periods. The 24-hour exposure time was the least effective, with a mortality rate of 27.32 as shown in the table (1), which indicates that the increase in the concentration of the extract increases its effect in the process of egg mortality. This can be explained by the higher concentration that may contain a greater number of active compounds that target the eggs and adults of the insect and thus lead to their death.

Concerning exposure periods, the data clearly show that the longer exposure period (96 hours) led to an increase in the percentage of egg mortality by showing that insects need sufficient time to be exposed to the extract and absorbed until it reaches the lethal dose. Also, the longer period allows the active compounds contained in the extract to achieve their full effect on insects. Accordingly, it can be concluded that the highest concentration of the extract (10 mg/mL) and the longest exposure time (96 hours) lead to the highest mortality rate for eggs and adults, as *A. millefolium* extract can be used in specific concentrations and periods of exposure to achieve maximum effectiveness and this is consistent with the study carried out by Al-Ghazali *et al.* (2011). A study by Al-Rubaie *et al.* (2011). The results of the current study showed a significant effect on the

Table 1. Effect of aqueous extracts (cold water – hot water) and organic extracts of ethyl alcohol, ethyl acetate and hexane of *A. millefolium and H. bacciferum* on the mortality rate of the Southern Cowpea beetle *C. Maculatus eggs.*

Plant type	Extraction method	Concentration on mg/mL	Exposure Time/hours				Plant type Effect	Extraction method Effect	Concentration Effect
			24	48	72	96			
A. millefolium	Cold water	0	0.00	0.00	0.00	0.00			
		2.5	22.06	26.30	35.83	47.30			
		5	25.72	34.68	40.98	52.87		Cold water = 25.91	
		7.5	30.71	38.12	46.78	59.07			
		10	35.96	40.48	49.83	63.03			0=0.00
	Hot water	0	0.00	0.00	0.00	0.00			
		2.5	27.80	31.87	37.70	51.92			
		5	32.08	37.01	45.02	53.94	43.226		
		7.5	40.05	42.20	48.01	59.01			
		10	42.72	44.38	53.19	63.82			
	Hexane	0	0.00	0.00	0.00	0.00			
		2.5	34.61	48.99	54.09	56.11		Hot water= 28.59	
		5	47.71	53.00	58.85	63.15			
		7.5	56.37	57.05	63.89	68.01			
		10	60.41	62.94	65.00	71.93			2.5=35.82
	Ethyl alcohol	0	0.00	0.00	0.00	0.00			
		2.5	51.86	56.01	58.77	62.96			
		5	47.07	62.85	66.08	69.61			
		7.5	63.39	66.40	72.22	75.05			
		10	68.48	70.00	75.97	79.60			
	Ethyl acetate	0	0.00	0.00	0.00	0.00		Hexane =35.39	
		2.5	47.57	51.07	55.76	58.60			
		5	53.90	57.73	62.01	65.88			
		7.5	60.22	63.93	68.32	71.89			
		10	65.00	66.78	69.16	76.00			5= 42.56
H. bacciferum	Cold water	0	0.00	0.00	0.00	0.00			
		2.5	9.66	13.35	19.29	31.01			
		5	13.71	17.27	23.21	34.81			
		7.5	17.47	22.17	28.22	40.32			
		10	20.69	23.08	30.23	42.08			
	Hot water	0	0.00	0.00	0.00	0.00			
		2.5	13.07	15.86	21.91	33.16			
		5	17.94	20.85	27.20	38.82		Ethyl alcohol = 42.12	7.5= 45.73
		7.5	20.70	23.95	29.91	41.92			
		10	23.77	26.06	32.85	45.00			
	Hexane	0	0.00	0.00	0.00	0.00			
		2.5	17.74	20.30	25.48	36.98			
		5	21.15	24.05	30.14	39.89	25.309		
		7.5	24.77	27.01	32.11	43.88			
		10	27.83	32.06	39.20	51.01			
	Ethyl alcohol	0	0.00	0.00	0.00	0.00			
		2.5	23.86	27.19	30.77	42.56		Ethyl acetate = 39.40	10 = 49.34
		5	27.77	31.97	41.68	53.78			
		7.5	32.14	36.86	46.09	56.45			
		10	35.88	40.78	49.60	61.05			
	Ethyl acetate	0	0.00	0.00	0.00	0.00			
		2.5	20.30	23.00	30.20	40.95			
		5	24.93	27.80	39.79	50.05			
		7.5	28.15	30.43	41.99	54.07			
		10	30.94	34.09	45.61	57.00			
	Exposure Time Effect		27.32	30.60	35.86	43.29			
LSD (0.05)	Plants = 0.2036		Extraction type = 0.322		Concentration =0.322	Exposure time =0.288		Interference = 2.0365	

mortality rates of eggs of the southern cowpea beetle *C. maculatus* where there are significant differences between the concentrations of different treatments of *H. bacciferum* extract of organic extracts for each of (ethyl alcohol, ethyl acetate, hexane) with concentrations (0, 2.5, 5, 7, 10 mg/mL) on the egg mortality rate, the results indicated that the concentration of 10 (mg/mL) led to the highest mortality rate, where the percentage of organic egg depreciation (ethyl alcohol, ethyl acetate, hexane) respectively compared to the control treatment (0%). The mortality rate of eggs for ethanolic alcohol was 79.60%, ethyl acetate 76.00, and hexane 71.93%, and the results of Table (1) also showed significant differences between the treatments used for the effect of different exposure time on the destruction of eggs of the southern bean beetle. The 96-hour exposure time gave the highest mortality rate of 43.29% compared to the other exposure period. The 24-hour exposure time was the least effective at 27.32, suggesting that the increased concentration of the extract increases its effect on the Loss. This can be explained by the fact that a higher concentration may contain a greater number of active compounds that target the eggs and adults of the insect and thus lead to their death. The data clearly show that the longer exposure duration (96 hours) led to an increase in the rate of egg mortality and it was found that insects need sufficient time to be exposed to the extract and absorb it until it reaches the lethal dose. The longer duration also allows the active compounds contained in the extract to achieve their full effect on insects. Based on this, the results showed that the highest concentration of the extract (10 mg/mL) and the longest exposure time of 96 hours leads to the highest rate of egg lethality, where *A. millefolium* extract can be used in specific concentrations and duration of exposure to achieve maximum effectiveness, and this is consistent with the study carried out by Ibrahim *et al.* (2018) and a study carried out by Hassan *et al.* (2015).

The current study showed a significant effect of aqueous extracts (hot and cold) on the mortality rates of eggs of the southern cowpea beetle *C. maculatus*. It was noted through the above table that there are significant differences between the coefficients used for the effect of different concentrations of *H. bacciferum* extract (0, 2.5, 5, 7.5, 10 mg/mL) on egg destruction, and the results indicated that the concentration of 10 (mg /mL) led to the highest percentage of egg mortality 42.08% for cold water for *H. bacciferum* compared to other concentrations. Hot water of *H. bacciferum* extract demonstrated the highest egg mortality rate (45.00%). The results also showed significant differences between the treatments used for the effect of different exposure time on the egg mortality rate of the southern cowpea beetle *C. maculatus*. While 96-hour exposure period gave the highest egg mortality of hot and cold water compared to other exposure periods. While the 24-hour exposure period was the least effective as shown in Table (1).

The results show that the effectiveness of *A. millefolium* extract on the mortality rate of the southern cowpea beetle *C. maculatus* eggs is significantly affected by the concentration of the extract and the exposure time. The concentration (10 mg/mL) was the most effective in the destruction of eggs, suggesting that the increased concentration of the extract increases its effect on the process of destruction. This can be explained by the fact that a higher concentration may contain greater proportions of active compounds that target the insect's eggs and thus lead to their destruction.

Regarding exposure periods, the data clearly show that the longer exposure period (96 hours) led to an increase in the mortality rate of eggs. The reason for this can be that insects need enough time to be exposed to and absorbed the extract until it reaches the lethal dose. Also, the longer period allows the active compounds contained in the extract to achieve their full effect on insects.

It can be concluded that the highest concentration of the extract (10 mg/mL) and the longer exposure period (96 hours) lead to the highest percentage of egg mortality, as *A. millefolium extract can be used* in specific concentrations and exposure periods to achieve maximum effectiveness in the process of destruction of eggs and these results are consistent with the findings of Al-Hallaq *et al.* (2013), and the findings of Bunyan *et al.* (2017).

The results in Table 1 showed a significant effect of alcoholic extracts, ethyl alcohol, ethyl acetate and hexane on the rates of death of eggs of the southern bean beetle *C. maculatus*. As it is noted from the above table that there are significant differences between the coefficients used for the effect of different concentrations of *Achillea millefolium* extract of organic solvents (0, 2.5, 5, 7.5, 10 mg/mL) on egg destruction, and the results indicated that the concentration of 10 mg/mL led to the highest percentage of egg mortality, as the number of destruction reached 79.60 %, 76.00% and 71.93% respectively for each of ethyl alcohol, ethyl acetate, hexane as shown in Table 1 compared to other concentrations, either For the time effect, significant differences were also demonstrated between the parameters used for the effect of different exposure periods on the destruction of the eggs of the southern cowpea beetle. The 96-hour exposure period gave the highest egg mortality rate of 43.29% compared to other exposure periods. While the 24-hour exposure period was the least effective if the mortality rate reached 27.32%.

The results show that the effectiveness of organic extracts of *A. millefolium* and *H. bacciferum* in the destruction of eggs of the southern cowpea beetle *maculatus C.* They are greatly influenced by the concentration of the extract and the period of exposure. The high concentration (10 mg/mL) was the most effective in the destruction of eggs, suggesting that the increased concentration of the extract increases its effect on the process of destruction. This can be explained by the fact that a higher concentration may contain a greater number of active compounds that target the insect's eggs and thus lead to their destruction.

About exposure periods, the data clearly show that the longer exposure period (96 hours) led to an increase in the percentage of egg destruction, during which it was shown that insects need enough time to be exposed to the extract and absorbed until it reaches the lethal dose. Also, the longer duration allows the active compounds contained in the extract to achieve their full effect on insects.

It can be concluded that the highest concentration of the extract (10 mg/mL) and a longer exposure period (96 hours) lead to the highest rate of egg death, as *A. millefolium* extract can be used in specific concentrations and periods of exposure to achieve maximum effectiveness in the process of destruction of eggs and these results are consistent with the findings of Al-Ghazali, *et al.* (2011); Ibrahim *et al.* (2018); Hassan *et al.* (2015).

The low percentage of eggs laid on the treated grains may be because of the active substances of the extracts and their inhibition of the action of the chemical receptors of the host odor in the insect and preventing it from laying eggs (Ibrahim and Nasser, 2009).

Zarzis and Al-Jubouri (1998) also stated that many plant extracts affect female egg productivity because of containing compounds like the hormone moulting that lead to imbalance in growth and development and reduce female egg productivity. Or it may lead to the destruction of eggs because of the entry of extracts into the egg, which causes rapid killing of the embryo in the eggs through their direct cytotoxic activity through their influence through the outer shell and then lead to the failure of the embryonic development process, or through its effect on the muscle tissue of the fetus inside the egg as it causes a defect in the effectiveness of the outer shell of the egg and then the embryo loses the ability to hatch (Roxetine, 1991). These results are like those of Ibrahim and Naser (2009).) He pointed out that the treatment of cumin extract at a concentration of 1% gave the highest prevention rate for the eggs subjected, which amounted to 95.69%. It is similar to a study by Al-Husseini (2009) where it was indicated that the alcoholic extract of rue seeds hurt the productivity of adult khapra beetle and that the average number of eggs placed was inversely proportional to the concentration of the extract. Khalaf (2014) pointed out that the extract of dendritic enamel has a significant impact in reducing the number of the first generation, as the rate of members of the first generation reached 14.33% compared to the control treatment, which amounted to 92.66%. The hatchability ratio at 10 mg/mL was 20 and 13.33. And 16.66% for each of Datura extract, dendritic enamel and Jasmine Zaphyr respectively compared to the control treatment of 80%. He also mentioned that the effect of the extracts may be due to their entry into the egg through the opening of the peck or the eggshell when immersed in extracts and then the death of the embryo and incomplete development.

We conclude from this the superiority of the alcoholic extract of *H. bacciferum* and *A. millefolium* over the rest of the other extracts cold water - hot water - hexane - ethyl acetate on the death of the eggs of the southern cowpea beetle *C. maculatus.* The mortality rates of ethyl alcohol extract were 42.12%, ethyl acetate extract 39.40%, hexane extract 35.39%, hot water extract 28.59% and cold water extract 25.91% respectively. *H. bacciferum* surpassed *A. millefolium* extract in the egg mortality rates of the southern cowpea beetle *C. maculatus* . As for the effect of concentrations, the concentration exceeded 10% over the rest of the other concentrations, where the mortality rates reached 49.34%, followed by concentration 7, which amounted to 45.73%, followed by concentration 5, which amounted to 42.56, followed by concentration 2, amounted to 35.82.

The effect of aqueous extracts (cold water-hot water) and organic extracts of ethyl alcohol, ethyl acetate, and hexane of *Achillea millefolium and Heliotropium bacciferum* on the death of adults of the southern cowpea beetle *C. maculatus.*

Table (2) showed the significant effect of aqueous extracts (hot and cold) of the *plant H. bacciferum* on the mortality rates of adults of the southern bean beetle *C. maculatus*. It was noted from the table that there are significant differences between the concentrations of the different treatments of the extract of the plant *H. bacciferum* aqueous (cold-hot) (0, 2.5, 5, 7.5, 10 mg/mL) in the adult mortality rate. The concentration of 10 mg/mL for *A. millefolium and H. bacciferum* revealed the highest percentage of adult mortality, as the percentage of adult mortality (67.15% and 73.48%), respectively, Table (2).

Exposure time exhibited different effects in the adult mortality rate of the southern cowpea beetle *C. maculatus.* 96-hour exposure time gave the highest mortality rate for adults (57.43%) compared to other exposure time. The exposure time of 24 hours was the least effective, as the mortality rate was (38.06%) as shown in Table (2), which indicates that the increase in the concentration and exposure time of the extract increases its effect in the process of adult mortality of the insect. This can be explained by the fact that a higher concentration may contain a greater number of active compounds that target the insect's adults and thus lead to their death.

Adult mortality rate was higher at 96-hour after treatment which means that insects need sufficient time to be exposed to the extract and absorbed until it reaches the lethal dose. Also, the longer period allows the active compounds contained in the extract to achieve their full effect on insects. Furthermore, there are significant differences between the concentrations of different treatments of *H. bacciferum* extract for organic extracts for each (ethyl alcohol, ethyl acetate, hexane) with concentrations of (0, 2.5, 5, 7.5, 10 mg/mL) on the adult mortality rate. The concentration of 10 mg/mL led to the highest mortality rate, where the mortality rate for adults for ethanolic alcohol was 93.83%, ethyl acetate 89.45 and hexane 90.42%, respectively.

The results of the current study showed a significant effect of aqueous extracts (hot and cold) on the mortality rates of adults of the southern cowpea beetle *C. maculatus.* The concentration of 10 mg/ml led to the highest mortality rate for adults, as the mortality rate, was 63.91% for cold water for *H. bacciferum* compared to other concentrations, while hot water had the highest mortality rate for eggs 69.94% for *H. bacciferum*. The 96-hour exposure time showed the highest adult mortality rate of hot and cold

Table 2. Effect of aqueous extracts (cold water – hot water) and organic extracts of ethyl alcohol, ethyl acetate and hexane of *A. millefolium and H. bacciferum* on the adult mortality rate of the southern cowpea beetle *C. Maculatus.*

Extraction method	Concentration mg/mL	Exposure Time/hours				Plant type Effect	Extraction method Effect	Concentration Effect
		24	48	72	96			
A. millefolium								
Cold water	0	0.00	0.00	0.00	0.00			
	2.5	32.43	35.77	42.50	52.24			
	5	34.58	36.98	45.69	55.61		Cold water = 36.22	
	7.5	39.32	45.28	50.44	61.72			
	10	45.37	49.65	57.59	67.15			0= 0.00
Hot water	0	0.00	0.00	0.00	0.00			
	2.5	36.55	39.48	46.58	56.99			
	5	41.63	41.35	50.58	61.98	= 49.202		
	7.5	43.87	47.31	65.14	54.53			
	10	48.52	52.59	61.26	73.48			
Hexane	0	0.00	0.00	0.00	0.00			
	2.5	40.25	56.21	66.56	70.37		Hot water =39.57	
	5	44.44	60.38	69.88	81.72			
	7.5	47.36	62.41	78.52	86.65			
	10	52.60	74.91	82.30	90.42			2.5= 50.94
Ethyl alcohol	0	0.00	0.00	0.00	0.00			
	2.5	54.53	58.17	67.89	75.55			
	5	58.73	64.59	75.58	82.85			
	7.5	65.53	70.57	80.53	86.28			
	10	71.31	83.34	88.66	93.83			
Ethyl acetate	0	0.00	0.00	0.00	0.00			
	2.5	50.00	57.30	65.23	70.04		Hexane= 51.87	
	5	53.39	60.07	69.38	74.08			
	7.5	67.62	66.32	73.67	79.66			
	10	65.64	77.61	83.74	89.45			5= 57.55
H. bacciferum								
Cold water	0	0.00	0.00	0.00	0.00			
	2.5	28.09	32.13	38.96	48.69			
	5	30.98	34.83	42.01	52.06			
	7.5	36.03	40.62	47.79	58.22			
	10	41.98	46.07	54.01	63.91			
Hot water	0	0.00	0.00	0.00	0.00			
	2.5	32.73	35.32	43.23	52.83			
	5	36.98	37.31	46.94	57.86		Ethyl alcohol = 46.299	7.5= 61.96
	7.5	40.00	43.19	51.26	61.95			
	10	44.30	48.93	58.14	69.94			
Hexane	0	0.00	0.00	0.00	0.00			
	2.5	36.94	51.88	63.68	67.12	46.299		
	5	41.04	56.86	65.85	78.00			
	7.5	44.10	61.93	75.11	83.32			
	10	48.06	70.28	78.24	87.32			
Ethyl alcohol	0	0.00	0.00	0.00	0.00			
	2.5	51.02	54.83	63.95	71.76		Ethyl acetate= 53.83	10 = 68.64
	5	54.13	61.00	71.73	76.16			
	7.5	62.12	66.87	76.97	82.86			
	10	67.99	80.03	84.67	90.19			
Ethyl acetate	0	0.00	0.00	0.00	0.00			
	2.5	46.25	54.25	61.28	65.95			
	5	49.73	57.71	65.20	70.90			
	7.5	54.88	62.07	79.02	77.26			
	10	62.17	73.99	79.96	86.05			
Exposure Time Effect		38.06	44.21	51.39	57.34			
LSD (0.05) Plants = 0.265		Extraction type = 0.322		Concentration =0.419	Exposure time =0.375		Interference = 2.649	

water compared to other exposure periods. While the 24-hur exposure time was the least effective as shown in Table 2.
The results show that the effectiveness of *A. millefolium* extract in the death of adults of the southern cowpea beetle *C. maculatus* is significantly influenced by the concentration of the extract and the exposure time. The high concentration (10 mg/mL) was the most effective in the adult mortality rate, suggesting that the increased concentration of the extract increases its effect on death. This can be explained by the fact that the higher concentration may contain greater proportions of active compounds that target the insect's adults. Then led to an increase in the mortality rate of adult. The reason for this can be that insects need enough time to be exposed to and absorbed the extract until it reaches the lethal dose. Also, the longer period allows the active compounds contained in the extract to achieve their full effect on insects.
It can be concluded that the higher concentration of the extract (10 mg/mL) and the longer exposure time (96 hours) lead to the highest rate of egg destruction, as *A. millefolium* extract can be used in specific concentrations and exposure periods to achieve maximum effectiveness in the process of adult mortality and these results are consistent with the findings of Al-Hallaq *et al.* (2013), and the findings of Bunyan et al. (2017).
The results in Table (2) showed a significant effect of alcoholic extracts, ethyl alcohol, ethyl acetate, and hexane on the mortality rates of adults of the southern bean beetle *C.maculatus*. There are substantial differences between the treatment used for the effect of different concentrations of *Achillea millefolium* extract of organic solvents (0, 2, 5, 7, 10 mg/mL) on adult mortality, and the results indicated that the concentration of 10 mg/mL led to the highest mortality rate for adults, as the number of mortality reached 90.19%, 87.32% and 86.05% respectively for ethyl alcohol, hexane, ethyl acetate as shown in Table 2 compared to other concentrations, as for the effect of time, also showed significant differences between the coefficients used to affect different exposure periods on the death of adults of the southern bean beetle. The 96-hour exposure time gave the highest mortality rate for adults at 57.34% compared to other exposure periods. While the 24-hour exposure period was the least effective if the mortality rate reached 38.06%.
The results show the effectiveness of organic extracts of *A. millefolium* and *H. bacciferum* in the death of adults of the southern cowpea beetle *C. maculatus* They are greatly influenced by the concentration of the extract and the period of exposure. The high concentration (10 mg/mL) was the most effective in the death of adults, suggesting that the increased concentration of the extract increases its effect on the adult mortality rate. This can be explained by the fact that a higher concentration may contain a greater number of active compounds that target the insect's eggs and thus lead to their death.
It can be concluded that the highest concentration of the extract (10 mg/mL) and 96 hurs exposure leads to the highest mortality rate for adults, as *A. millefolium* extract can be used in specific concentrations and exposure periods to achieve maximum effectiveness in the adult mortality rate and these results are consistent with the findings of Al-Ghazali *et al.* (2011); Hassan *et al.* (2015) and Ibrahim *et al.* (2018).
The results of the study showed that the plant extracts used had a significant effect on the rates of adult mortality and this effect increases with increasing concentration and increasing the period of exposure to the extract. It was also noted that the superiority of the extract of *A. millefolium* for ethyl alcohol over the rest of the plant extracts used is also due to the effect of plant extracts used in the study to contain many effective groups such as alkaloids, terpenes, tannins, sapons, and flavonoids and contain oils An effective volatility that can spread and penetrate through the tissues of the insect in a similar way to the action of pesticides or may affect the extracts used in a manner in contact with the surface of the body of the insect by penetrating the insect through thin areas, causing paralysis and then death (Afifi,

2002). The effect of these extracts may be because they contain alkaline compounds and toxic substances or other active compounds that act as inhibitors of nutrition that lead to the death of insects, or the effect of the extracts may be due to their entry through the respiratory openings, affecting the nervous system and the digestive system, as *H. bacciferum* and *A. millefolium* contain a lot of active substances, Or the effect of plant extracts may be because they contain hormone-like compounds, which lead to an imbalance in the functions of cells and then their death. This is similar to the findings of Mohamed et al. (2008) that plant extracts from 27 plants selected from twenty plant families showed a clear effect on the mortality percentage of the larval IV of the red rusty flour beetle *T. castaneum* and the *granarium T.* mortality rate increased with increasing concentration. The study by Kim *et al.* (2003) also indicated that the methanolic extracts of 30 species of aromatic medicinal plants and five essential oils had a significant effect on the mortality rates of rice weevil beetle adults (*S. oryzae (L* and the cowpea beetle *Callosobruchus chinensis* (L.) These extracts caused 100% adult mortality within 1-4 days of treatment. The ethanolic extract of cloves, garlic, red pepper and black pepper achieved the highest death rate of the ethanolic extract of mint, cumin, cinnamon and black seed and this difference between the extracts in the effect may be due to the difference in the quality and quantity of active compounds contained in these plants and this result is similar to what Saljoqi and others (2006) The ethanolic extract of *Melia azedarach*, *myrtus communis*, lemongrass *cymbopogon citrates*, *Mentha longifolia* and *Pegnum harmala* all had a significant effect on the death rates of the rice weevil beetle *S. oryzae* L. The ethanolic extract of the azdrecht had the highest effect, followed by myrtle extract, mint extract, and lemongrass and rue extract was the least effective. He also mentioned (Al-Hadidi et al., 2014) that the plant powders of *Zingiber officinale* and nutmeg *M. fragrans Coriandrum sativum* led to an increase in the killing rate of the rusty flour beetle insect after 7 days of treatment and the cause of nutmeg powder the highest rate of death, followed by ginger and then coriander and cinnamon powder. The results of the study by Ali et al. (2014) also showed that the extract of the garlic plant *A. sativum* and turmeric *C. longa* cause of death of *adults T. castaneum* The death rates are directly proportional to the increased concentration and garlic extract had a higher effect than turmeric extract. The results of this study were also similar to Ismail and Al-Dulaimi (2017), who found that when they studied the effect of ethanolic extract for several plants, including cumin and black seed, to find out their effect on the rusty flour beetle insect, as they concluded that the mortality rate of the insect has increased with increasing concentration and duration of exposure, as it reached after 7 days 86.66 and 80% for each of the black seed and cumin respectively. It was noted that *the H. bacciferum* plant outperformed the extract of the *A. millefolium* plant in the adult mortality rates of the southern cowpea beetle *C. maculatus*. Where the percentage of *H. bacciferum* plant reached 49.202 compared to 46.299 for the *A. millefolium* extract. The effect of concentrations, the concentration exceeded 10% over the rest of the other concentrations, where the depreciation rates reached 68.64%, followed by concentration 7, which amounted to 61.96%, followed by concentration 5, which amounted to 51.87, followed by concentration 2, amounted to 50.94. As well as the superiority of the alcoholic extract over the rest of the other extracts of the plants *H. bacciferum* and *Achillea millefolium*, followed by ethyl acetate, hexane, hot water, and cold water.

A conclusion, *C. maculatus*, poses a significant threat to stored seeds, impacting their quality and marketability. Chemical pesticides, commonly used for control, present risks to human and animal health, prompting the search for alternative pest management strategies. Recent research has demonstrated the efficacy of plant extracts and bioactive compounds in controlling insect pests, leading to this study's focus on extracting bioactive compounds from *A. millefolium* and *H.*

bacciferum. The study identified various active compounds in the extracts, including alkaloids, terpenes, tannins, saponins, and flavonoids, through GC-Mass spectrometry. Testing different concentrations and extracts over varying time periods revealed that the extract from *H. bacciferum* had the most significant impact on the beetle's life performance metrics. Alcoholic extracts, particularly ethanol, proved most effective in causing mortality in both eggs and adults of *C. maculatus*, with the highest efficacy observed at a 10% concentration over 96 hours. Notably, the ethanol extract of *H. bacciferum* demonstrated substantial egg mortality at 74%, followed by ethyl acetate and hexane extracts. Cold water and hot water extracts exhibited comparatively lower mortality rates. Overall, the study suggests that alcoholic extracts from these plants could offer a safe and efficient alternative for reducing pest damage, providing a promising avenue for sustainable pest management in stored grains.

Authors Contribution

Wafaa Mashkoor Husein was responsible for conducting the experiments, carrying out the laboratory trails, conducting the bioassay tests, and drafting the manuscript as well as handling the data management, editing the manuscript, gathering references, and participating in writing the manuscript, While Abdulla Nezar Ali analyzed the results, managed the data, contributed to the experimental design, and wrote the manuscript. Before submission, all authors thoroughly reviewed and gave approval for the final version of the manuscript.

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Wafaa Mashkoor Husein[1] and Abdulla Nezar Ali[2*]

[1]University of Kufa, Faculty of Agriculture University of Kufa, Department of Plant Protection, Najaf Governorate, Iraq

Email: Wafaaw.alhusni@student.uokufa.ediu.iq

[2]University of Kufa, Faculty of Agriculture, Department of Soil and Water Science, Najaf Governorate, Iraq.

*Corresponding author

E-mail: abdullah.alqaseer@uokufa.ediu.iq