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Article

Investigation of The Biological Impact of Aqueous Extracts from *Chenopodium album* and *Achillea fragrantissima*, with Sexual Aggregation Pheromone, on The Death Rates of The Sixth Nymphal Instar and Adult Stages of *Periplaneta americana*

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Abstract: The biological impact of the aqueous extracts from *Chenopodium album* and *Achillea fragrantissima* was evaluated at various concentrations (0, 2.5, 5, 7.5, and 10%). Additionally, 2.5 g of aggregation pheromone was incorporated at a concentration of 10% during the sixth nymphal stage and in adult American cockroaches, *Periplaneta americana*, utilizing the spraying method on the insect diet. The proportion of insect mortality was assessed after 3, 6, 9, 12, and 15 days of treatment. The tests revealed substantial differences in the impact of the concentrations of the evaluated extracts on the mortality rate. The study demonstrated that a 10% concentration, combined with the aggregation pheromone, was the most effective, achieving a 100% mortality rate for both cold and hot water extracts after 6, 9, 12, and 15 days of treatment. The potential advantages of utilizing these extracts and incorporating the insect's sex pheromone into an integrated control program for this significant pest were examined.

Keywords: aqueous extracts , *Periplaneta Americana*, sixth nymphal

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1. Introduction

Fossil evidence suggests that P. Americana existed approximately 300 million years ago, with around 4600 species identified [1]. Cockroaches inhabit tropical and subtropical locations globally [2]. Several species inhabit temperate climates and exhibit adaptability to various settings. Their species inhabit crevices in human-built structures, beneath tree bark, and under decaying plant foliage [3]. Their mature colouration is yellowish-brown, they are nocturnal, and they exhibit either carnivorous or herbivorous dietary habits. They can endure for several weeks without sustenance and proliferate year-round under adverse conditions [4]. Cockroaches are classified within the order Blattodea and encompass numerous families: Polyphagidae, Blattellidae, Blaberidae, and Cryptocercidae.

P. Americana, a member of the Blattidae family, is the most prevalent cockroach species in Iraq and is the largest, attaining a length of approximately 30 mm [5]. Its behaviour, presence in unsanitary environments, and nutritional habits enable it to harbour and disseminate several infections [6], serving as a vector for bacteria, fungi, viruses, protozoa, and parasitic worms. It significantly contributes to the transmission of

diseases via food tainted with its excrement. Contact with cockroach bodies may result in digestive problems in humans [7].

Prior to initiating control measures against cockroaches, it is imperative to acquire comprehensive knowledge regarding their biology, behaviour, habitats, and reproductive practices, as recent studies suggest that non-target organisms may remain unaffected. This study was conducted to optimally utilise the aggregation pheromone against P. Americana, given the availability of *Chenopodium album* and *Achillea fragrantissima* in the southwestern Badia desert of Al-Muthanna Governorate, Iraq, and the presence of active compounds in their extracts.

2. Materials and Methods

Raising the P. Americana

Adults and nymphs of the insect were gathered from various residential units, particularly kitchens and health facilities at Al-Muthanna University. Cardboard traps, designed in layered formations resembling the letter W and available in various sizes, were utilised. Three traps were positioned in the specified locations at dusk and retrieved the following morning. A combination of powdered milk and crumbled biscuits served as an appealing lure in the traps [8]. Samples were collected daily for two weeks, subsequently transferred to the laboratory, and identified using taxonomic keys as *P. Americana*.

Adults were situated in circular plastic containers measuring 30 cm in diameter and 20 cm in height. The inner edge of each container was coated with a 2 cm thick layer of Vaseline to prevent insects from escaping. The containers were equipped with shelters constructed from thick paper, including three sheets folded many times in the form of the letter W and stacked vertically. A paper separator composed of identical paper material was positioned between each item and secured with a paper belt. The insects were nourished with a uniform blend of biscuits and milk powder, and water was supplied via tubes fitted with cotton at an angle to maintain moisture in the cotton [9].

Extraction of aggregation pheromone

Faeces from adult and nymph stages of the examined insect were collected from the bases of rearing containers using small brushes, and subsequently sieved through an 8 mm sieve to eliminate contaminants [10]. The maximum concentration influencing insect behaviour was achieved by weighing 2 g of faeces and including 25 ml of hexane as the organic solvent. The combination was heated to 55 °C in a water bath for one hour, after which the extract was cooled, filtered through filter paper, and stored for future use.

Collection and identification of plant samples

Specimens of *C. album* and *A. fragrantissima* were gathered at three locations in the western desert of Al-Muthanna Governorate in late March and early April 2023, coinciding with the completion of their flowering period. The samples were gathered in opaque polyethylene bags and subsequently transported to the laboratory of the Badia Research and Studies Centre at Al-Muthanna University, where they were stored at ambient temperature until utilised. Several samples were dispatched to the National Herbarium inside the Seed Testing and Certification Department of the Iraqi Ministry of Agriculture.

Preparation of extracts

The samples were dried, chopped into small pieces, and subsequently processed into a dry powder using an electric grinder. To create the cold water extract of the two plants individually, 20 g of dry powder was measured and placed in a 1-liter beaker, followed by the addition of 500 ml of distilled water. The mixture was then blended using an electric mixer for 10 minutes. The mixture was allowed to settle for 20 minutes, subsequently filtered through cloth, and the filtrate was concentrated in a centrifuge at 3000 rpm for 10 minutes. The filtrate was collected while the precipitate was disregarded. The filtrate exhibited a concentration of 10 mg/liter, from which concentrations of 2.5%, 5%, 7.5%, and 10% were formulated. The control treatment utilised only distilled water, while the boiled water extract was prepared similarly to the cold water extract, with the distinction that the distilled water was heated to 100°C [11].

Effect of aqueous extracts of C. *album* and *A. fragrantissima* on the mortality rate of sixth instar nymphs and adults of *P. Americana*

Four concentrations of aqueous extracts (cold and hot water) from C. album and A. fragrantissima were evaluated for their efficacy in suppressing the American cockroach in three scenarios. The aqueous extracts of cold and hot water from the two examined plants were prepared at the four specified concentrations and incorporated into the formulated diet (2.5 g of biscuit powder and 2.5 g of milk powder) for both adults and sixth instar nymphs of the insect, without the inclusion of the aggregation pheromone, and conducted in three replicates for each concentration individually. In the second experiment, a higher concentration (10%) of the extracts from the two plants was utilized individually, supplemented with the recommended concentration of the aggregation pheromone, which was prepared by combining 2 g of feces from adult and nymph stages of the insect with 25 ml of hexane [12], and conducted in three replicates. The control treatment involved the addition of pre-prepared insect food without any additional substances.

To evaluate the concentrations of the previously generated aqueous extracts, 2.5 g of the food paste was measured, and 5 ml of each concentration was added individually. This mixture was then positioned with a water tube at the center of the pre-prepared test basin for insect rearing. Subsequently, ten adult insects were released individually, with three replicates for each concentration and time interval, which included 3, 6, 9, 12, and 15 days. Concerning the impact of the aggregation pheromone on the mortality rates of insect nymphs and adults, 2 grams of insect feces were incorporated into the 10% concentration, deemed the most effective for inducing insect mortality for both cold and boiling water plant extracts. The identical procedures were implemented in the treatment, and the inside perimeter of the test tanks was smeared with Vaseline to inhibit insect escape during the testing duration. In the comparison treatment, a food paste made from pre-prepared natural ingredients was utilized without the addition of any other substances, and mortality rates were calculated for all treatments after the previously specified treatment durations. The mortality percentages were modified in accordance with the Abbott equation [13].

$$\% \text{ corrected for death} = \frac{\% \text{ death in treatment} - \% \text{ death in control}}{100 - \% \text{ death in control}} \times 100\%$$

The chemical identification of the active constituents of the plant extracts and the aggregation pheromone was conducted in the industrial research and development laboratories of the Ministry of Industry and the College of Pharmacy at the University of Karbala.

Statistical analysis

The trials employed a completely randomized design (CRD) done in the laboratory, with significant differences between means analyzed using the least significant difference (LSD) test at a probability level of 0.05. The SAS program (2004) was employed for the statistical analysis of the data.

Table 1. Results of chemical detection of active substances in *C. album* and *A. fragrantissima* plants

Active substance	**detector used**	**Change in colour**	**c. album**	**a. fragrantissima**
TANNINS TEST	Ferric chloride	Blue-green	+	+
CARBOHYDRATE	alpha-naphthol	Violet Red	+	+
GLYCOSIDES TEST	Benedict	precipitate	+	+

PHENOL TEST	Potassium ferrocyanide	Blue-green	+	+
RESINS TEST	Hydrochloric acid	Occurrence	_	_
FLAVONOIDS TEST	Ammonia	Yellow	+	+
SAPONIN TEST	Mercuric chloride	White precipitate	+	+
ALKALOID TEST	Drakendoff	Orange	+	+
PROTIN TEST	Ninhydrin	Purple and yellow	_	_
COUMARINS TEST	Sodium hydroxide	Green yellow shiny	+	+
TERPENES TEST	Salkovskiy	Red tan separator	_	+
STEROIDS TEST	Liebermann test	Red	_	_

3. Results and Discussion

Results

The data in Table (2) illustrate the effect of cold and hot *A. fragrantissima* plant extract on the sixth instar nymphs of P. Americana following the application of the aggregation pheromone. It was observed that a 10% concentration combined with the pheromone outperformed all other treatments, achieving efficacy rates of 86.012% and 87.213% after three days of exposure to the cold and hot water extracts, respectively. Mortality rates escalated with prolonged treatment duration, culminating in 100% and 94.816% after six days for the respective extracts, and continued to reach 100% after nine, twelve, and fifteen days for each extract. No mortality was recorded in the control group, and a direct correlation was established between mortality rates and both the concentrations and durations of the treatments administered.

The results in the same table demonstrated the impact of the interaction between concentrations and varying treatment durations, as the 10% + 2.5 gm aggregation pheromone treatment exhibited superior efficacy after 6, 9, 12, and 15 days, achieving a maximum insect nymph mortality rate of 100% across the three specified treatments. The same proportion of interaction was attained between the type of extract, concentration, and time after employing the highest concentration (10%) and 2.5 grams of aggregation pheromone following identical treatment periods, respectively. The triple interaction among the study factors (type of extract, concentration, and treatment duration) demonstrated that a 10% concentration combined with aggregation pheromone resulted in complete mortality of the treated subjects after 6, 9, 12, and 15 days of treatment using both cold and hot water extracts.

Table 2. Effect of varying doses of hot and cold extracts of *A. fragrantissima* and the inclusion of aggregation pheromone on the mortality rate of sixth-instar nymphs of *P. Americana* in a laboratory setting

Extract	**Concentration**	**Time (days)**					**Average**	**Extract rate**
		3	**6**	**9**	**12**	**15**		
Cold Extract	2.5	19.868	23.870	92.223	33.632	41.061	29.531	50.561
	5	25.981	32.157	38.156	43.872	49.735	37.980	
	7.5	29.626	34.950	41.321	46.934	55.053	41.575	
	10	34.797	41.020	45.814	52.120	58.835	46.513	

	+ %10 pheromone	86.012	100.00	100.00	100.00	100.00	97.202	
	Time rate	39.257	46.399	50.901	55.312	60.937	50.581	
	Concentration	3	6	9	12	15	Average	Extract rate
Hot Extract	2.5	24.020	29.002	34.875	39.969	46.741	34.921	54.963
	5	30.023	34.684	41.085	48.785	53.860	41.687	
	7.5	33.219	38.848	46.618	51.915	57.881	45.696	
	10	37.740	46.047	59.011	65.804	71.927	56.106	
	+ %10 pheromone	87.213	94.816	100.00	100.00	100.00	96.406	
	Time rate	42.443	48.679	56.318	61.295	66.082		
	Concentration	3	6	9	12	15	Average	
Concentration x time	2.5	21.944	26.436	32.049	36.801	43.901	32.226	
	5	28.002	33.421	39.621	46.329	51.798	39.834	
	7.5	31.423	36.899	43.965	49.425	56.467	43.636	
	10	36.269	43.534	52.413	58.962	65.381	51.312	
	+ %10 pheromone	86.613	97.408	100.00	100.00	100.00	96.804	
	Time rate	40.850	47.539	53.609	58.303	63.509		
L.S.D 0.01	Extract	Concentration	time	× Extract Concentration	Extract time ×	Concentration × time	Total overlap	
	0.131	0.208	0.208	0.294	0.294	0.465	0.658	

The data presented in Table (3) demonstrated the impact of aqueous extracts of C. album at various concentrations, combined with 2.5 g of aggregation pheromone, on the mortality rates of sixth instar nymphs of *P. americana*. The analytical results revealed significant differences among the study variables, with the treatment comprising 10% extract and 2.5 g of aggregation pheromone achieving mortality rates of 91.042% and 93.909% after three days for the cold and hot extracts, respectively, Mortality rates attained 100% after 6, 9, 12, and 15 days of treatment with cold and hot aqueous extracts, respectively, at the maximum concentration of 10% without the addition of aggregation pheromone. The peak mortality rates were 66.141% and 79.244% after 15 days of treatment for each extract, respectively. A direct correlation was noted between concentration factors and treatment duration, with the mortality rate escalating alongside prolonged treatment durations.

The interaction between concentration factors and treatment durations was not significant for the two treatments at a concentration of 7.5% after 3 days, nor for the treatment at a concentration of 2.5 g after 9 days, as well as for the same two concentrations after 6 and 12 days of treatment. The results indicated that the effect is considerable when a triple interaction occurs among the three research components. A 10% concentration given to 2.5 g of aggregation pheromone resulted in the total mortality of insect nymphs after 6, 9, 12, and 15 days of treatment with cold and hot water extracts, each applied individually.

Table 3. Effect of varying doses of hot and cold extracts of C. album and the inclusion of aggregation pheromone on the mortality rate of sixth-instar nymphs of P. Americana in a laboratory setting

Extract	Concentration	Time (days)					Average	Extract rate
		3	6	9	12	15		
Cold Extract	2.5	25.934	30.791	36.853	41.938	48.163	36.772	67.564
	5	32.926	39.051	44.949	52.068	57.105	45.220	
	7.5	37.121	42.960	48.104	55.049	62.728	49.192	
	10	41.931	47.994	53.930	59.895	66.141	53.978	
	+ %10 pheromone	91.042	100.00	100.00	100.00	100.00	98.208	
	Time rate	45.791	52.195	56.767	61.790	66.827	56.674	
	Concentration	3	6	9	12	15	Average	Extract rate
Hot Extract	2.5	31.169	36.175	41.874	47.968	54.071	42.251	61.426
	5	37.348	41.740	48.209	55.441	62.158	48.979	
	7.5	41.130	47.003	55.068	59.965	64.887	53.611	
	10	45.880	53.531	66.177	72.705	79.244	63.507	
	+ %10 pheromone	93.909	100.00	100.00	100.00	100.00	98.782	
	Time rate	49.887	55.690	62.266	67.216	72.072		
	Concentration	3	6	9	12	15	Average	
Concentration x time	2.5	28.552	33.573	39.364	44.953	51.117	39.512	
	5	35.137	40.396	46.579	53.755	59.632	47.100	
	7.5	39.126	44.982	51.586	57.507	63.808	51.402	
	10	43.906	50.763	60.054	66.300	72.693	58.743	
	+ %10 pheromone	92.476	100.00	100.00	100.00	100.00	98.495	
	Time rate	47.839	53.943	59.516	64.503	69.450		
L.S.D 0.01	Extract	Concentration	time	× Extract Concentration	Extract time ×	Concentration × time	Total overlap	
	0.089	0.141	0.141	0.200	0.200	0.317	0.448	

Table (4) presents the mortality rates of adult P. Americana following exposure to various concentrations of aqueous extracts of C. album throughout distinct treatment durations. With a concentration of 10% and the incorporation of 2.5 g of aggregation pheromone, the maximum efficacy in exterminating adult insects was 100% after 9, 12, and 15 days of treatment with the cold water extract, and the identical mortality rate was achieved after 6, 9, 12, and 15 days following treatment with the hot extract. The treatment with the greatest concentration (10%) that did not include the aggregation pheromone resulted in mortality rates of 53.620% and 64.120% after 15 days of treatment with the cold and hot extracts, respectively.

The results demonstrated the substantial impact of the studied components independently. A concentration of 10%, combined with the addition of 2.5 g of aggregation pheromone, resulted in the total eradication of adult insects. All treatments exhibited superior efficacy after 15 days of administration of the cold and hot extract in

comparison to other treatment durations. The study indicated that the interaction between the average treatment duration of 12 days and the concentration of 10% was not significant, whereas the dual interactions among the study factors (type of extract + treatment duration, type of extract + concentration, and concentration + treatment duration) significantly affected the mortality of adult insects. The situation is identical for the triple interaction.

Table 4. Effect of varying doses of hot and cold extracts of *C. album* and the inclusion of aggregation pheromone on the mortality rate of adults of *P. Americana* in a laboratory setting

Extract	**Concentration**	**Time (days)**					**Average**	**Extract rate**
		3	**6**	**9**	**12**	**15**		
Cold Extract	2.5	44.080	24.070	30.610	34.000	39.620	34.476	49.361
	5	26.270	29.880	36.210	42.760	46.010	36.226	
	7.5	29.710	35.020	40.130	46.020	50.790	40.334	
	10	35.010	40.160	43.800	50.050	53.620	44.528	
	+ %10 pheromone	72.980	83.230	100.00	100.00	100.00	91.242	
	Time rate	41.610	42.472	50.150	54.566	58.008		
	Concentration	3	6	9	12	15	Average	Extract rate
Hot Extract	2.5	25.050	31.170	34.910	38.990	44.370	34.898	55.262
	5	30.070	34.770	38.970	45.390	50.690	39.978	
	7.5	32.110	38.960	48.900	52.530	57.940	46.088	
	10	74.940	44.910	53.220	59.590	64.130	59.358	
	+ %10 pheromone	79.950	100.00	100.00	100.00	100.00	95.990	
	Time rate	48.424	49.962	55.200	59.300	63.426		
	Concentration	3	6	9	12	15	Average	
Concentration x time	2.5	34.565	27.620	32.760	36.495	41.995	34.687	
	5	28.170	32.325	37.590	44.075	48.350	38.102	
	7.5	30.910	36.990	44.515	49.275	54.365	43.211	
	10	54.975	42.535	48.510	54.820	58.875	51.943	
	+ %10 pheromone	76.465	91.615	100.000	100.000	100.000	93.616	
	Time rate	45.017	46.217	52.675	56.933	60.717		
L.S.D 0.01	Extract	Concentration	time	× Extract Concentration	Extract time ×	Concentration × time	Total overlap	
	2.513	3.974	5.620	5.62 NS	8.885	12.566		

The analysis of variance results in Table (5) indicate a significant impact of the cold and hot water extracts of *A. fragrantissima* at varying concentrations and treatment durations, particularly with the inclusion of 2.5 g of the aggregation pheromone from P. Americana adults at the highest concentration (10%) of both extracts. This treatment achieved a 100% mortality rate of adult insects after 9, 12, and 15 days when utilising cold and hot extracts, and reached 91.737% and 96.139% mortality at the same concentration

without the addition of the aggregation pheromone after 6 days of treatment with the cold and hot extracts, respectively. The results indicated a direct correlation between concentrations and treatment durations, as the mortality rates of adult insects escalated with their increase.

The results from the table indicated a significant effect of the binary interaction of the study factors, which comprised the extracts and concentrations utilised in the study. However, the triple interaction was not observed in certain treatments involving the 10% concentration after various treatment periods for both the cold and hot extracts.

Table 5. Effect of varying doses of hot and cold extracts of *A. fragrantissima* and the inclusion of aggregation pheromone on the mortality rate of adults of *P. Americana* in a laboratory setting

Extract	Concentration	Time (days)					Average	Extract rate
		3	6	9	12	15		
Cold Extract	2.5	17.126	20.242	25.523	30.942	36.812	26.129	47.466
	5	23.011	28.208	34.621	39.799	46.003	34.328	
	7.5	26.528	32.022	37.458	43.180	51.832	38.204	
	10	31.099	37.707	42.567	48.991	54.878	43.048	
	+ %10 pheromone	81.973	96.139	100.00	100.00	100.00	95.622	
	Time rate	35.947	42.864	48.034	52.582	57.905		
	Concentration	3	6	9	12	15	Average	Extract rate
Hot Extract	2.5	20.868	25.234	31.157	35.616	42.876	31.150	51.857
	5	26.096	30.864	36.795	45.217	50.238	37.842	
	7.5	30.125	36.069	43.565	48.144	54.052	42.391	
	10	34.040	42.873	55.660	63.227	68.045	52.769	
	+ %10 pheromone	83.926	91.737	100.00	100.00	100.00	95.133	
	Time rate	39.011	45.355	53.435	58.441	63.042		
	Concentration	3	6	9	12	15	Average	
Concentration x time	2.5							
	5	18.997	22.738	28.340	33.279	39.844	28.640	
	7.5	24.554	29.536	35.708	42.508	48.121	36.085	
	10	28.327	34.046	40.512	45.662	52.942	40.298	
	+ %10 pheromone	32.570	40.290	49.114	56.109	61.462	47.909	
	Time rate	82.950	93.938	100.000	100.000	100.000	95.378	
L.S.D 0.01	Extract	37.479	44.110	50.735	55.512	60.474		
	0.099	0.156	0.156	0.221	0.221	0.350	0.495	

Discussion

The current study's results indicated that the highest mortality rates of sixth instar nymphs of P. Americana occurred when the aggregation pheromone was combined with the maximum concentration (10%) of the aqueous extracts from *C. album* and *A. fragrantissima* , The cause may be ascribed to the influence of the toxic substances found in these extracts, as corroborated by the chemical analysis of the active compounds

(glycosides, phenols, alkaloids, etc. in Table 1), which indicates that the majority of these compounds are harmful to insects. Schal and Hamilton [14] identified 150 chemicals from the faeces of *P. americana*, of which 57 were classified as carboxylic acids, pertaining to the aggregation pheromone. The aggregation pheromone is thought to be a component of this combination [15] demonstrated that the aggregation pheromone extracted from the faeces of nymphs and adults of the same insect comprises a blend of volatile amines and steroid glycoside (a non-volatile compound), both of which elicit aggregation behaviour in nymphs and adults.

Researchers have demonstrated the efficacy of aggregation pheromone in cockroach control, as Miller et al. [16] confirmed that the addition of aggregation pheromone to hydramethylnon-treated baits enhanced cockroach attraction compared to baits lacking the pheromone. Belete [17] and Yuan et al. [18] indicated that the substances involved in chemical defence against insects are low molecular weight secondary organic compounds, primarily comprising phenols, terpenes, alkaloids, and others. They may exhibit anti-feeding behaviour [19] and possess insect growth inhibitory qualities, therefore serving as insecticides [20].

The elevated mortality rates of insect nymphs and adults can be attributed to the influence of compounds, particularly Tannins, present in the aqueous extracts of the two examined plants. Pizzi et al. established that Tannins are the second most abundant polyphenols in vascular plants, functioning primarily as protease inhibitors. They impede digestive enzymes in the insect intestine by binding to these enzymes and obstructing protein deposition. Additionally, their bitter flavour deters insect feeding. Alkaloids in plant extracts have a significant biological function in their consumption by treated insects, as they contain nicotine, a pyridine alkaloid that influences the central nervous system of insects. The research findings underscore the significance of utilising plant-derived pesticides, particularly extracts from wild flora.

4. Conclusion

This study, reported for the first time, indicated that aqueous extracts of Chenopodium album followed by Achillea fragrantissima showed high insecticidal activity against sixth-instar nymphs and adults of Periplaneta americana through time-(15 day) and concentration-dependent manner. The most significant finding was that using the highest extract concentration (10% w/v) combined with 2.5 g of aggregation pheromone caused total (100%) mortality in both developmental stages after 6–15 days treatment; this was even more effective than any used plant extracts alone. The phytochemicals found in the studied plants like tannins, phenols (Flavonoids), alkaloids and glycosides saponins and coumarin seem to be responsible for the bioactivity. This study provides clear evidence for the utility of combining plant bioactive compounds with aggregation pheromones to serve as an eco-friendly and sustainable avenue in controlling P. americana in integrated pest management programs. In the future, it is necessary to isolate and characterize specific active compounds that contribute to insect mortality, test modes of action, assess effectiveness in field conditions and target environmental conditions, as well as test for safety towards non-target organisms and ecosystem health.

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