

(ARTICLE)

ATTRACT-AND-KILL BAIT FORMULATIONS INTEGRATING WILD BOTANICAL EXTRACTS AND FECAL AGGREGATION PHEROMONES AGAINST *Periplaneta americana* L.

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ABSTRACT

In this work, bio insecticidal activity of thermal and cold aqueous extracts from two indigenous plants of desert area in Al-Muthanna Governorate, Iraq (*Chenopodium album* L. and *Achillea fragrantissima* (Forssk.)) , and Sch. Bipica, anti-american cockroach (*Periplaneta americana* L.) were evaluated. In addition, the synergistic enhancement obtained by the addition of aggregation pheromones from the feces to botanical baits prepared against 1:1 sex ratio of sixth instar nymphs and mature adults was examined. Phytochemical screening was performed in order to evaluate the presence of important secondary metabolites such as polyphenols, tannins, alkaloids, saponins, flavonoids, coumarins, glycosides and terpenes. Laboratory bioassays showed distinct concentration and time dependent toxicity trends with thermal extracts being more potent than cold extracts, and nymphs being more susceptible than adults. Crude aggregation pheromone supplemented baits significantly optimized bait performance, resulting in 100% mortality of nymphs in 6 days for both plant species. *A. fragrantissima* combinations and *C. album* had complete eradication (100%) after 6-9 and 9-15 days respectively. On the other hand, the highest mortality rates were observed in the 15th day for both nymphs (66.1% - 79.2%) and adults (53.6% - 64.1%) with 10% plant preparations that were not supplemented. These results suggest that the fecal aggregation pheromones are efficient behavioral stimulators that stimulate the discovery of baits and persistent ingestion, thus increasing the toxicological effect of plant bioactive constituents. Thus, pheromone-enhanced formulations of botanical (plant) baits are a useful and environmentally sound attract and kill strategy for an integrated pest management (IPM) approach to manage *P. americana* L.

Keywords: *Periplaneta americana*; *Chenopodium album*; *Achillea fragrantissima*; Aggregation Pheromone; Botanical Insecticides.

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INTRODUCTION

The cockroaches are an extremely adaptable group of insects that has survived in terrestrial ecosystems for more than 300 million years, comprising over 4,600 species worldwide (Adedara *et al.*, 2021; Abbott, 1925). Though mainly successful in tropical and subtropical areas, certain species are synanthropic and can successfully colonize anthropogenic habitats with structural crevices, organic residues and domestic spaces as main harborages (Adedara *et al.*, 2021; Adenusi *et al.*, 2018).

American cockroach (*Periplaneta americana* L.; Blattodea: Blattidae) is still the most prevalent indoor pest species in residential and institutional settings especially in Iraq (Al-Qaswini and Al-Essa, 2014; Albalawi *et al.*, 2023). In addition to its mechanical nuisance and contaminating effects, it is also a coprophagic species (ingests faecal matter), and often comes into contact with unsanitary microhabitats, making it an effective mechanical vector for many human pathogens such as pathogenic bacteria, enteric viruses, fungal propagules and parasitic helminthes (Adenusi *et al.*, 2018; Belete, 2018; Fazeli-Dinan *et al.*, 2022).

Staggering scientific interest has been paid to safer botanical alternatives due to the emergence of resistance to conventional synthetic insecticides and the rising concern about environmental safety of non-target organisms ([Adedara et al., 2021](#); [Fazeli-Dinan et al., 2022](#); [Divekar et al., 2022](#)). This work evaluates the insecticidal effects of the hot and cold aqueous extracts of Lamb's quarters (*Chenopodium album*) and Lavender cotton (*Achillea fragrantissima*) two wild desert plants growing in the Badia of Al-Muthanna Governorate, Iraq. Furthermore, we assess if the crude fecal aggregation pheromone can be incorporated into plant-based toxic baits to help develop an improved behavioral-toxicological attract-and-kill system against 6th-instar nymphs and adult *P. americana* L.

MATERIALS AND METHODS

2.1 Insect Colonization and Rearing

The W-shaped corrugated cardboard traps baited with powdered milk and ground biscuits 1:1 w/w were used to capture wild populations of *P. americana* from residential and sanitary facilities at Al-Muthanna University. Specimens were taxonomically verified with the help of standard morphological identification keys. The stock colonies were kept in smooth-walled plastic rearing containers (30 cm diameter × 20 cm height) fitted with a thin film of petroleum jelly on the inner upper rim of the container to prevent escape ([Gajger and Dar, 2021](#)). Rearing rooms were kept at 65% relative humidity (RH) and 27 °C. The insects were provided with a standard nutrition regimen consisting of a diet that includes milk powder and biscuit in equal proportions (1:1) and fresh water as needed ([Gajger and Dar, 2021](#)).

Uniform biological cohorts were chosen for bioassay procedures:

Nymphs: Healthy 6th-instar nymphs collected within 3–5 days after they ecdysed.

Adults: Unmated adult males and females (1–2 weeks post-emergence) combined in a strict 1:1 sex ratio. Only gravid females that contained oothecae (Egg cases) were excluded (to avoid physiological variations associated with nutritional needs during gestation and with metabolic sensitivity).

2.2 Aggregation Pheromone Isolation

Fecal deposits were regularly harvested from colony containers, passed through an 8-mm mesh sieve to remove debris, and air-dried ([Harborne, 1984](#)). A sufficient amount of crude pheromone extract was obtained by exactly 50 g of purified fecal material being homogenized in 250 mL of analytical grade n hexane and extracted in a controlled water bath for 60 minutes at 55 °C ([Miller et al., 1997](#)). Following extraction the mixture was cooled to ambient temperature, filtered through Whatman No. 1 qualitative filter paper, and then concentrated under reduced pressure to produce a standardized crude pheromone extract stock that was kept at 4 °C as a specialized bait additive according to [Harborne \(1984\)](#).

2.3 Botanical Processing and Aqueous Extraction

Whole aerial plant biomass of *C. album* and *A. fragrantissima* was harvested during the late flowering phase (March–April) from the Western Badia region of Al-Muthanna Governorate. The harvested vegetation was shade dried at room temperature (25 °C) and mechanically ground to a fine, uniform powder and prepared as follows:

Cold Water Extract (CWE): 20 g of the plant powder was macerated with 200 mL of sterile distilled water for 24 h under room temperature, filtered with clean cheesecloth and centrifuged at 3,000 rpm for 10 minutes. The clear supernatant was

used as a 10% (w/v) stock solution (100 g/L mass to volume ratio). Accurate working concentrations of 2.5%, 5.0%, 7.5% and 10.0% (v/v) were achieved by serial dilutions with sterile distilled water (Harvey *et al.*, 2007).

Hot Water Extract (HWE): Formulated utilizing identical plant-to-water proportions, with distilled water maintained at 100 °C during initial thermal maceration for 60 minutes prior to cooling, straining, and centrifugation (Harvey *et al.*, 2007).

2.4 Bioassays and Statistical Analysis

Toxic bait matrices were prepared by thoroughly blending 5 mL of each botanical extract concentration with 5.0 g of a dry nutritional food base (comprising 2.5 g ground biscuit and 2.5 g milk powder) (Jiang *et al.*, 2021). For pheromone-fortified treatments, 2.5 g of the crude aggregation pheromone stock extract was thoroughly homogenized into 5 mL of the 10% plant extract prior to incorporation into the food matrix.

Groups of ten test insects (either uniform 6th-instar nymphs or adults at a 1:1 sex ratio) were introduced into designated bioassay arenas containing the test baits. Three independent biological replicates were performed per treatment. Mortality data were recorded at 3, 6, 9, 12, and 15 days post-exposure. Cumulative mortality percentages were corrected against untreated negative controls using Schneider-Orelli's formula (Abbott, 1925):

$$\text{Corrected Mortality \%} = \frac{\text{Treatment Mortality\%} - \text{Control Mortality\%}}{100\% - \text{Control Mortality\%}} \times 100\%$$

Experimental data were evaluated using a Completely Randomized Design (CRD) arranged factorial via SAS statistical software. Mean separations were executed using the Least Significant Difference (LSD) test at significance thresholds of 0.01 and 0.05.

RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Qualitative phytochemical analysis verified that aqueous extracts from both *C. album* and *A. fragrantissima* contain a rich profile of bioactive secondary metabolites (Table 1). These non-volatile constituents—including polyphenols, tannins, alkaloids, saponins, flavonoids, coumarins, glycosides, and terpenoids—function naturally as chemical defense barriers protecting plants against herbivorous consumers (Divekar *et al.*, 2022; Harborne, 1984).

Table (1) Qualitative phytochemical screening of active constituents in *Chenopodium album* and *Achillea fragrantissima* extracts.

Active Constituent / Bioactive Class	Reagent / Detection Method	Color Change / Indicator	<i>C. album</i>	<i>A. fragrantissima</i>
Carbohydrates	Alpha-naphthol	Violet-Red	+	+
Tannins	Ferric chloride	Blue-green	+	+
Glycosides	Benedict's test	Precipitate	+	+
Resins	Hydrochloric acid	Occurrence	-	-
Proteins	Ninhydrin	Purple / Yellow	-	-
Flavonoids	Ammonia	Yellow	+	+
Saponins	Mercuric chloride	White precipitate	+	+
Phenols	Potassium ferrocyanide	Blue-green	+	+
Alkaloids	Dragendorff's reagent	Orange	+	+
Coumarins	Sodium hydroxide	Greenish-yellow shiny	+	+
Steroids	Liebermann test	Red	-	-
Terpenes	Salkowski test	Red-tan separator	+	+

3.2 Bioactivity against 6th-Instar Nymphs

Bait formulations consisting of 10% plant extract enriched with crude aggregation pheromone triggered significantly elevated mortality levels relative to unsupplemented preparations ($P \leq 0.01$). For both of *A. fragrantissima* (Table 2) and *C. album* (Table 3), pheromone-supplemented 10% formulations had achieved been complete nymphal mortality (100%) within 6 days across both cold and hot extraction protocols.

Win contrast, standalone 10% extracts without pheromone fortification achieved maximum 15-day nymphal mortality ceilings of only 66.1% (CWE) and 79.2% (HWE) for *C. album*. These findings confirm that fecal aggregation pheromones act as potent behavioral arrestants and feeding stimulants that heighten bait discovery and consumption rates (Miller *et al.*, 1997; Oonincx *et al.*, 2015).

Table (2) Effect of varying concentrations of hot and cold extracts of *A. fragrantissima* and aggregation pheromone fortification on mortality (%) of *P. americana* 6th-instar nymphs

Extract Type	Concentration (%)	3 Days	6 Days	9 Days	12 Days	15 Days	Mean Extract
Cold Extract	2.5	19.87	23.87	32.22	33.63	41.06	29.53
	5.0	25.98	32.16	38.16	43.87	49.74	37.98
	7.5	29.63	34.95	41.32	46.93	55.05	41.58
	10.0	34.80	41.02	45.81	52.12	58.84	46.51
	10% + Pheromone	86.01	100.00	100.00	100.00	100.00	97.20
Time Mean		39.26	46.40	50.90	55.31	60.94	50.58
Hot Extract	2.5	24.02	29.00	34.88	39.97	46.74	34.92
	5.0	30.02	34.68	41.09	48.79	53.86	41.69
	7.5	33.22	38.85	46.62	51.92	57.88	45.70
	10.0	37.74	46.05	59.01	65.80	71.93	56.11
	10% + Pheromone	87.21	94.82	100.00	100.00	100.00	96.41
Time Mean		42.44	48.68	56.32	61.30	66.08	54.96
LSD (0.01)	\multicolumn{2}{l}{ct: 0.208}	Conc: 0.131	Time: 0.208	Extract × Conc: 0.294	Extract × Time: 0.294	Conc × Time: 0.465	Triple Interaction: 0.658}

Table (3) Effect of varying concentrations of hot and cold extracts of *C. album* and aggregation pheromone fortification on mortality (%) of *P. americana* 6th-instar nymphs.

Extract Type	Concentration (%)	3 Days	6 Days	9 Days	12 Days	15 Days	Mean Extract
Cold Extract	2.5	25.93	30.79	36.85	41.94	48.16	36.77
	5.0	32.93	39.05	44.95	52.07	57.11	45.22
	7.5	37.12	42.96	48.10	55.05	62.73	49.19
	10.0	41.93	47.99	53.93	59.90	66.14	53.98
	10% + Pheromone	91.04	100.00	100.00	100.00	100.00	98.21
Time Mean		45.79	52.20	56.77	61.79	66.83	56.67
Hot Extract	2.5	31.17	36.18	41.87	47.97	54.07	42.25
	5.0	37.35	41.74	48.21	55.44	62.16	48.98
	7.5	41.13	47.00	55.07	59.97	64.89	53.61
	10.0	45.88	53.53	66.18	72.71	79.24	63.51
	10% + Pheromone	93.91	100.00	100.00	100.00	100.00	98.78
Time Mean		49.89	55.69	62.27	67.22	72.07	61.43
LSD (0.01)	\multicolumn{2}{l}{ct: 0.089}	Conc: 0.141	Time: 0.141	Extract × Conc: 0.200	Extract × Time: 0.200	Conc × Time: 0.317	Triple Interaction: 0.448}

3.3 Bioactivity against Adult Cockroaches

Adult mortality trends followed patterns parallel to those observed in nymphs, although adult specimens generally demonstrated higher physiological tolerance. The 10% botanical baits fortified with crude aggregation pheromone achieved 100% adult mortality between days 6 and 9 for *A. fragrantissima* (Table 5), and between days 9 and 15 for *C. album* (Table 4).

Unsupplemented 10% extracts recorded lower 15-day adult mortality rates (53.6% for CWE and 64.1% for HWE). Plant polyphenols and tannins are widely documented to function as digestive enzyme suppressants (Pizzi *et al.*, 2009; Price *et al.*, 2019), whereas plant alkaloids disrupt normal neuromuscular signaling pathways (Harvey *et al.*, 2007; Yuan *et al.*, 2020). The strong efficacy reported in pheromone-integrated treatments suggests a high synergistic effect between behavior and toxicity, calling for specific feeding assays in the future to determine the exact amount of feed consumed (Schal and Hamilton, 1990).

Table (4) indicates mortality (%) of adult *P. americana* at different concentrations of hot and cold extracts of *C. album* and aggregation pheromone (AP) fortification.

Extract Type	Concentration (%)	3 Days	6 Days	9 Days	12 Days	15 Days	Mean Extract
Cold Extract	2.5	24.00	24.07	30.61	34.00	39.62	34.48
	5.0	26.27	29.88	36.21	42.76	46.01	36.23
	7.5	29.71	35.02	40.13	46.02	50.79	40.33
	10.0	35.01	40.16	43.80	50.05	53.62	44.53
	10% + Pheromone	72.98	83.23	100.00	100.00	100.00	91.24
Time Mean		41.61	42.47	50.15	54.57	58.01	49.36
Hot Extract	2.5	25.05	31.17	34.91	38.99	44.37	34.90
	5.0	30.07	34.77	38.97	45.39	50.69	39.98
	7.5	32.11	38.96	48.90	52.53	57.94	46.09
	10.0	44.94	44.91	53.22	59.59	64.13	59.36
	10% + Pheromone	79.95	100.00	100.00	100.00	100.00	95.99
Time Mean		48.42	49.96	55.20	59.30	63.43	55.26
LSD (0.01)	\multicolumn{2}{1}{Extra ct: 2.513}	Conc: 3.974	Time: 5.620	Extract × Conc: NS	Extract × Time: 8.885	Conc × Time: 12.566}	

Table (5) Effect of varying concentrations of hot and cold extracts of *A. fragrantissima* and aggregation pheromone fortification on mortality (%) of adult *P. americana*

Extract Type	Concentration (%)	3 Days	6 Days	9 Days	12 Days	15 Days	Mean Extract
Cold Extract	2.5	17.13	20.24	25.52	30.94	36.81	26.13
	5.0	23.01	28.21	34.62	39.80	46.00	34.33
	7.5	26.53	32.02	37.46	43.18	51.83	38.20
	10.0	31.10	37.71	42.57	48.99	54.88	43.05
	10% + Pheromone	81.97	96.14	100.00	100.00	100.00	95.62
Time Mean		35.95	42.86	48.03	52.58	57.91	47.47
Hot Extract	2.5	20.87	25.23	31.16	35.62	42.88	31.15
	5.0	26.10	30.86	36.80	45.22	50.24	37.84
	7.5	30.13	36.07	43.57	48.14	54.05	42.39
	10.0	34.04	42.87	55.66	63.23	68.05	52.77
	10% + Pheromone	83.93	91.74	100.00	100.00	100.00	95.13
Time Mean		39.01	45.36	53.44	58.44	63.04	51.86
LSD (0.01)	\multicolumn{2}{l}{Extra ct: 0.099}	Conc: 0.156	Time: 0.156}				

CONCLUSIONS

The results in this investigation show that crude fecal aggregation pheromones can be combined with aqueous extracts prepared from *C. album* or *A. fragrantissima* to make a very effective attract and kill bait system for *P. americana*. Incorporation of aggregation pheromones greatly enhances bait efficacy by stimulating attraction and feeding behavior in insects. Bio-bait set-ups can be a valuable environmentally friendly alternative in integrated pest management (IPM) strategies for urban cockroaches. Further studies on chemical composition of the active fraction of pheromones as well as histopathological analysis of the insect gut tissues will give the researchers more insights into the precise mechanism of toxicity action.

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