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Efficacy of Local *Ageratum conyzoides* Leaf Extracts on Controlling the Poultry Pest *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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ABSTRACT

Ageratum conyzoides Linn (Asteraceae) is known to have an insecticidal effect against agricultural-pest insects, but its activity has never been tested for its efficacy against the poultry pest *Alphitobius diaperinus* (Panzer) in Indonesia. This study aimed to analyze the phytochemical compounds of local *A. conyzoides* L. leaf extract and verify its efficacy against *A. diaperinus* under laboratory conditions. Efficacy of the extract was measured based on % mortality, % repellency, and production inhibition on *A. diaperinus*. Data were analyzed using one-way ANOVA followed by Tukey's test. GC-MS analysis found that extract of local *A. conyzoides* L. leaves contained ten main compounds, with the highest percentage of peak area being phenolic compounds, followed by other important compounds, most of which were essential oils. High mortality was found in the C75 and C100 groups at 36 h post-treatment, at 77.6% and 96.2%, respectively. *A. diaperinus* gave a denial response to all extract treatments, but the highest % repellence was found in the extract treatment of C100. The number of larval emergences was influenced by the concentration level, where the C25 and C50 treatments were able to reduce the number of larval emergences by 58.52% and 70.37%, respectively. We concluded that these extracts of local *A. conyzoides* L. are efficacious as a bioinsecticide to *A. diaperinus*. Thus, the results of this study can be used as the basis for the development of local bioinsecticides for the control of the poultry pest *A. diaperinus*, especially in smallholder poultry farm systems.

Keywords: *Alphitobius diaperinus*; *Ageratum conyzoides*; Larval emergences; Mortality; Repellence

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1. Introduction

This *Alphitobius diaperinus* (Pancer, 1797) (Coleoptera: Tenebrionidae) is a very detrimental pest insect, and is responsible for declines in poultry production, as it acts as a dispersal agent for pathogenic micro-organisms. *A. diaperinus* acts as a vector for the spread of poultry diseases and parasites, such as Newcastle disease and *Avian influenza* [1]. In addition, *A. diaperinus* also acts as a carrier for *Salmonella bacteria*, *Escherichia coli*, and various viruses [2]. Generally, controlling pests in rural poultry farm settings uses synthetic insecticides and is applied in the period of seedling turnover. The synthetic insecticide is usually applied by fumigation and spraying on the floor and walls of the cage prior to litter change for the next maintenance period. This method is carried out to prevent direct contact of chemical insecticides with chickens [3]. However, pest management in this manner is considered inefficient due to the behavior of *A. diaperinus*; it is very fast to hide in places difficult to reach when the poultry cage is empty. Therefore, the spraying of synthetic insecticides is less targeted [4]. The most widely used synthetic insecticides to control the population of *A. diaperinus* are pyrethroid, cypermethrin, deltamethrin, or dimethyl phthalate [5]. Various studies and facts in the field prove that the continued and unmeasured use of synthetic insecticides has caused resistance in *A. diaperinus* populations and is reported to leave residues that are harmful to human health, the environment, and the livestock. This fact has prompted many across the world to develop alternative insecticides that are eco-friendly. Bioinsecticides made from plants are a very promising class of pesticides today. Currently, the negative impacts of synthetic insecticides and the emergence of resistance in insect populations have been reported widely [1, 6].

This problem has led experts to a great attentiveness in the development of bioinsecticides derived from plant materials. Plants are a source of various active substances that naturally act as personal protective means from pests. Today, the use of synthetic insecticides is increasingly limited and poses many environmental problems. Therefore, various active compounds of plants are widely studied and utilized as bioinsecticides. Bioinsecticides are active compounds extracted from natural materials and are applied in a way similar to synthetic insecticides; however, the advantage of these bioinsecticides is that they are generally safer and more eco-friendly for both humans and the environment. Bioinsecticides are more specific in terms of their activity, have no residual problems, and only kill target insects, so they are safe for other organisms in the environment. Bioinsecticides are easily available with local raw materials, and have simple production processes. The limitation of bioinsecticides is that they require repeated application to obtain optimal efficacy while improving plant protection [7]. Because of its safety though, this bioinsecticide is able to be applied at any time when needed, without having to wait when the chicken cage is empty.

One plant that has been widely studied as a source of bioinsecticides to control agricultural pests is *A. conyzoides* (Familia: Asteraceae). This type of plant belongs to agricultural weeds [8]. Plants from the family group Asteraceae have been widely studied and are known to contain a complex diversity of secondary metabolite compounds, thus making these plants a potential source of natural material, both as traditional medicine and as bioinsecticides. Qualitative phytochemical analysis has shown the presence of carbohydrates, amino acids, alkaloids, phenolics, terpenoids, tannins, steroids, glycosides, and saponins. Various phytochemical compounds are known to have insecticidal effects at varying levels [9, 10], depending on the quality of the plant and the environment in which it has bred.

Previously, the local *A. conyzoides* leaf extract has been evaluated for efficacy against a number of insect species by researchers in various countries. The research has shown that volatile organic compounds emitted by these aromatic plants alter the behavior of aphids *Aphis citricola* (Hemiptera: Aphididae) and beetles *Harmonia axyridis* (Coleoptera: Coccinellidae) [11]. *A. conyzoides* essential powders and oils have also been shown to have insecticidal activity against rice weevil *Sitophilus oryzae* (L) (Coleoptera: Curculionidae), the flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), as well as the lesser grain, *Rhyzopertha dominica* (F) (Coleoptera: Bostrichidae) [12]. Powders and leaf extracts of *A. conyzoides* have a histopathological effect on the male reproductive systems of the beetle *Dermestes maculatus* and the grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae) [13, 14]. However, information related to the insecticidal activity of *A. conyzoides* against poultry pest *A. diaperinus* remains unexplored. *A. conyzoides* is well known to poultry farmers in smallholder farming systems in Indonesia as a traditional medicine. In order to reveal the efficacy of the local *A. conyzoides* leaf extract, and to support the application of green technology in the field of pest control, the present study aimed to analyze the effect of local *A. conyzoides* leaf extract on mortality, repellence, and production capability of *A. diaperinus* under laboratory conditions. These results are expected to strengthen the potential of *A. conyzoides* as a bioinsecticide, especially in controlling the poultry pest *A. diaperinus*.

2. Materials and Methods

2.1 Insect preparation

A. diaperinus adults and larvae that were used here, were reared at the Laboratory of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia. Insects were reared in 10 plastic jars (20×30×10 cm) that contained 100 adult male-female of each, and 200 g of commercial chicken feed as culture medium. Every 5 days, the adult *A. diaperinus* were transferred to another jar, leaving the eggs left in the jar to hatch all with a relatively homogenous larvae emergence. The first generation of *A. diaperinus* was used for experiments.

2.2 Extract preparation

Fresh leaves of local *A. conyzoides* were taken from the Temu Gesang traditional medicine garden, Grabag Village, Magelang Regency, Central Java, Indonesia. The type of garden soil was mostly Latosol, located at an altitude of 750 m above sea level with a temperature range of 22°C-28°C. Samples of *A. conyzoides* were washed with distilled water and dried at room temperature for 3-5 days, then dried in an oven at 40°C. Dried leaves were ground into powder. Extraction was carried out by maceration of the powder for 3×24 hours using ethanol as a solvent, followed by filtration and evaporation using a rotary vacuum and evaporator to obtain a crude extract. The crude extract was then stored in a sterile brown (dark) glass bottle at 4 °C. This concentrated extract was assumed to be 100% extract and was then used as testing material. Extract concentrations of 75%, 50%, and 25% were obtained by dilution, using distilled water as a solvent.

2.3 Phytochemical and GC-MS Analysis

Extracts of the local *A. conyzoides* leaf were phytochemically analyzed qualitatively to determine the presence of secondary metabolites, using standard procedures [15]. Visible color changes or formation of precipitates were considered as indications of the presence of (+) or absence of (-) certain active compounds in this phytochemical test. In this case, the presence of active compounds of terpenoids, alkaloids, flavonoids, phenolics, saponins, steroids, and tannins was tested.

GC-MS analysis of the local *A. conyzoides* leaf extract was conducted using Shimadzu Gas Chromatography-Mass Spectroscopy (GCMS-QP2010). During the process, the oven was maintained at 110 °C with a holding for 2 min. The injector temperature was set at 250°C. The inlet temperature was 200°C and the source temperature was 200°C. The mass spectrum was taken at 70 eV, scan period 0.5 S, and fragments from 45-450 Da. The carrier gas used was pure helium gas with a flow rate of 0.8mL/min. The split ratio used was 1:10. The start time was set at 2.5 min and the end time was set at 93 min. The interpretation of the GC-MS mass spectrum was carried out using the NIST (National Institute Standard and Technology) database and the WILEY library.

2.4 Mortality testing

The effect of *A. conyzoides* extract on mortality was tested by using a complete randomized design, with five concentration treatments (C0%, C25%, C50%, C75%, and C100%). Each treatment was repeated five times, and in each repeat group, 25 adult *A. diaperinus* were put into a plastic box measuring 5×5×10 cm equipped with a ventilated cover, placed 1×1 cm filter paper dripped extract as much as 200 µL. Furthermore, 25 adult *A. diaperinus* were inserted into it for direct contact and exposure to extract volatile compounds. Commercial chicken feed was put in a maintenance box of 5 g. The entire treatment box was then stored in a dark room. Mortality was calculated at 12, 24, and 36 h post-exposure. When mortality in the control group reached 5% - 20%, then data from the treated group were corrected using the Abbott formula [16].

$$Mortality(\%) = \frac{(X - Y)}{(100 - Y)} \times 100, \quad (2.1)$$

where X is mortality in the treated sample and Y is mortality in the control.

2.5 Repellent activity

A Y-tube dual choice olfactometer was used to investigate the response of adult *A. diaperinus* to volatile compounds derived from the extract. Each arm of the olfactometer had a diameter of 2.5 cm and a length of 20 cm. Each arm of the Y branched passageway was connected by two glass tubes that could be opened to the lid, while the other passageway was the entrance for test insects. The procedure is similar to that described by Koschier et al. [17] with some modifications. The end of the glass tube in aisle A contained a 1×1 cm filter paper treated wtih 200 µL extract, while the end of aisle B contained a 1×1 cm filter paper treated with 200 µL distilled water, to act as a control. The orientation of the walking direction of each insect was observed and used as an indicator of repellence or acceptance of test extracts. The test duration to ensure the position of the insect did not reverse was 60 minutes, according to the average time recorded in the preliminary test. After 60 minutes, the number of insects that entered the control hallway and the treatment hallway was measured. The percentage of repellency was calculated by the following formula [18].

$$\%Repellence = \frac{(C - T)}{C} \times 100, \quad (2.2)$$

where C is the number of insects present in the control areas, and T is the number of insects present on the treated side.

2.6 Production capability testing

The production ability of *A. diaperinus* exposed to the extract was indicated by the number of larvae that emerged during the 4-week rearing period. The test was carried out using a completely randomized design with three levels of extract concentration (0%, 25%, 50%). Each treatment was replicated five times with 3 pairs of adult *A. diaperinus* in each experimental unit. The place of rearing was a plastic box (6 cm high and 5 cm in diameter) equipped with a ventilated lid and 10 g of commercial chicken feed. Maintenance and observations were carried out for 4 weeks. The larvae emergence count was recorded.

2.7 Statistical analysis

The mean of mortality, % repellence, and production capability due to different extract concentrations was analyzed by one-way ANOVA, followed by Tukey's test. However, if the assumptions of normality and homogeneity of the data were not achieved, Kruskal-Wallis tests were performed. Analysis was conducted using IBM SPSS Statistics for Windows version 23.

3. Results and Discussion

3.1 Phytochemical constituents of extracts

The local *A. conyzoides* ethanol extract was qualitatively tested to identify the presence or absence of selected chemical constituents. The results of the analysis (Table 1) showed ethanol extracts of local *A. conyzoides* contained alkaloid compounds, steroids, terpenoids, phenolics, tannins, and flavonoids.

Table 1. Phytochemical Analysis of The Local *A. Conyzoides* Leaf Extract[a].

Sample	Parameter	Description
Local *A. conyzoides* extract	Alkaloids	(+)
	Phytosterols	(+)
	Terpenoids	(+)
	Flavonoids	(+)
	Phenolic	(+)
	Saponins	(-)
	Tannins	(+)

Notes: [a]Sample analysis at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang on January 2021. (+): detected; (-): undetected

These results correspond to the composition of leaf extracts studied in many countries [19, 20], although there may be differences in quantity and composition due to differences in soil conditions and growing environment, species differences, cultivars, and interactions with other environmental factors such as varying temperatures. The results of chemical screening of the extract through GC-MS analysis detected a total of 35 compounds, of which 10 predominant compounds were found, with the highest peak area being Phenol, 4.6-di (1,1-dimethyl ethyl)-2-methyl-by 38.93%, followed by 9 other important compounds detected as essential oils Table 2.

GC-MS screening results in 10 active compounds from *A. conyzoides* extract were observed. This bioactive mixture with various targeted modes of action is thought to have a synergistically negative effect on *A. diaperinus*. According to Oriyomi [21], phytochemicals with the most important insecticide activity are alkaloids, flavonoids, phenols, steroids, and terpenoids. These groups of active compounds may work independently or synergistically, though synergistic effects will provide higher performance than the sum of each individual compound [22]. Synergistic effects can occur when the combination of bioactive substances in an extract exerts a greater effect than the sum effect of each individual component [23]. The phytochemical activity of extracts consisting of a combination of various metabolite compounds may contribute to the overall biological effect. Thus, synergistic active compounds will increase the effectiveness of insecticides [24].

Alkaloids are important secondary metabolites in plants that can act as insecticides. Alkaloids can affect insects through biological activities such as interfering with physiological and cellular processes through redox imbalances and hormonal regulation [25]. Alkaloids cause neurological effects in insects, which can reversibly inhibit the activity of acetylcholinesterase (AchE), an important enzyme that regulates synaptic activity. The mechanism of action of these alkaloids acts by binding to specific amino acids on the active side of AChE, causing inhibition and imbalances in nervous system function [26]. Inhibition of AChE by coarse extracts from various plant families exhibits insecticidal activity, including extracts from the Asteraceae family.

The presence of terpenoid compounds in plants indicates that the plant has the potential to be an antifeedant, a growth disruptor, and has a considerable toxic effect on insects. Terpenoid compounds are known to stimulate cytochrome P450 activity, which is essential for the production of insect hormones, as well as pheromones, reproductive development, and behavior regulation. This causes hormonal regulation of pheromones to be inhibited. Likewise, the presence of saponins is known to affect the development of insects, especially inhibiting their growth and molting [27].

Developmental disorders, in addition to causing insects to die, will also significantly decrease the population of adult insects, as egg production is also reduced. All terpenoid groups had strong eating prevention activities. The essential oil not only reduces the level of food consumption but also significantly reduces the growth rate of insects.

Table 2. List of Major Compounds Identified in The Local Extracts of *A. conyzoides* Using GC-MS Analysis.

No.	Compound Name	RT (min)	Chemical Formula	Peak Area (%)
1	1,2,3-Cyclohexanetriol	17.56	$C_6H_{12}O_3$	13.45
2	17-Octadecynoic acid	19.78	$C_{18}H_{32}O_2$	2.07
3	Diisooctyl phthalate	20.56	$C_{24}H_{38}O_4$	4.58
4	ß-Longipinene	21.43	$C_{15}H_{24}$	3.37
5	Precocene I	21.67	$C_{12}H_{14}O_2$	6.70
6	Germacrene D	22.12	$C_{15}H_{24}$	1.62
7	Caryophyllene oxide	24.51	$C_{15}H_{24}O$	1.11
8	Phenol, 4,6-di(1,1-dimethyl ethyl)-2-methyl-	26.33	$C_{13}H_{16}O_3$	38.93
9	Hexadecanoic acid, ethyl ester	33.14	$C_{18}H_{36}O_2$	7.28
10	Phytol	35.38	$C_{20}H_{40}O$	1.02

Table 3. Mean of Mortality 36 h Post-Treatment, % Repellence, and The Number of Larval Emergences on Various Concentration Extract.

Treatment (%)	Mortality (%)	Repellence (%)	Number of Larval Emergences
C_0	3.2 ± 3.346^{a}	1.00 ± 0.000^{a}	$135 \pm 43{,}008^{a}$
C_{25}	16.0 ± 2.828^{b}	66.37 ± 10.356^{b}	$56 \pm 13{,}255^{b}$
C_{50}	70.4 ± 4.561^{c}	68.13 ± 6.946^{b}	$40 \pm 22{,}554^{b}$
C_{75}	77.6 ± 4.561^{d}	67.16 ± 14.981^{b}	-
C_{100}	96.2 ± 2.828^{e}	79.62 ± 14.332^{c}	-

a b c d e Each value represents the mean of five replicates. Means (±SD) followed by the same letters within an average column are not significantly different (p-value < 0.05; Tukey's test).

Plant extracts, especially essential oils, with significant repellent activity against plant pests are an attractive alternative [28].

A. conyzoides contain procenase, which is a group that acts as a contact poison. Active compounds of the terpenoid group containing isoprene units can also act as food deterrents [29]. Another group of terpenoids that can cause insect death is essential oils. Essential oils can contain alkaloid compounds that can act as neurotoxins in insects. Essential oil toxicity occurs due to inhibition of AChE during exposure and usually requires very high concentrations to obtain significant inhibition of AChE [30].

Phenolic compounds are known to dissolve lipids from the cell wall, thereby affecting cytoplasmic membrane integrity, inducing cell lysis, inhibiting ATPase binding to the cell membrane, and eventually leading to death in insects. Similarly, tannin compounds are known to have a powerful damaging effect on insects, i.e., affecting growth and development by binding to proteins, causing irritation of the middle intestine, and reducing the absorption efficiency of nutrients. When ingested, tannins reduce protein digestibility, thereby lowering the nutritional value of food [31]. Therefore, tannins can also act as an antifeedant in various pest insects.

3.2 Evaluation of efficacy of extract *A. conyzoides* against *A. diaperinus*

Bioinsecticide activity in insects works through several modes of action, such as toxic effects, repellent, antifeedant activity, oviposition inhibition, inhibition of growth and development, attractant, infertility, and death. In this study, three parameters were investigated, namely mortality, repellent activity, and developmental inhibition as measured based on the number of larval emergences (Table 3).

3.2.1 Mortality

The mortality rate of *A. diaperinus* from observations of 3×12 h (Fig.1) showed that 12 hours after treatment there had been no significant mortality, where the mortality rate was still below 50%. More than 50% of insect deaths occurred after 24 h and were found only in the treatment groups of C75 and C100. Furthermore, observations at 36 h indicated an insect mortality rate of more than 50% was also found in C50. The percentage of deaths appeared to increase in line with the increase in the concentration of extracts. The highest mortality (96.2%) was found at 36 h post-exposure in the C100 group.

In this group, 50% of deaths occurred 24 hours after treatment, and the lowest concentration (C25) occurred after 36 h. Mortality rates ranging from 42% to 80% were found at 24 h post-treatment for C50, C75, and C100. In the control group, it appeared that viability was still high, and at 36 h post-treatment, the mortality rate was only 3.2%. Therefore, data was not converted using the Abbots formula. The results of these observations showed that exposure to *A. conyzoides* extract did not cause a direct killing effect, but rather acted through a slower process. *A. conyzoides* extract causes impaired eating ability so that *A. diaperinus* survival decreases, and a mortality rate of more than 50% was achieved after 24 h post-treatment.

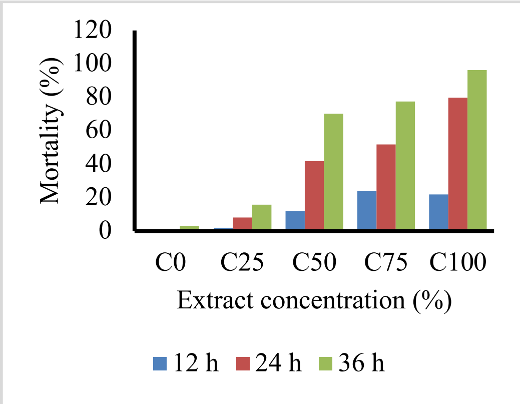

Fig.1. Mortality of *A. diaperinus* in various concentrations of Local *A. conyzoides* leaf extract.

The action of bioinsecticides generally do not kill directly, but rather through more diverse mechanisms.

According to Kortbeek et al. [32], in general, the effects of bioinsecticides occur gradually, which can begin with disruption of the digestive system and inhibition of insect growth or can accelerate the occurrence of malformations. Mortality data on observations 36 hours post-treatment, %repellence, and developmental barriers of *A. diaperinus* due to the treatment of *A. conyzoides* extracts are presented in Table 3. Statistical analysis of mortality data showed real differences in all treatments. There seems to be a correlation between the concentration of extracts and the percentage of insect deaths.

The higher the concentration of extracts tested the higher mortality appears to increase. When viewed from the results of GC-MS analysis, the local *A. conyzoides* leaf extract shows the dominance of phenol group compounds and various compounds that are classified as essential oils. In addition to terpenoids, phenolic compounds are identified as toxic compounds that play an important role in plants. Phenol has antioxidant properties and is insecticidal because phenol causes toxicity after being ingested by herbivorous insects [33]. In general, bioinsecticides derived from plant extracts have low toxicity but are able to control insect populations through a variety of means [34]. Use of plant extracts as a source of bioinsecticides is growing rapidly, including in members of the Asteraceae family. Although its toxicity may be several times lower than the acute toxicity of synthetic insecticides, the effects of subacute toxicity are also important because they can limit population development and spread (e. g., decreased fertility, low vitality, or low survival) as well as reduce post-harvest loss [35]. The effects of insecticide *A. conyzoides* have been tested on some insects and have been shown to be effective, such as with *Helicoverpa armigera* [36] and *Aedes aegypti* [37].

3.2.2 Repellent activities

One important group of secondary metabolites of plants is essential oils. Essential oils are the active compounds responsible for the aroma of plants. The results of insect behavior testing using a dual choice *Y*-olfactometer showed that treatment of high concentrations of extracts had a repellant effect. Statistical analysis showed that the concentration of extracts had a significant effect on insect response (p-value < 0.05). The highest repellence response was in C100, while the treatments of C25, C50, and C75 were no different. It seems that the specific aroma of the extract has caused insects to avoid and move away from the extract. This is understandable because the repellent effect is generally caused by the distinctive aroma of volatile compounds derived from bioinsecticides. The results showed that the volatile compounds of plants could come from fatty acid derivatives, terpenoid compounds, and phenols. It is these volatile compounds that have activity as repellents of herbivorous insects [38].

3.2.3 The number of Larval Emergences

In this study, reproductive barriers were measured based on the number of larval emergences during the three weeks of observation. The results showed that differences in the concentration of extracts had an effect on the number of larvae *A. diaperinus* produced (p-value < 0.05). Furthermore, the results of the Tukey's test (Table 3) showed the highest number of larval emergences was found in the control treatment. This is in stark contrast to the number of larvae in the treatment of C25 and C50. Exposure to C25 extract decreased the number of larvae by 58. 52% while in C50 there was a decrease of 70.37%. In this case, it appears that exposure to the extract causes a bottleneck in feeding ability for *A. diaperinus* and ultimately affects egg production.

Phenol compounds contained in plants, in addition to inhibiting the growth and development of insects, also play an indirect role as a deterrent to oviposition. This is in line with the results of a study on rhipicephalus microplus cow lice which found that *A. conyzoides* extract can inhibit egg hatching by up to 90% [39]. This egg hatching failure is caused by the presence of phenol and terpenoid compounds. In other studies, it was reported that *A. conyzoides* extract may play a role in affecting the oviposition and hatch power of *Plutella xylostella* eggs as well as causing damage to *Rhipicephalus* *microplus* oocytes (Acari: Ixodidae) [40]. Observations on horticultural plants [41] showed that high concentrations of *A. conyzoides* extract led to the disruption of stadia larvae of *Brassica juncea* pests. Metamorphosis is also inhibited because toxic compounds will damage nerve tissue and inhibit the process of larvae becoming pupae.

Overall, *A. conyzoides* have complete bioactive compounds, which are able to prevent the infestation of pest insects on plants, pests in stored post-harvest products (warehouse pests), as well as the poultry farming pest insect *A. diaperinus*. Volatile compounds decrease adult oviposition and appearance and various other sublethal effects such as weight loss, body size, decreased fertility, and altered behavior. Although plant-derived bioinsecticides cannot completely replace synthetic insecticides at this time, bioinsecticides can clearly contribute significantly to reducing the problems posed by synthetic insecticides. The three most important arguments in support of the use of bioinsecticides are: greater environmental safety, lower or no toxicity for vertebrates and humans, and prevention of the development of resistance.

4. Conclusion

This study proved that local *A. conyzoides* leaf extract has significant insecticidal activity against adult *A. diaperinus*. Thus, the results of this study can be used as the basis for the development of local bioinsecticides that are eco-friendly for the control of the poultry pest *A. diaperinus,* especially in smallholder poultry farm systems.

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