Original Research Article

Control of the maize pest beetle, *Anomala denuda* (Coleoptera: Scarabaeidae), using insecticidal plants *Ricinus communis* and *Azadirachta indica* in a laboratory setting

ABSTRACT

The aim of this study was to identify a natural alternative to synthetic insecticides for controlling the *A. denuda* beetle, a destructive corn pest. The investigation took place at Félix HOUPHOUËT-BOIGNY University's Natural Environments and Biodiversity Conservation Laboratory at a temperature of 17°C. Aqueous extracts of *Azadirachta indica* leaves and *Ricinus communis* seed capsules were tested for their effectiveness against the beetle. The reference pesticides, Cypercal 50EC and Viper 42EC, were used for comparison. The tests included three types of toxicity (inhalation, ingestion, and repulsion) with four repetitions each and a four-day exposure period. The ingestion test was the most effective. At 110 g/l, *A. indica* and *R. communis* extracts had corrected mortality rates of 87.41 ± 3.21 and 90.28 ± 1.71%, respectively. In the inhalation test, *A. indica* and *R. communis* extracts resulted in 80.92 ± 5.88% and 83.14 ± 7.57% mortality rates, respectively, at the same concentration. At the same concentration, *R. communis* extract repelled 60% of beetles, which was higher than *A. indica*'s 53.3%. The insecticidal effects of the extracts were similar to those of Cypercal in all tests. These results suggest that *R. communis* and *A. indica* extracts could be used to control *A. denuda* pests without relying on synthetic chemical pesticides. The keywords for this study are: toxicity test, insecticidal effect, *Azadirachta indica*, *Ricinus communis*, pest, *Anomala denuda*.

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1. Introduction

The Beetle *A. denuda* belongs to the family Scarabaeidae and the subfamily Rutelinae. Until now, only the larvae (white grubs) were known to cause significant damage to crops such as corn, rice, sugarcane, potato, beet, bean, tomato, and certain ornamental plants, as well as numerous weeds. These larvae can cause extensive damage, especially to young plants [1]. As for the adults, they are believed to feed very little, if at all, on leaves and flowers of plants [2]. However, during a nocturnal survey for stem and ear borers in an experimental maize plot measuring 2,527 m^2 in Songon, we unexpectedly observed a very high population of *A. denuda* adults, approximately 46,508 insects [3]. These authors noted that the adults of this beetle attack the reproductive organs of maize, causing an unacceptable 32% loss in maize production. These observations have sparked our interest in finding an alternative viable control solution, other than using synthetic chemical pesticides, to reduce its impact on maize production.

In this study, the insecticidal effect of aqueous extracts from two plants (*A. indica* and *R. communis*) will be tested on *A. denuda* adults in the laboratory. The objective of this study is to determine the plant and lethal concentration of the extract that would be most effective against this beetle.

Indeed, the choice to search for a biopesticide for the control of *A. denuda* is motivated by the fact that the use of biopesticides is a means of pest control that respects human health and the environment [4]. This choice is also justified by the fact that numerous control trials against several species of pest insects, using *A. indica* and *R. communis*, have yielded favorable results, as evidenced by the works of Tano et al. [5] , Monfankye [6], Ossey *et al*. [7], Makoundou et al. [8], and Obodji [9]. However, these studies were conducted on smaller beetles

(Ootheca mutabilis, 14-16 mm, and Coelaenomenodera lameensis, 2-5 mm), mosquitoes, and mostly Lepidopteran larvae. The question that arises here is whether the extracts of these two plants, although their insecticidal effect is proven, would have a confirmed insecticidal effect on *A. denuda*, which is a larger beetle (1.5-3 cm) and tougher compared to those already studied. To answer this question, two specific objectives have been established. 1. Evaluate the effectiveness of aqueous extracts of *A. indica* and *R. communis* on *A. denuda* adults in the laboratory to determine its LD50 (the duration in which 50% of the insects die). 2. Compare the efficacy of chemical insecticides and the tested extracts on the beetle to determine the most effective extract and its optimal concentration.

2. MATERIALS AND METHODS

2.1. Preparation of Aqueous Extracts

In this study, the method for preparing aqueous extracts was based on the protocol developed by Zirihi and Kra [10]. Firstly, the leaves of *A. indica* and the capsules of *R. communis* seeds were collected and dried in the shade for a period of three to four weeks. Subsequently, the leaves and capsules were ground into a fine powder using a blender. 100 g of the resulting powder for each type of organ was then diluted in 200 ml of distilled water. The mixture was homogenized in the blender for a duration of ten minutes, and subsequently filtered through muslin cloth. To ensure the purity of the extracts, two more filtrations were performed using Whatman filter paper (3 MM) and a funnel containing hydrophilic cotton. The obtained product was then placed in plates and subjected to concentration by evaporation in an oven maintained at 50 °C for 48 hours, until a dry residue was obtained. This dry residue served as the basis for preparing the various concentrations, specific to each type of organ of every plant species.

2.2. Concentration determination

The concentration levels were determined using the dry residue obtained from each organ type of the plant species. This residue was kept in an oven for 48 hours, and we chose the concentration of 80 g/l as the base because of its effectiveness against specific pests, including the oil palm pest (*Coelaenomenodera lameensis*) [7], the cowpea pest (*Ootheca mutabilis*) [5], and Coleoptera that are three to four times smaller than *A. denuda*. As *A. denuda* is larger, mobile, and subject to natural field conditions, we prepared three other concentrations by increasing the base concentration of 80 g/l by 10 g/l. These concentrations were 90 g/l, 100 g/l, and 110 g/l. In laboratory tests, we used four concentrations, including the 80 g/l base concentration, and two reference chemical insecticides, namely Cypercal 50EC (cypermethrin 50g/l) and VIPER 46 EC (16 Acetamiprid, 30 Indoxacarb). We designated the aqueous extract treatments of both plants as TA1: 80 g/l, TA2: 90 g/l, TA3: 100 g/l, and TA4: 110 g/l for *A. indica* leaves, and TR1: 80 g/l, TR2: 90 g/l, TR3: 100 g/l, and TR4: 110 g/l for *R. Communis* seed capsules. The chemical insecticide reference treatments were noted as Ti for Cypercal 50EC (cypermethrin 50g/l) and Tii for VIPER 46 EC (16 Acetamiprid-30 Indoxacarb). Finally, we designated the control group as T0.

2.3. Toxicity Test

The toxicity tests were conducted in rectangular plexiglass boxes measuring 12cm x 9cm x 8cm at a constant temperature of 17°C. Three toxicity tests were conducted for each laboratory test:

1. an inhalation toxicity test (11 boxes prepared with 4 replicates = 44 boxes);
2. an ingestion toxicity test (11 boxes prepared with 4 replicates = 44 boxes); and
3. a repellent toxicity test (11 boxes prepared with 4 replicates = 44 boxes).

Each of the 11 boxes contained 4 doses of *A. indica* aqueous extract, 4 doses of *R. Communis* aqueous extract, 2 doses of synthetic chemical insecticides, and a neutral control box. Finally, 20 adult *A. denuda* beetles were placed in each box (**Fig. 1**).

2.3.1. Inhalation toxicity test

To conduct the inhalation toxicity test, absorbent paper uniformly impregnated with various doses of *A. indica* and *R. Communis* aqueous extracts and reference chemical insecticides (Cypercal 50EC and Viper 40EC) is placed in the bottom of the boxes. The papers in the control boxes are left untreated. The boxes are immediately closed after introducing the insects. Dead insects are counted every 24 hours for 4 days and preserved in 70% alcohol. The mortality rate in the treated boxes (Mo) is expressed using Abbott's formula [11] as corrected mortality (Mc), taking into account the natural mortality observed in the control boxes (Mt). This formula can be written as:

$$\mathrm{Mc}\ (\%) = \frac{\mathrm{Mo} - \mathrm{Mt}}{20 - \mathrm{Mt}} X\ 100$$

Mc% = Percentage of corrected mortality; Mo = Percentage of mortality in the treated group; Mt = Percentage of mortality in the control group.

2.3.2. Ingestion toxicity test

To conduct the ingestion toxicity test, the same apparatus as that used for the inhalation test is utilized. The sole distinction is that the filter paper is replaced with a food bait (5 g of maize leaves). The baits are treated with various doses of aqueous extracts of the plants under investigation, as well as with synthetic insecticides in some cases. The food baits in the control boxes are left untreated. The enumeration of dead insects is carried out every 24 hours for four consecutive days. The mortality of insects in the treated boxes (Mo) is expressed using Abbott's formula [11] to calculate the corrected mortality (Mc), taking into account the natural mortality observed in the control box (Mt).

2.3.3. Repellent effect of extracts on blotting paper.

To evaluate the repellent effect of aqueous extracts on adult *A. denuda* beetles, we use the zone preference method on filter paper, as described by Mc.Donald *et al.* [12]. After a two-hour exposure to the various treatments, the number of insects present on the part of the filter paper soaked in insecticide is compared to the number of insects present on the untreated part. The percentage of repellency (PR) is calculated using the following formula:

$$\mathrm{PR} = \frac{\mathrm{Nc} - \mathrm{Nt}}{\mathrm{Nc} + \mathrm{Nt}} X\ 100$$

Nc: number for the untreated part

Nt: number for the insecticide-treated part.

The mean percentage of repellency to the insecticides was calculated and assigned, according to the classification by Mc.Donald *et al.* [12], to one of several repellency classes ranging from 0 to V.

2.4. Data analysis

Initially, Microsoft Office 2010's Excel software was utilized for data entry and chart creation. Subsequently, SPSS 21.0 software under Windows was utilized to carry out statistical analysis of the data. This analysis focused on the mean corrected mortality rates of the *A. denuda* beetle, induced by various doses of plant extracts tested. The mean corrected mortality rates were subjected to an analysis of variance (ANOVA), and the means were ranked into homogenous groups using the Student-Newman-Keuls (S-N-K) test, with a significance threshold of 0.05.

3. RESULTS

3.1. Evolution of corrected mean mortality rates according to exposure durations to the extracts

3.1.1. Results of the inhalation toxicity test

The analysis of the inhalation toxicity test results has revealed that plant extracts were effective against the *A. denuda* beetle at any dose used (Fig. 2). The efficacy of the plant extracts increased with the concentration. Therefore, after four days of exposure to different concentrations, the highest average corrected mortality rates were obtained with the highest concentration (110 g/l) of *A. indica* and *R. communis* extracts, which were 79.54% and 83.14% respectively (**Fig. 2**).

In contrast, chemical insecticides reached their LD50 on the first day (within 24 hours) with a corrected mortality rate of 53.33% for each. For the concentration of 110 g/l of the two tested plant extracts, the LD50 was reached on the second day (48 hours), with corrected mortality rates of 61.02% for *A. indica* and 50.81% for *R. communis*. Finally, the lowest tested concentrations (80 g/l, 90 g/l, and 100 g/l) reached their LD50 on the third day, i.e. after 72 hours of exposure (**Fig. 2**).

3.1.2.Results of the ingestion toxicity test

The results of the aqueous extract ingestion toxicity tests revealed that both plant extracts were effective against *A. denuda*. The mean corrected mortality rates varied with increasing doses of the tested aqueous extracts and the duration of exposure.
In contrast to the inhalation test, only the chemical insecticide Viper reached its LD50 after 24 hours of exposure with a mean corrected mortality rate of 53.33% (**Fig. 3**). However, the 110 g/l concentration of plant extracts and Cypercal reached their LD50 after 48 hours of exposure. The mean corrected mortality rates after these two days were 98.15% for Cypercal, 57.78% for *A. indica*, and 51.85% for *R. communis* at the 110 g/l concentration (**Fig. 3**).

3.1.3. Results of the repellency toxicity test

The results of the toxicity test by repellency are presented in the table (I), which shows the percentages of repellency observed for the different doses of the two biopesticides. After a 2-hour exposure, the aqueous extracts of the two plants generated the following percentages of repellency against the beetle *A. denuda*: for *R. communis*, the concentrations of 80, 90, 100, and 110 g/L resulted in respective repellencies of 6.67%, 53.33%, 53.33%, and 60%; for *A. indica*, the concentrations of 80, 90, 100, and 110 g/L resulted in respective repellencies of 0%, 33.33%, and 53.33%. The results demonstrate that the percentage of repellency varies according to the concentration of the aqueous extracts of the two plants. However, at a concentration of 110 g/L, the extract of *R. communis* proved to be relatively more repellent (PR=60%) than that of *A. indica* (PR=53.3%), although both extracts are considered moderately repellent according to the Mc.Donald classification (**Table 1**).

3.2. Bioefficacy of *A. indica* and *R. communis* aqueous extracts

3.2.1. Bioefficacy of *A. indica* and *R. communis* aqueous extracts by inhalation test

The study of the bioefficacy of plant aqueous extracts by inhalation showed that the extracts of the treated plants are effective. However, this efficacy is dependent on the tested plant and concentration. After 4 days of insect exposure to different treatments, the reference chemical insecticides recorded corrected mean mortality rates of 88.52±9.35% for Cypercal (Ti) and 84.07±8.27% for Viper (Tii). To date, the corrected mean mortality rates of *A. indica* aqueous extracts were 77.59±6.11% (TA1), 77.31±5.66% (TA2), 79.54±5.69% (TA3), and 80.92±5.88% (TA4) (**Fig. 4**). As for *R. communis*, its different concentrations induced corrected mean mortality rates of 74.90±5.54% (TR1), 75.36±5.38% (TR2), 76.2±5.08% (TR3), and 83.14±7.57% (TR4). The Anova test followed by the Newman-Keuls test at the 5% threshold revealed significant differences ($p < 0.05$) between the corrected mean mortality rates of different treatments. The Cypercal chemical insecticide treatment (Ti) was found to be the most effective. As for the second insecticide, Tii (Viper), its induced mean corrected mortality rate is statistically similar to that of the concentration of 110g/l of *R. communis* (**Fig. 4**).

3.2.2. Bioefficacy of *A. indica* and *R. communis* aqueous extracts by ingestion test

The study of the bioefficacy of aqueous plant extracts by ingestion has demonstrated their effectiveness. After four days of exposure of insects to different treatments, reference chemical insecticides recorded average corrected mortality rates of 98.15±1.51% for Cypercal (Ti) and 94.44±2.41% for Viper (Tii). Aqueous extracts of *A. indica* resulted in average corrected mortality rates of 82.78±3.39% (TA1), 85.19±1.71% (TA2), 85.19±1.71% (TA3), and 87.41±3.21% (TA4) (**Fig. 5**). As for *R. communis*, its different concentrations induced average corrected mortality rates of 82.78±3.21% (TR1), 83.61±2.74% (TR2), 84.91±0.54% (TR3), and 90.28±1.71% (TR4) (**Fig. 5**). The ANOVA test followed by the Newman-Keuls test at the 5% level revealed a significant difference ($p < 0.001$) between the average corrected mortality rates of different treatments. The chemical insecticide treatment Ti (Viper) was found to be the most effective, while Tii (Cypercal) resulted in a statistically similar corrected mortality rate to that of the 110 g/l concentration of *R. communis* (**Fig. 5**).

4. DISCUSION

To improve its production, the global agricultural landscape regularly uses synthetic chemical pesticides. Their massive, uncalibrated, and repetitive use in some cases can lead to a chemical imbalance of ecosystems due to the persistence of their active chemical agents. In addition to polluting the environment, these chemical pesticides are potentially toxic to humans and non-target organisms (useful insects such as pollinators, necrophagous dipterans, termites, and ants that are soil engineering insects, etc.) to varying degrees. The immeasurable negative impact of chemical pesticides on the environment is a major concern today. Although their use allows for improved agricultural yields by limiting the risk of yield losses, the question of an alternative solution is strongly considered to reduce the use of these synthetic chemical pesticides. In this study, we were faced with an invasive nocturnal beetle. According to the work of Boga *et al.* [3], the damage caused a 32% loss of yield. In this context, the objective was to find an effective bio-insecticide as an alternative treatment to synthetic insecticides against the invasive beetle *Anomala denuda*, a formidable corn pest. To do this, aqueous extracts of *A. indica* leaves and *Ricinus communis* seed capsules were tested against this beetle in the laboratory. In this study, the aqueous extracts were effective against the *A. denuda* species. And this effectiveness is a function of exposure duration, concentration, and plant. Indeed, concentrations of 100 and 110g/L of the two aqueous extracts take two days to cause the death of 50% of the tested insects. On the other hand, the other concentrations 80 and 90 g/l take 3 days in the inhalation and ingestion tests. As for the repulsion test, the results showed that the percentage of repulsion of the aqueous extracts of the 2 plants used varies according to the concentration used. However, the *R. communis* extract would have relatively higher repellent properties (PR = 60%) than that of *A. indica* (PR = 53.3%) at a concentration of 110 g/l, although both are moderately repellent. After 4 days of exposure, the ingestion test showed relatively effective results. For this test, aqueous extracts of *A. indica* and *R. communis* at a concentration of 110 g/l respectively induced mean corrected mortality rates of 87.41 ± 3.21 and 90.28 ± 1.71% against 80.92 ± 5.88 and 83.14 ± 7.57% for the inhalation test. The insecticidal effects induced by these 2 extracts were substantially identical to those induced by the chemical insecticide Cypercal regardless of the test.

Indeed, the insecticidal effect of *R. communis* has been reported by Adabie *et al.* [13] and Tano *et al.* [5] who reported the efficacy of *R. communis* respectively on Sitophilus zeamais (Coleoptera: Curculionidae), a maize pest, and Coelaenomenodera lameensis (Coleoptera: Chrysomelidae), a pest of oil palm. Aboua *et al.* [14] also mentioned a reduction in the infestation rate of eggplant fruits by Leucinodes orbonalis (Lepidoptera: Pyralidae) using aqueous extracts of *R. communis*. The efficacy of aqueous extracts of *R. communis* seed capsules is due to ricin, one of its main compounds. It is a glycoprotein present in the seeds and shells (capsules) of castor oil plant. Ricin has insecticidal properties and can cause mortality by inhibiting protein synthesis in insects. The aqueous extract of *A. indica* leaves also has insecticidal properties, which are attributed to the presence of bioactive compounds such as azadirachtin, nimbin, and salanin [15]. These compounds have been reported to have insecticidal properties against various pests, including Coleoptera, Lepidoptera, and Hemiptera [15].

In conclusion, this study demonstrates that aqueous extracts of *A. indica* leaves and *R. communis* seed capsules have significant insecticidal and repellent effects against the invasive corn pest *Anomala denuda*. These natural products could be considered as an alternative to synthetic chemical pesticides to control this pest and potentially reduce the negative impact of chemical pesticides on the environment and non-target organisms. Further studies are needed to evaluate the efficacy of these natural products under field conditions and to assess their impact on beneficial insects and the environment.

Fig. 1 Laboratory test setup

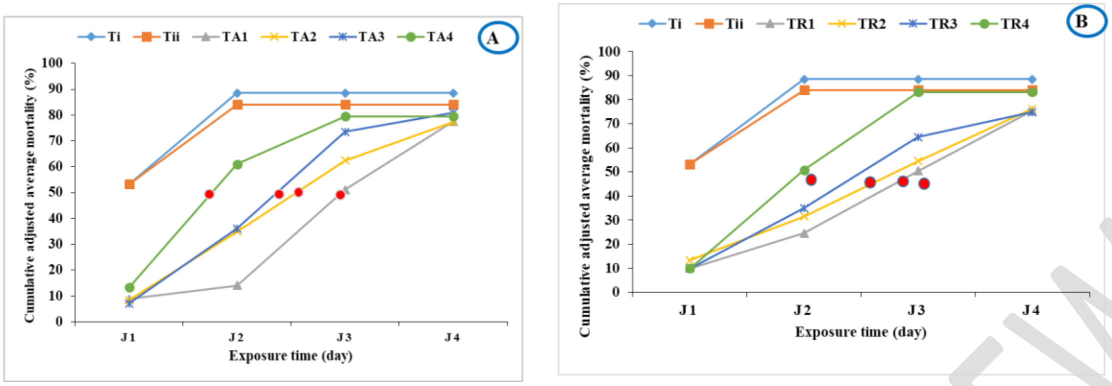

Fig.2 Corrected mortality rates by inhalation as a function of exposure duration to *Azadirachta indica* (A) and *Ricinus communis* (B) extracts.

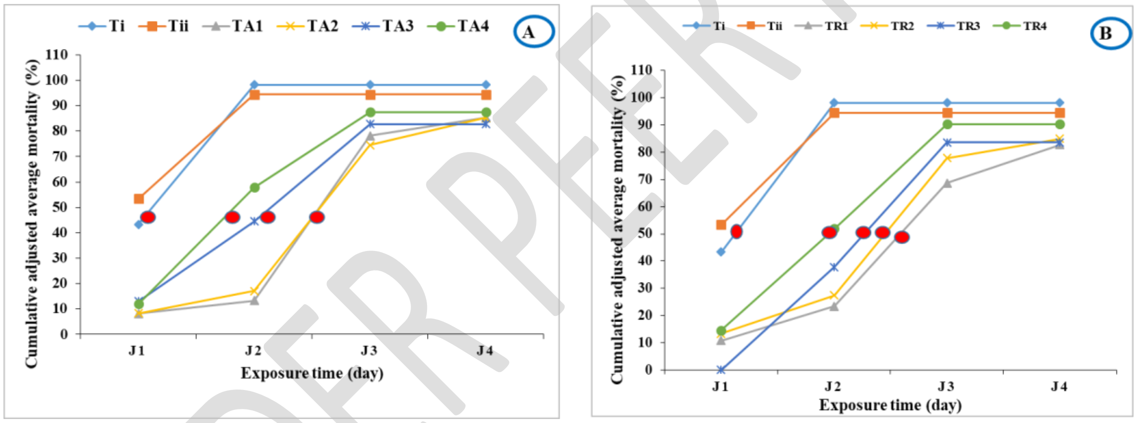

Fig. 3 Corrected mortality rates by ingestion test as a function of exposure duration to *Azadirachta indica* (A) and *Ricinus communis* (B) extracts.

● *DL50: Duration during which 50% of the mean corrected mortality rates were recorded.*

Table 1 Repellency rates of *A. indica* and *R. communis* extracts on *A. denuda*

Concentration (g/l)	**Classes**	**Repellency rates (%)**		**Repellency effect**	
		A. indica	***R. communis***	***A. indica***	***R. communis***
80	1	0	06,67	nul	nul
90	2	33,33	53,33	Faible	Modéré
100	2	40,00	53,30	Faible	Modéré
110	3	53,33	60,00	Modéré	Modéré

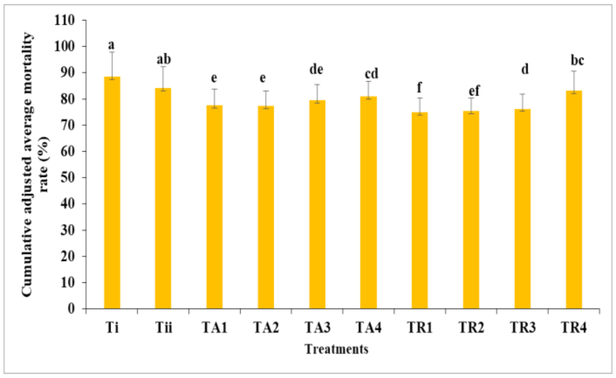

Fig. 4 Bioefficacy of chemical insecticides and tested extracts by inhalation (df = 9; P <

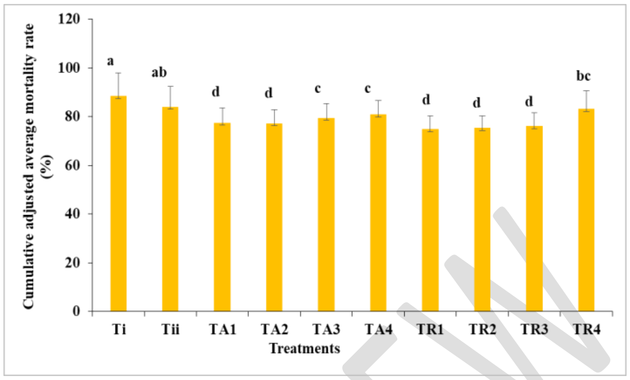

Fig. 5 Bioefficacy of chemical insecticides and tested extracts by ingestion (df = 9; *P* < .05)

Note: *Means followed by the same letters are not significantly different according to the Student Newman-Keuls test at a threshold of 5%.*

5. CONCLUSION

In this study, we aimed to find an effective bio-insecticide as an alternative treatment to synthetic insecticides against the destructive corn pest, *A. denuda*. To achieve this goal, aqueous extracts from *Azadirachta indica* leaves and *Ricinus communis* seed capsules were tested against this beetle, with the aim of determining the most effective plant and lethal concentration of the extract on the beetle.

After 4 days of exposure, the ingestion test proved to be relatively effective. In this test, the aqueous extracts of *A. indica* and *R. communis* at a concentration of 110 g/l induced corrected mean mortality rates of 87.41 ± 3.21% and 90.28 ± 1.71%, respectively, compared to 80.92 ± 5.88% and 83.14 ± 7.57% for the inhalation test. The insecticidal effects induced by these two extracts were substantially identical to those induced by the chemical insecticide, Cypercal, regardless of the test. As for the repellency test, the extract of *R. communis* had relatively higher repellent properties (PR = 60%) than that of *A. indica* (PR = 53.3%) at a concentration of 110 g/l, although both were moderately repellent.

Based on these results, the *A. indica* and *R. communis* extracts at a concentration of 110 g/l could be effectively used to combat *A. denuda* infestations with minimal reliance on synthetic chemical pesticides. However, field trials could help to better understand their potential to effectively control this pest. In conclusion, this study provides valuable information for the development of eco-friendly pest management strategies for sustainable agriculture.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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