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Direct and delayed effect of the plant *Cleome amblyocarpa* Barratte & Murb (*Capparidaceae*) on the two species of (Blattodea) *Blattella germanica* (Linnaeus, 1767) and *Shelfordella lateralis* (Walker, 1868)

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Abstract. Finding substantial substitution of chemical pesticides to control cockroaches, which were proved to link with multiple health issues, has become the major target of many researchers. In fact, subjecting this pest to the effects of toxic plants extracts is considered ideal to primarily avoid jeopardizing human health. This study is divided into two objectives. The first is to confirm the toxicity of the ethanolic extract from the plant *Cleome amblyocarpa* Barratte & Murb, on two species of urban cockroaches; which are *Blattella germanica* (Linnaeus, 1767) and *Shelfordella lateralis* (Walker, 1868), while the second is to verify the effect of the attractive odor of the extract on the feeding behavior of the two already mentioned species. Therefore, the findings demonstrated that the extract causes a mortality rate of 60% of *B. germanica* after 30 days of treatment at a high concentration of (3g/l). The mortality rate in *S. lateralis* does not exceed 6.7%. Further, findings exhibited that the proportion of *B. germanica* attracted by the smell of the plant soaked in hexane at a specified time (15, 30 and 60 minutes) was estimated at 40%, whereas that of *S. lateralis* exceeded 60%. The results provide the presence of toxic elements within the examined extract, suggesting the potential for developing bio-insecticides derived from *Cleome amblyocarpa* for utilization in the pesticide industry.

Keywords: *Blattella germanica*, *Cleome amblyocarpa*, feeding behavior, *Shelfordella lateralis,* toxicity.

Introduction

Cockroaches are classified within the arthropod phylum, fall under the class of Insecta, Blattodea. Actually, more than 4500 species of cockroaches have been globally recognized (Hashemi-Aghdam and Oshaghi, 2015). So, a relatively small number, of cockroach species, have adapted to domestic habitats (Robinson, 2005). The majority of cockroach species are not household pests; in fact, the major pest species constitute less than 1.0% of all cockroach species (Rehn, 1945).

These pests are abundantly present in human environments, which is a result of the availability of appropriate conditions of humidity and temperature in some places such as food stores, restaurants, restrooms and culinary places (Nedelchev *et al.*, 2013). In fact, urban cockroaches carry diseases; as a consequence, they can contaminate foods consumed by humans with pathogenic organisms and cause asthma, especially for children (Hashemi-Aghdam and Oshaghi, 2015). Moreover, cockroaches excretions likes feces, molded skins and saliva can cause severe allergic symptoms (Sookrung and Chaicumpa, 2010).

Despite acknowledging their toxicity to human health, chemical compounds such as fipronil, imidacloprid and sulfonamide (Cutler *et al.,* 2017) have been used to control cockroaches; hence, to avoid the consequences of contracting diseases resulting from contact with this creature. However, the use of these products is being gradually avoided these years, this is due to the increasing resistance to common insecticides (Ko *et al.,* 2016). The previous facts contribute to the constant increase of the necessary of alternative methods such as biological struggle which replace environmentally harmful chemicals (Cutler *et al.,* 2017).

In effect, more than 2000 plant species with insecticidal activity have already been identified (Shilaluke and Moteetee, 2022). *Cleome amblyocarpa* is used in traditional medicine by the nomads of the Sahara as an analgesic for neuralgic pain (Khlifi *et al.,* 2021). In Saudi Arabia the whole plant is used to treat scabies, rheumatic fever and inflammation. While in Mauritania, roasted leaves are cooked into a food taken for kidney and back infections and as an aphrodisiac (Schmelzer and Grurib-Fakim, 2013).

Regarding Algeria, entomological investigations and studies related to plants extracts are scarce. Information is still fragmentary, incomplete or even non-existent (Aouissi *et al.*, 2021, Farhi *et el.,* 2022, Deghiche-Diab *et al.,* 2022). This study fills in an important gap in the knowledge of North African biodiversity in general.

The aim of the current study is first to test the toxicity of the ethanolic extract of *Cleome amblyocarpa* on two species of urban cockroaches, namely *Blattella germanica* and *Shelfordella lateralis*, in addition to examining the effect of this plant soaked in hexane in the food attractiveness of the two species, *Blattella germanica* and *Shelfordella lateralis.*

Materials and methods

1. The investigated target species of Blattodea

❖ *Blattella germanica* are cosmopolitan insects, closely associated with human accommodation and activities. They depend on the warm climate of homes and other habitat. Some abiotic factors such as food and water also affect their population (Gul *et al.,* 2017; Gul *et al.*, 2022).

❖ *Shelfordella lateralis,* the Turkestan cockroach, also known as the rusty red cockroach, is a lively mainly external cockroach, native to an area from North Africa to Central Asia (Kim and Michael, 2013). This species is commonly found in fields, houses and especially in steam tunnels (Gul *et al.,* 2017).

Breeding

The mass breeding of the individuals of the studied cockroaches (*B. germanica* and *S. lateralis*) was carried out in plastic boxes with a perforated lid so that the cockroaches did not escape from the boxes. The cockroaches were fed dog kibble, which was provided approximately weekly. To ensure the humidification of the environment, the boxes were equipped with tubes filled with water and plugged with cotton; the water was changed every four days.

2. The investigated target plant species

Cleome amblyocarpa: is a perennial green grass belonging to the Capparidaceae family (Ozenda, 1958). The plant used in this study was collected in the year of 2018 from Bousaâda region, located in the state of M'sila; Algeria.

Extraction of plant active ingredients

The powder of the plant *C. amblyocarpa* is macerated into 150 mL of distilled water and 350 mL of ethanol, then left to cool with stirring for 48 h. The obtained mixture is filtered using Whatman filter paper (3 mm). Then, in order to obtain an exact estimate of the quantity of plant matter dissolved in the aqueous extracts, the latter were concentrated by evaporation in an oven heated to 50°C for 48 hours, until a dry residue was obtained, of which the quantity is expressed in g.

3. Toxicity test

The larvae of *B. germanica* and *S. lateralis* are separated and arranged in groups within three repetitions contain 10 individuals in each box (13x11x5 cm), which contain dog food as well as water tube with added concentration from the extract of *C. amblyocarpa* (1g/l, 2g/l and 3g/l The control group is watered with pure water. Each experiment was repeated three times and followed for 30 days; the number of mortalities is recorded daily to determine lethal concentration and time (LC/ LT).

4. Food attractiveness test

In a flask containing 30 ml of hexane, 25 g of the leaves of *C. amblyocarpa* emerge; after 15, 30 and 60 min of incubation under laboratory conditions. A piece of filter paper emerges in each extract to perform the food attractiveness tests on the larvae of two species (*B. germanica* and *S. lateralis*) by the use of a tube of form (Y) for the tests in the various times 15 individuals were used.

5. Statistical analysis

Regarding the results acquired from the toxicological test done, calculations were conducted following the mathematical methods defined by Finney (1971). The lethal time (LT50% and LT90%) and lethal concentration (LC50% and LC90%) were determined for the extract used.

For the feeding behavior, we compared the results obtained from ethological tests conducted in an olfactometer using Monte-Carlo simulations, based on a Chi-2 (χ^2) test at the threshold $p=0.05$ (Vaillant and Derrij, 1992). The results were also analyzed using the analysis of variance at a threshold variation criterion (ANOVA). Additionally, the View stat software on iMac was used for all these calculations.

Results

1.Effect of the ethanoic extract of C. amblyocarpa on the mortality of B. germanica and S. lateralis larvae

Mortality rate. Figure 1 provides the rates of the mortality of *B. germanica* and *S. lateralis* obtained after the 5th, 15th and 30th day of exposure to different concentrations of *C. amblyocarpa*.

B. germanica larvae: the use of a concentration of 1 g/l causes a mortality of 6.7% after 5 days of exposure and increases as a function of time to reach 30% after 30 days. A mortality of 20% to 46.7% was recorded after 5 and 30 days of exposure for the 2 g/l concentration. The 3 g/l concentration causes a mortality of 20% at the end of the 5th day to reach 60% at the 30th day. The analysis of variance indicates significant variations in mortality rates after 30 days of treatment among the different concentration. (Fobs = 2.06; P < 0.0001***) (Fig. 1).

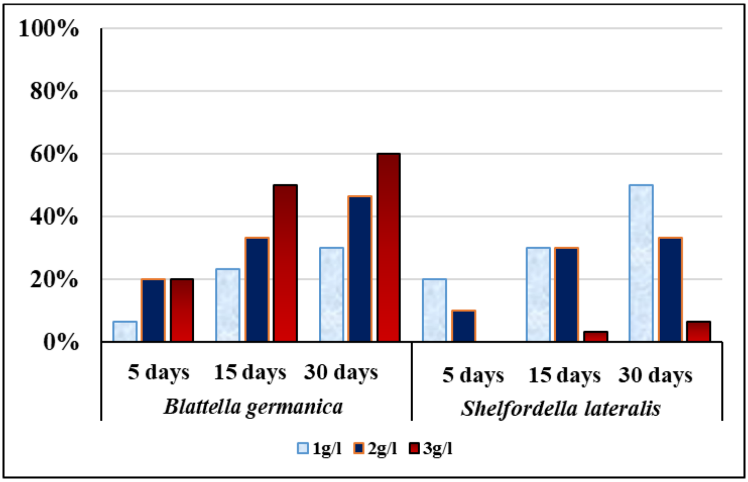

Figure 1. Mortality rate of *B. germanica* and *S. lateralis* larvae by different concentrations of ethanoic extracts of *C. amblyocarpa*.

S. lateralis larvae: the use of a concentration of 1 g/l causes a mortality of 20% after 5 days of exposure and increases as a function of time to reach 50% after 30 days. A mortality of 10% to 33.3% was recorded after 5 and 30 days of exposure for the 2 g/l concentration. The 3 g/l concentration causes a mortality of 0% at the end of the 5th day to reach 6.7% at the 30th day. The analysis of variance demonstrates a significant variation between the mortality rates registered after 30 days of treatment with the different concentrations (F obs = 0.34; P = 0.72) (Fig. 1).

Toxicological parameters. Table 1 presents the toxicological parameters of the larvae of *B. germanica* and *S. lateralis* treated with different concentrations of the ethanolic extract of *C. amblyocarpa*.

Table 1. The toxicological parameters of different species treated with *C. amblyocarpa*.

B. germanica			
Lethal concentrations			
Exposure time	**5 days**	**15 days**	**30 days**
Regression	Y= 3.55+1.47X R= 0.93	Y=4.22+1.48X R=0.96	Y=4.45+1.63X R=0.99
LC50%(g/l)	0.98	0.52	0.33
LC90%(g/l)	1.85	1.39	1.12
Lethal times			
Concentrations	**1g/l**	**2g/l**	**3g/l**
Regression	Y=2.67+1.26X R=0.97	Y=3.55+0.85X R=0.99	Y=3.20+1.41X R=0.99
LT50%	6.35	5.50	3.58
LT90%	17.55	24.82	8.80
S. lateralis			
Lethal concentrations			
Exposure time	**5 days**	**15 days**	**30 days**
Regression	Y=4.66-7.98X R=0.83	Y=4.66-2.46 R=0.77	Y=5.12-2.99X R=0.92
LC50%(g/l)	0.958	0.879	1.040
LC90%(g/l)	0.816	0.517	0.678
Lethal times			
Concentrations	**1g/l**	**2g/l**	**3g/l**
Regression	Y=3.45+0.95X R=0.97	Y=3.77-0.19X R=0.96	Y=-2.94+4.62X R=0.95
LT50%	5.111	0.0015	5.576
LT90%	16.667	$0.00000018*10^{3}$	7.357

To achieve a 50% mortality rate of *B. germanica* larvae after 5 days, the concentration needs be equal to 0.98 g/l. On the other side, ensuring the mortality of 90% of the larvae in the same period necessitates the use of a concentration of (1.85g/l) of this insecticide. Further, 50% of these larvae can be eliminated after 15 days when a concentration of 0.52 g/l is applied, while 90% require the use of a concentration of (1.39g/l) in 15 days. For a mortality of the order of 50% of these larvae, the necessary concentration is (0.33 g/l), and for a mortality of 90% of the larvae a concentration of (1.12 g/l) is necessary, after 30 days (Tab. 1).

Concerning *S. lateralis* larvae, and in order to insure 50% mortality of these spices after 5 days, the concentration should be equal to (0.958 g/l). whereas (0.816 g/l) of this whereas (0.816 g/l) this insecticide ensures the mortality of 90% of the larvae in the same period. More, 50% of these larvae can be eliminated after 15 days when a concentration of (0.879 g/l) is applied, while 90% require the use of a concentration of (0.517g/l). For a mortality of the order of 50% of these larvae, the necessary concentration is (1,040g/l), and for a mortality of 90% of the larvae, a concentration of (0.678 g/l) is necessary after 30 days (Tab. 1). Thus, a positive correlation was observed between the mortality rates recorded and the exposure time and/or the concentration of the extract used against the larvae of *B. germanica* and *S. lateralis* (Tab. 1).

2. Effect of the hexanoic extract of C. amblyocarpa on the food attractiveness of B. germanica and S. lateralis

The percentage of *B. germanica* attracted to the odor of the foods tested at the specified time (15.30 and 60 minutes) is estimated at 40% (Fig. 2). However, the percentage of *S. lateralis* attracted by the smell of the foods tested at the specified time (15.30 and 60 minutes) is estimated at 60% (Fig. 2).

The detection time. Table 2 provides a summary of the statistical analysis conducted on the latency times of *B. germanica* and *S. lateralis* larvae in detecting the tested odor.

B. germanica larvae seem more attracted to the *C. amblyocarpa* extract with hexane for 15 min (2.454 ± 0.997), and they take longer with the *C. amblyocarpa* hexane extract 30 minutes (10.893 ± 4.757). The statistical analysis of the mean detection times reveals significant differences (F=2.290; P = 0.038*) (Tab. 2).

S. lateralis larvae seem more attracted to the *C. amblyocarpa* extract with hexane for 30 min (0.756 ± 0.342). The statistical analysis of these detection times reveals significant differences (F=1.891; P = 0.080*) (Tab. 2).

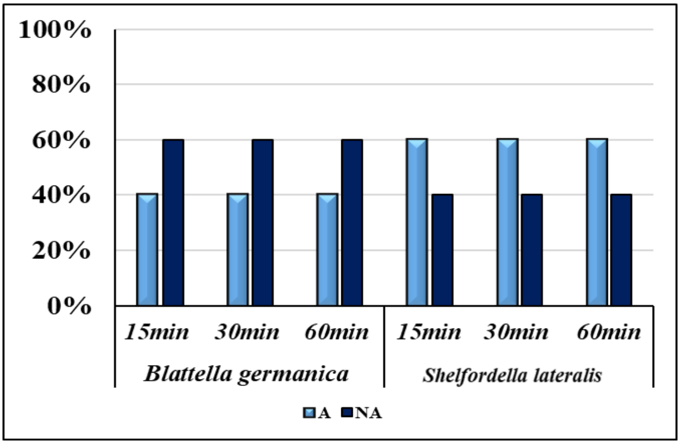

Figure 2. Food attractiveness rate of *B. germanica* and *S. lateralis* (NA: not attracted; A: attracted)

Table 2. Detection time of larvae to *C. amblyocarpa.*

***B. germanica* larvae**			
Time	**15min**	**30min**	**60mins**
Detection	2.454±0.997	10.893±4.757	8.933±4.417
F	2.461	2.290	2.023
P	0.027*	0.038*	0.063
***S. lateralis* larvae**			
Time	**15min**	**30min**	**60mins**
Detection	12.301±6.505	0.756±0.342	2.118±0.684
F	1.891	2.214	3.096
P	0.080	0.045*	0.008*

(*: Significant)

The arrival time. Table 3 illustrate the statistical analysis of the different latency times taken by larvae of *B. germanica* and *S. lateralis* to arrive at the tested odor. When the filter paper is soaked in the extract for 60 minutes, the majority of *B. germanica* larvae were noted to move towards a vacuum, i.e. they are not attracted by the smell of the *C. amblyocarpa* extract at an average of (49.040 ± 11.231). Statistical analysis of these mean latency times of indicates significant differences (F= 4.366; P = 0.001*) (Tab. 3).

Larvae of *S. lateralis* are more attracted to the smell of the hexanoic extraction of *C. amblyocarpa* at 60 min (4.782 ± 2.189). Statistical analysis suggests that there is a significant difference between the means arrival time (F = 2.185; P = 0.046*) (Tab3).

Table 3. Arrival time of larvae to *C. amblyocarpa* extract

***B. germanica* larvae**			
Time	**15min**	**30min**	**60mins**
Arrival	32.239±11.666	24.632±6.917	49.040±11.231
F	2.763	3.561	4.366
P	0.015*	0.003*	0.001*
***S. lateralis* larvae**			
Time	**15min**	**30min**	**60mins**
Arrival	6.800±2.134	11.255±7.852	4.782±2.189
F	3.186	1.433	2.185
P	0.007*	0.145	0.046*

(*: Significant)

Discussion

In his attempt to control harmful insects such as urban cockroaches, man deploys considerable efforts, and seeks new methods of physical, biological or chemical control in order to limit their proliferation (Kim *et al.,* 1995; Lyon, 1997).

The application of chemical methods to fight against harmful cockroaches cause harmful consequences on the environment and on man (Amin *et al.,* 2022). This is why the world is starting to look for another plant-based alternative to protect the globe with its different components. Plant products act on the sensory system and cause behavioral effects on insects, they include pheromones, extracts and oils of plants, plant growth regulators and insect growth regulators. Bio-insecticides work in harmony with integrated pest management programs (Veer and Gopalakrishnan, 2016). The effects of secondary plant metabolites responsible for insecticidal activity are described as suppression of calling behavior, growth retardation, toxicity, disruption of mating, deterrence of oviposition, inhibition of Diet and Reduced Fecundity and Fertility (Veer and Gopalakrishnan, 2016).

In Algeria, this type of research focuses on the use of plant extracts. It has gradually begun to develop in recent years with great concentration on aqueous plant extracts against urban pests, through several recent works (Benhissen *et al.,* 2018; Habbachi *et al.,* 2019; Boublata *et al.,* 2020; Saadane *et al.,* 2021; Hedjouli, 2022). This study, which uses ethanoic plant extracts as a means of control of the pests *B. germanica* and *S. lateralis*, focuses on the toxicological effect of different concentrations of ethanoic extracts from the leaves of the *C. amblyocarpa* plant, with the study of food attractiveness.

Based on the results obtained, we demonstrated that the leaves *C. amblyocarpa* affects the larval mortality of *B. germanica* and *S. lateralis*. The mortality rates of these larvae increase with the different exposure times and the concentrations used of the ethanolic extract of this plant. The results also indicated that the mortality rate of *B. germanica* larvae is greater than the mortality rate of *S. lateralis* larvae, so that *B. germanica* is more sensitive to this bio-insecticide.

These results are similar to a recent work by Hedjouli (2022). They demonstrated that the aqueous extract of *C. amblyocarpa* has an effect on the mortality of adults (male and female) of *B. germanica*. Further, he stated that the mortality rates in both sexes vary according to the duration of exposure and the concentrations used of *C. amblyocarpa*. They found that the values of LC 16%, LC50%, LC84, LC90%, LT16%, LT50%, LT84% and LT90% decrease with the increase of the treatment time and/or product concentration. The results also indicate that female mortality is greater than that of males. Therefore, females are more sensitive to this bio-insecticide. Habbachi *et al.* (2019) showed that the aqueous extract of *C. amblyocarpa* at different concentrations act on the time of mortality of *Drosophila melanogaster* larvae as a function of the concentration applied. More, the concentration of 35 μg/ml has a weak larvicidal activity. For the three concentrations (70, 100 and 200 μg/ml), 50% of the population is killed after 15 days of treatment. Kemassi *et al.* (2018) revealed that the aqueous extract from the leaves of *C. amblyocarpa*, acts on the mortality of adult males and females of *Schistocerca gregaria*. They added that a mortality percentage of 76.67% and 86.67% is noted in male and female larvae respectively. As for the signs of intoxication, they are observed in treated individuals, including decreased activity mobility, intense defecation, unusual water loss in the form of diarrhea, blockage and/or difficulties during moulting. Another study carried out by Korichi-Almi (2016) on larvae and adults of *Ectomyelois ceratoniae*, which were treated with different concentrations of the aqueous extract of *C. amblyocarpa*. The study Showed the existence of a positive correlation between the time and the mortality rate. Korichi noted that at the 7th day of the treatment, mortality rates reach 42.2%, 73.3% and 82.2% respectively for the 5%, 10 and 15% concentrations.

Cockroaches display advanced chemical communication capabilities, as described by Cornnette (1997). Their feeding behavior progresses through a series of distinct behavioral sequences triggered by the detection of odors. Initially, they engage in odor detection behavior, followed by moving towards the source of the scent, whether larvae or adults. Dajoz (1998) explains that a chemical composition enables cockroaches to locate food through olfactory attraction or repulsion. Within the genus, dietary preferences remain consistent, with cockroaches consistently being omnivorous, consuming both carbohydrates and proteins.

An extraction with hexane on *C. amblyocarpa* was carried out in order to obtain the apolar molecules. These extracts were tested on *B. germanica* and *S. lateralis* larvae. The latter are well attracted according to their concentration. The more the concentration of the extract increases, the more the attraction of the two cockroaches increases. *S. lateralis* larvae were noticed to be more attracted to the extract than *B. germanica* larvae.

These results show that *C. amblyocarpa* affect treated *S. lateralis* larvae. The number of *S. lateralis* attracted by the extracts is found much higher than that of *B. germanica*. Our results are comparable to those of Elbah (2017), who tested the effect of aqueous extracts of *Peganum harmala* and *Daphne gnidium* at sub-lethal concentrations on the gregarious behavior of *B. germanica*. The two products tested act on the perception of adult cockroaches; *B. germanica* treated and tested in an olfactometer did not significantly detect the odors tested.

The behavior of insects and all animals is controlled by interactions between neurons within their nervous systems. Insecticides have been selected, and sometimes designed, for their remarkable ability to kill insects (Souto *et al.,* 2021), and most of them attack specific sites in the insect's nervous system. It is therefore not surprising that insecticides can affect behavior at levels but that do not cause mortality. This study was important to fill a gap in Algerian biodiversity, specifically by bringing an entomological investigation related to plants extract. However, further studies are more than needed in order to better understand the underlying mechanisms related to animal behavior.

Conclusions

In this study, the purpose of the first experiment was to know the mortality rate of the larvae of these two cockroaches treated with the extracts of *Cleome amblyocarpa*. It was observed that the mortality rate on the 30th day at different concentrations (1 g/L, 2 g/l and 3 g/l) in *B. germanica* is 60%, whereas this rate is 6.7% in *S. lateralis*. The mortality is positively correlated with the different concentrations used, the duration of exposure and the different species. In the second experiment, the aim was to find out which of these species is most attracted to *Cleome amblyocarpa* soaked in hexane for a duration of (15, 30 and 60 minutes). The results showed that *S. lateralis* is well attracted by the extracts because the ratio of reproduction to odor exceeds 60%, whereas it is just 40% in *B. germanica*.

The results of this work prove the presence of toxic substances in the studied extract which may lead to the development of bio-insecticides based on *Cleome amblyocarpa* to be used in the pesticide market.

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Availability of data and material: The data and material that support the findings of this study are available from the corresponding author Benhissen S, upon reasonable request.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: The conduct of this study was approved by the local ethics committee of the University of Mohamed Boudiaf-M'sila.

The ethics of research involving animals: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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