South Asian Journal of Experimental Biology

ISSN: 2230-9799 Vol. 4, Issue 5, Page 215-221 http://www.sajeb.org

REGULAR ARTICLE

Determination of impact of *Azadirachta indica* L. leaves on adults and eggs of *Callosobruchus maculatus* Fabricius, the largest predator of stored cowpea (*Vigna unguiculata* Walp) by an applicable method by farmers

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ARTICLE INFO

Article History:
Received: 8 Sep 2014
Revised: 2 Oct 2014
Accepted: 8 Oct 2014

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Keywords: *C. maculatus*, eggs, adults, *A. indica*, leaves, *V. unguiculata*

ABSTRACT

The effect of neem leaves has been tested in the laboratory on eggs and adults of cowpea weevil (*C. maculatus*). Different formulations of this plant were applied to these forms of *C. maculatus* Fab. Grinding fresh contact sheets induced significant lethal effects from 96.12% to 100% on eggs; whereas 100% of mortality was recorded at the end of eight days of application to three adults with higher doses. Fumigation on turn proved less effective than contact on eggs. It induced a maximal effect of 95.73% mortality with the larger dose (D4: 0.02912g/cm^3). On adults, we recorded highest mortality (100%) from the 7th day of the show with the highest dose. The aqueous extract of neem leaf powder was less effective than all other formulations on the eggs as well as adults of this insect; with a higher mortality rate (74.99%) observed on the eggs with the application of the concentration C2. On adults we recorded a maximum effect (100% mortality) from the 13th day of the application with the highest concentration (C1). These mortalities would be related to the support of several active molecules contained in neem as established in literature.

1. Introduction

The fact of looking for food self-sufficiency requires farmers to diversify their crops. Thus they make use of leguminous plants such as groundnut and cowpea. The latter is a remedy in search of protein, which is a essential substance for the development of living beings. Cowpea is one of the most protein-rich legumes; Alzouma (1995) estimated 20-25% of its dry weight. So farmers can do without very expensive and not always available for farmers vegetable protein. In West Africa, the rainy season lasts three months and requires farmers to store their crops in order to have it at disposal if necessary. Something not always is easy because of pests. The seeds of *Vigna unguiculata* may suffer damage by 90% after 6 months of storage without treatment (Seck, 1992). This damage is largely opens *Callo-*

sobruchus maculatus, as dreaded insect can produce several generations in cowpea stocks. To mitigate the damage, some farmers use synthetic insecticides, which often cause ecological imbalances. To correct these imbalances, many researchers are looking in search of bioactive substances of plant origin (Al-Hazmi, 2013; Faye et al., 2014; Mbaye et al., 2014). In the same movement, we tested several formulations (contact, fumigation and aqueous extract) from the leaves of *A. indica* against *C. maculatus* for better storing cowpeas.

2. Materials and Methods

2.1. Biological material

The specimen of *C. maculatus* used in the experimentation comes from a sample of cowpea infested bought at Tilène market in Dakar. To rid the seeds of cowpea used for mass rearing and as a carrier for biological testing, infestation, they are made to stay for 72h in the freezer. We used neem leaves picked early in the morning before sunrise. Harvesting was done in the months of December and January. A portion of the harvested leaves was used immediately in the test location and the fumigation of other portion was dried on trays to be used in tests with extracts.

The breeding of the insect *C. maculatus* occurs in glass jars (10.5 cm and 8 cm in diameter) on cowpea variety “bay ngagne” removed from infestation. Sexual adults are introduced per couple in jars containing cowpea fully covering their bases. After 24h of contact with the seed weevils are retrieved. Infested seeds were then tracked and the adults that emerged were used either for adulticide tests, or to maintain mass rearing. In this way the strain is maintained in the laboratory of *C. maculatus*.

2.2. Treatment impacted on C. maculatus

2.2.1. Contact crushed fresh leaves

After harvest, the early morning before sunrise, fresh leaves of *A.. indica* were ground using a mortar. The ground material of these sheets was filled into jars 10.5 cm in diameter and 8 cm high with wire mesh lids. In each jar, 12 adult *C. maculatus* asexual were introduced. Four weight of leaves are used (2g, 4g, 8g et16g). For each weight, three repetitions were performed and rehearsals are always accompanied by white witnesses. For each repetition, the crushed leaves and insects are mixed in a single jar. Dead weevils were counted at intervals of 24 hours. Are counted dead weevils, which all the affected legs and antennae do not perform any movement of legs or antennae. The method used for ovicidal test is the same as for tests adulticides. In each jar, a bearing 12 seeds each egg *C. maculatus* are mixed with fresh crushed leaves of *A. indica*. At the end of three weeks each jar is opened and the eggs were peeled from seed coat and the latter are crushed to expose the existence of larvae. This process will allow us to calculate the mortality rate embryo by the following formula:

ME = (Number of unhatched eggs X 100) / total number of eggs

This parameter is reported as a percentage and corrected by the following formula of Abbott (1925):

Mc = (Mo - Mt) x 100 / (100-MT)

Mc = adjusted mortality

Mo = observed mortality

Mt = control mortality

The same formula is also used to quantify the rate of adult mortality.

2.2.2. Fumigation of fresh leaves crushed

As previously fresh leaves of this plant are harvested early in the morning immediately introduced into jars small (8 cm in diameter and 5 cm high) equipped with mesh cover. Each jar is then placed in a box of larger size (10.5cm in diameter and 8 cm in height) containing 12 adults of *C. maculatus* asexual. Large jars are automatically closed after the introduction of small jars with lids and without roasting are enhanced by closure of the adhesive tape. Always four weight crushed leaves are used. For each weight of leaves used three repetitions are performed and a white crushed leaves without witness. Dead insects were counted each day. Death is counted any insect lying on his back and making no movement of legs or antennae after shaking.

In the same way as the above are achieved ovicide test. Seeds each with an egg substitute adults in large jars. After two weeks the jars are open and the hatched and unhatched eggs were counted. This occurs crushing seeds for the state of larvae if they exist. This allows us to calculate the mortality rate per embryo Abbott's formula. Mortality rates are presented in tables or graphs.

2.2.3. Aqueous extract

The leaf powder of *A. indica* was used. We performed a solid-liquid extraction with the solvent used is tap water. 200 g leaf powders were extracted with 1 L of tap water. The resulting mixture was placed in a refrigerator for 5 days to compensate for any fermentation of the latter. After these five days of contact, the solution obtained is filtered through a sieve of household reinforced scrim. The aqueous extract is stored in a quart bottle. The latter is placed in the refrigerator and will be used as needed. Three solutions of different concentrations are obtained by the following method:

C1 = 40 ml of the solution obtained with extraction (0.2 g of powder per cm^3)

C2 = C1 + 20 ml of tap water (0.13 g of powder per cm^3)

C1 + C3 = 40 ml of tap water (0.1 g of powder per cm^3)

Adults aged up to 72 hours, from mass rearing are used in the experiment; it is the same, for a Petri dish. In each Petri dish, we place the Whatman paper. With a micropipette is used to spread 1 ml prepared homogeneously on Whatman paper and 12 adults asexual deposited there. Three replicates and two control (blank control and solvent control) are made for each extract concentration given. For the solvent control, 1 ml of tap water is spread on Whatman paper and 12 adults are deposited; about the white light, we have direct adult weevils on Whatman paper. The dead are accrued daily. The rate of adult mortality is calculated and then corrected with Abbott's formula. Cowpea seeds each carrying a dozen eggs are sprayed with a micropipette with 1ml of each solution concentration. Three replicates and two control (blank control and solvent control) are made for each concentration. For the solvent control, we sprinkled the seeds with the solvent (water), by cons for the blank control, the seeds are not treated. Results are used in tables and graphs and ANOVA tests have allowed us to make comparisons.

3. Results

3.1. Biological impact of contact tests

The test contact ground fresh leaves of *A. indica* gave mortality statistically equal to $p \leq 0.5$ on eggs *C. maculatus*. All doses induced more than 50% mortality. And the lowest dose (D1 (0.00273g/cm^3)) and the highest dose (D4 (0.0218g/cm^3)) all showed maximum mortality 100%, while intermediate doses (D2 (0.00546g/cm^3) and D3 (0.0109g/cm^3)) revealed a mortality rate of 96.12%.

Doses (g/cm^3)	Corrected egg mortality (in %)
D1 (0.00273g/cm^3)	100^a
D2 (0.00546g/cm^3)	$96,12^a$
D3 (0.0109g/cm^3)	$96,12^a$
D4 (0.0218g/cm^3)	100^a

Table 1: Percentage corrected mortality of eggs for testing contact *Azadirachta indica*. Values followed by exposing the same alphabetical letter are statistically equal.

On the first day of contact, all doses gave more or less significant mortalities on adults with a higher percentage with the dose D2 (0.00728 g/cm^3) (19.49%). Correcting these mortalities revealed the highest ineffective dose on the second day of application. When other doses (D1 (0.00364 g/cm^3), D2 (0.00728 g/cm^3), D3 (0.01456 g/cm^3)) give respectively kills 40.18% and 33.96% and 15.1%. This trend continued until the fifth day of application where the correction of mortality showed a differential efficiency with the application of different doses; and D2 dose (0.00728 g/cm^3) was more effective against adults of *C. maculatus* with 51.25%. The seventh day all doses gave the same mortality (88.67%) except D2 (0.00728 g/cm3) which gave 44.47%. Maximum efficiency (100% mortality) was observed with the three highest doses from the eighth day of application, while the lowest dose only gives 88.67%. The latter induces 100% mortality on the ninth day.

3.2. Biological impact of fumigation

The neem leaves have a very high toxicity opposite eggs of *C. maculatus*. Thus we see that only the lowest dose, D1 (0.00364g/cm3) did not give more than 50% mortality. It appears from these tests that are based on mortality induced doses. Thus increasing the dose leads to an increase in the mortality induced eggs *C. maculatus* by this dose. It is notable that higher doses have led the greatest mortality. Doses D1 (0.00364g/cm^3), D2 (0.00728g/cm^3), D3 (0.01456g/cm^3) and D4 (0.02912g/cm^3) respectively give the following deaths: 32.44; 59.46; 79.73 and 95.73%.

On the first day of testing fumigation, the three lower doses gave the same mortality (8.33%), while

Doses (g/cm^3)	Corrected egg mortality (in%)
D1 (0.00364g/cm^3)	$32,44^a$
D2 (0.00728g/cm^3)	$59,46^b$
D3 (0.01456g/cm^3)	$79,73^b$
D4 (0.02912g/cm^3)	$95,73^c$

Table 2: Percentage of corrected mortality of eggs for testing fumigation *A. Indica.*

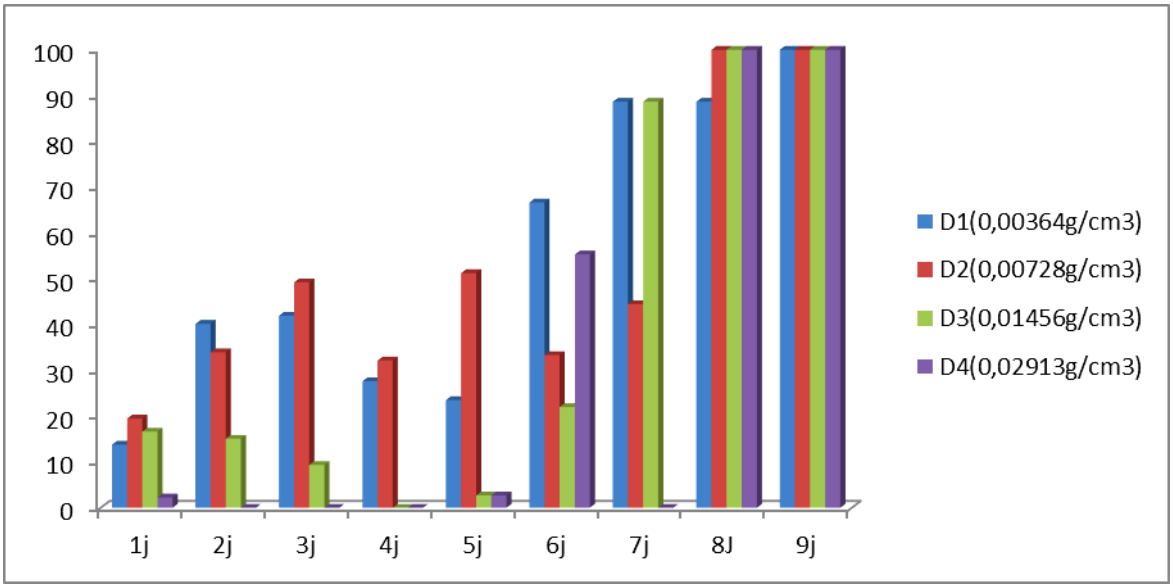

Figure 1: Percentage of adults' mortality corrected for contact tests with *A. indica.*

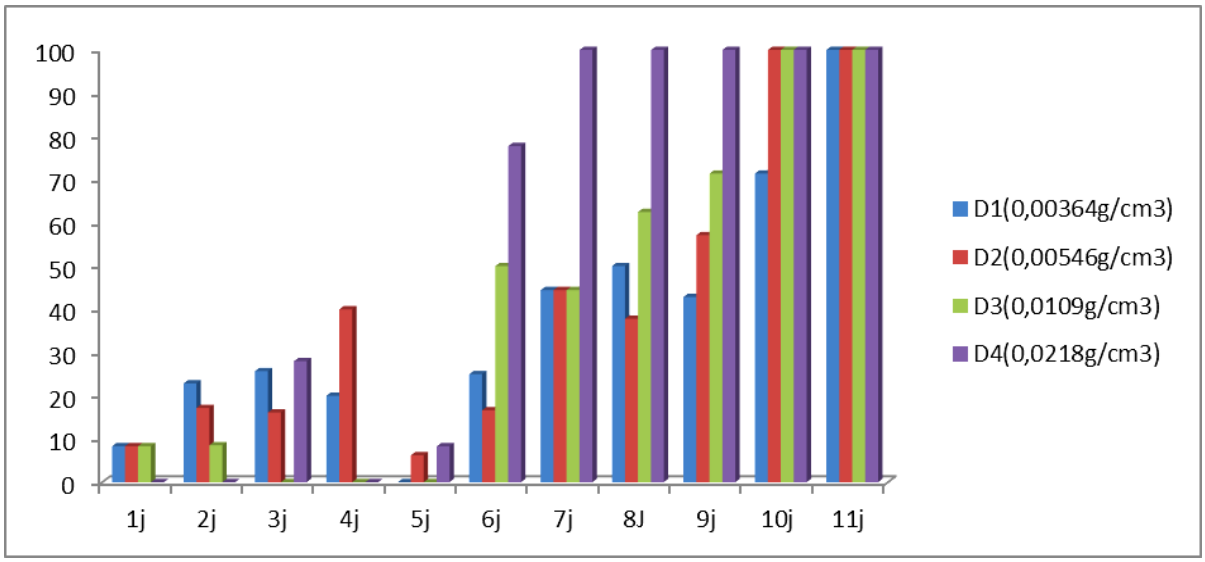

Figure2 : Percentage of adult mortality corrected for fumigation tests with *A. Indica.*

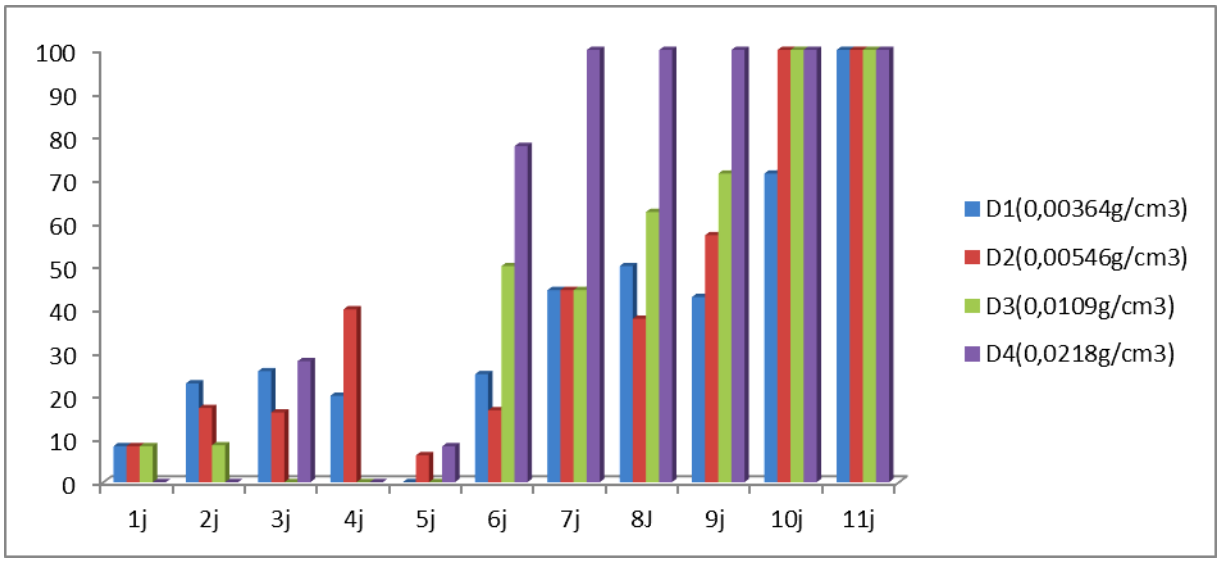

Figure 3: Percentage corrected mortality of adult normal form for the tests with the aqueous extract of the powdered leaves of *A. indica*.

the highest dose has no effect on the adult *C. maculatus*. We also note from the second to fourth day of the tests that the efficiency of *A. indica* is decreasing following the dose. This trend has reversed in the fifth day of the experiment, and the highest dose gave greater toxicity vis-à-vis the adult *C. maculatus* until the tenth day when the three highest doses induced all 100% mortality. The lowest dose did not show a maximum effect but at the eleventh day of the application.

3.3. Biological impact of aqueous extract

Table 3 shows a greater efficiency of the C2 concentration on eggs of *C. maculatus*, with 74.99% of mortality. Other concentrations, C1 and C3, respectively, show kills 43.99% and 45.02%. Only the concentration C2 gave over 50% mortality. And we observe two sets of data statistically significant and different at $p < 0.05$.

The figure highlights a very disproportionate efficacy of the aqueous extract of *A. indica* leaf powder on adults of *C. maculatus*. Thus until the second day of application, the concentration C2 gave higher mortality. This trend is reversed in the third day of testing with more substantial effects other concentrations (C1 and C3). Fourth to Sixth day, the effectiveness of the application of these concentrations on adults of *C. maculatus* decreases proportionally according to the concentrations. The lower concentration is more effective than the other between the sixth and tenth day, with greater efficiency in the eighth and ninth days of application (58.33%). Against by the eleventh and twelfth days, only C2 was effective on adults of *C. maculatus*. The last days of the application are characterized by a value proportional to the concentrations of efficiency. 100% of mortalities observed in the fifteenth day of application for all concentrations.

Concentrations	Corrected egg mortality (in %)
C1(0.2 g/cm^3)	43,99^a
C2(0.13 g/cm^3)	74,99^b
C(30.1 g/cm^3)	45,02^a

Table 3: Percentage mortality corrected for testing eggs with the aqueous extract of powdered leaves of *Azadirachta indica*.
Values followed by exposing the same alphabetical letter are statistically equal.

4. Discussion

The efficiency of *A. indica* on the external forms of *C. maculatus* was tested in a laboratory. Thus, three formulations were applied to these forms of insect. The first three days of testing, the contact of fresh crushed leaves was more effective than the other two applications (aqueous extract and fumigation) on adults of cowpea weevil. This trend is reversed on the sixth day of application for the fumigation. This study shows that aqueous extract of leaves powder *A. indica* proved less effective than other formulations (fumigation and contact with crushed fresh leaves). As for eggs, the test contact was more effective than other applications, while aqueous extract induced the lowest mortality on the outer shape of *C. maculatus*.

All doses applied by contact with fresh crushed leaves on eggs of the insect gave greater than 95% mortality. The effectiveness of this application reminiscent obtained by Faye et al (2012, 2014). Indeed, their work showed mortalities eggs *C. maculatus* between 70% and 90% with the application of ground contact of fresh leaves *Crateava religiosa* (Faye et al., 2012). As with these authors, our results showed a higher efficiency of formulation on eggs of *C. maculatus* with the application of the lowest dose (D1 (0.00364g/cm^3)) and the highest dose (D4 (0.02912g/cm^3)). This same observation is found by other authors (Faye et al, 2014) with the application of the same formulation. They have shown with the use of *Senna occidentalis* mortality of 45% with the lowest dose (D1 (0.00364g/cm^3)) and 40% with the highest dose (D4 (0.02912g/cm^3)). These results may lead us to think that the contact sheet plants insecticide or repellent effect and becomes more efficient with the application of specific doses such as D1 (0.00364g/cm^3) and (D4 (0.02912g/cm^3). D other authors have also shown notable effects of neem powder on the activity of *C. maculatus*. This is the wake Radha and Murugan (2011) showed a reduction in seed damage cowpea up to 12% by the application of this plant powder. powder contact leaves of other plants such as *C. religiosa* was evaluated on other insects. thus Mbaye et al (2014) showed mortalities of adult *Dermestes ssp* no more than 50% after 13 days of application with the powdered leaves of *C. religiosa* (8g/2kg.) in our study, the contact of fresh crushed leaves gave 100% mortality on adult *C. maculatus*. On the same insect Wahedi et al (2013), have identified mortality of 2.50 ± 1.29 with 10 adults on the application of neem seed powder. This difference of effect on these two plants of these two insects was linked to a difference in active molecules contained in both plants and or to a difference in size of these two insects.

Fumigation was very effective from the seventh day of the experiment with 100% mortality with the application of high dose D4. This trend has been reinforced with additional doses on the following days. Our results are in the same movement as Faye et al (2014). These researchers have demonstrated the efficacy of *S. occidentalis* on adults as well as eggs of *C. maculatus*. According to study their greater efficiency compared to that of adults eggs. The same is observed in study. This greater sensitivity of adult *C. maculatus* fumigation could see the presences of antennas are very sensitive organs environmental insects, so the active molecule in the fresh leaves of plants. Other researchers have also shown the efficacy of the fumigation of different herbal formulations on different insects. In this Fredros et al., 2007, reported LC50 effect with the application of 11ppm of neem oil on *Anopheles gambiae* after 8 days of exposure. It is the same for Ketoh et al., 2005, which showed very significant mortality of adult *C. maculatus* exposed to the essential oil of *Ocimum basilicum* (25µl / g) after 12 hours of fumigation. Very remarkable ovicidal effects on *C. maculatus* are highlighted by Ketoh et al., 2005, as well as Kiendrebeogo et al., 2006.

The aqueous extract of neem was very effective (74.99%) with the application of C2 on the eggs of *C. maculatus*, while C1 and C3 gave respectively 43.99% and 45.02%. We also increased the efficacy of lower concentration (C3) between the 6th and the 10th day of the experiment, while the following day it is the highest concentration (C1) which was more effective. Several other researchers have found an effectiveness of this plant on insect pests of stored products. It is in this context that Odeyemi and Ashamo, 2005, highlighted the effectiveness of *A. indica* on control of *Trogoderma granarium*. They thus obtained mortalities 60.00 ± 2.24% of adults with the application of neem leaf extract (500 mg / ml), while the extract containing neem seeds (500 mg / ml) induced mortality of 55.08 ± 3.62% of the adult insect. To this end the witnesses showed 36.42 ± 4.62 mortalities% for adult *Trogoderma granarium*. They also detected emergences of adults 48.26 ± 4.52 with the application leaf extract of *A. indica* (500 mg / ml), then with the neem seeds (500 mg / ml), they get 42.62 ± 4.60 emergences for adults of this insect. They recorded the emergences of 73.20 ± 4.83 adults with the witness. Wahedi et al., 2013, obtained 4.50 ± 1.29 kills on 10 adults of *C. maculatus* with the application of 1.5ml of aqueous extract of neem seeds within 24 hours of exposure. By analyzing these results, neem is very effective defense against stored product pest attack. This effectiveness can be explained by neem its composition active molecules. Thus azadirachtin contained in neem and many other molecules have demonstrated efficacy against insect pests of crop inventories (Gauvin et al., 2003).

5. Conclusion and Perspectives

Processes undertaken in our study are inspired from peasant activities against pests of food stocks. Thus we applied several formulations of *A. indica* leaves on eggs and adults of *C. maculatus*. Our study revealed that contact with fresh crushed leaves was more than other formulations (fumigation and aqueous extract) on eggs as well as adults of this insect. We plan to determine the impact of these formulations on the proliferation of the insect and other insects in stored commodities stocks. In addition, we intend in the future to verify the impact of these applications on the survival and fertility of survivors from ovicidal testing *C. maculatus*.

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