# *Original Research Article*

**Insecticidal and repellent activitiesof *Azadirachtaindica A. (Juss)* oil against fifth instar larvae and adults of *Sahlbergellasingularis*(Hemiptera: Miridae)**


ABSTRACT

*Sahlbergellasingularis* is one of the insects that cause the most damage to cocoa trees in Togo. The management of this pest is mainly based on chemical control with its negative consequences on human health and the environment. To find an alternative to the use of synthetic chemical pesticides, the insecticidal and repellent activity of neem oil was tested in vitro on $5^{th}$ instar larvae and adults of *S. singularis* at concentrations of 0. 5; 1; 1.5; 2, and 2.5 $\mu l.ml^{-1}$. The results showed an increasedmortality rate with increasing concentrations of neem oil for both $5^{th}$ instar larvae and adults. The $LC_{50}$ calculated for the larvae and the adults were 0.89 and 0.98 $\mu l.ml^{-1}$ respectively. Neem oil has a weak repellent effect on both $5^{th}$ instar larvae and adults with average repellent rates of 23.60% and 28.8% respectively. These results show that neem oil can be an alternative to the use of synthetic chemical pesticides in the control of *S. singularis*.



*Keywords: Azadirachtaindica,* oil*, Sahlbergellasingularis,* larvae, adults, insecticide, repellent


## 1. INTRODUCTION

Originally from the Amazon, the cocoa tree is a plant widespread in the world and known as the food of the gods [1]. In several countries in general and Togo in particular, it is considered a cash crop [2]. Most of the world's cocoa production, 70%, comes from Africa [3] and occupies an important place in the economy of the producing countries in West Africa. In Togo, cocoa production occupies 15,414 households [4], and its contribution to Togo's GDP was estimated at 1.3% in 2010 [5]. National cocoa production is estimated at more than 10,000 t with a total export of 9,127 t in 2020 [6]. Cocoa production faces many constraints, including lack of maintenance of orchards, poor management of shade, poor management of soil fertility and old orchards, and especially poor management of insects. , diseases, and pests [3].

Among these factors, diseases and insects cause the most loss. Indeed, the cocoa tree is a plant strongly attacked by insects and diseases. Mirids (Distantiellatheobroma and *Sahlbergellasingularis*) are the main insect pests of cocoa in West Africa in general and in Togo in particular. They are responsible for losses ranging from 20 to 75% [7, 8]. There are several methods of controlling these insects, but chemical control is the most widely used [9].

It is known that the use of synthetic chemical pesticides pollutes groundwater, water sources, plants, and the ecosystem [10]. In cocoa production, the production and use of synthetic chemical fertilizers and pesticides are major causes of environmental pollution [11]. In addition, analysisof bean samples revealed the presence of organophosphates and synthetic pyrethroids [12, 13]. These pesticides are thus ingested by consumers and are found in blood, serum, and sweat [1415]. Exposure to synthetic chemical pesticides is associated with cancer of the blood, liver, prostate, pancreas, uterus, and other types of cancers [14,16].

Not only are these pesticides dangerous to the environment and humans, but they also cause the phenomenon of mirid resistance [17,18].

The search for alternatives to these synthetic chemical pesticides is therefore important. In this context, the use of local agro-resources is a serious avenue. Indeed, many plants have insecticidal properties that can be used in the control of cocoa mirids. Among these plants, neem (*Azadirachtaindica*) is known and used for its insecticidal properties [19].

The scientific question guiding our study is this: What is the spectrum of insecticidal activity of neem oil? The objective of our work is therefore to contribute to theproduction of cocoa in Togo that respects the environment and human health. This will specifically assess the insecticidal and repellent properties of neem oil on stage five larvae and adults of *S. singularis*.

## 2. MATERIAL AND METHODS

### 2.1- Material

#### 2.1.1. Collection and rearing of *S. singularis*

The larvae were collected using fine brushes in boxes 11 cm of diameter on plots of certified organic cocoa that had not undergone any phytosanitary treatment. They are collected very early in the morning between 6 a.m. and 8 a.m. In the laboratory, the different larval stages were placed in different boxes measuring 45x30x30 cm. The upper end of the boxes was covered with a plastic net to facilitate ventilation. These larvae were used for the tests after an acclimatization period of 24 hours at ambient laboratory temperature (t: 27+/-2°C; RH: 80%). Adults are obtained by rearing part of the collected larvae. The larvae are placed in boxes measuring 45x30x30 cm and then fed with young leaves, branches, and cocoa pods until the emergence of adults. The inside of the boxes was lined with blotting paper. The homogeneous batches of emerged adults were then used for the tests after an acclimatization period of 24 hours at laboratory temperature.

#### 2.1.2- Neem oil

The neem oil was provided by BIOPHYTO, a Beninese company specializing in the research and exploitation of neem. The neem oil supplied is certified ORGANIC by ECOCERT, a certification organism.

### 2.2- Method

#### 2.2.1- Biological tests

#### 2.2.1.1. Contact toxicity tests

The tests took place in vitro under laboratory conditions following a completely randomized experimental design with five (05) replications. Five (05) concentrations of neem oil were tested after presumptive tests to circumscribe the limit ranges of toxicity of the oil and Tween 80: 0.5; 1; 1.5; 2 and 2.5 µl.ml$^{-1}$. Neem oil was diluted in distilled water by adding Tween 80 as an emulsifier at a concentration of 0.1% non-toxic to insects. The solutions are prepared daily just before the tests.

A lot of twenty (20) insects were removed using a fine brush and then placed in a 45x30x30 cm box containing absorbent paper. The solution was then sprayed on the insects using a manual pressure sprayer of 15 ml capacity. After this operation, the insects are transferred to another box 11 cm in diameter containing pieces of young pods and young leaves placed on the absorbent paper lining the bottom of the box to serve as food for the treated insects. The upper end of the boxes was covered with a net. Each treatment, therefore, consists of a box 11 cm indiameter containing twenty (20) insects. The absolute control consists of distilled water. The positive control consists of IMIDA 30 EC, a synthetic chemical insecticide composed of 30 g/l of imidacloprid, at a concentration of 3.3 µl.ml$^{-1}$.

The mortality rate of the insects subjected to the various treatments was evaluated 24 h after the treatment.

Adjusted mortality was calculated using Abbott's formula [20]: MC=(Me-Mt)/(100-Mt)*100

Mc=Adjusted mortality; Me=sample mortality and Mt=untreated control mortality.

#### 2.2.1.2. Repellency test

This test was carried out according to the preferential zone method on filter paper described by McDonald [21]. A disc of Wattman n°2 filter paper of 15 cm diameter was used. The filter paper was cut into two equal parts. The five concentrations of oil (0.5; 1; 1.5; 2 and 2.5 µl.ml$^{-1}$) were prepared by dilution in acetone. A volume of 0.5 ml of each solution was spread uniformly on one half of the disc and the other half received acetone only.

After fifteen minutes, the time necessary for the complete evaporation of acetone, the two halves of the discs were re-welded using adhesive tape.

The filter paper disc thus reconstituted was placed in a box 16 cm in diameter and a lot of twenty (20) insects wereintroduced into the center of the disc. Five replications were performed for each concentration of the solution. After two hours, the number of insects present on the part of the filter paper treated with neem oil (Nt) and the number of those present on the part treated only with acetone (Nc) was recorded.

The percent repellency (PR) was then calculated using the following McDonald's formula [21]: PR=(Nc-Nt)/(Nc+Nt) *100.

The PR helped to classify the solutions according to the repellent classes of McDonald et al. [21] from 0 to V.

### 2.2.2- Analysis of results

The results obtained were statistically analyzed using SPSS. 21 and the Student-Newman-Keuls test permitted discrimination between the means obtained.

The LC50s were calculated using the probit regression method.

## 3. RESULTS AND DISCUSSION

### 3.1- Results

#### 3.1.1- Insecticidal effect of A. *indica* seed oil on 5th instar larvae and adults of *S. singularis*

Mortalities induced by neem seed oil on 5th-instar larvae and adults of *S. singularis*are presented in the following table.

**Table 1:** Average mortality of 5th instar larvae and adults of *S. singularis*after treatment with *A. indica* seed oil

| | Mortality rate (%) | |
|---|---|---|
| **Concentration (µl.ml $^{-1}$)** | **5th instar larvae** | **Adults** |
| **0** | 0th | 0f |
| **0.5** | 39±4.54d | 29±5.4e |
| **1** | 54±3.98c | 48±5.4d |
| **1.5** | 67±5.05b | 57.5±5.4b |
| **2** | 78±6.21b | 68.5±5.4b |
| **2.5** | 100±00a | 100±5.4a |

Within the same column, the means assigned the same letter do not differ statistically from each other (Newman- Keuls test, p=0.05).

The results show increasing mortality of all stages with increasing concentrations of neem seed oil. The concentration of 2.5µl.ml $^{-1}$caused the death of 100% of both 5th-instar larvae and adults. However, the concentration of 1 µl.ml $^{-1}$ caused the death of 54±3.98% of the 5th instar larvae whereas more than 1.5 µl.ml $^{-1}$caused the death of 57.5±5.4% of adults (Table 1)

The $LC_{50}$ calculated using the probit regression methodis given in the following table.

**Table 2**: $CL_{50}$ per contactneem seed oil tested on5th instar larvae and adults of *S. singularis*

| Stage | $LC_{50}$ (µl.ml $^{-1}$) |
|---|---|
| **5th instar larvae** | **0.89** |
| **Adults** | **0.98** |

The results show that neem oil acts differently in the developmental stages of *S. singularis.* The calculated $LC_{50s}$ increase according to the increasing stages of development of the insect. The $LC_{50s}$ were 0.89 and 0.98 µl.ml $^{-1}$ respectively for 5$^{th}$ instar larvae and adults of *S. singularis*(Table 2)

### *3.1.2-* Repellent effect of *A. indica* seed oil on 5$^{th}$ instar larvae and adults of *S. singularis*

The table below shows neem seed oil's repellency percentages (PR).

**Table 3: RepellencyPercentageof different concentrations of *A. indica* seed oil tested on 5$^{th}$ instar larvae and adults of *S. singularis.***

| Repellency Percentage(RP) | | |
|---|---|---|
| **Concentration (µl.ml $^{-1}$)** | **5$^{th}$ instar larvae** | **Adults** |
| **0.5** | 10 | 12 |
| **1** | 10 | 24 |
| **1.5** | 24 | 28 |
| **2** | 36 | 40 |
| **2.5** | 38 | 40 |
| **Mean PR** | **23.6** | **28.80** |

The results of our work show an increasing repellencypercentage (RP) with increasing concentrations of neem seed oil for both 5$^{th}$ instar larvae and adults of *S. singularis.* The average RPs of neem seed oil on 5$^{th}$ instar larvae and adults are 23.6 and 28.8%, respectively (Table 3).

These results allowed us to classify neem oil according to McDonald's repellency classes which are presented in the table below.

**Table 4:** Mean Repellency Percentage (MRP), McDonald classes, and properties of A. *indica* seed oil

| Stage | MRP | McDonald's class | Properties |
|---|---|---|---|
| **5$^{th}$ instar larvae** | **23.6** | **II** | **Weakly repellent** |
| **Adults** | **28.8** | **II** | **Weakly repellent** |

The mean repellency percentages (MRP) calculated were 23.6 and 28.8 for 5$^{th}$ instar larvae and adults of *S. singularis,* respectively. These repellency percentages place neem seed oil in McDonald's Class **II**. Then, the neem seed oil is weakly repellent against both 5$^{th}$ instar larvae and adults of *S. singularis*(Table 4).

### 3.2- Discussion

Observed mortality increased with neem oil concentrations for both 5th instar larvae and adults of *S. singularis*. These results show that neem oil was toxic to both larvae and adults. However, calculated LC50s revealed that neem oil is more toxic to larvae than adults. This toxicity is due to the seed oil's composition and mechanism of action. Indeed, the biological properties of neem are attributed to more than 135 active phytochemical constituents, including alkaloids, carotenoids, flavonoids, ketones, phenolics, steroids, and triterpenoids, which have been isolated from different parts of the neem plant. These compounds include azadirachtin, gedunin, isomargolonone, margolone, margolonone, nimbidine, nimbin, nimbolide, and salannine [22]. Among these compounds, azadirachtin which is the main active ingredient of neem oil is the cause of 90% of the effects on insects [23]. Azadirachtin and other compounds in neem products exhibit various modes of action against insects, such as anti-appetence, growth inhibition, fertility suppression, disruption of oviposition, changes in morphology, and inhibition of development [24]. The contact toxicity obtained in our study can be explained by the direct effect of azadirachtin on the cells and tissues of the insect. Indeed, azadirachtin is absorbed by cells and causes inhibition of cell division and protein synthesis. These effects manifest as mild muscle paralysis, midgut cell necrosis, loss of gut nidi (regenerative cells), and lack of midgut enzyme production [25]. Azadirachtin also affects insect respiration and digestion. This action has been demonstrated in *Anticarsiagemmatalis* [26]. Our results are similar to those of many studies conducted on the insecticidal effects of neem oil on mirid larvae and adults. Indeed, neem oil and the ethanolic extract of neem oil are effective against *S. singularis* and lead to mortality rates ranging from 72.5 to 97.5% by contact and 32.5 to 70.0% by ingestion [27]. Some studies demonstrated that an aqueous extract of neem oil inhibits larval molting with an inhibition rate of more than 50% [28].Moreover, this plant has an interest in the control of cocoa mirids because all its parts can be used because they contain azadirachtin.

Calculated average repellent rates revealed that neem oil has a weak repellent effect against both 5th instar larvae and adults of *S. singularis*. The repellent effect of neem oil is due to azadirachtin [29, 30, 24]. Other studies have shown that neem oil has a repellent effect on several insects including *Triboliumcastaneum* and *Bemisiatabaci* [30,31]. The weak repellent effect of neem oil in our study could be explained by the concentration of the solutions used. Indeed, our results revealed that the rate of repellency is increasing with increasing concentrations of neem oil.

These results,, therefore, show that neem oil can be an alternative to synthetic chemical pesticides in the fight against *S. singularis*.

## 4. CONCLUSION

The constraints of cocoa cultivation are numerous. Among these constraints, insect pressure, particularly *S. singularis*, causes the most loss. Chemical control of these insects leads to environmental pollution and endangers the lives of consumers. The search for alternatives to these synthetic chemical pesticides is essential. Thus, our work consisted in investigating the insecticidal and repellent properties of neem oil against 5th instar larvae and adults of *S. singularis*. Neem oil was insecticidal and weakly repellent against these stages of *S. singularis*. It can therefore be an alternative in the control of this insect. Our study will continue with field tests to conclude on the effectiveness of neem oil.



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