Journal of Asian Scientific Research

Online ISSN 2223-1331

Vol 1 No 5

September, 2011

Asian Economic and Social Society
www.aessweb.com

Journal of Asian Scientific Research, 1(5), pp.271-284
.
2011

Effect of Azadirachtin A systemic injection on *Coelaenomenodera lameensis* Berti and Mariau (Coleoptera: Chrysomelidae) an oil palm (*Elaeis guineensis L*) pest

Abstract

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Coelaenomenodera lameensis is the major pest of oil palm. The fight against this pest is achieved by using several methods including the injection of systemic insecticide in the trunk of the palm. The tests were conducted at the agro-industrial units of Toumanguié, located 90 km from Abidjan. Azadirachtin A was used in systemic treatment at four concentrations: 0.1, 0.2, 0.4 and 0.6 g/ml. Chiffon sleeves were placed on the palms to assess the effect of products on *C. lameensis*. Systemic injections were made during the dry and rainy seasons. The efficacy of azadirachtin was assessed by reference to thiamethoxam, an insecticide used in the fight against this pest. The effective minimal concentration was 0.4 g/ml of azadirachtin. This concentration generated the cumulated mortality rates of 38.92% in dry season and 19.28% in rainy season on the adults after 28 days of treatment. The percentages of efficiency achieved in the dry season on the larvae 7 days after treatment were 74.75% (stage 1), 57.23% (stage 2), 7.14% (stage 3) and 5.31 % (stage 4). In rainy season, the efficacy of azadirachtin on larvae was less than 29%. Azadirachtin can be used in dry season, at the beginning of the infestation period, to fight against *C. lameensis*.

Key words: Elaeis guineensis, Coelaenomenodera lameensis, systemic injection, Azadirachtin A.

Introduction

Côte d'Ivoire is the eighth world producer and the main African exporter of oil palm (Boutin, 2007). The annual production of oil palm was estimated at more than 276 000 tons in 2003 (Anonymous, 2003). The country ensures 60% of exports of Economic Community Of West African States (ECOWAS). Oil palm takes part for 50 billion FCFA in the gross domestic product of the country (Anonymous, 2003). The production of oil palm, however, is insufficient with a deficit of 150 000 tons in the space of the West African Monetary Union and 500 000 tons for the ECOWAS. Unfortunately, the oil palm is the target of diseases (viral, fungal infections, mineral deficiencies) and pests (rodents, insects) (Jacquemard, 1995). The insect that causes the greatest damage is *Coelaenomenodera lameensis* (Mariau, 2001; Koua, 2008). Leaf miner larvae feed on the parenchyma. They bore visible galleries on the upper surface of the leaflets. Heavy defoliation may decrease from 30 to 50% of production for two to three years (Lecoustre, 1988). Many control methods are used to reduce the population of this pest: biological control, selection of resistant varieties and chemical control by terrestrial, air or systemic way (Mariau et al., 1973, 1979; Philippe et al., 1979, 1983; Lecoustre et al., 1980; Philippe, 1990; Yawson et al., 2009; Koffi et al., 2009; Niamouké et al., 2011). Only chemical control can now maintain populations of this insect to a tolerable economic threshold by the injection of systemic insecticides in the trunk of the palm tree (Mariau, 2001). Monocrotophos, organochlorine that was working, was removed in 2004 from the list of pesticides registered in Côte d'Ivoire because of its toxicity (Anonymous, 2004). Thiamethoxam, is currently, the only systemic insecticide used against *C. lameensis* (Niamouké et al., 2011). The need to find a biodegradable and nontoxic systemic product, has guided our choice of a substance extracted from neem seeds: azadirachtin A in the formulation of Suneem 1%. Insecticides extracted from neem and used systemically have been used to fight against several pests: Thuya leaf miner (*Argyresthia thuiella*), the white pine sawfly (*Diprion similis*), the pine of Litchi (*Tessaratoma papillosa*) and the ash borer *Agrilus planipennis* (Helson et al., 2001; Schulte et al., 2006; Helson et al., 2008).

This study evaluates the efficacy of azadirachtin A on harmful forms of *C. lameensis* compared with that of thiamethoxam.

Material and methods

Study area

The tests were carried out in the administrative area of south Comoé, on agro-industrial units of Toumanguié (latitude: 05-15N, longitude: 0003-56W, altitude: 7 m) located at 90 km of Abidjan, in Côte d'Ivoire. The agro-industrial unit has 2 959 ha of oil palm plantation. The locality presents a wet tropical climate, with an annual temperature varying from 24 to 28°C, a relative humidity ranging between 78 and 90% and a photoperiod of 12 h. Annual rainfall varies between 1400 and 1800 mm. The region has two rainy seasons each year (April to mid-July and September to November) and two dry seasons (mid-July to August and December to March). The study was conducted from August 2009 to August 2010.

The tests were conducted on a block of 100 ha made up of four parcels of 25 ha. The study focused on palms tree of "*Tenera*" variety aged 12 years old and having an average size of 5 m.

Experimental device

The device was a block of Fischer with 6 treatments and 3 repetitions. Fifty four palm trees were treated. Fifteen quadrats (45 palm trees) received treatment and three (9 palm trees) served as controls. For each concentration of chemical product, 9 palm trees were treated. Controlled infestation in muslin sleeves (150 cm x 60 cm) allowed obtaining the four larval stages of *C. lameensis*: thirty couples aged 20 days (sexually mature) were placed on leaflets covered with sleeves for 3 days. These couples have been moved to 3 other sleeves fixed on the same palm successive intervals of 6, 9 and 12 days. These movements have resulted in a single larval stage by sleeve (Table I).

Determination of the concentrations of products

Both products were used: Suneem 1% and Actara ® 240S. The Suneem 1% is a commercial preparation based on azadirachtin extracted from the seeds of neem (*Azadirachta indica* A. Juss). It is a limonoid composed primarily of the following molecules: azadirachtin, salanine and melandriol (Adote, 1994).

Four volumes (10, 20, 40 and 60 ml) were collected from a liter of Suneem 1%. These volumes correspond to concentrations of 0.1, 0.2, 0.4 and 0.6 g / ml of azadirachtin A. The concentration of 0.04 g / ml of thiamethoxam, minimum effective concentration of *C. lameensis* (Niamouké et al., 2011), was obtained from a commercial preparation of 1 liter of Actara ® (240 g ai), diluted in 5 liters of water. The injected volume was 24 ml per palm. Azadirachtin treatments were noted: Az 0.1 (0.1 g / ml), 0.2 Az (0.2 g / ml), 0.4 Az (0.4 g / ml) and 0.6 Az (0.6 g / ml). The reference control, treatment (0.04 Th), a concentration of 0.04 g / ml of thiamethoxam. The untreated control is designated T_0.

Systemic injection in the oil palm trunk

One day before injection, a control was done to determine the number of live larvae per stage. Two palm leaves (coated sleeves) by the larval stage, were cut and galleries open to extract the live larvae. Then, the leaf presents the stumps of palm were removed to facilitate the perforation of the stipe. This was done in two places diametrically opposite (180 °) at a height ranging from 0.5 to 1 m above ground. Two holes to a depth of 15 cm and 1.2 cm in diameter were performed on two sites previously stripped using a drill motor. The holes were inclined at 45 ° down, to prevent release of the product. The volumes of insecticide were introduced into the holes using graduated syringes 50 ml. Half volume of product was injected into each of the two holes. After a period of 30 to 60 minutes, required for penetration into the trunk, the holes were plugged by means of pins by the method of Mariau et al. (1979). Also, to prevent entry of rain water in the trunk, the tar was applied around the injection holes. A check was carried out the seventh and 14th days after injection. This consisted to cut two fins (coated sleeves) by concentration and larval stage; the galleries were opened to extract the larvae of *C. lameensis* for their enumeration. For each larval stage, the percentage of average efficiency of products (P_{em}) were calculated and corrected by the Henderson-Tilton formula (1955):

$$Pe_m = \frac{\Sigma\, x_i n_i}{\Sigma\, n_i}$$

$$xi = \left(1 - \frac{Tap \times Cav}{Tav \times Cap}\right) \times 100$$

ni: number of sleeves, $\mathbf{P_{em}}$: average percentage efficiency corrected; **xi**: efficiency percentage corrected, **Tap**: number of individuals living in the palm after treatment, **Cav**: number of individuals living in the control palm before treatment; **Tav**: number of individuals living in the palm trees before treatment; **Cap**: number of individuals living in the control palm trees after treatment

Effects of azadirachtin on adults of *C. lameensis*

For each concentration of azadirachtin, four batches of 30 adults have been established. These adults were aged 20 days and sexually mature.

A Lot 1 of insects have been introduced into the sleeves No. 1 on the palm arranged one day before injection. Those of Lot 2 were placed in the sleeves No. 2 seven days after injection. Insects of Lot 3 were introduced into the sleeves No. 3 placed on other palms of the same palm fourteen days after injection. The same procedure was followed with insects of Lot 4, inserted into the sleeves No. 4 twenty-one days after injection. For the first lot, the number of dead insects was noted 1, 7, 14, 21 and 28 days from the date of injection products in the stipes of palm trees. For the three other lots, the number of dead insect has been raised 1, 7, 14; 21 and 28 days, from the date on which the insects in the sleeves. For each lot, the average mortality rates were calculated and corrected by Abbott's formula (1925):

$$M = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

$$Mc_m = \frac{\Sigma\, xini}{\Sigma\, ni}$$

$$xi = \frac{Mo - Mt}{100 - Mt} \times 100$$

M: Rate of mortality; $\mathbf{M_{cm}}$: average mortality corrected; **xi**: mortality corrected; **ni**: number of sleeves, **Mo**: mortality rate observed in the test; **Mt:** observed mortality rate in the control.

Statistical analysis

Data processing was performed using Statistica software version 6.0. An analysis of variance (ANOVA) revealed significant differences between the data. The test of Student-Newman-Keuls at 5% was used to classify the means into homogeneous groups.

Results

Pourcentages efficacy of azadirachtin on larvae of *C. lameensis*

• *Seven days after systemic injection*

In dry season, the percentage efficiency of azadirachtin on larvae ranged from 33.35% (0.1 Az) to 76.43% (0.6 Az) for S1; 25.89% (0, Az 1) to 60.19% (0.6 Az) for S2; 4.73% (0.1 Az) to 7.21% (0.6 Az) for S3 and 4.62% (0.1 Az) to 5.36% (0.6 Az) for S4.

The action of thiamethoxam (0.04 Th) resulted in 100% efficiency (S1); 98.17% (S2); 85.84% (S3) and 66.64% (S4) (Figure 1A). Statistical analysis showed highly significant differences, in terms of the effectiveness of treatments ($F = 4417.9$, $df = 19$, $p < 0.001$).

In rainy season, the efficacy of azadirachtin on larvae ranged from 18.07% (0.1 Az) to 32.24% (0.6 Az) for S1, 15.69% (0.1 Az) to 27.43% (0.6 Az) for S2; 4.68% (0.1 Az) to 4.80% (0.6 Az) for S3 and 4.55% (0.1 Az) to 4.76% (0.6 Az) for S4. Treatment with thiamethoxam (0.04 Th) was effective up to 71.07% (S1), 63.44% (S2), 37.19% (S3) and 33.41% (S4) (Figure 1B). Statistical analysis applied to the percentages of treatment efficacy on larvae showed highly significant differences ($F = 1271$, $df = 19$, $p < 0.001$).

• *Fourteen days after systemic injection*

In dry season, the lowest efficiency percentage (3.81%) was obtained on S4 with 0.1 Az; the highest (79.16%) was recorded on S1 at 0.6 Az. For thiamethoxam, the percentages of efficiency were 74.98% (S4) 90.30% (S3) and 100% for S1et S2 (Figure 2A). Statistical analysis showed highly significant differences, in the effectiveness of treatments ($F = 2601.29$, $df = 19$, $p < 0.001$). In rainy season, the lowest efficiency percentage (3.70%) was recorded on S4 at 0.1 Az; the highest (21.80%) was obtained for S1 at 0.6 Az. For thiamethoxam, the percentages of efficiency were 37.63% (S4), 41.01% (S3), 67.78% (S2) and

75.94% for S1 (Figure 2B). Statistical analysis applied to the percentages of efficacy showed highly significant differences ($F = 2786.82$, $df = 19$, $p < 0.001$).

• *Overall efficiency percentage to 7 and 14 days after systemic injectio*n

Seven days after injection, the overall efficiency was higher in the dry season (39.36%) than in the rainy season (21.50%) (Figure 3). Statistical analysis revealed a significant difference between the percentage of effectiveness on larvae according to season ($F = 8.41$, $df = 1$, $p < 0.05$). Fourteen days after injection, the overall efficiency was 36.04% in dry season and 18.08% in the rainy season (Figure 3). Statistical analysis also showed that the percentages of efficacy were significantly different from one season to another ($F = 6.05$, $df = 1$, $p < 0.05$).

Effect of azadirachtin on adults of *C. lameensis*

• *Adults inserted into the sleeves one day before the systemic injection*

In the dry season, the cumulative mortality observed increased marginally from 1st to 28th day after systemic injection. The lowest rate was 22.23% (0.1 Az) and the highest 42.11% (0.6 Az). Thiamethoxam (0.04 Th), showed mortality rate rose sharply to reach a value of 100% at the 21st day after injection (Figure 4A). Statistical analysis revealed highly significant differences between the cumulative mortality rate at day 28 after systemic injection ($F = 13.076$, $df = 4$, $p < 0.001$).

In rainy season, the cumulative mortality rates were also low at the first 28 days after systemic injection. The lowest rate was 9.87% (0.1 Az) and the highest 22.51% (0.6 Az), the 28th day. Treatment with thiamethoxam (0.04 Th) caused a cumulative mortality ranged from 27.44% (seventh day) to 68.26% (28th day) (Figure 4B). Statistical analysis indicated highly significant differences between the cumulative mortality rate at day 28 after systemic injection ($F = 6438.61$, $df = 4$, $p < 0.001$).

• *Adults inserted into the sleeves 7 days after systemic injection*

In dry season, the cumulative mortality rate ranged from 7.40% (0.1 Az) to 29.87% (0.6 Az) at day 28. As for thiamethoxam, it has generated a cumulative mortality rate of 100% from the seventh (Fig. 5A). Statistical analysis showed highly significant differences between the cumulative mortality rate at day 28 after systemic injection ($F = 19979.88$, $df = 4$, $p < 0.001$).

In rainy season, the cumulative mortality rate obtained under the effect of azadirachtin ranged from 7.31% (0.1 Az) and 17.98% (0.6 Az), 28 days after the introduction of adults in the sleeves. Treatment with thiamethoxam (0.04 Th) induced mortality rates ranging from 32.63% cumulative (seventh day) to 65.61% (28th day) (Figure 5B). Statistical analysis indicated highly significant differences between the cumulative mortality rate at day 28 after systemic injection ($F = 6221.01$, $df = 4$, $p < 0.001$).

• *Adults inserted into the sleeves 14 days after systemic injection*

In the dry season, the cumulative mortality obtained at day 28 (42 days after systemic injection of azadirachtin) ranged from 7.40% (0.1 Az) to 9.87% (0.6 Az). Statistical analysis revealed no significant difference between the mortality rate caused by azadirachtin ($F = 0.56$, $df = 3$, $p = 0.66$). In the presence of thiamethoxam, the cumulative mortality rate rose sharply to reach a value of 100% on day 14 (Figure 6A).

In rainy season, the cumulative mortality rate due to azadirachtin ranged from 7.31% (0.1 Az) and 8.78% (0.6 Az). Statistical analysis showed no significant difference in mortality rates observed with azadirachtin ($F = 0.40$, $df = 3$, $p = 0.75$). The effect of thiamethoxam resulted in cumulative mortality rates ranging from 12.63% (7th day) to 45.61% (28th day) (Figure 6B).

• *Adults inserted into the sleeves 21 days after systemic injection*

In dry season as during the rainy season, the 28th day (49 days after systemic injection) azadirachtin caused mortalities comparable to those of controls without treatment; cumulative mortality rates were therefore zero (Figure 7). In the dry season, thiamethoxam (0.04 Th) resulted in cumulative mortality rates between 34.25% (day 7) and 89.52% (28th day), while in wet season rates obtained ranged between 16.63% (7^{th} day) and 32.61% (28^{th} day).

Discussion

In our experimental conditions, all tested concentrations of azadirachtin caused the death of larvae and adults of *C. lameensis* after systemic injection. The highest concentrations, 0.4 and 0.6 g / ml of azadirachtin, gave the percentages of efficiency and mortality rates highest on the larvae and adults.

Level of larvae, the percentage of efficiency above 70% was obtained on stage 1, respectively, with 0.4 and 0.6 g / ml of azadirachtin. Those between 50 and 70% were recorded on stage 2. The percentages of efficacy were less than 10% for stages 3 and 4.

The effect of azadirachtin on larval insects including *Nezara viridula (*Hemiptera: Pentatomidae) (Riba et al., 2003), *Mamestra brassicae* (Lepidoptera: Noctuidae) (Seljåsen and Meadow, 2006) and *Tessaratoma papillosa* (Hemiptera: Pentatomidae) (Schulte et al., 2006) is due to inhibition of the secretion of juvenile hormone and therefore the interruption of moults. This hypothesis could be invoked to explain the action of azadirachtin on larval *C. lameensis*. The review of the development of larvae fed on stems sprayed or treated with injections of azadirachtin, the same authors found that the percentage of moults for the transition to the next stage were lower for stages 1 and 2 than for stages 3 and 4. This could explain the higher mortality rate in the first two stages. Helson et al. (2001) have shown that systemic injection of azadirachtin in white pine (*Pinus strobus*) resulted in larval mortality rates (83-100%). Azadirachtin was ineffective on adults of *C. lameensis* with a cumulative mortality rate below 40% in dry season, 28 days after systemic injection.

The low percentages of efficacy of azadirachtin observed on the larvae of stages 3 and 4 and low levels of adult mortality are related to the detoxification process set up by the insect. Indeed, Seri-Kouassi (2004) and Smirle et al. (2010) reported that the contact of certain insects such as *Callosobruchus maculatus* (Coleoptera: Bruchidae), *Aphis pomi* and *Aphis spiraecola* (Hemiptera: Aphididae) with insecticides induce the secretion of esterase to detoxify these molecules.

Thiamethoxam induced efficiency percentages ranging from 34 to 100% of the larvae and the cumulative mortality rate between 33 and 100% of adults of *C. lameensis*. These results are similar to those of Niamouké et al. (2011) that were 96% of the larvae and 89% of adults of the same insect.

In the rainy season, the percentages obtained on the effectiveness of different larval stages and adult mortality rates were low compared to those of the dry season. This is linked to a high dilution of the product injected by the presence of a large amount of water in the sap of the palm. Indeed, during the rainy season, the volume of the sap becomes more important because of the high water availability in the rhizosphere and the atmosphere and reducing the phenomenon of evapotranspiration (Heller et al., 2004).

From the 21st day of introduction of the adults in the sleeves, mortality rates were statistically equal to those of controls. This led us to assume that the length of the residual effect of azadirachtin in *E. guineensis*, in our experimental conditions is less than 21 days. However, a longer duration of residual effect of azadirachtin injection (77 days) was observed in *Pinus strobus* by Helson et al. (2001). The persistence of azadirachtin is higher in systemic applications. His remaining life as a foliar spray was 4 to 8 days (Ascher, 1993).

Conclusion

The experiments showed that the minimum effective concentration of 1% Suneem was 0.4 g/ml of azadirachtin A. The percentage of efficiency above 50%, seven days after systemic injection, was obtained on the larval stages 1 and 2, in the dry season. Those recorded on stages 3 and 4 were below 10%. In rainy season, the percentage of the highest efficiency (29%) was obtained on a stage 1. On adults of *C. lameensis* aged 20 days, cumulative mortality, although low was two times higher in the dry season (39%) than in rainy season (19%). In the rainy season, the effect of injected products was mitigated because of the presence of too much water in the sap of the palm. Treatment applied at the beginning of the infestation period and in the dry season, is expected to obtain better performance. The duration of residual effect of azadirachtin A in the oil palm was less than 21 days. The effects of azadirachtin were less than those caused by thiamethoxam. But unlike the thiamethoxam is a synthetic insecticide, azadirachtin is a natural substance non-toxic and readily biodegradable. The efficacy of azadirachtin injection system could be improved by increasing its concentration in the formulation of Suneem 1%.

Acknowledgement

We thank the PALMCI (Palm of Côte d'Ivoire) for supporting this study. Special thanks to the SENCHIM (Chemical Industries of Senegal) for the supply of Suneem1%.

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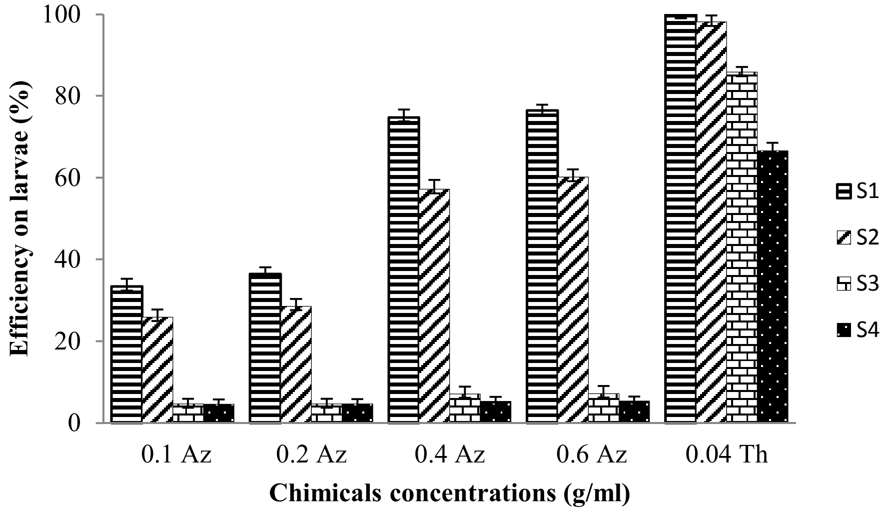
Appendix

Table 1: Establishment of controlled infestations to have the four larval stages of *C. lameensis* for systemic processing

Saison	Laying rank	Duration of laying	Larval stage (S)	treatment date
Dry	1st	03/12/09 to 05/12/09	stage 4	
	2nd	12/12/09 to 14/12/09	stage 3	26/01/10
	3rd	21/12/09 to 23/12/09	stage 2	
	4th	02/01/10 to 04/01/10	stage 1	
Rainy	1st	01/04/10 to 03/04/10	stage 4	
	2nd	10/04/10 to 13/04/10	stage 3	25/05/10
	3rd	19/04/10 to 21/04/10	stage 2	
	4th	01/05/10 to 03/05/10	stage 1	

A



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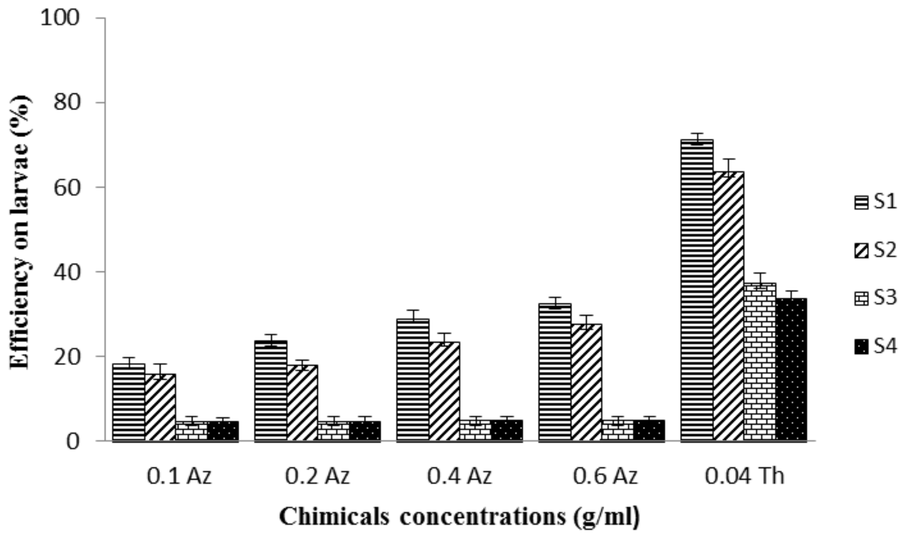
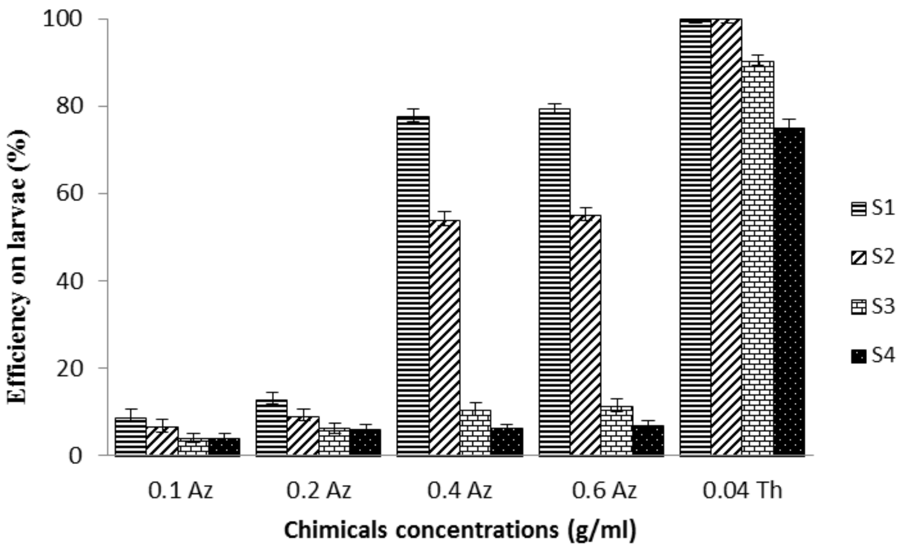


Figure 1: Efficacy of azadirachtin and thiamethoxam on larvae of *C. lameensis* 7 days after systemic injection according to the seasons

A: dry season; B: rainy season (0.1 Az: 0.1g/ml d'Azadirachtin; 0.04Th: 0.04 g/ml de Thiamethoxam)

(S1: larval stage 1; S2: larval stage 2; S3: larval stage 3; S4: larval stage 4)

A



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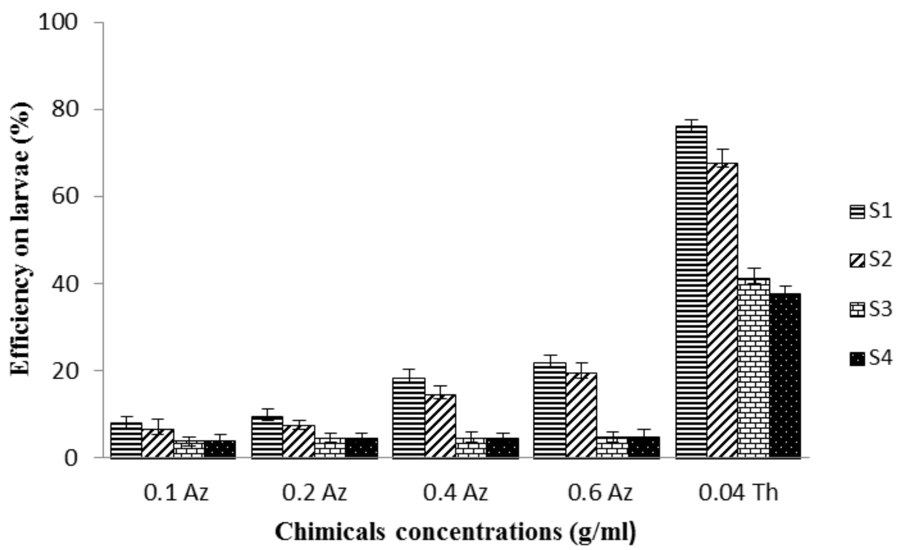


Figure 2: Efficacy of azadirachtin and thiamethoxam on larvae of *C. lameensis* 14 days after systemic injection according to the seasons

A: dry season; B: rainy season (0.1 Az: 0.1g/ml d'Azadirachtin ; 0.04Th: 0.04 g/ml de Thiamethoxam)

(S1: larval stage 1; S2: larval stage 2; S3: larval stage 3; S4: larval stage 4)

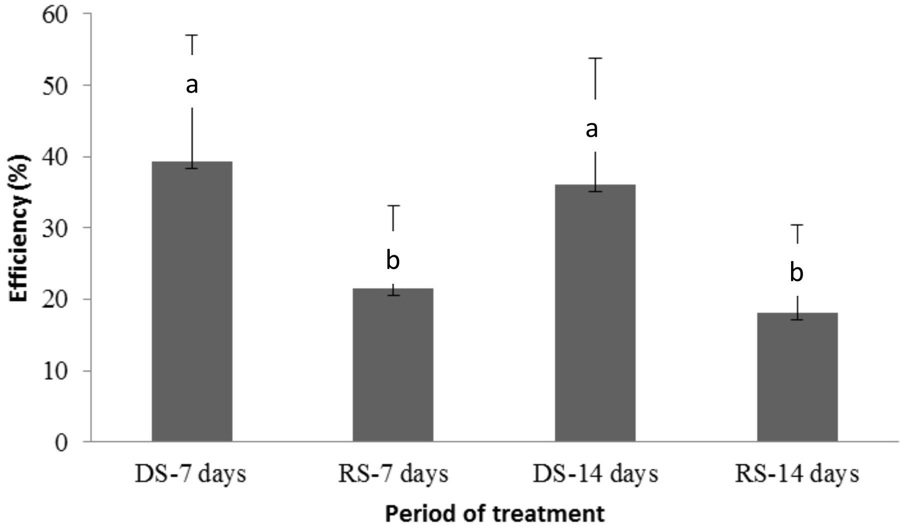


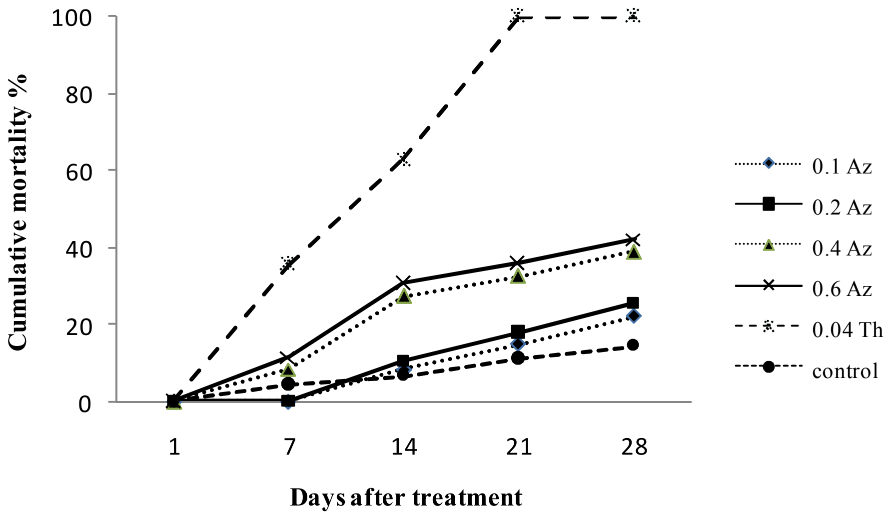
Figure 3: Average efficiency of treatment on the larvae of *C. lameensis* depending on the season at 7 and 14 days after treatment

DS: dry season; RS: rainy season

Efficiency control at 7 days after treatment: F = 8.41 df = 1, $p < 0.05$

Efficiency control at 14 days after treatment: F = 6.05 df = 1, $p < 0.05$

A



B

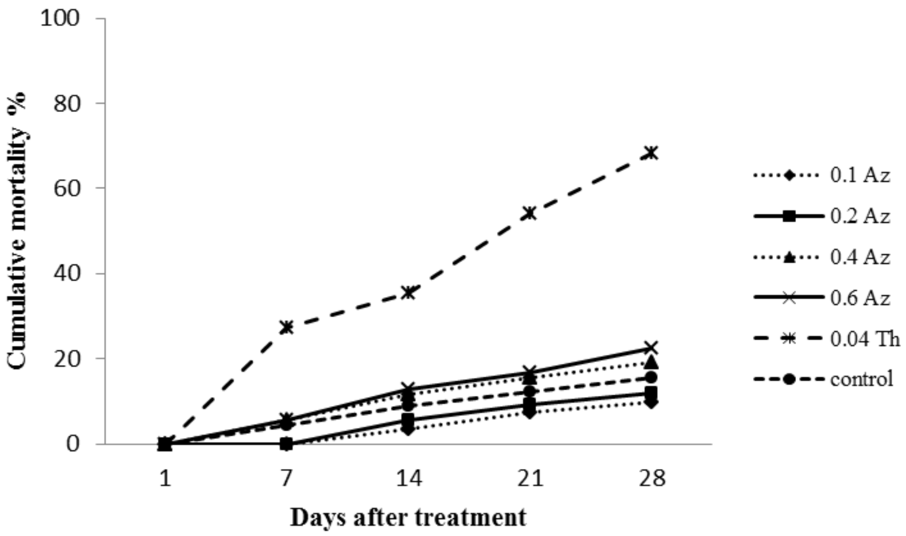
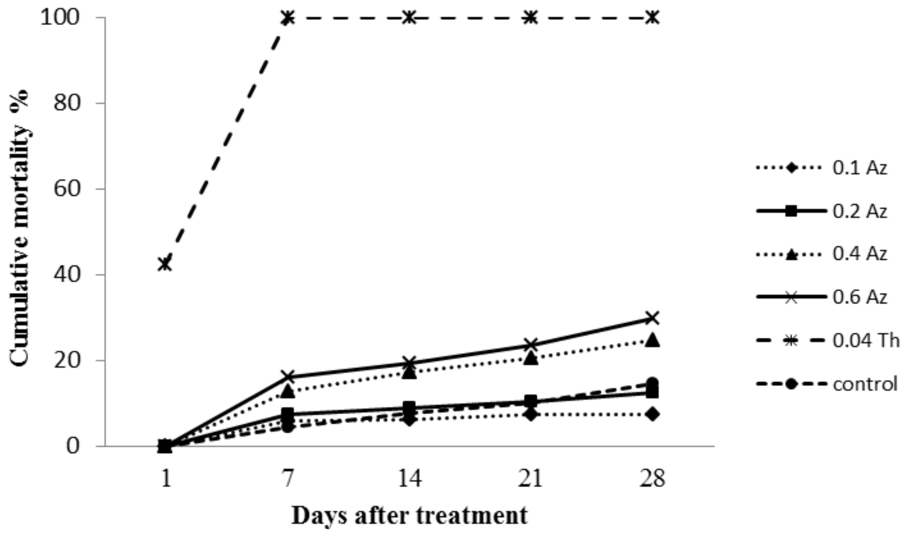


Figure 4: Cumulative mortality of adult of *C. lameensis* aged 20 days after systemic injection of azadirachtin and Thiamethoxam in dry and rainy season

A: dry season; B: rainy season

A



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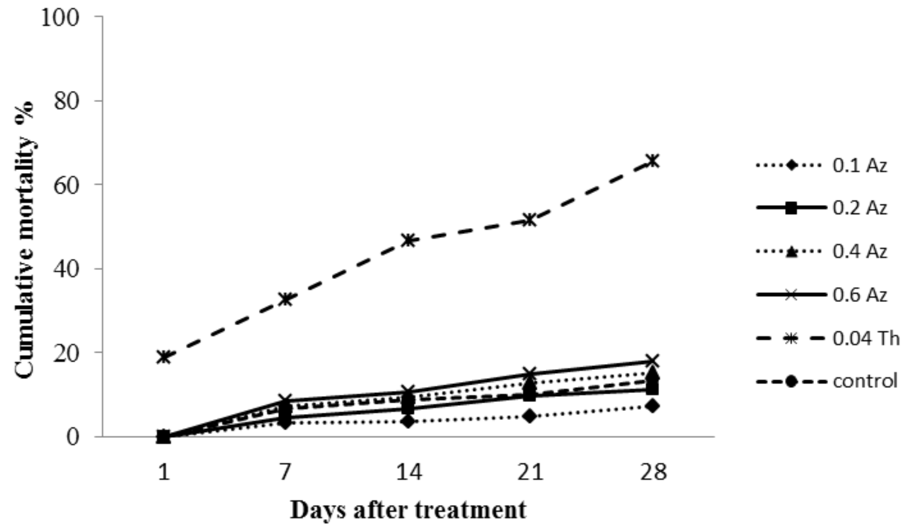


Figure 5: Cumulative mortality of adult forms of *C. lameensis* aged 20 days introduced into breeding sleeves 7 days after systemic injection of azadirachtin and Thiamethoxam in dry and rainy seasons

A: dry season; B: rainy season

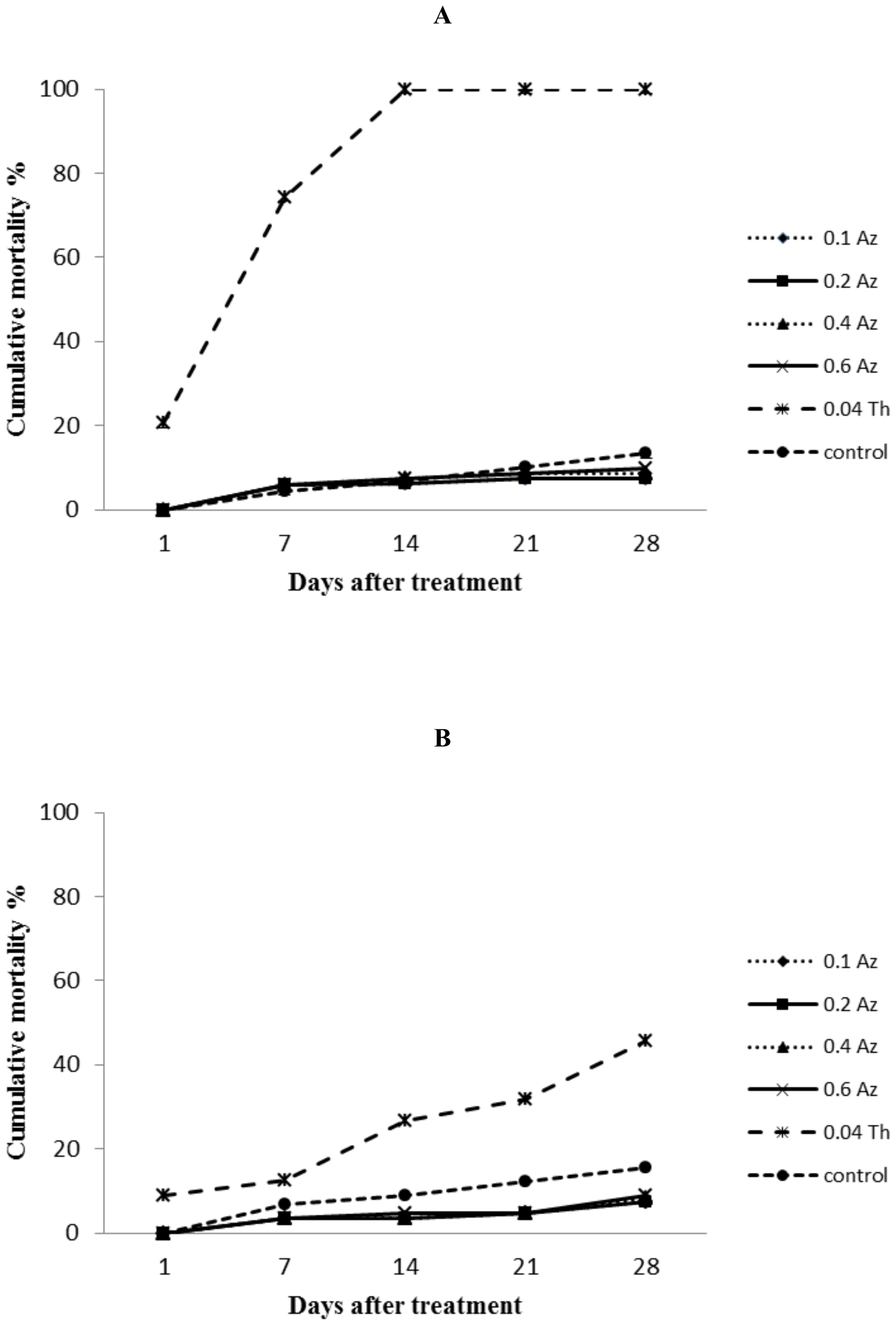
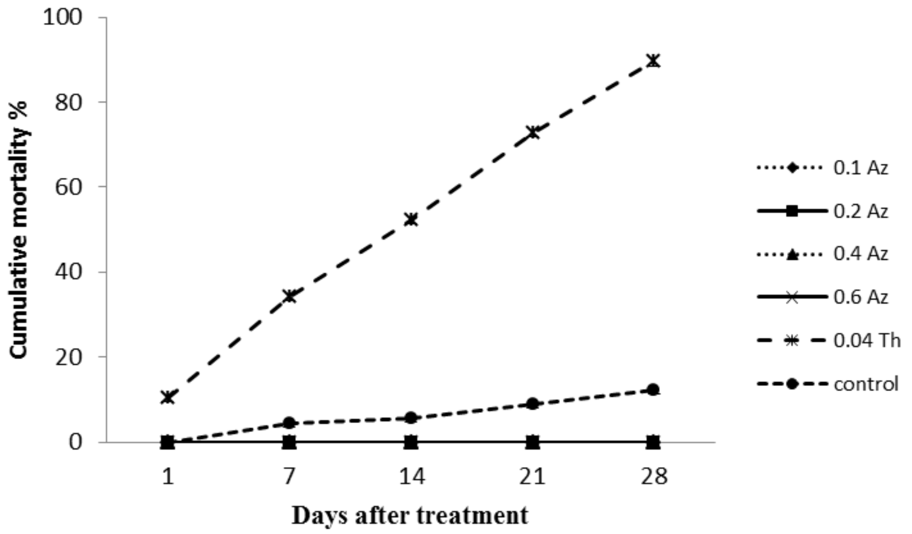


Figure 6: Cumulative mortality of adult forms of *C. lameensis* aged 20 days introduced into breeding sleeves 14 days after systemic injection of azadirachtin and Thiamethoxam in dry and rainy seasons

A: dry season; B: rainy season

A



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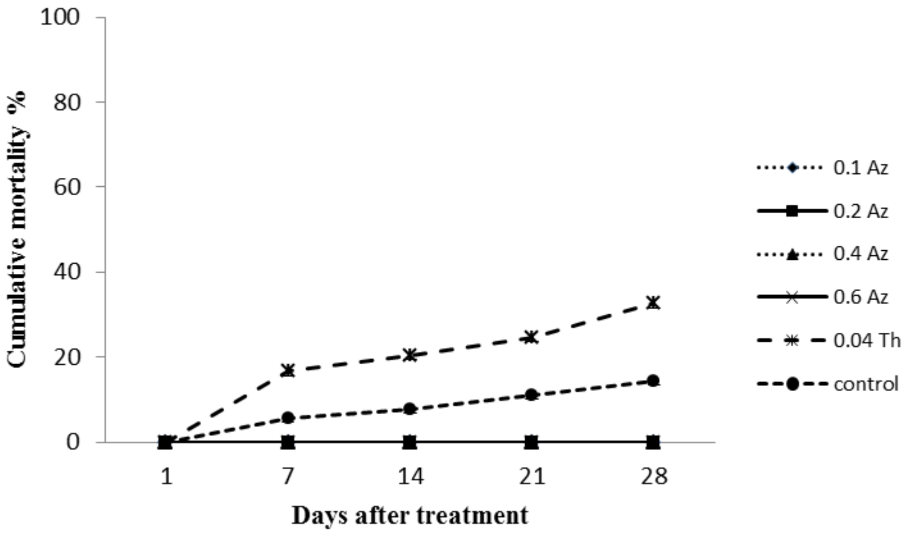

Figure 7: Cumulative mortality of adult forms of *C. lameensis* aged 20 days introduced into breeding sleeves 21 days after systemic injection of azadirachtin and Thiamethoxam in dry and rainy seasons

A: dry season; B: rainy season

Aims and Scope

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Mas-Colell, A and J. Silvestre (1991) “A Note on Cost-Share Equilibrium and Owner- Consumers” Journal of Economic Theory Vol.54, No.1,pp. 204-14.

Appendix: At the end of the paper

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