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Biological control against the cowpea weevil (*Callosobruchus chinensis* L., Coleoptera: Bruchidae) using essential oils of some medicinal plants

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Abstract: Chickpea (*Cicer arietinum* L.) is a valuable foodstuff but unfortunately this legume is prone to insect attacks from the chick pea weevil (*Callosobruchus chinensis* L.). This serious pest damages the chickpea and causes decreases in the yield and in the nutritional quality. Biological control is being used to deal with this problem. We tried different doses of the essential oils of three new medicinal plants, namely *Salvia verbenaca* L., *Scilla maritima* L., and *Artemisia herba-alba* Asso to limit the damage of the chick pea weevil pest, and to protect consumer's health. To determine the effect and efficiency of the oil, the tests were conducted using the different biological parameters of fertility, longevity, and fecundity, under controlled temperature and relative humidity (28°C and 75%). The effectiveness of organic oils was demonstrated. We tested these oils on the germination of seeds. The obtained results showed that the tested plant oils have a real organic insecticide effect. The essential oil of *Artemisia* proved most effective as a biocide; achieving a mortality rate of 100%. A significant reduction in longevity was observed under the effect of 30 µl of *S. maritima* (1.3 days) and *S. verbenaca* (2.8, 4.6 days), respectively, for males and females compared to 8 and 15 days for the control. For fecundity, an inhibition of oviposition was obtained using 30 µl of *Salvia* and *Scilla* essential oils. The test on the seed germination using different essential oils, showed no damage to the germinating seeds. The germination rate was 99%. These findings suggest that the tested plants can be used as a bio-insecticide for control of the *C. chinensis* pest of stored products.

Key words: *Artemisia herba-alba*, biological control, *Callosobruchus chinensis, Cicer arietinum, Salvia verbenaca, Scilla maritima*

Introduction

Chickpea is a legume which occupies large areas in Algeria. It is a legume which offers high protein and high energy value in human nutrition. But every year there have been considerable quantitative and qualitative losses (60%) (Labdi 1995) especially in stocks of chickpeas. The losses are primarily due to the attacks of insect pests, particularly the Chinese beetle (*Callosobruchus chinensis* L.). This pest is a potentially ubiquitous cosmopolitan beetle which can infest its host plant *Cicer arietinum* L. both in the field and in stocks. Within the framework of plant health protection, the use of insecticides had always been the solution (Hall 1970; Haubruge *et al*. 1988; Relinger *et al*. 1988). But use of insecticides has had bad consequences, such as increased resistance (where increasingly insatiable species have appeared), an imbalance of the ecosystem (the massive and random destruction of the harmful and useful insects), and disturbances of the environment as there is a risk of toxicity due to the problems of residues. Several authors have brought up these problems in their work. These authors include: Greathead 1992, Mullié and Keith 1993, Gwinner *et al*. 1996, Gilliom *et al*. 1999, Wania *et al*. 1999, Panisset *et al*. 2003, Provost *et al*. 2003, Dauguet *et al*. 2006, and Carlos 2006.

The alternative solution currently recommended, is the exploitation of our phytogenetic resources by the judicious use of medicinal plants. Within the framework of the biological fight against such attacks, several works have been undertaken by several researchers such as Thiam and Ducommun 1993, Shaaya *et al*. 1991, Vincent *et al*. 2000, Isman 2006, Arnason *et al*. 2008, Chîasson *et al*. 2008, Regnault-Roger *et al*. 2008, Allahvaisi *et al*. 2011, and Abd-Elhady 2012.

In this study, we used the essential oils of three medicinal plants. The Algerian ecotypes were tested for the first time: *Artemisia herba-alba* Asso (Asteraceae), *Scilla maritima* L. (Liliaceae), and *Salvia verbenaca* L. (Lamiaceae), in different doses (10, 20, and 30 µl), as organic insecticides against the Chinese weevil. This study also deals with what happens to the germination of chickpea after the application of these essential oils.

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Materials and Methods

Rearing of the insect

The strain of *C. chinensis* is originally from (Mascara) Algeria and was reared in the Zoology Laboratory of the research unit on Biological Systems and Geomatics. The emerged adults, aged 0–24 h, were obtained from a mass rearing achieved in glass jars filled with chickpeas. There were about 300 seeds per jar and a high amount of adult males and females of different ages. The experimental device was presented throughout the test in an oven under a controlled temperature (28°C), relative humidity (72±5%), and a photoperiod of 12 h : 12 h (L : D). The substrate used is composed of grains of the chickpea variety FLIP 93–93C obtained directly from the experimental farm of the University of Mascara.

Obtaining essential oils of plants

In May 2013, the three following, flowering plants were collected directly in the surrounding area of the study: *A. herba-alba, S. maritima* and *S. verbenaca*. Hydrodistillation was used to obtain the essential oils from a significant amount of *Artemisia* and *Salvia* leaves, and *Scilla* bulbs (Kéita *et al*. 2001). The oils were collected in sealed glass containers and refrigerated in the dark at 4°C until their use.

Seeds treatement by oils

In this study, 200 chickpea seeds distributed in 10 Petri dishes are mixed with different doses of oils (10, 20, and 30 µl) for each of the plants used. A *C. chinensis* pair, aged 0–24 h, was added to each Petri dish. It should be noted, that for each plant and each amount we redid the same experiment by always using a control.

The study of the effect of the oils on longevity

For each plant tested and for each 10, 20, and 30 µl; 10 repetitions were carried out to estimate the life span of the males and females (0–24 h). A male and a female were put in limp of Kneaded each containing 20 chickpea seeds impregnated with oil. The individuals were under daily control.

The study of the effect of the oils on fecundity

Twenty essential oil-treated chickpea seeds and an insect pair were insulated in limp of Kneaded to estimate the number of eggs laid by the female. The experiment was repeated each time with the same amount seeds and a pair.

The study of the effect of the oils on fertility

The goal of the test was to determine the percentage of eggs hatched after a treatment with those various essential oil amounts already tested in the fecundity parameter.

The study of the effect of the oils on germinative faculty

We aimed at detecting a possible action on the germinative faculty of chickpea seeds, from the essential oil substances of the three tested plants. We started our experiments using:

- a pilot batch including 100 chickpea seeds in germination without any treatment;
- three other batches were used for germination, each batch had 100 impregnated seeds with 30 µl of the essential oils from *A. herba-alba*, *S. maritima* and *S. verbenaca*.

Statistical analysis

All the results obtained were subjected to analysis of variance. To classify the insects for all the parameters of the study, Static software, version 5 was used.

Results and Discussion

The effect of the essential oils on longevity

According to the results seen in figures 1a, b, and c, the individuals which underwent a treatment with the various amounts of essential oils showed a longevity which varied appreciably with the sex, the amount, and the plant, when compared with the control. The effect of essential oils on the various biological parameters are shown in figure 1a. The amount of 20 µl of *Artemisia* oil had a lethal effect on the males and the females. The effect was very significant: $F_c = 5.37$ and $F_{th} = 3.86$ (F_c – F calculated, F_{th} – F theoric). There was 100% mortality noted with the use of *Artemisia* oil after just a few minutes of treatment. These results meant we were saved from studying other parameters such as fecundity and fertility. Ketoh *et al*. (2006) showed that monoterpene *β*-pinene and the pepiritone contained in *A. herba* have insecticidal activity against *C. maculatus*. A lethal effect was not produced from the oil of *S. maritima* and *S. verbenaca* (Fig. 1b, c), but there was a remarkable reduction in the life span of the insects. We noted a longevity of 1.3 days, respectively, for the males and the females under the effect of 30 µl of *Scilla*, and 2.8 and 4.6 days, respectively, for the males and the females treated by the oil of *Salvia*. The controls had a longevity of 9.8 and 12.6 days. The results were highly significant $F_c = 9.02$ and $F_{th} = 3.86$. Koumaglo *et al*. (1998) showed that essential oil of *Cymbopogon schoenanthus* Spreng. had a toxic effect on the various developmental stages as well as on the adults of *C. maculatus*. Mansour (1997) showed that 0.5% of Neem azal-S produced 100% mortality in the various developmental stages of the insect.

The effect of the essential oils on fecundity

The essential oils of the various plants seemed to have a direct effect on the egg laying. It was noticed, as seen in figures 2a, b, and c, that the average of the egg layings under the effect of the treatment with the oils of the various plants, was weak by comparison with the control (39.8%). The

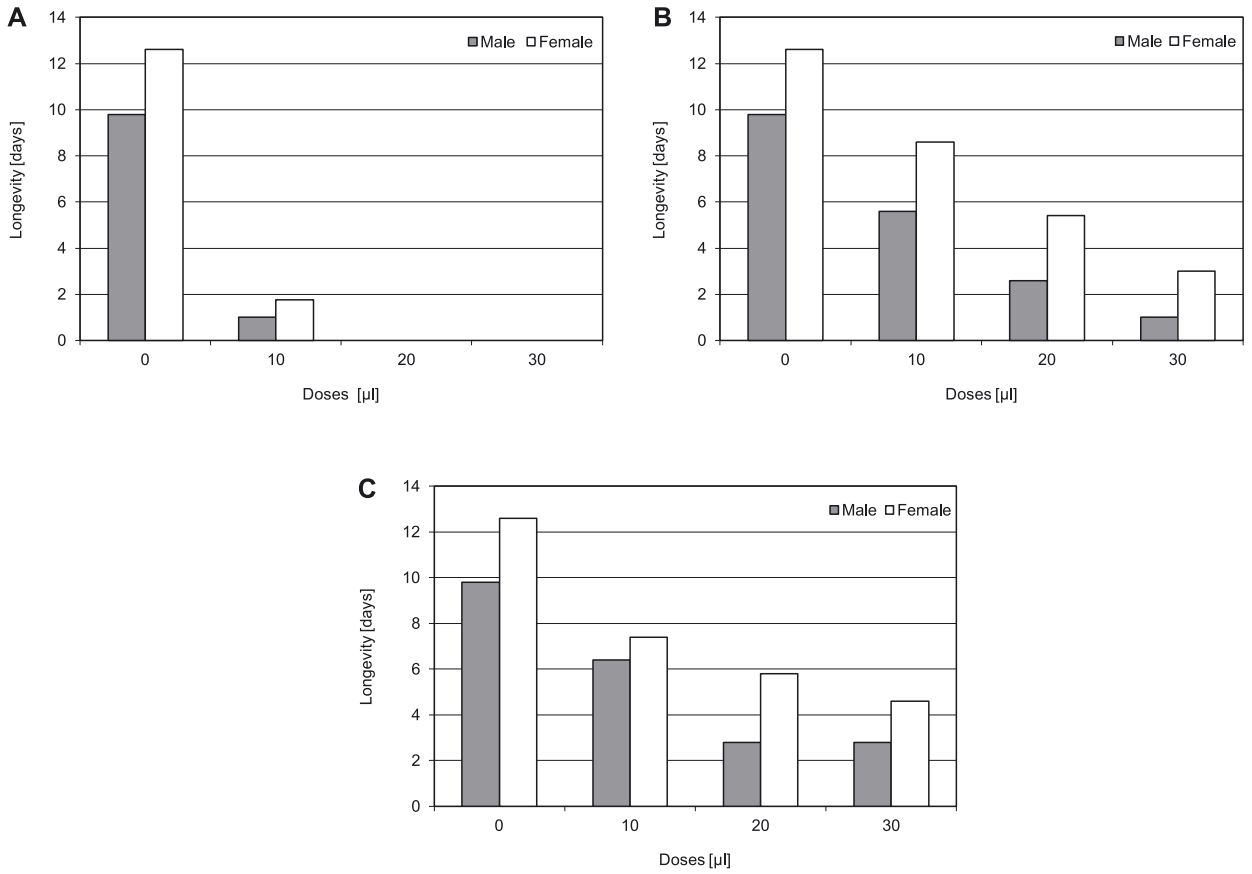

Fig. 1. Effect of the essential oils of (A) *A. herba-alba,* (B) *S. maritima,* and (C) *S. verbenaca* on the longevity of *C. chinensis*

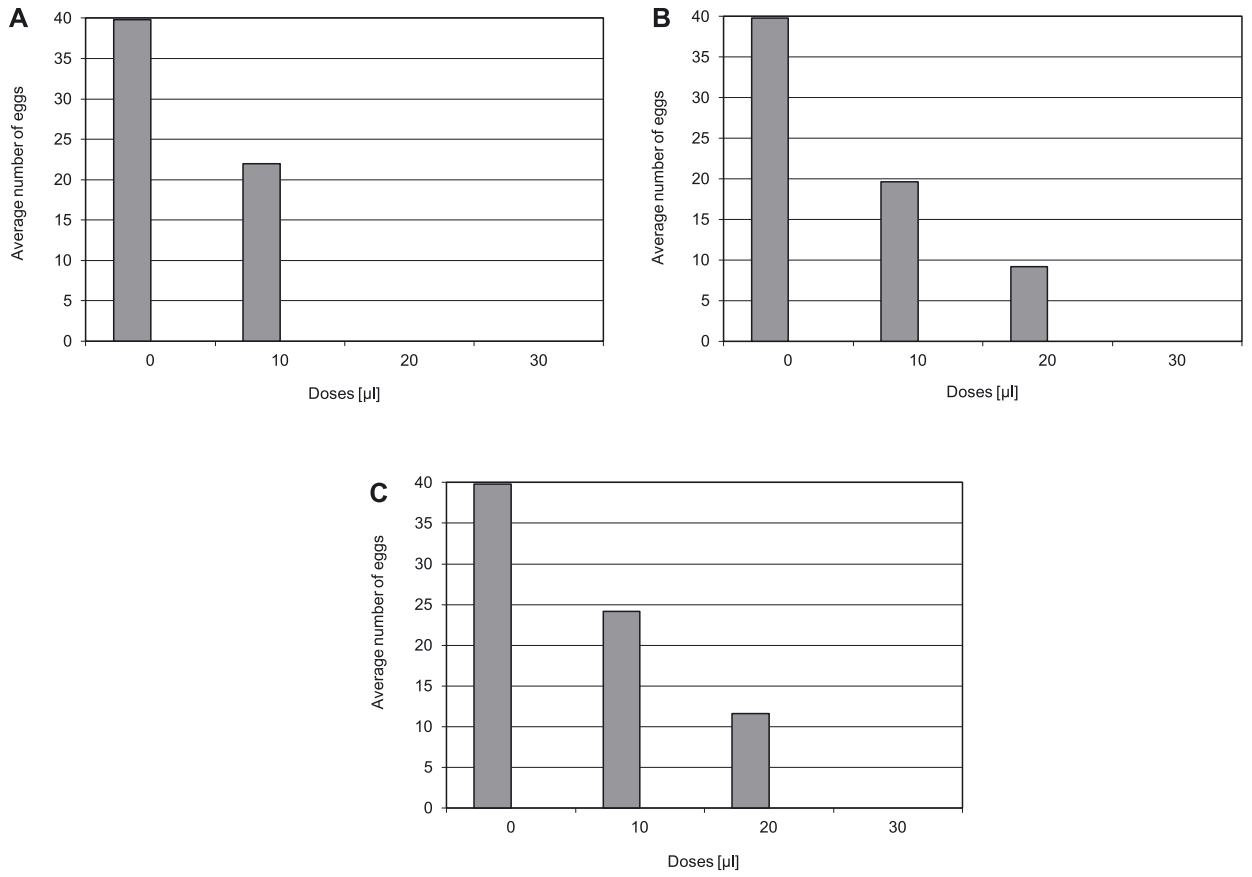

Fig. 2. Effect of the essential oils of (A) *A. herba-alba,* (B) *S. maritima,* and (C) *S. verbenaca* on the fecundity of *C. chinensis*

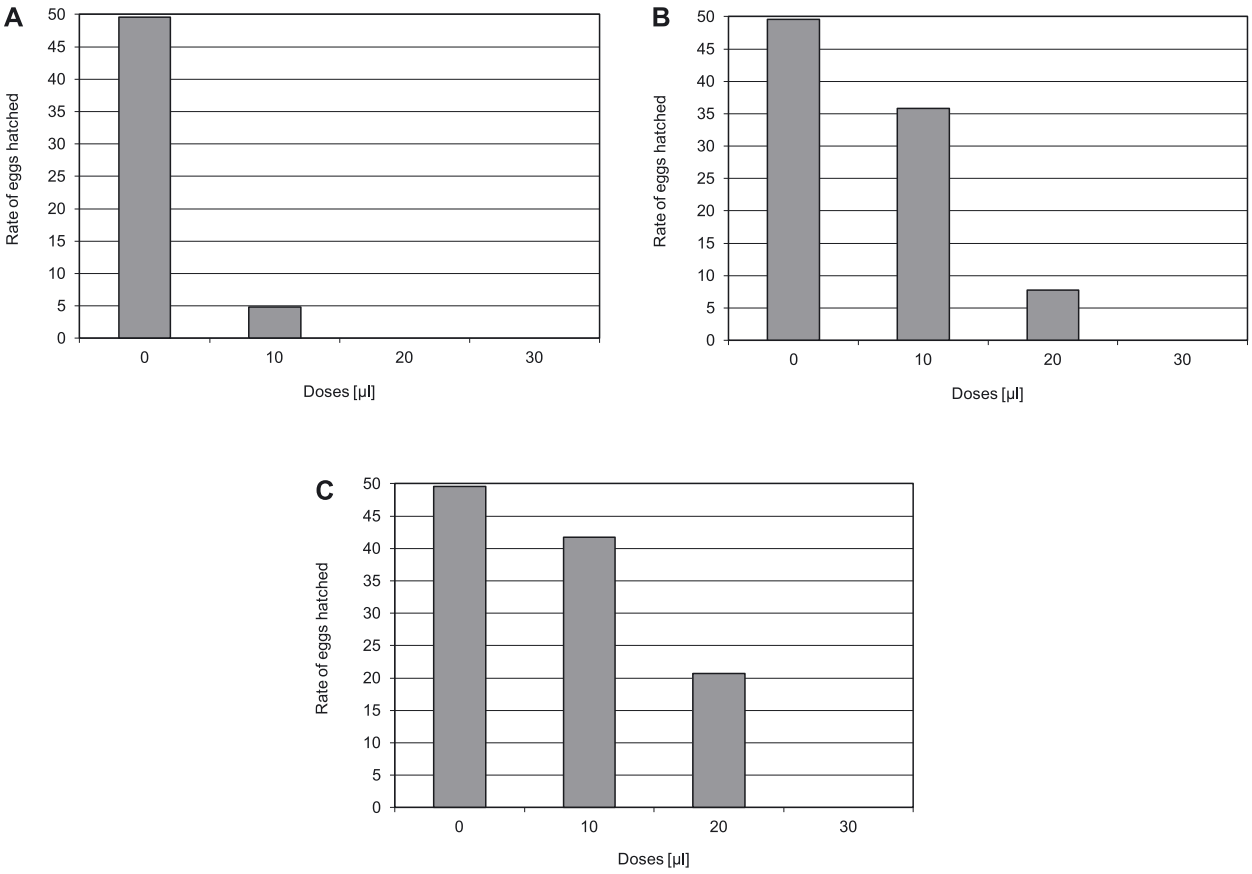

Fig. 3. Effect of essential oils of (A) *A. herba-alba*, (B) *S. maritima*, and (C) *S. verbenaca* on the fertility of *C. chinensis*

weakest egg laying was recorded for the individuals treated by *Scilla* (9.2%) followed by *Salvia* (11.6%) in the amount of 20 µl. On the other hand, with a treatment of 30 µl of the same plant oils, the egg laying was completely inhibited (0%). The reduction of fecundity is not only related to the reduction of the egg laying period and to the reduction in the longevity of the females but it can also be due to a disturbance of the vitellogenesis process. The ovicide action of the shoots of *Nicotiana tabacum* L., *Ocimum gratissum* L. (Ofuya 1990) and neem powder (*Azadirachta indica* A. Juss.) (Seck *et al*. 1991) is shown on the niébé beetle by various authors. When comparing the beetle of the chickpea our results were in agreement with the above-mentioned authors' results concerning the comparison of the beetle prey of the chickpea. According to Seri-Kouassi *et al*. (2004), the essential oil treatments of *O. gratissum* on the females of *C. maculatus* resulted in a very significant reduction in the egg laying, compared to the control. Various researchers showed that flavonoides significantly reduced the egg laying and the fertility of *C. chinensis*. Jacob and Sheila (1990) affirmed that the treatment with neem oil used against *C. chinensis* reduced mortality to more than 60%.

The effect of the essential oils on the fertility

The fertility according to the results illustrated in figures 3a, b, and c, was really influenced by the application of essential oils used on the chickpea seeds. At 20 µl of the essential oils of *Salvia* and *Scilla*, the rate of eggs hatched was 20.69 and 7.74%, respectively, compared to a rate of 49.6% for the control. The use of 10 µl of *Artemisia* oil, showed that for the average number of eggs laid by a female (22), the fertility was significantly lowered to 4.87% (Fig. 3a). It was useless to study the fertility of a 30 µl treatment of *Salvia* and of *Silla* because we have already noted the inhibiting effect of these oils on egg laying. The essential oils of the different plants great influenced the fertility of the *C. chinensis's* eggs. This fertility obviously varies with the concentration, as proven by the statistical analysis: F_c = 7.08 and F_{th} = 2.88. These results are highly significant. Osekre and Anery (2002) tested the effect of some powders of the shoots of the trees, and the aromatic plants which contain essential oils, and the rates were more or less high. Their test showed a positive effect on the niébé beetle. Pandy and Singh (1995) confirmed that the Neem powder reduced the damage of the beetle. Al Lawati *et al*. (2002) proved the great inhibiting capacity of the powder extract of *Annona squamosa* L. on *C. chinensis*. Several manioc and bean plants tested on the Coleopters of the Bruchidae type, showed an insecticidal and ovicide effect (Monge *et al*. 1988; Glitho *et al*. 2008). Ofuya (1990) observed ovicide activity in the niébé beetles when powder of d'Og shoots was used.

The effect of the essential oils on the germinative capacity of chickpea seeds

We also studied the effect of the essential oils of the various test plants on the germinative capacity of chickpea seeds. After a period of 30 days of seed germination, no

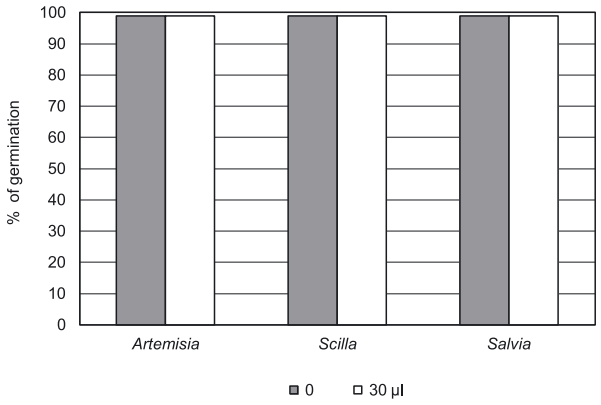

Fig. 4. Effect of different essential oils on the germination of chickpea seeds

negative effect of the test oils was noted on the germinative ability of the seeds (Fig. 4). Almost all of the seeds germinated 99%. We noted that in spite of the biocide effect of the oils on the beetles, there was no significant difference observed between the germinative capacity of the control seeds and that of seeds impregnated with various essential oils. Pacheco *et al*. (1995) as well as Rajapakse and Vanemden (1997) showed the effectiveness of the various oil treatments on *C. maculates*. They reported that the effect of the oils reduced longevity and reduced egg laying without affecting the viability of the seeds.

Conclusions

Oils were the research objects of many scientists trying to find ways to reduce the losses caused by insects of food products (Varma and Pandy 1978; Mahgoub 1992; Gbolade and Adebayo 1994; Ramzan 1994; Hall and Menn 1999; Kellouche and Soltani 2004; Negahban *et al*. 2007; Kailash and Bhanwar 2013).

Our use of the oils extracted starting from the three new, tested, medicinal plants (*S. maritima, S. verbenaca,* and *A. herba-alba*) as bio-insecticidal against the chickpea beetle, led to satisfactory results. The oil of *A. herba-alba* showed agreat biocide purpose on the insects: 100% mortality was recorded after only a few minutes of exposure. The oil of *S. maritima* and *S. verbenaca* at an amount of 30 µl, significantly reduced the longevity of the beetles and inhibited the egg laying of the females. These encouraging results persuaded us to check if these insecticidal plants influence the germination capacity of chickpea seeds intended for seed. Once again, our results showed that the seeds treated once with strong amounts of the noted essential oils, do not present any harmful effects on the germination of the seeds. Our results corroborate with those of several authors. Rajapakse (1996) showed the same effect of *Piper nigrum* oil on the niébé beetle. Don-Pedro (1989) used essential oils of citruses.

We recommend further study on the use of other amounts and other plants in order to have an insecticidal range available for constant use when this pest appears. The safe guarding of this invaluable food product is very important. Thus any attempt to study the use of natural substances in this field is very justified.

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