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Research Paper

Efficiency of botanical extracts on bee pests in the north-western of Benin

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ABSTRACT

Beekeeping is an alternative source of income for beekeepers and is included in the forestry management plan of Benin. The decrease in honey production was reported by beekeepers recently due to the proliferation of the honeybees *Apis mellifera adansonii* pests in beehives. The purpose of this study was to improve quantitatively and qualitatively the honey produced in Benin by developing bee pests control strategies. Trials were conducted in 3 townships (Tanguieta, Materi and Cobly) of Atacora district. The reduction of bee pests was investigated in four management systems: untreated beehive, beehive treated with insecticide Sumithion 50 EC (Fenitrothion l/ha), beehive treated with *Hyptis suaveolens* (Hyptis) leaf extract and beehive treated with *Azadirachta indica (*neem) leaf extract in a completely randomized block design (CRBD). The proportion of eliminated bee pests (imagos and larva) in the hives treated with neem and Hyptis leaf extracts was significantly higher (76 and 84%), respectively than that eliminated in the hives treated with Sumithion 50 EC (40%). The leaf extract of neem gave the best control on bee pests and appeared to be an attractive subtract for beehive colonization. In conclusion, neem leaf extract can be used by beekeepers to control honeybees pests.

Keywords: *Apis mellifera adansonii*, honeybee's pests, bio-insecticide, *Azadirachta indica*, *Hyptis suaveolens.*

INTRODUCTION

The honey and other bee products such as propolis, wax, pollen, brood, royal jelly and bee venom are among the groups of Non Timber Forest Products (NTFP) of animal origin. Indeed, honeybees collect large amounts of food that they store in anticipation of hard times. For thousands of years, humans harvest these food stocks. He made a craft beekeeping (van't Leen et al., 2005). In Benin in general and in North-Western Benin in particular, beekeeping is an alternative source of income for farmers in paucity period. It is a part of the forestry management (Kokoye, 1991) and represents an agricultural activity which provides honey and other products to the people.

In most of the developing countries in general and in Benin in particular few data are available on the control of honeybees pests. The few works in beekeeping in Africa refers to the relation between plants and bees and the inventory of bees pests carried out in Egypt (Hussein, 2001), Nigeria (Agwu and Akambi, 1985), Benin (Mensah and Ilco, 1996; Mensah and Koudjou, 1999; Adjinakou, 2000; Mensah et al., 2003, 2004, 2005; Donou, 2007; Sossou, 2008), Burkina Faso (Guinko et al., 1992a, b; Sawadogo, 1990, 1993; Nombre, 2003) and Togo (Lobreau-Callen et al., 1986) where many problems prevent the development of beekeeping. The main problem which impairs the development of beekeeping in Benin is not only the decrease of the yield in the honey production and other beehive products but also the reduction of the beehive number due to the proliferation of some diseases and the migration of some pests of the honey bees (*Apis mellifera adansonii*) and beehives

Figure 1: Location of study area.

products (Mensah et al., 2004). Among bees and beehives products enemies, arthropods (bee pests) are the main issue (Mensah et al., 2003). The works of Mensah et al. (2003, 2007), Hodonou (2005), Pomalegni et al. (2007) and Donou (2007) orientated on the natural enemies of bees and beehives and reported 39 Arthropods species. Bee enemies contribute to decrease in the immunizing system responses of the bee (Gregory et al., 2005). They are also active vectors in the transmission of virus and bacteria (Yang and Cox-Foster, 2005, 2007). Knowing that the main elements that can contaminate honey and other bee products come either from the environment or beekeeping practices (Bogdanov, 2006), a beekeeping directed to the use of botanical extracts can provide a solution to (i) reduce the population of pests and (ii) protect natural resources and food products of chemical contamination (Charleston et al., 2005). Among the biological insecticides, extracts of neem (*Azadirachta indica*) and Hyptis (*Hyptis suaveolens*) demonstrated power for biological control of various noxious insects (Raja et al., 2005).

MATERIALS AND METHODS

Experimental sites

The experiments were conducted in the north-western of Benin in the townships of Tanguiéta, Matéri and Cobly located in the district of Atacora (Figure 1). The area extended between the parallels 10° and 11°30' Northern latitude and meridian lines 0°45' and 2°10' longitude East. Three villages were selected per township. Beekeeping, degree of beehive infestation by the bee pests and the diversity of the hives were the beehives selection criteria. Two different experiments were conducted. For experiment 1, beehives were treated after their colonization by bee pests and for experiment 2, beehives were treated before their colonization by bee pests. The both experiments were carried out in each of the three townships at the same period.

Experimental design

The experimental design in each township was a randomized complete block design (RCBD) with 3 replications (villages) and 4 treatments (Figure 1). The variants were: untreated beehive; Beehive treated with the insecticide, Sumithion 50 EC (Fenitrothion) at 0.64 $l.ha^{-1}$; Beehive treated around with *H. suaveolens* (Hyptis) leaf extract at 31.8 $kg.ha^{-1}$; Beehive treated around with *A. indica* (neem) at 31.8 $kg.ha^{-1}$. Regarding the experiment 1, seven beehives were used. Three were destroyed for counting the number of pests before the establishment of the experiment and four others used for pesticides application. Concerning experiment 2, four new beehives colonized by bees were installed.

Preparation and application of *Hyptis suaveolens* and *Azadirachta indica* leaf extract

H. suaveolens and *A. indica* leaf extracts were prepared according to the modified method of Kossou et al. (2000). The modification is related to the non addition of oil to the prepared solution in order to avoid the desertion of the hives. The extracts were applied around the hives within a radius of 5 m or an area of 157 m^2. The support of the hive and all plants and grasses nearby were treated. Six applications with one per week were realized. The extracts were applied at night at 8 pm to limit the bee stings.

Counting and identification of bee pests

For experiment 1, the population of the bee pests was evaluated at the beginning and at the end for the

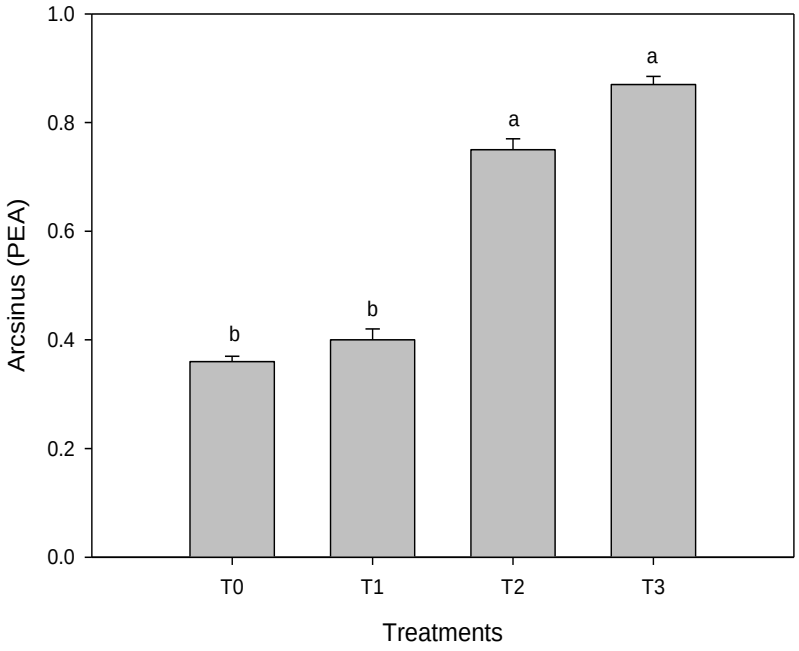

Figure 2: Average of Proportion of Eliminated Arthropods of all pests species: imagos and larva T0: Control; T1: Sumithion; T2: Hyptis; T3: neem.

experiment and for experiment 2, the population of pests was evaluated only at the end of the experiment.

The initial population of bee pests was counted at night by destroying the beehives using light, smoker, loupe and protection clove. The collected bee pests were identified at the insect museum of Biological Control Center for Africa at the International Institute of Tropical Agriculture, Cotonou, Benin using reference collection. Bee pests were collected with three beekeepers. This allowed us to carefully examine honeycombs, brood combs and inside of the hive searching imago bee pests. The counting methods involved opening the hive and counting all the observed bee pests. However, the larvae were also collected, counted and photographed. Some of them were raised in the laboratory in order to obtain imago for good identification. Furthermore, the location of the pests in the hive was recorded as well as, the destroyed hive products.

In such a context, it is necessary to develop the control methods of the honeybees pests. The main purpose of this work is to evaluate the technical efficiency of some botanical extracts on the infestation of the beehives by bee pests in the North-Western of Benin. The counting methods were the same for both tests.

Statistical analysis

ANOVA of 2 criteria (treatment and township) of classification of SAS (SAS Institute Inc., Release 9.0., Cary, NC, USA) were performed on the Proportion of Eliminated Arthropods (PEA) bee pests in general including larva and on their individual species according to the following formula. The data were transformed in order to respect the normality of the population.

$$PEA = \frac{\text{Number of bee pest at the beginning} - \text{Number of bee pest at the end}}{\text{Number of bee pest at the beginning}}$$

The Student-Newman-Keuls test was used to compare mean values of PEA. The software SigmaPlot (Systat Software, Inc. (2008) was used to design graphs.

RESULTS

Effect of *Hyptis suaveolens*, *Azadirachta indica* and sumithion extracts on the bee pests in the hives

Experiment 1

Effect of Hyptis suaveolens, Azadirachta indica and Sumithion on bee pests

The proportion of eliminated bee pests including imagos *and larva* was significantly different among treatments after the application of different products (P=0.001). It also significantly varied from one township to another (P<0.0001) and not for the interaction between townships and treatments (P=0.44). The proportion of eliminated bee pests (imagos and larva) in the hives treated with neem leaf extract was significantly higher (P=0.017) than that eliminated in the hives treated with Sumithion 50 EC at 0.64 $l.ha^{-1}$ (P=0.038) (Figure 2).

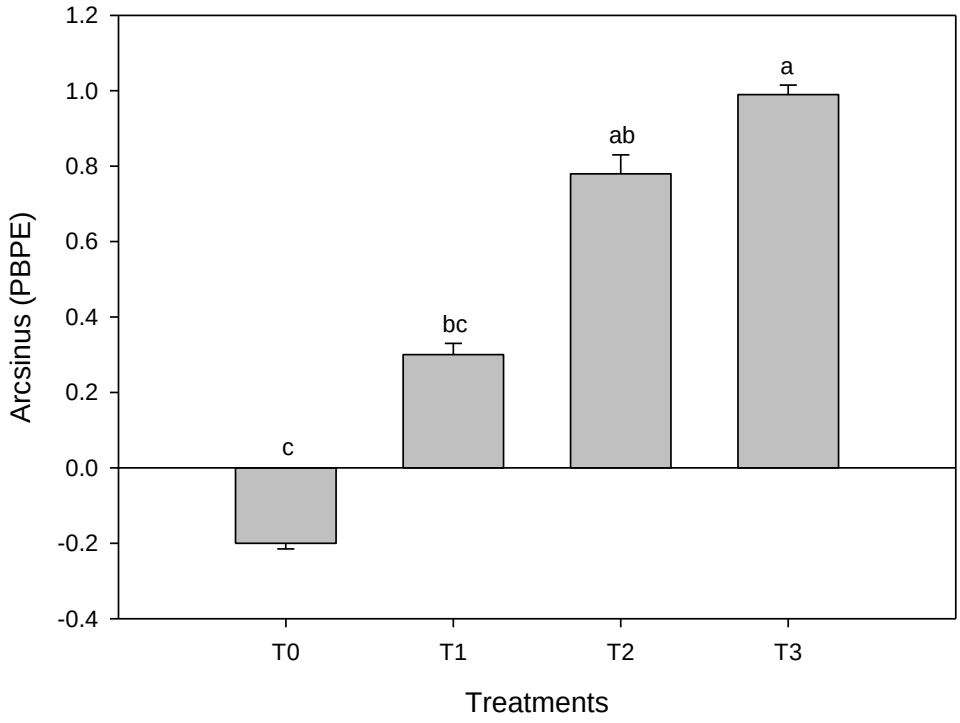

Figure 3: Mean proportion eliminated bee pests (imagos and larva) eliminated from the hives per treatment T0: Control; T1: Sumithion; T2: Hyptis; T3: neem.

Effect of Hyptis suaveolens, Azadirachta indica and Sumithion 50 ECon all bee pests at imago stage

Slight significant difference was obtained among treatments for the number of imago bee pests in the hives treated with Sumithion 50 EC and those obtained in untreated hives ($p<0.05$). The treatments T1, T2 and T3 had a positive control effect when compared to untreated hives (Figure 3). Among these treatments, the biopesticides *H. suaveolens* and *A. indica* leaf extracts were more effective than the chemical insecticide and among the both biopesticides. *A. indica* leaf extract was more efficient in reducing pests which colonize and infest hives. Thus, the neem leaf extract appeared in this particular case as a biopesticide well appropriate to control imago bee pests.

Effect of Hyptis suaveolens, Azadirachta indica and Sumithion 50 EC on the orders of major insects

After the application of the different products on bee hive, there was significant difference among treatments for coleopteran (P=0.0001) and for hymenoptera as well (P=0.0001). The difference was more significant when the eliminated Coleoptera, hymenoptera and other insects proportion in the bee hive treated with *H. suaveolens, A. indica* and Sumithion 50 EC was compared with the untreated bee hives. It was also observed that the leaf extract of *A. indica* controlled other insect's orders (Diptera, Isoptera, Lepidoptera and Hemiptera) than *H. suaveolens* and Sumithion (Figure 4). Indeed, Coleoptera and Lepidoptera were most dominant. As a matter of fact, other insect's orders were minority. They represent together 26.8% (6.7% for each insect order) of Arthropods identified against 40% for Hymenoptera and 33.33% for Coleoptera.

Effect of Hyptis suaveolens, Azadirachta indica and Sumithion 50 EC on the colonization of hives, the survival and the disertation of bees

During experiment one, no case of mortality of honeybees was noticed in the neighborhoods, nor inside the hives after products application. However, only one case of desertion was observed with the hives treated with Sumithion 50 EC. The rate of desertion was estimated to be 11.1% as against 0% for *H. suaveolens* and *A. indica.*

Experiment 2

Effect of Hyptis suaveolens, Azadirachta indica and Sumithion 50 EC on the Arthropods imagoes

In this experiment, the reduction of bee pests was significantly different between township (P=0.010) and

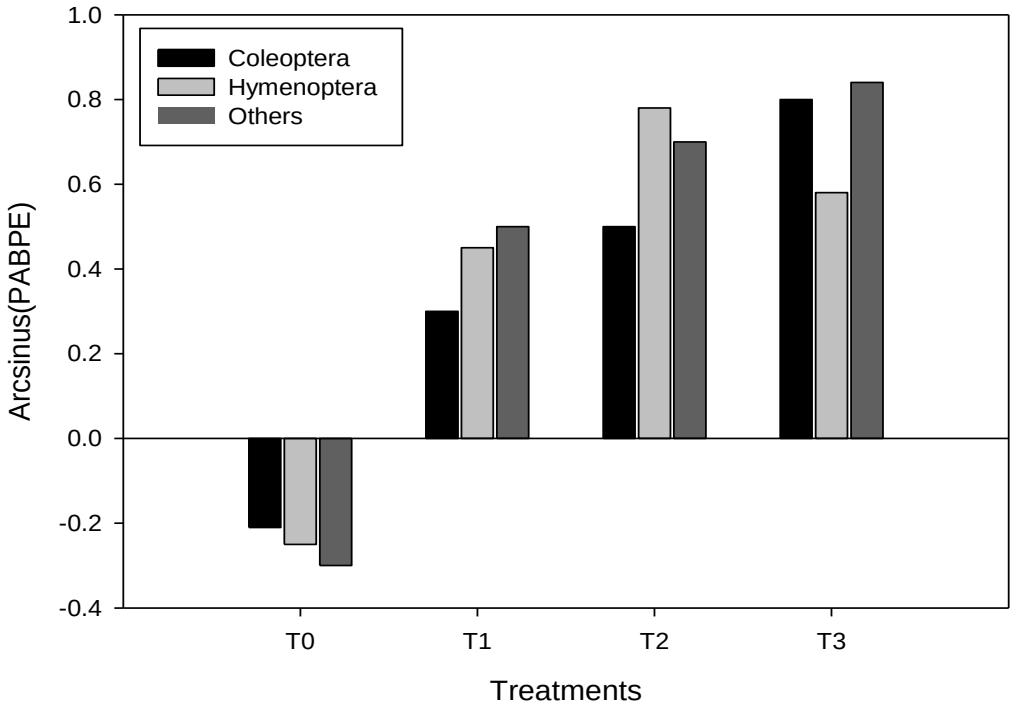

Figure 4: Eliminated proportion of imago of Coleoptera, Hymenoptera and others bee pests T0: Control; T1: Sumithion; T2: Hyptis; T3: neem.

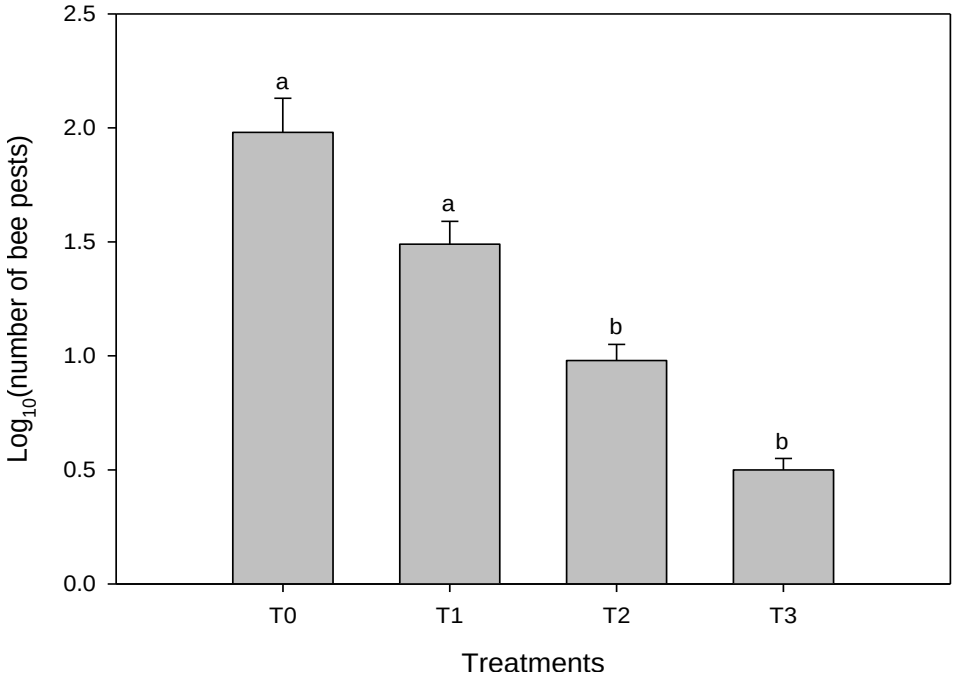

Figure 5: Numbers of bee pest counted in the hives treated with T0: Control; T1: Sumithion; T2: Hyptis; T3: neem.

treatments (P=0.000). The interaction between township and treatments was not significant for the number of imago bee pests counted in the hives (P=0.130) (Figure 5).

The number of bee pests in the bee hive treated with *H. suaveolens, A. indica* leaf extract and untreated bee hive was significantly different with P value of 0.0037 and P=0.000 respectively.

According to Student-Newman-Keuls test, the number of

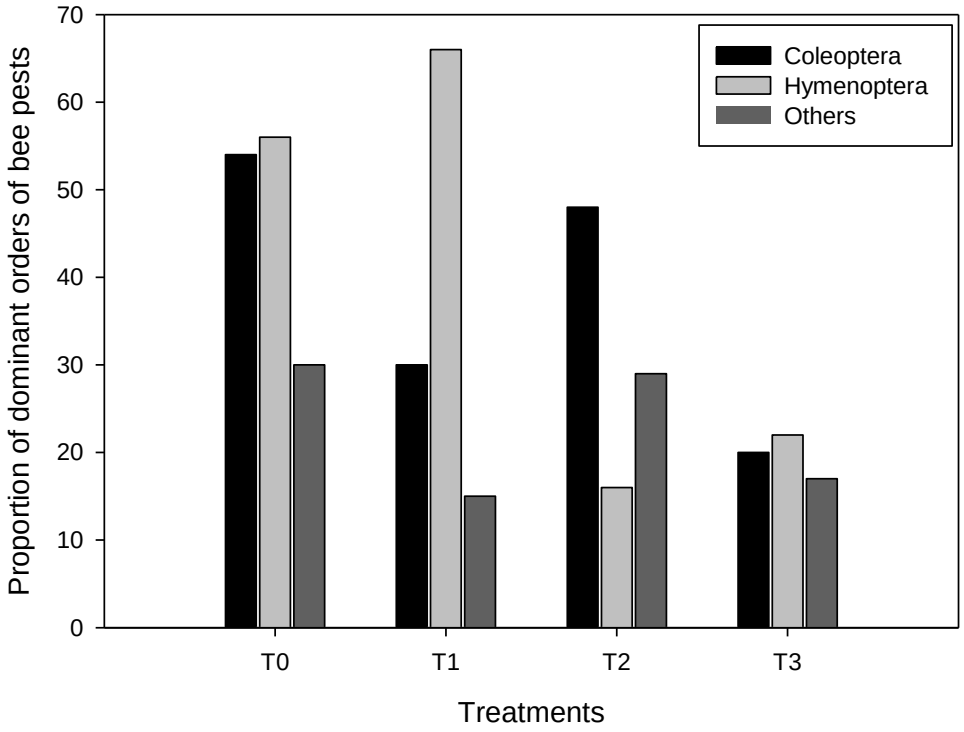

Figure 6: Proportion of Coleoptera, Hymenoptera and other pests in bee hives T0: Control; T1: Sumithion; T2: Hyptis; T3: neem.

bee pests was significantly higher in untreated bee hive and treated with Sumithion 50 EC and significantly low in bee hives treated with *H. suaveolens* and *A. indica* leaf extract. No significant difference was obtained between bee hives treated with *H. suaveolens* and *A. indica* leaf extract (P=0.3799).

Effect of Hyptis suaveolens, Azadirachta indica and Sumithion 50 EC on the dominant orders of bee pests

The number of Coleoptera in the hive was not significantly different between the treatments (P= 0.8766) while that of Hymenoptera was (P=0.0430). Treatments T2 and T3 reduced the proportions of hymenoptera in the treated hives. The highest proportions of Hymenoptera were found for the untreated beehives (control) and beehive treated with Sumithion. Concerning the other orders, the difference between the average proportions of insects was not significant (P=0.6281) (Figure 6).

Effect of Hyptis suaveolens, Azadirachta indica and Sumithion 50 EC on the colonization of hives, survival and dissertation of bees

Significant differences were obtained for average number of colonized hives among treatments (P=0.025). The colonization of hives by bees was slow and significantly low in untreated and treated hives with Sumithion 50 EC comparatively to hives treated with *H. suaveolens* and *A. indica* in which the colonization by bees was fast and significantly higher. The leaf extracts of *H. suaveolens* and *A. indica* better facilitated colonization of hives than Sumithion 50 EC (Figure 7).

DISCUSSION

The Coleoptera, Hymenoptera, Diptera and other insects were the main observed orders of bee pests. This result is consistent with those found by Hennou (2010) and Gbedomon (2011). The reflexion of developing methods to control bee pests based on the use of the botanical extracts is typically from Benin. However, the use of botanical extracts and other materials and products in the control of vegetable and food crop pests began decades ago. Several studies have been carried out in this field (Roy and Pande, 1991; Fatope et al., 1995; Djibode et al., 1996; Prakash and Rao, 1997; Yehouenou, 1997; Kossou et al., 2000; Bachabi, 2003; Illoba et al., 2006; Ruiu et al., 2008).

The results of both experiments showed that it is necessary to treat the neighborhoods of hives so as to prevent the colonization of hives by bee pests. The tested products *H. suaveolens* leaf extract, *A. indica* leaf extract and Sumithion 50 EC reduced the population of pests in bee hives. However, the effectiveness was variable among the three pest control products.

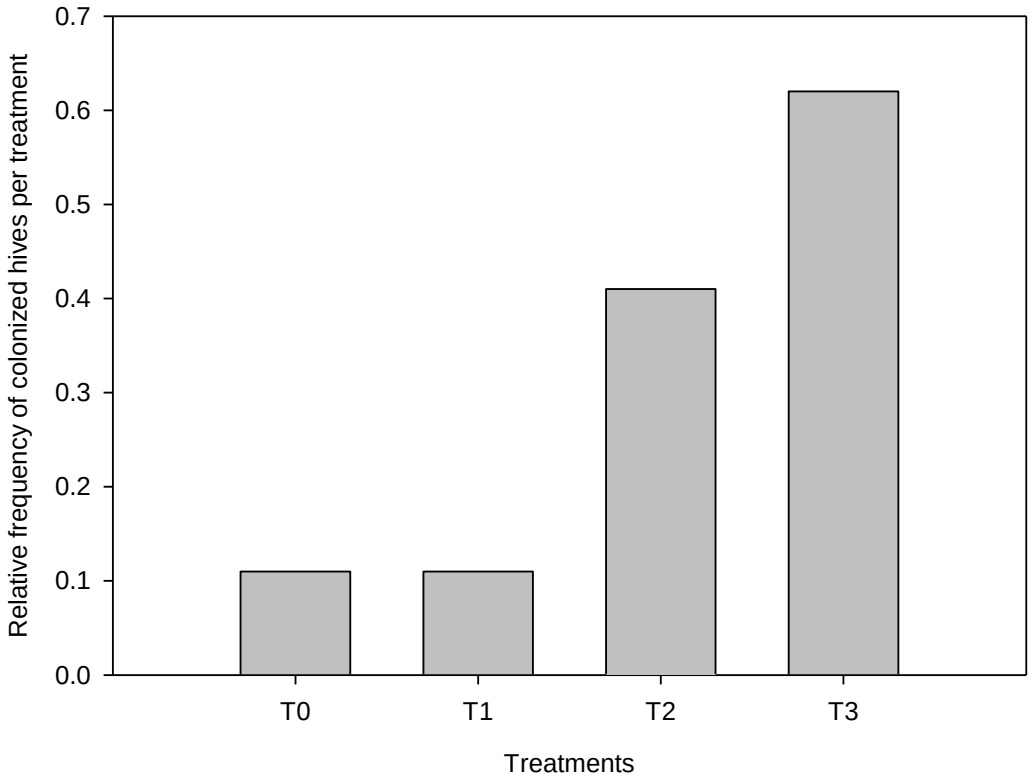

Figure 7: Relative frequency (number of hives colonized per treatment divided by the total number of colonized hives) of the hives colonized per treatment - T0: Control; T1: Sumithion; T2: Hyptis; T3: neem.

When the hives were colonized by the bees before product applications, *H; suaveolens* and *A. indica* leaf extract appeared very efficient. This result justifies the higher proportion of eliminated arthropods using botanical extracts compared to the lower proportion of eliminated arthropods using Sumithion 50 EC.

Comparing both botanical leaf extracts, they were not significantly different in efficiency. The proportion of eliminated insects using *A. indica* leaf extract is greater than that eliminated in the case of hives treated with *H. suaveolens* leaf extract. It appeared that the *A. indica* leaf extract was more effective than the *H. suaveolens* leaf extract. Our results confirmed that of Kossou et al. (2000), Ruiu et al. (2008) who demonstrated the repellent effect of *H. suaveolens* and *A. indica* on Coleoptera, Hymenoptera and Diptera in cowpea growing fields. Schmutterer (1995) working on the same field reported that neem leaf extracts were effective against more than 400 species of arthropod pests and nematodes in several crops of Asia, Africa and the America. According to these authors, *A. indica* leaf extract do not necessarily kill insects, but prevent crops from pests' damages. Except the repellent effect of *H. suaveolens* and *A. indica* on bee pests, they accelerated the rate of hives colonization by honey bees comparatively to Sumithion 50 EC with which 11.1% of hives were deserted.

In the case of non-colonized hives by bees before the application of *H. suaveolens* and *A. indica,* leaf extracts and sumithion 50 EC, the results were quite similar to those obtained when hives were colonized by bees before the application of plant extracts and sumithion 50 EC. In both cases, *A. indica* leaf extract stimulated the colonization of hives by bees. According to the work of Pomalegni et al. (2007), most bee pests are the less in terms of quantity of honey harvested. Our results showed that *H. suaveolens* and *A. indica* leaf extracts prevent the infestation of hives by bee pests than chemical insecticide sumithion 50 EC which play a static role and prevents the reinfestation of hives.

When we consider the effectiveness of the three applied products on the bee pest orders, we noticed that the leaf extract of *A. indica* was more effective on the different orders of insects. Our results corroborate those of Soukossi (1986) and Ruiu et al. (2008) who found that the component of *A. indica* is effective against several order of insects in general and against Coleoptera, Lepidoptera and Orthoptera in particular. Insecticidal effects with active substances of A. indica like Azadirachtine ($C_{35}H_{44}O_{16}$); Saline one ($C_{35}H_{44}O_9$) and Nimbin ($C_{30}H_{30}O_9$) were reported by Behi (2000).

Furthermore, our results are in conformity with the works of Ruiu et al. (2008) who demonstrated that Azadirachtine is effective against Hymenoptera larva and other order of insects. Indeed, the leaf extract of *H. suaveolens* better controlled Hymenoptera than the leaf

extract of *A. indica* in our work. The chemical insecticide sumithion, appeared more efficient on Dipterous, Arachnid, Heteroptera and Lepidoptera than Coleoptera and Hymenoptera. On the notice of the product Sumithion 50 EC, butterflies, beetles, trips, worms, aphids and borers were the target crop pests. During our work, the susceptible bee pests to the insecticide Sumithion 50 EC were Diptera, Heteroptera butterflies, Coleoptera, Isoptera and Hemiptera.

The property of *A. indica* leaf extract to facilitate the colonization of the hives by the honeybees is confirmed by the high population of bees in the bee hives treated with this particular product. This result can be justified by the smell of neem leaf extract which attracted bees and facilitated the colonization of the hives and protect bees from attack and invasion of enemies of arthropods. Scientific researches must continue on this successful and promising field of research to detect the active substance of *A. indica* leaf that attracts bees. The leaf extract of *A. indica* is in one hand the efficient biopesticide against bee pests and in another hand, has a good capacity to drain the bees and facilitate the colonization of the hives by the bees which is a very important stage for bee-keeping.

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