Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 13(3): 1613-1620, June 2019

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Toxicity of the powdery formulation of *Hyptis spicigera* (Lamiaceae), *Azadirachta indica* (Meliaceae) and *Vepris heterophylla* (Rutaceae) Single and in combination on *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae)

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ABSTRACT

The red flour weevil, *Tribolium castaneum* (Herbst 1797) is a pervasive pest, polyphagous which is becoming increasingly resistant to the normal insecticides. Finding alternative insecticides to those because of their inefficiency pollute the environment and poison consumers is an emergency. An ethno-botanical survey conducted with about 300 farmers in the Far North region of Cameroon identified 15 local plants insecticides associated with food during storage in combination with two or three others. Among these plants, the most used are *Hyptis spicigera* (Lamiaceae) 78%, *Azadirachta indica* (Meliaceae) 76.6% and *Vepris heterophylla* (Rutaceae) 28,33% which is more used in mountain area. The powders of these single plants are insecticides on *T. castaneum*, however *A. indica* single or in combination with *H. spicigera* gives better insecticidal efficacy 73.33% and 66.67% mortality respectively.

Keywords: Tribolium castaneum, stocked food stuff, combination, vegetal powder, insecticide.

INTRODUCTION

Cereals are a staple food for populations in developing countries (Guèye et al., 2011, Nanfack, 2015) accounting about 75% of the food calories intake, the least costly form to the populations (Hoogland and Holen, 2001)). In general, these cereals are produced seasonally and at a fixed period due to harsh climatic conditions. Storage is thus the only way that allows a quasi-permanent availability of these commodities on the market and ensures the seeds for the upcoming agricultural seasons (Ngamo, 2000). However, high pest pressure and lack of control over protection methods cause significant losses of food during storage (Tapondjou et al., 2002). In general, these pests cause losses by significantly reducing

DOI: https://dx.doi.org/10.4314/ijbcs.v13i3.32

the quality and quantity of stored food (Bounechada and Arab, 2011). Losses from insect pests in Africa range from 25 to 30% (FAO, 2012).

Among pests which attack flour are the red flour worm *Tribolium castaneum* (Herbst 1797), which is the most pervasive pest, the most polyphagous, and the most resistant to the usual insecticides such as Malathion, pyrimiphos methyl (Guedes et al., 1996; Andric et al., 2015). Synthetic chemical insecticides used to control these pests become increasingly serious problems: food contamination, consumers' intoxication which is a major public health problem (FAO, 2004) and environmental pollution (WHO, 2008; Khaliq et al., 2014). The abusive and uncontrolled use of these chemical pesticides is among others factors, a factor favoring the development of resistant strains of these pests (Goudoum et al., 2010). There is therefore a need to explore alternative solutions such as the formulation of new molecules from plant origin that are less aggressive for humans and less polluting for the environment. Several plants have been promoted as alternatives to harmful chemical insecticides to cereal pests (Tamgno and Ngamo, 2014). The active ingredients for which insects develop the fastest resistance are active ingredients based on a single component. Meanwhile solely active plants may have more. The contact toxicity of powders of neem leaves, almonds, shells and cakes is lethal to *Sitophilus oryzae*, *S. zeamays*, *Ryzopertha dominica* (Tamngo and Ngamo. 2014). Certainly, plant mixtures are alternatives for which insects would resist less.

The objective of this study was to find out plant sources of ingredients for alternative insecticides among those used in combination by the farmers of Far-North Cameroon for the formulation of biopesticides to be used for the protection of foodstuffs during storage.

MATERIALS AND METHODS

Study area and investigation

The Ethno-botanical investigations were conducted in two divisions of the Cameroon's Far-North Region: Mayo Danay and Mayo-Kani. The sub-divisions investigated were: Doukoula, Kai-kai, Kalfou, Vele, Wina, Yagoua (Mayo Danay Division) and Guidiguis, Kaele, Lara, Mindif, Moutourwa, Touloum (Mayo Kani Division) (Figure 1). In these localities, the investigations were carried out in markets and households. Shopkeepers and heads of households were interviewed using a survey map. Thus, the names of the plants introduced in combination in the granaries were noted, an herbarium constituted and their names noted in vehicular languages.

Powder formulation of tested plants for insecticidal efficacy.

Almonds of *A. indica* and leaves of *H. spicigera* and *V. heterophylla* were harvested respectively in the localities of Maroua, Yagoua and Moutourwa in the dry season. These plant materials were dried in the shade for two weeks and separately grinded into powder. The powder formulation was obtained by mixing in a test tube equal masses of the two and / or three powders to be considered for the combination followed by rigorous stirring of the tube for three minutes to homogenize the mixture.

Breeding insects

A population of *T. castaneum* extracted from a stock of attacked maize collected at the local market of Dang (Ngaoundere-Cameroon) was raised on six lots of 250g of millet flour mixed with 5% yeast in 1200 ml pots of volume at the Laboratory of Applied Zoology of the University of Ngaoundere. Each pot was infested with 10 adults of *T. castaneum*. The infested pots were placed in an incubator Uniscope SM9082 set at 30°C. After a week stay on this flour, the adults were removed and the laid eggs developed and produced larvae and then adults of known ages.

Biological test

The powders of plants alone and the balanced combinations of two and three plants produced are as follows.

Three powders of single plants: *H. spicigera*; *A. indica*; *V. heterophylla*; Three powder combinations of two plants: *H. spicigera* + *V. heterophylla*, *H. spicigera* + *A. indica* and *A. indica* + *V. heterophylla*. And a combination of the powders of three plants: *H. spicigera* + *A. indica* + *V. heterophylla*. The application of the formulation was done by dusting the larvae in a test tube with 1g of powder formulation, 15 repetitions for each treatment were made. 48 hours later, sieving was done. The dead larvae were extracted and the mortalities established, then the survivors put back in breeding on healthy flour where their growth was followed and noted.

Statistical analysis

Data obtained with the bioassay were subjected to analysis of variance (ANOVA) under the null hypothesis that the compiled data did not show significant differences. In case of rejection of this null hypothesis by an F test, a post test by the procedure of Duncan with the software XLSTAT 2007 version 8.04 was made to see if there are significant differences between the different levels of insecticidal toxicity of the different single and combination powders.

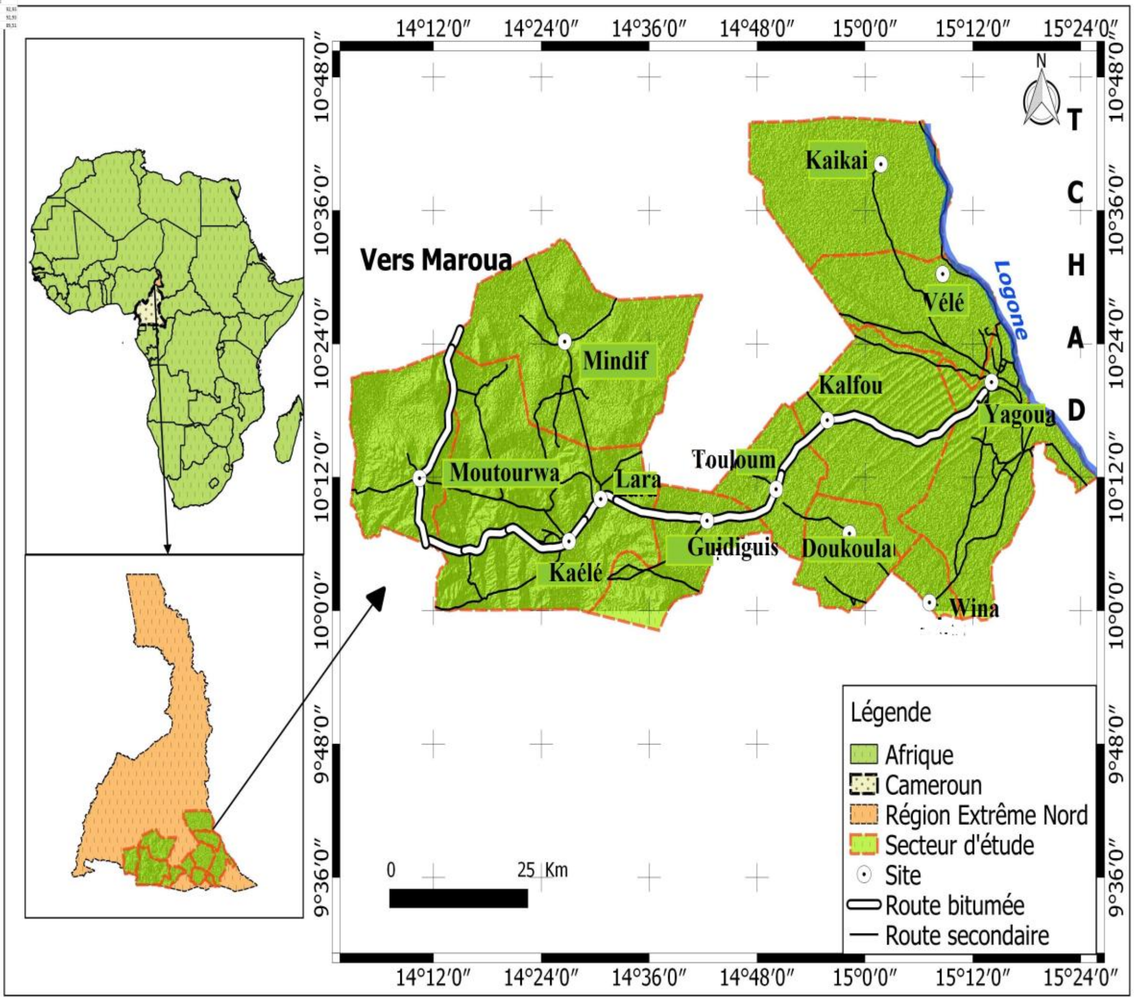

Figure 1: Location map of the boroughs surveyed.

RESULTS

Diversity of insecticide plants used in combination to protect food stored in the Far North of Cameroon.

During the survey, 300 participants aged between 32 and 70 years answered the questionnaire. 15 plants were listed as shown in Table 1.

This table indicates that the plants mostly used in the storage of commodities in the study area belong to the families of Lamiaceae, Meliaceae, Solanaceae and Liliaceae with respectively 234, 230, 207 and 132 farmers who use them, ie 78%, 76.67%, 69% and 44%. Those that are moderately used belong to the families of Poaceae (86 peasants: 28.67%), Rutaceae (85 peasants: 28.34%), Balanitaceae (42 peasants: 14%), Myrtaceae (35 peasants: 11.67%) and Anonaceae (30 farmers = 10%). The families of Caesalpiniaceae (12 farmers: 4%) and Fabaceae (02 farmers: 0.67%) are the least used. These single and combination plants are used to optimize the protection of foodstuffs.

Evaluation of the insecticidal efficacy of powder formulations of single or combined plants on larvae III of *Tribolium castaneum*.

The mortality analysis of *T. castaneum* larvae shows a toxicity of the powders of *H. spicigera, A. indica* and *V. heterophylla* after two days of exposure. This mortality is a function of the plants (Figure 2). The dead larvae out of 15 at baseline were: 11 with Neem (73.34%), 09 with *H. spicigera* (60%), 07 with *V. heterophylla* (46.67%) and 00 for the control (Figure 2). All larvae that survived exposure to the powders of different plants did not reach the adult stage. This was the case observed with *A. indica* powder where no adult were observed. On the other hand, all the larvae that survived treatment with *H. spicigera* powder arrived at the end of the cycle. *A. indica* almond powder was the most effective among these individually tested powders

The powders of the various plants in combination also showed significant toxicity on *T. castaneum* larvae. The highest mortality rate was obtained with *H. spicigera* + *A. indica* (66.67%) and the lowest with *H. spicigera* + *V. heterophylla* (26.67%). Similarly, no emergence of adults was observed with *H. spicigera* + *V. heterophylla* and *A. indica* + *V. heterophylla* combinations (Figure 2). The combination that was more effective is that of *H. spicigera* with *A. indica* (neem) saw the induced mortality (10 dead larvae is 66.67%).

According to Berenbaun (1989), three possible situations can arise when at least two compounds are put together: synergy or additive effect, antagonism and neutrality. For the plants combined in the present study, no synergistic effect was observed. On the other hand, the antagonism was observed with the combinations *H. spicigera* + *V. heterophylla* and *A. indica* + *V. heterophylla* which induce a mortality considerably lower than the average of those induced separately by the same powders.

Table 1: Importance of the plants mentioned, the parts used and the localities of use.

N	Plants	Famillies	Users	Parts used
1	*Hyptis spicigera* Lam.	Lamiaceae	234	Whole plant
2	*Azadirachta indica* A. Juss	Meliaceae	230	Seeds and Leaf
3	*Capsicum frutescens* L.	Solanaceae	207	Fruits
4	*Occimum gratissimum* L.	Lamiaceae	200	Whole plant
5	*Alium sativum* L.	Liliaceae	132	Fruits
6	*Sorghum bicolor* (L.) Moench	Poaceae	86	Stem
7	*Vepris heterophylla* (Engl.) Letouzey	Rutaceae	85	Leaf
8	*Panicum meliaceum* L.	Poaceae	54	Stem

9	*Balanites aegyptiaca* (L. Del.	Balanitaceae	42	Leaf and branches
10	*Eucalyptus* sp	Myrtaceae	35	Leaf and branches
11	*Nicotiana tabacum* L.	Solanaceae	32	Leaf
12	*Anona senegalensis* Pers.	Annonaceae	30	Leaf
13	*Piliostigma thonnigii* (Schmumach)	Caesalpiniaceae	12	Leaf
14	*Allium cepa* L.	Liliaceae	09	Whole plant
15	*Dalbergia sissoo* Roxb.	Fabaceae	02	Leaf

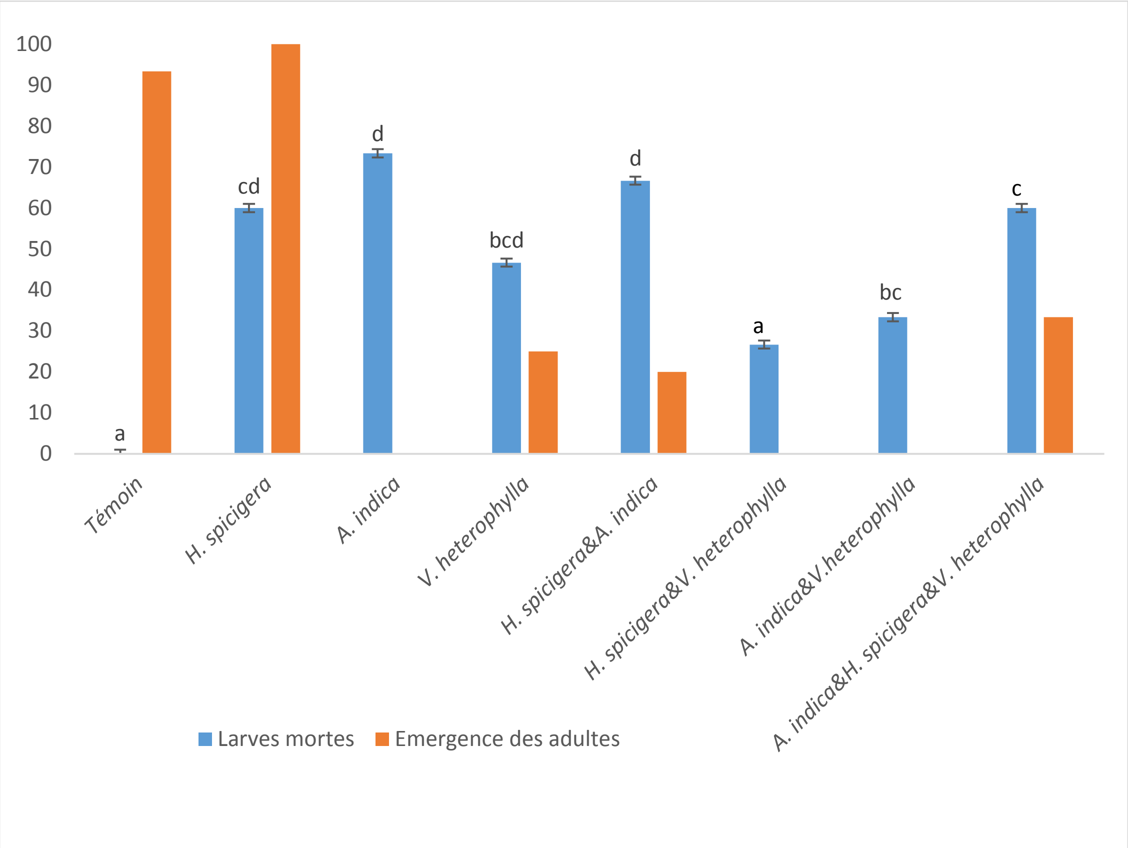

Figure 2: Larval mortality and adult emergence of *Tribolium castaneum* obtained with different treatments. (Mortality values followed by the same letter are not significantly different for p = 0.0003 from the F statistic and a post-test following the Duncan procedure).

DISCUSSION

The insecticidal efficacy of *A. indica* (Neem), *H. spicigera* and *V. heterophylla* is no longer in doubtful (Tamgno et al., 2014, Goudoum et al., 2015, Ngamo et al., 2016, Kouninki et al., 2017) and these plants are presented as important sources of botanical pesticides. The differences between the insecticidal activities of the powders of single and combination of plants alone are explained by the composition and the chemical nature of the compounds assets they contain. *V. heterophylla* is rich in flavonoids and quinoline alkaloids (Ngassoum et al., 2007) whose insecticidal activity has been proven by Ngamo et al., 2007 and Kouninki et al., 2017, while *H. spicigera* is rich in terpene compounds (Ngassoum et al., 2007).

Likewise, pyrethrins, acts by disrupting the functioning of the sodium channels at the level of neuronal membranes preventing the transmission of nerve impulses, the alkaloids present in many plants act by modifying the electrical activity of the neurons. These active compounds act on the nervous system and kill the insect by inhibiting the production of acetylcholine (Koné, 2009)

Terpenic compounds act on neurons by inhibiting the activity of acetylcholinesterase, an enzyme that destroys acetylcholine after nerve impulse transmission (Ryan and Byrne, 1988). This explains the absence of emergence with *V. heterophylla* powder in combination with that of neem and *H. spicigera*

Azadirachta indica shows a maximum mortality of 45.63% *T. castaneum* after 24h exposure at a dose of 2.5%. Indeed, *A. indica* belongs to the family Meliaceae whose insecticidal activity is the work of anti-appetizers and growth regulators they contain and which act according to at least 12 modes of action (Faye, 2010a). The main active compounds are limonoids. Azadirachtin is the predominant compound with primary target sites ecdysone responding through which it causes a stop larval development and a blocking of moults (Seck, 2009). The moulting does not take place, or else, there is aberration in the development of the insect (Marco et al., 1990). According to Cassier et al.(1987), azadirachtin inhibits the biosynthesis of chitin, a key component of insect exoskeleton. As anti-appetizing, it acts on the mouth receptors and other sensory organs of insects and thus causes the repulsion of processed foods. In addition, it inhibits the mitotic division of cells in larval stages and affects the physiology of insects' muscles and fats (Mordue & Blackwell, 1993). Which could justify the lack of emergence in our study. Due to these widely varying effects and modes of action, attempts to select for azadirachtin resistance in the laboratory have failed (Schmutterer, 1988). The development of resistance therefore seems unlikely.

The balanced mixture of the powders of the plants does not necessarily give an additive effect. Such is the case of *H. spicigera* and *V. heterophylla* in the present study which give an antagonistic effect in a balanced mixture. This effect was also observed during the work of Ngatanko and Ngamo, 2015 who showed that the proportional mixing of 190 ppm of the essential oil of *H. spicigera* with 680ppm of *V. heterophylla* causes 100% mortality of adult *Sitophillus oryzae* while a balanced mixture gives an antagonistic effect. The effectiveness of the mixtures of the plant powders would therefore be a function of the type of mixture produced (proportional or balanced) and of the species considered

Conclusion

The objective of this research was to find out plants which are sources of ingredients which can be used as alternative to harmful pesticides to protect stocked food stuffs. The field work and laboratory analysis indicated that, the most commonly used plants suitable for protection against pests in the study area are *A. indica*, *H. spicigera* and *V. heterophylla*. Tested on *T. castaneum,* the strongest toxicities were obtained with *A. indica* powder and the combination of *A. indica* + *H. spicigera*, thus making these plants to be the most efficient among the tested.

In a bid to further researches on the topic, it is judicious to suggest the following perspectives:

- Ameliorate the mixture proportions of powders to be tested (proportionate mixing)
- Assess the afterglow of the most efficient plants and mixtures
- Make the Nano formulation from plants extract
- Find out formulation support to be used in the protection of flour
- Enlarge the test on all the stages of development of *T. castaneum*.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All the authors of this article have actively taken part in its writing.

ACKNOWLEDGMENTS

The authors of this article will like to thank the Applied Zoology Laboratory of the University of Ngaoundéré and all those who from far or near contributed to the elaboration of this work.

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