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)

Evélé Nathanael AYIKI¹, Béranger Raoul TAMGNO³, Chantal MADOU¹,², Jérémie VATSOU¹*¹, Martin Benoit NGASSOU⁴ and Léonard Simon NGAMO TINKEU¹

¹University of Ngaoundéré, Faculty of Sciences, Department of Biological Sciences, B.P. 454 Ngaoundéré-Cameroon.
²Agricultural Research Institute for Development (IRAD), Garoua Multipurpose Station, B.P. 415 Garoua-Cameroon.
³Institute of Fisheries and Aquatic Sciences, University of Douala, Douala, Cameroon.
⁴Department of Applied and Environmental Chemistry, University of Ngaoundéré, PO BOX 455 Ngaoundéré-Cameroon.
*Corresponding author; E-mail: jeremievatsou@gmail.com; Tel: (+237)697464257 / (+237)653319705

**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.

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**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...
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 (Tamngno 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 Guidiguie, Kaelie, 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, i.e. 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 (2 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 Berenbaum (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.

<table>
<thead>
<tr>
<th>N</th>
<th>Plants</th>
<th>Families</th>
<th>Users</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hyptis spicigera Lam.</td>
<td>Lamiaceae</td>
<td>234</td>
<td>Whole plant</td>
</tr>
<tr>
<td>2</td>
<td>Azadirachta indica A. Juss</td>
<td>Meliaceae</td>
<td>230</td>
<td>Seeds and Leaf</td>
</tr>
<tr>
<td>3</td>
<td>Capsicum frutescens L.</td>
<td>Solanaceae</td>
<td>207</td>
<td>Fruits</td>
</tr>
<tr>
<td>4</td>
<td>Occimum gratissimum L.</td>
<td>Lamiaceae</td>
<td>200</td>
<td>Whole plant</td>
</tr>
<tr>
<td>5</td>
<td>Alium sativum L.</td>
<td>Liliaceae</td>
<td>132</td>
<td>Fruits</td>
</tr>
<tr>
<td>6</td>
<td>Sorghum bicolor (L.) Moench</td>
<td>Poaceae</td>
<td>86</td>
<td>Stem</td>
</tr>
<tr>
<td>7</td>
<td>Vepris heterophylla (Engl.) Letouzey</td>
<td>Rutaceae</td>
<td>85</td>
<td>Leaf</td>
</tr>
<tr>
<td>8</td>
<td>Panicum meliaceum L.</td>
<td>Poaceae</td>
<td>54</td>
<td>Stem</td>
</tr>
<tr>
<td></td>
<td>Plant Name</td>
<td>Family</td>
<td>No.</td>
<td>Part of Plant</td>
</tr>
<tr>
<td>---</td>
<td>------------------------------------------------</td>
<td>----------------------</td>
<td>-----</td>
<td>----------------</td>
</tr>
<tr>
<td>9</td>
<td><em>Balanites aegyptiaca</em> (L. Del.)</td>
<td>Balanitaceae</td>
<td>42</td>
<td>Leaf and branches</td>
</tr>
<tr>
<td>10</td>
<td><em>Eucalyptus sp</em></td>
<td>Myrtaceae</td>
<td>35</td>
<td>Leaf and branches</td>
</tr>
<tr>
<td>11</td>
<td><em>Nicotiana tabacum</em> L.</td>
<td>Solanaceae</td>
<td>32</td>
<td>Leaf</td>
</tr>
<tr>
<td>12</td>
<td><em>Anona senegalensis</em> Pers.</td>
<td>Annonaceae</td>
<td>30</td>
<td>Leaf</td>
</tr>
<tr>
<td>13</td>
<td><em>Piliostigma thonningii</em> (Schumach)</td>
<td>Caesalpiniaceae</td>
<td>12</td>
<td>Leaf</td>
</tr>
<tr>
<td>14</td>
<td><em>Allium cepa</em> L.</td>
<td>Liliaceae</td>
<td>09</td>
<td>Whole plant</td>
</tr>
<tr>
<td>15</td>
<td><em>Dalbergia sissoo</em> Roxb.</td>
<td>Fabaceae</td>
<td>02</td>
<td>Leaf</td>
</tr>
</tbody>
</table>

### DISCUSSION

The insecticidal efficacy of *A. indica* (Neem), *H. spicigera* and *V. heterophylla* is no longer in doubt (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).

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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).
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 moulding 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 Sitophilus 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.

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