# POPULATION SUPPRESSION EFFECTS OF RWANDAN MEDICINAL PLANT, TETRADENIA RIPARIA (HOCHST.) CODD (LAMIACEAE) ON STORED GRAIN AND BEAN INSECTS

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## ABSTRACT

Tetradenia riparia (Hochst.) Codd (Lamiaceae) is a perennial mint whose dried leaves are used as an infusion in Rwandan folk medicine for many conditions in which microorganisms play a major role. Laboratory studies by others have confirmed the antimicrobial properties of this plant against Candida albicans, Shigella dysenteriae, and Streptococcus pyogenes. Since antimicrobial preparations also often have insecticidal or population regulatory properties, the leaves of this plant were examined as a possible insect protectant in wheat and dry edible beans (Phaseolus vulgaris L.). Multigenerational growth and development bioassays were completed for the two main causes of loss in dry edible beans, the bean weevil, Acanthoscelides obtectus Say and the mexican bean weevil, Zabrotes subfasciatus Bohem with concentrations (w/w) of 0, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 10.0. At the highest concentration (10.0%) was there a significant decrease in the number of eggs laid. At $\geq$ 2.0%, there was a significant decrease in the proportion of beans used for oviposition and subsequent emergence. Beans stored with T. riparia were also subjected to human sensory evaluation by a trained panel in Rwanda. The panel found beans stored with T. riparia as acceptable as those stored with conventional methods (1% actellic), which is currently used as the sole commercial protectant against storage insects in Rwanda.

## INTRODUCTION

Traditional medicine and traditional insect management in most cultures are a rich source of ideas for sustainable pest management. These practices are a focus in our laboratory for two reasons. First, within the culture that developed the practice, these traditional on-farm procedures may require only minor changes to be used successfully on a large scale. Second, these traditional practices may be a source of novel techniques and of genetic material that codes for defensive phytochemicals which may be adapted for insect management in the USA. Laboratory studies need to determine if, indeed, these traditional products and techniques are efficacious and environmentally safe. This study was based on both a search for plant products we can use in the USA and ideas that can be adopted by farmers and managers of large scale warehouses in Rwanda.

Pest management solutions for a developing country can often be found within the borders of the country itself. One possible source of such solutions is traditional medicine (antimicrobial treatments) used by the people (Dunkel 1988). The most important plant in Rwandan pharmacopea is a perennial mint, umuruvumba, Tetradenia riparia (Hochst.) Codd. Most farmsteads contain one or more of these shrubs (Dunkel, personal observation). In Rwanda, umuravumba is used against malaria, yaws, helminths, gastroenteritis, dental abscesses, and other disorders (Van Puyvelde et al. 1975; Van Puyvelde 1976). An infusion is made of the leaves and it is taken orally or applied as moist leaves under the tongue. The whole leaf contains hundreds of compounds, including 8(14),15-sandaracopimaradiene-7alpha,18-diol, a diterpenol. In laboratory tests it was shown to have good antimicrobial activity for fungi, bacteria, and protozoa of medical importance (Van Puyvelde et al., 1986). A leaf fraction containing this compound is currently being mass produced in Rwanda at Centre de Recherche sur la Pharmacopee Medecine Traditionnelle (CURPHAMETRA). The question was posed by CURPHAMETRA as to the possibility of other fractions or the essential oil having insect control properties.

The main sources of protein and calories for the people of Rwanda are dry beans (Phaseolus vulgaris L.). The main grain is sorghum. In 1984-1986, we conducted an extensive national survey of these commodities, on-farm, in cooperative silos and hangars, and at OPROVIA warehouses (Dunkel et al. 1988a). This survey indicated that the most common storage insects causing loss in Rwanda were the bean bruchid, Acanthoscelides obtectus Say and the two grain insects, the lesser grain borer, Rhyzopertha dominica (F.) and the rice weevil, Sitophilus oryzae (L.). The main insecticide used is actellic (pirimiphos methyl). It is used by 50% of the farmers for beans and by 28% of the farmers for sorghum (Dunkel et al. 1988). At the national government warehouses of Rwanda (=OPROVIA= Office National pour le developpement et la Commercialisation des Produit Viviers et des Productions Animales), actellic has been used prophylactically since 1983. The survey also indicated populations of these two grain insects at OPROVIA warehouses may already show significant resistance to actellic, the main insecticide used in Rwanda. In 1988, populations of A. obtectus and S. oryzae with significantly increased resistance to actellic were identified in OPROVIA Warehouses (Sriharan et al. 1990).

Due to the suspected resistance problem, it was recommended that alternative, more sustainable insect protectant procedures be developed for the postharvest system in Rwanda (Dunkel et al. 1988). A search is underway to identify low input, sustainable storage structures (Hanegreefs et al. in press) or plant preparations which are or can be locally produced (Dunkel et al. 1990; Weaver et al. 1990, 1991). One of these plants, Ocimum canum Sims, is used by a small percentage of Rwandan farmers for protection against storage insects (Dunkel et al. 1988a). Laboratories indicate that it has easily volatilized, strongly insecticidal properties (Weaver et al. 1990, 1991).

We tested the general hypothesis that the most common traditional antimicrobial in Rwanda can play a role in storage insect management in Rwanda. We tested the specific hypothesis that: T. riparia has population suppression effects on Rwandan stored grain and bean insects and these effects can be used as an economical, sensory acceptable alternative to actellic in Rwandan warehouses.

## MATERIALS AND METHODS

**Plant material preparation-** Leaves of T. riparia were collected from the area surrounding Butare, Rwanda and dried in a 40°C oven for 24 hr. The dried leaves were then crushed and shipped via courier to Montana State University. Upon receipt, the plant material was stored at -20°C until used. Two lots of plant material were used in the following experiments. The first was collected and shipped in February, 1990 (Lot A) and the second in June, 1990 (Lot B). All experiments were begun within 14 days of the receipt of the plant material. Plant material was received in a crushed condition (mode particle size 3-5mm, particle size range 0.75-15.0 mm). Freshly milled preparations were prepared in an electric coffee grinder for 40s just prior to initiation of experiment (mode particle size 0.3-6mm, particle size range 0.15-1.1mm).

Essential oil was prepared using a large scale Clevenger-type steam distillation apparatus at CURPHAMETRA (Center for Ethnopharmacology) in Butare, Rwanda. The yield of this procedure was 0.07% from fresh leaves. Shipping and storing conditions were as for the leaves.

**Insect rearing and experimental conditions**- All four species were reared at 27$\pm$ 1° C and 65$\pm$5% relative humidity under a photoperiodic regime of 12:12 light:dark. Experiments were conducted under similar conditions. The mexican bean weevil, Zabrotes subfasciatus Bohem and A. obtectus were reared on a diet of Pinto beans (Phaseolus vulgaris L.). R. dominica, and S. oryzae were reared on a diet of 96:2:2 w/w (weight/weight) soft white wheat:whole wheat flour:brewers yeast.

**Gas chromatographic/mass specroscopy analysis**- Relative ion chromatograms of the essential oil from leaves of T. riparia were obtained under the following conditions: Initial T=50 °C; Initial hold=4min: Ramp=5°C/min; Final T=280°C; Final hold=10min; Column=30m DB5 (J and W Scientific Folsom CA), 0.25 mm internal diameter, 0.25 $\mu$ film thickness; 260 ° C injection port; 290°C detector T. EI-MS were obtained on VG Analytical VG 70EHF operating at 70eV with a source T of 200°C.

**Oviposition and egg development studies**- Leaves of T. riparia were added to ten replicates of 20g dried Pinto beans (approximately 50 beans; 16.07% moisture content determined by oven dry at 110°C) at dosages of 0.1, 0.5, 1.0 and 10.0% w/w or dosages of 1.0, 2.0, 3.0, 4.0 and 10.0% w/w.. Ten control replicates, without T. riparia, were also prepared for each experiment for both the milled and crushed preparations. The plant material was placed in a 10 dram plastic shell vial and mixed by shaking. Five male and five female Z. subfasciatus (0-3 da after emergence from bean) were added to each vial, and it was sealed with a perforated lid. Adults were allowed to oviposit until death, which occurred within 17da for all individuals. At 25 days, individual beans were examined to count the number of hatched and unhatched eggs.

For A. obtectus, a reduced scale experiment was required because this species did not affix its eggs directly to the bean pericarp as Z. subfasciatus does, but lays them loose among the plant material and beans. Dosages were prepared as percentage by weight of three beans only, to which four male and four female A. obtectus (1-2da after adult emergence from bean) were added. Insects were sexed according to Halstead (1963). Preliminary experiments with our stock culture indicated that this number of beans had no negative effect on oviposition of four mating pairs when compared to a larger quantity of beans. Insects were removed from the oviposition chamber after 6da. Hatched and unhatched eggs were counted at 15da. The 6da oviposition period encompassed the peak oviposition period of A. obtectus, 2-5da after adult eclosion in our preliminary experiment. Females lived approximately 13da, but laid less than 25% of their eggs after 6da post-emergence. Plant material and insects were placed in a 3 ml glass vial sealed with perforated filter paper taped to the sides of the vial with transparent tape. Ten replicates of each dosage and of a control were used.

**Essential oil bioassays**- A 9.0 cm diam. Whatman #1 filter paper was placed in a 10cm diam. glass Corning petri dish. A 1.0 ml aliquot of the appropriate dilution of the essential oil of T. riparia was delivered to the filter paper. The control was diluant (absolute ethanol) only. The ethanol was allowed to evaporate for 20 minutes prior to the addition of the insects. Mortality was evaluated if the insect was immobile and did not react to probing with a blunt dissecting probe. Moribundity was assessed by viewing those insects that were on their backs and ambulating very weakly. These insects were subsequently righted and viewed carefully. Those that immediately fell on their backs again as a result of intoxication, were classified as moribund. At higher dosages all moribund insects subsequently died with the passage of time. Recovery rarely occurred at lower dosages. Five males and five females were used in each replicate with Z. subfasciatus; 10 adults of unknown sex were used for the other species. Counts of mortality/moribundity were conducted at 24 hrs. To obtain data for a probit analysis of the effect on S. oryzae, a count was also required at 48 hrs.

**Sensory evaluation**- A trained panel of twenty Rwandans (13 males and 7 females ages 24 to 47) evaluated the cooked samples in standard evaluation booths. Beans were stored for two weeks with crushed T. riparia leaves or other insecticidal plant preparation. Control beans were obtained from OPROVIA and therefore had been treated with actellic (pirimiphos methyl 1%). Prior to cooking, the insecticidal plant material was removed. Beans were cooked in the traditional manner with water and salt. After 3 hrs of cooking, the beans were served to the panel with a score card.

**Statistical analysis**- Data were subjected to two way analysis of variance. Comparisons of paired means were made with t-tests. If the two-way analysis of variance (ANOVA) indicated no significant interaction occurred between preparation and dose, a one way analysis of variance was conducted and individual dosages were compared to the control if dosage was significant in the ANOVA. Count data were normalized by square root transformation and proportion/percent data were normalized by arcsine transformation prior to analysis (Sokal and Rohlf, 1981). Possible linear correlations were determined using regression analysis (Sokal and Rohlf, 1981). All statistical analyses were conducted using MSUATAT Version 4.12 (Lund, 1988)

The essential oil dose-response bioassay data were subject to probit analysis (Matsumura, 1975) after Abbott's formula was used to adjust for control mortality (Abbott, 1925). Since the dominant response of the moribund insects was to die, moribundity and mortality were pooled for statistical analysis.

## RESULTS

The gas chromatogram of the essential oil which we used for the bioassays has 206 distinct peaks (Figure 1). One of these we recognize as linalool which is a readily volatilized compound (Weaver et al. in press). The GC-MS analysis indicated negligible variability between essential oil samples at the initiation and completion of the experiments. Similarly, there was little variability between the lot of essential oil used for bioassay with S. oryzae exclusively and the one used for the three other species. The slopes of the probit lines were: Z. subfasciatus Y=4.2748X + -0.4514; A. obtectus Y=3.0726X + 0.7829; R. dominica Y=2.0227X + 2.1048; S. oryzae Y=5.0029X + -5.3060 (Figure 2). This indicates the two bruchid populations tested were relatively homogeneous and the grain insects, particularly S. oryzae, were more heterogeneous. The LC50 for these species showed wide variation between species (Table 1). The most sensitive species was Z. subfasciatus. The LC50 of S. oryzae was six times that of Z. subfasciatus.

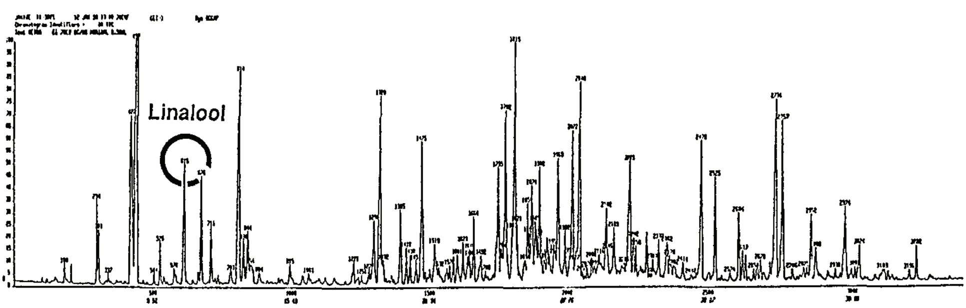


Figure 1. Relative ion chromatogram of the essential oil obtained by steam distillation from leaves of Tetradenia riparia (Hochst.) Codd grown in Rwanda (Prefecture Butare) and obtained September 1989 after 6 months in a crushed condition at ambient temperatures. Peak number 615 is linalool.

Oviposition studies indicated that beans to which 2.0% w/w T. riparia leaves had been added, there was a significant decrease in the number of eggs laid by A. obtectus (Figure 3) with

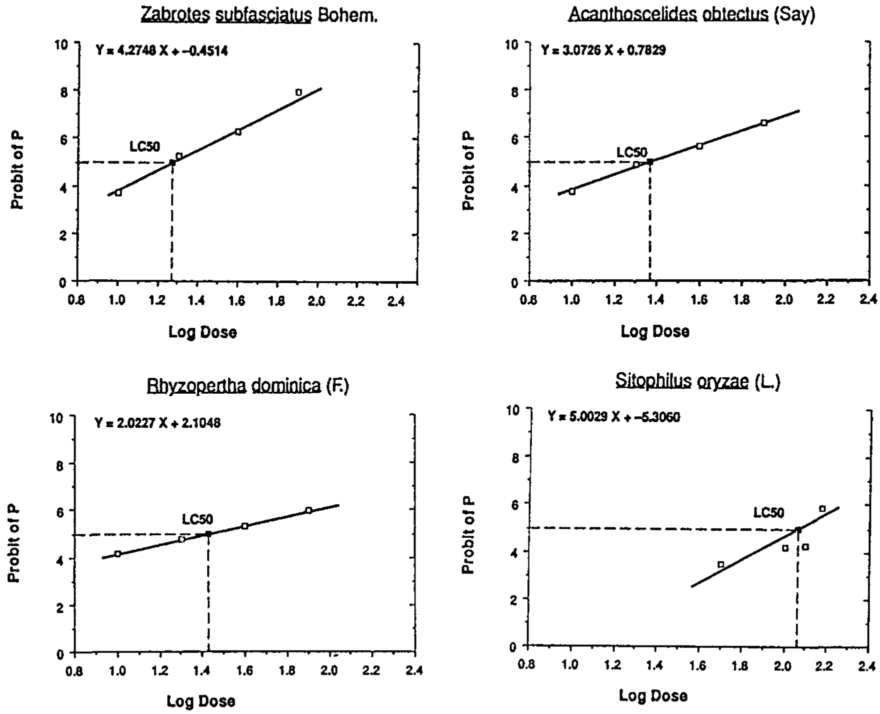


Figure 2. Dose-response curves induced by the essential oil of Tetradenia riparia (Hochst.) Codd for four species of stored product Coleoptera in filter paper bioassays (n=10; 10 insects/rep; 27±2°C; 65±8% relative humidity; 12L:12D). Zabrotes subfasciatus Bohem., Acanthoscelides obtectus Say, and Sitophilus oryzae (L.) were 0-2 da post-adult eclosion. Rhyzopertha dominica (F.) were 0-3 da post-adult eclosion.

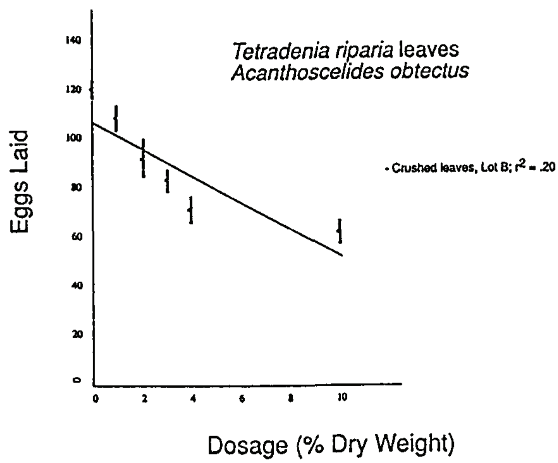


Figure 3. Eggs laid (mean ± standard deviation) by Acanthoscelides obtectus Say exposed to dosages of dry, crushed leaves of Tetradenia riparia (Hochst.) Codd (n=10; 4 males + 4 females 0-2 da post-adult emergence; 6 da oviposition period).

a similar effect at 4.0% for Z. subfasciatus (Table 2). Analysis of egg development with Z. subfasciatus indicated only at 10% w/w did the plant leaves significantly decrease the proportion of eggs hatched (Table 2). The number of beans oviposited on by Z. subfasciatus, however, was significantly reduced at the 2% dose of T. riparia (Figure 4). At 10% w/w T. riparia, the number of beans oviposited on was decreased by 71.5%. There was some variation between plant lots, but within plant lot (only Lot A was tested), there was no significant difference between whether the material was milled or crushed.

In sensory evaluation studies, 55% of the panel preferred beans that had been stored with T. riparia versus 45% of the panel that preferred beans stored the conventional way with actellic (Table 3). When asked to evaluate properties that contributed to positive and negative aspects of the samples, the scores were similar to the conventional insecticide (actellic) treated beans. Texture and odor were more positive than the conventional beans.

## DISCUSSION

Insect management materials used for these experiments are either grown in most farmsteads (the leaves) or prepared in Butare, Rwanda in a pilot scale facility (the essential oil) for producing a traditional medicine. Both products, therefore, are available to Rwandans without the expenditure of foreign exchange. The essential oil has potential for use as a protective coating applied to beans and sorghum stored in government warehouses. Target studies with this material are presently underway in our laboratory. Nontarget studies are planned.

In the Rwandan open marketing system and in the household as the beans are prepared for cooking or planting, we found in an earlier study (Dunkel et al. 1988b) that the number of emergence holes in an individual bean is important. Those with more than one emergence hole are discarded and therefore represent a complete loss. The leaf preparations dramatically decreased this type of loss. Tetradenia did not prevent development of bruchid populations, even at the highest concentrations used. The efficacy of the leaf material in concentrating the reproductive effort of the insects, however, will significantly decrease loss in the marketing system and in the triage process in the household.

These low input, sustainable methods would be unimportant if the beans would lose their acceptability after cooking or be harmful to consumers. Clearly, after a short storage period, protection with T. riparia leaves did not negatively affect sensory properties of the consumed product. Studies are underway to evaluate the product after longer term storage with the leaf preparations.

Studies on leaves of T. riparia indicate that the crushed leaves would be appropriate for reducing loss in beans stored in the most common on-farm structure in Rwanda, the open, dung-lined basket. The essential oil, after efficacy and non-target testing, may be appropriate for use in long-term storage at the national warehouses.

## CONCLUSIONS

- Essential oil of Tetradenia riparia has potential for use against A. obtectus, Z. subfasciatus, and R. dominica.

- Mammalian toxicity studies of the essential oil need to be completed before further insect studies are undertaken.

- Crushed dried leaves of T. riparia provide some protection against bruchids when relatively large quantities are added to stored beans. Sensory properties of these beans are acceptable to Rwandan consumers.

- For Rwanda, and other areas where the plant is cultivated in homesteads for medicinal purposes, crushed leaves could be used in combination with another medicinal plant, Ocimum canum which provides a fumigative effect.

- For Rwanda, the essential oil, product with medicinal properties, has possibilities for use as a protectant in government storages.

Table II. Mean oviposition, hatch, and percentage of beans oviposited upon ($\pm$ standard deviation) by Zabrotes subfasciatus Bohem. with dried leaves of Tetradenia riparia (Hochst.) Codd incorporated among the beans (10 reps; 20g beans; 5 males + 5 females; $28 \pm 1$ °C; $65 \pm 5$% relative humidity; 12L:12D; oviposition allowed until death of parents).

| Dosage % wt/wt | Eggs Laid | Proportion hatched | Percentage of beans oviposited upon |
|---|---|---|---|
| 0.0 | $135.8 \pm 28.6^{a}$ | $0.945 \pm 0.041^{a}$ | $84.4 \pm 7.9^{a}$ |
| 0.1M | $160.0 \pm 27.8^{a,*}$ | $0.960 \pm 0.014^{a}$ | $89.6 \pm 7.3^{a}$ |
| 0.5M | $145.8 \pm 26.9^{a}$ | $0.932 \pm 0.040^{a}$ | $85.8 \pm 6.1^{a}$ |
| 1.0M | $156.6 \pm 13.3^{a}$ | $0.932 \pm 0.053^{a}$ | $87.1 \pm 7.3^{a}$ |
| 10.0M | $66.6 \pm 36.8^{b;*,1}$ | $0.790 \pm 0.122^{b,*}$ | $18.5 \pm 17.2^{b,*}$ |
| 0.1C | $147.2 \pm 38.8^{a}$ | $0.943 \pm 0.020^{a}$ | $87.1 \pm 7.3^{a}$ |
| 0.5C | $147.0 \pm 22.6^{a}$ | $0.948 \pm 0.022^{a}$ | $86.8 \pm 5.4^{a}$ |
| 1.0C | $156.4 \pm 17.5^{a}$ | $0.909 \pm 0.034^{a,*}$ | $85.8 \pm 4.2^{a}$ |
| 10.0C | $93.8 \pm 23.5^{b,*;2}$ | $0.820 \pm 0.088^{b,*}$ | $13.3 \pm 3.7^{b,*}$ |

C = crushed leaves; M = milled leaves

Plant material supplied in February, 1990 (=Lot A) was used in this experiment. Insects placed in test chambers at 0-3 da post-adult emergence from bean.

[a] $P \leq 0.05$; Tukey-Kramer test.
[*] $P \leq 0.05$; pairwise t-tests.
[1] $P \leq 0.05$, pairwise t-test.
[2] $P \leq 0.05$, pairwise t-test.

| Dosage % wt/wt | Eggs Laid | Proportion hatched | Percentage of beans oviposited upon |
|---|---|---|---|
| 0 | $121.7 \pm 18.6^{a}$ | $0.970 \pm 0.011^{a}$ | $78.86 \pm 8.81^{a}$ |
| 1 | $109.3 \pm 24.2^{ab}$ | $0.959 \pm 0.017^{a}$ | $71.66 \pm 8.33^{ab,*}$ |
| 2 | $119.4 \pm 12.4^{a}$ | $0.953 \pm 0.020^{ab}$ | $59.80 \pm 15.34^{b,*}$ |
| 3 | $125.1 \pm 18.1^{a}$ | $0.956 \pm 0.021^{a}$ | $58.68 \pm 19.72^{bc,*}$ |
| 4 | $120.3 \pm 28.1^{a}$ | $0.903 \pm 0.077^{bc,*}$ | $40.01 \pm 19.25^{c,*}$ |
| 10 | $90.2 \pm 15.6^{b;*}$ | $0.860 \pm 0.029^{c;*}$ | $7.32 \pm 3.00^{d,*}$ |

Plant material was used in a crushed condition and supplied in June, 1990 (= Lot B). Insects placed in test chambers at 0-2 da post-adult emergence from bean.

[a] Means followed by same letter not significantly different; $P \leq 0.05$; Tukey's HSD test.
[*] Means significantly less than those for untreated control; $P \leq 0.05$; pairwise t-tests.

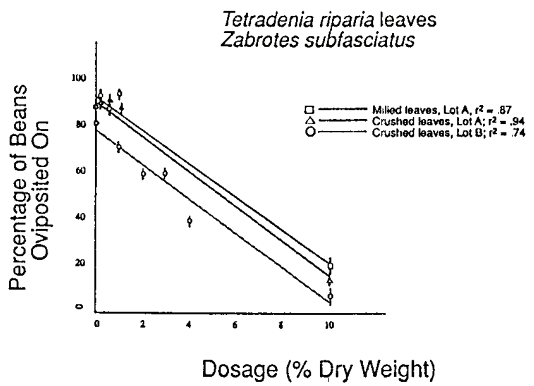


Figure 4. Percentage of beans oviposited (mean $\pm$ standard deviation) on by Zabrotes subfasciatus Bohem. when exposed to dosages of dry leaves of Tetradenia riparia (Hochst.) Codd (n=10; 5 males + 5 females 0-3 da post-adult emergence). Comparisons made between leaf preparations (crushed vs. milled) and plant supply (Lot A vs. Lot B).

Table III. Summary of results of human sensory preference measured by a trained Rwandan panel for beans cooked after 2 weeks exposure during storage to three plant products: crushed leaves of Tetradenia riparia (Hochst)CODD; neem seed extract (Margosan-O); and crude pyrethrin extract. The control was beans treated in the usual manner for storage at national warehouses (1% actellic).

| Bean Treatment | % preference for sample | % acceptability of sample |
|---|---|---|
| Actellic vs neem | 70–30 | 90–70 |
| Actellic vs *T. riparia* | 45–55 | 85–75 |
| Actellic vs pyrethrin | 55–45 | 100–79 |

| Bean Treatment | *% Appreciation of certain sample properties*[2] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Positive effects | | | | Negative effects | | | |
| | Ta | O | Te | A | Ta | O | Te | A |
| Actellic[1] | 54 | 8 | 23 | 15 | 15 | 15 | 23 | 47 |
| Pyrethrin | 41 | 17 | 25 | 17 | 0 | 8 | 42 | 50 |
| *T. riparia* | 56 | 11 | 33 | 0 | 11 | 11 | 22 | 56 |
| Neem | 56 | 11 | 22 | 11 | 11 | 22 | 11 | 56 |

[1] Actellic, in this experiment was considered the control because all beans sold by OPROVIA are treated with actellic.

[2] Ta = taste; O = odor; Te = texture; A = appearance


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# EFFETS DES REGULATEURS DE CROISSANCE D'UNE PLANTE MEDICINALE, *TETRADENIA RIPARIA* (HOCHST.) CODD (*LAMIACIAE*) SUR LES INSECTES DES HARICOTS ET DES GRAINS STOCKES

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**Résumé**

*Tetradenia riparia* est une menthe vivace dont les feuilles séchées sont utilisées en infusion par la médecine populaire au Rwanda dans le traitement de nombreuses maladies et autres cas où les micro-organismes jouent un rôle important. Des études de laboratoire entreprises par VanPuyvelde et ses collaborateurs ont confirmé les propriétés antimicrobiennes de cette plante sur *Candida albicans*, *Shigella dysenteriae* et *Streptococcus pyogenes*. Les préparations antimicrobiennes présentent également souvent des propriétés insecticides ou régulatrices sur la croissance des insectes, les feuilles de cette plante ont été étudiées en tant qu'agent protecteur potentiel du blé et des haricots stockés (*Phaseolus vulgaris* L.). Des mesures biologiques portant sur la croissance et le développement de plusieurs générations ont été entreprises sur des grains, *Rhyzopertha dominica* F. et , *Zabrotes subfasciatus* Bohem, aux concentrations (w/w) de 0 ; 0,1 ; 0,5 ; 1,0 et 10 %. Une nette diminution (P = O,O5) du taux de croissance larvaire et du développement a été observée chez *Z. subfasciatus* à 10 %. Des études portant sur la fécondité, la fertilité et la sensibilité des adultes ont également été entreprises. Des mesures biologiques faites en boîtes de Pétri à l'aide d'huile essentielle de *T. riparia* montrent que les adultes de Z. subfaciatus présentent un taux de mortalité élevé à 30 microlitres d'huile par cm2 de papier filtre comme substrat. Des études simultanées de chromatographie en phase gazeuse et de spectrométrie de masse montrent 206 pics distincts dans l'huile essentielle de cette plante. Le linalool est présent en petite quantité ainsi que d'autres substances défensives de la plante telles que la limonème et l'alpha-pinène