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Full Length Research Paper

Insecticidal potency of *Hyptis spicigera* preparations against *Sitophilus zeamais* (l.) and *Tribolium castaneum* (herbst) on stored maize grains

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Food situation has remained insecure and unpredictable in sub-Saharan Africa, leading to high levels of cyclic famine and poverty. This has been exacerbated by perennial pest problems, which cause substantial post-harvest food grain losses. It is against this background that this study was designed to investigate the use of *Hyptis spicigera* preparations for pest control and hence generate effective biological control options compatible with the prevailing farmers' circumstances. Laboratory and on-farm studies were conducted in order to assess the insecticidal potency of *Hyptis spicigera* extracts on maize weevils, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and red flour beetle (*Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Fumigant toxicity was assessed at four rates (0, 2, 4, 6, 8, 10 µl/l air) in space fumigation whereas repellence was evaluated in choice bioassay system at five rates (0, 0.2, 0.4, 0.6, 0.8 and 1.0% w/w). Contact toxicity was evaluated by the perforated jar method using dry powder, fresh leaves and oils while antifeedant activity was studied by the flour disk method. The results established that both whole plant parts and steam-distilled essential oil extracts possess strong insect repellent activity at reasonably low doses. Following exposure of newly emerged adult insects to 12 h of fumigation using essential oils at a dose of 25 µl/vial, 70% mortality was recorded for *H. spicigera* and 0% in the control. Whole plant powder had high potency compared to polar extracts which were not significantly efficacious to the insects. Hexane extract also showed the highest feeding deterrence activity compared to either ethyl acetate or water extracts. *Hyptis* oil extract on maize was also the most effective treatment at reducing insect infestation and seed damage compared to other treatments in the field. Whole plant extracts and essential oils described could be useful for managing field populations of *S. zeamais* and *T. castaneum*. Therefore, this study introduces an innovative approach to the use of traditional plant-based pesticides for grain protection in Kenya.

Key words: *Hyptis spicigera,* bioactivity, antifeedant, toxicity, repellancy, stored-product insects.

INTRODUCTION

Common insect pests threaten storage of grains especially in subsistence agriculture with its poor storage facilities. Peasant farmers predominantly store their staple cereal (maize and sorghum) grains in traditional granaries and a combination of traditional granary and bags whereas legume (beans, cowpea, green gram and groundnut) grains are almost exclusively stored in bags, with a mean storage duration of 5 and 3½ months for the cereal and legume grains, respectively. Global post-harvest grain losses caused by insect damage and other bioagents range from 10 to 40% (Raja et al., 2001). Currently, fumigants such as phosphine and methyl bromide are used as quick and effective tools for insect control in food commodities. Despite their significance in assuring quailty, several fumigants have been withdrawn or discontinued on grounds of environmental safety, cost, carcinogenicity, ozone depletion, insect resistance and other factors (Chaudhry, 2000; Shaaya and Kostyukovsky,

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2006).

There has been growing interest in the use of natural plant products for protection of agricultural commodities due to their low mammalian toxicity and low persistence in the environment (Raja et al., 2001). Although the molecular complexity, limited environmental stability and low activity of many biocides from plants, compared to synthetic pesticides, are discouraging, however advances in chemical and biotechnology are increasing the speed and ease with which man can discover and develop secondary compounds of plants as pesticides. These advances, combined with increasing need and environmental pressure, are greatly increasing the interest in plant products as pesticides. The use of "insecticidal" plants is especially prevalent in developing countries, where plants grown locally (which are renewable resources) are cheaper for subsistence farmers to use than are synthetic chemical pesticides. Commercially, however, only a few of these plants, especially those containing pyrethrins, rotenoids, and alkaloids, have been used as sources of bioinsecticides (Githinji and Kokwaro, 1994).

Plants in the family Labiatae are aromatic herbs with enormous socio-economic value in flavoring, cosmetics, perfumery, confectionery and medicinal preparations (Magness et al., 2006). Essential oils produced by these plants are a complex mixture of terpenes, sesquiterpenes, their oxygenated derivatives and other aromatic compounds. The actual chemical composition is a function of species, chemotype, climate, soil conditions and geographical location (Shaaya and Kostyukovsky, 2006). *Hyptis spicigera* L. is an erect hairy aromatic herb commonly found in the bushlands of southern Sudan, and western Kenya. Literature survey has revealed that *H. spicigera* is used as a trap plant against *Striga hermonthica* weed as well as an insect repellant in grain stores (Pendleton, 2007). It is also used as a relief to stomach ache and as a source of flavoring for pharmaceuticals. The objective of this study was to evaluate the toxicity, repellency and antifeedant activity of *H. spicigera* extracts against two major coleopteran pests of stored grains.

MATERIALS AND METHODS

Collection and preparation of test plants

Seeds of *H. spicigera* L., was supplied by Dr. Leo Onek and grown in Kibos Agricultural Field Station for three months. At maturity, samples of fresh aerial parts were harvested, placed in labeled bags and transported to Egerton University, Kenya, within 24 h. The samples were subdivided into three fractions, in which the first fraction was subjected to experimental tests while fresh. The second fraction was shade-dried and ground to a powder in a two-stroke engine hammer mill to produce whole plant powder. The third fraction was subjected to steam distillation and the oils collected over hexane. The oil was concentrated by blowing over a stream of nitrogen gas to produce oil extract.

Sourcing and rearing of test insects

Two insect species, maize weevils, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), were obtained from the International Centre for Insect Physiology and Ecology, Nairobi, Kenya. The insects were reared in Egerton University insectary (30℃, 85% r.h. and 12 h light/12 h dark photoperiods) on wheat grains at 14% moisture content throughout the research period. Western hard red spring wheat was obtained from the National Plant Breeding Research Center, Njoro, Kenya. Laboratory experiments were carried out at Egerton University, Njoro while on-field study was done at farmers' grains stores at Rachuonyo District, Western Kenya.

Contact toxicity of fresh leaves and powders

About 100 g of disinfected maize seeds were placed in plastic jars. 10 adult unsexed weevil (*S. zeamais*) or *T. castaneum*) were placed in each jar and covered with tight netted cloth. *Hyptis* preparations were placed in each jar at rates (in grams) and mixed with grains. Treatments were *Hyptis* dried powder and *Hyptis* fresh leaves, applied at rates (% w/w) of 0, 1, 2, 3, 4, and 5, with 4 replicates. Commercial insecticide, Actelic Super, was used as a positive control. Data on insect mortality was taken at 1, 3, 5, 7, 14, and 21 days after exposure. Any living adult insects were removed on day 14 to prevent overlap of generations during F1 counting.

Contact toxicity of crude oil extracts

Plant parts of *H. spicigera* (leaves, stems and roots) were collected dried and extracted twice using methanol (polar) and hexane (non-polar) separately. The oil extracts were concentrated to dryness, and 50 mg of each extract was applied to filter papers and placed at the bottom of a Petri dish. 10 live unsexed insects were placed in Petri dishes and covered with a lid and replicated 4 times. The treatments were: filter paper with methanol extract; filter paper with hexane extract; and 4 controls – methanol treated filter paper, hexane treated filter paper, filter paper treated with pyrethrum-based insecticide and untreated filter paper.

Fumigant toxicity of essential oils

The fumigant toxicity of *H. spicigera* essential oils against *S. zeamais* and *T. castaneum* was evaluated in space fumigation according to Shaaya et al. (1991). 20 unsexed adults of each test insect species were introduced into meshed metallic cages with a small amount of food and suspended from a hook in 3.4 L flat bottom glass fumigation chambers. Essential oil was applied to provide dosages of 0, 1, 5, and 10 (µl/l air) on small pieces of Whatman No. 1 filter paper and then suspended in the chamber slightly below the cage. Magnetic stirrers were used to ensure even distribution of fumigant over a 24 h exposure time. Adult mortality was recorded 24, 72, 120 and 160 h after treatment, and percentage adult mortality was computed according to Asawalam et al. (2006).

Repellency tests

Repellent effects of *H. spicigera* powder and oil against *S. zeamais* and *T. castaneum* was performed by a choice bioassay system based on that described by Ogendo et al. (2003) but with modifications. 5 basins were arranged for each treatment and floors of each basin divided into quadrants with marker pen. About 200 g of disinfected maize seeds were placed in all the 4 quadrants of each basin followed by treatments in only 2 opposing quadrants. The treatments were grams dried powder and millitres oil extracts of *H. spicigera*, applied at rates (% w/w for powder and % v/w for oils) of 0, 2, 4, 6, 8, and 10. A commercial insecticide, Actelic Super, was

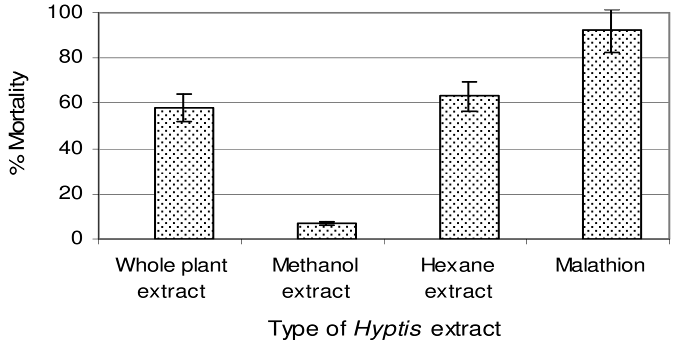

Figure 1. Toxicity of *H. spicigera* extracts on *S. zeamais*.

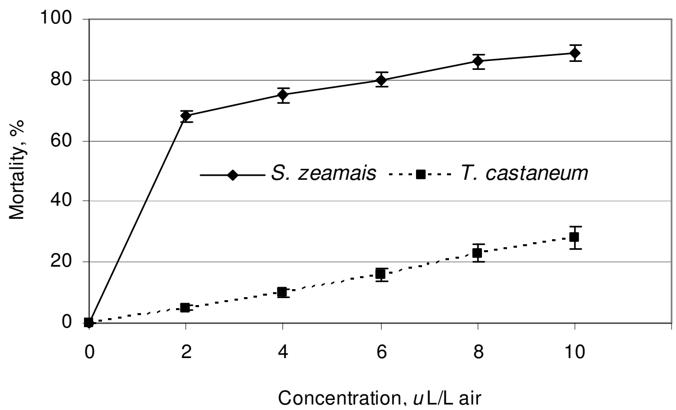

Figure 2. Percentage adult mortality of two insect pests 7 days after treatment with *H. spicigera* oil.

used as positive control. 10 adult unsexed weevils were then placed at the central circle of each basin and allowed to settle out. Data on number of insects in each quadrant was taken every 15 min for the first 1 h and then every hour for the next 11 h. Percentage repellency was calculated using the Talukder and Howse (1995) formula, PR = 2 (C – 50).

Antifeedant activity of *H. spicigera* extracts

The antifeedant bioassay was performed according to a method developed by Xie et al., 2006; Omar, et al., 2007). *Hyptis* dried powder was extracted for 24 h using hexane, ethylacetate and water. Only 1 ml of *Hyptis* extract solution containing the oil extract at a concentration of 0.5% w/w was added to 200 mg of hard red spring wheat flour and mixed using a magnetic stirrer. 10 aliquots (100 µl each) of the stirred suspension were placed in a 100 × 15 mm plastic Petri dish and allowed to dry overnight in air at room temperature. The next day, 5 of the flour disks were weighed and placed in a new Petri dish with 25 insects. The 6 treatments were: hexane extract, ethylacetate extract, water extract and negative controls (hexane, ethylacetate, and water only) each with *T. castaneum.* The Petri dishes were then sealed with para film paper and kept at 30°C and 85% r.h. for 3 days. Finally, the uneaten parts of the flour disks were weighed and recorded. The experiments were replicated 4 times.

Field evaluation of insecticidal potency of *H. spicigera*

The insecticidal potency of *H. spicigera* preparations was evaluated in two farmers' granaries in Lambwe and Oyugis field sites and a simulated granary at Egerton University, Njoro. Each 20 kg bag of maize was given known quantities of different preparations of *H. spicigera* and placed as far apart as possible from each other in each granary. The treatments were *Hyptis* powder (200 g/bag) mixed thoroughly with maize; *Hyptis* fresh leaves (1 kg/bag) in stratum; *Hyptis* oil extract (50 ml/bag) sprayed on maize grains; *Hyptis* oil extract sprayed on gunny bag; commercial insecticide, Super Actellic (5 g/ 90 kg bag), as positive control; and no additive as negative control. Data on insect incidence and grain damage rating (assessed as the grain weight change and seed viability) was collected every month for 4 months by sampling from every bag using the spear method.

Data analysis

Statistical analysis of the laboratory experiments was undertaken using one-way ANOVA ($p < 0.05$) after log transformation of the data. This included the data on percent insect mortality, repellence and flour consumption. Differences between treatment means were determined using Tukey's Studentized (HSD) test (Gomez and Gomez, 1984; Talukder and Howse, 1995).

RESULTS

Toxicity of *H. spicigera* fresh leaves, powders and crude oil extracts

The insecticidal effects observed as knockdown effects and reduced insect activity which was mostly limited to movement of appendages only, indicated that *Hyptis* essential oils possess toxicity to weevils. Both whole plant powder and hexane (essential oils) extracts possess strong insecticidal activity at reasonably low doses. *Hyptis* powders, oils and Malathion powders had percentage mortality of 65, 36 and 92% respectively. Although *Hyptis* preparations showed high insecticidal toxicity, methanol extracts were not significantly efficacious to the weevils (Figure 1).

Fumigant toxicity of *H. spicigera* essential oils

The results of space fumigation tests showed that *Hyptis* oil had high toxicity against adult *S. zeamais* but low effect on *T. castaneum.* The concentration of fumigant applied and time of exposure significantly influenced the percentage adult mortality of both the insects tested (Figure 2). At a concentration of 2ul/l air, *Hyptis* oil caused 68% mortality of *S. zeamais* in 2 days whereas *T. castaneum* showed very low mortality of 3%.

Repellency of *H. spicigera* preparations

Insect repellency was detectable 1 h after exposure and the results indicated that *S. zeamais* and *T. castaneum* differ in their susceptibility to *H. spicigera* extracts and

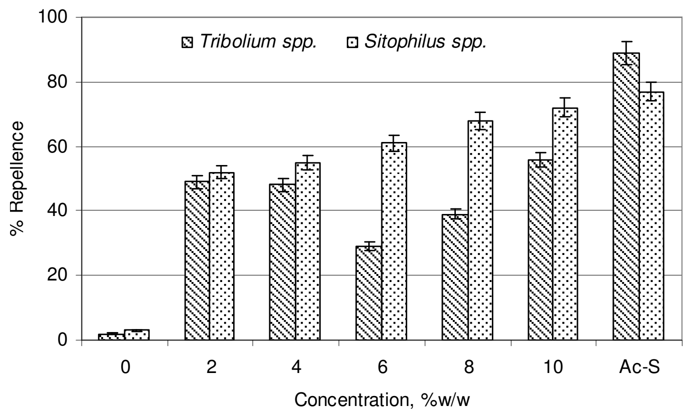

Figure 3. Effects of various doses of *Hyptis* powder on insect repellency for 12 h.

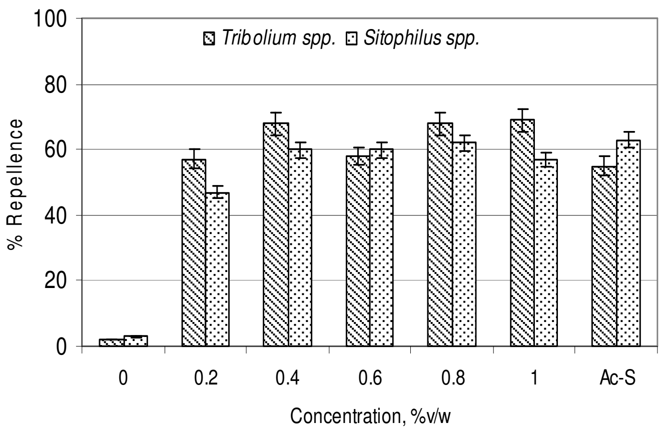

Figure 4. Effects of various doses of *Hyptis* spp oil extract on insect repellency for 12 h.

doses under laboratory conditions (Figures 3 and 4). In a two-choice bioassay (non versus treated), the grains treated with *H. spicigera* extracts had significantly fewer insects than untreated grains. *H. spicigera* oil extracts had higher PR values which were less dose dependent compared to the crude powder. The PR values for *Hyptis* powder against *T. castaneum* and *S. zeamais* were 49 - 56 and 52 - 72% respectively. For the *Hyptis* oil extract, the PR values against *T. castaneum* and *S. zeamais* were 57 - 69 and 47 - 57% respectively. In both cases, the commercial repellant had PR values of 55 - 89 and 63 - 77% respectively. The PR values for untreated controls were below 3% hence were not significant.

Antifeedant activity of *H. spicigera* extracts

Flour disks prepared using the crude extracts (ethyl acetate and hexane fractions) exhibited significantly high inhibition of diet consumption at 0.50% (w/w), but the water extract was found to be phagostimulatory. There was no significant difference at 0.5 and 1.0% (w/w) of extract concentration (Figure 5 and 6). Hexane extracts showed the highest feeding deterrence compared to either ethyl acetate or water extracts.

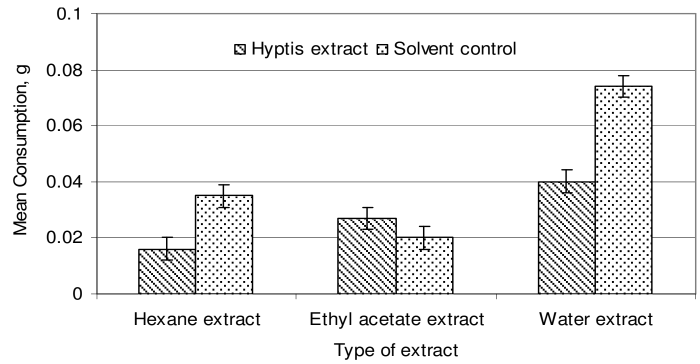

Figure 5. The effects of various *H. spicigera* extracts on feeding of *Tribolium spp* for 3 days.

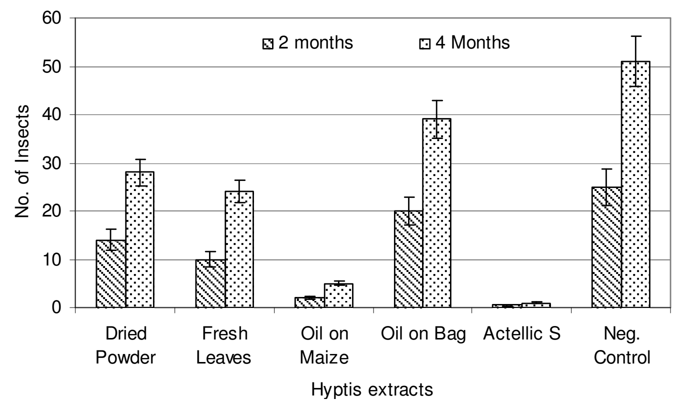

Figure 6. Effect of various *Hyptis* preparations on insect infestation rate of stored maize after 2 and 4 months.

On-field trials on insecticidal potency

Storage bioassays were run to assess the long-term effect of *Hyptis* dry leaf powder, fresh leaf stratum, oil extract on gunny bag and oil extract on grains. *Hyptis* oil extract on maize was the most effective treatment at reducing insect infestation and seed damage compared to other treatments (Figures 7 and 8). Seed viability tests also concluded that *Hyptis* oil extract, like Actellic Super, have no significant effect on the seed germination rate. At 4 months of storage, a rate of 85% germination was recorded in seeds treated with *Hyptis* oil extract and protected from insects, compared with 92% for untreated seeds that were not exposed to insects, as well as 48.5% for untreated seeds that were exposed to insects.

DISCUSSION

Documented information has shown the existence of significant intra-species variations in chemical composition

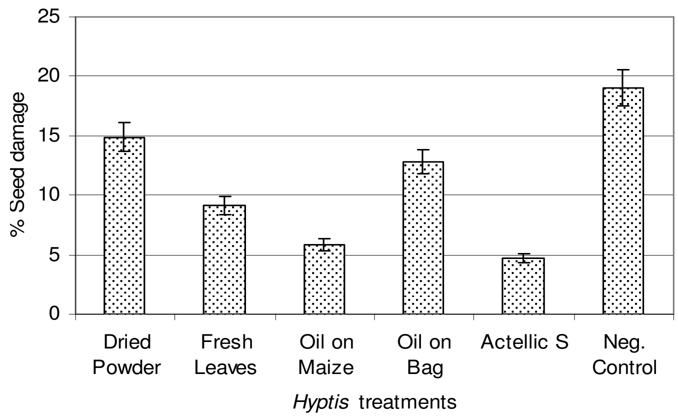

Figure 7. Effects of various *Hyptis* treatments on maize seed damage after 4 months of storage.

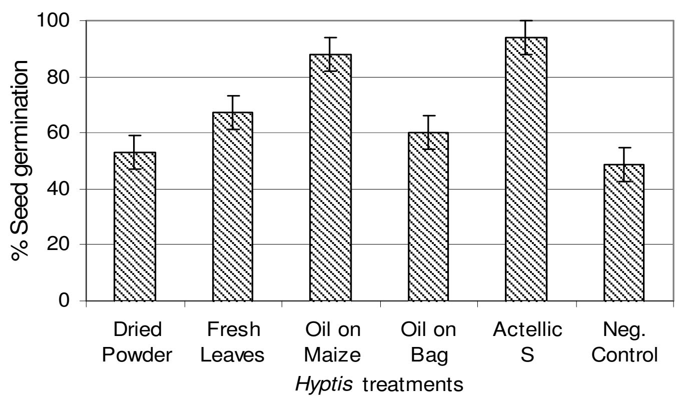

Figure 8. Effects of various *Hyptis* treatments on maize seed viability after 4 months of storage.

of many herbs which is dependent on season, time and stage of harvest and geographical origins (Tchoumbougnang et al., 2006). This intra-species variation makes a strong case for comparative bioactivity studies involving collections from various geographical origins in the region. From the choice bioassay tests, *Hyptis* preparations exhibited strong dose- and exposure time-dependent repellence against the insects tested. Both whole plant powder and steam-distilled oil extracts possess strong insect repellant activity at reasonably low doses.

The fact that *Hyptis* oil at a concentration of 0.2ul/l air was potent enough to achieve 68% kill of *S. zeamais* 7 days after treatment offers a practical potential. It is also evident here that data on adult insect mortality need to be collected for at least 1 week after treatment in order to measure final efficacy. The differential inter-specific insect responses to *Hyptis* oil could be attributed to compound structure-activity relationships and physiological-structural induced cellular changes resulting in poisoning of insects by blocking octopamine receptors (Priestly et al., 2006). Results of our study compare favourably with other investigations in which essential oils from Labiatae family plants produced significant fumigant activity against stored-product insects (Shaaya and Kostyukovsky, 2006).

To date, numerous plant extracts are known to possess strong feeding deterrent activity (Arnason et al., 1987). This is a frequent finding in studies on plant bioactivity against insects because the extracts contain non-polar essential triterpenoids. The crude extracts were more active than the isolated compounds. This may be the result of synergistic effects of the compounds in a mixture (Xie et al., 1996) and/or the presence of other unidentified active compounds. The concentration used to observe antifeedant effects was much higher than for the commercially available products such as Margosan-O, active at a 3.75 ppm azadirachtin level or toosendanin at 20 ppm (Xie et al., 1996). Thus, the commercial application of *H. spicigera* extracts as a stored-product antifeedant may not be practical. Nonetheless, the antifeedant bioassay provides a rapid and inexpensive method for screening novel compounds available in small quantities to assess their activity as insect antifeedants.

Results from field experiments were more or less consistent with laboratory experiment results but this does not negate the importance of conducting field experiments to validate the efficacy of a botanical where laboratory testing indicates some activity. These results suggest that the use of *Hyptis* oil extracts might be more effective at reducing maize infestation by *S. zeamais* and *T. castaneum* than the use of *Hyptis* dry leaf powder or fresh leaf stratum. This might have very practical consequences for small-scale farmers, who may be persuaded to replace the traditional application of *Hyptis* fresh leaf stratum with oil extract on grains, obtaining a more effective protection of their stored commodity.

CONCLUSION

The results from this field trial are of practical consequence for local farmers, who can improve their traditional method of maize protection by using oil extracts of *H. spicigera* instead of the dry powder. Nevertheless, the antifeedant bioassay technique employed in this study clearly allows a fast screening and isolation of bitter compounds present at low concentrations in the active crude extracts. In conclusion, whole plant extracts and essential oils described could be useful for managing field populations of *S. zeamais* and *T. castaneum*. Therefore, this study introduces an innovative approach to the use of traditional plant-based pesticides for grain protection in Kenya.

These findings show very strong extension potential. Upscaling to involve more farmers should be carried out to confirm the practicability, adoptability and sustainability of the new technology. Cost-benefit analysis for one farming year is needed to add value to the new technology. Finally, processing of the new technology for national performance trials (NPT) needs to be done. This involves making available *Hyptis* insecticide preparations, the technical report on its efficacy, cost-benefit analysis, and a draft of farmers' manual.

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