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Bioactivity of cypress leaf powder (*Cupressus macrocarpa*) on cowpea weevil (*Callosobruchus maculatus* Fabr. Coleoptera: Bruchidae) and maize weevil (*Sitophilus zeamais* Motschulsky, Coleoptera: Curculionidae) in stored maize grains in Cameroon

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Abstract

Cowpea weevil (*Callosobruchus maculatus*) and maize weevil (*Sitophilus zeamais*) are very destructive pests of cowpea and maize respectively. Synthetic chemicals have been used extensively to control these pests. The present study investigated the potential of cypress leaf (*Cupressus macropcarpa*) powder as a biological agent against these pests. Three doses (g) of cypress leaf powder (2, 5 and 10g) were exposed to 10 adult insects in triplicate and mortality was observed every 24hrs for 3days. A repellency test was also conducted in triplicate using 3 doses (g) (2, 5, and 10g) of cypress leaf powder. The experimental design was Completely Randomised Design (CRD). The experiments were conducted in a laboratory (28 ± 2 ^{o}C and 80 ± 10% RH) in the Farm house of the Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon The results revealed that, the cypress leaf powder did not cause any significant mortality ($p > 0.05$) compared to the controlled for both insect pests. The repellency test revealed that the cypress leaf powder showed a very high potential as a repellent agent ($p < 0.05$) with up to 50 – 60% of both insect pests repelled just after 10mins of exposure to the smallest dose (2g). About 90% repellency was observed for both pests from the highest dose (10g) within 10mins of exposure making 10g a perfectly repellent dose. Our research supports strongly that cypress leaf powder has a strong repellency effect on both pests and little or no potential to cause significant mortality. Thus cypress leaf powder is a good candidate to incorporate in integrated pest management (IPM) programmes for control of cowpea and maize weevil in stored grains by poor-resourced farmers and store keepers in Cameroon and the world at large. Thus, appropriate technology transfer systems should be developed and passed on to poor-resources farmers to promote a direct preparation of indigenous pesticides at farm-house level.

Key words: *Cupressus macrocarpa, Sitophilus zeamais, Callosobruchus maculatus*, IPM, CRD, repellency

Introduction

Cowpea [*Vigna unguculata* (L.) Walp.] is a staple food for many countries in Africa including Cameroon. It serves as human food and fodder for livestock (Nwaogu et al., 2013). Like other crops, cowpea is pruned to attack by a wide spectrum of pests and diseases (Jibrin et al., 2013). Insects attack almost all parts of this plant. The most important insect pest of cowpea is the cowpea weevil *Callosobruchus maculatus*, Fabr (Coleoptera: Bruchidae) able to cause about 90% - 100% loose if unchecked from field to storage (Ajayi and Wintola, 2006).

Maize (*Zea mays* L.) is an out-breeding heterogeneous crop close to being a staple food for many impoverished African countries contributing about 20% – 30% of total carbohydrate consumption (Ristanovic, 2001). Maize is equally attacked by a wide array of pests and diseases. Among the insect pests, maize weevil *Sitophylus zeamais* Motschulsky (Coleoptera: Curculionidae) stands out as the most damaging pest of maize causing about 80% - 100% damage in stored grains (Obeng-Ofori and Akumoah, 2000; Akob and Ewete, 2007). *Sitophilus zeamais* is a cosmopolitan pest of sound and wholesome grains in the tropics and temperate regions of the world. Field survey and modeling have shown that *S. zeamais* density is directly proportional to grain damage and weight loss (Holst et al., 2000).

These two major insect pests have threatened the economy of many African countries as they attack the staples that can be afforded by many. Infestation can result in reduced quantity and quality of grain; ranging from unsuitability of grain for consumption, weight loss to reduced viability (Singh and Abdullahi, 2011). Several control measures have been adopted over the decades to curb the problem of insect infestation. These include improving storage organs, use of chemical agents or synthetic insecticides, biological, physical, genetic and legislative control methods etc (Srivastava, 1988). The use of chemical agents or synthetic insecticides is overwhelming over physical or traditional methods due to ease of application and their ability to guarantee immediate results (Oni, 2011). The incessant use of these insecticides (Afreh-Nuamah, 1984; Achiri, 2013) has raised a public and environmental outcry on the residual effect of synthetic pesticides. Concerns are centered about their effect on human health, non-target organisms; wide spread environmental hazards and the insect's ability to develop resistance to some of these insecticides over time (Yusuf et al., 2006) and the cost usually associated with purchasing them. Thus the need for eco-friendly and cheaper pest control method is inevitable (Asawalam et al., 2008). Many bacteria, fungi, animal and plant derivatives have been exploited for this purpose. Plant derivatives have been overwhelmingly exploited due to their availability and ease to work with (Adedire et al., 2011) ether as dust/powders or extracts in different solvents. Plant products have been proven to contain some natural toxins that can serve as botanical insecticides with an added advantage that they have a natural ability to breakdown rapidly and not producing toxic residues (Alan et al., 2009). Many plant families have anti-insect compounds such as: Amaranthaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Meliaceae, Pperaceae, Poaceae, Rosaceae, Solanaceae and other (Potenza et al., 2002).

In line with these researches in search of cheap and eco-friendly insect control methods, we evaluate the potential of cypress leaf powders (Cuppressaceae) which is abundant in the Northwest Region of Cameroon. Cypress is planted is this region of Cameroon for many purposes: it is planted around residence and institution for aesthetic value and to guarantee some sort of security. It is shaped by gardeners into different structures: effigies, maps, animals, labels and other structures to behold. It is also planted for wood used in construction and fuel. It can grow to heights of about 20m as well as being dwarfed by the owners for different reasons. Preliminary studies showed that the leaves of this plant are interspaced with maize in bands in and around smoked kitchens in the North West Region of Cameroon. This experiment is designed to evaluate the mortality and repellency effect of the leaf powders against these two stored product pests. The experiment was conducted during the period on March, April and May of 2014.

Materials and method

Insect culture

Insects used to establish a laboratory colony of *C. maculates* and *S. zeamais* came from a batch of infested cowpea and maize purchased from the Bamenda Main Market (North West Region, Cameroon). 2kg each of cowpea and maize grains was collected independently and stabilized by heating for 10mins at 50 ^{o}C and then allowed to cool in order to kill all insects and pre-infested grains. Broken and holed grains were removed from the lot. The sterilized grains were stored in glass jars covered with muslin cloth. 50 adults of cowpea and maize weevils were introduced into the sterilized cowpea and maize grains respectively and stored in a laboratory (28 ± 2 ^{o}C and 80 ± 10% RH) in the Farm house of the Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon. The insects were removed after 1week, during which eggs would have been laid. The jars were allowed undisturbed for 8weeks for the F1 and F2 adult emergence; these were used as test insects.

Leaf powder preparation

The fresh leaves were harvested in Bamenda (North West Region of Cameroon) and kept in an open, clean room to air dry without sunlight for 3 weeks. Dried leaves were ground to powder using a grinding mill. The powder was stored in an air-tight container until time for use.

Data was collected on

- Mortality (contact toxicity)
- Repellency and the repellency dose required to repel 50% (RD_{50}) of the insects and the fiducial limits estimated

Contact toxicity (mortality test)

Four different doses of cypress leaf powder (0g, 2g, 5g, and 10g) were introduced into clean sterilized glass jars containing 100g of uninfected sterilized cowpea grains. 10 unsexed adult cowpea weevils were introduced into each jar. Mortality count was recorded every 24hrs for 3 days dead insects were removed from the set. An insect was considered dead or moribund if no movement was observed after being probed with a camel hair brush. The experimental design was as Completely Randomised Designed (CRD) with treatments in triplicates. The experiment was repeated for maize weevil. Analysis of Variance (ANOVA) was used to test for significant differences between the mean (± standard deviation) of mortality count from the different doses. Means were separated using Duncan Multiple Range Test (DMRT) at 95% level of confidence using SPSS (version 16.0).

Repellency test

The repellency effect of the plant powder against cowpea weevil and maize weevil was assayed using the method of preferential zone on a filter paper described by McDonald et al., (1970) with some minor modifications. A petri dish was lined with a Whatman filter paper (No. 10). The paper was divided into 3 equal zones along the diameter of the petri dish using a line drown with an HB pencil. 10 unsexed adult insects were starved for 24hrs in a clean glass jar. 30g of sterilized cowpea grains were placed at the center of the two extreme zones of the petri dish. Plant powders (0g, 2g, 5g and 10 g) were place at one heap of grain at one of the extreme zones in the petri dish. 10 starved adult cowpea weevils were placed at the center of the central zone of the divide and the number of insects moving into the two extreme zones was recorded after 10mins. The experiment was conducted in triplicate for each dose of the plant powders in CRD. The process was repeated for maize weevil using maize. Percent repellency was calculated using the formula proposed by Alzouma 1992;

$$PR = \frac{NC - NT}{NC + NT} \times 100$$

Where:

NC – number of insects in the controlled zone (no plant powder)

NT – number of insects in the treated zone (plant powders available)

PR – percent repellency. The PR was ranked in six different classes as described by McDonald et al (1970) as shown below:

Percent Repellency (PR) classes ranked by McDonald et al., (1970)

Class	**PR proportion (%)**	**Description**
O	PR < 0.01	Not repellant
I	0.1 < PR ≤ 20.0	Fair repellant
II	20.1 ≤ PR ≤ 40	Moderate repellant
III	4.01 ≤ PR ≤ 60	Good repellant
IV	60.1 ≤ PR ≤ 80	Very repellant
V	80.1 ≤ PR ≤ 100.0	Perfect repellant

Source; McDonald *et al*., 1970

Percent repellency less than one was considered zero (Obeng-Ofori and Akuamah, 2000). Data from repellency test was analyzed using chi square test to assess the repellency activity of the various powder doses of cypress leaf and the susceptibility of the different weevils.

PR_{50} was calculated using Finney (1971) method based on the probit regression of mortality as a function of the logarithm of plant powder doses. All analysis was done using SPSS (version 16.0).

Results

Contact toxicity

Table 1 and Table 2 show the mean (± SD) of the mortality counts from the contact toxicity test for cowpea weevil and maize weevil respectively. There was no significant difference in the mean mortality from the different doses of cypress plant powders for cowpea weevil and maize weevil. Chi square comparison of mortality susceptibility of cowpea weevil and maize weevil to different doses of cypress leaf powder revealed no significant differences in mean mortality over the different days of exposure (Figure 1)

Repellency

Table 3 show the mean (±SD) of cowpea weevil and maize weevil repelled by different doses of cypress leaf powder after 10 minutes of exposure. The chi-square test was conducted on counts. The result reveals that the highest concentration (10g) of leaf powder had a strong repellent effect: 9.0 ± 1.00 and 9.0 ± 1.00 for cowpea weevil and maize weevil respectively described according to McDonald et al (1970) as perfectly repellent. The percent repellency is thus greatest for the highest dose as shown in figure 2 for both stored-product pests. PR ranged from 'perfect repellent' (> 80%) to 'moderate repellent' ($20.1 \leq PR \leq 40.0$).Though the repellency effect was not significantly different for stored-grain pest, it is clear that more plant powder s required maize weevil to elicit a similar response in cowpea weevil as revealed even in the PR_{50} and PR_{90} (Table 4). The chi-square test comparing the repellency susceptibility of cowpea weevil and maize weevil against the different doses of cypress leaf powder reveals that the was no difference ($\chi^2 = 0.057$, df = 3, p = 0.996) in level of repellency susceptibility for both store-grain insects (Figure 3).

Discussion and Conclusion

The utility of botanicals for protection against pest depends upon its mortality rate in target organisms, reduce fecundity, growth rate and behavior alteration. The effect of botanicals has been well documented in affecting these parameters.

Two parameters; mortality and repellency were tested in this experiment against two major pests of stored grains. Incorporation of different doses of cypress leaf powders into cowpea and maize grain and introduction of *C. maculatus* and *S. zeamais* respectively provided a simple and rapid bioassay of plant materials.

The results revealed that cypress leaf powder did not show any significant potential to cause mortality in cowpea and maize weevil after 3 days of storage. This indicated that the plant powders (2, 5 and 10g) did not have some toxic substances required to negatively impact the survival of the adults of cowpea and maize weevils or that the period of exposure had to be extended or that the potency of the toxic substances within cannot be elicited in powder form. However, other works with some plant powders have shown high degree of mortality causation. In Nigeria, Epidi et al., (2008) reported that *Dracaena arborea* and *Vitex grandifolia* leaf powders were able to effectively suppress about 60% - 70% of the survival of these two major pests over a period of 3 – 7 days with doses of 1 – 3g.

Most botanical assays have been documented using extracts in different solvent (aqueous or oils). Perhaps the understanding of the mortality potential of cypress leaf powders may be revealed if extracted in aqueous or oil base. Though our results revealed an almost insignificant mortality rate, it is clear that cowpea weevil was more susceptible to cypress leaf powder than the maize weevil.

The results further revealed a very efficacious repellency potential of cypress leaf powder against cowpea and maize weevil with all doses repelling at least 50% of the insects within 10mins of exposure. The smallest PR (57.0& and 60.0%) for maize and cowpea weevil respectively was recorded by 2g of cypress leaf powder and the highest PR (90%) for both insects was recorded by 10g of cypress leaf powders. The cypress leaf powders seem to be more of a repellent than a causation of mortality with a parallel increase of repellency with dose (g). The repellency experiment also revealed that there was no clear cut significant differences in repellency susceptibility between cowpea and maize weevil, more cypress leaf powder was required to repelled 50% (PR_{50}: 1.20g) and 90% (PR_{90}: 10.4g) of maize weevil compared to 1.04g and 8.15g to cause a similar effect for cowpea weevil respectively. This degree of repellency is often reported from botanicals extracted in essential oils; having observed such degree of repellency from cypress leaf powder which requires little or no skill and low cost to prepare makes cypress leaf powder an attractive candidate in the management of stored-grain insect pests like cowpea weevil and maize weevil. The above results support strongly that cypress leaf powder have a repellency effect on these two insect pests. Though the mode of action and the constituent active ingredient elucidating this repellency behaviour was not revealed in this study, the repellency behavior of both stored-grain insect pests may be due to suffocation and inhibition of different biosynthetic process of the insect's metabolism (Don-Perdo, 1989).

Botanical toxicants are broad-spectrum in pest control, safer to apply and can be easily produced by small-scale poor resource farmers and therefore likely to be less expensive (Owusu-Akyaw, 1991; Baba et al., 1992). Obeng-Ofori and Akuamah (2000) posit that thorough investigation into use of botanicals especially developing them into powdered dusts for use by resource-poor farmers who cannot afford synthetic pesticides should be a priority. Botanical pesticides thus represent an important potential for integrated pest management programmes in developing countries as they are cheap, available and sourced from local material.

Conclusion

This high level of repellency observed compels us to strongly recommend cypress leaf powder or dust as cheap, easily available, eco-friendly good alternative to synthetic insecticides against *C. maculates* and *S. zeamais*. Full incorporation of this plant powder could further minimize the use of synthetic chemicals. Moreover, application of this powder is not likely to have harmful residues against mammals since they are rapidly degraded to non-toxic derivatives. Thus, appropriate technology transfer systems should be developed and passed on to poor-resource farmers to promote a direct preparation of indigenous pesticides at farm-house level from cypress leaf.

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Table 1. Mean (±SD) mortality count from contact toxicity bioassay of cypress leaf powder against cowpea weevil

Dose (g) plant powder	**Mean mortality count and the days of exposure**		
	1 Day	**2 Days**	**3 Days**
0	0.0^{a} (± 0.00)	0.0^{a} (± 0.00)	0.0^{a} (± 0.00)
2	0.3^{a} (± 0.38)	0.3^{a} (± 0.58)	0.7^{ab} (± 0.58)
5	1.3^{a} (± 1.52)	0.0^{a} (±0.00)	0.3^{a} (± 0.58)
10	0.3^{a} (± 0.57)	0.3^{a} (± 0.00)	1.0^{b} (± 1.00)

Mean mortality count of 10 cowpea weevil per dose of cowpea leaf powder in triplicate. Mean in the same column with the same superscripts are not significantly different according to DMRT ($p < 0.05$), SD – Standard deviation.

Table 2. Mean (±SD) mortality count from contact toxicity bioassay of cypress leaf powder against maize weevil

Dose (g) plant powder	**Mean mortality count and the days of exposure**		
	1 Day	**2 Days**	**3 Days**
0	0.0^{a} (± 0.00)	0.0^{a} (± 0.00)	0.3^{a} (± 0.58)
2	0.3^{a} (± 0.38)	0.0^{a} (± 0.00)	0.7^{a} (± 0.58)
5	0.7^{a} (± 1.16)	0.3^{a} (±0.58)	1.0^{a} (± 1.00)
10	0.7^{a} (± 1.16)	0.7^{a} (± 0.58)	1.0^{a} (± 1.00)

Mean mortality count of 10 maize weevils per dose of cowpea leaf powder in triplicate. Mean in the same column with the same superscripts are not significantly different according to DMRT ($p < 0.05$), SD – Standard deviation.

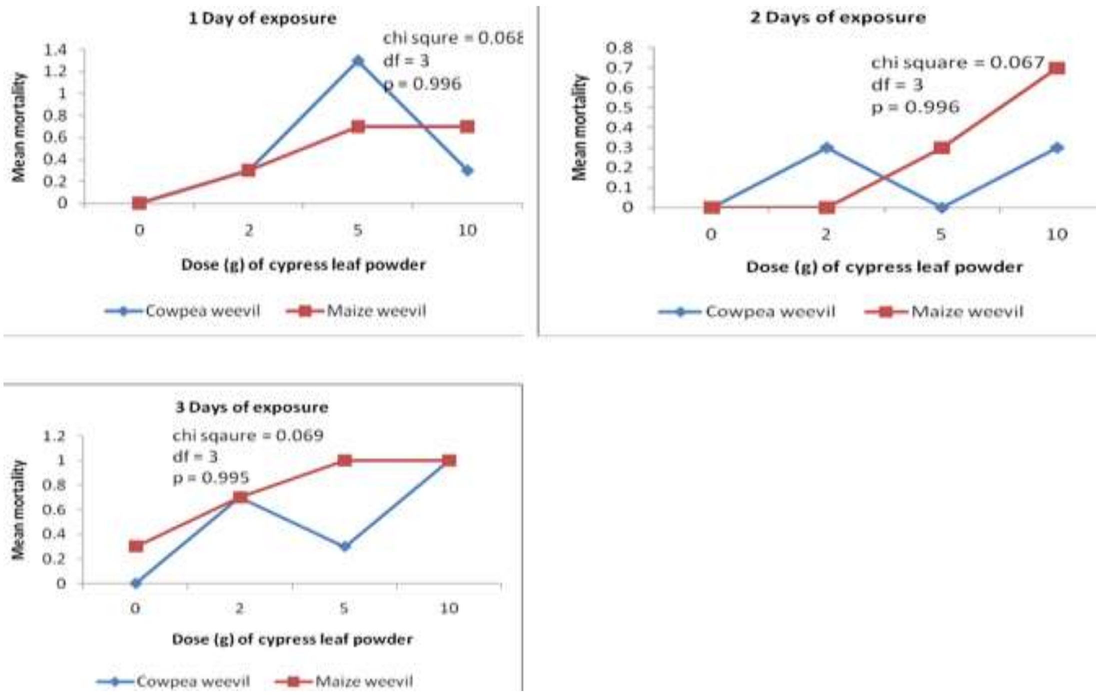

Figure 1. Comparison of mortality susceptibility of cowpea weevil and maize weevil to different doses of cypress leaf powder (Chi square test for comparison of mortality of 10 cowpea weevils and 10 maize weevils exposed to different doses of cypress leaf powder. Chi square test was conducted on (x + 5): where x – is the number of dead or moribund insects and 5 is a constant added to improve the reliability of the chi square analysis).

Table 3. Repellency caused by cypress leaf powder against cowpea weevil and maize weevil after 10mins in petri test of preferential zone

Insect type	Dose (g) of cypress leaf powder	Mean (±SD) number of insects in the controlled zone	Mean (±SD) number of insects in the treated zone	χ^2 = 17.366, df = 3, p = 0.001
	0	4.3 ± 0.58	4.7 ± 0.58	
C. maculatus	2	6.0 ± 1.00	3.3 ± 1.16	
	5	7.0 ± 1.00	1.7 ± 0.58	
	10	9.0 ± 1.00	0.3 ± 0.58	
				χ^2 = 12.803, df = 3, p = 0.005
	0	4.3 ± 1.50	4.3 ± 1.53	
S. zaemais	2	5.7 ± 0.58	3.3 ± 0.58	
	5	6.7 ± 0.58	2.3 ± 0.58	
	10	9.0 ± 1.00	0.7 ± 0.57	

Repellency caused by cypress leaf powders against cowpea weevil (*C. maculates*) and maize weevils (*Sitophilus zeamais*) after 10mins in petri dish test of preferential zone

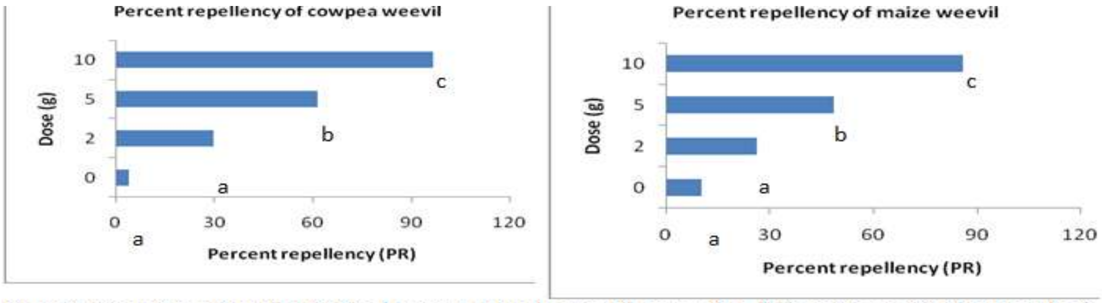

Figure 2. Mean Percent repellent (PR) of cowpea and maize weevil exposed to different doses (g) of cypress leaf powder after 10mins (Mean PR of 10 insects after 10mins of exposure to different doses of cypress leaf powder. Means in the same chart with the same letters are not significantly different using DMRT at p = 0.05)

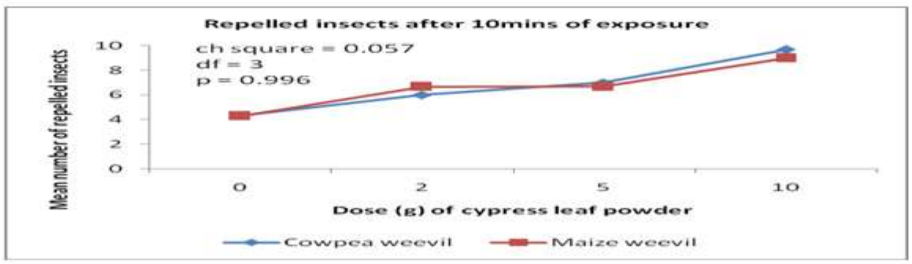

Figure 3. Comparison of susceptibility of repellency effect of different doses of cypress leaf powder between cowpea weevil and maize weevil after 10mins of exposure

Table 4. PR_{50} and PR_{90} for cowpea weevil and maize weevil after 10mins of exposure to cypress leaf powder

	PR	**PR value g/10insects**	**95% fiducial confident interval**	
			Lower limit	**Upper limit**
Cowpea weevil	PR_{50}	1.043	1.418	2.464
	PR_{90}	8.150	1.172	12.764
Maize weevil	PR_{50}	1.20	2.185	2.963
	PR_{90}	10.44	7.689	18.044