__Original Research Article__

Cypress (*Cupressus Macrocarpa*) Leaf Powder Modulates Metabolism of Maize Weevil *Sitophilus zeamaiz* Motschulsky (Coleoptera: Curculionidae)

ABSTRACT

Aims: Maize grains are severely damaged by maize weevil *Sitophilus zeamais* Mostchulsky (Coleoptera: Curculionidae). Exploiting botanicals against synthetic insecticides as a control strategy has gained prominence in recent decades. The aim of the study was to investigate the potential of cypress (*Cupressus macrocarpa*) to exert some control on *S*. *zeamais*.
Methodology: Maize and *S*. *zeamais* were collected from Muea market in Buea, Cameroon. The maize was heat sterilized and all debris removed. *Sitophilus zeamais* was reared for three generations before use. Ten *S*. *zeamais* were stored with 100 g of maize and 0 g, 2 g, 5 g, and 10 g of shade-dried cypress leaf powder was added in three replicates. Data was collected weekly for 5 weeks and analyses were done using MS Excel (2016) and PAST (ver. 4). The study was conducted in a laboratory of the Teaching and Research farm in the University of Buea, Cameroon, in a randomized complete design.
Results: Mortality of *S*. *zeamais* was significantly affected (F = 126.67, df = 3, 8, p < .001) by different doses of cypress leaf powder. The mean mortality was 0%, 40.47%, 50.6% and 70.67% for control, 2 g, 5 g and 10 g of cypress leaf powder, respectively. Grain damaged (%) and holed grain were also influenced by cypress leaf powder. The highest grain damaged and holed grain were from the control treatment. A strong positive correlation (r = 0.814, p < .001) was observed between grain damaged and holed grain while a strong negative correlation was observed between mortality and holed grain (r = -0.926, p < .001), and grain damaged (r = -0.913, p < .001).
Conclusion: Based on the findings of this study, it is revealed that cypress leaf powder can alter some metabolic activities of *S*. *zeamais*, thus it can play a role in an integrated pest management program (IPM) of *S*. *zeamais* in maize storage.

Keywords: cypress, maize, holed grain, damaged grain, IPM, metabolic, mortality, *Sitophilus zeamais*.

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important staple in the world, particularly in Africa where it is commonly grown by smallholder farmers [1][2]. Like many stored food grains, damage of stored maize grains is a very serious global problem [3]. The situation is worse in developing countries with poor-resource farmers and inadequate storage facilities. Africa is suffering from 20-30 % postharvest losses [4]. with some farmers experiencing 100% postharvest losses in severe infestations. The principal objective of storage is to guarantee food security and that agricultural products are preserved for the realization of price stabilization, industrial input provision, enhancement of a nations reputation and seeds for planting [5]. These objectives are hardly attained in developing nations due to a plethora of factors amongst which insect pests play a vital role, causing, in some cases, irreparable damage, resulting in major economic losses [6]. Maize grains are vulnerable to many insect pests during storage. Insect pests from the order Coleoptera and Lepidoptera are the most damaging to stored maize grains [7]. [8] and [9] mentioned that lesser grain borer *Rhyzopertha dominica* (Fabricius), weevil complex *Sitophilus* spp. khapra beetle *Trogoderma granarium* Everts, red flour beetle *Tribolium casteneum* (Herbst), larger grain borer *Prostephanus truncatus* Horn and *Callosobruchus* spp. are very important coleopterous pests. The maize weevil *Sitophius zeamais* Motchulsky (Coleoptera: Curculionidae) is one of the major primary pests of stored maize in Africa [1][10]. Females lay eggs into the grain which on hatching, the larva feeds towards the inside of the grain until pupa. The adults emerge and eat their way towards the testa causing exit holes resulting into insect damaged grain [11]. *Sitophilus zeamais* infestation results into severe losses due to direct consumption of maize grains, in addition, their activity increases temperature and moisture conditions thus accelerating the growth of mould such as mycotoxins [12], leading to quality deterioration. It is estimated that about 50% maize grain loss is due to *S. zeamias* infestation [13]. Consequently, the control of this pest is imperative. Presently, farmers rely heavily on the use of synthetic insecticides to control *S. zeamias* in Africa. In traditional farm stores in Africa, the use of synthetic insecticides for grain protection has been partially successful [14]. However, questions have been raised about the safety and prolonged use of synthetic pesticides as pests control strategy - synthetic pesticides treadmills have resulted into negative environmental impacts, harm on non-target species, development of resistance in some insect species, increased cost of application, direct hazards from direct exposure and high residues in food and erratic supply of these insecticides in developing countries [10][13][14][15][16]. These problems have rekindled the interest in the re-evaluation of traditional botanical pests control methods which are cheaper, readily available and environmentally friendly [17]. Researches on botanical on *S. zeamais* are many and varied, with emphasis on plant types and plant parts (roots, rhizomes, bulbs, stems, tree bark, leaves, fruits, seeds) and methods of delivery (ash, powder, fumigant, in solvents) (Table 1). In line with previous studies (e.g Table 1), the current study evaluates the antifeeding and mortality potential of cypress leave powder on the maize grain weevil *Sitophilus zeamais*.

Table 1. List of some plants in Africa evaluated for their insecticidal properties on *Sitophilus zeamais*

Scientific name	**Common name**	**Family**	**Part(s) evaluated**	**Manner of exposure**	**Reference**
Azadirachta indica	Neem	Meliaceae	Seeds	Powder	[18]
Jatropha curcas L.	Physic nut	Euphorbiaceae	Leave, bark, seeds	Powder	[19]
Citrus sinensis L.	Orange	Rutaceae	Peels	Powder	[19]
Capsicum frutescens L.	Chilli	Solanaceae	Fruits	Powder	[20][21]
Allium cepa	Onion	Liliaceae	Bulb	Powder	[20]
Piper guineense	Black pepper	Piperaceae	Seeds	Powder	[13][20][21]
Allium sativum L.	Garlic	Liliaceae	Bulb	Powder	[22]
Nicotiana tabacum L.	Tobacco	Solanaceae	Leaves	Powder	[22]
Ageratum	Billygoat	Asteraceae	Leaves	Solvents	[23]

conyzoides	weed				
Lantana camara	Lantana	Verbenaceae	Leaves	Solvents	[23]
Chromolaena odorata	Siam weed	Asteraceae	Leaves	Solvents	[23]
Curcuma longa L.	Turmeric	Zingiberaceae	Rhizome	Solvents	[24]
Gnetum africanum	Eru	Gnetaceae	Leaves	Solvents	[24]
Ocimum basilicum	Basil	Lamiaceae	Leaves	Powder	[25]
Ricinodendron heudelotii	Njangsa	Euphorbiaceae	Leaves, bark, roots	Powder	[26]

2. MATERIAL AND METHODS

This study was conducted in one of the laboratory of the Teaching and Research Farm, House of the Faculty of Agriculture and Veterinary Medicine of the University of Buea. It is located at the foot of mount Cameroon in Fako Division, South West Region of Cameroon, at latitude/longitude 4^0 9' 33" N/9^0 14' 12" E. It was conducted at an average temperature of 27 ^{o}C and 65% relative humidity. The experiment was laid out in a complete randomized design (CRD) with 4 treatments and 3 replicates. The study was conducted in 2014.

2.2. Maize and insect collection

1.5kg of dry maize was bought from traders in the Muea market located in Buea municipality, south west region, Cameroon. Cracked, broken grains and other debris were sorted out from the others. The maize grains were heat sterilized at 40-50 ^{o}C for 20 mins to kill any residual eggs. Adults *Sitophilus zeamais* were obtained from the Muea market and reared under ambient condition of 12:12 photoperiods, temperature of 27 ^{o}C and humidity of 65%. The insects were stored in sterilized maize grains for 70 days during which time three generations must have passed.

2.3. Plant powder preparations

Fresh plant material (leaves) of *Cupressus macrocarpa* Wilma (Pinales: Cupressaceae) were collected from Bamenda, north west region of Cameroon. The leaves were shade-dried in one of the rooms of the teaching and research farm house of the faculty of agriculture and veterinary medicine of the University of Buea for 2 weeks to avoid volatilization of the active ingredients. On drying, the leaves were ground into powder by a Victoria grinding mill and parked into a black polythene bags for future use.

2.4. Experimental setup, Data Collection and data analyses

2.4.1. Mortality test

Four different doses of cypress leaf powder (0g, 2g, 5g, and 10g) were measured in the laboratory using a chemical balance and introduced into different containers holding 100 g of sterilized maize grains. 10 adult weevils were introduced in the grains and mortality was checked weekly for 3 weeks. The insects were considered dead on failure to respond to three probing using a blunt dissecting probe [15]. At the end of the third week, a cumulative mean mortality was calculated using equation 1.

$$Cumulative\ mean\ mortality\ (\%) = \frac{\sum_1^i x_i}{n} \times 100 \quad \ldots\ldots\ldots\ldots \text{eq. 1.}$$

Where n is the number of maize weevil per trial (10) and x_i is the mean weekly mortality of maize weevil per treatment in the *i*th week.

2.4.2. Percent Holed and Percent Damaged grain

Ten unsexed adult weevils were stored in 100 g of maize with the four different doses. Two weeks after treatment (DAT) the adult live insects were removed from treated and control containers. The number of grains adult exit holes and damaged grain was counted after 4 weeks after infestation for the calculations of percentages of grain damaged and holed grains. Percentages were calculated using equation 2 (percent damaged grain) and equation 3 (percent holed grain).

$$Percent\ damaged\ grain\ = \frac{Number\ of\ damaged\ grains \times 100}{Total\ number\ of\ grains} \text{ .... eq. 2.}$$

$$Percent\ Holed\ grain\ = \frac{Number\ of\ Holed\ grains}{Total\ number\ of\ grains} \times 100 \text{ .... eq. 3.}$$

2.5. Data analysis

The experiments were arranged in a Completely Randomized Design. Normality and homogeneity of variance tests were conducted using Kolmogorov-Smirnov test in SPSS (ver 23) and Glantz' modification of Levene's test on Micrsoft Excel add-in Daniel's XL Toolbox version 7.3.4 (http://www.xltoolbox.net), respectively, prior to one-way Analysis of Variance (ANOVA) test for statistical significance. The Bonferroni-Holm post hoc test on the MS Excel add-in was used separate the significantly different means at an alpha (α) level of 0.05. A correlation analysis was done to ascertain how the parameters vary concurrently. A hierarchical cluster analysis was used to understand to evaluate the similarity of the treatments considering all parameters simultaneously. The Paired group (UPGMA) algorithm and the Euclidean similarity index were used. The correlation and cluster analyses were conducted using Paleontological Statistics (PAST ver. 3.25).

3. RESULTS AND DISCUSSION

3.1. Mortality test

The weekly mortality of *S. zeamais* as a result of exposure to different doses of cypress leaf powder is shown in table 2. The mortality was significantly different (F = 14.55, df = 3,8, p = .0013) after week 1. About 3, 2, and 1 weevil died in 10 g, 5 g and 2 g of cypress leaf powder, respectively in the first week (Table 2). The number of weevils that died in the second week from cypress leaf powder treatments were not significantly different although different from the control treatment (F = 13.83, df = 3, 8, p = .0016). After week 3, the mean mortality of *S. zeamais* was significantly highest from 10 g of cypress leaf powder compared to 2 g of cypress leaf powder and control treatments (F = 15.0, df = 3, 8, p = .0012). It is clear from the findings in table 2 that cypress leaf powder exhibits some significant mortality to *S. zeamais*. No *S. zeamais* mortality was recorded from control treatment. [17] reported that cypress leaf powder showed some repellency to maize weevil *S. zeamais*. The current study suggests that, in addition to repellency effect, cypress leaf powder has some significant mortality potential on *S. zeamais*. Many botanicals are known to exhibit some mortality and repellency to *S. zeamais*. For instanc, [25] reported that basil (*Ocimum basilicum*) leaf, bark and an admix of leaf and bark powder killed and repelled *S. zeamais*.

The cumulative mean percentage mortality of *S. zeamais* after three weeks of exposure to different doses of cypress leaf powder is shown in figure 1. There was a significant difference in the cumulative mean mortality (%) of *S. zeamais* (F = 126.67, df = 3, 8, p < .001). The cumulative mean mortality (%) was 0%, 40.47%, 50.67% and 70.67% for control, 2 g, 5 g and 10 g of cypress leaf powder, respectively (Figure 1). The cumulative mean mortality revealed that *S. zeamais* mortality was dose dependent. The higher the dose of the cypress leaf powder, the higher the cumulative mean mortality of *S. zeamais*. More *S. zeamais* died in 10 g of cypress leaf powder treatment than from the other treatments. In fact, the cumulative mean mortality from 10 g of cypress leaf powder treatment was almost twice that from 2 g of cypress leaf powder treatment. It seems that *S. zeamais* mortality is plant-type, plant-part and dose dependent. [27] reported a progressive increase in *S. zeamais* mortality with increasing dose (0.1, 0.2.0.3 and 0.4 g/50g of maize grain) of powdered seeds of black pepper *Piper guineense* after three weeks of exposure. The current study is on par with those of [23][25].

Table 2. Mean mortality (±sd) of maize weevil weeks after treatment with cypress leave powder

Dose (g)	Week 1	Week 2	Week 3
0	0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
2	1.3 ± 0.57 b	1.7 ± 0.57 b	2.3 ± 0.57 b
5	1.7 ± 0.57 bc	1.7 ± 0.57 b	2.7 ± 0.57 bc
10	2.7 ± 0.58 c	2.0 ± 0.00 b	3.0 ± 0.0 c

Mean mortality of 10 maize weevil treated with different doses (g) of cypress leaf powder. Means in the same column with the same letter(s) are not significantly different by Bonferroni-Holm post hoc test at p < 0.05. sd – standard deviation

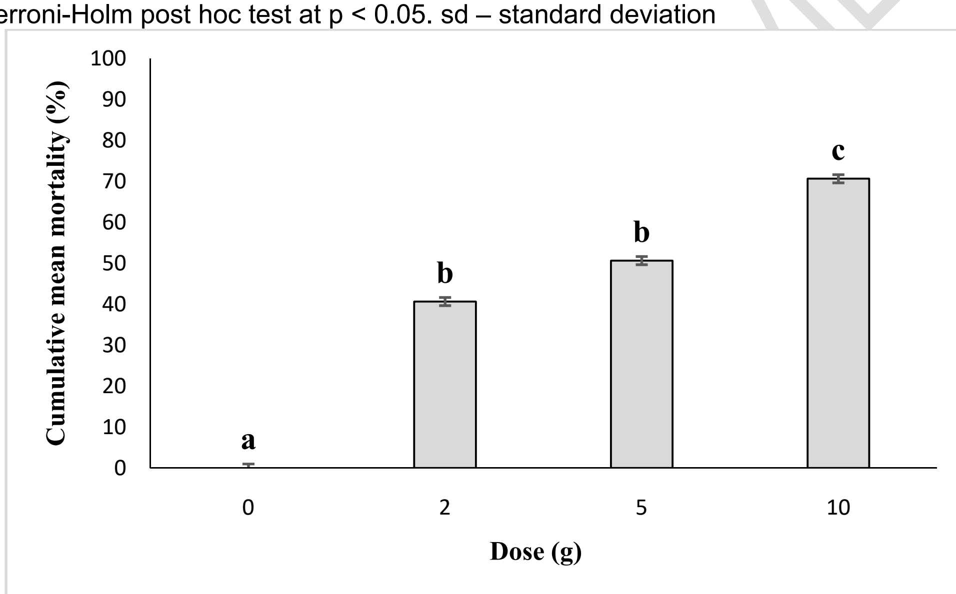

Figure 1. Cumulative mean percentage mortality of *Sitophilus zeamais* after three weeks of exposure to different doses of cypress (*Cupressus macrocarpa*) leaf powder. Means with the same letter(s) are not significantly different according Bonferroni-Holm post hoc test (α = .05).

3.2. Grain damaged (%)

The percentage maize grain damaged by *S. zeamais* exposed to different doses of cypress leaf powder is shown in figure 2. A one-way ANOVA revealed that there was a statistical significant difference ($F = 32.72$, df = 3, 8, $p < .001$) in maize damaged (%) by *S. zeamais*. The highest grain damaged was 71.0% from control treatment, followed by 60.66%, 52.33% and 43.66% from 2 g, 5 g and 10 g of cypress leaf powder, respectively (Figure 2). Some significant amount of grain damaged was observed from all treatments, although that of 10 g of cypress leaf powder was below 50%. This shows that cypress leaf powder prevented quality degradation of maize grains. The current study is in accordance with that of [9] who reported decreasing maize grain damage by *S. zeamais* with increasing dose of powders of

Lippia javanica dried leaves and *Spirostachys Africana* wood. According to [20] quality deterioration of maize grains has severe economic implications especially for poor-resourced farmers in the developing countries.

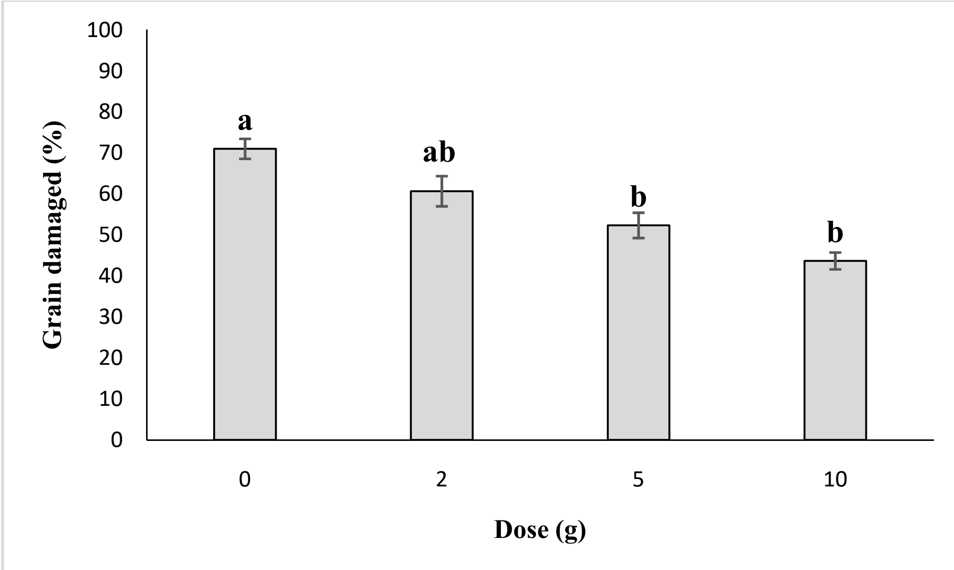

Figure 2. Mean maize grain damaged (%) by *Sitophilus zeamais* after five weeks of exposure to different doses of cypress (*Cupressus macrocarpa*) leaf powder. Means with the same letter(s) are not significantly different according Bonferroni-Holm post hoc test (α = .05).

3.3. Percent holed grain (%)

The percentage of holed maize grains due to *S. zeamais* activity after exposure to different doses of cypress leaf powder is shown in in figure 3. There was a significant difference (F = 17.26, df = 3, 8, *p* = .0007) in holed grains (%). The least holed grain was 49.67% from 10 g of cypress leaf powder treatment. It was followed by 54.67%, 67.0% and 86.0% from 5 g and 2 g of cypress leaf powder, and control treatment, respectively. A similar pattern to grain damage (%) was observed for holed grain (%). A significant percentage of maize grain had holes, although that for 10 g of cypress leaf powder treatment was below 50%; all others were above 50%. Indeed, the percentage holed grains from control treatment was twice that from 10 g of cypress leaf powder treatment. Holed grain (%) showed an inverse relationship to dose of cypress leaf power. Holed grains is an indication that the grain content has been consumed by *S. zeamais*. Such holed grains do not eventually germinate. [27] reported that germination of stored maize grains increased with increased doses of *Piper guineense*, due to reduced *S. zeamais* activity. Inability of stored maize grains to germinate due to holed grains falls short of one of the fundamental principles of storage [5]

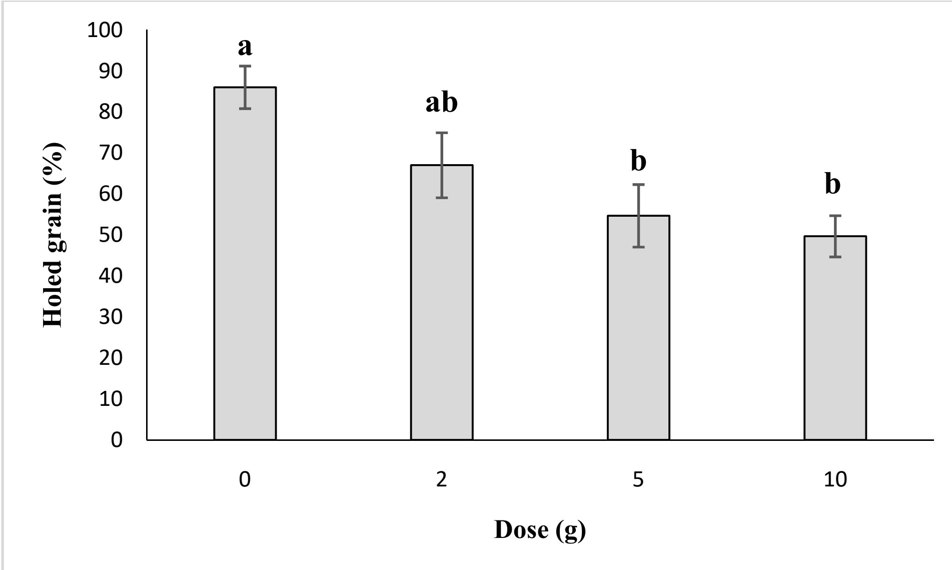

Figure 3. Mean holed grain (%) of maize by *Sitophilus zeamais* after five weeks of exposure to different doses of cypress (*Cupressus macrocarpa*) leaf powder. Means with the same letter(s) are not significantly different according Bonferroni-Holm post hoc test (α = .05).

3.4. Correlation matrix for three variables measured

A correlation analysis was conducted to ascertain how the measured parameters vary together. The correlation matrix is show in figure 4. A strong positive correlation [r (18) = 0.814, $p < .001$] between maize grain damaged and holed grain was observed. On the other hand, there was a strong negative correlation between cumulative mortality and grain damaged [r (18) = -0.913, $p < .001$]. In addition, a strong negative correlation was observed between cumulative mortality and holed grain [r (18) = -0.926, $p < .001$] (Figure 4). The correlation matrix reveals two key points; (i) increased mortality of *S. zeamais* results into less and less maize grain damage (%) and holed grains (%), in other words, there is an indirect proportional relationship between *S. zeamais* mortality and maize grains (%), and (ii) there is a direct proportional relationship between grain damage (%) and holed grain (%), in other words, any attempt to minimize grain damaged can equally minimize holed grain (%). The finding of the current study is in accordance with that of [27] who reported increased mortality of *S. zeamais* due to its exposure to powder of *Piper guineense* resulted into decreased maize grain damaged and eventually higher germination rates. Similar findings have been reported by [28] even though inert materials were used.

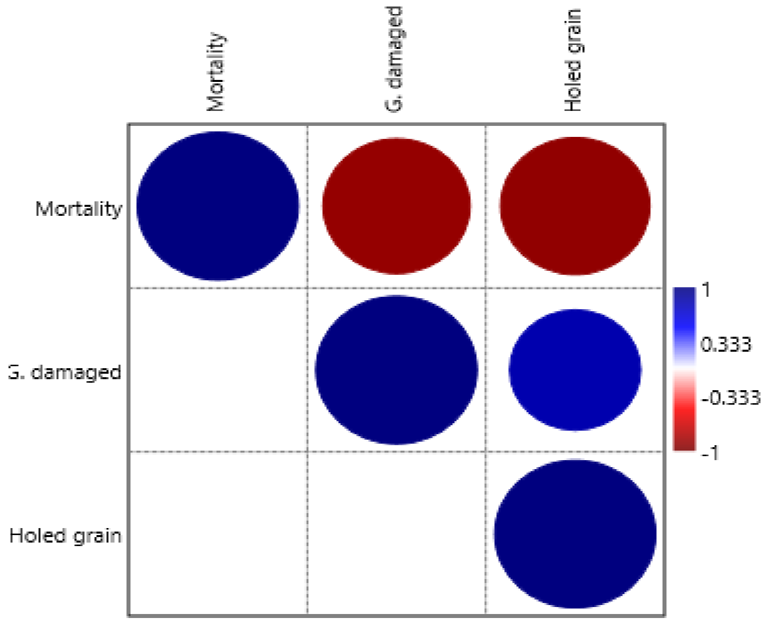

Figure 4. Correlation matrix of some measured parameters of *Sitophilus zeamais* activity after exposure to different doses of cypress (*Cupressous macrocarpa*) leaf powder in stored maize

3.5. Similarity

The result of the hierarchical cluster analysis is shown in the dendrogram in figure 5. Two distinct clades were observed; one containing cypress leaf powder treatments and the other containing control treatment. The observations of 10 g of cypress leaf powder were most conserved. The dendrogram clearly shows that results from 2 g and 5 g were more similar than 2 g and 10 g of cypress leaf powder. The findings from the similarity study (cluster analysis) further support the idea that an active component or components in the cypress leaf powder actually influenced the metabolism of *S. zeamais* resulting into higher mortality and a reduced maize grain damaged (%). The results are consistent with those of other studies [10][13][14][17]. and purports that cypress leaf powder can play a phenomenal role in the management of *S. zeamais*.

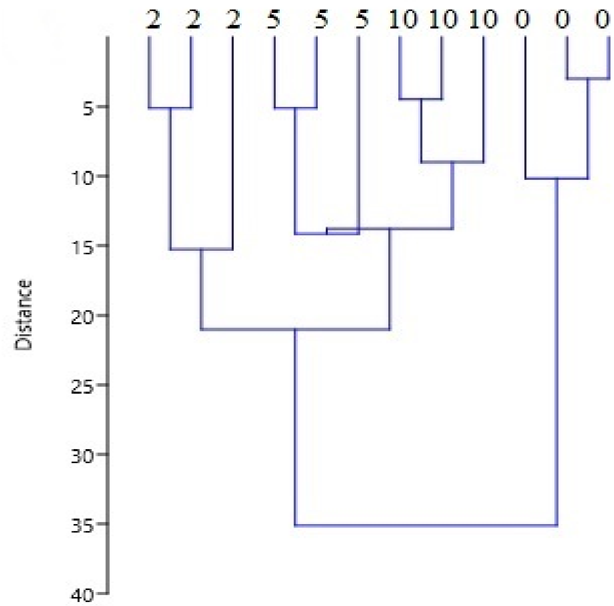

Figure 5. Similarity between different doses of cypress (*Cupressus macrocarpa*) leaf powder treatments on the activities of maize grain weevil *Sitophilus zeamais*.

4. CONCLUSION

The main goal of this study was to evaluate the potential of cypress leaf powder to alter some metabolic processes of *S. zeamais*. The study has shown that mortality of *S. zeamais* due to cypress leaf powder exposure is dose-dependent. In addition, it also demonstrated that increasing mortality of *S. zeamais* will eventually reduce maize grain damage. The evidence from this study suggest that cypress leaves adds to the growing list of botanicals that can play a tremendous role in an integrated pest management program for *S. zeamais*.

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