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Survival analysis of maize weevil *Sitophilus zeamais* Motschulsky 1855 (Coleoptera: Curculionidae) exposed to some medicinal plant powders

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ABSTRACT

Maize weevil (*Sitophilus zeamais*) is a very destructive pest of stored maize, accounting for about 15 – 25 % of loss, and sometimes 100% in severe infestation. Synthetic pesticide is often the first step of control, which is often costly, scarce, detrimental to the environment and human health. As a result, more environmentally friend options like the use of botanicals are encouraged, in line with the United Nation Sustainable Development Goals. The purpose of this study was to assess how fast to kill and how hazardous are four medicinal plant powders on maize weevil. The study was conducted in June-July, 2020 in the Food Science laboratory of Catholic University of Cameroon, Bamenda. Plant powders of pawpaw *Carica papaya* seeds, cypress *Cupressus macrocarpa* leaves, piper *Piper nigrum* seeds and a control (no-input) were assessed. Mortality was observed every 48 hr for 192 hr (8 days). The data was subjected to survival analysis using SPSS ver. 23. The proportion of Event (mortality) and Censored (no mortality) at varied amongst treatment with Event in piper significantly higher ((χ^2 = 26.2, df = 7, P < 0.001) than Censored, and the reverse was the case for the other treatments. Only the piper treatment

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had 50% lethal time (LT_{50}) of 144 hr. The Kaplan-Meier survival probability curve of piper had many sharp drops unlike the flat curves for control and pawpaw. Hazard ratios were 0.553, 2.47and 0.819 for pawpaw, piper and cypress, respectively. We proposed the possibility of exploring the angle formed between the line linking both ends of the survival curve and the horizontal line linking the end of the survival curve and the y-axis to assess the degree of hazardousness. The findings of this study revealed that piper powder killed the maize weevil very fast and was also the most hazardous treatment. We strongly recommend the integration of piper seed powder in the management of stored maize in order to prolong the shelve life, and maintain the quality and quantity of stored maize.

***Keywords*:** maize weevil, medicinal plants, piper, survival analysis, hazard ratio, *Sitophilus zeamais*, *Zea mays*

1. INTRODUCTION

Maize (*Zea mays* L.), a member of the family Poaceae or Gramineae, is a kind of grass related to rice, wheat, barley, and oat, ranking second in area cultivated, first in production and productivity in order of world grain production [1]. In fact, maize constitute one of the most important ingredients for compounding animal feed. It is an important staple food in sub-Saharan Africa, animal feed and also has varied industrial uses [2]. This implies that maize is and will always have a very high demand. Due to the ever-increasing demand for high quality and safe food by the consumers, there is a need to maintain and protect maize grain from insect damage and fungal infection especially at post-harvest [3, 4].

Postharvest loss is a measurable quantitative, qualitative loss across the supply chain, or the postharvest system, from the time of harvest till its consumption [5, 6]. Postharvest loss of maize has been estimated to be between 15% and 26%, with severe cases reaching 80 – 100% [7].

The greatest portions of these losses occur in the standing crops and during storage and are mainly due to insect infestation. Like many stored food grains, damage of stored maize grains is a very serious global problem [8]. The situation is worse in developing countries with poor-resource farmers and inadequate storage facilities. Africa is suffering from 20-30 % postharvest losses with some farmers experiencing 100% postharvest losses in severe infestations [9].

Among insect pests, maize weevil (*Sitophilus zeamais* Motsch.) and Angoumois grain moth (*Sitotroga cerealella*) are the most important insects in stored maize in Nepal ([10, 11]. The most economically important and widely occurring postharvest losses due to insect pests of stored maize include the maize weevil (*Sitophilus zeamais* Motsch.), a reddish brown to black snout weevil [12]. *S. zeamais* Motsch. is an internal feeding pest of maize. It is among the most destructive pests in stored grain, especially corn in tropical regions [13]. Adult female of weevils causes damage by boring into the kernel and laying eggs (ovipositing). Then, larvae and pupae eat the inner parts of the kernel, resulting in a damaged kernel, and reduced grain weight (quantity) and quality [14].

The adults emerge and eat their way towards the testa causing the characteristic exit holes resulting into insect damaged grain [15]. The infestation elevates temperature and moisture content in the stored grain mass, which accelerate fungal growth, including toxigenic species such as *Aspergillus flavus* [16] leading to quality deterioration.

As a result, the control of this pest is essential. Currently, farmers rely heavily on the use of synthetic insecticides to control *S. zeamias* in sub-Saharan Africa, with very minimal use of traditional knowledge like the use of botanicals. In traditional farm stores in Africa, the use of synthetic insecticides for grain protection has been partially successful [17]. Nevertheless, questions have been raised about the safety and incessant 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 [17-20, 30-32]

These problems have reignited the interest in the re-evaluation of traditional knowledge - botanical pests control methods, which are relatively cheaper, easy-to-make, readily available and environmentally friendly [4]. Researches on botanical or medicinal plants as control measure of *S. zeamais* are varied. Achiri et al. [21] has reviewed this phenomenon 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).

Thus, in-line with other researches, the primary objective of this study was to evaluate the ability of pawpaw (*Carica papaya*) seeds, piper or bush pepper or black pepper (*Piper nigrum*) seeds and cypress (*Cupressus macrocarpa*) leaves to cause mortality to adult maize weevil. In this study, we were interested in (1) how fast these medicinal plants will kill the maize weevil, (2) how hazardous these medicinal plants will be to the maize weevils and (3) how feasible will it be to use the acute angle formed between the line linking both ends of the survival curve and the horizontal line running from the end of the survival curve to the y-axis to estimate or infer hazardousness of the tested medicinal plants.

2. MATERIAL AND METHODS

2. 1. Study site

The study was conducted in the Food science laboratory of Catholic University of Cameroon, Bamenda (CATUC), in June-July of 2020. Bamenda is the regional capital of North West Region Cameroon. It is a city council called the Bamenda City Council (BCC) made up of three councils. Bamenda is 1250m above sea level and it is situated between 9°58'16''N, 6°3'14''E and 10°14'16'N, 5°51'8" E. The annual rainfall is 2567 mm and average temperature is 23 °C, ranging between 15 – 32 °C. There are two seasons, the rainy (March – October) and dry (November to February) season. The vegetation in this western highland city is mostly savanna with shrubs dotted here and there [22].

2. 2. Maize and insect collection

1.5kg of dry maize (CHC202) was bought from traders in a local food market located in Bamenda municipality, North west region, Cameroon. Cracked, broken grains and other debris were sorted out from the others. The maize grains were heat sterilized at 40-50 °C for 20 minutes to kill residual eggs. Adults *Sitophilus zeamais* were obtained from infested maize in warehouse in the same local market reared in laboratory conditions (approximately, 12:12 photoperiods, temperature of 27 °C and humidity of 65%). The insects were stored in sterilized maize grains for 70 days during which three generations must have passed.

2. 3. Plant powder preparations

Fresh leaves of *Cupressus macrocarpa* Wilma (Pinales: Cupressaceae) were collected from Bamenda (Bambili), North West Region of Cameroon. Seed from ripe pawpaw (*Carica papaya*) were collected from roadside fruit vendors in Bamenda municipality. Fresh seeds of the spice, piper (*Piper nigrum*), commonly called 'bush-pepper' was obtained from a local market in Bamenda. These plant materials were shade-dried; 2 weeks for the leaves and 3 weeks for the seeds to avoid volatilization of the active ingredients. After these periods, the plant materials were ground into powder using a Victoria grinding mill, packed into a black low density polythene bag, and stored in the refrigerator for future use.

2. 4. Mortality test

The mortality test was conducted using the same glass vial as was for F1 generation emergence. Each glass vial contained 5% w/w (100g) of plant powder and maize and 10 adult maize weevils were added into the vials. The glass vials were covered with a muslin cloth and tightly held with a rubber band. The mortality of the maize weevil was assessed every 2 days, for a period of 8 days (i.e 48, 96, 144, and 192 hours). The insects were considered dead on failure to respond to three probing using a blunt dissecting probe [23]. There were four replicates per treatment, arranged in a completely randomize design on a table in the laboratory. The glass vials were separated from each other by 30 cm. After every day of data collection, the positions of the glass vials were rearranged randomly. This exercise was repeated twice under the same laboratory conditions and the average results of the two trials taken into consideration.

2. 5. Data analysis

Survival analysis, which is generally a set of methods for analyzing data where the outcome variable is the time until the occurrence of an event of interest (mortality), was conducted. Mean mortality time was subjected to one-way ANOVA and significantly different means were separated using the *posthoc* test Tukey's Honestly Significant Difference test (Tukey HSD). Kaplan-Meier survival curves were used to estimate median survival times (LT_{50}: median survival time is the time at which 50% of adult maize weevil survived) 192 hours after exposure to the insecticides and their respective 95% confidence intervals (CI) were determined.

Hazard ratios after 192 h were estimated using Cox regression to estimate the probability of mortality of maize weevil occurring in the insecticide-treated leaves compared to the control (no-input) maize at any given time. In this analysis, two measurements were used to described the weevils: 'Event' and 'Censored'. An Event means that mortality has occurred and Censored means the insect was alive at the end of the study duration. All analyses were done using Statistical Package for Social Sciences (SPSS. ver. 23, 2015).

In addition, we propose the possibility of using the angel (ɵ) formed by the line running from end to end of the survival curve and a horizontal line from the end of the survival curve to the y-axis, as a measure of the toxicity of the treatments. The angel (ɵ) defined by tan^{-1} function (equation) for piper is discussed.

$$\theta = tan^{-1}\left(\frac{m1 - m2}{1 + m1.m2}\right)$$

where: m1 is the slope of the line joining the two ends of the survival curve, m2 is the slope of the horizontal line joining the end of the survival curve and the y-axis.

3. RESULTS

3. 1. Proportion of Event and Censored outcomes of maize weevil exposed to medicinal plant powder

The proportion of Event and Censored outcomes of maize weevil is reported in Figure 1. The proportion was significantly different in the control (χ^2 = 23.3, df = 7, P < 0.001), pawpaw (χ^2 = 19, df = 7, P < 0.05) and piper (χ^2 = 26.2, df = 7, P < 0.001). However, the proportion was not significantly different for cypress (χ^2 = 1.6, df = 7, P > 0.05). At the end of the study time, the Event outcome for the control, pawpaw, piper and cypress were 17.5%, 42.5%, 87.5% and 48.7%, respectively.

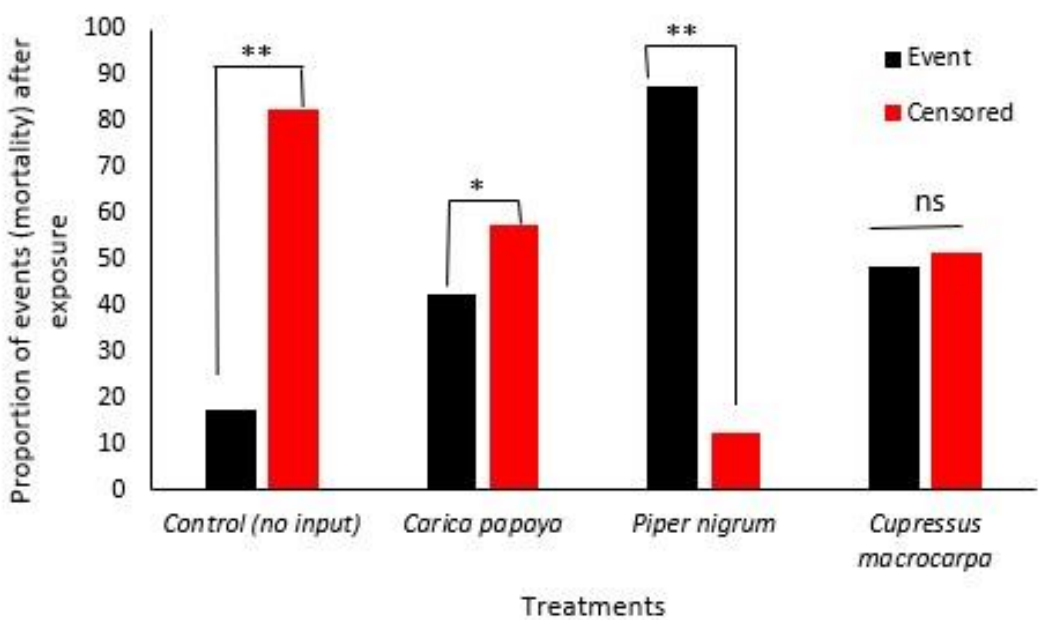

Figure 1. Proportion of Event and Censored outcomes of maize weevil exposed to medicinal plant powders. (ns – not significantly different, * - α = 0.05, ** - α = 0.01)

3. 2. Mean mortality time and Lethal time (LT_{50}) of maize weevil exposed to medicinal plant powder

The mean mortality time of maize weevils exposed to different medicinal plants are shown in Table 1. The highest mean mortality time was observed from the control (177.6 hr) and this differed significantly (F = 12.3, df = 3, 15, P < 0.05) with that of piper (136.8 hr). The mean mortality time for pawpaw and cypress were 176.4 h and 164.4 hr respectively.

The 50% lethal time: the time it takes for 50% of the weevil to die is presented in Table 1. The LT_{50} for piper was 144.0 hr. No LT_{50} was recorded for control, pawpaw and cypress treatments.

Table 1. Mean mortality time and 50% lethal time (LT_{50}) of maize weevil exposed to medicinal plant powders.

Treatments	Mean mortality time (hr)			LT_{50} (hr)		
	Mean ± s.e	95% CI		LT_{50} ± s.e	95% CI	
		Lower bound	Upper bound		Lower bound	Upper bound
Control	177.6±6.66a	164.55	190.65	-	-	-
Carica papaya	176.4±5.36a	165.90	186.91	-	-	-
Piper nigrum	136.8±8.18b	120.77	152.83	144.0±16.33	111.99	176.01
Cupressus macrocarpa	164.4±7.374ab	147.95	178.85	-	-	-

Means within a column with the same letter(s) are not significantly different (Tukey HSD, $\alpha = 0.05$). Some treatments do not have LT_{50} values because mortality did not reach 50.0%

3. 3. Survival probability of maize weevils and hazard ratios of medicinal plant powders

The mortality of the maize weevil was recorded every 48th hour for 192 hours (8 days). Figure 2 shows the Kaplan-Meier survival curve for each medicinal plant. For all treatments except the control, mortality was observed on the 48th hr. The steepness of the slope for the control started at the 96th hour with 82.5% of the weevils surviving till the end. The steepness of the slope of pawpaw began at the 48th hour with a survival probability of 0.975 (97.5%). A small vertical drop was observed at the 96th hour and at the end of 192nd hour, the survival probability was 0.575 (57.5%) for pawpaw. A similar trend was observed for cypress, although the survival probability at the end of the 192nd hour was lower, 0.526 (52.6%). A sharp vertical drop was observed at the 48th and 96th hours for piper, giving a survival probability of 0.875 (87.5%) and 0.6 (60.0%), respectively. This sharp drop continued throughout the study with a survival probability of 0.125 (12.5%) at the 192nd hour.

Table 2. Hazard ratio of medicinal plant powders on maize weevil

Treatments	Hazard ratio	95% CI		*P* values
		Lower bound	Upper bound	
Control	-	-	-	-
Carica papaya	0.553	0.137	0.78	0.012
Piper nigrum	2.47	1.41	4.32	0.002
Cupressus macrocarpa	0.819	0.43	1.58	0.051

Omnibus test for hazard ratios (Chi square $\chi^2 = 38.38$, df = 3, $P < 0.0001$). CI – confidence interval

The hazard ratios of the medicinal plant tested on maize weevil is shown in Table 2. There significant differences (Omnibus test for hazard ratios: χ^2 = 38.38, df = 3, $P < 0.0001$) in the hazard ratios. The hazard ratio in this study ranged from 0.553 from pawpaw to 2.47 from piper. The hazard ratio for cypress was 0.819.

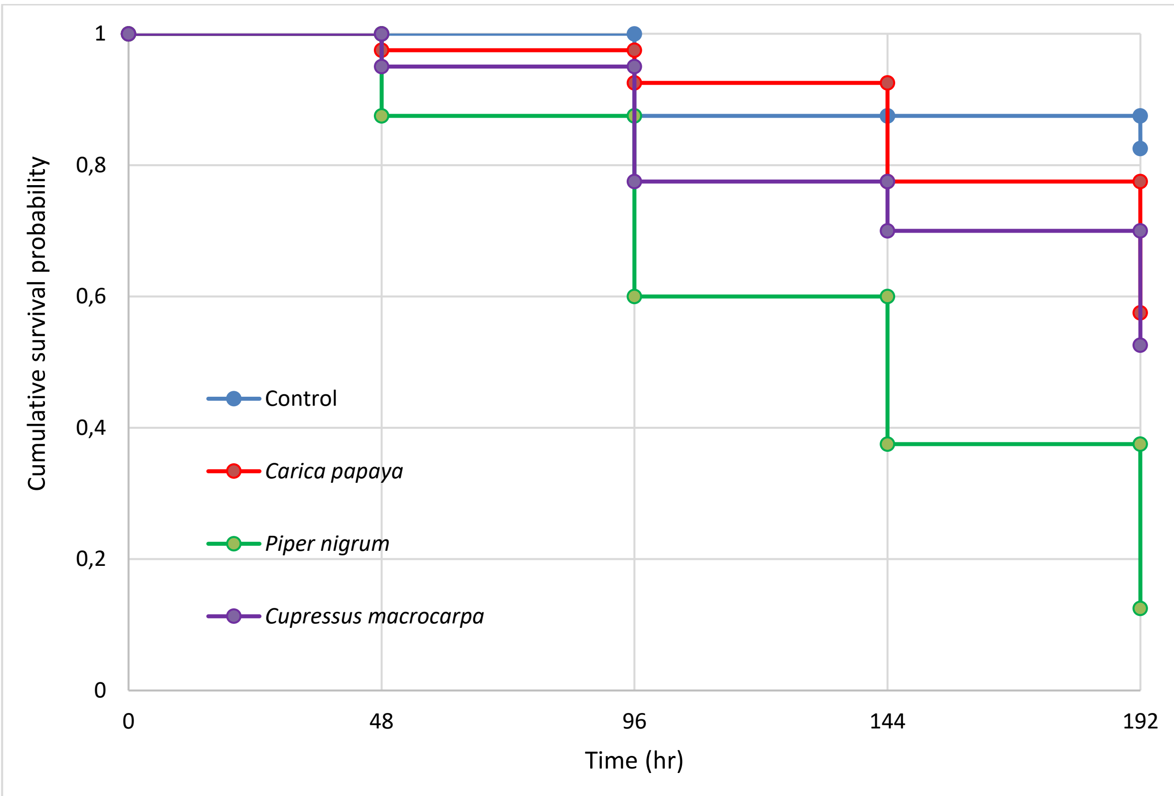

Figure 2. Kaplan-Meier survival curves for maize weevil exposed to some medicinal plant powders for 192 h. Log Rank (Mantel-Cox) for LT_{50} (Chi square χ^2 = 45.577, df = 3, $P < 0.0001$)

3. 4. Possibility of proposed angle (ɵ) from survival curve to estimate hazardousness of medicinal plant powders

Figure 3 shows the angles formed between the line joining the two ends of the survival curve (rise – r1) and the horizontal line joining the end of the survival curve and the y-axis (run – r2). Three important observations were made on the possible outcomes of these angle: (1) If no event occurred during the study time, the angle between r1 and r2 will be zero, (2) if the angle is formed, it will always be an acute angle, and (3) survival curves with very low survival probability at the end of the study will have large angles, i.e the lower the survival probability, the higher the angle formed. Consider Figure 3(c), survival probability of curve of piper, the slope of the line r1 (m1) and r2 (m2) can be used to estimate the angle between them using the $\tan^{-1}$ function.

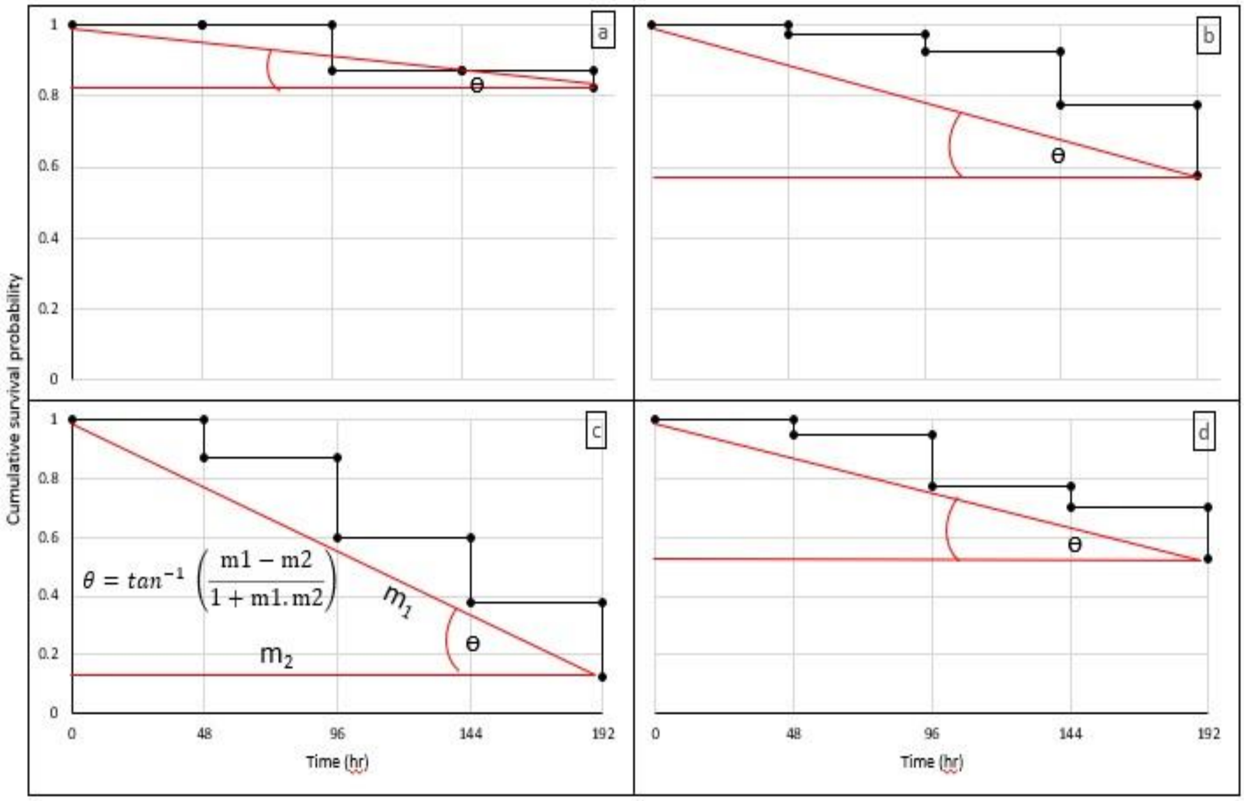

Figure 3. Proposed angle (ɵ) for estimation of hazardousness of medicinal plant powders. (a) control (no-input), (b) *Carica papaya*, (c) *Piper nigrum* and (d) *Cupressus macrocarpa*

4. DISCUSSION

The proportion of Event and Censored outcomes of the maize weevil is a good indicator of the potency of the medicinal plant powder. In this study, the Censored outcome for the control was extremely higher than all other treatments. This was expected since the control treatments had no inputs. The lowest Censored outcome was observed from piper and followed by cypress. Worthy of mention is that the Censored outcome (survival probability) for cypress was 4-fold higher than that of piper. This indicates that piper has the ability to cause more mortality than the other treatments.

Many medicinal plant powders have been reported to significantly interfere with many biological processes of maize weevil [24, 25] Secondary metabolites in these plant materials such as phenols and terpenoids have been implicated to account for antifeedant or direct toxicity of insects [26]. Piper is known to possess a high concentration of secondary metabolites such as total phenol, total flavonoid and piperine in the pericarp of piper seeds (Lee et al., 2020). Which could have accounted for the high potency of piper.

In line with the Event and Censored outcome proportions, the mean mortality time and LT_{50} are good parameters to assess the potency and how fast a medicinal plant or botanical powder will cause mortality of the maize weevil. Piper had the shorter mean mortality time compared to the other treatments.

In addition, only piper has a value for LT_{50}, implying that for the study duration, only the maize weevil subjected to the piper treatments attained 50% mortality. This appears to be a survival advantage for insect that were exposed to pawpaw and cypress compared to those exposed to piper.

The survivor function representing the probability that an individual survives from the time of origin to sometime beyond time t. It is usually estimated by the Kaplan-Meier method. The log-rank test may be used to test for differences between survival curves for groups, such as treatment arms (in this case, medicinal plant powders) [27]. The Kaplan-Meier survival curve in the current study varied for the different treatments. The curve was flat for the control and pawpaw (i.e it stays close to 1.0) which suggest a high survival and the curve dropped sharply for piper (i.e towards 0), suggesting a poor survival [28]. This suggest that piper causes mortality very fast, compared to all other treatments. Hazard function gives the instantaneous potential of having an event at a time, given survival up to that time. It is used primarily as a diagnostic tool or for specifying a mathematical model for survival analysis [29]. The hazard ratio values varied amongst the treatments. The hazard ration was ~2.5 times higher in piper treatments than in the control. In other words, there is an ~71.4% increase in the expected hazard for piper treatment compared to the control. The value is 22.6% for cypress. This degree of hazard suggest that piper is a good candidate to manage maize weevil.

Furthermore, we explore the possibility of using the angle (ɵ) formed between the line (r1) linking the ends of the survival curve and the horizontal line (r2) joining the end of the survival curve and the y-axis. With observations made, it can be inferred that as the medicinal plant hazardousness increases, the angle increase.

5. CONCLUSION

Medicinal plant powders have natural chemicals with insecticidal properties and are used as sustainable alternatives to synthetic or chemical pesticides for crop protection and preservation of animal feed to avoid negative or side effects of synthetic insecticides. Mortality was observed from plant powder treatment. The current study has demonstrated that piper (bush pepper or black pepper) seeds, pawpaw seeds and cypress leaf powders can be used in integrated pest schemes for maize weevil, with piper being the most promising. The current study highlights the toxicity of piper with regards to how fast it can kill the maize weevil and the degree of hazardousness. In addition, we propose the possibility of exploring the angle (ɵ) formed between the line (r1) linking the ends of the survival curve and the horizontal line (r2) joining the end of the survival curve and the y-axis to assess hazardousness of medicinal plant powders. The current study is in-line with many other studies that have explored and demonstrated a huge potential of botanicals to control many storage and field pests. The medicinal plants exploited in this study are readily available, easy-to-make and can serve as first step for maize weevil management, especially for storage in cottage systems.

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