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Evaluation of Leadwood (*Combretum imberbe*) against rusty red grain beetle (*Cryptolestes ferrugineus*) on stored grains

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Abstract
The rusty red grain beetle, *Cryptolestes ferrugineus* (Stephens) is a pest of high economic importance. It is reported to infest stored products causing huge economic losses. This study evaluated *Combretum imberbe* wood ash against *C. ferrugineus* on stored cereals and pulses at 28±2ºC and 70±5% RH in the laboratory at the University of Botswana. The bioassay was conducted using three concentrations (1g, 3g and 5g/50g food commodity) of *C. imberbe* wood ash powder. Each treatment was replicated 5 times. The toxic effect was evaluated 3 months following application and probit analysis was used to determine the LC_{50} values for the treatments. At the highest concentration *C. imberbe* was able to cause 50% mortality of beetles on broken maize and broken sorghum over the period of the experiment. When treatments were applied to pulses, the highest mortality was achieved on broken Bambara groundnuts, ground Bambara groundnuts and broken cowpeas. The type of stored food commodity did not significantly affect the effectiveness of *C. imberbe* wood ash powders. This study showed that *C. imberbe* wood ash powder can be used in combination with other control measures to achieve effective control of *C. ferungineus* and reduce the application of synthetic insecticides to stored food commodities.

Keywords: *Cryptolestes ferrugineus*, *combretum imberbe*, bio-efficacy, stored grain pest, botswana

Introduction

Cryptolestes ferrugineus S., rusty red grain beetle (Coleoptera: Laemophloeidae), is a cosmopolitan grain pest (Jian *et al*., 2007) [1] of high economic importance. It is regarded among the most debilitating beetle pests of stored grains, and a threat to the food supply chain worldwide. *C. ferrugineus* causes significant economic losses and food wastage. The insect is known for its ability to adapt to different environmental conditions due to its unique behavior, and reproductive capabilities (Bharathi *et al*., 2023) [2]. Its primary hosts are stored grains including maize, barley, wheat, groundnuts, beans, and other dried produce. Both the larvae and adults feed on the germ and endosperm of the grain thus causing a loss of quality and reducing germination (Ajayi & Peter, 2016) [3]. Heavy infestations cause extensive damage by causing large quantities of grain to spoil, and by introducing fungal spores into the stored grain (Bharathi *et al*., 2023) [2].

C. ferrugineus is one of the insect pests infesting stored food products in Botswana (Allotey *et al*.,2012) [4]. Hot and dry conditions prevalent in Botswana are favorable to the development of *C. ferrugineus* (Allotey *et al*., 2017) [5]. In Botswana, control of stored grain pests is heavily reliant on the use of synthetic insecticides and fumigants. The use of chemical insecticides leads to development of resistance in storage insect pests, dangerous to human health, environmental consequences and are very expensive (Rather *et al*., 2014) [6]. Therefore, efforts to promote the use of environmentally friendly, low risk insecticides as alternatives are continually being made. Along with biological and physical control strategies, the use of plant powders in integrated pest management (IPM) is an essential component that reduces utilization of chemical insecticides (Barzman *et al*., 2015) [7].

Plant powders are of great potential since they possess insect repellent and anti-feedant properties, specific, non-toxic to humans and animals, bio-degradable, non- pest resistance and naturally available (Pavela, 2016) [8]. One of the promising plant derivatives are those from *Combretum imberbe.* The Combretaceae family consists of 600 species of trees (Masoko and Eloff, 2007) [9]. Plants belonging to this family are found in tropical and subtropical regions, mostly in Africa and India. Five genera are commonly found in southern and western Africa. Its wood ashes are normally used as natural stored grains protectants in small-scale farms of Africa and India (Chikukura *et al*. 2011) [10].

With synthetic insecticides facing challenges like high costs, environmental damage, and the development of pest resistance, plant powders offer a promising and sustainable alternative for pest management (Aktar *et al*., 2019) [11]. Plant powders are highly effective against certain pests, yet they do not harm humans, most beneficial insects, and other non - target organisms. Plant powders are biodegradable, have diverse mechanismss of action, and are often more readily available than their synthetic counterparts (Lengai *et al*., 2020) [12].

Because of their low toxicity to humans, plant extracts are suitable for use in integrated pest management (IPM) programs, especially where pests have developed resistance to other insecticides. The application of plant powders as a component of an IPM program can reduce environmental pollution and delay the expression of resistance to other insecticides (Ogendo *et al*., 2012) [13]. However, little is known about their effects when applied against *C. ferrugineus* infesting grain storages in Botswana. Considering the damage caused by the *C. ferrugineus* on food stores, it is necessary to undertake studies to determine the effectiveness of plant powders against this pest and make recommendations for their effective use. Determination of the effectiveness of plant powders is an important component for the development of non-synthetic pest management. Despite its impact, little or no research has been carried out to evaluate the effectiveness of plant powders against *C. ferrugineus* on stored produce in Botswana. Therefore, the purpose of this study is to determine the efficacy of *C. imberbe* wood ash powder against *C. ferrugineus* on stored cereals and pulses in Botswana.

Materials and Methods

The bioassay was conducted in the Biological Sciences laboratory at the University of Botswana in Gaborone, Botswana (24°39'37"S 25°55'51"E Alt: 990 m). Infested food stuffs were collected from the Seed Multiplication Unit (SMU) warehouse, Sebele old library, Plant Protection Division in Sebele, and selected homesteads and farms in the greater Gaborone area. The food commodities were collected by scooping a handful (40 grams) of grains in polythene bags, then transferring to glass jars labelled to indicate the place of collection, date, name of the infested commodity and type. These were then stored in the Department of Biological Sciences insectary, University of Botswana. The conditions in the insectary were kept at temperatures of 28±2ºC and 70±5% RH with alternating 12 hours light and dark cycles (Beckel *et al*., 2007) [14].

Insect identification

Different insect infested food products were weighed then sieved using standard sieves of mesh sizes; 25 microns for flour and ground food stuff and 45 microns for grains and seeds (Humboldt, Chicago, lllinois, USA) to obtain live insects. The different species of insects from each infested food commodity were identified using the insect identification keys (Biege & Partida, 1976) [15] and then counted separately using a digital hand counter before use in the bioassay.

Preparation of the purchased food commodities

Purchased food commodities (ground and whole) were placed in trays and sterilized in the hotbox Gallenkamp oven (SG96/03/206) at 80ºC for 2 hours. Other equipment such as the glass vials and plastic petri dishes was also thoroughly washed, and oven dried in order to prevent cross infestation.

Preparation of plant powders

Combretum imberbe dried wood was collected from Bobonong (Geographical coordinates, 21.9814˚S, 28.4280˚E), Botswana. Dried wood of *Combretum imberbe* which is popularly known as elephant trunk was burnt and ashes collected. The ashes were sieved through a mesh size of 150- 600µm to obtain a fine powder (Nenaah & Ibrahim, 2011) [16]. The material was then sieved through 150-600 µm mesh sieve to get the fine powder, which were kept in airtight plastic bags and stored at room temperature in the insectory (Ahmed *et al*., 2003; Hossain *et al.*, 2014; Akunne and Ononye, 2015) [17, 18, 19].

Bioassay methods

C. imberbe wood ash powder was used in the bioassay. The *C. imberbe* wood ash powder was applied at three levels (1, 3 and 5 g/50g food commodity) (Anita *et al*., 2012) [20], on 50 grams of the broken and ground maize, sorghum, rice, beans, cowpeas and Bambara groundnut kept in two-liter glass jars, each with five replicates for each treatment and control (without the *Combretum imberbe* ashes) in a completely randomized design. Ten adults (five males and five females) of *Cryptolestes ferrugineus* were introduced into the treatments and control. Adult emergence was monitored and recorded weekly for three months (Jagadeesan *et al*., 2013) [21]. This experiment was carried out under ambient temperatures.

Assessment of mortality

C. ferrugineus adults were observed daily under a binocular microscope. The number of adults on each treatment were recorded immediately before application of treatments. A camel brush was used to stimulate individual beetles. Adults that were incapable of walking were recorded as dead. The adults were assessed on weekly basis following treatment. Results were expressed as percentage mortality and corrected for untreated mortality using Abbott's formula (Dawidar *et al*., 2012) [22].Untreated mortality was also documented.

Statistical analysis

Mortality data were transformed to arcsines while concentrations were transformed to $\log_{10}$ (x+1) prior to analysis (Najem *et al*., 2020) [23]. Data was analysed using Log_{10} concentration versus probit mortality regression and analysis of variance (ANOVA). LC_{50} values were estimated from the probit lines. LC_{50} values were used to compare mortalities caused by the different treatments. Statistical analysis was performed using SPSS version 25 software. Tukeys Honestly Significant Difference test was used to separate the means.

Results

Relationship between *C. imberbe* wood ashes powder concentration and mortality of *C. ferrugineus* adults on broken and ground cereals.

Figure 1 shows a positive curvilinear relationship between log concentration and probit mortality caused by *C. imberbe* on broken and ground maize. The results depict an LC_{50} value of 0.45 (equivalent to 1.82g/50g) (correlation coefficient: 0.9863) on broken maize. *C. imberbe* achieved 0.54 (equivalent to 2.47g) (correlation coefficient: 0.9868) on ground maize. A positive curvilinear relationship between log concentration and probit mortality caused by *C. imberbe* when applied to broken sorghum and ground sorghum (correlation coefficient: 0.9806 and 0.9860 respectively). *C. imberbe* ashes achieved LC_{50} value of 0.53 (equivalent to 2.39g/50g) on broken sorghum. *C. imberbe* ashes achieved an LC_{50} of 0.62 (equivalent to 3.17g/50g) on ground sorghum. A positive curvilinear relationship between log concentration and probit mortality caused by *C. imberbe* on broken and ground rice when an LC_{50} value of 0.60 (equivalent to 2.98g/50g) (correlation coefficient: 0.9958) on broken rice. *C. imberbe* achieved 0.60 (equivalent to 2.98g/50g) (correlation coefficient: 0.9991) 3 months after application of *C. imberbe* ashes to ground rice.

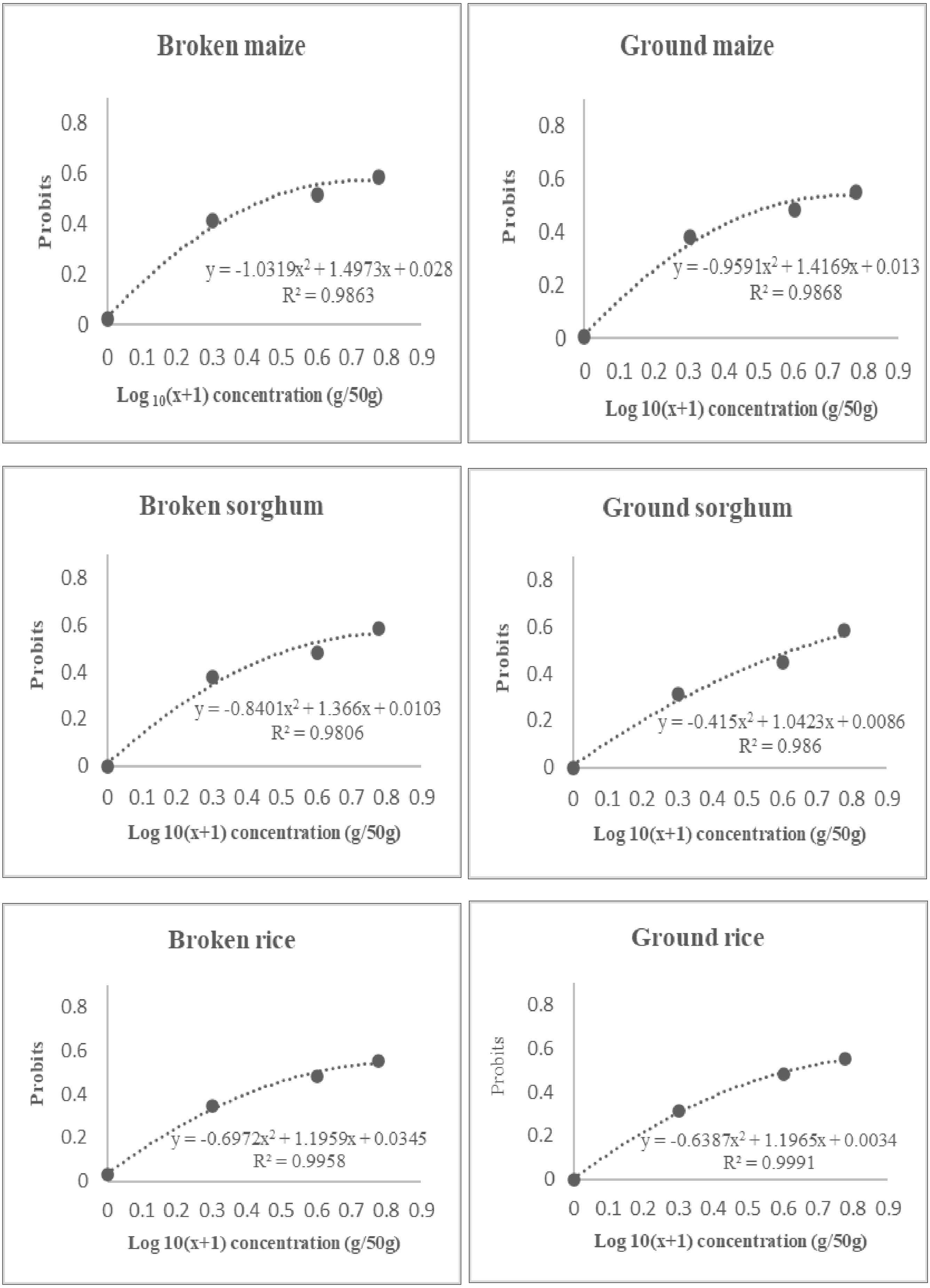


Fig 1: Probit mortality of *C. ferrugineus* adults three months following application of serial concentrations of *C. imberbe* ashes on broken and ground cereals.

Mortality of *C. ferrugineus* adults on cereals treated with serial concentrations of *C. imberbe* wood ash.

Table 1 shows the mortality of *C. ferrugineus* on cereal commodities following treatment with serial concentrations of *C. imberbe* wood ash. The results show that the mortality of *C. ferrugineus* increased as *C. imberbe* concentrations were increased. The highest mortalities were achieved following application of 5g/50g *C. imberbe* wood ash to broken maize and broken sorghum. The highest mortality (50%) was achieved following application of the highest concentration (5g/50g food commodity) of *C. imberbe* wood ash to broken maize which was significantly different from the 48% mortalities achieved on ground maize, ground sorghum, broken rice and ground rice.

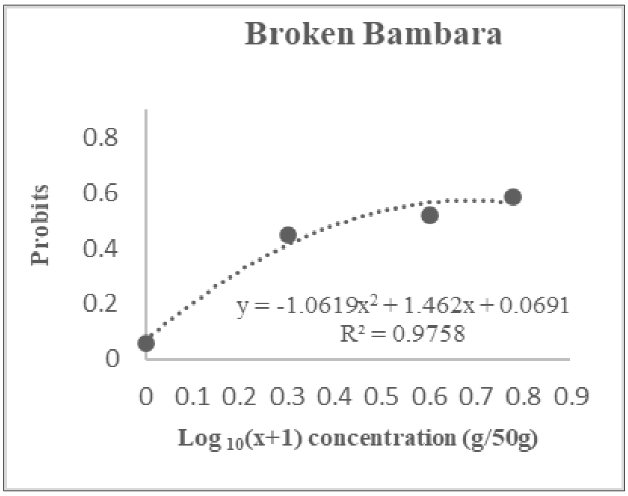
Table 1: Mortality of *C. ferrugineus* on cereals following treatment with serial concentrations of *C. imberbe* wood ash.

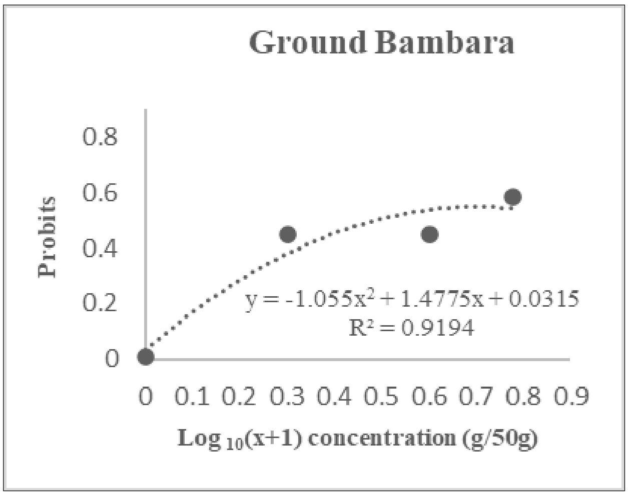
Concentration (Grams)	Food commodities	Mortality ± SE
0	Broken maize	8 ± 5.66^{e}
	Ground maize	4 ± 2.83^{c}
	Broken sorghum	0 ± 0.00^{a}
	Ground sorghum	0 ± 0.00^{a}
	Broken rice	10 ± 8.49^{f}
	Ground rice	2 ± 1.41^{a}
1	Broken maize	40 ± 2.65^{l}
	Ground maize	38 ± 0.71^{k}
	Broken sorghum	38 ± 0.71^{k}
	Ground sorghum	34 ± 4.24^{i}
	Broken rice	36 ± 0.00^{j}
	Ground rice	34 ± 4.24^{i}
3	Broken maize	46 ± 2.83^{o}
	Ground maize	44 ± 0.00^{n}
	Broken sorghum	44 ± 0.00^{n}
	Ground sorghum	42 ± 1.41^{m}
	Broken rice	44 ± 0.00^{n}
	Ground rice	44 ± 0.00^{n}
5	Broken maize	50 ± 0.00^{q}
	Ground maize	48 ± 1.41^{p}
	Broken sorghum	50 ± 0.00^{q}
	Ground sorghum	48 ± 1.41^{p}
	Broken rice	48 ± 1.41^{p}
	Ground rice	48 ± 1.41^{p}

Means followed by the same alphabet do not differ significantly at ($P\leq0.05$) by Tukey's Honestly Significant Difference test.

Relationship between *C. imberbe* wood ashes powder concentrations and mortality of *C. ferrugineus* adults on broken and ground pulses.

Figure 2 shows a positive curvilinear relationship between log concentration and probit mortality caused by *C. imberbe* on broken and ground Bambara groundnuts. It also shows an LC_{50} value of 0.42 (equivalent to 1.63g/50g) (correlation coefficient: 0.9758) on broken Bambara groundnuts. *C. imberbe* achieved 0.48 (equivalent to 2.02g) (correlation coefficient: 0.9194) 3 months on ground Bambara groundnuts. A positive curvilinear relationship between log concentration and probit mortality caused by *C. imberbe* (correlation coefficients: 0.9750 and 0.9751). *C. imberbe* achieved an LC_{50} of 0.42 on the probit scale (equivalent to 1.63g) on broken cowpeas. *C. imberbe* also achieved LC_{50} value of 0.48 (equivalent to 2.02g) on ground cowpeas. A positive curvilinear relationship between log concentration and probit mortality caused by *C. imberbe* applied to broken and ground beans (correlation coefficient: 0.9762 and 0.9676 respectively). *C. imberbe* ashes achieved LC_{50} value of 0.48 (equivalent to 2.02g/50g) on broken beans. It also shows that *C. imberbe* ashes achieved an LC_{50} of 0.57 (equivalent to 2.72g/50g) 3 months following application to ground beans.





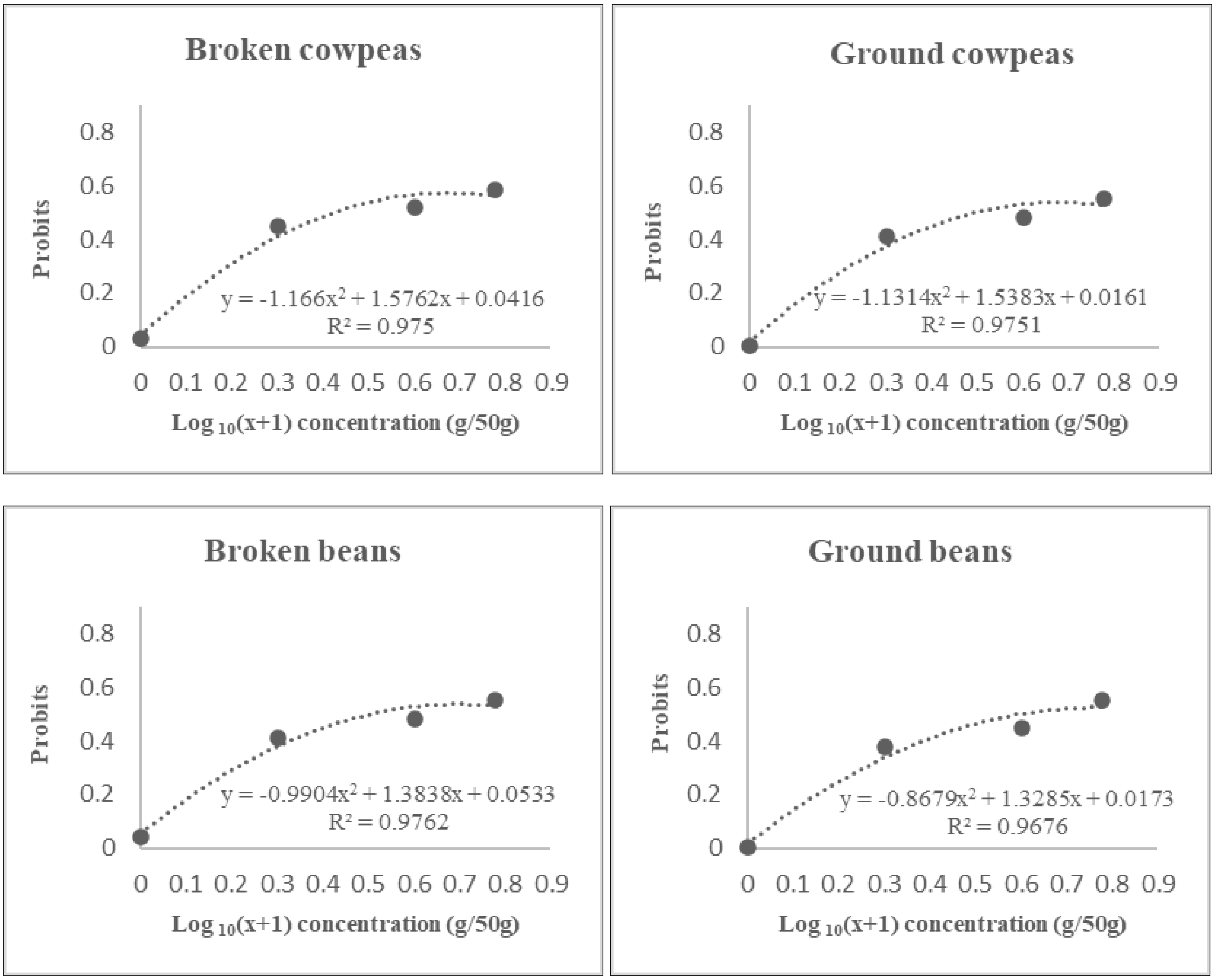


Fig 2: Probit mortality of *C. ferrugineus* adults three months following application of serial concentrations of *C. imberbe* wood ashes on broken and ground pulses.

Mortality of *C. ferrugineus* adults on pulses treated with serial concentrations of *C. imberbe* wood ash.

Table 2 shows the results of average % mortality of *C. ferrugineus* in the pulse commodities following treatment with serial concentrations of *C. imberbe* wood ash. The results show that the mortality of *C. ferrugineus* increased with rises in *C. imberbe* wood ash concentrations. The highest mortalities were achieved following application of 5g/50g *C. imberbe* wood ash to broken Bambara groundnuts, ground Bambara groundnuts and broken cowpeas. The highest concentration of *C. imberbe* caused 50% mortalities on Broken Bambara groundnut, ground Bambara groundnut and broken cowpeas. The 48% mortality achieved following the application of the highest concentration (5g/50 food commodity) of *C. imberbe* on ground cowpeas was not significantly different ($P < 0.0005$) from the mortalities achieved by a lower concentration of 3g/50g food commodity on broken beans and ground beans.

Table 2: Mortality of *C. ferrugineus* on different pulses treated with serial concentrations of *C. imberbe* wood ash.

Concentration (g/50g)	Food commodities	Mortality ± SE
0	Broken Bambara groundnuts	14±5.66[h]
	Ground Bambara groundnuts	6±2.83[d]
	Broken cowpeas	10±8.49[f]
	Ground cowpeas	4±2.83[a]
	Broken beans	2±1.41[b]
	Ground beans	12±2.83[g]
1	Broken Bambara groundnuts	42±1.41[m]
	Ground Bambara groundnuts	42±1.41[m]
	Broken cowpeas	42±1.41[m]
	Ground cowpeas	40±1.41[l]
	Broken beans	38±0.71[k]
	Ground beans	40±1.41[l]

3	Broken Bambara groundnuts	46 ± 2.83^{o}
	Ground Bambara groundnuts	42 ± 1.41^{m}
	Broken cowpeas	46 ± 2.83^{o}
	Ground cowpeas	44 ± 0.00^{n}
	Broken beans	48 ± 1.41^{p}
	Ground beans	48 ± 1.41^{p}
5	Broken Bambara groundnuts	50 ± 0.00^{q}
	Ground Bambara groundnuts	50 ± 0.00^{q}
	Broken cowpeas	50 ± 0.00^{q}
	Ground cowpeas	48 ± 1.41^{p}
	Broken beans	48 ± 1.41^{p}
	Ground beans	48 ± 1.41^{p}

Means followed by the same alphabet do not differ significantly at ($P \leq 0.05$) by Tukey's Honestly Significant Difference test.

Discussion

The results of this study demonstrate that *C. imberbe* wood ash has some insecticidal effect on *C. ferrugineus*. Studies have shown that *C. imberbe* wood ash can effectively suppress pest populations and reduce damage to stored produce. A study by Chikukura *et al*. (2011) [10] revealed that *C. imberbe* wood ash was effective at reducing cowpea pest populations and was relatively more effective than other plant powder-based treatments. The results also demonstrated that mortality of *C. ferrugineus* was concentration dependent as more beetles were killed at high concentration levels. These results are consistent with those of Suleiman *et al*., (2012) [24] who found that the mortality of *S. zeamais* on stored sorghum was dependent on the amount of plant powder applied. Tendai (2021) [25] observed that *C. imberbe* contains osdienen, an essential oil documented to affect survival of insects. *C. imberbe* ash has also been documented to cause an obstruction of inter-granular spaces which hinders the movement of adult beetles and this leads to less or shallower infestation (Boeke *et al*., 2001) [26]. Since the movement of the adults is hampered, their latitude of movement and of meeting conspecifics is limited and their rate of multiplication will thus be lower. Filling the inter-granular space might serve as good protective medium for stored seeds. However, relatively irregular size cannot be evenly mixed with the *C. imberbe* ash, therefore less effective than on fine food commodities. The applied ash does not only hamper beetle movement, but it can also do physical damage to the adult beetles which is one of the mode of action.(Boeke *et al*., 2001) [26]. When the adult beetles move over or through the ash, their bodies, especially the layer of epicuticle on the adult's abdomen are removed. The abrasive particles of ash can remove the protective wax layer of the beetle's cuticle, making it easier for water to evaporate leading to dehydration and death (Wakil *et al*., 2025; Sagumaran, 2022) [27, 28]. Clogging of insect spiracles and tracheae or blocking of the lateral stigmates, all essential for respiration, cause suffocation of the adult and enhance mortality (Boeke *et al*., 2001) [26].

Suffocation could be a mechanism of the effect of ash on eggs, adults and larvae. Thick layers of ash reduce the available oxygen and could interfere with the respiratory ability of eggs, larvae and adults. According to Chikukura *et al*. (2011) [10], *C. imberbe* ash gave satisfactory level of protection following application to beans, and reduced the number of offspring than the control. *C. imberbe* wood ash was found to be more effective than most other botanicals including onion peels and dry chili pepper. The application of *C. imberbe* wood ash powder can be an effective, natural method for controlling *C. ferrugineus* at the small holder farmer level.

Conclusions and Recommendations

The present study found that *C. imberbe* wood ash can be used to effectively reduce *C. ferrugineus* populations on stored grains although more frequent treatments may be required. Higher concentrations than those tested in this study may give better results, especially causing higher beetle mortalities. *C. imberbe* is naturally occurring and the extracts are easy to handle, inexpensive and safe for stored product pest management. The products do not pose any harm to the environment, humans and non-target organisms. They do not have risk of insecticide resistance development and residual poisoning problems. Results of this study would encourage further research that will lead to the use of *C. imberbe* wood ash for management of *C. ferrugineus* and replace questionable synthetic insecticides in stored food pest management. From the results of this study, it can be concluded that *C. imberbe* wood ash when used alone cannot offer effective and timely control of *C. ferrugineus*. However, with a completely unique mechanism of action it can be used as a resistance management component of *C. ferrugineus* management programs, and reduce the application of synthetic grain protectants. The population in this study did not exhibit any signs of resistance development, therefore the use of *C. imberbe* wood ash powder can be safely recommended.

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