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Masego Kgosietsile Mfuma
Department of Biological Sciences, University of Botswana, P/Bag 0022, Gaborone, Botswana

Ntebaleng Makate
Department of Biological Sciences, University of Botswana, P/Bag 0022, Gaborone, Botswana

Mitch Mosimanegape Legwaila
Botswana University of Agriculture and Natural Resources, P/Bag 0027, Gaborone, Botswana

Corresponding Author:
Masego Kgosietsile Mfuma
Department of Biological Sciences, University of Botswana, P/Bag 0022, Gaborone, Botswana

Bio-efficacy of *M. oleifera* leaf powder against *Cryptolestes ferrugineus* (Stephens) on stored cereals and pulses in Botswana

Masego Kgosietsile Mfuma, Ntebaleng Makate and Mitch Mosimanegape Legwaila

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Abstract
Cryptolestes ferrugineus S. (Coleoptera: Cucujoidae; Laemophloeidae), is a pest of high economic importance as it attacks stored cereals and pulses in storage, causing enormous economic losses. Insecticides and fumigants are often used to minimize the damage they cause, and their extended use generates serious problems such as the emergence of resistant pest populations. This study evaluated the effectiveness of *M. oleifera* leaf powders against *C. ferrugineus* on stored cereals in the laboratory at the University of Botswana. Using three dosages of *M. oleifera* leaf powder; 1 g, 3 g and 5 g, a bioassay was conducted against *C. ferrugineus* at 28±2 °C and 70±5% RH. Each treatment was replicated 5 times. The toxic effect was evaluated 3 months following application of *M. oleifera* leaf powders. Probit analysis was used to determine the LD_{50} and LD_{90} values for the treatments. The lowest dosage of *M. oleifera* was able to cause 90-100% mortality over the period of the experiment. The type of stored food commodity did not significantly affect the effectiveness of *M. oleifera* leaf powder. This study showed that *M. oleifera* leaf powder can be used to achieve effective control of *C. ferungineus* and reduce damage to stored grains and pulses under storage conditions. These findings may be helpful in the selection of botanical source insecticides and in developing effective integrated pest management strategies.

Keywords: *Cryptolestes ferrugineus*, *Moringa oleifera*, bio-efficacy, stored grain pest, Botswana

Introduction
The rust-red grain beetle, *Cryptolestes ferrugineus* (Coleoptera, Cucujoidea: Laemophloeidae), is a destructive pest of stored grain and legume products worldwide (Bharathi *et al*., 2023) [1]. It is considered among the most destructive beetles of stored grain and legume products (Suleiman & Rugumamu, 2017) [2], and a significant threat to the global food supply chain. *C. ferrugineus* causes significant economic losses, increases production costs and food wastage (Wubetu & Hiruy, 2020) [3]. Both the larvae and adults feed on the germ and endosperm of the grain (Ajayi & Peter, 2016) [4] causing a loss of quality and reducing germination. Heavy infestations cause extensive damage by causing large quantities of grain to spoil, and by introducing fungal spores into the stored food produce (Bharathi *et al*., 2023) [1].

Allotey *et al*. (2012) [5] reported that *C. ferrugineus* is one of the predominant insect pests infesting stored food products in Botswana. It's presence in Southern Africa has also been reported by Nukenine (2010) [6]. Hot and dry conditions prevalent in Botswana are favorable to the development of storage pests and their control is heavily reliant on the use of synthetic insecticides and fumigants. The use of chemical insecticides leads to development of resistance as well as deleterious human health and environmental consequences, and are very expensive (Rather *et al*., 2014) [7]. 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 derivatives in integrated pest management (IPM) is an essential component that reduces application of chemical insecticides (Barzman *et al*., 2015) [8]. Botanical source insecticides are of great potential since they possess insect repellent and anti-feedant properties, are specific, non-toxic to humans and animals, bio-degradable, non- pest resistance and naturally available. Some of the most promising botanical source insecticides are those based on *Moringa oleifera* L.

Moringa oleifera L. (Moringaceae) a native to India, Africa, Arabia, South Asia, South America, the pacific and Caribbean Islands, is a tree widely distributed in the tropics (Race *et al*., 2012) [9]. It is of significant economic importance because of the several industrial and medicinal applications, and the various products (i.e. food and animal feed) which can be derived from its leaves and fruits. *M. oleifera* has insecticidal and microbial properties against a wide variety of pests (Nisar *et al*., 2021) [10], and these properties are attributed to the presence of the functional bioactive compounds, such as phenolic acids, flavonoids, alkaloids, phytosterols and organic acids (Dhakad *et al*., 2019) [11]. These properties can play an important role in developing an Integrated Pest Management (IPM) for control of storage insect pests.

With the increasing costs and risks posed by synthetic insecticides, botanical source insecticides offer a great deal of promise for pest management. They are highly effective against certain pests, yet do not pose the serious environmental hazards associated with conventional insecticides.

Because of their low toxicity to humans, beneficial and non-target organisms, botanical source insecticides are suitable candidates for use in integrated pest management (IPM) programs, especially where pests have developed resistance to synthetic 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) [12]. However, little is known about their effectiveness when applied against *C. ferrugineus* infesting grain storages in Botswana. Considering the damage caused by the *C. ferrugineus* in storage facilities, it is necessary to undertake studies to determine the effectiveness of plant powders, and make recommendations for their effective use. 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 evaluate the effectiveness of *M. oleifera* leaf powders 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 different places around Gaborone, Seed Multiplication Unit (SMU) warehouse, Agriculture shop, the old library in Sebele, Department of Crop Protection in Sebele (Coordinates), households in and around Gaborone, Mogoditshane, Tlokweng, Oodi, Ramotswa and Molepolole, supermarkets, the University of Botswana insectary in the Department of Biological Sciences and farms (Moshupa, Ramotswa and Mmathethe).

Infested 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 an established insectary in the Department of Biological Sciences, 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) [13].

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, llinois, USA) to obtain live insects. The different species of insects from each infested food commodity were identified using the insect identification keys 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 80 °C for 2 hours. This was carried out to effectively destroy organisms responsible for spoiling the food commodities and to increase their shelf-life. Other equipment such as the glass vials and plastic petri dishes were also thoroughly washed, and oven dried in order to prevent cross infestation.

Preparation of plant powders

M. oleifera fresh leaves were collected from Bobonong (Geographical coordinates, 21.9814˚S, 28.4280˚E), Botswana. The leaves were air dried in the laboratory at room temperature of 25 ˚C to 30 ˚C and relative humidity of 65% to 70%; followed by grinding using a 5 speed Brabantia table blender (1.5L glass jug) for 60 seconds at 10 rpm. The materials were then sieved through 150-600 µm mesh sieve to obtain a fine powder (Anita *et al*., 2012) [14], which were kept in airtight plastic bags and stored at room temperature in the insectary (Akunne & Ononye, 2015) [15]. Two kilograms of *M. oleifera* leaf powder was obtained for use in the experiment.

Bioassay methods

The leaf powders were applied at three levels (1 gram, 3 grams and 5 grams). The treatments were separately applied to 50 grams of the broken and ground maize, sorghum and rice kept in two litre glass jars, each with five replicates for each treatment and control. The jars were shaken to allow the plant powders to adequately mix with the whole food commodity. The experiment was arranged in a completely randomized design. Ten adults (Five males and five females) of *C. ferrugineus* were introduced into the treatments and control (Dhakad *et al*., 2019) [11]. 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) [16]. Untreated mortality was also recorded.

Statistical analysis

Probit analysis was used to analyse mortality results. Mortality data were subjected to arcsine transformation while the dosages were transformed to $\log_{10}(x+1)$ prior to analysis (Najem *et al*., 2020) [17]. Data was analysed using

Log_{10} dosage versus probit mortality regression and analysis of variance (ANOVA). LD_{50} and LD_{90} values were estimated from the probit lines. Relative susceptibilities of *C. ferrugineus* adults were compared using LD_{50} and slope of probit lines. Data were also analysed as a repeated measure using mixed model procedure (PROC MIXED) (Littell *et al.*, 1998) [18]. Least square means were compared between varieties based on LSD and considered significant at P = 0.05.

Results

Relationship between *M. oleifera* leaf powder dosages and mortality of *C. ferrugineus* adults on broken and ground cereals

A positive curvilinear relationship between log dose and probit mortality due to applications of *M. oleifera* (Correlation coefficients: 0.9942 and 0.9925) is shown in Figure 1. The results indicate that *M. oleifera* achieved LD_{50} values of 0.4 on the probit scale (Equivalent to 1.51g) on both broken and ground maize when assessed 3 months following application. LD_{50} values of 0.39 (equivalent to 1.46 g/50 g) (Correlation coefficient: 0.9974) and 0.42 (Equivalent to 1.63g) (Correlation coefficient: 0.9818) were achieved following application of *M. oleifera* on broken and ground sorghum commodities respectively. There was a positive curvilinear relationship between log dose and probit mortality (Correlation coefficient: 0.9868 and 0.9983) following application of *M. oleifera* leaf powder to ground and broken rice respectively. *M. oleifera* leaf powder achieved LD_{50} values of 0.54 (Equivalent to 2.47g/50g) and 0.51 (Equivalent to 2.24g/50g) on ground and broken rice respectively.

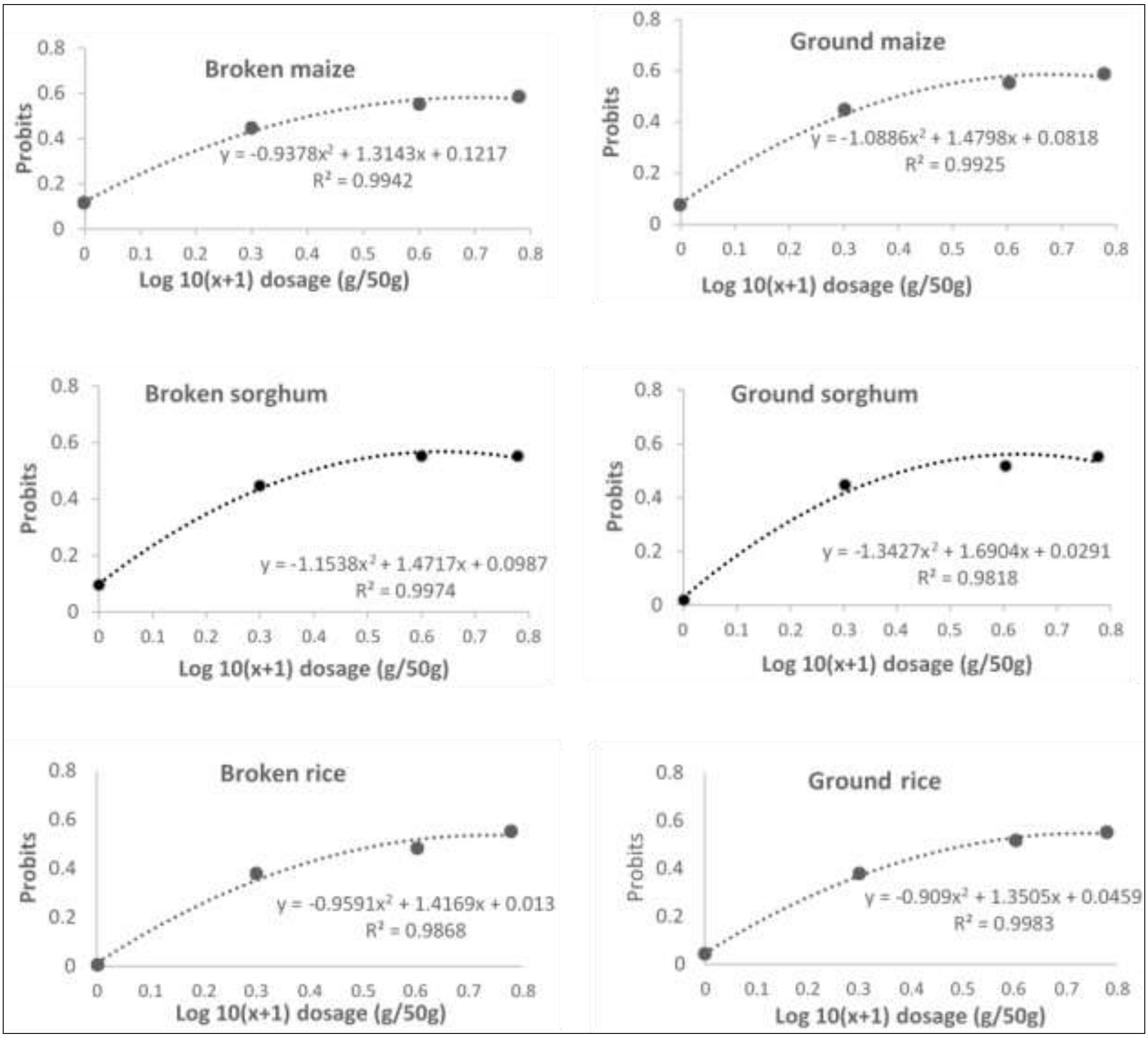

Fig 1: Probit mortality of *C. ferrugineus* adults on ground and broken cereals three months following application of different dosages of *M. oleifera* leaf powder

Table 1: Effect of *M. oleifera* dosages and stored cereal commodity type on *C. ferrugineus* mortality.

	Means ±SE					
Conc.	Ground maize	Crushed maize	Ground rice	Crushed rice	Ground sorghum	Crushed sorghum
0g	55.2±3.87^{C}	70±1.89^{BC}	54±0.74^{B}	62±1.66^{B}	58±1.09^{B}	68±1.01^{B}
1g	86±1.80^{AB}	92±0.87^{AB}	88±0.89^{A}	88±0.89^{A}	92±0.87^{A}	92±0.87^{A}
3g	98±0.45^{A}	98±0.45^{A}	94±0.57^{A}	96±0.56^{A}	96±0.55^{A}	98±0.45^{A}
5g	100±0.00^{A}	100±0.00^{A}	98±0.45^{A}	98±0.45^{A}	98±0.45^{A}	98±0.45^{A}

Averages in the column followed by the same letters are not significantly different (Tukey's honestly significant difference test ($p< 0.05$).

Averages in the row followed by the same letter are not significantly different (Tukey's honestly significant difference test ($p< 0.05$).

Table 1 shows the effect of *M. oleifera* leaf powder dosages and stored cereal commodity type on *C. ferrugineus* mortality. The highest mortality 90- 100% that occurred on ground maize following application of 3 g was not significantly different ($F_{(11,28)}$ =10.44; $p<0.0001$) from the 92% mortality achieved on crushed maize following application of 1 g *M. oleifera* leaf powder. The highest mortalities of 90-100% achieved on ground and crushed rice following treatment with 3 g *M. oleifera* leaf powder were not significantly different ($F_{(11, 28)}$ =16.84; $p<0.0001$) from the 88% mortalities achieved after treatment with the lowest dosage of 1 g *M. oleifera* leaf powder. The 98% mortalities achieved with application of the lowest dosage of 1 g *M. oleifera* leaf powder on ground and crushed sorghum were not significantly different ($F_{(11,28)}$ =16.15; $p<0.0001$) from the mortalities achieved with the highest dosage of 5 g *M. oleifera* leaf powder on the same food commodities.

Relationship between *M. oleifera* leaf powder dosages and mortality of *C. ferrugineus* adults on broken and ground pulses

A positive curvilinear relationship between log dose and probit mortality following application of different dosages of *M. oleifera* leaf powder (Correlation coefficients: 0.9821 and 0.9602) is shown on Figure 2. Results indicate that LD_{50} values of 0.41 (Equivalent to 1.57 g) and 0.39 (Equivalent to 1.45 g) were achieved three months following application of *M. oleifera* leaf powder to ground and broken Bambara groundnuts respectively. Results show a positive curvilinear relationship between log dose and probit mortality due *M. oleifera* on crushed and ground cowpeas. LD_{50} values of 0.44 (Equivalent to 1.75 g/50 g) (Correlation coefficient: 0.9896) and 0.38 (equivalent to 1.40 g) (correlation coefficient: 0.9601) were recorded on ground and broken cowpeas respectively. A positive curvilinear relationship between log dose and probit mortality due to *M. oleifera* leaf powders on ground and broken beans (Correlation coefficient: 0.9915 and 0.9601 respectively) is shown. LD_{50} values of 0.45 (Equivalent to 1.82 g/50 g) and 0.37 (Equivalent to 1.34 g/50 g) were achieved on ground and broken bean commodities respectively.

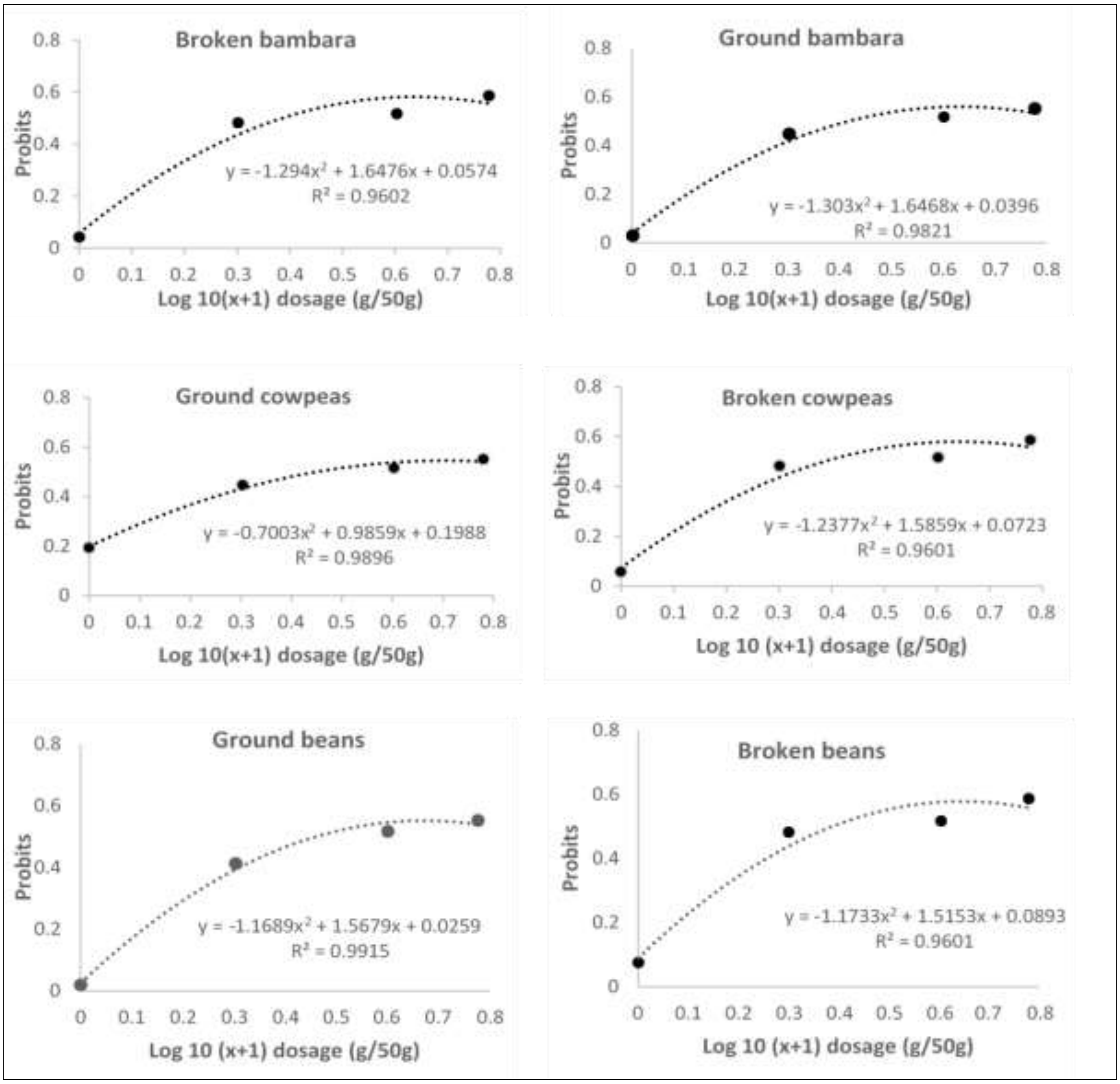

Fig 2: Probit mortality of *C. ferrugineus* adults on ground and broken pulses three months following application of different dosages of *M. oleifera* leaf powder.

Table 2: Effect of *M. oleifera* dosages and stored pulses commodity type on *C. ferrugineus* mortality.

	Means ± SE					
Conc.	**Ground cowpeas**	**Crushed cowpeas**	**Ground Bambara**	**Crushed Bambara**	**Ground beans**	**Crushed beans**
0g	60±1.58^{B}	64±2.09^{B}	60±1.29^{B}	62±2.27^{B}	58±1.09^{B}	66±1.65^{B}
1g	92±0.87^{A}	94±0.92^{A}	92±0.87^{A}	94±0.92^{A}	90±0.74^{A}	94±0.92^{A}
3g	96±0.56^{A}	96±0.56^{A}	96±0.56^{A}	96±0.56^{A}	94±0.57^{A}	96±0.56^{A}
5g	98±0.45^{A}	98±0.00^{A}	98±0.45^{A}	100±0.00^{A}	98±0.45^{A}	100±0.00^{A}

Averages in the column followed by the same letters are not significantly different (Tukey's honestly significant difference test ($p< 0.05$).

Averages in the row followed by the same letter are not significantly different (Tukey's honestly significant difference test ($p< 0.05$).

Table 2 shows the effect of *M. oleifera* leaf powder dosage and stored pulse commodity type on *C. ferrugineus* mortality. High mortalities of 92 and 94% were achieved with the lowest dosage of 1g *M. oleifera* leaf powder on ground and crushed cowpeas respectively, and were not significantly different ($F_{(11,28)}$ =14.99; $p<0.0001$) from the 98% mortalities achieved on the same commodities following application of 5 g *M. oleifera* leaf powder. The highest mortalities of 98 and 100% achieved on ground and crushed Bambara groundnut following treatment with the highest dosage (5 g) of *M. oleifera* of leaf powder were not significantly different ($F_{(11, 28)}$ =9.60; $p<0.0001$) from the 92 and 94% mortalities achieved after treatment with the lowest dosage of 1 g *M. oleifera* leaf powder on ground and crushed Bambara groundnut respectively. High mortalities (90 -100%) that were achieved with application of the lowest dosage of 1 g *M. oleifera* leaf powder on both ground and crushed beans were not significantly different ($F_{(11,28)}$ =13.16; $p<0.0001$) from the mortalities achieved with the highest dosage of 5 g *M. oleifera* leaf powder on the same food commodities.

Discussion

Botanical source insecticides have been suggested as possible alternatives to synthetic insecticides for controlling stored product pests by affecting survival and nutrition (De Oliveira *et al*., 2020) [19]. In this work, *M. oleifera* leaf powders were assessed for the control of *C. ferrugineus* infesting stored cereal and pulse commodities. Results showed that *M. oleifera* leaf powder had insecticidal properties against *C. ferrugineus* on crushed and ground food commodities. These results concur with those of Mahpara (2019) [20] who demonstrated that *M. oleifera* leaf powder was very effective against several pests of stored produce. On sorghum, treatments with *M. oleifera* achieved equal mortalities on both ground and crushed sorghum. The differences in types of cereal commodities did not have an effect on the effectiveness of *M. oleifera* leaf extracts. Similarly, the type of pulse commodity did not have any influence on the survival of the pest.

In order to evaluate the effectiveness of a bio-insecticide like *M. oleifera*, other mechanisms of action apart from mortality have to be taken into consideration. *M. oleifera* leaf powder has several mechanisms of action which include; feeding deterrence, reduction of oviposition, ovicidal activity and toxicity to immature stages (Ogunwolu & Odunlami, 1996) [21]. Nisar *et al*. (2021) [10] demonstrated that *M. oleifera* was highly toxic to *T. granarium*, while Anjum *et al*. (2019) [22] demonstrated repellent action of *M. oleifera* leaf extract against the pest. Iqbal *et al*. (2018) [23] found that extracts of various parts of *M. oleifera* caused high mortality of adults and larvae of *T. granarium* and showed repellent properties. The insecticidal activities are attributed to presence of phytochemicals including tannins, steroids, flavonoids, terpenoids, anthraquinones and alkaloids (Lengai *et al*., 2020) [24]. Mahpara (2019) [20] demonstrated that *M. oleifera* leaf powder was very effective against *Callosobruchus maculates* on cowpea in storage. Nisar *et al*. (2021) [10] found that *M. oleifera* leaf extracts were highly effective against *Odontotermes obesus* in storage. Other studies showed the insecticidal and repellence activity of *M. oleifera* leaf extracts against many insects including *Anopheles gambiae*, *S. littoralis*, and *Tribolium castaneum* (Iqbal *et al*., 2018) [23].

According to Ileke & Ogungbite (2014) [25] powdered plants can adequately protect stored food commodities against storage insects since they are able to block the spiracle of the insects ultimately leading to suffocation. *M. oleifera* leaf powder has also been shown to be an effective oviposition deterrent. Anita *et al*. (2012) [14] demonstrated in a study on *C. maculatus* that *M. oleifera* was an oviposition deterrent and significantly reduced adult emergence, and these properties were dosage dependent. These properties were highly welcome since outbreaks of populations from subsequent life stages would be greatly reduced thereby reducing damage to the commodity (Anita *et al*., 2012) [14]. Furthermore, Nukenine (2010) [6] suggested that the bitter taste of *M. oleifera* leaves and the presence of biochemical constituents contributed to the feeding deterrent properties and contributed to increased mortalities. The results that lower dosages of *M. oleifera* can be used to achieve high mortalities of *C. ferrugineus* are highly welcome since this would reduce the amount of active ingredient applied to the food commodity. *M. oleifera* leaf powders do not necessarily need to cause high mortalities to confer protection of the commodity from damage since it possesses multiple modes of action. Low dosages of *M. oleifera* leaf powder can be safely used to protect the stored food commodities provided long exposure periods are allowed. These results are in agreement with Adenekan *et al*. (2013) [26] who found that, *M. oleifera* is a suitable alternative to synthetic insecticides for the control of stored commodity pests.

Conclusions and Recommendations

The present findings have important implications in the practical control of *C. ferrugineus* in storage facilities. The plants source studied 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 a pose risk of insecticide resistance development and residual poisoning problems.

From the results of this study, it can be concluded that *M. oleifera* leaf powders can offer effective management of *C. ferrugineus* at minimal levels of the active ingredient provided long exposure periods are allowed. These findings would encourage further research that will lead to the use of *M. oleifera* leaf powder for management of *C. ferrugineus* and replace synthetic insecticides in stored food pest management. The population in this study did not exhibit any signs of resistance development, therefore the use of *M. oleifera* leaf powder can be safely recommended. With a completely unique mechanism of action, *M. oleifera* leaf powder can be used as a resistance management component of integrated *C. ferrugineus* management programs.

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