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Research Article

Evaluation of the Insecticidal Efficacy of *Aloe vera* L. Leaf Powder on Adult Bean Weevil (*Callosobruchus maculatus (*F.))

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ABSTRACT

In Sub-Saharan Africa, *Callosobruchus maculatus* is a significant pest that grossly reduces cowpea yield, leading to economic crop losses and shortages in cowpea supply. Traditional plant powders prepared from indigenous plant parts have been used to provide ecologically tolerable and potentially safe pesticides against this pest. This study evaluated the insecticidal efficiency of *Aloe vera* leaf powder against adult *C. maculatus* in cowpea. The toxicity of *A. vera* leaf powder was assessed using contact toxicity method in cowpea variety black eye at 48 hours and 30 days post treatment. Seed damage rate of *C. maculatus* was assessed at six treatments (0g/20g, 0.5 g/20 g, 1.0 g/20 g, 1.5 g/20 g, 2.0 g/20 g and 2.5 g/20 g cowpea seeds) using a randomized design, estimating parameters like percentage damage, seed weight loss and adult exit hole after 30 days. The study found no mortality of *C. maculatus* after 48 hours of infested cowpea seed exposure to *A. vera* leaf powder. However, a concentration dependent increase in toxicity and decrease in seed damage was observed after 30 days exposure to *A. vera* leaf powder. Furthermore, maximum mortality of 10.33%, resulting in a 3.33% seed damage reduction, was observed at 2.0 grams of *A. vera* treatment. The results suggest that *A. vera* has a low insecticidal effects and low efficacy in protecting against *C. maculatus* seed damage rates. The bioactive chemicals of the *A. vera* leaf extracts should be explored to create a potent biopesticide for preventing cowpea loss and enhancing food security.

Keywords: *Aloe vera;* Insecticidal; *Callosobruchus maculatus;* Leaf Powder; Cowpea

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INTRODUCTION

Cowpea (Plate III), (*Vigna unguiculata* (L.) Walp) constitute an important edible legume crop for both human and livestock due to its high protein content (Diouf, 2011). Cowpea is a major cash crop largely grown by small- to medium- scaled farmers in tropical and sub-tropical regions (Machacha *et al.,* 2012). According to Oyerinde *et al.* (2013), insect pests and disease infestations have a significant negative impact on its production, potentially resulting in crop losses and discontinuity in the supply of cowpea in Africa. As the main stored pest of cowpea in the tropics, the cowpea weevil; *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) (Plate II), is the most widespread field-to-store pest of legume seeds. The mature *C. maculatus* insect lays its eggs on the surface of cowpea seeds, which hatch and penetrate the seed's cotyledons causing qualitative and quantitative crop loss both in the uncultivated and stored cowpea (Olayemi *et al.*, 2022). The primary cause of seed damage rate impacted by *C. maculatus* is mainly the lack of advanced storage and processing facilities particularly in the Sub-Saharan Africa. Substantial losses caused by *C. maculatus* are

manifested by seed perforation and reductions in weight, market value, nutritional value and germination ability of seeds (Oluwafemi, 2012). Controlling *C. maculatus* thus becomes imperative for ensuring food security, reducing poverty, preventing protein related malnutrition and increasing the market value of cowpea seeds.

In order to combat food insecurity, synthetic chemical pesticides are currently the preferred strategy for the control of stored-cowpea insects (Epidi and Odili, 2009). But the extensive use of these chemicals has resulted to a number of toxicities and health risks for humans, other animals and the environment, as well as the emergence of resistant forms of the pests (Rahman *et al.,* 2009). Furthermore, the residual chemical impacts to cooked cowpea seed a persistent chemical odor and flavour (organoleptic quality) (personal observation; Sharma and Meshram, 2006). Consequently, a lot of botanical- based products have gained popularity since they are easily accessible, safer and less expensive than chemical- based preservatives for controlling *C. maculatus* in cowpea storage. In sub-Saharan Africa, common botanical indigenous plants used are *momordica balsamina* leaf powder (Usha rani *et al.*, 2014; Ribeiro *et al.*, 2014), chilli pepper, and neem (*Azadirachta indica*) leaf powder (Oshomah and Degri, 2016). Due primarily to their phytochemical contents, *Aloe vera* leaf extracts have been documented in numerous studies for the control of a variety of pest and spoilage causing microbes in stored products (Agarry *et al.*, 2005; Yadav, 2015; Mallavadhani, 2016). The absence of scientific data to support the efficacy, mode of action, and safety of botanical products has prevented their wide commercial application in the management and control of storage pests.

For millennia, people have used and appreciated the heath, aesthetic, therapeutic, and skin-care benefits of the *Aloe vera* plant (Plate I). It is primarily found growing mainly in the dry regions of Africa, Asia, Europe and America. High concentrations of vitamins, enzymes, minerals, sugars, hormones and amino acids are among the nutritional constituents of *A. vera* leaf exudates and gel (Amar *et al.*, 2008). Additionally, phenolic compounds, lignin, saponins, salicylic acids, glycosides (aloins), 1,8 dihydroxy-anthraquinones (aloe emodin), β- 1,4 acetylated mannan, mannose phosphate, and alprogengluco protein are reported to be present in *A. vera* (Sharma, 2014). Additional substances with antibacterial properties include urea nitrogen, cinnamonic acid, sulphur and lupeol (Agarry *et al.*, 2005). Numerous biological activities of *A. vera* such as its cytotoxicity against ovarian cell lines and its antimicrobial, antifungal, antibacterial, antioxidant, and insecticidal effects, have been reported by activity- based studies (Mallavadhani, 2016). These activities are primarily attributed mainly to Aloin A, with smaller amounts of its C-10 epimer, Aloin B also contributing (Balasubramanian and Narayanan, 2013; Yadav, 2015). Additionally, it has been reported that *A. vera* gel can be used to enhance and lengthen the post-harvest shelf life of fruits such as sweet cherry, grapes, kiwifruits, blueberry, and apple slices (Vieira *et al.*, 2016). The peel, gel and exudates of the leaf contain the majority of the bioactive compounds identified in *A. vera* (Vieira *et al.*, 2016).

Therefore, research into *A. vera* leaf powder's insecticidal potential is necessary as it may serve as a potential safe and effective bio-pesticide substitute for chemical postharvest treatments. The majority of locally grown plants that are used locally to control stored pest have little to no detrimental biological activity, yet there is paucity of research on them. In order to achieve this, the study assessed the insecticidal efficacy of *Aloe vera* leaf powder on adult cowpea weevil (*Callosobruchus maculatus*) by determining the toxicity of the powder on *C. maculatus* and assessing the rate of seed damage following *Aloe vera* leaf powder treatment using indices like seed weight loss, adult exit hole and mortality.

MATERIALS AND METHODS

Plant Collection, Identification and Preparation

Aloe vera leaves were collected from a botanical garden in Dutse, Jigawa State-Nigeria in June 2017. The Department of Biological Sciences, Federal University Dutse Herbarium authenticated the leaves obtained and voucher samples with number FSC/ZOO/13/0009 were deposited for future reference. Dutse, Jigawa state falls under Sudan savannah, an area well known for its two distinct seasons; the wet season which runs from May to September and the dry season, which runs from October to April. The area receives 600-800 mm of precipitation annually, with an average yearly temperature of 26ºC.

After washing any sand or dirt with borehole ground water, the leaves were cut into pieces and allowed to air dry for three weeks at room temperature. The dry sample was grounded into a fine powder using pestle and mortar, sieved, packaged in a polythene bag and stored in a cool and dry place until required.

Collection of Cowpea Seeds

Non infested cowpea seeds were purchased from a local market in Dutse, Jigawa State and the variety was identified at the Department of Biological Sciences, Federal University Dutse. The cowpea seeds were stored and packed in an air tight container for 10 days to ensure that they are free of pest infestation prior to its use for insect culture.

Insect Culture

Callosobrochus maculatus was collected from an infested stock of bean purchased from a local market in Dutse, Jigawa State and identified by an entomologist. The insects were reared at a temperature of 25 ± 2˚C and relative humidity of 65 ± 5 % in a transparent glass jars covered with muslin cloth containing whole cowpea seed (100g) as described by Jose (2014).

Insecticidal effect of *Aloe vera* Leaf Powder

Contact Toxicity Assay

Toxicity of *Aloe vera* leaf powder against *C. Maculatus* was determined with the contact toxicity method using the completely randomized design as described by (Adesina, 2010; Udo, 2000).Twenty grams (20g) of cowpea was measured into six (6) transparent glass jars and different *A. vera* leaf powder treatments; 0.5g, 1.0g, 1.5g, 2.0g and 2.5 gram were added while the control contained no treatment. The glass jars were covered and tumbled several times to ensure homogenous mixing of the powder with the cowpea seeds and left at room temperature for one (1) hour. Five (5) pairs of adult *C. maculatus* (male and female) (1-2 days old) were carefully introduced into the different treatments glass jars and control. The containers were then covered and placed on the laboratory benches for observation.

The experiment was replicated three times and cumulative mortality was recorded at 48 hours and 30 days after infestation respectively. Insects were considered dead on failure to respond to three probings using a blunt dissecting probe. After 48 hours and 30 days, live adult insects were removed and percentage mortality was calculated as follows.

$$Percentage\ mortality = \frac{number\ of\ dead\ insects}{total\ number\ of\ insects\ introduced}\ x\ 100$$

Assessment of Seed Damage rate

Seed damage assessment was carried out using the method described by Adetumbi and Olakojo (2010). The live insects were reintroduced back into the treatment containers and percentage seed damage was calculated 30 days after infestation as follows:

Percentage seed damage= number of seed damage/number of seed stored x 100

Adult Exit Hole

The total number of seeds with adult exit holes in the different treatments and undamaged cowpea were counted after 30 days treatment.

Percentage weight loss

Percentage weight loss was determined 30 days post *Aloe vera* leaf powder exposure as follows:

Percentage weight loss =

$$\frac{\text{initial weight of seeds} - \text{final weight of seed}}{\text{initial weight of seed}}\ X\ 100$$

Data Analysis

Data collected were subjected to one way analysis of variance (ANOVA), while seed damage, exit holes and weight loss were subjected to Tukey's post hoc test where significant differences existed and $p<0.05$ was considered significant.

RESULT

As shown in Table 1, no mortality was observed after 48 hours of *Aloe vera* leaf powder treatment. Toxicity was however observed 30 days after treatment (Table 1) as evidenced by mortality of bruchid observed. Significantly, lowest mortality percentage ($p<0.05$) of *C. maculatus* was observed at the highest *Aloe vera* treatment (2.5g) (5.67±2.08%) and highest mortality was observed at 2.0 g (10.33±4.58), 30 days after infestation when compared to the control (2.33±1.56%).

The efficacy of *Aloe vera* leaf powder against *C. maculatus* seed damage in cowpea stored for 30 days assessed using weight loss, percentage seed damage and exit hole are presented in Table 2. A concentration dependent decrease in *C. maculatus* seed damage rate was observed in the *A. vera* leaf treated cowpea seeds stored for 30 days. Significant reduction in seed damage ($p<0.05$) caused by *C. maculatus* became obvious at treatment dose of 1.5 g (9.33±7.51%) of *Aloe vera* powder. However, *C. maculatus* caused maximum seed damage of 14.00±9.17% at the lowest treatment (0.5g/20g cowpea seed) which resulted in weight loss and exit hole number of 3.13±1.94% and 3.78±7.37 respectively. Maximum decrease of percentage seed

damage (3.33±1.53%), number of exit hole (3.00±2.65%) and percentage weight loss (0.87±0.31%) was observed in cowpea seeds stored for 30 days treated with 2.0 g *A. vera* leaf powder.

Table 1. Toxicity of Aloe vera Leaf Powder against Adult Cowpea Weevil

Quantity (g)	Mortality 48hrs (%)	Mortality 30 days (%)
Control	No mortality	2.33±1.36[a]
0.5	No mortality	6.33±1.15[c]
1.0	No mortality	7.33±1.15[c]
1.5	No mortality	8.33±6.66[c]
2.0	No mortality	10.33±4.58[d]
2.5	No mortality	5.67±2.08[b]

Values expressed are mean ± standard deviation, n=10 number of weevils. Grams of cowpea seeds= 20 g. Values with different superscripted letters within a column are statistically significant; p<0.05.

Table 2. Seed damage rate by *Callosobrochus maculatus* in Cowpea Seeds stored for 30 days and Treated with *Aloe vera* Leaf Powder

Quantity (g)	Seed damage (%)	Exit hole (n)	Weight loss (%)
Control	11.00±5.20[a]	4.67±4.00[a]	3.80±1.30[a]
0.5	14.00±9.17[a]	3.78±7.37[b]	3.13±1.94[a]
1.0	13.00±12.49[a]	3.57±10.02[b]	2.87±2.54[b]
1.5	9.33±7.51[b]	2.33±6.24[c]	2.30±1.95[c]
2.0	3.33±1.53[c]	1.00±2.65[d]	0.87±0.31[d]
2.5	5.00±5.57[d]	1.56±5.03[d]	1.13±1.10[d]

Values expressed are mean ± standard deviation, n=10 number of weevils. Grams of cowpea seeds = 20g. Values with different superscripted letters within a column are statistically significant; p<0.05

DISCUSSION

Callosobrochus maculatus is the most destructive pest of stored cowpea, posing addition treats to food security and poverty and malnutrition rates of Sub-saharan Africa. Conventional chemical synthetic insecticides used for the control of stored product pest are associated with health concerns to humans, environment and development of resistant strain of the pest (Sharma and Meshram, 2006), which necessitates the search for safer alternatives. Plant bio-pesticides have gained popularity especially in developing countries as a result of their large content of bioactive compounds, availability and assumed safety (Abdallah *et al.*, 2017). Many botanical products such as leaves, seeds, roots, bark, oils and essential oils have been reported as potential biopesticides in many stored products (Aliyu, 2006; Mulungu *et al.*, 2007; Usha rani 2014).

This study indicates low toxicity of *Aloe vera* leaf powder against cowpea weevil and/or increase tolerance after its exposure. This is contrary to findings of many studies where mortality of up to 70% of *A. vera* extract against *C. maculatus* and *Sitophilus oryzae* (Mallavadhani *et al.*, 2016) were observed. *A. vera* leaf powder could have the characteristics of a slow acting insecticides where insecticidal effects are dependent on exposure period rather than the dosage. A longer study period using the leaf powder could therefore have produced a higher toxic effect. Insects breathe by means of trachea which usually opens at the surface of the body through spiracles (Adedire *et al.,* 2011). These spiracles might have been blocked by the bioactive compounds in the leaf powder thereby preventing respiration via trachea leading to suffocation. Furthermore, probable mechanism of toxic responses observed from *A. vera* leaf powder could be through alterations of relevant life traits or neurotoxic effects i.e fecundity, immune capacities, reproduction, oviposition and feeding or compromise to neurone and metabolic tools (Sushree *et al.*, 2017).

Furthermore, contrary to our study, most studies evaluated solvent extracts of *A. vera* leaf. Solvent extracts are reported to contain more concentrated and soluble constituents that could have insecticidal effects against *C. maculatus*. This therefore calls for isolation of bioactive constituents of *A. vera* leaf powder and their characterization with the application of NMR, HPLC or GC-MS in order to produce more potent *A. vera-based* bio pesticide. Efficacy of *A. vera* extracts against *C. maculatus* could

be attributed to ovicidal effects, reduced oviposition, and reduced adult longevity (Joana and Daniel, 2010).

The low toxicity of the *A. vera* leaf powder could therefore be as a result of low amounts of bio-constituents reported in the doses studied as opposed to the concentrated contents found in plant extracts and volatile oils. The reported bioactive components of *A. vera* leaf that could have potential insecticidal effects mainly includes salicylic acids, carbohydrates, glycosides and anthraquinones which can impact a negative survival environment for the adult cowpea weevil (Dales, 1996). Mallavadhani (2016) reported the repellency and toxicity effect aloin A contained in *A. vera* has and demonstrated a 58% mortality against *S. oryzae*. In addition, judging by the numerous biological activities reported in many studies such as antimicrobial, antifungal, antibacterial, antioxidant and cytotoxicity against ovarian tumour cell lines (Amar *et al.*, 2008), the concentration of Aloin in the different plant parts of *A. vera* is reported to be responsible for contact toxicity against *S. oryzae* while repellency was due to other compounds (Liang *et al.*, 2013).Correlations between cowpea weevil density and stored grain damage have been reported by Jose (2014). The significant reduction in percentage seed damage, weight loss and adult exit holes in the treated cowpea seeds could therefore be attributed to contact toxicity effects and potential to reduce adult pest longetivity (Joana and Daniel, 2010).

CONCLUSION

Aloe vera leaf powder showed no toxicity against cowpea weevil within 48 hours of treatment with contact toxicity observed after prolonged exposure. Low mortality of cowpea weevil observed demonstrated poor insecticidal effects of *Aloe vera* leaf powder and thus its low efficacy in protecting stored cowpea seeds against cowpea weevil.

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Appendix I

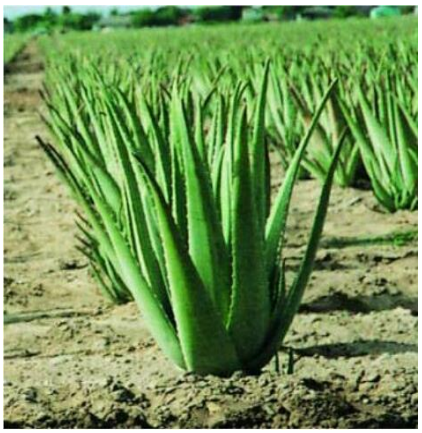

Plate I. Aloe vera plant (Kabir *et al*., 2020)

Plate II. Dorsal View of the male (on the left side) and the female (on the right side) of *C. maculatus.* Source: Christopher and Lawrence (2014).

Plate III. Damaged stored cowpea seeds infested with Cowpea weevil Source: International Institute for Tropical Agriculture (IITA), Kano State.