


Sustainable management of two *Sitophilus* species infesting wheat grains using crude extracts from botanicals

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Received: 30-10-2024; Accepted: 02-08-2024; Published: 17-08-2024

DOI: [10.21608/EJAR.2024.243557.1452](https://doi.org/10.21608/EJAR.2024.243557.1452)

ABSTRACT

This study investigates the bioactivities of the leaf extract of *Acalypha godseffiana* (Muell Arg.) and the stem bark extract of *Alstonia boonei* (De Wild) against *Sitophilus oryzae* (Linnaeus) and *Sitophilus zeamais* (Motschulsky) at different concentrations (2.5, 5.0, 7.5, 10.0, and 12.5% %). The relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested food (ECI) of both *Sitophilus* species were assessed using extract-treated wheat flour discs. The contact toxicity and effects of both extracts on the adult emergence of both weevils were also determined. Control experiments were set up for all the bioassays. Phytochemical compounds (tannins, saponins, anthraquinones, cardiac glycosides, flavonoids, and alkaloids) in each botanical extract were checked. Except for anthraquinones, which was lacking in *A. boonei* but marginally present in *A. godseffiana*, both extracts contain all the tested phytochemicals in either low, moderate, or high amounts. The LC₅₀ values showed that both extracts had higher toxicity to *S. zeamais* at 24 and 48 h and to *S. oryzae* at 72 and 96 h. Both extracts at 12.5% significantly decreased adult emergence and evoked higher inhibition in adult emergence of both *Sitophilus* species relative to controls. RGR, RCR, and ECI generally decreased as extract concentrations increased. At 12.5%, *A. boonei* and *A. godseffiana* considerably decreased the RCR of *S. oryzae* and *S. zeamais*, respectively. Additionally, the ECI of both species was significantly lower relative to controls. Conclusively, the two extracts evoked insecticidal, anti-nutritional, and significantly reduced adult emergence of both *Sitophilus* species relative to controls.

Keywords: *Acalypha godseffiana*, *Alstonia boonei*, *Sitophilus* species, phytochemical compound, toxicity

INTRODUCTION

For ages, insect pests have played a significant role in preventing several countries from achieving self-sufficiency in food production. For instance, in Nigeria, insect pest such as *Sitophilus oryzae* (L.) and *Sitophilus zeamais* (Motschulsky) have been identified as major constraint against large scale production of cereal crops such as *Oryza sativa* (L.), *Sorghum bicolor* (L. Moench), *Zea mays* (L.) and *Triticum spp* (L.) among others. They have resulted in direct product loss and indirect economic cost through the consumption of grains, formation of exit holes for adult emergence, consumer complaints, and pest control costs among others (Ogunbite and Oyeniyi, 2014; Gbaye *et al.*, 2015). Both the adult and larvae stage of both species are voracious eaters capable of eliciting up to 100% loss if left unchecked for several months. The need to reduce losses associated with infestations of both species has resulted in indiscriminate use of synthetic insecticides, especially during large scale storage by farmers and crop merchants. This has in turn led to various adverse effects on human and the environment, thus giving impetus for the need to search for a safer and eco-friendly means of control (Oyeniyi and Ajayi, 2019).

For the management of most insect pests, botanical pesticides derived from plants have long been promoted as an effective substitute for the majority of synthetic insecticides (Oyeniyi and Ajayi, 2019; Oyeniyi *et al.*, 2021). Consequently, several plant materials have been investigated for their insecticidal efficacy against many stored product pest (Abbasipour *et al.*, 2011; Gbaye *et al.*, 2015; Oyeniyi *et al.*, 2015a, b; Salehi *et al.*, 2019; Otunola, 2022). Despite several works that has been reported on the insecticidal efficacy of several botanicals, there is need to still search for more botanicals with greater efficacy for better management of insect pests on stored grains. Nigeria, a tropical nation, is thought to be highly endowed with numerous insecticidal active plants. Notable among them are *Acalypha godseffiana* (Muell Arg.) and *Alstonia boonei* (De wild) (Oni *et al.*, 2019).

A. godseffiana commonly known as copperleaf is a tropical and subtropical shrub which belongs to family *Euphorbiaceae* (Ogundaini, 2005). Several authors have reported the efficacy of pressed juice or boiled decoction of *A. godseffiana* leaves in the traditional treatment of several ailments such as gastrointestinal disorder, skin infection, hypertension, malaria among others (Ikewuchi and Ikewuchi, 2009a, b). *Alstonia boonei* is, however, a large deciduous, tropical-forest tree belonging to the dogbane family, *Apocynaceae*

(Adubasim *et al.*, 2018). It is frequently used to treat a variety of conditions, including rheumatoid arthritis, snakebites, worms, venereal disease, and insect pest management (Akinmurele *et al.*, 2023). Both plants are abundantly available in Nigeria (Odugbemi, 2006).

Nutritional indices have been used extensively by several authors in describing feeding, growth and food utilization of different insects (Xie *et al.*, 1996; Oyeniya *et al.*, 2024). Huang *et al.* (2002) investigated the insecticidal properties of different botanical derivatives and their impact on the nutrition of *Sitophilus zeamais* and *Tribolium castaneum*. Similarly, Abbasipour *et al.* (2011) investigated the potential of *Datura stramonium* L. as a bioactive agent for the management of *T. castaneum* infesting wheat flours. In spite of various works that has been done on the ethno-medicinal, chemical and toxicological properties of both botanicals, there is little information on the insecticidal and anti-nutritional effects of both botanicals on the feeding, growth and food utilization of *S. zeamais* and *S. oryzae* infesting wheat grains. The potential effect of insect species and exposure time on the rate of adult emergence, nutritional and insecticidal response of the two *Sitophilus* species treated with *A. godseffiana* and *A. boonei* also remained to be explored. This study, therefore, seeks to investigate the effects of two plant extracts (*A. godseffiana* and *A. boonei*) on the post-embryonic survival, nutritional and insecticidal response of the two *Sitophilus* species infesting wheat grains.

MATERIAL AND METHODS

The bioassays were carried out at Biology Laboratory III of the Biology Department, Federal University of Technology Akure (FUTA), Nigeria from March to September, 2022.

Sourcing of plant materials and wheat seeds:

Clean wheat grains were bought from Oba market, Akure, Ondo state, Nigeria and thereafter disinfested for four weeks at -4°C in the freezer. The disinfested wheat was allowed to equilibrate in the laboratory for 2-3 days to prevent mouldiness (Gbaye and Holloway, 2011; Oyeniya, *et al.*, 2015a). The leaves of *A. godseffiana* were obtained from Obanla, opposite of School of Agricultural and Agricultural Annex, FUTA, Ondo state whereas *A. boonei*'s stem bark was obtained from a sawmill near road-block area, Akure, Ondo State. The voucher specimens of both plant materials were authenticated and deposited at FUTA Herbarium (*A. boonei*: 0390; *A. godseffiana*: 0391). Both plant materials were dried under laboratory conditions for 7 days at ambient temperature ($28 \pm 2^\circ\text{C}$) and relative humidity ($75 \pm 5\%$). The plant parts were ground into fine powder using Mascot Mixer Grinder (AN ISO 9001:2000; Titan Scales, Thane, Maharashtra, India) and the powders were further sieved (180 μ mesh size) before being stored in pre-labelled separate plastic containers with tight lids for subsequent use.

Insect Culture:

Adult *S. zeamais* and *S. oryzae*, used in this study were obtained from the existing cultures in the Research laboratory, Department of Biology, Federal University of Technology, Akure, Ondo state, Nigeria. Each *Sitophilus* species has been maintained on wheat grains for more than ten (10) generations. Both insect species were reared on disinfested wheat grains inside 1.5 liters plastic containers. Each container was covered with perforated lid and muslin cloth to allow for proper aeration and prevent the escape of the insects. The plastic containers used for insect culture were stored in a culture cage at ambient temperature ($28 \pm 2^\circ\text{C}$) and relative humidity ($75 \pm 5\%$). The insects introduced were sieved out after 10 days and then observed for the F₁ generation to emerge. Adults of the two species of insects used in contact tests were 2–3 weeks old after emergence. Similarly, adult weevils (2-3 weeks old) starved for 48 h prior to the experiments were used for the nutritional bioassay (Huang *et al.*, 1999).

Preparation of plant extract and wheat flour:

Methanolic extracts of *A. godseffiana* and *A. boonei* were carried out using cold extraction method. Two hundred grams (200 g) of each plant powder was soaked separately in 1.5 litres extraction bottle containing 800mL of absolute methanol for 72 h. Each botanical mixture was periodically stirred for 30 minutes every 24 h with a handheld glass rod. With muslin cloths, the filtrate was separated from the residue after 72 h. A double layer Whatman No. 1 filter paper was used to further filter the filtrate. Each filtrate was concentrated using a rotary evaporator at 40 to 45 °C with rotary speed of 3 to 6 rpm for 4 h (Gbaye *et al.*, 2021). The crude extract obtained for each plant was air-dried for 10 min in order to remove traces of solvents. From the main stock solution for each plant extract, different concentrations of 2.5, 5.0, 7.5, 10.0 and 12.5% were made in methanol (solvent). The various concentrations were made using small glass bottles and graduated syringes. After each dilution, the syringe was rinsed with the solvent while different syringes were used for different plant part extracts.

Wheat seeds were grounded into fine powder using electric milling machine. The wheat flour was further sieved (180 μm mesh) and disinfested in the oven at 60°C for 90 min to kill insects' eggs and any developmental stages. The disinfested wheat flour was later used for flour disks bioassay.

Phytochemical Screening:

The methanolic extracts of the two plant materials were subjected to phytochemical screening to determine the phytochemicals that are present both in the leaf and stem bark of *A. godseffiana* and *A. boonei*, respectively. Each botanical extract was also screened for the presence of tannins, saponins, anthraquinones, cardiac glycosides, flavonoids and alkaloids using the various methods proposed by Selvakumar *et al.* (2019).

Contact toxicity bioassay:

The method of Awan *et al.* (2012) was used for contact toxicity test but with little modification. One milliliter of different concentrations of each plant extract was evenly spread out into Petri-dish and each Petri-dish was then exposed for 1h to ensure the evaporation of the volatile solvent. Ten pairs of adult insects were then introduced into each Petri-dish, covered and left for 1 h to allow for proper contact between the insect and the plant extracts. Two control treatments were set up, one with neither solvent nor extract (untreated control: control A) and one with solvent alone (solvent control: control B). Twenty grams of wheat grains were then weighed into 170 mL plastic containers. After 1 h, the insects were transferred to pre-labelled 170 mL plastic containers containing the wheat grains. The technique described by Bandara and Saxena (1995) for sexing and handling of coleopterans was used in this experiment. Four replicates were set up for each concentration and both controls in a Completely Randomized Block Design. Adult mortality was assessed after 24, 48, 72, and 96 h post-treatment. Both dead and live insects were removed on the fourth day and the experiments were left for 42 days to allow for adult emergence of F1 generation and the number of emerged adults was counted. Inhibition rate (%IR) in adult emergence was calculated using the method described by Tapondju *et al.* (2002).

$$\%IR = \frac{C_n - T_n}{C_n} \times \frac{100}{1}$$

Where C_n represents the number of adult insects that emerged in the control and T_n represents the number of adult insects that emerged in the treated grains. Untreated control was used in calculating the inhibition rate as described by Ashamo and Akinawonu (2012).

Nutritional studies:

Flour discs were prepared according to the method of Xie *et al.* (1996) and Huang *et al.* (2002) with slight modifications. Aliquots of 200 μ L of the stirred suspension of wheat flour in water (10 g in 30 mL) was pipetted onto a polystyrene Petri dish using a Micropipette (model number: EN ISO 8655) and allowed to dry at ambient temperature overnight under laboratory condition to produce uniform flour discs. The weights of the flour discs ranged from 36 to 46mg, and the moisture content range from 12 to 14%. The range of weights and moisture contents of wheat flour discs was determined using an electric weighing balance and an MB35 Halogen Moisture Analyser (OHAUS/USA). Flour disc were then treated with different concentration (2.5, 5.0, 7.5, 10.0 and 12.5%) of *A. godseffiana* and *A. boonei* extracts. 10 μ L of each methanol solution of each concentration was added to each disc and ordinary methanol was added to the control disc. The solvent was allowed to evaporate for 1h and two discs of the same treatment were placed in each Petri-dish. The Petri-dish and lids containing the discs were independently weighed, and ten group-weighed, unsexed adults of both species of weevils were added separately to each Petri-dish. Four replicates were set up for each concentration and control. Ten unsexed adult weevils were therefore added to each Petri dish to make a total of four Petri dishes and 40 weevils per treatment. The ten weevils were weighed together since the weight of individual weevil was too small to be measured by the balance. Similar method was used by Huang *et al.* (1999) and Oyeniya *et al.* (2024). After 72 h, the Petri-dishes with flour discs and live insects were weighed again, and survival insects were recorded. The weights of two flour discs treated with *A. godseffiana* and *A. boonei* but without insects were also recorded. Weights of the discs used in the experiments were then corrected for change in the weight of the control flour discs due to evaporation of the chemicals and absorption of water. Nutritional indices were calculated as previously described by Huang *et al.* (1997):

$$\text{Relative Growth Rate (RGR)} = \frac{A - B}{B \times \text{Days}}$$

Where:

$$A = \frac{\text{Mean weight of live insects on the third day after treatment (mg)}}{\text{number of live insects on the third day after treatment}}$$

$$B = \frac{\text{original weight of insects (mg)}}{\text{original number of insects}}$$

$$\text{Relative Consumption Rate (RCR)} = \frac{D}{(B \times \text{Days})}$$

Where:

$$D = \frac{\text{biomass ingested (mg)}}{\text{number of live insects on the third day}}$$

$$\text{Efficiency of Conversion of Ingested food (ECI) (\%)} = \left(\frac{RGR}{RCR}\right) \times \left(\frac{100}{1}\right)$$

Statistical analysis:

Abbott (1925) formula was used to correct all data on adult mortality counts using control mortality. All data on adult emergence, percentage inhibition in adult emergence and nutritional indices were later checked for normality based on Shapiro-Wilk test before being subjected to one-way analysis of variance (ANOVA) at $\alpha = 0.05$. Treatment means were separated using Tukey's Test. To estimate the concentration of each botanical extract lethal to 50% of each insect species, data on adult mortality were submitted to probit and log transformation. General Linear Model (GLM) was used to investigate the effect of insect species, concentration and exposure time (Duration) on the response of weevils to each botanical extract. The Statistical Package for Social Sciences (SPSS 22.0) software was used for all analyses.

RESULTS

Phytochemical composition of both botanicals:

Table 1 shows the qualitative screening of phytochemicals present in the leaf and stem bark extract of *A. godseffiana* and *A. boonei*, respectively. Both plant extracts were screened for the presence of alkaloid, cardiac glycosides, flavonoids, antraquinones, saponins and tannins. Saponins were observed to be highly present in *A. boonei* but moderately present in *A. godseffiana*. Also, flavonoids, alkaloid and cardiac glycosides were moderately present in *A. boonei* but showed a low occurrence in *A. godseffiana*. Antraquinones was however slightly present in *A. godseffiana* but totally absent in *A. boonei* while tannins were moderately present in *A. godseffiana* but slightly present in *A. boonei*.

Table 1. Phytochemicals in methanolic extract of the leaf and stem bark of *A. godseffiana* and *A. boonei*

Phytochemicals	Botanical	
	<i>A. godseffiana</i>	<i>A. boonei</i>
Tannins	++	+
Saponins	++	+++
Antraquinones	+	-
Flavonoids	+	++
Cardiac glycosides	+	++
Alkaloids	+	++

+ = slightly present; ++ = moderately present; +++ = highly present; - = absent.

Contact toxicity of *A. godseffiana* and *A. boonei* on adult *S. oryzae* and *S. zeamais*:

The lethal concentration of *A. boonei* and *A. godseffiana* extracts required to achieve 50% mortality in *S. oryzae* and *S. zeamais* at 24, 48, 72 and 96 h post-treatment is shown in Table 2. Lower amount of both plant extracts was needed to achieve 50% mortality of *S. zeamais* (*A. godseffiana* – 24 h: 49.06 mg/ml, 48 h: 75.91 mg/ml; *A. boonei* – 24 h: 35.43 mg/ml, 48 h: 26.88 mg/ml) relative to *S. oryzae* (*A. godseffiana* – 24 h: 990.22 mg/ml, 48 h: 115.51 mg/ml; *A. boonei* – 24 h: 201.89 mg/ml, 48 h: 42.65 mg/ml) at 24 and 48 h post-treatment. However, at 72 and 96 h post-treatment, lesser amount of both plant extracts was required to achieve 50% mortality of *S. oryzae* (*A. godseffiana* – 72 h: 38.03 mg/ml, 96 h: 25.26 mg/ml; *A. boonei* – 72 h: 25.09 mg/ml, 96 h: 25.24 mg/ml) relative to *S. zeamais* (*A. godseffiana* – 72 h: 72.96 mg/ml, 96 h: 85.20 mg/ml; *A. boonei* – 72 h: 31.10 mg/ml, 96 h: 31.51 mg/ml). However, both plant extracts were significantly ($p < 0.05$) more toxic to *S. zeamais* than *S. oryzae* at 24 h post-treatment only based on their lower fiducial limits.

Table 2. Lethal concentration (LC₅₀) (mg/ml) of *A. godseffiana* and *A. boonei* extract against adult *S. oryzae* and *S. zeamais*

Botanical	Insect spp.	Duration (H)			
		24	48	72	96
<i>A. godseffiana</i>	<i>S. oryzae</i>	990.22 (137.23-1851.21)	115.51 (36.15-194.87)	38.03 (16.34-59.72)	25.26 (12.60-133.94)
	<i>S. zeamais</i>	49.06 (31.19-131.98)	75.91 (34.16-1154.96)	72.96 (26.70-1501.03)	85.20 (31.12-7609.78)
<i>A. boonei</i>	<i>S. oryzae</i>	201.89 (78.20-1910.37)	42.65 (25.35-134.66)	25.09 (18.69-41.38)	25.24 (18.64-42.30)
	<i>S. zeamais</i>	35.43 (26.71-56.33)	26.88 (22.02-36.05)	31.10 (24.25-44.95)	31.51 (24.15-46.94)

Values in parenthesis represents 95% Fiducial limits and overlapping fiducial limits are not significantly different from each other ($p > 0.05$).

Effect of *A. godseffiana* and *A. boonei* on emergence and percentage inhibition rate of adult *S. zeamais* and *S. oryzae*:

The effect of two botanical extracts (*A. boonei* and *A. godseffiana*) on the adult emergence of two *Sitophilus* species is shown in Figure 1. Generally, the adult emergence of *S. zeamais* was significantly ($p < 0.0001$) reduced by *A. boonei* ($F_{6, 21} = 48.906$) and *A. godseffiana* ($F_{6, 21} = 217.529$). Equally, the adult emergence of *S. oryzae* was significantly ($p < 0.0001$) reduced by *A. boonei* ($F_{6, 21} = 191.271$) and *A. godseffiana* ($F_{6, 21} = 58.761$). Also, *A. boonei* significantly reduced adult emergence of *S. zeamais* at concentrations of 7.5, 10 and 12.5% relative to both controls while all the experimental concentrations of *A. godseffiana* evoked a significant ($p < 0.0001$) reduction in the adult emergence of *S. zeamais* relative to ordinary control (control A). For *S. oryzae*, In comparison to the ordinary control (control A), adult emergence was significantly ($p < 0.0001$) reduced by all experimental concentrations of *A. boonei* and *A. godseffiana*.

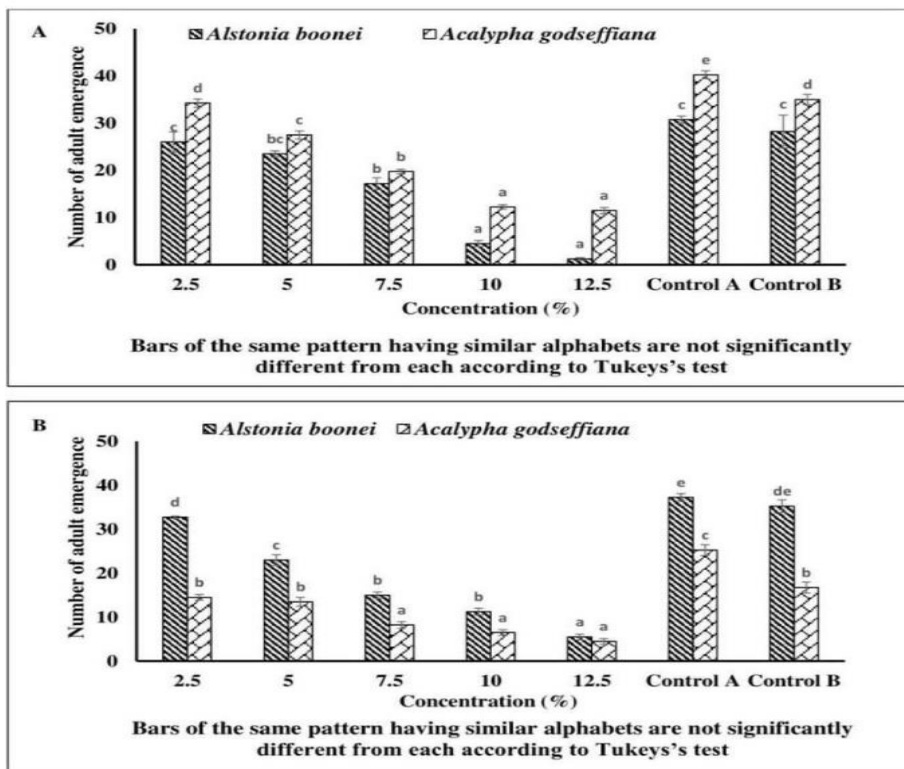


Fig. 1. Number of adult emergence of (A) *S. zeamais* and (B) *S. oryzae* treated with botanical extracts

The percentage inhibition in adult emergence of both *Sitophilus* species by *A. boonei* and *A. godseffiana* increased with increasing concentration of each extract (Figure 2). There was significant ($p < 0.0001$) inhibition in adult emergence of *S. zeamais* exposed to all the experimental concentrations of *A. boonei* ($F_{5, 18} = 243.805$) and *A. godseffiana* ($F_{5, 18} = 187.320$) relative to control. Also, at concentrations of 10 and 12.5%, both plant materials evoked the highest inhibition rate (*A. boonei*: 85.38 and 95.91%; *A. godseffiana*: 69.54 and 71.29%) and they were significantly ($p < 0.0001$) different from the inhibition rate at 2.5, 5 and 7.5%. For *S. oryzae*, the adult emergence was significantly ($p < 0.0001$) inhibited by *A. boonei* ($F_{5, 18} = 172.427$) and *A. godseffiana* ($F_{5, 18} = 102.605$) relative to control. Also, *A. boonei* at a concentration of 12.5% elicited the highest inhibition rate of 85.39% that was significantly ($p < 0.0001$) different from that of other concentrations. Relative to other concentrations, *A. godseffiana* at 12.5% also evoked the highest inhibition rate of 82.29% that was not significantly ($p = 0.352$) different from 73.72% evoked at 10% (Figure 2).

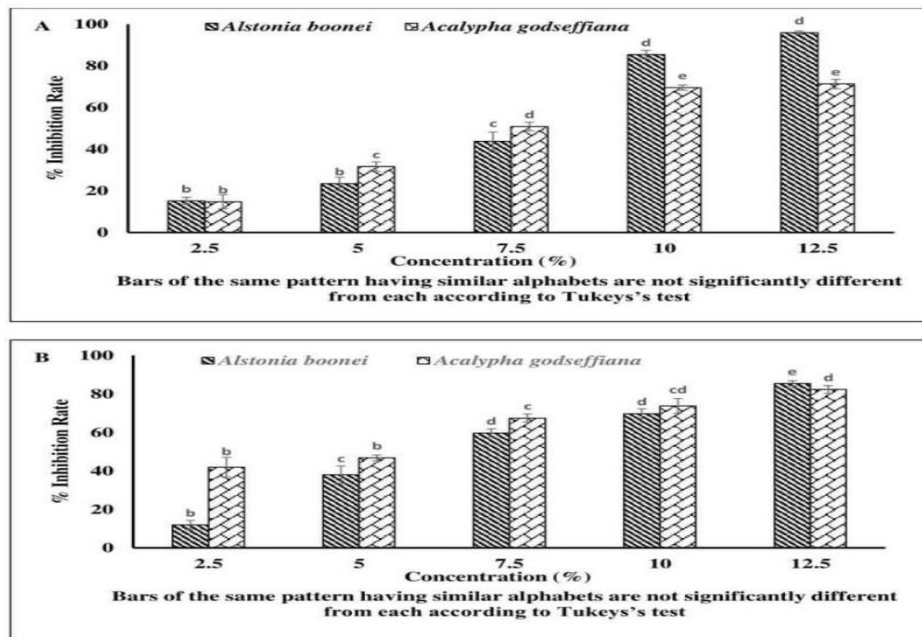


Fig. 2. Percentage inhibition in adult emergence of (A) *S. zeamais* and (B) *S. oryzae* treated with botanical extracts

Nutritional indices of two *Sitophilus* species exposed to flour disc treated with *A. godseffiana* and *A. boonei*:

The nutritional indices of *S. oryzae* and *S. zeamais* exposed to the flour discs treated with the extracts of *A. boonei* (Table 3) and *A. godseffiana* (Table 4) reveals that both plant extracts influenced the nutritional physiology of both *Sitophilus* species. The highest relative growth rate (RGR), relative consumption rate (RCR) and efficiency of conversion of ingested food (ECI) was observed in weevils exposed to control flour discs. Similarly, as the experimental concentration increases, RGR, RCR and ECI generally decreased, irrespective of the weevil species. The only exception was observed in ECI of *S. oryzae* exposed to flour discs treated with *A. boonei* (Table 3). Also, there was no significant difference ($p > 0.05$) in the RGR of both *S. oryzae* and *S. zeamais* exposed to botanical-treated (*A. boonei* and *A. godseffiana*) and untreated flour discs (Table 3 and 4). The consumption rates of *S. oryzae* exposed to flour discs treated with *A. boonei* (Table 3) and *S. zeamais* treated with *A. godseffiana* (Table 4) at concentrations of 7.5, 10 and 12.5% were significantly lower relative to those exposed to 2.5, 5.0 and control flour discs. However, the consumption rate of *S. zeamais* and *S. oryzae* exposed to flour discs treated with different concentrations of *A. boonei* (Table 3) and *A. godseffiana* (Table 4), respectively, were not significantly ($p > 0.05$) different from each other. All the experimental concentrations of both plant extracts were able to significantly ($p < 0.05$) reduce the ECI of both weevil species relative to their controls (Table 3 and 4). Also, the least significant ECI was observed at the highest experimental concentration of 12.5%, regardless of plant extract and weevil species.

Table 3. Nutritional indices (mean \pm S.E) of *S. oryzae* and *S. zeamais* exposed to *A. boonei* treated flour disc

Insect species	Concentration (%)	RGR(mg/ml/day) (mean \pm S.E)	RCR(mg/ml/day) (mean \pm S.E)	ECI (%) (mean \pm S.E)
<i>S. oryzae</i>	Control	0.057 \pm 0.014 ^d	0.331 \pm 0.017 ^e	17.221 \pm 0.063 ^e
	2.5	0.046 \pm 0.005 ^d	0.321 \pm 0.014 ^{de}	14.330 \pm 0.017 ^b
	5.0	0.045 \pm 0.005 ^{cd}	0.285 \pm 0.013 ^c	15.789 \pm 0.014 ^c
	7.5	0.042 \pm 0.004 ^{bcd}	0.256 \pm 0.014 ^{ab}	16.406 \pm 0.007 ^d
	10.0	0.034 \pm 0.002 ^{abcd}	0.213 \pm 0.071 ^{ab}	15.962 \pm 0.010 ^c
	12.5	0.030 \pm 0.030 ^{abcd}	0.212 \pm 0.067 ^{ab}	14.151 \pm 0.003 ^a
<i>S. zeamais</i>	Control	0.027 \pm 0.013 ^{abcd}	0.229 \pm 0.019 ^{abc}	11.790 \pm 0.041 ^f
	2.5	0.015 \pm 0.006 ^{abc}	0.227 \pm 0.015 ^{abc}	6.608 \pm 0.026 ^e
	5.0	0.012 \pm 0.004 ^{ab}	0.205 \pm 0.014 ^{ab}	5.854 \pm 0.022 ^d
	7.5	0.011 \pm 0.001 ^{ab}	0.200 \pm 0.014 ^{ab}	5.514 \pm 0.037 ^c
	10.0	0.008 \pm 0.008 ^a	0.173 \pm 0.013 ^a	4.624 \pm 0.022 ^b
	12.5	0.004 \pm 0.003 ^a	0.166 \pm 0.011 ^a	2.410 \pm 0.002 ^a

Means in the same column followed by the same letters are not significantly ($p > 0.05$) different according to Tukey's test.

Table 4. Nutritional indices of *S. oryzae* and *S. zeamais* exposed to *A. godseffiana* treated flour disc

Insect species	Concentration (%)	RGR(mg/ml/day) (mean ± S.E)	RCR(mg/ml/day) (mean ± S.E)	ECI (%) (mean ± S.E)
<i>S. oryzae</i>	Control	0.027±0.011 ^a	0.223±0.021 ^b	11.955±0.153 ^f
	2.5	0.025±0.014 ^a	0.217±0.012 ^{ab}	11.457±0.064 ^e
	5.0	0.018±0.018 ^a	0.213±0.099 ^{ab}	8.404±0.047 ^d
	7.5	0.017±0.012 ^a	0.211±0.014 ^{ab}	8.017±0.040 ^c
	10.0	0.016±0.009 ^a	0.211±0.012 ^{ab}	7.524±0.059 ^b
	12.5	0.004±0.004 ^a	0.208±0.053 ^{ab}	1.902±0.021 ^a
<i>S. zeamais</i>	Control	0.020±0.008 ^a	0.255±0.017 ^{cd}	7.843±0.032 ^f
	2.5	0.015±0.006 ^a	0.239±0.016 ^{cd}	6.276±0.028 ^e
	5.0	0.014±0.009 ^a	0.231±0.016 ^c	6.061±0.023 ^d
	7.5	0.006±0.009 ^a	0.135±0.012 ^{ab}	4.444±0.042 ^c
	10.0	0.006±0.012 ^a	0.129±0.018 ^{ab}	3.101±0.065 ^b
	12.5	-0.002±0.013 ^a	0.115±0.035 ^a	-1.739±0.055 ^a

Means in the same column followed by the same letters are not significantly ($p > 0.05$) different according to Tukey's test.

DISCUSSION

The increased understanding of the potential negative effects linked to numerous synthetic insecticides has consistently fueled the usage of pesticides derived plants. The majority of farmers in poor nations have therefore turned to the use of botanicals for pest management in order to avoid the potential dangers connected with the majority of synthetic chemicals. Notable among plants whose potent medicinal properties have been proven include *A. boonei* and *A. godseffiana* (Abiola, 2020; Osuntokun and Ajiga, 2020). Consequently, this study examined the bioactivity of the extracts from the leaves and stem barks of *A. godseffiana* and *A. boonei*, respectively, against *S. oryzae* and *S. zeamais* infesting wheat grains.

Both plant extracts resulted in adult mortality of both *Sitophilus* species irrespective of the experimental concentration and exposure time. This confirms the insecticidal effect of *A. boonei* and *A. godseffiana* against both *Sitophilus* species, thus corroborating the previous findings of Oyeniya (2018). The ability of both botanicals to result in weevil mortality may be linked to the presence of considerable amount of established insecticidal compounds in both extracts. For instance, saponins were moderately and highly present in *A. godseffiana* and *A. boonei*, respectively. Similarly, tannins was moderately present in *A. godseffiana* while alkaloids and flavonoids were identified in moderate amount in *A. boonei*. These phytochemical compounds have been demonstrated to possess insecticidal and antifeedant effects against a variety of stored product insect pests (Chaieb, 2010; Ogungbite and Oyeniya, 2014; Kosini and Nukenine, 2017; Oyeniya and Ajayi, 2019). It is possible that the direct contact of the toxic phytochemicals in the plant oils with weevils' cuticle could have resulted in the weevils engaging in behaviours that involve excessive physical contact in order to remove the oils. It is also possible that the insecticidal compounds in both plant extracts could have possibly interacted with *Sitophilus* cuticular components, making the weevils' structure to become weakened and more susceptible to physical abrasion. Basically, the polysaccharide chitin, cuticular lipids, and cuticular proteins are the main structural components of an insect's cuticle (Fang *et al.*, 2021). The cuticular components of *Spodoptera litura* (Fabricius) had been reported to have undergone cuticular alteration in response to alkaloids (Sun *et al.*, 2012). Additionally tannins were reported to form complexes with proteins and polysaccharides found in insect cuticle (Lattanzio *et al.*, 2005; Gbaye and Holloway, 2011; Petchidurai *et al.*, 2023). Ultimately, many insect species are known to die from physical abrasion of their cuticle due to their inability to prevent excessive water loss from the damaged cuticular structures (Johnson *et al.*, 2011). The weevils may have eventually died through repeated cuticle abrasion caused by the toxic phytochemicals in both plant extracts.

However, none of the two botanical extracts was able to induce complete (100%) weevil mortality regardless of the experimental concentration and exposure time. This is in contrast to the previous findings by Ileke (2014) where extract of *A. boonei* stem bark evoked 100% mortality in weevils reared on maize grains after 96 h post-treatment. The inability of both extracts to induce complete mortality may be ascribed to difference in host food used in this study. Wheat is known to possess higher nutritional value than other cereals (Preedy, 2011; Wong and Lee, 2011; Oyeniya *et al.*, 2021; Gbaye *et al.*, 2021). Likewise, It has also been argued that wheat contains more nutrients in all parts of the grain kernel, including the bran, germ, and endosperm (Baba and Malik, 2020) and this might have provided immense energy to the developing larvae of both weevils to fight the toxins in both extracts at adult stage.

Also, this study showed that the insecticidal effect of both extracts varied with plant type, experimental concentration, exposure time, insect species, and their behaviours. Generally, the mortality of the weevils varied with the concentrations of the extracts and the exposure time. Also, the lethal concentration

(LC₅₀) values of both botanical extracts confirmed their higher toxicity to *S. zeamais* than *S. oryzae* at 24 and 48 h post-treatment. On the contrary, the extracts were more toxic to *S. oryzae* at 72 and 96 h post-treatment. Insects belonging to different species are known to respond differently to botanical-based insecticides (Oyeniya *et al.*, 2015a; Oyeniya *et al.*, 2021; Oyeniya *et al.*, 2024). An insect species might be more tolerant to the toxic effects of one plant extract and less tolerant to that of another plant extract (Oyeniya 2018). In this study, the lesser tolerance of *S. zeamais* to both extracts at 24 and 48 h as well as its slightly higher tolerance at 72 and 96 h relative to *S. oryzae* could be due to the rate of cuticular penetration and flight activity of both species. Similarly, the high toxicity of both extracts to *S. zeamais* at 24 and 48 h post-treatment indicate that the weevils experienced acute effects shortly after exposure to the extracts and this could be as a result of faster penetration rate of the cuticle. On the contrary, *S. oryzae* could have experienced delayed response due to slower penetration rate of the cuticle. The rate of response of each species to the chemical composition of each extract and the characteristics of the cuticle of each *Sitophilus* species could have resulted in the differences observed in their rate of susceptibility. Earlier, the characteristics of the cuticle of *Sitophilus zeamais* and *S. oryzae* had been reported to play a major role in their insecticidal responses to *Newbouldia laevis* (Seem) (Ogunbite and Oyeniya, 2014). Similarly, the variation in the insecticidal response of *Tribolium castaneum* and *T. confusum* to *Dennettia tripetala* (G. Baker) had earlier been linked to two major bioactive compounds in the plant, linalool and 2-phenylnitroethane (Oyeniya *et al.*, 2024).

Although the two species share similar morphology, *S. zeamais* is larger and more active in flight than *S. oryzae*, and its flight activity is highly temperature-dependent (Vásquez-Castro *et al.*, 2009; Cao *et al.*, 2024). Due to differences in size and flying habits between the two species, *S. oryzae* often encountered pesticide resistance cases earlier than *S. zeamais* (Jian, 2019). In this study, the reduced flight activity, size and mobility of *S. oryzae* within the plastic container could be responsible for their higher tolerance to the toxic effect of both extracts within the first two days of exposure. Reduced flight activity, size and mobility could have also reduced the rate at which *S. oryzae* pick up the botanical extracts. Higher tolerance of *S. oryzae* to insecticides had earlier been linked to its lower flight activity (Vásquez-Castro *et al.*, 2009). The greater tolerance of *Callosobruchus maculatus* (F) reared on different cowpea varieties to botanical powders had also been linked to reduced body size (Oyeniya *et al.*, 2015a).

In addition, both plants extracts at higher experimental concentrations significantly reduced the adult emergence of both *Sitophilus* species relative to their controls and this shows agrees with earlier findings by Ashamo *et al.* (2013) and Oyeniya (2018). However, at the highest experimental concentration, *A. boonei* evoked higher reduction in the adult emergence of *S. zeamais* relative to *A. godseffiana*. In contrast, slightly lesser adults of *S. oryzae* emerged in grains treated with *A. godseffiana* than *A. boonei*. The variations in the emergence of the weevils based on the type of plant materials and insect species agreed with earlier studies by Oyeniya and Ogunbite (2014) as well as Gbaye *et al.* (2015). Similarly, both plant extracts at the highest concentrations (10 and 12.5%) significantly inhibited adult emergence of *S. zeamais*. Only *A. boonei* significantly inhibited adult emergence of *S. oryzae* at 12.5%. Results on the percentage inhibition and adult emergence imply that *A. boonei* exhibited higher efficacy than *A. godseffiana* in inhibiting adult emergence of both species of *Sitophilus*. The differences in the number of adult emergence and percentage inhibition in adult emergence of both species by the extracts could be linked to the variations in the phytochemical components of *A. godseffiana* and *A. boonei* (Oyeniya, 2018). Higher levels of bioactive compounds were observed in the stem bark of *A. boonei* relative to the amount in the leaf extract of *A. godseffiana*. Plants are known to store many useful phytochemical compounds in their stem before onward transmission to other parts (Ashamo and Akinnawonu, 2012). This may have been responsible for the higher concentration of phytochemical compounds in the stem bark of *A. boonei* when compared to the leaf extract of *A. godseffiana*. Consequently, higher concentration of flavonoids, alkaloids and saponins observed in *A. boonei* relative to *A. godseffiana* could have been responsible for the results obtained in this study.

The rates of insect feeding, growth, and food consumption are frequently described using nutritional indices (Huang *et al.*, 2002; Wahba *et al.*, 2023). In this study, regardless of the weevil species and botanical extract, the relative growth rate (RGR), consumption rate (RCR) and efficiency of conversion of ingested food (ECI) generally decreased with increasing concentration of the extract and this agrees with the findings of Wahba *et al.* (2023). The only exception was observed in ECI of *S. oryzae* fed with *A. boonei*-treated flour discs. The fact that *A. boonei* and *A. godseffiana*, at the highest experimental concentration, significantly reduced the feeding rate of *S. oryzae* and *S. zeamais*, respectively, points to each extract's inhibitory effects on the respective weevil species. Although the growth rates of both species were not significantly different from their controls, the observed modest decline in the growth rates of both species could be linked to the reduction in consumption rate (Abbasipour *et al.*, 2011; Oyeniya *et al.*, 2024). At the highest concentration, the food use (ECI) for both species was significantly lower than that of the controls, regardless of plant extract. This implies

that both species might have been subjected to post-ingestion toxicity from both plant extracts, which could explain why mortality rose with extract concentration (Huang *et al.*, 1997). Therefore, the decrease in growth rate and food utilization of both *Sitophilus* species could be attributed to the decrease in consumption rate caused by the post-ingestion toxicity of both extracts.

This study indicates that it might be possible to infer the mechanism behind the anti-nutritional effects of both plant extracts on the nutritional physiology of each species. For instance, the nutritional effects of *A. boonei* and *A. godseffiana* extracts on *S. oryzae* and *S. zeamais*, respectively, may primarily be through feeding deterrent and food utilization as a result of the observed significant decreases in their consumption and usage of ingested food (Huang *et al.*, 1997; Abbasipour *et al.*, 2011). When *S. zeamais* and *S. oryzae* were fed flour discs treated with *A. boonei* and *A. godseffiana* extracts, respectively, the nutritional effect of the both extract might only be ascribed to minor post-ingestion toxicity as a result of non-significant reduction with concentration (Tripathi *et al.*, 2001; Abbasipour *et al.*, 2011; Oyeniya *et al.*, 2024).

CONCLUSION

Several findings from the present study confirmed the effectiveness of the stem bark extract of *A. boonei* and leaf extract of *A. godseffiana* as both contact and anti-nutritional poison against *S. oryzae* and *S. zeamais* infesting wheat grains. *A. boonei* stem extract at a 12.5% concentration may be utilized to successfully inhibit adult emergence in both *Sitophilus* species, while both extracts at a 12.5% concentration might significantly reduce adult eclosion of *S. oryzae*. Also, *A. boonei* proved to be more effective than *A. godseffiana* at preventing the adult emergence of both *Sitophilus* species. It may also be concluded that food utilization and feeding deterrence may be the main causes of the anti-nutritional effects of *A. godseffiana* and *A. boonei* extracts on *S. oryzae* and *S. zeamais*, respectively. The ability of both extracts to evoke weevil mortality could also be linked to post-ingestion toxicity of the extracts. This study has therefore established the insecticidal and anti-nutritional effects of *A. godseffiana* and *A. boonei* extracts on both *Sitophilus* species infesting wheat grains.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

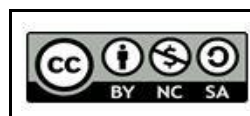
Conflict of Interest: The authors declare no conflict of interest

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