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Research Article

Effect of Botanical Extracts on Proximate Contents of Stored Sorghum Infested by *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) in Nigeria

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Abstract

Proximate and mineral analyses of sorghum grain treated with ethanolic leaf extracts of *Euphorbia balsamifera* Aiton, *Lawsonia inermis* L., *Mitracarpus hirtus* (L.) DC and *Senna obtusifolia* L were determined before and after being infested by *S. zeamais*. Twenty grams of sorghum were treated with the aforementioned botanicals at the concentrations of 25, 50 and 100mg/g. The results showed that moisture content was not significantly different ($p > 0.05$). Ash was higher in treated grains than in untreated ones and vice versa for crude fibre. Per cent crude lipid in treated grains ranged from 6.50 ± 0.00 to 7.17 ± 0.17% and increased with increased in concentrations. The amount of crude protein decreased drastically in untreated samples compared to treated grains where it varied between 8.75 ± 0.01 to 8.99 ± 0.41%. Carbohydrate content in the sorghum grains was significantly different ($p < 0.05$) among the botanical treatments. Findings of this study have revealed that all the botanical extracts have potentials of reducing effect of *S. zeamais* on the nutritional value of sorghum grains with *E. balsamifera* as the most effective.

Introduction

The role of agriculture remains significant in the Nigeria economy despite the strategic importance of the oil sector. Agriculture provides primary means of employment for Nigeria and accounts for more than one third of total gross domestic product (GDP) and labour force [1]. More than 70% of the working adult populations in Nigeria are employed in the agricultural sector directly or indirectly and over 90% of Nigeria's agricultural output comes from peasant farmers who dwell in the rural areas where 60% of the population live [1]. The major cereal crops in Nigeria are rice, maize, sorghum, wheat, and millet. Sorghum is the staple food crop that is economical to grow in dry areas of many regions of Africa where the climatic conditions are unfavourable for the growth of other crops [2].

Sorghum is produced for human nutrition and animal feeds all over the world, and over half (55%) of it is used for human consumption in Asia and Africa [3]. It is the major crop for many poor farmers, especially in Africa, Central America and South Asia [4]. Grain sorghum is used for flours, porridges and side dishes, malted and distilled beverages as well as popped grain. It is the main source of calories and protein in some regions of Africa and Asia [5]. Sorghum is the primary food crop in virtually all parts of northern Nigeria [6].

Despite its nutritional value, sorghum production has been hindered by biotic and abiotic constraints. Among the biotic constraints, insect pests are the major devastating factors attacking the grains during storage [7]. Once infestation is established, insect pests cause gradual and progressive damage leading to losses in weight, nutritional, organoleptic and aesthetic quality of grains [8]. Goftishu and Belete [9] emphasized that *S. zeamais* damage leads to quantitative and qualitative deterioration of sorghum grains. These losses could be influenced by the storage time and population of the insects involved in the infestation. The qualitative loss is attributed to change in biochemical components such as carbohydrates, fats and proteins [10,11].

Qualitative loss arises primarily from the alteration of the physical appearance and chemical constituents of the grains with insect frass and debris that could lead to detectable reduction in important nutrients such as sugar, proteins, fats, minerals and vitamins [12]. In order to reduce these losses, some botanicals of various forms have been tested by some researchers against the weevils in maize grains [10]. However, there is limited information on effects of botanicals on nutritional quality of sorghum grains infested by *S. zeamais* or any other insect pest [11]. Considering the economic importance of sorghum and the damage caused to it by *S. zeamais*, the present study was aimed at assessing the qualitative loss in the infested sorghum grains treated with different botanical powders and to determine the effectiveness of the botanicals in protecting the grain quality.

Materials and Methods

Sample Preparation

Twenty gram of sorghum grains in the plastic bottles was treated with 25.00, 50.00 and 100.00 $mgml^{-1}$ of ethanolic leaf extracts of *E. balsamifera, L. inermis, M. hirtus* and *S. obtusifolia*. Another 20g of the grains was placed in separate bottle without any treatment. All treatments were arranged in a completely randomised

design (CRD) with 3 replicates. Ten adults of *S. zeamais* were introduced into the bottles and allowed them to stay for 14 days in an incubator at 30°C and 70% R.H. after which they were removed. The grains were maintained in the same condition in the incubator for 6 months and then sieved to remove the powders and any other unwanted particles. The sieved samples were then washed with deionised water and ovum dried at 65°C for 6 hrs. The dried samples were ground into fine powders using laboratory stainless steel mortar and pestle. Clean sorghum grains were earlier obtained and ground into fine powder before infestation (as untreated un-infested). The powders were placed in well-labelled bottles separately and kept in the laboratory for proximate and mineral analyses.

Proximate Analysis of Sorghum Grains Treated with the Botanicals and Infested by *S. zeamais*

To assess the qualitative losses caused to sorghum grains by *S. zeamais*, the ground samples were analyzed for moisture, ash, crude fibre, crude lipid, crude protein and carbohydrate based on the recommendation of the Association of Official Analytical Chemists [13]. All analyses were conducted in triplicates.

Determination of Moisture Content

Five grams of the sample powders were weighed into pre-weighed crucible (W_1) and placed into a drying oven at 105°C for 24 hrs. The crucible was removed, cooled in a desiccator and re-weighed. The processes of drying, cooling and re-weighing were repeated until a constant weight (W_2) was obtained. The moisture content was determined as:

$$\%\,Moisture = \frac{W_1 - W_2}{Weight\ of\ sample\,(5g)} \times 100$$

Where: W_1 = Weight (g) of sample
W_2 = Constant weight (g) of crucible + sample after drying

Determination of Ash Content

Five grams of the powdered samples was weighed into pre-weighed crucibles (W_1) and placed into a muffled furnace at 550°C for 8 hrs. The ash was cooled in a desiccator and weighed (W_2). The weight of the ash was determined by the difference between the powdered sample, pre-weighed crucible and the ash in the crucible. Percentage ash was calculated as:

$$\%\,Ash = \frac{W_2 - W_1}{Weight\ of\ sample\,(5g)} \times 100$$

Where: W_1 = Weight (g) of empty crucible
W_2 = Weight (g) of crucible + ash

Determination of Crude Fibre

One gram of the powdered sample was placed in a beaker and boiled in 150cm^3 of 1.25% H_2SO_4 solution for 30 min. The boiled sample was washed 3 times with 30cm^3 of hot deionised water and filtered through Whatman No. 1 filter paper. The residue was scrapped back into the beaker with a spatula and boiled again in 150cm^3 of 1.25% NaOH solution for another 30 min. The boiled sample was washed as in the acid digestion but the last wash was done with cold deionised water, and washed three times with 25cm^3 of acetone and filtered as above. The residue was carefully transferred into a weighed crucible where it was dried in the oven at 105°C to a constant weight (W_1). It was thereafter burnt to ash in a muffle furnace at 550°C, cooled in a desiccator and weighed (W_2). The percentage crude fibre was calculated as:

$$\%\,Crude\,Fibre = \frac{W_2 - W_1}{Weight\,of\,sample\,(1g)} \times 100$$

Where: W_1 = Weight (g) of crucible + sample after washing and drying
W_2 = Weight (g) of crucible + sample ash

Determination of Crude Lipid

Crude fat was determined by solvent extraction gravimetric method described by Ilodibia *et al.* [14]. Two gram of the powdered sample was wrapped in a Whatman No. 1 filter paper and put in a thimble. The thimble was put in a soxhlet extractor and extracted into a pre-weighed extraction flask containing 200cm^3 of petroleum ether. The upper of the reflux flask was connected to a water condenser. The solvent (petroleum ether) was heated, boiled, vaporised and condensed into the reflux flask filled. Soon the sample in the thimble was covered with the solvent until the reflux flask filled up and siphoned over, carrying its oil extract down to the boiling flask. This process was allowed to go on repeatedly for 4 hrs before the defatted sample was removed, the solvent recovered and the oil extract was left in the flask. The flask (containing the oil extract) was dried in the oven at 60°C for 30 min to remove any residual solvent. It was cooled in the desiccator and weighed. The weight of fat extract was determined by difference and calculated as a percentage of the weight of sample analyzed thus:

$$\%\,Crude\,Fat = \frac{W_2 - W_1}{Weight\,of\,sample\,(2g)} \times 100$$

Where: W_1 = Weight (g) of empty extraction flask
W_2 = Weight (g) of flask + oil (fat) extract

Determination of Crude Protein

Crude protein was determined using the micro-Kjeldahl whereby 2g of sample was weighed along with 20cm^3 of distilled water into a micro-Kjeldahl digestion flask [15]. It was shaken and allowed to stand for some time. Fifteen gram of NaSO4 and 1g of $CuSO_4$ as catalysts were added followed by addition of 20cm^3 conc. H_2SO_4. Some glass beads were added as anti-bump. The flask was heated under a fume cup board for 4 hrs and then allowed to cool. The content was transferred into a 50cm^3 volumetric flask and diluted to the mark with water. An aliquot of 10cm^3 of the digest was transferred into another micro-Kjeldahl flask along with 20cm^3 distilled water and placed in the distilling outlet of the micro-Kjeldahl distillation unit. A conical flask containing 20cm^3 of boric acid indicator was placed under the condenser outlet. A 20cm^3 of 40% NaOH solution was added to the content in the Kjeldahl flask by opening the funnel stop cock. The distillation started and the heat supplied was regulated to avoid sucking back. When all the available distillate was collected in 20cm^3 boric acid, the distillation stopped.

The nitrogen in the distillate was determined by titrating with 0.01M of H_2SO_4. The nitrogen content of the sample is given by the formula:

$$\%N = \frac{TV \times Na \times 0.014 \times V_1}{G \times V_2} \times 100$$

Where: TV = Titre value of acid (cm^3);
Na = Concentration or normality of acid;
V_1 = Volume of distilled used for distilling the digest (50cm^3);
V_2 = Volume of aliquot used for distilling the digest (10cm^3); and
G = Weight of sample (2g).
The crude protein was calculated as % Crude Protein = % N x 6.25

Determination of Carbohydrate

The carbohydrate content was obtained by subtracting the values of moisture, ash, crude fibre, crude fat and crude protein from 100. Thus;

% Carbohydrate = 100 % - (% Moisture + % Ash + % Crude fibre + % Crude fat + % Crude protein).

Data Analysis

All data were collected in triplicates and subjected to analysis of variance (ANOVA) using Graph Pad Prism (version 7.03). Significantly different means were separated by using Bonferroni's multiple comparisons test and Fisher's LSD. All analyses were carried out at $p < 0.05$.

Results

Proximate composition in grains treated with ethanolic extracts and infested by *S. zeamais* varied. Percentage moisture ranged from 10.20 ± 0.01 to 10.22 ± 0.01% in treated grains at the concentrations of 25.00, 50.00 and 100.00 $mgml^{-1}$. However, a higher moisture level was recorded in the control where it reached 14.18 ± 0.04% (Table 1). Variation in concentrations of ethanolic extracts of the botanicals did not cause any significant difference ($F(2, 36) = 0.1185$, $p = 0.8886$) in moisture content among the treatments. However, a highly significant difference ($F(5, 36) = 7660.00$, $p < 0.0001$) existed among the treatments. Bonferroni's multiple comparisons test showed that all grains treated with the botanicals had significantly lower moisture content than the control.

Table 1: *Proximate composition of sorghum treated with different concentrations of ethanolic extracts of the botanicals and infested by S. zeamais*

Treatments	Conc. ($mgml^{-1}$)	% Organic Compounds (Mean ± S. E.)					
		Moisture	Ash	Crude Fibre	Crude Lipid	Crude Protein	Carbohydrate
E. balsamifera	25.00	10.22 ± 0.01^{b}	1.73 ± 0.07^{ab}	3.00 ± 0.00^{bc}	6.83 ± 0.17^{a}	8.81 ± 0.05^{a}	69.41 ± 0.17^{a}
	50.00	10.21 ± 0.00^{b}	1.87 ± 0.07^{ab}	2.67 ± 0.33^{c}	7.00 ± 0.00^{a}	8.84 ± 0.05^{a}	69.42 ± 0.27^{a}
	100.00	10.20 ± 0.01^{b}	1.93 ± 0.07^{a}	2.00 ± 0.00^{c}	7.17 ± 0.17^{a}	8.90 ± 0.02^{a}	69.83 ± 0.07^{a}
L. inermis	25.00	10.20 ± 0.01^{b}	1.67 ± 0.07^{ab}	3.33 ± 0.33^{b}	6.67 ± 0.44^{a}	8.79 ± 0.04^{a}	69.35 ± 0.27^{a}
	50.00	10.21 ± 0.01^{b}	1.67 ± 0.07^{ab}	3.00 ± 0.00^{bc}	6.83 ± 0.33^{a}	8.80 ± 0.05^{a}	69.42 ± 0.27^{a}
	100.00	10.21 ± 0.01^{b}	1.80 ± 0.00^{ab}	2.33 ± 0.33^{bc}	7.00 ± 0.29^{a}	8.85 ± 0.05^{a}	69.81 ± 0.59^{a}
M. hirtus	25.00	10.22 ± 0.01^{b}	1.53 ± 0.07^{b}	3.67 ± 0.33^{b}	6.67 ± 0.17^{a}	8.78 ± 0.06^{a}	69.13 ± 0.16^{a}
	50.00	10.22 ± 0.01^{b}	1.67 ± 0.07^{ab}	3.33 ± 0.33^{b}	6.67 ± 0.17^{a}	8.78 ± 0.14^{a}	69.33 ± 0.27^{a}
	100.00	10.20 ± 0.02^{b}	1.73 ± 0.07^{ab}	2.67 ± 0.33^{bc}	7.00 ± 0.00^{a}	8.82 ± 0.01^{a}	69.57 ± 0.38^{a}
S. obtusifolia	25.00	10.22 ± 0.01^{b}	1.53 ± 0.07^{b}	4.00 ± 0.00^{b}	6.50 ± 0.29^{a}	8.73 ± 0.04^{a}	69.02 ± 0.33^{a}
	50.00	10.21 ± 0.00^{b}	1.60 ± 0.00^{b}	3.67 ± 0.33^{b}	6.67 ± 0.17^{a}	8.76 ± 0.12^{a}	69.09 ± 0.40^{a}
	100.00	10.21 ± 0.01^{b}	1.67 ± 0.07^{ab}	2.00 ± 0.00^{c}	6.83 ± 0.17^{a}	8.80 ± 0.04^{a}	69.50 ± 0.12^{a}
Untreated un-infested	0.00	10.27 ± 0.07^{b}	1.99 ± 0.01^{a}	1.53 ± 0.27^{d}	7.17 ± 0.33^{a}	8.99 ± 0.41^{a}	70.06 ± 0.28^{a}
Control	0.00	14.18 ± 0.04^{a}	0.67 ± 0.07^{c}	14.00 ± 0.58^{a}	6.00 ± 0.29^{b}	5.79 ± 0.06^{b}	59.37 ± 0.37^{b}

Conc. = Concentration

Means in the same column followed by different letter superscript are significantly different at $p < 0.05$ by the Bonferroni's Multiple Comparisons Test and Fisher's LSD.

Ash content was highest in *E. balsamifera* treatments which followed by *L. inermis, M. hirtus* and *S. obtusifolia* (Table 1). Two-way ANOVA showed that a significant difference was recorded in ash content among the botanicals and within the concentrations as well. Bonferroni's test showed that at 25.00$mgml^{-1}$, grains treated with *E. balsamifera* had higher ash content than in those treated with the other botanicals,

though statistically the same as in *L. inermis*. Similar trend as recorded in 50.00mgml^{-1} application rate. At 10.0mgml^{-1}, the percent ash in grains treated with of *E. balsamifera* had the highest among the botanicals, and statistically the same as in the untreated un-infested grains.

Percentage crude fibre in the treated grains ranged from 2.00 ± 0.00 to 4.00 ± 0.00%. The crude fibre decreased in the following order: *S. obtusifolia* > *M. hirtus* > *L. inermis* > *E. balsamifera*, at the different concentrations (Table 1). Analysis of variance indicated a highly significant difference (F (5, 36) = 585.00, $p < 0.0001$) in percent crude fibre among the botanical treatments. Also, the difference among the three concentrations was significant (F (2, 36) = 6.25, $p = 0.0047$). Bonferroni's multiple comparisons test revealed that at each of 25.00 and 50.00mgml^{-1}, *E. balsamifera* treatments had significantly lower crude fibre than the rest, while it was the same as in *S. obtusifolia* and less than in *L. inermis* and *M. hirtus*.

The crude lipid in treated and infested grains varied from 6.50 ± 0.29% to 7.17 ± 0.17% (Table 1). There was a highly significant difference in crude lipid among the botanical types (F (5, 36) = 7.182, p < 0.0001). Fisher's LSD showed that the difference was between each of the treatments and control. The crude lipid was lowest in the control, while it was statistically the same in all the treatments. Further, the varying concentrations did not lead to any significant difference (F (2, 36) = 1.114, p = 0.3394).

The crude protein in treated and infested grains followed a decreasing order of percentage *E. balsamifera* > *L. inermis* > *M. hirtus* > *S. obtusifolia* (Table 1). Analysis of variance showed that the percent crude protein in the grains was highly significantly different among the botanicals (F (5, 36) = 150.30, $p < 0.0001$), but insignificant within the concentrations (F (2, 36) = 0.1022, $p = 0.9031$). The crude protein in all the botanical treatments at each of the three concentrations was statistically the same and significantly higher than in the control (Bonferroni's test).

The carbohydrate content in the treated grains ranged from 69.02 ± 0.33 to 69.83 ± 0.07%. The untreated un-infested and control had 70.06 ± 0.28 and 59.37 ± 0.37%, respectively (Table 1). There was a highly significant difference in percent carbohydrate among the grains treated with ethanolic extracts. Bonferroni's multiple comparisons test showed that the difference was between each botanical type and the control only. Furthermore, no significant difference in percentage carbohydrate in the grains among application rates of the botanicals.

Discussion

The effectiveness of the selected botanical extracts in reducing organic compounds in treated sorghum corroborates Suleiman and Abdullahi [11] who reported that powders *E. balsamifera, L. inermis, M. hirtus* and *S. obtusifolia* applied at 2.5. 5.0 and 10.0 x 10^4 ppm decreased loss of organic compounds in treated maize grains.

The high moisture content in the control (untreated infested rains) corresponds to the findings of Okoroafor and Job (2017) [16] who reported an increase in moisture content of yellow and white local maize varieties after 12 months of storage. Suleiman and Abdullahi [11] recorded similar results when leaf powers of *E. balsamifera, L. inermis, M. hirtus* and *S. obtusifolia* were applied. This increase in moisture content

of damaged grains could be due to the fact that grain is a living organism that respires and emits moisture that moves within the grain mass [11]. Prolonged stay of insects in the control could also increase respiratory activities in the grain and add more moisture content. This phenomenon was explained by USAID [17] that biological factors such as insects and moulds respire and can add to the moisture being released and migrating through the stack. Paudyal *et al*. [18] also stated that insect infestations might have possibly increased moisture content of maize stored in bags treated with deltamethrin for six months. As there were few weevils in the treated grains, respiratory activities were less and hence, had less moisture content.

The loss of ash content in sorghum treated with the selected botanicals was concentration dependent. Ash content was lost most in grains treated with low concentration of the botanicals, but better than the control. This shows that the weevil's feeding and reproductive activities might have reduced the mineral content of the grains [19].

It was found that the untreated sorghum grains had more crude fibre than the treated grains, which concurs with Suleiman and Abdullahi [11] who reported that sorghum grain treated with botanical powders of *E. balsamifera, L. inermis, M. hirtus* and *S. obtusifolia* had less crude fibre than the untreated grains. The increase in fibre content in untreated infested grains was as a result of feeding activities of the weevils on endosperm hollowing out the grain leaving only the bran, which is largely fibre. This finding agrees with Bamaiyi *et al.* [19] who noted that insect infestations decreased the nutritional quality of grains and increased the relative level of dietary fibre. Botanical concentration also affected crude fibre level in the stored sorghum. Higher concentration seemed to preserve more nutritional quality as there were low infestations, hence lower level of fibre than grains treated with lower concentration.

Findings of this study revealed that the loss in lipid content in sorghum grains was significantly reduced by ethanolic leaf extracts of the selected botanicals. This is consistent to Danjumma *et al*. [10] who recorded more crude lipid in maize grains treated with some botanical powders than in untreated control. There was drastic loss in crude lipid in untreated sorghum compared to the treated grains, which supported Suleiman and Abdullahi [11] *S. zeamais* infestations resulted in substantial reduction in crude lipid content of stored sorghum grains. Similarly, it was reported that there was a reduction in crude lipid in white and yellow local maize varieties damaged by *S. zeamais* after 12 months of storage [16]. The reduction in crude lipid of untreated grains might probably be due to mass infestations by S. zeamais which were actively feeding on germ and endosperm of the grain.

Total protein content in sorghum grains treated with the test extracts was less than in untreated grains. It was found that higher concentration of methanolic and ethanolic extracts of *E. balsamifera* were the most effective botanicals which exhibited similar trend to the check in reducing protein loss of the grains. This was because the botanicals must have killed most of the weevils early before feeding on the grains, and served as antifeedants to the few that survived shortly, while in the control they survived for longer period and fed much on the grains. Therefore, as insect infestations increased, feeding activity increased and probably a decrease in the protein content of grains. Bamaiyi *et al*. [19] found that the endosperm component of maize grains contains 90% of seed protein, which is readily damaged by insects, and sorghum has protein

content ranging from 7 to 15% [20]. Crude protein in white local maize was reduced from 9.65% to 8.86% in undamaged and damaged grains, respectively [16].

Findings of this study showed that the untreated grains lost more carbohydrate than the treated ones, probably due to the fact that the insects were freely feeding on the untreated grains while the botanicals inhibited feeding and hence reduced feeding activities. Carbohydrates are important components of the diet for most insects as usual respiratory fuels, converted to lipid, provide carbon skeleton for the synthesis of various amino acids and the cuticle characteristically contains the polysaccharide chitin [21]. Consumption of more carbohydrate in the untreated grains was observed by Chapman [22] that insects feeding on stored products can use a wide range of carbohydrates. This study has revealed that the botanicals preserved carbohydrate content of stored sorghum which was significantly the same as the untreated un-infested grains regardless of their concentration levels.

Conclusion

The present study has uncovered that ethanolic leaf extracts of *E. balsamifera, L. inermis, M. hirtus* and *S. obtusifolia* reduced losses in proximate composition of sorghum grains and hence, could serve as alternative means of protecting sorghum from nutritive deterioration due to *S. zeamais* infestations thereby contributing in tackling malnutrition.

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