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CHEMICAL FACTORS RESPONSIBLE FOR THE RESISTANCE OF SOYBEAN TO STORAGE INSECT PESTS

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ABSTRACT

The non-preference for soybean (SB) grains to storage insect pests in comparison to cowpea (CP) was investigated for chemical factors that may be responsible for the relatively higher resistance. From this study, chemical factors that made soybean resistant to storage insect pests compared to cowpea include its lower soluble carbohydrate, safe moisture content for storage, stability of the fat content before and even after prolonged storage and hydrolysis. Although there were significant differences (P= 0.05) in the protein content of soybean and cowpea, these were inconsistent. Protein is therefore not a contributory factor to resistant of soybean; so is the ash content. Chemical composition of seed coat has no value in preventing storage insect infestation of soybean.

Keywords: *Soybean, Resistance, Storage insect pests and cowpea*

INTRODUCTION

One of the world's oldest cultivated crops and an important leguminous crop grown in the tropics (Duke, 1990) is soybean, *Glycine max* (L.) Merrill (Weiss 1983). It is a versatile crop which found usage internationally as human food, animal feed, medicinal, raw materials in industries and other purposes. Food made from soy flour is an excellent reliever for people suffering from high blood pressure, heart diseases etc. Accordingly to Branford and Ferris (2000) soybean is the most important agricultural commodity in the world market. Consequently, it could be a good substitute for crude oil as source of foreign exchange for third world countries like Nigeria. The annual yield of soybean according to Borget (1992) is put at between 400-3,800 kg/ha.

Although, there were conflicting reports about the susceptibility of soybean grains to insect pests under storage (Cornes, 1973; Weiss, 1983; Williams, 1986; Jackai *et. al*., 1990; Akem, 1991; and Ofuya 2001), Okunade (2008) reported that the damage level of soybean grains by *C. maculatus* and *Tribolium castaneum* is less than the threshold of 5%. Okunade (2008) therefore concluded that the grain is resistant to pest under storage and the reasons for this are chemical and physical factors borne by the grain. It is therefore the goal of this research work to investigate the chemical factors that confer resistance to soybean grains against insect pests under storage.

MATERIALS AND METHODS

Seven and six varieties of soybean and cowpea respectively were used for this investigation. The soybean varieties include four improved, i.e. (TGx 1485-1D, TGx 1440-1E, TGx 1448-2E and TGx 1740-2F) and three local varieties: Landrace 1 (Kano State), Landrace 2 (Benue State) and Landrace 3 Oyo/Kwara States); while the cowpea varieties include three moderately resistant varieties to CM i.e. (IT 89KD-288, IT 90K-2772 and IT 95K-207-15) and three susceptible (Danborno, Danmisira and Danila) varieties.

The improved soybean varieties, the

moderately resistant cowpea and Danila (susceptible) varieties were obtained from Ibadan and Kano stations of the International Institute of Tropical Agriculture (IITA) while the Landrace 1, 2 and 3 of soybean as well as Danborno and Danmisira (susceptible) varieties were obtained from local farmers. Comprehensive analyses of the whole grains of these varieties were conducted for protein, carbohydrate, ash and moisture content.

(a) ***Determination of quantities of protein***

Biuret reagent used for this was prepared by mixing (in 500 ml distilled water) 18.0 g of Sodium potassium nitrate (Rochelles salt), 3.0 g of copper sulphate and 5.0 g of potassium iodide – and made up to a volume of 1litre with 0.2 M Sodium hydroxide (NaOH). Into 10 mg of each grounded samples of the varieties, 100 ml distilled water was added. About 2.0 ml of this was taken and 1.0 ml of water added, followed by 2.0 ml of Biuret reagent to make up 5.0 ml water. Each was replicated three times. The mixtures were allowed to stand for 30 minutes while the amount of protein present in each sample was taken using the UV photo spectrometer at 540 nm (Hallaway, 1976). Results obtained for protein content were analyzed using SAS (1998 Edition) and means separated using the SNK at P=0.05.

(b) ***Determination of quantities of carbohydrate***.

i. Molisch Test (Soluble Sugars) About 1.0 g of each ground sample varieties was dissolved in 100 ml distilled water and made up to a strength of 1% sample. Then 2 ml of the solution was placed in test X-Naphthol (alcoholic naphthol) and 0.5 ml conc. H_2S0_4. The resultant purple solution was poured into an evaporating dish and steam dried. Amount of sugar was determined by weighing the residue in the tube. Each was replicated three times and the results treated as in (a) above.

ii. Anthrone Test (for insoluble Carbohydrate) To prepare the Anthrone reagent, 0.4 g Anthrone was placed in 0.5M 100 ml H_2SO_4 + 5.0 ml 0.2 M Ferric Chloride. About 2.0 ml of 1% sample solution (used for Molisch test above) was placed in a test tube and 1.0 ml of Anthrone reagent added to it +5 drops of sample solution. The solution was transferred into an evaporating dish, steam dried and the residue weighed to know the amount of insoluble carbohydrate (Hallaway, 1976). Each was replicated three times and results were treated as in (a) above.

(c) ***Determination of moisture content***

Washed crucibles were dried to a constant weight in an oven at 100^0 C, then cooled in a desiccators and re-weighted (W1). About 2.0 g ground samples of soybean and cowpea were placed in separate moisture dish (W2) while the crucibles containing the samples were kept in the oven at 1000C for 24 hours and weighted. This was returned to the oven and re-weighted after 3 hours to a constant weight (W3).

Thus:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

W_1 = Weight of oven-dried empty desiccators

W_2 = Weight of moisture dish + sample

W_3 = Final weight of moisture dish + sample after drying

Each sample was replicated three times. Results were analyzed as in (a)

(d) ***Determination of the relative quantities of ash content***

From the ground samples of each varieties of soybean and cowpea, 5.0 g each were taken and placed in separate crucibles and 15mls of 0.5 M HCl was added and allowed to evaporate in a stream both at 50^0C. The residue were later scraped and weighted. These were returned into the crucibles and placed in Muffle Furnace at 50^0 C for three hours for ashing to take place. After removal, they were left for 1 hour before transferring to desiccators with sufficient desiccants for 48 hours in other to remove excess moisture. This was later removed and weighed for percentage ashing. Each was replicated three times. Results obtained were analyzed as in (a) above.

e. ***Determination of Composition of Seed Coat***

Seed coat of all the varieties of soybean and cowpea were carefully removed and the proximate analysis of each carried out in the laboratory in three replicates each using the method described by AOAC (1980). Results obtained were similarly analyzed as in (a) above. (f) Hydrolysis using Dichlorvous as solvent Dichlorvous, an anticholinesterase used for controlling stored products pests of most coleoptera was used to emulsify samples of soybean and cowpea. The brand used was VIP, obtained from African Agro Products Ltd, Kano. About 25.0 g of ground samples were weighed and wrapped inside a condensable timbles. Then 25.0mls of Dichlorvous was placed in a flat bottom flask and raised to 600C for hydrolysis to take place. Each sample was replicated three times. Thereafter, 50.0 mls of the liquid content were taken and steam dried at 40^0C for 1 hour. The residue left, the emulsifier liquid, as well as the percentage fat present in the sample was also determined by taking the weight. Results were treated as in (a) above.

RESULTS

Upon the addition of NaOH into the mixture of Sodium potassium nitrate, copper sulphate and potassium iodide, blue colouration was formed and when the mixture was allowed to stand for 30 minutes, different colours (purple, deep blue, yellow etc.) were formed indicating different quantity of protein present in each tube.

Table 1 shows the relative protein content in the varieties of soybean and cowpea. There were significant differences (P=0.05) between the Soybean and Cowpea while the first three varieties of Soybean (TGx 1485-1D, TGx 1440-1E and TGx 1448-2E) differed significantly from the last four. There were no significant differences (P=0.05) between the Soybean varieties and moderately resistant Cowpea which differed from susceptible Cowpea (soluble carbohydrate: Molisch Test) – Tables 2 and 3.

Moreover, the insoluble carbohydrate in soybean compared favourably with those of cowpea (Anthrone test). In comparison however, there were no significant differences between soluble and insoluble carbohydrate content of soybean and cowpea (susceptible) while those of moderately resistant cowpea significantly differed (P=0.05).

The purple colour shown by all the samples at the end of the test is an indication that carbohydrate is present in all the samples. Table 4 shows the relative amount of moisture content of whole and decorticated grains of soybean and cowpea varieties. There were significant differences between the whole and decorticated grains of the two groups of crop, with a border line drawn among the three groups of crops; Soybean, moderately resistant Cowpea, and the susceptible Cowpea. In the whole grains, the mean moisture content of soybean varieties were much more lower than those of the cowpea while the reverse is the case in the decorticated grains.

Table 1: Quantities of protein and fat in soybean and cowpea varieties

Crop	Varieties	Protein (540 mm)	% Fat (from Hydrolysis)
Soybean	TGx 1485-ID	0.18^{d}	0.18^{h}
	TGx 1440-IE	0.23^{d}	0.23^{gh}
	TGx 1448-2E	0.25^{d}	0.26^{fgh}
	TGx 1740-2F	0.32^{d}	0.32^{efg}
	Landrace 1	0.34^{d}	0.34^{def}
	Landrace 2	0.36^{d}	0.36^{de}
	Landrace 3	0.38^{bc}	0.38^{cde}
Cowpea (Moderately Resistant)	IT 89KD-288	0.35^{abc}	0.59^{b}
	IT 90K-277-2	0.35^{abc}	0.65^{ab}
	IT 95K-207-15	0.35^{ab}	0.72^{a}
C. Cowpea (Susceptible)	Danborno	0.34^{abc}	0.44^{cd}
	Danmisira	0.35^{ab}	0.48^{c}
	Danila	0.39^{a}	0.64^{ab}
		SE $\pm$ = 0.138; CV = 9.46%	SE+ = 0.18 CV = 11.2

Note: - Means followed by the letter in the column are not significantly different (P=0.05) from one another.

Table 2: Quantities of carbohydrate in soybean and cowpea varieties

Varieties	% Carbohydrate content	
	Molisch Test (soluble carbohydrate	Anthrone Test (Insoluble carbohydrate
Soybean		
TGx 1485-ID	0.09[d]	0.12[a]
TGx 1440-IE	0.11[d]	0.12[a]
TGx 1448-2E	0.18[d]	0.13[a]
TGx 1740-2F	0.26[cd]	0.14[a]
Landrance 1	0.25[cd]	0.16[a]
Landrance 2	0.24[cd]	0.20[a]
Landrance 3	0.11[d]	0.14[a]
Cowpea(moderately resistance)		
IT89KD-288	0.08[d]	0.06[a]
IT90K-277-2	0.10[d]	0.03[a]
IT95K-207-15	0.11[d]	0.05[a]
Cowpea (Susceptible)		
Danborno	0.38[bc]	0.30[a]
Danmisira	0.42[b]	0.30[a]
Danila	0.63[a]	0.25[a]
SE±	0.22	0.28
CV (%)	31.23	75.79

Note: Means followed by the same letters in the same column are not significantly different (P=0.05).

Table 3: Comparison of soluble carbohydrate (Molish Test) and Insoluble carbohydrate (Anthrone Test) by grain groups.

Grain Characteristics	Mean	
	Soluble Carbohydrate	Insoluble Carbohydrate
Soybean (Resistant)	0.17[b]	0.14[b]
Cowpea (Moderatelhy resistant)	0.09[b]	0.05[c]
Cowpea (Susceptible)	0.48[a]	0.28[a]
SE ±	0.22	0.28
CV(%)	31.23	75.79

Note:- Means followed by the same letter in the same column are not significantly different (P=0.05) level of significance.

The percentage ash content of the cowpea and soybean varieties as presented on Table 5 shows that there were no significant differences (P=0.05) in ash content of all the samples tested. This is an indication that ash content may not have much bearing on the resistance of the grains to insect attack.

Table 6 shows the relative chemical composition of seed coat of the tested varieties. This shoes that there were no significant differences (P=0.05) in the composition of the seed coat of soybean varieties and that of cowpea in terms of all the six components except for carbohydrates where the susceptible cowpea varieties were significantly different (P=0.05) from the moderately resistant cowpea. This is in agreement with that of Weiss (1993) on tropical pulses.

It indicates that seed coat made no contributions in respect of resistance of this crop to insect infestation in storage. However, based on the grains characteristics, the result is presented on Table 7, and it indicated that the resistant crop (soybean) and the moderately resistant (cowpea) compared favourably well with each other (P=0.05) except in terms of carbohydrate, ash and crude fibre that were not significantly different.

However, chemical constituent within each crop group were not significantly different (P=0.05) in some cases like protein, fats, moisture content (for moderately resistance) and protein, fats, moisture content and ash (for susceptible). The percentage fats (from the hydrolysis) presented on Table 8 indicated that the mean values for soybean were smaller than those of cowpea while the values for moderately resistant cowpea varieties were higher than those of the susceptible one except for Danila that compared favourably well with the moderately resistant varieties. This mean value for soybean varieties is the least (0.29) followed by the susceptible cowpea (0.52) and then the moderately resistant cowpea (0.66).

The weight of the samples left were comparable (almost the same) with the amount of fat while the fats were very fine, light, smooth and without impurities; no curding or wax was noticed when it was exposed for one week.

Table 4. Amount of moisture content in soybean and cowpea varieties.

Varieties	Mean % Moisture content	
Soybean	Whole Grain of Soybean	Decorticated grains of soybean
TGx 1485-ID	2.33[d]	4.52[bc]
TGx 1440-IE	2.63[d]	4.14[bc]
TGx 1448-2E	3.11[d]	4.44[c]
TGx 1740-2F	2.89[d]	4.26[bc]
Landrance 1	2.81[d]	4.20[c]
Landrance 2	3.14[d]	4.78[c]
Landrance 3	2.85[c]	4.17[b]
Cowpea (moderately resistant)		
IT89KD-288	12.98[d]	5.63[b]
IT90K-277-2	3.96[d]	4.36[bc]
IT95K-207-15	4.81[d]	3.98[c]
Cowpea (Susceptible)		
Danborno	12.98[a]	5.63[b]
Danmisira	10.30[b]	9.34[a]
Dqnila	8.57[c]	8.44[a]
SE±	0.80	0.59
CV (%)	19.16	10.29

Note:- Means followed by the same letters in the same column are not significantly different (P=0.05)

Table 5: Quantities of ash content in soybean and cowpea varieties

Soybean	Mean % Ash Content
TGx 1485-ID	5.04[a]
TGx 1440-IE	4.90[a]
TGx 1448-2E	4.64[a]
TGx 1740-2F	10.53[a]
Landrace 1	13.63[a]
Landrace 2	9.17[a]
Landrace 3	8.57[a]
Cowpea (Moderately resistant)	
IT 89KD-288	11.88[a]
IT 90K-277-2	11.98[a]
IT 95K-207-15	8.92[a]
Cowpea (Susceptible)	
Danborno	11.41[a]
Danmisira	12.27[a]
Danila	12.6[a]
SE± = 1.417	
CV (%) = 31.17	

NOTE: Means followed by the same letters in the column are not significantly different (P= 0.05)

DISCUSSION

From the results, boarder lines seems to have been drawn for the protein content in soybean, moderately resistant and susceptible cowpea varieties and this is traceable to genetic factors of the crop (the improved and local varieties). The local soybean variety (Landrace 3) that was significantly related to cowpea varieties could be due to environmental and edaphic factors. The results are comparable with that of Weiss (1983) who observed that protein content of cowpea was about 23.40% while that of soybean was between 20.60 – 50.30%. Proteins are essential for cell divisions, as enzymes, hormones, metabolic process etc.

Although significant differences were observed in the protein content of the three groups of crops tested, but there was no consistency to come to a conclusion on their positive impact on crop resistance to insect pests. Consequently, protein content is not a reliable factor of resistance in soybean. The results showed that both soluble and insoluble carbohydrates are present in soybean and cowpea.

Chippendale (1978) reported that carbohydrates are indispensable to the structure and functions of all insect tissues and are required at all levels of cellular organization, nucleus cytoplasm, cell membrane, extracellular haemolymph and supporting tissues. They are also required for carrying out the various metabolic processes in young and ageing insects (Puri and Sharma, 1984).

Table 6: Chemical composition of seed coat of soybean and cowpea varieties

	% Composition					
Variety	Carbohydrate	Protein	Fats	Moisture Content	Ash	Crude Fibre
Soybean						
TGx 1485-ID	51.91 abcd	35.27 a	7.78 a	2.17 a	2.87 ab	3.63 a
TGx 1440-1E	45.74 cd	37.46 a	9.56 a	3.94 a	3.30 ab	3.29 a
TGx 1448-2E	47.91 abcd	37.17 a	8.38 a	3.25 a	3.30 ab	3.78 a
TGx 1740-2F	50.07 abcd	35.81 a	7.52 a	3.31 a	3.30 ab	3.62 a
Landrance 1	46.72 abcd	36.10 a	9.78 a	3.67 a	3.73 ab	3.92 a
Landrance 2	50.39 abcd	35.44 a	7.20 a	3.38 a	3.59 ab	3.60 a
Landrance 3	50.42 abcd	36.96 a	6.48 a	3.03 a	3.12 ab	3.89 a
Cowpea (Moderately resistant)						
IT 89KD-288	46.11 bcd	38.14 a	8.13 a	3.13 a	2.61 ab	4.44 a
IT 90K-277-2	44.26 d	37.13 a	8.16 a	3.24 a	2.41 b	4.69 a
IT 95K-207-15	46.19 bcd	37.16 a	8.12 a	3.18 a	3.17 ab	4.21 a
Cowpea (susceptible)						
Danborno	55.22 a	35.03 a	3.01 a	3.60 a	3.14 ab	3.72 a
Danmisira	55.52 ab	34.31 a	3.79 a	3.18 a	3.20 ab	3.57 a
Danila	54.11 abc	30.14 b	3.60 a	4.25 a	3.39 ab	4.14 a
SE+	1.55	1.27	1.37	0.78	0.57	0.69
CV (%)	4.85	4.51	26.62	18.15	9.98	12.26

Note:- Means followed by the same letters in the same column are not significantly different (P=0.05)

Table 7: Chemical composition of seed coat of varieties of soybean and cowpea based on grain characteristics

	Means (%)					
Grain Characteristics	Carbohydrate	Protein	Fats	Moisture Content	Ash	Crude Fibre
Resistant (Soybean)	49.02 b	36.32 a	8.10 a	3.25 a	3.31 a	3.68 b
Moderately resistant (cowpea)	45.52 c	37.48 a	8.17 a	3.18 a	2.73 b	4.45 a
Susceptible (Cowpea)	54.95 a	33.16 b	3.47 b	3.68 a	3.24 a	3.81 b
SE (±) / cowpea)	0.60/0.15	0.50/0.77	0.49/0.74	0.30/0.47	0.23/0.35	0.24/0.37
CV (%)	5.04	4.97	23.58	19.57	11.40	10.78

Note:- Means followed by the same letter in the same column are not significantly different (P=0.05)

Consequently, sample with lower carbohydrate (soybean in this case), will be less susceptible to insect infestation because insects don't fling comparative advantage with them. Moreso, insect's ability to maximally utilize specific carbohydrate depends on a combination of processes like gestation, digestive hydrolysis, absorption and metabolism (Chippendale, 1978). In addition, the soluble carbohydrate is much lower in soybean than in cowpea. This explains why soybean is resistant to insect pests under storage compared to cowpea. That the moisture content of cowpea was significantly higher than that of soybean (whole and decorticated) is consistent with the findings of

Weiss (1983) and is characteristic of oil seeds.

However, that there were no significant differences between decorticated Landrace 3 soybean and decorticated moderately resistant and Danborno cowpea is traceable to a number of factors such as environmental, the condition of the grain utilized, the stage at harvest and the storage duration. Moisture is a critical factor in storage of any commodity. Okunade (2006) reported that if grains are dried to safe moisture content (SMC), over 80% of the storage problems are solved and such grains will not be easily predisposed to attack by storage pests like insect among others. The higher the SMC, the higher the rate of insect pest attack would be. Soybean, being an oil seed, enjoys a lower SMC hence are safe for storage and hence described as having SMC as compared with cowpea.

Although, little moisture may be absorbed by the grains from the environment, but the absorbed moisture will not be high enough to predispose soybean (an oil seed) to attack by insect pests under storage. This explains why soybean is less prone to attack by storage insect pests than cowpea. That decorticated grains had higher mean MC than whole grains is traceable to the fact that the outer protective covering of the grain, which precludes it from absorbing moisture from the atmosphere, had been removed. It is therefore possible that decorticated grains could absorb some moisture from the environment under storage.

The mean values for ash content of cowpea are higher than those of soybean, but there were no statistical differences between them. This fact might be traceable to the relative hardness of the two crops whereby soybean was harder than cowpea. Weiss (1983) observed that in terms of minerals removed (in kg) per ton of seed from the soil, soybean removed more nitrogen, phosphorus and potassium than any of the other common leguminous crops like groundnut, cowpea, bambara groundnut and common beans. Since soybean is harder than any of these crops, it could be implied that the issue of mineral consumption from the soil is responsible for the hardness of soybean and not the ash content. Hence, ash content is not a contributory factor for resistance of soybean to storage insect pests compared to cowpea. Wolf and Cowan (1971), reported that seed coat constitutes 8.0% of the whole grains. This is made up of carbohydrate (56%), protein 80%, fats (1%) and ash 4.3%. Fats and oils have been reported as being good insecticides against storage insect pests of cereals and leguminous crops (Singh *et al*., 1978; Rani and Osmani, 1984; Groot, 2001; Lale, 2002). The results indicated that the composition of seed coat in soybean and cowpea were comparable; and confirms that chemical composition of seed coat played no role in protecting the crop against attach by insect pests. This is consistent with the findings of Edde and Amatobi (1998) on cowpea seeds against *C. maculatus*.

The resistance of soybean to storage insect pests is therefore within the grain itself and not on the surface. Although there were gaps between the mean percentage fat in soybean and cowpea varieties, the fact that they are not statistically difference indicates that there are some other factors responsible for this. Among this could be the availability of the fats and oils components as being fixed (not available) and non-fixed (available). However from these results, to some extent, the fixed fats/oils in soybean and cowpea were comparable. Since these results confirmed that soybean oil is good and not curd-up for days even under low temperature, it is possible that insect pests could not successfully attack soybean under storage because of the nature of the fats and oils it contained.

Moreso, Borget (1992) reported that the oils in soybean contained an average of 15-20% of poly-unsaturated fatty acids. This must have made it difficult for insects to penetrate soybean as their respiratory system could be blocked by excess oil and they die in the process from asphyxiation. Hydrolysis, which according to Grant (1969) and Stenesh, (1975) is the decomposition reaction of a substance with water that leads to the separation of the elements of water H, and OH is less in soybean than that of cowpea. This indicates that soybean is harder, and more resistance than cowpea and hence could not be easily broken/attack/decomposed compared to cowpea. The soybean oil in the non-refined form, according to Lof (1995) contains a natural anti-oxidant and could be kept well. This natural anti-oxidant could further preclude insect attack on this crop compared to cowpea.

CONCLUSION

From the findings in this work, it becomes clear that the chemical factors responsible for the resistant of soybean to insect pest under storage include soluble carbohydrate, moisture content, and fat while protein content, seed coat and ash content play no role in this aspect.

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