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Inherent susceptibility of elite rice varieties to post harvest damage by *Sitophilus zeamais* Mostch. (Coleoptera: Curculionidae)

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ABSTRACT
Four elite rice varieties: Nerica-1, Nerica-3, Cisadane and OS-6 were evaluated for their degree of susceptibility to infestation by Sitophilus zeamais in the laboratory. The experiment was carried out at ambient temperature (24± 3^0C); relative humidity (70-85%) and 12:12 hour photoperiod. All rice varieties were evaluated using no-choice and free-choice tests. The primary and secondary metabolites in the rice samples were analyzed using standard analytical procedures. Mean F_1 adult emergence (57.8) and grain damage (33.3%) was highest on Nerica-3. The most preferred variety for feeding and oviposition by S. zeamais was Nerica-3 with high susceptibility index (SI=5.16). Small grain size and comparatively higher moisture content was inferred as the likely factor which conferred susceptibility on Nerica -3

Key words: Susceptibility, Grain damage, Nerica, Rice, *Sitophilus zeamais*

INTRODUCTION

Rice is the most important food crop for more than half of the world's population. Current global rice production is put at 520 million tonnes which is sufficient to provide 20% of the total human calorie requirement (Sohl, 2005).

Insect pests are major among the principal causes of crop losses in the field and in storage. The use of synthetic insecticides has proved to be very effective among several methods available for the control of insect pest. However, environmental and toxicological side-effects during and after application have led to intense search for alternative methods of controlling storage pest. The adverse effects of synthetic insecticides, resulting from their misuse, include human poisonings, destruction of natural enemies of pests, insecticide resistance, crop pollination problems, domestic animal poisonings, contaminated livestock products and fish and wildlife losses (Pimentel et al., 1980) and contamination of underground water and rivers. As a result of these limitations, efforts have been put in place to exploit non-toxic methods of protecting grains.

One of the ways of reducing post harvest losses of grains is breeding for varietal resistance. This strategy is particularly appropriate for use by resource poor, small scale farmers in Africa who cannot afford the capital to purchase expensive insecticides (Oghiake et al. 1993). The factors that confer resistance to the grains against infestation by storage insects are varied. A plant may be resistant to insect attack either because it is less preferred, tolerant or has antibiosis. Non preference refers to plant characteristics that lead insects away from a particular host. Antibiosis refers to all adverse effects on the insect life cycle which results from feeding on a resistant host -plant variety while tolerance includes all plant responses resulting in the ability to withstand insect infestations.

Rice varieties exhibit varying degrees of susceptibility to damage by insects (Ashamo, 2006). Some works have been done on the resistance or susceptibility of different varieties of rice to attack by the rice weevil, *Sitophilus zeamais* L. but there are still a lot of varieties waiting to be tested for resistance to S *zeamais*. This becomes expedient as new varieties are developed to satisfy local requirements of taste and utility This study therefore investigated the resistance status of selected rice varieties against *S. zeamais* in the laboratory.

MATERIALS AND METHODS

The study was conducted at ambient temperature (24±3^{o}c) and relative humidity (70-85%) in the Entomology Department Laboratory of the Nigerian Stored Product Research Institute (NSPRI), Abule-Oja Lagos Nigeria.

Rice Varieties

Four elite varieties of rice comprising two Nerica and two *Oryza sativa* species were evaluated for resistance to .*S. zeamais.* The rice varieties were obtained from the Africa Rice Centre (formerly WARDA) at the International Institute of Tropical Agriculture (IITA) Ibadan and these were Nerica-1, Nerica-3, Os-6 and Cisadane. A susceptible local rice variety-*Tuwo* obtained from Iddo market was used to culture *S. zeamais* for the experiment. Clean rice samples were sorted out by handpicking of damaged seeds and other debris, and thereafter disinfested in an oven at 55^{o}c for 24 hours. The grains were then aerated for two hours to stabilize at ambient conditions.

Insect Culture

Stock cultures of *S. zeamais* were obtained from the insectaries of NSPRI Lagos, where they have been isolated from all kinds of insecticides for several years and raised on the local variety-*Tuwo*. Kilner jars of 1kg capacity were used for the culture. The temperature and other weather parameters were maintained at laboratory conditions, using a thermo-hydrograph. Muslin cloth was placed over the open end of the kilner jar to allow for ventilation and also prevent the escape of weevils. Cultures of *S. zeamais* were established by adding 50 adult *S. zeamais* of mixed sexes to 200g of rice in the kilner jars. After three weeks, the adults were removed from the culture. New adults of 7-14 days old that emerged from the culture were used to maintain the cultures. Freshly emerged adults from the culture were used for the experiment.

Physical Characteristics of Rice Grains

The rice varieties were studied for their physical features. The seed weight was measured by weighing 100g sample of each variety on a sensitive weighing balance. Relative kernel size was determined by measuring average length of each grain using micrometer screw-gauge. The relative size was also determined by a count of a total number of rice grains contained in a 10g sample of each variety.

Resistance Evaluation by No Choice Experiments

A 20g sample of each rice variety was weighed into10cl capacity plastic cups. Five pairs of 7 days old adult *S zeamais* were carefully introduced into each of the samples in four replicates. Another set of rice samples which were not infested was used to correct for moisture. The adult insects were sexed based on the description by Reddy (1951), Halstead (1962) and Proctol (1971). Thereafter, the plastic cups were covered with muslin cloth and held tight with rubber band to allow ventilation and prevent escape of insects. The experiment was laid out under laboratory conditions in a Completely Randomized Design (CRD). After 14 days of infestation, the insects were sieved and the number dead and alive was recorded. Insects that did not respond to probe by pin nor move for 24 hours were counted as dead. The set-up was left undisturbed in the laboratory and thereafter inspected daily for emergence of F_1 adults. The susceptibility of each variety of rice to attack by S. *zeamais was* evaluated on the basis of percentage adult mortality, adult emergence, final weight loss and percentage grain damage. The weight of the infested rice samples was determined using the following formula.

$$\text{Percentage weight loss (w \%)} = \frac{X_1(cf) - X_2(cf) \times 100}{X_1(cf)}$$

Where: X_1 = is the initial weight of infested rice samples,

X_2 = final weight of infested rice samples

cf = the correction factor for moisture.

The index of susceptibility (Dobie, 1974) was determined as follows:

Index of susceptibility (SI) = (Natural log F ×100)/D

Where: F= Total number of insects counted, D= Median developmental period in days. Median developmental period is defined as the time from the middle of oviposition period until the emergence of 50% of the F_1 generation.

Grain damage was assessed after complete emergence of adult insects by separating grains into damaged and undamaged categories and expressed as percentage of total grains.

Resistance Evaluation by Free- Choice Experiments

A free choice chamber was used for this experiment. The chamber was improvised using a 60cm diameter bowl. The bottom of the bowl was covered with a cardboard which had been subdivided into twelve chambers with a separating stick. Each variety had three replicates in the chamber. The samples were laid out in CRD. One hundred and twenty adult *S. zeamais* were introduced at the centre of the chamber. The set-up was thereafter covered with a muslin cloth, held tight with a thread and left under laboratory conditions. The number of insects in each chamber was counted after 7 days.

Proximate Analysis of Rice Samples

The procedure for proximate and mineral analysis was as described by AOAC (1984). The moisture content was calculated using the air-oven method as described by Onyieke and Ayalogu (2003). The crude protein was obtained by using the micro-kjeldal method and multiplying the percentage nitrogen by a protein conversion factor which is 6.25. The secondarily metabolites were analyzed using the method of Horwitz (1985) and Pharmacognosy Manual (2003).

Data Analysis

All data collected were analyzed appropriately using one way analysis of variance. Means were thereafter separated using Duncan Multiple Range Tests (p=0.05) (SAS, 1996)

RESULTS

Table 1 shows the physical characteristics of the rice varieties evaluated in this study. OS6 (31.7g) was significantly ($p<0.05$) bigger and heavier than other varieties. Nerica-3 was comparatively longer and more slender than the other varieties although this difference was not significant ($p>0.05$) (Table 1).

Table 1: Physical parameters of rice varieties.

Variety	Seed weight (mg)	Seed length (mm)	Shape
N1	29.0 ± 0.15a	6.97 ± 0.24a	Fairly robust
N3	28.7 ± 0.07a	7.08 ± 0.47a	Slender
So6	31.7 ± 0.06b	6.08 ± 0.58a	Robust
Cisadane	28.9 ± 0.07a	6.15 ± 0.00a	Robust

Means followed by the same letter in the column are not significantly different ($p>0.05$) Duncan Multiple Range Test

Mortality of adult *S. zeamais* on the tested rice samples after 14 days was significantly higher in OS6 (97.5) > Cisadane but lowest in Nerica-1 (25%) < Nerica-3 (12.5%) under no-choice test (Table 2). Thus grain damage was significantly higher ($p<0.05$) in Nerica-3 (33.3%) and Nerica-1 (25.4%) compared to Cisadane and OS6 (Table 2). Also under free choice, the mean number of adult *S. zeamais* that migrated was significantly ($p<0.05$) higher in Nerica-3 (9) and Nerica-1 (7) than OS6 (2.7) and Cisadane (0.7).

Table 2: Mean percentage weight loss and grain damage (mean ± SE) of rice due to infestation by *S .zeamais*

Variety	% mortality	% grain damage	No of insects in free choice
N1	2.50 ± 2.50a	2.54 ± 0.36 b	7.00± 1.44a
N3	12.50 ±6.29a	3.30 ± 0.60b	9.00 ± 0.96a
SO6	97.50 ± 2.50b	0.19 ± 0.04a	2.67 ± 0.29b
Cisadane	82.5 ± 7.50b	0.16 ± 0.04a	0.67 ± 0.29b

Means followed by the same letter along the vertical column are not significantly different at p=0.05 from each other using Duncan multiple range test

Mean emergence of F1 adult *S. zeamais* on the tested rice varieties varied significantly ($p<0.05$) (Table 3). Whereas, no adult emerged from OS-6 and Cisadane ().5), mean number of F1 of adult that emerged was highest ($p<0.05$) in Nerica-3 (57.8) followed by Nerica-1 (37.5). There was however no significant

Table 4: Proximate analysis of rice samples

Variety	No of Adult emerged	I.S	Moisture content	% ash	% protein
N1	37.50	4.65	12.12	16.09	5.0
N3	57.75	5.16	11.98	29.70	9.31
SO6	0.00	0.00	10.27	14.14	4.41
Cisadane	0.50	0.94	11.31	21.36	6.69

(p<0.05) difference in the mean weight of F1 adult emergent, the host rice variety notwithstanding. Similarly the mean developmental period which ranged from 32.0 days in Cisadane to 34.0days in Nerica-3 and Nerica-1 was not significantly (p<0.05) different. However, Nerca-3 has the highest susceptibility index (SI=5.16) > Nerica-1 (SI=4.65) > Cisadane (0.94). > OS-6 (0).

Table 3: Mean F1 adult emergence, mean weight of emergent and mean developmental period of *S. zeamais* and index of susceptibility of rice varieties.

Variety	Number of emerged adults	Weight of emergent (mg)	Mean Developmental period (days)	Index of susceptibility
N1	37.50 ± 6.17b	2.1 ± 0.07b	34 ± 0.95b	4.65± 0.22a
N3	57.75 ± 13.14c	2.2 ± 0.70b	34 ± 1.08b	5.16 ± 0.33a
SO6	0.00 ± 0.00a	0.0 ± 0.00a	0.00 ±0.00a	0.00 ± 0.00b
CISADANE	0.50 ± 0.50a	2.1 ± 0.00b	32 ± 0.00b	0.94 ± 0.00b

Means followed by the same letter along the vertical column are not significantly different at p<0.05 from each other using Duncan's multiple range tests

Table 5: Toxic secondary metabolite contents of rice varieties

Variety	Alkaloids	Flavonoids	Glycosides	Terpenoids
N1	+	-	-	-
N3	+	-	-	-
S06	+	-	-	-
Cisadane	+	-	-	-

The proximate content of the rice varieties evaluated in this study also varied significantly (p<0.05) (Table 4). Although percentage protein and ash were comparatively higher in Nerica-3, no definite pattern and order was observable in the composition of the proximate content. However, the moisture content was comparatively higher in Nerica-3 > Nerica-1 > Cisadane > OS-6 varieties. The only secondary metabolite detected was alkaloid and this was present in all the tested varieties (Table 5).

Discussion and Conclusion

The results obtained in this study showed that Nerica-3 and Nerica-1 rice varieties are susceptible to damage by *S. zeamais* in the store. Although several factors relating to physical and biochemical properties of the rice grains could be responsible, it is inferred from this study that the comparatively smaller grain size of Nerica-3 and Nerica-1 might be major among the factors responsible for the susceptibility of these varieties to *S. zeamais* (Ogunwolu and Idowu, 1994; Ogunwolu, and Odunlami, 1996). It is well known that larger grain sizes are indicators of resistance against infestation and damage by *S. zeamais* (Guadrups et al., 2001). This result also agrees with Omoloye and Amodu (2006) who reported that smaller-grained sorghum were comparatively more susceptible to infestation by *S. zeamais* than larger grains.

No positive relationship could be established between abundance of selected proximate and mineral content of rice and infestation. However, it appears that higher moisture level rather than the nutritional composition of the rice varieties contributed significantly to the susceptibility of Nerica-3 and Nerica-1 to *S. zeamais* in the store. Yet, this does not preclude the role of nutritional and mineral content in conferring resistance to rice grains. Indeed, it is well reported that abundance of selected primary metabolites were positively correlated to infestation and damage by *S. zeamais* (Nwana and Akibo-Betts, 1982).

Similarly, no positive relationship could be established between abundance of selected secondary metabolites of rice grains and infestation by *S. zeamais*. However, it is found in this study that all the tested rice samples contained only alkaloid while other secondary metabolites such as flavonoids, glycosides and terpenoids were not detected in all the samples. Although it is possible that non-detection of these metabolites might be due to inability of the analytical equipment used, this result suggests that the mechanisms and the factors responsible for the biochemical mediated resistance in rice against *S. zeamais* could be more than the identified groups. For example, Omoloye and Vidal (2007) had identified 24-methylene-cholesterol as potential factor of resistance in *Oryza glaberrima* varieties against a major field pest of rice. This suggests that more work is needed to identify the mechanisms and factors involved in the resistance of rice against *S. zeamais*.

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