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Management of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) using Nigerian Raw Diatomite

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ABSTRACT: Maize weevil, *Sitophilus zeamais*, whose management has largely been via traditional control practices, is a key pest of stored maize grains causing severe losses. This study explored the use of Nigerian Raw Diatomite (RD) in the management of the weevil. A 3-replicated split plot designed laboratory experiment was conducted for 6 months duration. The treatments were Raw Diatomite (RD) at the rate of 250, 500, 750 and 1000 mg/kg and a control (untreated) across four improved maize grain varieties. Data collected were on mortality, F_1 progeny produced, grain weight damage, and loss. Data were analyzed with variance analysis and significantly different means were separated using Turkey Kramer HSD test at $P<0.05$. Results showed that the control had the least weevil mortality across the varieties throughout the periods of assessment. Highest weevil mortality was recorded with increase in dose rates of RD and progresses to 100% - largely at the 14th day post-treatment. Control recorded the highest weight loss and grain damage of 16.5% and 38.1%, respectively when compared to all the other treatment rates on the most tolerant variety (SAMMAZ 25). The study revealed that RD had a promising potential to substitute synthetic insecticides and can be incorporated into the integrated pest management strategy against maize weevil. We therefore suggest that further work be done to refine the Nigerian RD so as to standardize the most effective dosage application rate for management of insect pests of stored maize and other cereals.

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Maize (*Zea mays* L.), which belongs to the grass tribe Andropogoneae, and family Gramineae (Poaceae), is an important food, cash, and industrial crop (Adebayo and Omoloye, 2016). Nigeria produces about 7.7 million tonnes of maize grains annually, representing 0.9% of the world production (Amudalat, 2015). Despite, occupying less land area than either rice or wheat, maize gives higher average yield per unit area (5.5 t/ha), thereby fostering the drive towards global food security (Sasson, 2012). Maize is the most widely cultivated cereal crop in Nigeria after guinea corn and millet, and provides families with the much needed nutrients such as carbohydrates, proteins, fats, vitamin B and minerals (Tongjura *et al*., 2010). Both in the field and storage, insects are the major cause of maize grain losses (Dubale *et al*., 2012; Simbarashe *et al*., 2013). The maize weevil, *Sitophilus zeamais* Motschulsky is a very serious primary pest of stored maize grains which cause severe losses in stored maize grain in Africa (Tongjura *et al*., 2010). It is estimated that *S. zeamais* accounts for about 10 - 40% of the total damage to stored maize grains worldwide. Grain weight losses of 12 - 80% attributed to maize weevil have been documented in untreated grains stored in traditional structures in the tropics (Muzemu *et al*., 2013). The indiscriminate use of insecticides for the control of stored-product insect pests is of global concern due to its attendant hazards to man and the environment (Okrikata and Anaso, 2019). The current trend in stored-product pest control is to use reduced-risk or low-toxicity insecticidal materials as replacements/substitutes for conventional grain protectants, mainly organophosphates. In this respect, inert dusts, including diatomite, have received considerable attention during the past two decades (Athanassiou *et al*., 2006). Diatomite also called Diatomaceous earth (DE) is a promising alternative to residual insecticides and has been shown to be effective against stored product insect pests (Kabir, 2013). It has extremely low mammalian toxicity, and its use is compatible with other reduced-risk integrated pest management-based control methods in storage facilities (Athanassiou *et al*., 2006). DEs are 'Generally Regarded as Safe' by the USA Environmental Protection Agency (Anon., 1991). They are of natural origin and can be applied with similar technology to that needed for residual pesticides (Subramanyam and Roesli, 2000). As a result, several DE formulations are now commercially available (Subramanyam and Roesli, 2000), and a

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number of studies showed that they are quite effective against a wide range of stored-product insect species (Vayias *et al.,* 2006; Athanasiou and Korunic, 2007). Such studies are scarce in Nigeria and thus this study evaluates the impact of Nigerian Raw Diatomite (RD) on the mortality and progeny development of *S. zeamais* a cross 4 maize varieties and the associated effect on grain injury.

MATERIALS AND METHODS

Study Site: The experiment was conducted from July to December, 2018 (6 months) in the Laboratory of the Department of Crop Protection, Modibbo Adama University of Technology, Yola, Nigeria. Yola is located in the Northern Guinea Savannah Agro-Ecological Zone of Nigeria at latitude 9^o 10N, longitude 12^o 35E, and altitude 185.5m. The minimum and maximum temperatures, and relative humidity are 20^{o}C and 48^{o}C; 48% and 65%, respectively (Ezra *et al*., 2016).

Sources of Maize Varieties and Raw Diatomaceous Earth: Four maize grain varieties were acquired from the Institute for Agricultural Research (IAR) Samaru, Zaria, Kaduna State, Nigeria viz; SAMMAZ 16, SAMMAZ 20, SAMMAZ 25 and SAMMAZ 29 while, RD was obtained locally from DE mines in Bularafa village, Gujba Local Government Area, Yobe State, Nigeria. The RD was supplied in a form of crude soft chalky rock. The rock was milled finely in the laboratory using mortar and pestle and then sieved through a mesh of 0.20 mm (Laboratory Test Sieve - BS410/86) to obtain a powdery consistency. The fine powder was analyzed for silicon (SiO_2 - 28.7%) content in the Mineralogy Laboratory of the Department of Geology, Modibbo Adama University of Technology, Yola.

Sample Preparation and Insect Culture: The experimental bottles and maize varieties were examined, cleaned and sterilized thermally in a hot-air oven (Hot Air Circulated Oven - OV95c) at 60^{o}C for 1 hour to kill any pest and pathogen that might be present, and afterwards allowed to equilibrate for 24 hours in the laboratory before the commencement of the bioassays (Medugu, 2012). Population of *S. zeamais* was obtained from naturally infested maize grains obtained from a local grain merchant in Yola. The insects were reared on a susceptible local maize variety "commonly called Saksi" in two 1-litre transparent plastic buckets and routinely maintained to provide weevils of similar age for the study. Each bottle contained 100 adults *S. zeamais* per 500 g grains. The bottles were then covered with muslin cloth to allow for aeration and to prevent escape of the weevils. All the introduced parents *S. zeamais* in each bottle were removed seven days after oviposition. The set up was then kept at laboratory conditions on an open air shelf. Emerged F_1 progenies (0 - 14 days old) were then used for the bioassays.

Experimental Design and Bioassay: The bioassay was laid in a split-plot design with three replications under ambient laboratory conditions of 26 - 38°C and 48 - 65% temperature and relative humidity, respectively. Two lots of 200 g of each of the 4 maize varieties were treated with 0.0 (control), 250, 500, 750 and 1000 mg/kg of RD. Each lot was placed in 500 ml capacity bottles, and then capped and shaken manually for approximately 2 minutes to achieve uniform distribution of RD in the entire grain mass. Thereafter, 4 samples of 50 g of treated maize grains was then taken from each lot, and placed in 250 ml capacity glass bottles. In each bottle, 20 *S. zeamais* adults (0 – 14 days old) were then introduced, the bottles were then covered with muslin cloth fitted with rubber band to allow gaseous exchange. The bottles were then kept at laboratory conditions.

Adult Mortality of S. zeamais: Adult mortality in both treated and untreated (control) bottles were assessed at 3, 7 and 14 days after infestation (DAI) by weevils. All adult insects were removed from each bottle, and the dead and living insects were counted and recorded (an insect is assumed dead when there is no movement on probing with a pin). After 3 and 7 DAI count, live insects were returned to their respective bottles, while dead insects were discarded. However, after the 14 DAI counts, both live and dead insects were discarded. The bottles containing the grains were then kept under same condition for observation of progeny development and grain damage.

Progeny Production of S. zeamais: After removing all the introduced adult insects as described above, each bottle was kept under the same experimental conditions to assess the emergence of F_1 progenies. The number of F_1 progenies in each bottle was counted after additional 40 days. To do this, the content of each bottle was poured onto a tray and every emerging progeny was removed, counted and recorded on each assessment day.

Maize Grain Damage: After F_1 progeny count, 20 grains were randomly taken from each jar to assess the percentage of grain damage [i.e. grains with weevil emergence hole(s)]. This was expressed as a proportion of the total number of seeds sampled (Abebe *et al*., 2009);

$$\%GD = \frac{G_T - G_D}{G_T} x100$$

Where % GD = percentage Grain damage; G_T = Total number of grains; G_D = Number of damaged grains

Maize Weight Loss: Grain weight loss due to weevil feeding was expressed as percentage weight loss, and was determined by count and weight method as describe by Lale (2002);

$$\% \text{ Weight loss} = UaN - (U + D)/UaN \times 100$$

Where: Ua = average weight of one undamaged grain, N = total number of grains in the sample, U = weight of undamaged fraction in the sample, D = weight of damaged fraction in the sample.

Data Analysis: Before variance analysis, mortality data were corrected using Abbott's formula (Abbott, 1925), and data on mortality, seed damage and weight loss were arc-sine transformed while, data on progeny production was square root $\sqrt{(x + 0.5)}$ transformed. The transformed data were subjected to analysis of variance (ANOVA) using the GLM procedure of Statistix 8.0. Significantly different treatment means were separated using Tukey-Kramer "Honestly Significant Difference" (HSD) test at 5% level of probability.

RESULTS AND DISCUSSION

Effect of RD Treatment on Adult Mortality of S. zeamais on Different Maize Varieties: Table 1 showed that mortality of *S. zeamais* was significantly affected by the various doses of RD vis-à-vis exposure time. In the treatments with RDs, mortality of *S. zeamais* started on the 3rd day after treatment across the varieties, and by the 14th day, statistical parity was observed between 250 and 750 mg/kg treatments on SAMMAZ 16 and SAMMAZ 29 varieties. The accumulated mortality in the 14th day was >75% at 250 mg/kg, >85% at 500 mg/kg, >95% at 750 mg/kg, and 100% at 1000 mg/kg of RD while, mortality was <10% in the control bottles on all the 4 maize varieties. Of the 4 maize grain varieties, SAMMAZ 25 had the highest weevil mortality across the dose rates. Treatment with1000 mg/kg RD across the varieties effected 100% weevil mortality within 14 days after treatment (DAT). Significantly ($P<0.05$) lower mortality was recorded with grains treated with the lowest dose rate (250mg of RD). Findings from this study therefore showed that the Nigerian RD was toxic to adult *S. zeamais*, causing significant mortality to the weevils. However, the toxic action of the RD was slowest at the lowest used rate (250 mg/kg) within 7 days post-treatment. Toxicity of RD was noted to increase with increasing exposure period for all the treatments. The increase in adult mortality according to ascending exposure period and concentration could be attributed to the increase in the quantity of active ingredients picked by the insects. The exposure time is crucial for the effectiveness of DEs, because insects' movement increases the contact of the cuticle with the dust particles (Athanassiou *et al.,* 2006). That RD induced > 50% mortality of the weevils from the 7th day post-treatment corroborates the findings of Demissie *et al.* (2008), and Jean *et al*. (2015) who used a commercial DE formulation (Silicosec) against *S. zeamais*. These authors reported that Silicosec caused 100% mortality of *S. zeamais* within 7 days exposure period, at the rates of 1% and 2%, respectively. The results of this study also suggest that relatively higher dose of this product is required to kill *S. zeamais* completely within 14 days. Our findings are in agreement with those of Athanassiou *et al*. (2006) and Wakil *et al*. (2006) who showed that adult mortality increases with increase in dose rate and exposure period to DEs, and that efficacy varied between grain types. A very important finding of this study is that the RD used affects *S. zeamais* in ways similar to commercial DE formulations. The death of insects caused by DEs could be attributed to dehydration provoked by the abrasiveness of the small particles of this inert dust and by adsorption of oils from the body of the insects (Fields and Korunic, 2000; Kavallieratos *et al*., 2006; Okrikata and Anaso, 2019), which breaks the layer of wax on the epicuticle, exacerbating the fatal loss of water as reported by Subramanyam and Roesli (2000), and Okrikata and Anaso (2008). Ibrahim *et al.* (2012) observed that DE reduced the production of progenies by ovicidal and larvicidal activities, increasing adult mortality and, reducing oviposition. Jean *et al*. (2015) showed that though adult weevils were killed by exposure to DEs, some oviposition could still take place, and this may lower the suppression of progeny production. This may explain the emergence of progeny at the highest RD rate in the current study.

Effect of RD Treatment on Progeny Production of S. zeamais on Different Maize Varieties: The dynamics of emergence of adult *S. zeamais* F_1 progeny on the maize varieties is presented in Figure 1. Results indicated that adults emerged earlier (21 days after treatment - DAT) on SAMMAZ 16 and SAMMAZ 29 varieties. First adult emergence on SAMMAZ 25 variety was recorded 3 days later. Complete adult emergence on SAMMAZ 16 and SAMMAZ 20 varieties were noted after 54 DAT. Duration of emergence on SAMMAZ 29 variety was shorter than the two other varieties. The number of daily emergence was also lower in SAMMAZ 25 when compared to SAMMAZ 16 and SAMMAZ 29 varieties. However, higher number of progenies was recorded on day 33, 36 and 39 on all the three varieties.

SAMMAZ 16 and SAMMAZ 29 varieties produced higher number of progenies on each assessment day in comparison to SAMMAZ 20. Progeny emergence started on day 27 on SAMMAZ 25 with a cumulative average of 152.9 which was the least (Figure 2). The highest number of adults emerged on SAMMAZ 29 (cumulative average of 230.0) followed by SAMMAZ 16 (221.4), and then SAMMAZ 20 (190.7). Efficacy of protectants is determined by mortality of adults and/or immatures which is confirmed by suppression of progeny production (Hertlein *et al*., 2011). Suppression of progeny production was higher in the treated grains across the 4 maize varieties in all exposure periods (3, 7 and 14 days). This could be due to higher mortality of parents in the treated grains. This agrees with previous studies that showed that, suppression of progeny emergence and infestation by maize weevils can be achieved by using DE (Lorini and Beckel, 2006; Wakil *et al.* 2006). Among the treated grains, SAMMAZ 29 had higher adult emergence. SAMMAZ 25 on the other hand exhibited some resistance as the F_1 progeny emergence period was shorter (52 days) and the numbers of adult weevils produced were fewest (152.9).

Table 1. Percentage mean mortality of *S. zeamais* on maize varieties

	Dose	SAMMAZ 16			SAMMAZ 20			SAMMAZ 25			SAMMAZ 29		
Treatment	rates (mg/kg)	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT
Untreated	0.0	1.7^{d}	1.7^{d}	6.7^{d}	1.7^{e}	5.0^{d}	5.0^{d}	2.2^{d}	7.9^{d}	8.6^{d}	1.7^{d}	5.0^{d}	8.3^{d}
RD	250	13.3^{c}	13.7^{c}	75.0^{c}	6.7^{d}	45.0^{c}	81.7^{bc}	26.2^{c}	51.6^{c}	77.4^{c}	21.7^{bc}	45.0^{c}	85.0^{bc}
	500	23.3^{b}	56.7^{b}	85.0^{b}	15.7^{c}	65.0^{b}	93.3^{ab}	23.6^{c}	69.9^{b}	96.7^{ab}	23.3^{bc}	65.0^{b}	95.0^{b}
	750	23.2^{b}	53.3^{b}	86.7^{b}	21.7^{b}	61.7^{b}	95.0^{ab}	40.3^{b}	69.3^{b}	98.8^{ab}	26.7^{b}	61.7^{b}	95.0^{b}
	1000	33.3^{a}	68.3^{a}	100.0^{a}	31.7^{a}	76.7^{a}	100.0^{a}	69.2^{a}	79.0^{a}	100.0^{a}	36.7^{a}	76.7^{a}	100.0^{a}
SE±		1.07	0.83	1.22	1.34	0.93	0.58	0.17	1.32	1.47	1.14	0.45	0.78
CV (%)		9.28	3.86	9.35	4.82	5.06	1.86	17.2	20.52	36.13	5.20	2.44	7.12
$HSD_{(0.05)}$		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.004	0.0147	0.0381	0.0001	0.0001	0.0001

Means followed by the same superscript(s) along the same column are not significantly different (P<0.05) from each other using Tukey-Kramer HSD test; ***RD*** *– Raw Diatomite;* ***DAT*** *– Days after treatment.*

*Effect of RD Treatments on Maize Grain Injury of Different Maize Varieties***:** The mean percent grain damage of the maize varieties caused by *S. zeamais* is presented in Table 2. The table shows that maize grain damage caused by *S. zeamais* at different dose rates were significantly (P<0.05) different. A significant (P<0.05) reduction in grain damage on each of the varieties was obtained in the treated grains. Across varieties, significantly (P<0.05) higher grain damages were observed on the untreated grains (Table 2). The highest percentage grain damage among the treated grains was observed on the lowest dose rates treated grain varieties; SAMMAZ 16 (54.3%), SAMMAZ 20 (51.8%), SAMMAZ 29 (55.1%) and the least among them was SAMMAZ 25 with 34.8% grain damage. Results on the assessment of treatments and varieties on weight loss due to *S. zeamais* infestation in both treated and untreated grains are also shown in Table 2. The trend followed a pattern similar to that of grain damage.

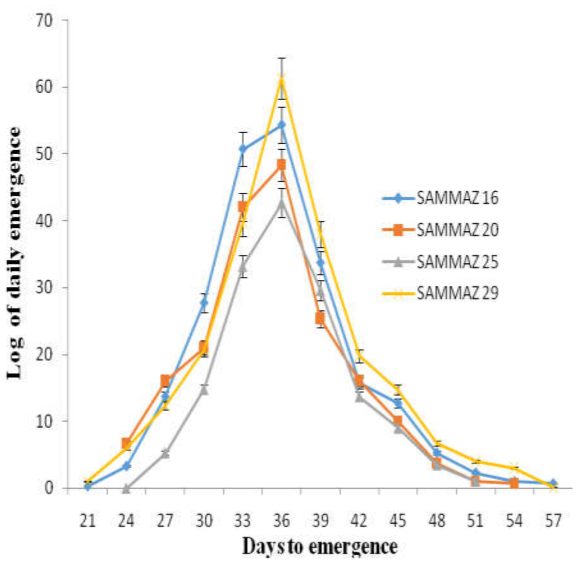

Fig 1. Dynamics of progeny emergence of *S. zeamais* from four maize varieties

Most of the treatments (particularly the higher dose rates – 500 – 1000 mg/kg), significantly (P<0.05) suppressed weight loss when compared to the untreated. Treatment with the lowest rate of RD (250 mg/kg) was largely ineffective in reducing weight loss caused by *S. zeamais*. There was lesser weight losses recorded on grains treated with higher dose rates of RD (750 and 1000 mg/kg). The highest weight loss on treated grains was observed on SAMMAZ 29 (42.0%), and the least was on SAMMAZ 25 (15.0%).

Studies by Wakil *et al*., 2006 and Vardeman *et al*., 2007 reported that commercial DE formulations provided complete control of *S. zeamais* at dose rate range of 500 to 1000 ppm. However, none of the dose rates used in the present study completely stopped grain damage.

Significant differences in levels of grain damage were however noted among the used dose rates on all the grain varieties. Damage was relatively lower on all the varieties treated with RD, while at lower dose rate, varieties sustained heavy damage with > 50% grain damaged at 250 mg/kg RD. The emergence of some progenies in all the treated grains explains the presence of grain damage in the treatments. According to Fields and Korunic (2002), effective DEs should have > 80% SiO_2, a pH below 8.5, and a tapped density < 300 g/L. The Nigerian RD used in this study has a lower level of SiO_2 (28.7%) content - the component responsible for insecticidal effect, higher pH value 9.5, and tapped density of 312.5. On the other hand, most commercial DE products contain other substances such as silica aerogel or baits, which either improve physical properties or enhance efficacy (Subramanyam and Roesli, 2000).

The aforementioned could be the reasons for lower performance of even the highest dose of RD used in the present study. Grain injury was significantly reduced on the maize varieties treated with each dose of RD after 3, 7, and 14 days of storage. Matti and Awaknavar (2009) observed no grain damage in sorghum treated with Protect-It (a commercial formulation of DE) at the dosage of 0.1 g. In all the varieties treated with RD, damage observed on grains may be attributed to the increase in population of weevils. Generally, this result suggests that the grain injury of the maize varieties was not only affected by each dose rate of RD but also due to inherent variations among the maize varieties used for the study.

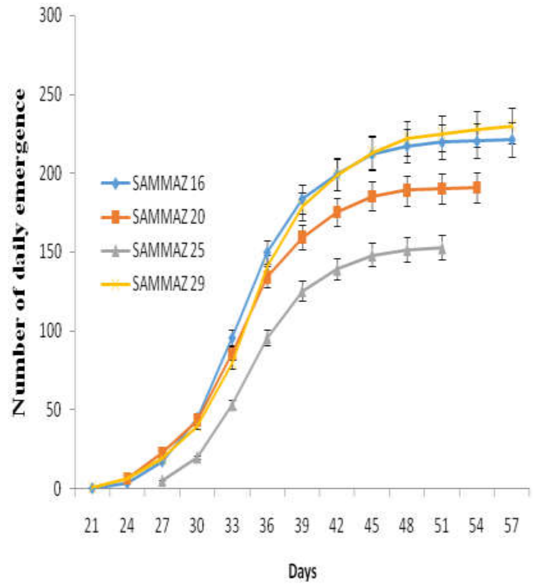

Fig 2. Cumulative emergence curves of *S. zeamais* from four maize varieties

Table 2. Mean percent grain damage and weight loss by activities of *S. zeamais* on different maize varieties treated with different dose rates of RD

Treatment	Dose rates (mg/kg)	SAMMAZ 16 GD	SAMMAZ 16 WL	SAMMAZ 20 GD	SAMMAZ 20 WL	SAMMAZ 25 GD	SAMMAZ 25 WL	SAMMAZ 29 GD	SAMMAZ 29 WL
Untreated	0.0	58.1^{a}	41.4^{a}	55.6^{a}	32.2^{a}	38.1^{a}	16.5^{a}	58.9^{a}	44.0^{a}
RD	250	54.3^{ab}	39.4^{a}	51.8^{ab}	29.9^{a}	34.8^{b}	15.0^{b}	55.1^{ab}	42.0^{a}
	500	51.0^{bc}	33.8^{b}	48.5^{bc}	23.2^{bc}	31.8^{bc}	11.1^{c}	51.8^{b}	36.7^{b}
	750	48.9^{cd}	30.9^{bc}	46.4^{cd}	19.2^{c}	29.8^{c}	9.1^{d}	49.6^{c}	33.8^{bc}
	1000	44.6^{de}	27.7^{cd}	42.2^{de}	16.1^{cd}	25.8^{d}	7.3^{e}	45.3^{d}	30.9^{cd}
SE±		0.83	1.07	1.34	1.22	1.42	0.65	0.58	0.93
CV %		3.86	9.28	4.82	9.35	7.33	11.56	1.86	5.06
$HSD_{(0.05)}$	0.0001	0.0001	0.0001	0.0001	0.0001	0.5815	0.0001	0.0001	0.0001

*Means followed by the same superscript along the column are not significantly different from each other using Tukey-Kramer (HSD) at (P<0.05); **RD** – Raw Diatomite; **GD** – Grain damage; **WL** – Weight loss.*

Conclusion: Findings from this study indicate that the Nigerian RD at higher dose rates was effective against *S. zeamais* and can provide substantial level of control of *S. zeamais* despite low SiO_2 content. Efficacy varied with dose, exposure period and grain type. To achieve higher mortality or prevention of grain damage, use of dose rates higher than 1000 mg/kg of RD is suggested. The information obtained from the present study will assist in devising management strategies against this legendary pest of maize as well as other cereals.

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