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Effect of X-ray irradiation on the F₁ generation of *Sitophilus zeamais* Motschulsky and the germination rate of maize grain

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Abstract

Background: The use of X-ray irradiation in the control of the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) infesting stored maize was tested at 60, 70 and 80 KeV as part of the international concerted efforts to save maize production and enhance food security. Investigations were done in the laboratory at mean temperature and relative humidity of 29.2 °C and 75.7%, respectively using three varieties of maize. The possibility that X-ray irradiation can affect the viability of maize grains when planted after weevil control process was also evaluated in the laboratory. Standard methods were used to achieve the specific objectives, and X-ray machine snap constituted the exposure time for each dose.

Results: The results revealed 10% mortality of adult weevils after 24 h and 40% mortality after a period of 7 days. The result indicates moderate effect on mortality. Progressive increase in mortality was recorded as dose increased from 60 to 80 KeV. X-ray irradiation at the doses tested did not significantly ($P > 0.05$) restrict *S. zeamais* emergence from maize grains and did not also significantly protect grains against damage and flour production. There was no difference in the pattern of daily emergence of new progenies and pattern of accumulated emergence. The relationship between X-ray irradiation doses and weight of emerging progenies require more specific analysis. Absence of significant difference between the viability of irradiated grains and un-irradiated grains strongly suggests that X-ray irradiation did not adversely affect seed viability, and this gives irradiation technique an opportunity of higher acceptability in agriculture.

Conclusions: We recommend > 80 KeV of X-ray irradiation or its incorporation into integrated pest management system in order to achieve effective post-harvest control of the pest.

Keywords: X-ray irradiation, Maize weevil, Viability of maize, Mortality

Background

Maize (*Zea mays* L.) grain plays crucial role in human nutrition and unequivocally, it is a source of raw materials for industries (Donald, Gabriel, & Emmanuel, 2008; Olotuah, 2013; Temitope, 2014). Maize grain is critical in the realisation of food security and therefore should

be protected against insect pest attack. The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is a major pest of maize grains in storage. It has the capacity to cause qualitative, economic and agronomic problems through its biological activities on maize (Compton & Sherington, 1999; Khairnar, Adsule, & Thakur, 1996; Thanda & Kevin, 2003). Different control strategies are available to tackle this insect pest in storage. They however differ in merits and limitations, and these differences should form important considerations

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for farmers who wish to reduce storage losses due to *S. zeamais* attack. The use of conventional synthetic insecticides and resistant varieties constitute the major weapons in the fight against infestation and damage by *S. zeamais*. Chemical insecticides cause health and ecological problems (Ashouri & Shayesteh, 2009; Fields, 2006; Udo, 2005), whereas resistance in maize is liable to breakdown due to overreliance (Nwosu & Adedire, 2019). It is therefore necessary to identify alternatives that will address these deficiencies.

Irradiation of seeds or food materials in order to cope with insect pest infestations of stored products is gaining popularity in different countries of the world. Irradiation is free from residues, and the technique is desirable to managers seeking to minimize insect pest infestations while the product is in storage (Espo, Eyidozehi, & Ravan, 2015; Hallman 2013; Salem, Fouda, Abas, Ali, & Gabarty, 2014). The technology extends the shelf life of different fruits and vegetables and maintains the quality of the product for a prolonged period of time. The technique is convenient, safe and attractive and does not significantly change the quality of the stored product (Espo et al., 2015; Salem et al., 2014). The specific objectives were to determine the (i) effect of X-ray irradiation on the mortality of *S. zeamais* adults, (ii) effect of X-ray irradiation on the number of *S. zeamais* emerged progenies, (iii) efficacy of X-ray irradiation technique in the protection of stored maize against damage and loss by *S. zeamais* and (iv) effect of X-ray irradiation on grain viability when the grains were planted in the laboratory.

Methods

Experimental site

The experiment was carried out in the Crop Protection Laboratory of the Department of Crop and Soil Science,

Faculty of Agriculture, University of Port Harcourt and at the Radiology Department, University of Port Harcourt Teaching Hospital, Rivers State, Nigeria. The average temperature and relative humidity of the sites were 29.2 °C and 75.7%, respectively.

Insect culture

A population of parental maize weevil pest from already-infested maize grains were collected from Choba Market, Port Harcourt and introduced in fresh maize samples in four different containers. The initial population of *S. zeamais* used to start the culture was authenticated by an expert. They were left for 8 days to feed, mate, and lay eggs in the fresh maize samples. On the 8th day of infestation, the parental weevils were sieved out of the plastic containers (height 25 cm; diameter 15 cm), dead or alive and the set-up was kept under the same experimental conditions (29.2 °C and 75.7%) until new insects emerged in about 60 days. Newly-emerged adults, 1 to 7 days old were used for the bioassay.

Irradiation of maize varieties at the radiology department

The X-ray machine used was a high-digital snap and record machine which measures radiation doses in Kilo electron volts (KeV). The machine was handled by an expert at the Radiology Department of University of Port Harcourt Teaching Hospital. Three-hundred (300) grams of each maize variety was separately irradiated (in a snap as exposure time) at 60, 70 and 80 KeV. The action was displayed by a computer connected to the X-ray machine. The irradiated samples were used immediately for the experiments without further storage. The maize varieties were yellow maize, flour maize and dent maize obtained from Jos, Nigeria. They were properly

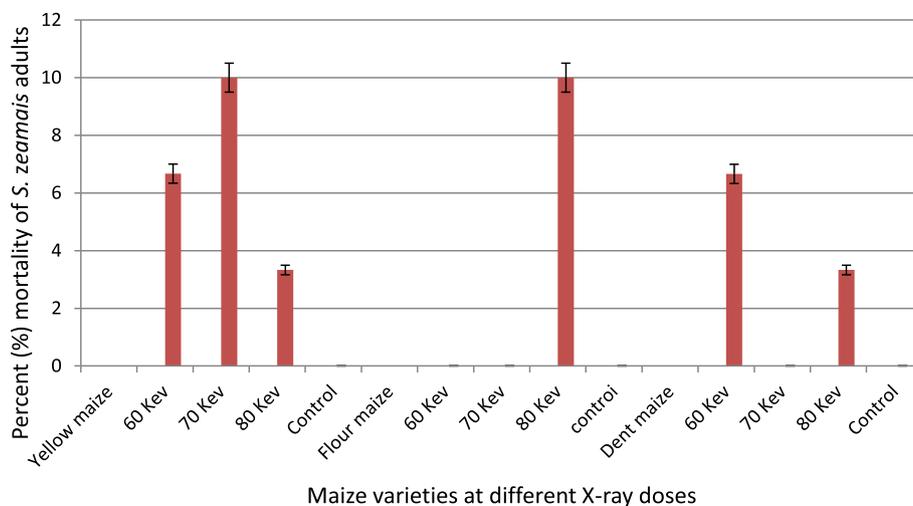


Fig. 1 Effect of X-ray exposure dose (KeV) on the mortality of *Sitophilus zeamais* Motschulsky adults after 24 h

Table 1 Regression coefficients on the yellow maize, flour maize and dent maize varieties for effect of X-ray irradiation on the mortality of *S. zeamais* after 24 h of exposure

Variety	Coefficients ^a		Unstandardized coefficients		Standardized coefficients	t	Sig.	Collinearity statistics	
			B	Std. error				Beta	Tolerance
	Model								
Yellow maize	1	(Constant)	.712	3.784		.188	.868		
		Kev	.082	.062	.681	1.317	.319	1.000	1.000
Flour maize	1	(Constant)	– 1.226	5.164		– .237	.834		
		Kev	.071	.085	.510	.839	.490	1.000	1.000
Dent maize	1	(Constant)	.580	3.489		.166	.883		
		Kev	.037	.057	.412	.639	.588	1.000	1.000

^a Dependent variable: mortality

cleaned to remove any unwanted material before irradiation.

Determination of the effects of X-ray irradiation on mortality, adult emergence, seed damage and flour production caused by *S. zeamais*

An amount of 20 g of each of the maize varieties separately irradiated/ exposed to 60, 70 and 80 KeV in a snap were put in a plastic container (11 cm diameter and 4.5 cm height) in three replicates and a control designated, laid on a laboratory bench in a randomized complete block design. Five pairs (5♀ + 5♂) of newly emerged (1 to 7 days old) *S. zeamais* adults (not irradiated) were introduced to each of the transparent plastic containers for feeding and oviposition. Male and female weevils were sexed morphologically with the aid of a stereoscope

according to Halstead (1963). *Sitophilus zeamais* adults with comparatively shorter, wider and rougher rostrum were identified as male, while those having longer, narrower, smoother and shinier rostrum were identified as female. Each container was covered with muslin cloth (held in place with cut container-lid) to allow for ventilation and to prevent exit of the weevils and entry of intruders. After 7 days of introduction, the number of dead adults was counted and recorded. Dead adults were those that did not respond to a probe with a pin. Percent mortality was calculated using the standard formula:

$$\frac{\text{Number of dead } S.zeamais \text{ adults} \times 100}{\text{Total number of } S.zeamais \text{ adults}}$$

All dead and live *S. zeamais* adults were sieved out immediately after mortality count leaving behind their

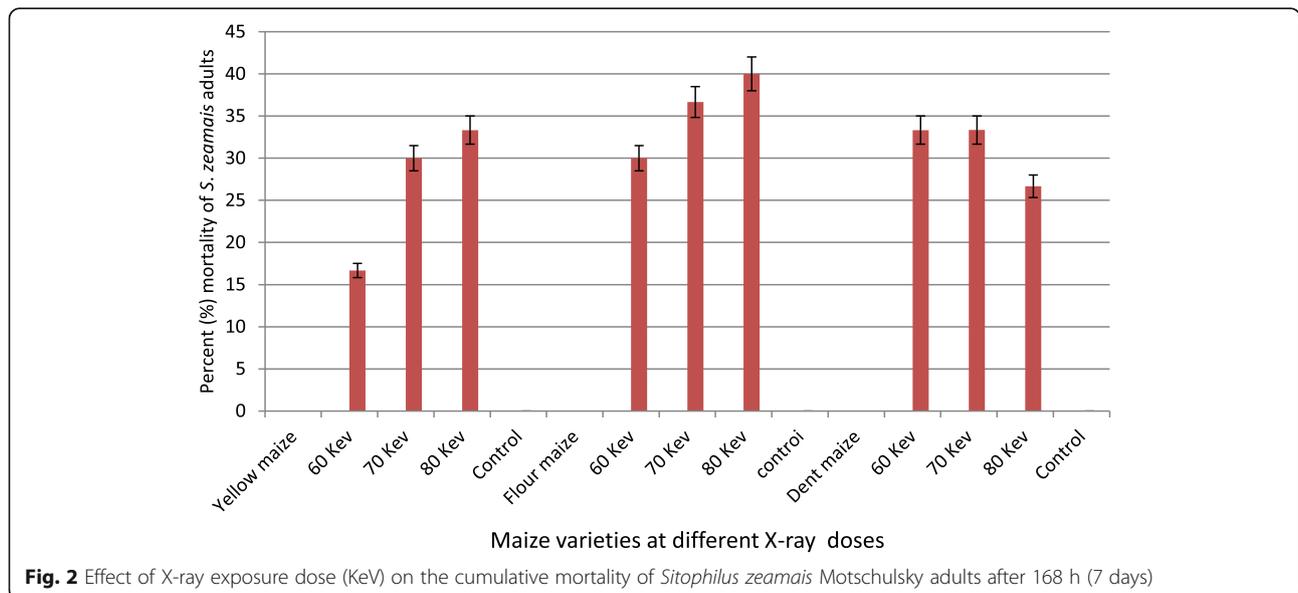


Fig. 2 Effect of X-ray exposure dose (KeV) on the cumulative mortality of *Sitophilus zeamais* Motschulsky adults after 168 h (7 days)

Table 2 Regression coefficients on the yellow maize, flour maize and dent maize varieties for effect of X-ray irradiation on the mortality of *S. zeamais* after 168 h (7 days) of exposure

Variety	Coefficients ^a		Unstandardized coefficients		Standardized coefficients	T	Sig.	Collinearity statistics	
			B	Std. error				Beta	Tolerance
	Model								
Yellow maize	1	(Constant)	2.453	14.248		.172	.879		
		Kev	.191	.233	.502	.820	.498	1.000	1.000
Flour maize	1	(Constant)	.842	4.102		.205	.856		
		Kev	.460	.067	.979	6.846	.021	1.000	1.000
Dent maize	1	(Constant)	.470	2.507		.187	.869		
		Kev	.499	.041	.993	12.148	.007	1.000	1.000

^a Dependent variable: mortality

eggs. The infested grains were maintained under the same laboratory conditions as the insect cultures. Thirty days after *S. zeamais* introduction, the number of emerged adult from grains were counted daily until emergence of F₁ progenies stopped. Association between the weight of emerged adults and X-ray intensity (KeV) was sought electronically using scatter plots. Damaged seeds were also recorded. Damage was characterized in terms of holes and tunnels and was expressed in percent. The weight of maize flour due to *S. zeamais* activity in the grain was directly weighed on an electronic weighing machine.

Determination of the effect of X-ray irradiation on seed viability

A total of 10 seeds per variety which were irradiated with X-ray separately at 60, 70 and 80 KeV, but not infested, were randomly chosen from the 20 g sample

and used for the agronomic experiment. The seeds were placed separately on a moistened Whatman filter paper (110 mm diameter) inside a transparent plastic container (height 4.5 cm; diameter 11 cm), and 10 ml of distilled water was added. The experiment was monitored daily to augment moisture level for a period of 7 days. On the 7th day, the number of germinated seeds was recorded and compared statistically with the control. There were three replications.

Statistical analysis

Mortality and progeny emergence data were subjected to regression analysis using the Statistical Package for the Social Sciences (SPSS) version 17.0. Data on percent seed damage and weight of flour caused by the insect were subjected to analysis of variance (ANOVA), and significantly different means were separated using least significant difference (LSD) at $\alpha = 0.05$.

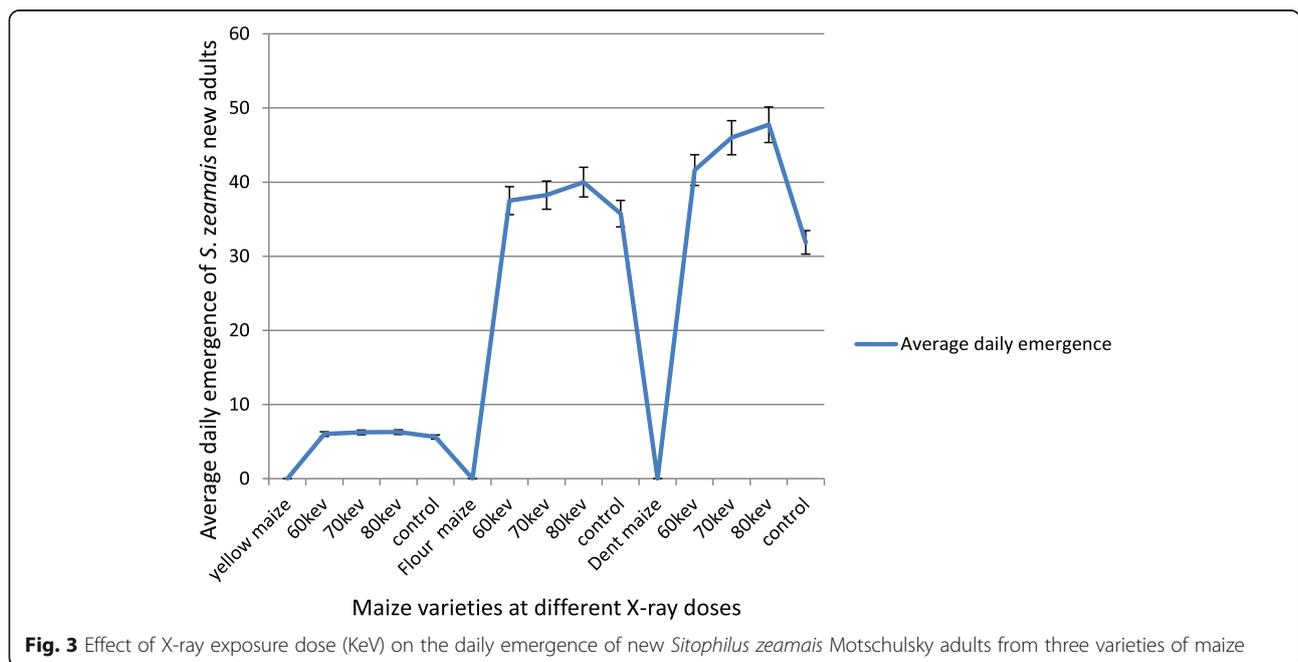


Fig. 3 Effect of X-ray exposure dose (KeV) on the daily emergence of new *Sitophilus zeamais* Motschulsky adults from three varieties of maize

Table 3 Regression coefficients on the yellow maize, flour maize and dent maize varieties for effect of X-ray irradiation on the daily emergence of new progenies of *S. zeamais* after exposure

Variety	Coefficients ^a		Unstandardized coefficients		Standardized coefficients	t	Sig.	Collinearity statistics	
	Model		B	Std. error				Beta	Tolerance
Yellow maize	1	(Constant)	245.346	34.052		7.205	.019		
		Kev	- 3.371	.558	- .974	- 6.042	.026	1.000	1.000
Flour maize	1	(Constant)	6.557	3.315		1.978	.187		
		Kev	.453	.054	.986	8.340	.014	1.000	1.000
Dent maize	1	(Constant)	.918	3.319		.277	.808		
		Kev	.627	.054	.993	11.533	.007	1.000	1.000

^a Dependent variable: daily emergence

Results

Effect of X-ray irradiation on the mortality of *S. zeamais* adults

Figure 1 presents the results of the effect of X-ray irradiation on the mortality of *S. zeamais* adults after 24 h (Yellow maize: $Y = 0.712 + 0.082X$ KeV; Flour maize: $Y = - 1.226 + 0.0071X$ KeV; Dent maize: $Y = 0.580 + 0.037X$ KeV). Table 1 presents the regression coefficients for observations on yellow maize, flour maize and dent maize varieties, respectively. In all the varieties, increase in X-ray dose increased mortality in 24 h. The range of X-ray exposure rates investigated killed maximum of 10% of the adult weevils after 24 h. In the flour maize variety, there was no adult mortality at 60 and 70 KeV X-ray exposure doses. Figure 2 shows the effect of X-ray dose (KeV) on the cumulative mortality of *S. zeamais* adults after 168 h (Yellow maize: $Y = 2.453 + 0.191X$ KeV; Flour maize: $Y = 0.842 + 0.460X$ KeV; Dent maize: $Y = 0.470 + 4.99X$ KeV).

Table 2 presents the regression coefficients for observations on yellow maize, flour maize and dent maize varieties, respectively. In all the varieties, increase in X-ray dose increased mortality after 168 h. After 168 h (i.e. 7 days) of exposure to different X-ray doses, at most 40% of the adult weevils were killed. In the yellow and flour varieties in particular, increase in X-ray exposure rates (from 60 to 80 KeV) led to a progressive increase in the mortality of the adult insects.

Effect of X-ray irradiation on *S. zeamais* adult emergence from grains

The effect of X-ray irradiation on daily emergence of new *S. zeamais* progeny is summarized in Fig. 3 (Yellow maize: $Y = 245.346 - 3.371X$ KeV; Flour maize: $Y = 6.557 + 0.453X$ KeV; Dent maize: $Y = 0.918 + 0.627X$ KeV). Table 3 presents the regression coefficients for observations on yellow maize, flour maize and dent maize

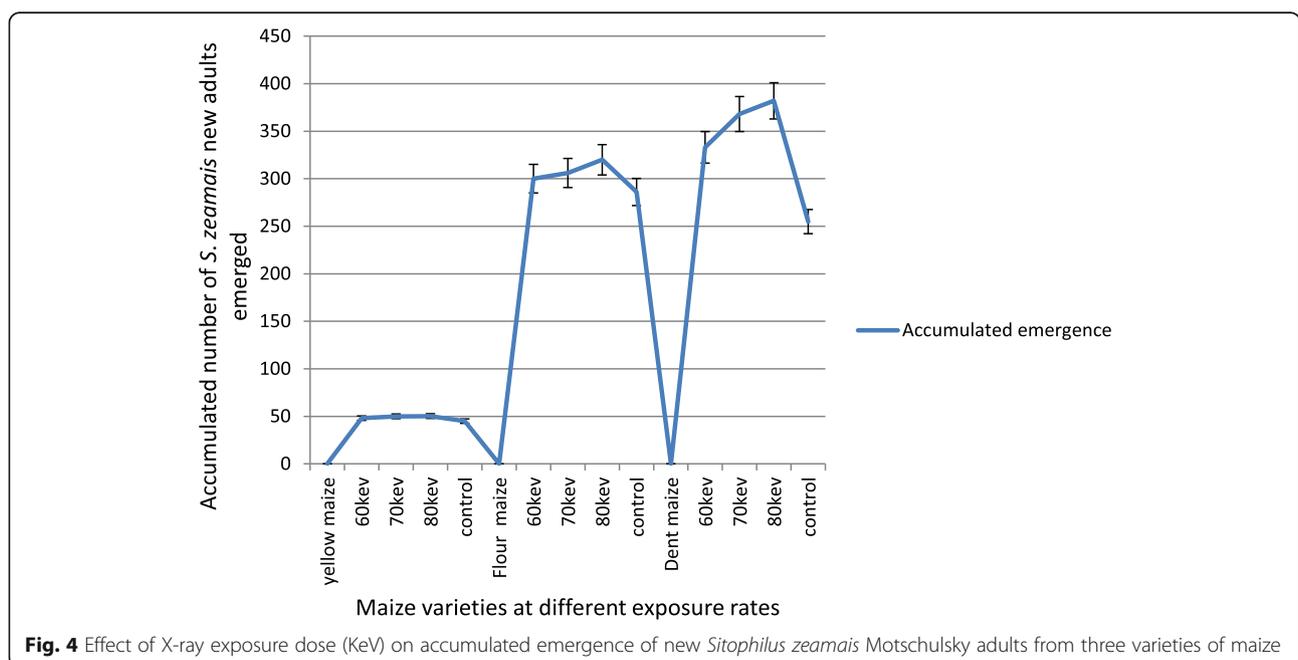


Fig. 4 Effect of X-ray exposure dose (KeV) on accumulated emergence of new *Sitophilus zeamais* Motschulsky adults from three varieties of maize

Table 4 Regression coefficients on the yellow maize, flour maize and dent maize varieties for effect of X-ray irradiation on the accumulated emergence of new progenies of *S. zeamais* after exposure

Variety	Coefficients ^a		Unstandardized coefficients		Standardized coefficients	t	Sig.	Collinearity statistics	
	Model		B	Std. error				Beta	Tolerance
			Yellow maize	1	(Constant)	32.262	1.459		
		Kev	.244	.024	.991	10.198	.009	1.000	1.000
Flour maize	1	(Constant)	52.508	26.518		1.980	.186		
		Kev	3.624	.434	.986	8.341	.014	1.000	1.000
Dent maize	1	(Constant)	282.275	14.443		19.545	.003		
		Kev	1.143	.237	.960	4.828	.040	1.000	1.000

^a Dependent variable: accumulated emergence

varieties, respectively. In the yellow maize variety, increase in X-ray dose reduced the daily emergence of new *S. zeamais* progeny. The result shows that the doses tested did not reduce the daily emergence of new progenies in flour maize and dent maize varieties. The daily emergence data appeared relatively uniform in the yellow maize variety and in all the three varieties tested; there was no significant difference in the rate of progeny emergence between the X-ray irradiated samples and control. Accumulated emergence (Fig. 4) was not adversely affected by X-ray irradiation too (Yellow maize: $Y = 32.262 + 0.244X$ KeV; Flour maize: $Y = 52.508 + 3.624X$ KeV; Dent maize: $Y = 282.275 + 1.143X$ KeV). Table 4 present the regression coefficients for observations on yellow maize, flour maize and dent maize varieties, respectively.

Association between X-ray exposure dose and weight of emerged adults

Figure 5 presents the results of association between X-ray exposure dose and weight of *S. zeamais* adults

at emergence. The result showed weak positive association between X-ray exposure dose and weight of the insect at emergence.

Effect of X-ray irradiation on seed protection and flour suppression against *S. zeamais*

Table 5 shows the results on the effect of X-ray irradiation on percent seed damage and flour production. X-ray irradiation caused reduction in seed damage and flour production. There was no significant difference ($P > 0.05$) between the percent seed damage and flour production across all doses tested and in comparison with untreated seeds (i.e. control at 0 KeV).

Effect of X-ray irradiation on the viability of maize grains

Table 6 shows the effect of X-ray irradiation on the germination rate of maize grains planted in the laboratory. At the lowest dose of 60 KeV, at least 60% of the seeds germinated, reaching its peak in the flour maize variety with 86.67% germination. At highest test dose of 80 KeV, at least 80% of the irradiated seeds germinated.

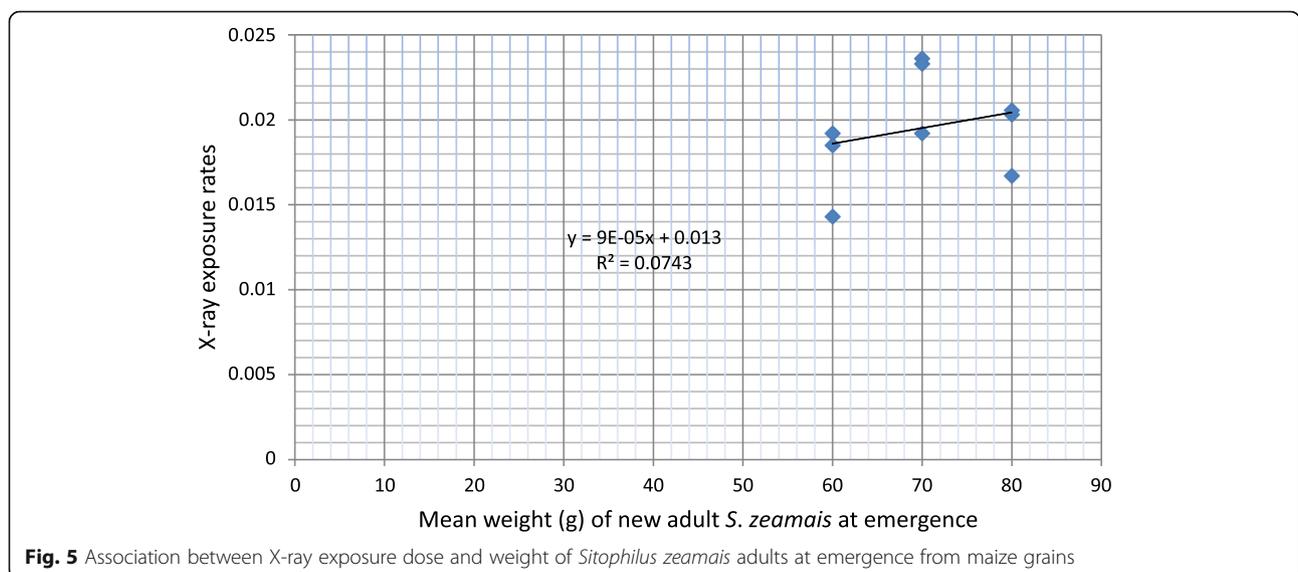


Fig. 5 Association between X-ray exposure dose and weight of *Sitophilus zeamais* adults at emergence from maize grains

Table 5 Effect of X-ray irradiation on percent seed damage and flour production caused by *S. zeamais* Motschulsky

X-ray exposure dose (KeV)	% Damage	Quantity of flour (g/ 20 g maize)
Yellow maize		
60.00	22.95 ± 1.50 ^a	1.855 ± 0.00 ^a
70.00	23.33 ± 0.05 ^a	1.924.00 ± 0.00 ^a
80.00	23.33 ± 1.80 ^a	2.00 ± 0.00 ^a
Control*	31.71 ± 4.70 ^a	2.14 ± 0.00 ^a
Flour maize		
60.00	17.67 ± 1.00 ^a	1.505 ± 0.001 ^a
70.00	18.19 ± 2.50 ^a	1.621 ± 0.00 ^a
80.00	17.24 ± 3.02 ^a	1.895 ± 0.00 ^a
Control*	24.29 ± 0.05 ^a	2.021 ± 0.00 ^a
Dent maize		
60.00	24.76 ± 4.00 ^a	2.232 ± 0.001 ^a
70.00	18.10 ± 5.00 ^a	1.892 ± 0.00 ^a
80.00	28.10 ± 1.10 ^a	2.026 ± 0.00 ^a
Control*	32.29 ± 2.50 ^a	2.026 ± 0.001 ^a

Data are means ± SEM of three replications

Mean values in a column with same letter are not significantly different by LSD ($\alpha = 0.05$)

* Not irradiated with X-ray

Discussion

This study has investigated the possibility of X-ray irradiation to restrict the survival and development of *S. zeamais*, while evaluating the effect of X-ray exposure on seed viability. This step is fundamental because any effective strategy against the maize weevil pest should

Table 6 Effect of X-ray irradiation on the viability of un-infested maize grains in Port Harcourt, Nigeria during 2019

X-ray exposure rate (KeV)	% Germination
Yellow maize	
60.00	80.00 ± 0.00 ^a
70.00	70.00 ± 0.00 ^a
80.00	73.33 ± 5.00 ^a
Control*	90.00 ± 0.00 ^a
Flour maize	
60.00	86.67 ± 12.500 ^a
70.00	70.00 ± 6.30 ^a
80.00	50.00 ± 13.00 ^b
Control*	73.33 ± 0.00 ^a
Dent maize	
60.00	60.00 ± 0.00 ^a
70.00	53.33 ± 0.00 ^a
80.00	60.00 ± 0.00 ^a
Control*	50.00 ± 0.00 ^a

Data are means ± SEM of three replications

Mean values in a column with same letter are not significantly different by LSD ($\alpha = 0.05$)

* Not irradiated with X-ray

give the seeds an opportunity to germinate after the weevil control process. With the analysis done, it is now possible to understand the patterns of response of irradiated grains to some insect activities. The analyses of results revealed moderate performance of X-ray irradiation in protecting stored maize against *S. zeamais* infestation. Moderate insect mortality caused by X-ray irradiation in the present study suggests good impact because irradiation is a phytosanitary measure. Irradiation does not kill insect pests right after application (Donahaye, 2000). In other words, high radiation tolerance of many storage insect pests (Follett et al., 2013) suggests that higher doses will be required to cause high mortality in storage insect pests. This agrees with the recommendation of this study. From all indications, it is evident that irradiation is not a direct-impact treatment, and so, 40% mortality at low doses (60–80 KeV) in 7 days is an indication of good performance because higher doses will be quicker in action and ultimately cause higher mortality. Progressive increase in mortality recorded as dosage increased from 60 to 80 KeV suggests that X-ray irradiation might control the insect at higher doses. Further studies are required to confirm specific lethal dose that will ensure maize security with high degree of confidence. This current assertion is supported by Follett and Hennessey (2007). Irradiation is a viable alternative to chemical insecticides for many crops because chemical insecticides are toxic to life (Espo et al., 2015; Follett et al., 2013; Hallman 2013). Irradiation is a quarantine treatment option and part of the major benefits is sterility or prevention of insects from further development,

feeding or damage (Follett et al., 2013). The same authors reported that with stored product pests, it is not clear what the target is, whether immediate mortality, sterility or arrested feeding. As part of contribution to knowledge, we evaluated the impact of X-ray irradiation on direct mortality of weevil and examined its effects on emergence of new progenies, maize grain damage and flour production. Espo et al. (2015); Hallman (2013) and Salem et al. (2014) have reported effective management of stored-product insect pests using ionizing radiation, gamma irradiation and gamma and ultraviolet irradiation, respectively to affect reproductive capacities. Interestingly, it has been reported that irradiation of seeds or food commodities to tackle insect pest infestations is safe and convenient (Espo et al., 2015). Already, many countries of the world have adopted its application (Espo et al., 2015; Hallman, 2013; Salem et al., 2014), probably because it is convenient and has potentials for integrated pest management.

Furthermore, the analysis of results suggests that at the doses tested, X-ray irradiation did not restrict *S. zeamais* emergence from maize grains, but it reduced grain damage and flour production from seeds, though not significantly. Therefore, evaluation of higher doses and or incorporation into integrated pest management system is strongly recommended, provided the safety limit is not exceeded. Due to day-specific fluctuations, Lopes, Araújo, Santos, Santos, and Sousa (2016) recommended the analysis of accumulated emergence as against daily emergence. In this study, we considered the two, and did not find any difference in pattern. The relationship between X-ray exposure dose and weight of emerging progenies require more specific analysis. It is notable that X-ray irradiation did not significantly reduce the viability of maize grains when planted in the laboratory. Absence of significant difference between the viability of the irradiated grains and the un-irradiated ones strongly suggests that X-ray irradiation did not adversely affect seed viability. This is another factor in favour of irradiation in insect pest control.

Conclusion

Greater than 80 Kev of X-ray irradiation or its incorporation into integrated pest management system is required to achieve effective post-harvest control of *S. zeamais* infesting maize grains.

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Authors' contributions

This research was carried out in collaboration with all authors. LCN conceived, designed the study and wrote the manuscript. SPB collected

data. KDI and UOA checked data analysis and reviewed the manuscript draft. The authors approved the final manuscript.

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Availability of data and materials

Data collected and analyzed during the current study are available from the corresponding author on sensible request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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