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Effect of X-ray irradiation and oven-drying on the bionomics of *Acanthoscelides obtectus* say (Coleoptera: Chrysomelidae) infesting common bean in storage: Can X-ray irradiation affect seed viability after pest control process

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Abstract
Effectiveness of X-ray irradiation at 60, 70 and 80 KeV solely and in combination with oven-drying against *Acanthoscelides obtectus* Say infesting common bean seeds in storage were investigated in the laboratory at temperature and relative humidity of 29.7 ºC and 76.5%, respectively. The results showed that the maximum number of eggs oviposited per day by *A. obtectus* is 2.20 and this is comparatively low for bruchinae females. Restricted fecundity in the female individuals of *A. obtectus* is strongly attributed to effect of X-ray irradiation. The results further revealed that X-ray irradiation caused 37% maximum mortality of *A. obtectus* adults after 24 hours. Mortality was highly significant (minimum of 86.67%) after 7 days of exposure. In combination with oven-drying, there were significant reductions in daily and accumulated emergence of new progenies of *A. obtectus*, seed damage and powder production. The study recommends integrated application of X-ray irradiation and oven-drying in order to cope with *A. obtectus* infestation in stored bean. X-ray irradiation did not adversely affect the viability of bean seeds after the pest control process.

Keywords: bruchinae, fecundity, seed damage, integrated application, stored bean

Introduction
Common bean (*Phaseolus vulgaris* L.) is an annual leguminous crop which is unquestionably important in food security (Nwosu *et al*., 2018; Rodriguez-Gonzalez *et al*., 2019) [19, 23]. The genetic diversity, taxonomy, ecology, biodiversity and distribution of *P. vulgaris* are fairly well-documented (Debouck, 1993; Freytag, 2002; Beebe *et al*., 2014) [4, 7, 2] and many varieties are available for human use. Apart from the direct consumption of seeds, many useful products are possible. Bean seeds serve as raw material for industries where it is processed into other useful products of high demand. It provides self-sufficiency services for individuals who engage in bean business and delicacies. Dry beans are produced as grain for consumption, whereas, green beans are bred for the edible green pods. The widespread consumption of dry beans has been reported especially in developing countries where they constitute the main sources of protein (Lopes *et al*., 2016) [13]. The common bean is a well-known pulse with variable size and colour and unequivocally, pulses are indispensable in ensuring food security, good health and poverty alleviation (Larochelle *et al*., 2016) [12]. Most literature says that bean is a preferred source of protein in developing countries because it is more affordable than meat, fish and dairy products. This is not entirely correct, because consumers of seeds of the common bean cherish it because of high level palatability and plant-based protein which has received more favourable appraisal than animal protein. It is notable that in sub-Saharan Africa, beans are cultivated mainly by resource poor farmers. Under these low input/ precarious conditions, seeds of common bean are inevitably susceptible to attack by insect pests and pathogens, and this is discomfited by environmental stress conditions (Miklas *et al*., 2006) [16].

In the field, *Phaseolus vulgaris* crop faces multiple stresses; however, the seeds must be stored after harvest for prolonged consumption, for planting during the next season and to secure more gains when price rises. In storage, bean seeds are prone to infestation and damage by by beetle insects such as *A. obtectus* Say (Coleoptera: Chrysomelidae: Bruchinae) (Ofuya, 2001; Gad *et al*., 2019) [21, 8]. Attack by *A. obtectus* culminates in considerable losses in the quality and quantity of the stored-product and the farmers are compelled to sell their production early in the season when prices are low. *A. obtectus* (dry bean bruchid) and *Zabrotes subfasciatus* (Mexican bean bruchid) are the most important insect pests of stored bean seed (Ebinu *et al*., 2016) [5] and therefore deserve serious attention. In the present study, *A. obtectus* infestation of stored bean seeds was investigated for possibilities of effective control using techniques neglected especially in this region of the world.

Many tactics have been employed to control *A. obtectus* infesting bean seeds in storage with different degrees of efficacy (Rodriguez-Gonzalez *et al*., 2019) [23]. Commercially, the insect pest is effectively controlled using conventional synthetic insecticides (Mulungu *et al*., 2007) [18]. Notably, the use of conventional synthetic insecticides is effective, but environmental implication, high cost and rising fears of direct poisoning when consumed have discouraged its application (Daglish *et al*., 1993) [3]. On a small scale level, there are a variety of measures employed in the on-farm protection of stored bean (Mulungu *et al*., 2007) [18]. On-farm chemical control measures do not often have the desired effect due to a lack of information and illiteracy among subsistence farmers (Baier and Webster, 1990) [1]. Traditional on-farm control techniques such as the application of ash, vegetable oil and dust or physical measures such as bean tumbling or heat treatment are beneficial (Daglish *et al*., 1993) [3].

In recent times, irradiation of stored products using gamma rays and X-rays against insect pest infestations have increased and have been approved and used in many countries of the world (Hallman, 2013) [10]. Considerable studies have been done on the effects of irradiation on agricultural products at doses recommended for insect pest control (≤5000 KeV), with only few negative effects reported (Gao *et al*., 2004; Li *et al*., 2007) [9, 14]. This study investigated the effectiveness of X-ray irradiation in the control of *A. obtectus* infesting bean seeds in storage with the justifications that bean serves as a cost-effective protein source and people especially in this part of the world consume it because apart from being nutritious, it is affordable and palatable. The recent alarming price of rice in Nigerian markets (about 200% increases in four years) must have increased bean consumption. Apart from that influence, the global publicity on the health benefits of plant-based proteins favours bean (Larochelle *et al*., 2016) [12]. It has been forecasted that increases in bean consumption will lead to increases in bean production (Nwosu *et al*., 2018) [19]. Increases in bean consumption and production also justify the current attention accorded to bean protection by way of irradiation. The insect pest, *A. obtectus* causes severe qualitative and quantitative losses in stored bean and requires more effective management strategies. The present study adds to existing information on the use of oven-drying and X-ray irradiation in the control of *A. obtectus* infestations of stored bean at doses already found safe for stored product protection (Hallman *et al*., 2013; Salem *et al*., 2014; Espo *et al*., 2015) [10, 24, 6]. Mohapatra *et al*. (2015) [17] reported that physical treatments (such as oven-drying) and irradiation of seeds are gaining popularity due to bad effects of chemical insecticides. Therefore, the specific objectives were to determine the (i) effect of X-ray irradiation on oviposition by *A. obtectus* females (ii) effect of X-ray irradiation on the mortality of *A. obtectus* adults (iii) integrated effect of X-ray irradiation and oven-drying on the emergence of new progenies from seeds of stored bean (iv) effectiveness of X-ray irradiation and oven-drying in combination, against seed damage and powder production by *A. obtectus* and (v) determine the effect of X-ray irradiation on seed viability of bean in the laboratory after pest control process.

Materials and Methods

Experimental sites

The experiment was carried out at the Crop Protection Laboratory of the Department of Crop and Soil Science and at the Radiology Department of University of Port Harcourt, Nigeria during 2019. The laboratory received adequate ventilation to support optimum performance of the *A. obtectus* insects. The average room temperature and relative humidity of the sites were 29.7 ºC and 76.5%, respectively.

The common bean varieties used for the study

The three common bean varieties used in this study were Kampala bean, Sweet beans and Red kidney bean. The bean varieties were obtained from the rural farmers in Jos, Plateau State, Nigeria and located approximately in the centre of Nigeria (9.9201'N, 8.8931'E). The bean varieties were cleaned before they were irradiated. The samples for bioassay were standardized after cleaning before they were used. Clean seeds were standardized by keeping it in a deep freezer at -20 ± 2 ºC for 7 days to disinfest them and then stored at 4ºC to avoid infestation (Sulehrie *et al*., 2003) [25]. Disinfested seeds were exposed to ambient laboratory conditions for 21 days in muslin-covered plastic containers for acclimatization after which they were packaged for immediate irradiation.

X-ray irradiation of experimental seeds

This exercise was carried out in the Radiology Department of University of Port Harcourt Teaching Hospital. A total of 300 g of each variety was separately irradiated with the X-ray machine and displayed in the computer connected to the machine. The irradiated samples were carefully handled and taken to the laboratory immediately for bioassay to prevent contamination of seeds. Each of the three varieties was exposed to 60, 70 and 80 Kilo electron volts (KeV). Irradiation was handled by an expert in the Radiology Department.

Insect culture

Acanthoscelides obtectus adults used to start the culture came from naturally infested bean seeds procured at Choba market, Port Harcourt, Rivers State, Nigeria. The *A. obtectus* adults were reared on a susceptible local bean variety. The bruchids were reared in a transparent plastic container (height 25 cm and diameter 15 cm) at laboratory mean temperature and relative humidity of 29.7 ºC and 76.5%, respectively. Fully developed individuals were introduced into non infested bean for the bruchids to lays eggs and then were sieved out after 7days. After completing their life cycle, the new adults emerged. Then, the newly-emerged bruchids of uniform age (≤ 5 days) were used for the experiment.

Effects of X-ray irradiation on oviposition and mortality of *Acanthoscelides obtectus* adults

Twenty grammes (20 g) of each variety of common bean (clean and un-infested) exposed separately to 60, 70 and 80 KeV of X-ray radiation were put in a plastic container (11 cm diameter and 4.5 cm height) in three replicates and an un-irradiated control was designated. The experimental design was randomized complete block design (i.e. primarily interested in X-ray radiation and oven-drying, but not variety). Five pairs (5♀ + 5♂) of newly-emerged (1 to 5 days old) *A. obtectus* adults (un-irradiated) were introduced to each of the plastic containers for feeding and egg-laying. The individuals have been sexed in accordance with Gad and Abied (2019) [8] since males have larger eyes and longer antennae than females. Each container was covered with muslin cloth (held in place with cut container-lid) to allow for aeration and prevent escape of the insect and entry of unwanted organisms. After 7 days from introduction, the number of eggs laid by *A. obtectus* on the seeds of each common bean variety was recorded. The number of dead adults was counted, recorded and discarded. Dead adults were those that did not respond to a probe with a pin.

Combined effects of X-ray irradiation and oven-drying on adult emergence, rate of seed damage and powder production by *Acanthoscelides obtectus*

The same procedure used to determine the effects of X-ray irradiation on oviposition and mortality of *A. obtectus* adults was employed, except that the varieties used were oven-dried at 40°C for 2 hours and the number and the weight of emerged adults were recorded daily from day 20 to day 50 after insect introduction. On the 50th day (terminal day of bioassay), the percent seed damage and quantity of powder produced due to insect activities in the seed were recorded. Damaged seeds were seeds that had holes and/ or eggs (Janzen, 1977) [11].

The effect of irradiation on seed viability

Ten seeds per variety, irradiated with X-ray separately at 60, 70 and 80 KeV, but un-infested, were randomly selected from the 20 g sample of common bean varieties and were used for the seed viability test. The irradiated seeds were placed separately on a Whatman filter paper (110 mm diameter) inside a plastic container (11 cm in diameter). Then, about 10 mL/day of distilled water was used to moisten the filter paper and throughout the whole experiment duration. The experiment was routinely maintained until 8 days and thereafter, the number of germinated seeds was recorded and compared statistically with the control (un-irradiated seeds). The seed viability experiment had three replications.

Statistical analysis

The biological parameters of oviposition, mortality and progeny emergence evaluated are dependent of the increasing doses (0, 60, 70 and 80 Kev) and therefore regression analysis was applied using the Statistical Package for the Social Sciences (SPSS) version 17.0. Bar charts were applied to reveal patterns showed by the bruchids in response to treatment by X-ray radiation in terms of oviposition, mortality and emergence of new progenies. Data on rate of seed damage and loss allowed by X-ray radiation were subjected to analysis of variance (ANOVA) upon significance of the F-test, treatment means were separated using least significant difference (LSD) at $\alpha = 0.05$.

Results

Effects of X-ray irradiation on oviposition and mortality of *Acanthoscelides obtectus* adults

The effects of different X-ray exposure doses (60, 70 and 80 KeV) on the mean number of eggs laid per day on three bean varieties by *A. obtectus* (Red kidney bean: Y = 1.045+0.005XKev; Sweet bean: Y = 1.120+0.005XKev; Kampala: Y = 1.780+0XKev) showed that in all varieties, increase in X-ray dose did not reduce the mean number of eggs laid per day. However, the results further showed that the maximum number of eggs laid per day per female is 2.2. Figure 1 shows the effect of X-ray exposure doses on the total number of eggs laid on three bean varieties by *A. obtectus* after 168 hours (7 days) (Red kidney bean: Y = 21.939+0.114XKev; Sweet bean: Y = 23.526+0.104XKev; Kampala: Y = 37.390-0.008XKev). In the result, no consistent pattern was observed in terms of relationship between increased X-ray exposures with oviposition after 7 days. In the regression trend, there was evidence in Kampala bean variety that increases in X-ray exposure dose reduced the total number of eggs laid after a period of 7 days.

The effects of different X-ray doses on the mortality of *A. obtectus* adults after 24 hours (Kampala bean: Y = -0.043+0.348XKev; Sweet bean: Y = 1.764+0.284XKev; Red kidney bean: Y = 2.000+0.200XKev) are summarized in Figure 2. No consistent pattern was observed in terms of relationship between increased X-ray exposures with mortality. The range of X-ray exposure doses investigated in this study killed at most 37% of *A. obtectus* adults after 24 hours. Three varieties were considered, to evaluate consistency among materials of different genetic strength. The effects of different X-ray doses on the cumulative mortality of *A. obtectus* adults after 168 hours (7 days) (Kampala bean: Y = 0.129+0.045XKev; Sweet bean: Y = 1.033+0.280XKev; Red kidney bean: Y = 0.323+0.054XKev) are shown in Figure 3. After a longer period of 7 days, the mortality caused by the exposure doses increased significantly (P = 0.26, 0.22 and 0.40, respectively for Kampala bean, Sweet bean and Kidney bean). The range of X-ray exposure doses investigated in this study killed at least 86.67% of the adults after 168 hours (7 days).

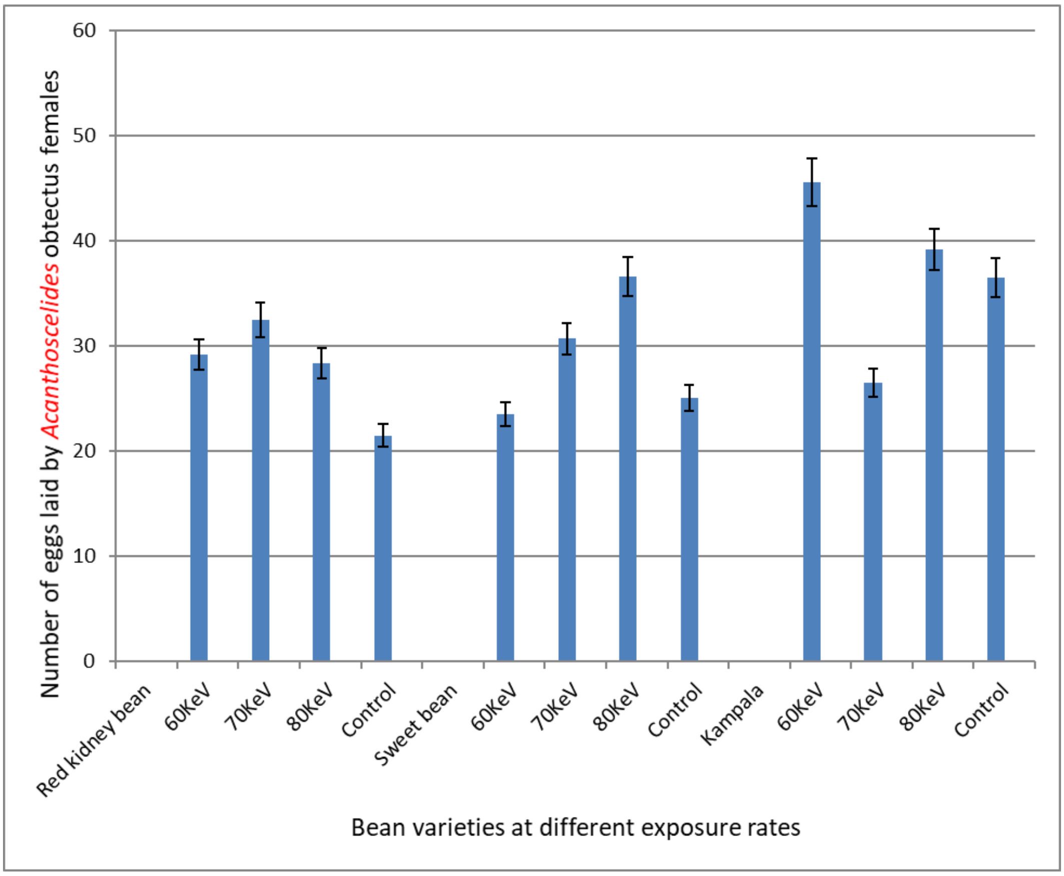

Fig 1: Effect of different X-ray exposure doses (KeV) on the total number of eggs laid on three bean varieties by *Acanthoscelides obtectus* female adults after 168 hours (7 days)

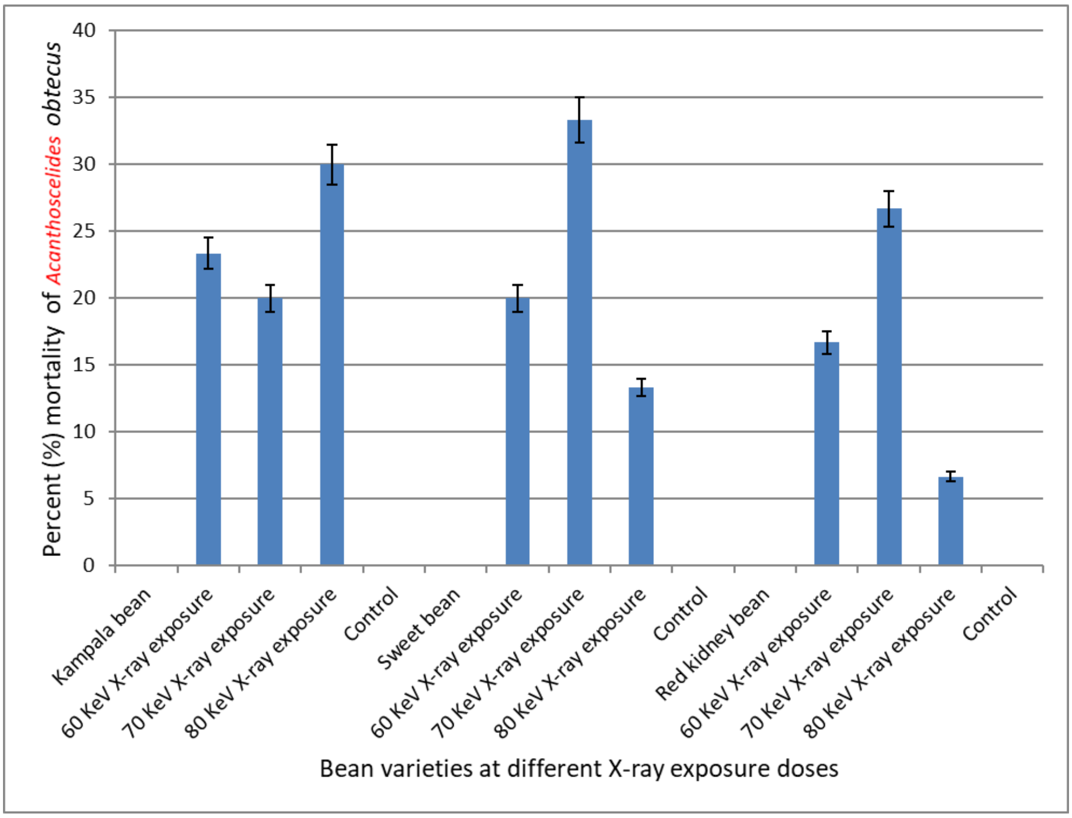

Fig 2: Effect of different X-ray exposure doses (KeV) on the mortality of *Acanthoscelides obtectus* adults after 24 hours.

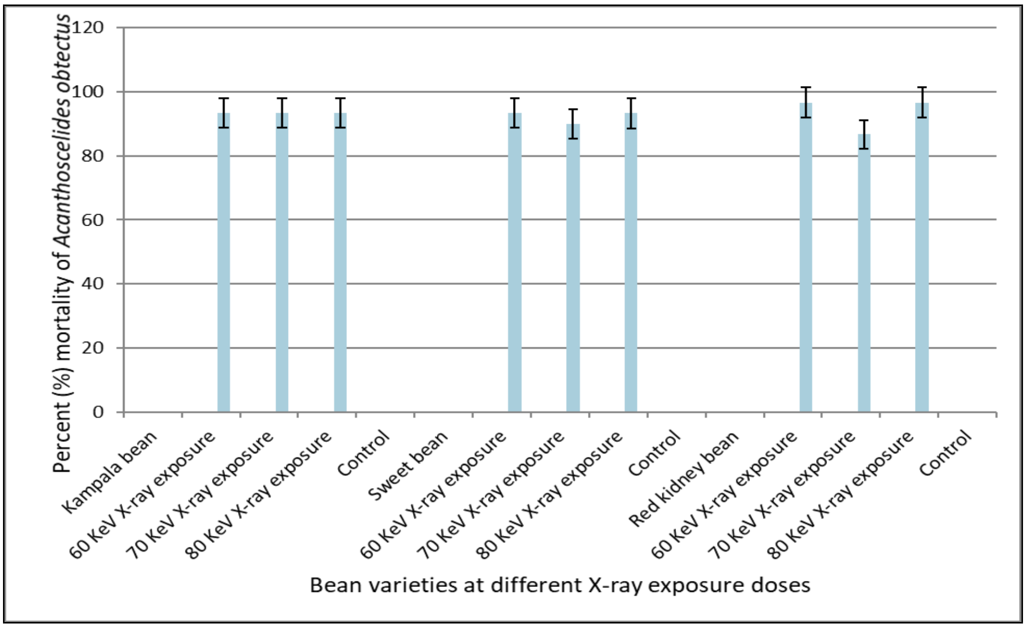

Fig 3: Effect of different X-ray exposure doses (KeV) on the cumulative mortality of *Acanthoscelides obtectus* adults after 168 hours (7 days).

Combined effects of X-ray irradiation and oven-drying on adult emergence, rate of seed damage and powder production by *Acanthoscelides obtectus*

The combined effects of the different X-ray exposure doses and oven-drying on the daily emergence of new progenies of *A. obtectus* (Red kidney bean: no emergence; Sweet bean: Y = 5.839-0.009XKev; Kampala: Y = 2.149+0.11XKev) show that when irradiation was supplemented with oven-drying of seeds, combined treatment effect reduced significantly the daily emergence of new progenies from seeds of Sweet bean. There was no daily emergence of new progenies from seeds of irradiated and un-irradiated red kidney bean. In the pattern, no daily pyramidal congestion was noticed. Figure 4 shows the combined effects of the different X-ray doses and oven-drying treatment on the accumulated emergence of new progenies of *A. obtectus* (Red kidney bean: no emergence; Sweet bean: Y = 87.581-0.130XKev; Kampala: Y = 32.230+0.164XKev). When irradiation was supplemented with oven-drying of seeds, combined treatment effect reduced significantly accumulated emergence of new offspring from seeds of Kampala bean variety. There was no accumulated emergence of new progenies from seeds of irradiated and un-irradiated red kidney bean.

Table 1 shows the result of combined ability of X-ray irradiation and oven-drying in the protection of seeds of common bean against damage and powder production by *A. obtectus*. The result shows that increase in the dose of X-ray (60 – 80 KeV) increased protection (i.e. reduced seed damage) and reduced powder protection caused to the seeds by the insect pest. Red kidney variety was not damaged by the insect pest and no powder was produced. The result showed significant protection ($P<0.05$) by combined effect of X-ray irradiation and oven-drying.

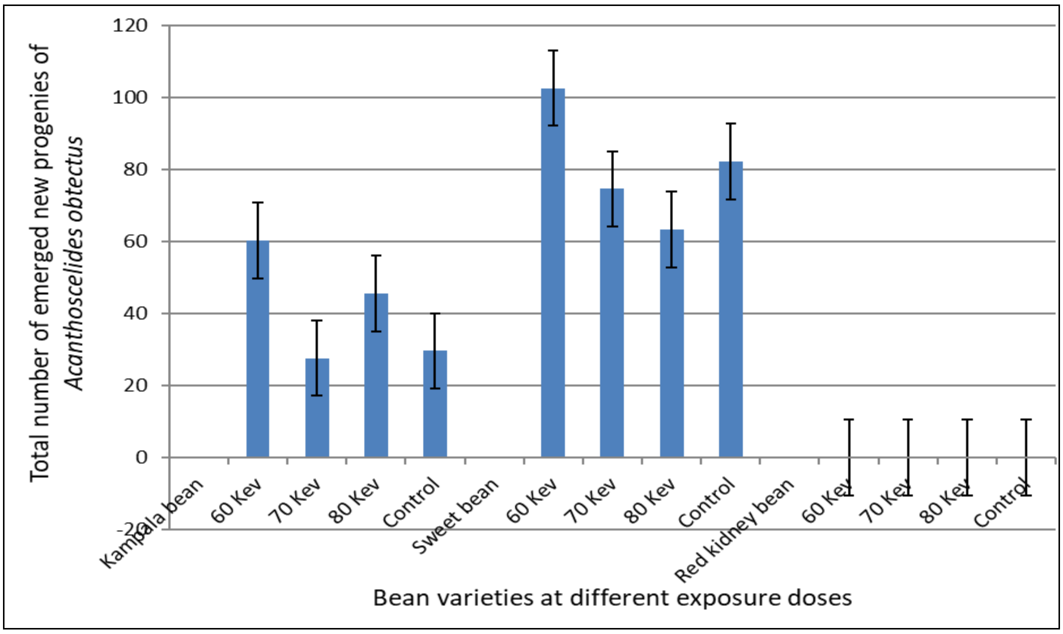

Fig 4: Combined effects of different X-ray exposure doses (KeV) and oven-drying on the accumulated emergence of new progenies of *Acanthoscelides obtectus*.

Table 1: Combined effects of X-ray irradiation with oven-drying on percent seed damage and powder production

X-ray exposure dose (KeV)	% damage		
	Red kidney bean	Sweet bean	Kampala bean
Control*	0.000±0.000[a]	57.000±0.000[a]	64.910±7.500[a]
60.000	0.000±0.000[a]	21.000±1.400[b]	26.920±1.000[b]
70.000	0.000±0.000[a]	18.700±0.500[bc]	6.840±5.200[c]
80.000	0.000±0.000[a]	10.160±0.010[c]	6.690±0.500[c]
	Quantity of powder (g)		
	Red kidney bean	Sweet bean	Kampala bean
Control*	0.000±0.000[a]	0.140±0.000[a]	0.200±0.001[a]
60.000	0.000±0.000[a]	0.004±0.001[b]	0.006±0.001[b]
70.000	0.000±0.000[a]	0.003±0.000[b]	0.002±0.000[c]
80.000	0.000±0.000[a]	0.001±0.000[c]	0.002±0.000[c]

*Not irradiated with X-ray (0 Kev)
Data are means ± SEM of three replications
Mean values in a column with same letter are not significantly different by LSD (α = 0.05).

The effect of irradiation on seed viability

Table 2 summarizes the results on the effect of X-ray irradiation on the viability of un-infested seeds of common bean. The red kidney bean showed 100% seed viability after exposure to X-rays of different doses. There was no significant difference (*P*<0.05) between the performance of the exposed seeds and the unexposed seeds (control) of the variety. Similar results were obtained for sweet bean variety. However, the kampala variety showed low seed viability and the observation on control experiment showed that the low viability was not due to impact of radiation.

Table 2: Influence of X-ray irradiation on the viability of un-infested seeds of common bean in Port Harcourt, Nigeria during 2019.

X-ray exposure dose (KeV)	% germination		
	Red kidney bean	Sweet bean	Kampala bean
Control*	100.000±0.000[a]	100.000±0.000[a]	53.330±0.000[a]
60.000	100.000±0.000[a]	76.670±12.500[a]	46.670±0.000[a]
70.000	100.000±0.000[a]	86.870±6.300[a]	63.330±0.000[a]
80.000	100.000±0.000[a]	76.670±13.000[a]	56.660±0.000[a]

*Not irradiated with X-ray (0 Kev)
Data are means ± SEM of three replications
Mean values in a column with same letter are not significantly different by LSD (α = 0.05).

Discussion

The use of conventional synthetic insecticides and its consequences on the environment has compelled search for alternative non-toxic insect pest control tactics. Irradiation (using Gamma or X-ray radiation) has become a recognized technique in controlling storage insects because of its residue-free benefits over chemical insecticides (Hallman *et al*., 2013; Salem *et al*., 2014; Espo *et al*., 2015) [10, 24, 6]. Irradiation technology has gained acceptability in food protection as alternative because it can extend the shelf life of the product, and maintain quality over a longer period of time (Mastrangelo *et al*., 2010) [15]. It has been recorded that irradiation does not significantly change the quality of the food material or stored seeds. Ionizing radiations such as Gamma rays and X-rays are used for the disinfestations of bulk grains under storage conditions (Hallman *et al*., 2013; Espo *et al*., 2015) [10, 6]. Current food production and processing industry plans to implement food irradiation primarily, involving electron beams and X-ray radiators. The radiation sources used for irradiation of stored-products against insect pests do not make food radioactive and this is a superior reason in favour of X-ray irradiation (Mastrangelo *et al*., 2010) [15].

The analysis of results showed that the maximum number of eggs oviposited per day by *A. obtectus* females is 2.20 eggs and this is comparatively low for bruchinae females. It has been reported that bruchinae females have the capacity to lay many eggs within 24 hours (Onekutu *et al*., 2015) [22]. In this present study, no such high fecundity was observed and this is strongly attributed to effect of X-ray irradiation. Mastrangelo *et al*. (2010) [15], Hallman (2013) [10], Salem *et al*. (2014) [24] and Espo *et al*. (2015) [6] reported effective control of stored-product insect pests using radiation. In this study, X-ray irradiation of seeds on its own did not considerably reduce the rate of egg-lying by *A. obtectus* females. In the analysis, no consistent pattern occurred in terms of relationship between increased X-ray exposure dosage with oviposition by the female insects after 7 days. Non-uniformity of absorbed dose due to possible permeability differences of varietal seed coat could be responsible for the inconsistency observed.

The analysis of results further revealed that doses of X-ray investigated (on its own) did not significantly kill *A. obtectus* adults; only a maximum of 37% died after 24 hours. However, this level of mortality within 24 hours of exposure is encouraging. This is because most direct-impact insecticides against stored products rarely kill more than 37% within 24 hours and this has been confirmed by Nwosu and Adedire (2019) [20]. After 168 hours (7 days), the range of X-ray exposure doses investigated in this study killed at least 86.67% of the adults and this is considerable. The impressive result reported here is strongly attributed to effect of X-ray radiation and time after exposure. Espo *et al*. (2015) [6] recognized the use of gamma and ultraviolet irradiation as effective and environment-friendly in pest control.

Results showed good concerted impact of X-ray and oven-drying on the daily emergence of new progenies of *A. obtectus*. When irradiation was supplemented with oven-drying of seeds, combined treatment effect reduced considerably both daily and accumulated emergence of new progenies from seeds of sweet bean. As revealed, new progenies did not emerge from seeds of red kidney bean. The study recommends integrated application of X-ray irradiation and oven-drying in order to help to cope with *A. obtectus* infestation in stored bean. In the study, the pattern of daily emerged new progenies of *A. obtectus* under combined effects of X-ray exposure doses and oven-drying unequivocally suggest that the emerging adult insects did not experience day-specific upsurge. Therefore, the recommendation to integrate application of X-ray irradiation and oven-drying in order to help to cope with *A. obtectus* infestation in stored bean should be taken seriously.

The analysis of results shows that the progressive increase in the dose of X-ray exposure from 60 – 80 KeV increased seed protection (i.e. reduced seed damage) and reduced powder production by the insect. The result revealed good protection of bean seeds by the combined effect of X-ray irradiation and oven-drying treatment. Therefore, the use of effect of X-ray irradiation and oven-drying should be popularized in the management of infestation and damage by *A. obtectus*.

It is interesting to note that X-ray irradiation did not affect adversely the viability of seeds of common bean. In fact, red kidney bean showed 100% seed viability irrespective of exposure to X-ray of different doses. The general absence of significant difference between the viability performance of the irradiated seeds and the un-irradiated seeds (control) is a clear indication that X-ray irradiation did not impact negatively on seed viability. This agronomical information is useful to farmers seeking to irradiate seeds for longer storage and thereafter, plant during the next crop season. Observation on control experiment revealed that the low seed viability recorded for kampala bean is attributable to effect of genetics. This is because even the un-irradiated seeds (i.e. the control experiment) did not show high germination potential too. In conclusion, it was possible to understand the bionomic patterns of *A. obtectus* as influenced by X-ray irradiation and integrated effect of X-ray irradiation and oven-drying. In the storage industry, the use of irradiation to minimize infestation and damage caused by insect pests has been said to be effective, convenient and safe. The recommendation to combine irradiation and oven-drying in other to have very good control of the insect pest should be taken seriously.

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