

STERILIZATION OF GRAINS USING IONIZING RADIATION: THE CASE IN GHANA

Richard Enu

AngloGold Ashanti [Obuasi Mine] Business Development Department
[Farms Unit]

Patrick Enu

Methodist University College Ghana

Abstract

Grains are important dietary components, providing many nutrients including vitamins, minerals, protein, and complex carbohydrates. The objective of the study therefore was to determine the minimum dose of Gray that would be required to kill insects on or inside the grains without affecting the viability for experimental work and there by render the grains free of infestation for laboratory experiments. In this investigation, maize and cowpea seeds were infested with *Sitophilus zeamais* and *Callosobruchus maculatus* respectively for 52 days each. The cultures were further divided into fifty grams (50g) each and put into 42 plastic containers. The seeds were then irradiated in a Co-60 gamma cell with doses 40Gy [Gray], 80Gy, 150Gy, 200Gy, 300Gy and 500Gy. The irradiated seeds were then examined for the number of insects alive in each case and the effect of the gamma irradiation on viability of the grain seeds. It was concluded that both *Sitophilus zeamais* and *Callosobruchus maculatus* were susceptible to gamma doses between 200Gy-500Gy. As much as 100% mortality was recorded for both insect at some doses. Germination tests subsequently carried out showed that gamma irradiation had no effect on seed viability.

Keywords: Sterilization, Grains, and Ionizing Radiation

Introduction

Maize and cowpea are important food grains and are very important dietary components, providing many nutrients including vitamins, minerals, protein, and complex carbohydrates. In the world, rice is the leading grain followed by wheat and maize but in sub-Saharan Africa, maize, rice and wheat are the major ranked staple foods for about 200 million people today. These products are stored as dry seeds and form an enormous reserve of food (FAO, 2001).

A national survey conducted in 1998 by M.O.F.A revealed that about 60% of grains cultivated in Ghana, are maize and the remaining percentage is made up of rice, millet, cowpea and sorghum. However, vast quantities of stored grains and legumes are lost annually as a result of insects attack. It is estimated that for large areas of the world, as much as 30% of the harvested foods are destroyed by insects (Hall, 1970).

In most of the developing countries, like Ghana, Nigeria, Togo and other West African countries which lie in the tropics, where the favourable climatic conditions allow, insects and other pests breed and spread rapidly. Losses of stored seeds are caused by insects, such as the larger grain borer, *Prostephanus truncatus*, maize weevil, *Sitophilus zeamais*, rice weevil, *Sitophilus oryzae* and the cowpea weevil, *Callosobruchus maculatus*. Consequently, storage pests, especially insects, compete with man for nutrients and cause quality deterioration which makes stored grains harmful to consume (Wilbur and Mills, 1985).

In addition, fungal growth is promoted under this condition, leading to the growth of moulds and the production of mycotoxins which contaminate the grains. A mould causes loss in weight, nutrients and deterioration in colour and flavour. Also mycotoxins contain poisonous chemical compounds produced mainly by two fungal genera: *Aspergillus* produces aflatoxins which are carcinogenic and *Fusarium* produces fumonisins and poses a health threat to humans (Wilbur and Mills, 1985).

Acceptable insect disinfestations usually include chemical fumigation, heat and cold treatment, or combination of these treatments. Chemical fumigants widely used include methyl bromine and phosphine but the former is considered to pose a health risk and is being phased out in many countries. Also the continuous and indiscriminate use of chemicals has resulted in the development of resistance strains of pests that are not killed. Ionizing irradiation has therefore been proposed as a good alternative to methyl bromide and other fumigants for pest control (Ignatowicz, 2004).

Ionizing radiation is a physical technique of food sterilization that has the potential to protect such grains from insect's infestation and microbial contamination. Ionizing irradiation include the use of ultraviolet rays, x-rays, and gamma rays.

Due to the need for better conservation and control of insects with modern, advanced, high-efficiency and low cost technology with little side effects, gamma radiation has become the most viable solution. This process consists of disinfecting grains with a determined dose of radiation, which inhibits reproduction, and even causes the death of the infesting insects.

Recent work shows that ionizing radiation is a promising new tool for insect control and can supplement the existing chemical control measures. Not only does radiation offer a method for complete control of infestation, it

also ensures partial protection against re-infestation through reproductive sterilization (I.A.E.A, 2004). The increasing concern about hazards to health through residual toxicity in chemical methods of control is an additional reason for increasing interest in radiation disinfestations of grain. Ionizing radiation is best alternative because;

- it has less effect on the quality of grains and has the ability of killing insects.
- it can cause death at all stages: egg, larva, pupa and adult.
- it can penetrate 20-50cm into a solid or whole grain.
- it penetrates the grain completely; therefore it can be very effective in killing eggs inside the grain.
- it produces no significant difference in terms of smell, taste and appearance

Ionizing irradiation kills insects by producing ions or free radicals (charged molecules that are very reactive). There have been several researches on the sterilization of grains by ionizing radiation. A dose of 500Gy caused 100% mortality of *Callosobuchus chinensis* (L) (Bhuiya *et al*, 1991). Also, further studies showed that 200Gy killed about 99.9% of adult *Sitophilus zeamais* and *Sitophilus oryzae* within 21days (Bhuiya *et al*, 1991). However, most of these studies focused on grain preservation and storage with less emphasis on the viability of the grains after the irradiation process.

Therefore, the objective of the study is to determine the minimum dose of Gray that would be required to kill insects on or inside the grains without affecting the viability for experimental work and there by render the grains free of infestation for laboratory experiments.

THEORETICAL FRAMEWORK AND REVIEW OF LITERATURE REVIEW GRAIN

Grains are generally classified as the seeds of cereal and legume plants. Cereals are characterized by their smallness, hardness and low water content. Most of them belong to the family of grasses (*Gramineae* or *Poaceae*). Grains have been the staple human diet from prehistoric times because of their wide cultivation, good keeping qualities, blend flavour and great variety, each of the cereals has characteristic properties and uses. The cultivation of grains for human consumption was probably developed around 10,000 B.C. [Before Christ]. It signified the commencement of the era of stable civilization from the primitive unsettled nomadic life. Ground cereal converted into bread for meal revolved soon thereafter. Grains have been modified and improved by centuries of cultivation and selective breeding. Grains consist of four essential parts, namely;

- the husk, hull, the outer covering loosely attached to the grain.
- the bran or the outer coat of the grain itself.

- the germ or embryo the endosperm which contains nutrients comprising a considerable volume of starch, a small amount of protein and a little fat.

There are numerous varieties of grains of which the most important are rice, wheat, maize, millets, oats and barley.

FOOD VALUE CEREAL GRAINS

The whole grains of all cereals have a similar chemical composition and nutritive value. They are classified as carbohydrate rich foods, for their average carbohydrate content is 70% per 100 gm. They provide energy and also some protein which is usually of good quality. The protein content of grains varies from 11.8 per cent for wheat to 8.5 per cent for rice per 100 gm. Whole cereals are good sources of calcium and iron but they are totally devoid of ascorbic acid and practically devoid of vitamin A activity. Yellow maize is the only cereal containing appreciable amounts of carotene. Whole grain cereals also contain significant amounts of B group of vitamins. For a balanced diet, cereals should be supplemented by other proteins, minerals and vitamin A and C found in nuts, seeds, milk, fruits and fresh green vegetables.

IMPORTANCE OF CEREAL GRAINS

- **Health benefits**

Grains are rich in fiber and this helps to reduce the risk of coronary heart disease.

- Grains also, reduce constipation when consumed.

- **Nutrients**

- Vitamins B (thiamin, riboflavin, niacin, and folic acid) play a key role in metabolism – they help the body to release energy from protein, fat, carbohydrates and are very essential for the functioning of a healthy nervous system.

- Iron contained in grains is used to carry oxygen in the blood.

Grains serve as sources of minerals, such as magnesium, iron and selenium. Magnesium is a mineral used in building bones and releasing energy from muscles.

MAIZE (ZEA MAYS L.)

Origin and Distribution of Maize

Maize is agreed to have originated from Central America over 61000 years ago, primarily Mexico and the Caribbean. Later, maize spread first through North and South America and after the discovery of America by

Columbus further spread to Europe and other areas in the world where it is now grown.

In Africa, maize is thought to have originated from Nigeria and was present before Columbus's voyages. It was taken to Europe in 1493, and later introduced back into Africa and spread through the continent by different routes. After 1900, different types of maize were brought to Africa by USA and from South America through South Africa. The predominant maize types adapted to the shorter growing season (Raemaekers, 2001).

Description

Maize, *Zea mays L.* belongs to the family *Poaceae*, and is an annual monocotyledonous plant. Maize has been classified to belong to eight genera. Three of these are found in America and the remaining five are Asiatic in distribution (Raemaekers, 2001).

Production of Maize

Maize is one of the most important grains after wheat and rice in the world. Maize is grown in more diverse areas of the world than any other major crop. About 60% of world output is produced by developed countries. The U.S.A alone grows over 40% of the world's total production on about 21% of the world's total area cropped. Hungary and Romania are the second largest producers in Eastern Europe.

In Africa, the total annual production is about 25million tones, which represents 6% of the world's production. The southern and eastern Africa countries produce 64% of all Africa maize. South Africa is the largest producer followed by Kenya, Zimbabwe Tanzania, Malawi, Zambia and Ethiopia.

West and Central Africa account for 25% of the annual maize production in Africa, with Nigeria being the major producer. Nigeria produces about 39% of the total West Africa production, with Cameroon, Cote d'Ivoire, Ghana and Benin playing an important role in maize production. In northern Africa, production accounts for 15% of the Africa total annual production with Egypt being the major producer. Egypt produces 92% of the regional maize (Raemaekers, 2001).

Insect Pests of Maize

Maize grains are frequently stored before it is properly dried and without insecticide treatment, insect damage to stored grain can cause losses of 50% or more. Problems with storage pests result because of high temperatures and intermediate humidity, which encourage insect population to grow. The grain moth, *Sitotroga cerealla* and the grain weevils, *Sitophilus spp.* are the common pests. The larger grain borer, *Prostephanus*

truncatus which also infest maize is a new introduction to Africa (Raemaekers, 2001).

Uses of Maize

Since ancient times, maize has been a source of food, feed, commodity, constructional material, medicines and fuel. The grain, stalk, leaves, cobs, tassel and silks all have commercial value in most areas. Maize is increasing becoming an industrial raw material for the production of starch, gluten, oil, flour and grits, alcohol for further processing into whole range of products. (Raemaekers, 2001). In Africa, maize is used for human feed, with exception of small amount fed to animals (less than 10%). Maize is consumed green as roasted or boiled, dried grain is processed into porridge, fermented paste for “kenkey” and” banku”.

COWPEA (VIGNA UNGUICULATA (L.) WALPERS)

Origin and Distribution of Cowpea

Cowpea, (*Vigna unguiculata (L.) Walpers*), originated in Africa, it was domesticated in the Neolithic age. Studies published to date, confirm that cowpea was domesticated in West Africa where a large number of primitive cultivars and semi-wild forms can be found. Cowpea spread to Asia about 2300BC [Before Christ]. Very specialized forms were bred from the African varieties (Raemaekers, 2001).

Description

Cowpea belongs to the family *Papilionaceae* (or *Fabaceae*) and the order *Leguminosales* (*Fabales*) and it is an herbaceous annual (Raemaekers, 2001).

Production Regions

Cowpea is cultivated mostly in the tropical, subtropical and warm temperate regions of the world. However, cowpea is chiefly grown in the sub-Saharan lowlands, in East Africa, and from Ethiopia to the Cape. Nigeria and Niger produce about 50% of the world production. Other African countries producing cowpea include Burkina Faso, Ghana, Kenya, Uganda, Malawi and Senegal (Raemaekers, 2001).

Insect Pests of Cowpea

The major pests of cowpea are *Callosobrunchos maculatus* and *Callosobruchus chinensis*. These insects cause both qualitative and quantitative losses to stored grains (Raemaekers, 2001).

Uses of Cowpea

Cowpea is mostly used in the dry grain form. It is an ingredient for soups and stews. In Ghana cowpea is used to prepare “Koose”. The grain is first and foremost softened by cooking, after the seed coat is removed and the seeds grounded to a fine paste. Onion is added to the paste and seasoned with spices and rolled into balls for frying in oil (Raemaekers, 2001).

INSECT DAMAGE TO CEREAL GRAINS

Insect pests impose their damage on stored products mainly by direct feeding. Some species feed on the endosperm causing loss of weight and quality, while other species feed on the germ, resulting in poor seed germination and low viability.

MAIZE WEEVIL (SITOPHILUS ZEAMAIIS)

Scientific classification of Sitophilus zeamais

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera

Family: Curculionidae

Subfamily: Dryophthorinae

Genus: *Sitophilus*

Species *Sitophilus zeamais* (maize weevil)

BIOLOGY OF SITOPHILUS ZEAMAIIS (MAIZE WEEVIL)

Maize weevil, *Sitophilus zeamais*, is a species of weevil that is usually found in maize crops. The larvae damage maize crops by developing inside an individual grain. The larvae then feed from the inside of the grain until maturity. The adult then reproduces, releasing more crop-damaging larvae. The maize weevil is a danger to both growing crops and stored maize (Parker, 2010).

Distribution

The *S. zeamais* are virtually cosmopolitan throughout the warmer parts of the world and are proficient fliers allowing it to distribute itself more easily. Its main endemic areas are areas that are tropical and temperate, where maize is grown. The maize weevil is slightly larger than the rice weevil (Parker, 2010).

Reproduction

The female lays the eggs inside the grain by chewing a minute hole in which each egg is deposited, followed by the sealing of the hole with a

secretion. The eggs hatch into tiny grubs which stay and feed inside the grain and are responsible for most of the damage. Mature larvae are plump, legless and white, about 4 mm long. Pupation takes place inside the grain. The adult beetle emerges by biting a circular hole through outer layers of the grain. Each female is capable of laying 300-400 eggs, and the adults live for five [5] to eight [8] months and are capable fliers (Parker, 2010).

Development

The larva is mostly 4 mm in length, white and legless. Larvae feed on the internal contents of the maize while developing and takes about 18 to 23 days. Later, the larvae become pupae, and transform into the adult weevil. This process takes about 6 days and during this stage, the pupae do not eat or move (Parker, 2010).

Maturation

The adult emerges by cutting a small circular hole in the grain and the whole process begins all over again. This takes about 30 to 45 days to complete. The adult maize weevil will also feed on the maize during its lifespan, which is approximately 5 to 8 months long, before dying (Parker, 2010).

Control

The damage it can inflict upon a crop can be overwhelming. The weevils infest foodstuffs and the safest method for the eradication of the pest is removal. Also, contaminated maize should be sealed in heavy plastic and disposed off. Grain storage facilities should be inspected regularly for signs of infestation. Another method is to freeze or super heat grain stores, although this method may be impractical for larger facilities and may damage the germ layer required for seeds to be utilized for planting. Also, good ventilation is required to reduce the amount of available moisture in the grain to reduce the ability of the weevils to reproduce (Parker, 2010).

BIOLOGY OF COWPEA BEETLE (*CALLOSOBRUCHUS MACULATUS*)

Scientific Classification

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera

Family: Chrysomelidae

Genus: *Callosobruchus*

Species: *Callosobruchus maculatus*(cowpea beetle)

Description

The adults are 3mm long and are reddish-brown in colour. Their wing covers (known as elytra) are black and grey and have two black spots. Also, the larva is whitish, and C-shaped, with a small head.

Reproduction and Maturation

The eggs are glued to the bean or the pod. On hatching the larvae bore into the seed where it makes a translucent 'window' in the seed before pupating. The larval and pupal stages are spent inside the bean. The adult emerges through the "window" leaving a neat round hole. Infestations can begin in the field. The cowpea beetle readily attacks dried beans; thus this beetle can be a serious storage pest.

Damage

The larval stage of the cowpea beetle tunnels and develops within the beans. The cowpea beetle may consume nearly the entire bean contents. Pupation occurs in the beans and adults emerge through a round hole in the seed coat. Damage is a combination of the feeding and contamination.

Control

Proper hygiene offers the most convenient means of controlling the insect. Since field infestations begin from beans, potential sources of beetles should be eliminated in production areas. Possible sources of cowpea beetles include broken sacks of seed beans left over from planting; seed beans left in planting hoppers; cull beans used in animal feed programmes in a production area; small collections of beans remaining on or in a harvester following harvest; and small piles of beans remaining in or around the field after harvest or in a warehouse area.

IRRADIATION

Food irradiation is the process of applying electromagnetic radiation, a form of energy that includes visible and ultraviolet light, to foods. There are three sources of radiation:

- Gamma rays, this uses radioactive isotopes including cesium 137 and cobalt 60.
- High-energy electrons
- X-rays

The three types of ionizing radiation have the same effects on food; however there are some differences in how they work. For example, electron beams and X-ray radiators are operated by electricity and do not use radioactive isotopes, example cobalt 60. Current food production and processing industry plans for implementing food irradiation primarily

involve electron beams and X-ray radiators. The radiation sources used in food irradiation do not make food radioactive.

Unit of Measure for Irradiation Dose

The dose of radiation is measured in the SI unit known as Gray (Gy). One Gray (Gy) dose of radiation is equal to 1 joule of energy absorbed per kg of food material. In radiation processing of foods, the doses are generally measured in kGy (1,000 Gy).

There are different modes of food irradiation; the mode used depends on the purpose:

- Low doses below 1 kilo gray (kGy) are used to control insects, trichinae (parasitic worms) in pork, and ripening or sprouting of fruits and vegetables.
- Medium doses (1-10 kGy) are used to control both spoilage-causing microorganisms (such as molds) and bacterial pathogens such as *Campylobacter* and *Salmonella*. Low to medium doses are effective against food borne protozoans such as *Cyclospora*.
- High doses (greater than 10 kGy) are used to control microbial contamination of spices and with only enough heating to deactivate enzymes, to make other foods storable at room temperature that is shelf-stable. High doses also can significantly reduce food borne viruses (Anonymous, 2010).

History of Irradiation

Initially, most of the irradiations made use of X-rays, which are produced when electrons from an electron accelerator are stopped in materials. The discovery of X-rays and radioactive substances by W.K. Roentgen in 1895 and Becquerel in 1896 led to further research of the biological effects of these "radiations." The early discoveries laid the foundation for food irradiation (Brynjolfsson, 1989).

However, no commercial development of this use occurred then, due to the inability to obtain ionizing radiation in quantities needed and at costs that could be afforded (Urbain, 1989).

In the late 1940s and early 1950s further research was to study the potential of five [5] different types of radiation, that is ultraviolet light, X-rays, electrons, neutrons, and alpha particles for food preservation. Studies at that time concluded that only cathode ray radiation (electrons) had the necessary characteristics of efficiency, safety, and practicality. X-rays were considered to be impractical because of the very low conversion efficiency from electron to X-ray that was possible at that time. Ultraviolet light and alpha particles were considered to be impractical because of their limited ability to penetrate matter.

Following further studies and research other kinds of ionizing irradiation were made available. As described by Urbain (1989), the first sources were machines that produced high energy electron beams of up to 24 million electron volts. Around the same period, man-made radio nuclides such as Cobalt-60 and Cesium-137 (which in their radioactive decay emit gamma rays) became available through the development of atomic energy and these promoted the research in food irradiation.

After 1950 most studies in food irradiation were government sponsored because of military concern for irradiation of food. The early research was done to sterilize food by the Quartermaster Corps of the U.S. Army at the Food and Container Institute in Chicago.

Advantages of Ionizing Irradiation

Irradiation can provide considerable advantages as summarized by Urbain (1989). Irradiation:

1. Preserves food to a varying extent as determined by the treatment. Food irradiation is particularly effective in controlling food borne spoilage microorganisms. All organisms present in the food can be inactivated to secure long-term preservation, or a fraction of them can be inactivated to secure limited extension of product life. Meats, seafood, fruits, vegetables, cereal grains, and legumes are some of the foods that can be preserved.
2. Decontaminates food of pathogenic bacteria, yeast, molds, and insects. This decontamination can improve the hygienic quality of the foods and prevent the potential health hazards. Meats and seafood can be decontaminated of bacteria and parasites; cereal grains, legumes, fruits, and dried fish of insects; spices and vegetable seasonings of bacteria and insects.
3. Controls maturation, senescence, and sprouting of fresh fruits and vegetables.
4. Alters chemical composition for quality improvement. The chemical composition of cereal grains and legumes can be altered so as to improve their quality. This is regulated by the dose (i.e., amount of radiation absorbed by the food).
5. Produces no toxic residues in foods. This is accomplished by limiting the energy level of the radiation employed, and also by selecting the type of radiation. The lethal action of ionizing radiation on living organisms was traced to alteration of the DNA molecule.
6. Products formed in foods by irradiation were identified and determined to be of no toxicological significance to the consumer of irradiated foods.

7. Maintains full nutritive value of foods. Studies have shown no changes in macronutrients and only insignificant ones in the micronutrients (vitamins). Irradiated foods were shown to be wholesome.

MATERIALS AND METHODS

Experimental Site and Design

The experiment was carried out at the Entomology laboratory in the Crop Science Department at University of Ghana. Two (2) kilograms of each grain: maize and cowpea used for the experiment were bought from Madina market. Experimental design was Randomized Complete Block Design. The equipments used for the experiment were sterilized for three hours (3hrs) at temperature of 70⁰C in oven as shown in Fig. 1



Fig. 1: Heat sterilization of bottles

Sitophilus Zeamais Culture

The maize was first heat sterilized for three hours (3hrs) at a temperature of 60⁰C by putting the maize in an aluminum baking tray placed in an oven. The maize was then allowed to cool for thirty minutes. Two kilogrammes of maize were weighed and placed in four sterilized bottles. The four bottles were filled with five hundred grams of maize and infested with fifty (50) adult *Sitophilus zeamais* each and covered with a piece of cloth tied with rubber band. The insects were obtained from an old culture in the Entomology laboratory at the Crop Science Department at University of Ghana. The insects were cultured in a controlled environment at 27 ± 2⁰C, 60-70% relative humidity (r. h) at the Entomology laboratory of the Department of Crop Science. The culture was left for fifty two (52) days. The culture was further subdivided into fifty grams (50g) each and placed into thirty smaller bottles for the irradiation. The number of insects in each fifty grams (50g) bottle was counted [see results and discussion of findings].



Fig 2: *Sitophilus zeamais* culture.

***Callosobruchus Maculatus* Culture**

The beans were first heat sterilized for three hours (3hrs) at a temperature of 60 °C by putting the cowpea in an aluminum baking tray placed in an oven. The beans were then allowed to cool for thirty minutes. Two kilogrammes of beans were weighed and placed in four sterilized bottles. The four bottles were filled with five hundred grams of beans and infested with fifty (50) adult *Callosobruchus maculatus* each and covered with a piece of cloth tied with rubber band. The insects were obtained from an old culture in the Entomology laboratory at the Crop Science Department at University of Ghana. The insects were cultured in a controlled environment at 27 ± 2°C, 60-70% relative humidity (r. h) at the Entomology laboratory of the Department of Crop Science. New adults were added every ten (10) days since the adults have a short life span. The culture was further subdivided into fifty grams (50g) each and placed into thirty bottles smaller bottles for irradiation. The number of insects in each fifty grams (50g) bottle was counted. [see results and discussion of findings].



Fig: 3 *Callosobruchus maculatus* culture

Irradiation of Maize and Cowpea Seeds

The second phase of the experiment was carried out at Ghana Atomic Energy Commission (G.A.E.C) at Kwabenya in Accra, Ghana. The treatments were six (6), replicated three (3) times and three (3) controls. The samples were taken to Kwabenya and irradiated with doses 40Gy, 80Gy, 150Gy, 200Gy, 300Gy and 500Gy in each case. After radiation the samples were brought back to the Entomology laboratory at the Crop Science Department at University of Ghana.

Germination Test (Petri-Dish Analysis)

Maize Germination Test

Filter paper was placed in Petri-dishes. The filter paper in the Petri dish was moistened with distilled water. Twenty five (25) sterilized (irradiated) maize seeds were randomly selected from each gamma level. The maize seeds were soaked in water for a minute to enhance germination. The maize seeds were placed on the filter paper in the Petri-dishes and covered with another Petri dish and kept in the Entomology Laboratory in the Crop Science Department. Germinated seeds were counted after five days and were recorded.

Cowpea Germination Test

Filter paper was placed in Petri-dishes. The filter paper in the Petri dish was moistened with distilled water. Twenty five (25) sterilized (irradiated) cowpea seeds were randomly selected from each gamma level. The cowpea seeds were soaked in water for a minute to enhance germination. The cowpea seeds were placed on the filter paper in the Petri-dishes and covered with another Petri dish and kept in the Entomology Laboratory in the Crop Science Department. Germinated seeds were counted after five days and were recorded.

RESULTS AND DISCUSSION OF FINDINGS

RESULTS

All data were subjected to analysis of variance using the Genstat 9th Edition. The Least Significant Difference (L.S.D.) was used to separate treatment means. Microsoft excel was used to draw graphs.

The results were attained by counting and recording the number of insects alive weekly for five (5) weeks. The means of the number of insects alive were used to draw tables below. Graphs were plotted for the percentage mortalities of insects alive for the five (5) weeks of observation. Also, the number of seeds that germinated was counted and their percentages determined.

Number of Insects Alive after Sterilizing on Maize

The number of insects alive in each week was counted and the means were used to draw Table 1. At week 0, week 1, and week 2, some of the *Sitophilus spp.* was still found alive in the grains at all the different levels of gamma rays. However, no insect was found alive in the 3rd, 4th and 5th weeks for dose 500Gy. Also no insect was found alive at dose 300Gy in the 5th week. Complete sterilization therefore occurred for dose 300Gy and 500Gy at week 5 and week 3 respectively.

Table 1: Number of Insects Alive after Five (5) Weeks of Sterilizing Maize

Dose (Gy)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
0(control)	25	24	31	37	43	49
40	25	24	28	33	39	43
80	23	19	16	15	14	14
150	24	16	10	7	5	4
200	22	13	9	7	5	3
300	20	9	5	2	2	0
500	16	5	2	0	0	0
L.s.d	1.71	1.52	1.54	1.62	2.03	1.44

L.s.d values at p<0.05

Effect of Ionizing Irradiation on Mortality Of *Sitophilus Zeamais*

From Fig 4, 100% mortalities were recorded for treatment 300Gy and 500Gy in the 3rd and 2nd week respectively. However, dose 200Gy recorded 96% mortality in the 4th week and 5th week respectively. Most of the mortalities were recorded in the first 3 weeks after irradiation and later the rate declined as seen in Fig. 4 below.

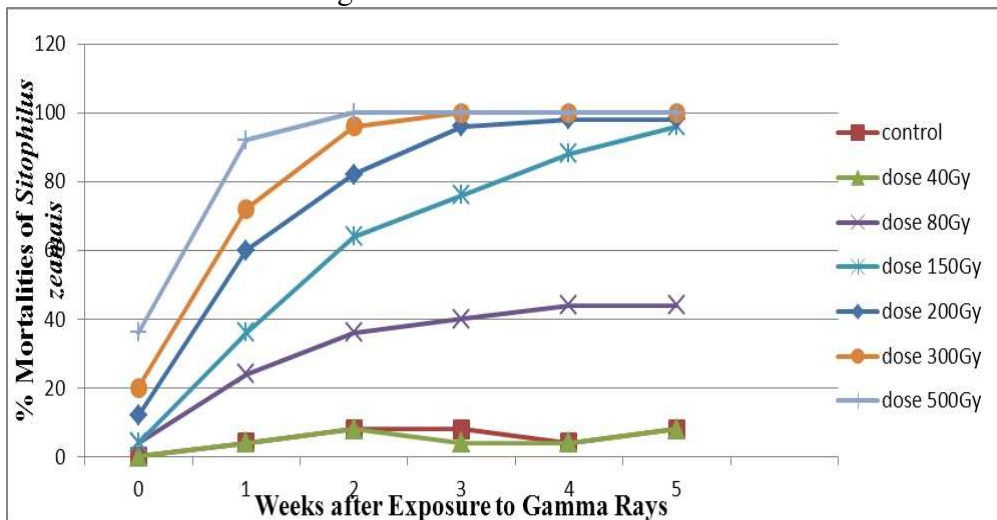


Fig. 4. Percentage mortality recorded weekly following exposure to radiation at different doses

Number of Insects Alive after Sterilizing on Cowpea

The number of insects in each week was counted and the means were used to draw Table 4.2. At week 0, and week 1, some of the *Callosobruchus maculatus*. were still found alive in the grains at all the different levels of gamma rays. However, no insect was found alive in the 2nd, 3rd, 4th and 5th weeks for dose 500Gy. Also no insect was found alive at dose 300Gy in the 4th and 5th weeks. However complete sterilization occurred for dose 300Gy and 500Gy in week 4 and week 2 respectively.

Table 2: Number of Insects Alive after Five (5) Weeks of Sterilizing Cowpea

Dose (Gy)	Week0(6hrs)	Week1	Week 2	Week 3	Week 4	Week 5
0(control)	23	23	28	34	37	41
40	24	21	23	26	29	31
80	23	23	21	18	13	10
150	24	15	10	7	3	2
200	19	13	7	5	3d	1
300	17	8	3	1	0	0
500	13	3	0	0	0	0
L.s.d	2.13	2.55	2.12	1.90	1.92	2.20

L.s.d values at p<0.05

Effect of Ionizing Irradiation on Percentage Mortality of *Callosobruchus Maculates*

Fig 5, 100% mortalities were recorded for treatment 300Gy and 500Gy in the 4th and 2nd week respectively. However, dose 200Gy recorded 92% mortality in the 5th week.

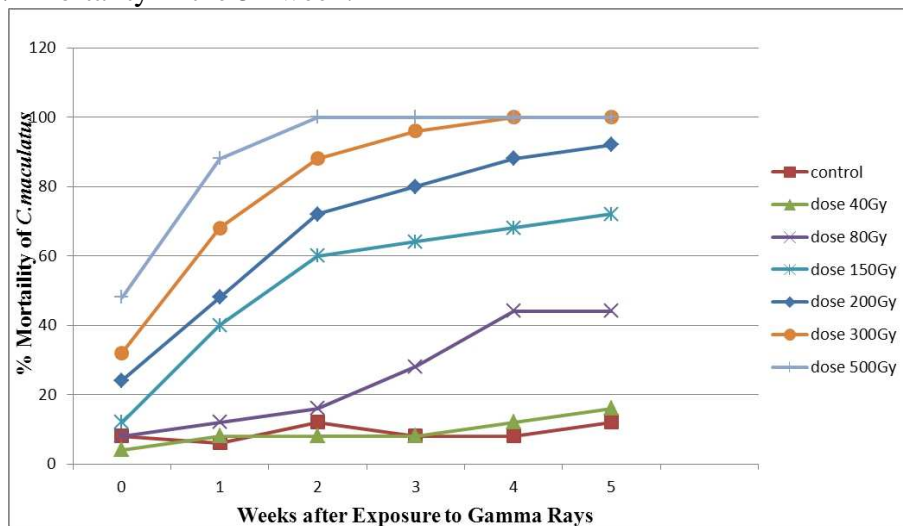


Fig 5. Percentage mortality recorded weekly following exposure to radiation at different doses.

Effect of Gamma Irradiation on the Percentage Viability of Maize and Cowpea Seeds

Seeds from both irradiated and the control all germinated within five days, irrespective of the dose used. No significant differences were observed among the irradiated samples in each case. Also, no significant difference was observed for the control and dose 40Gy for both grains. However, there were significant differences between the control and doses above 40Gy [see table 3 below].

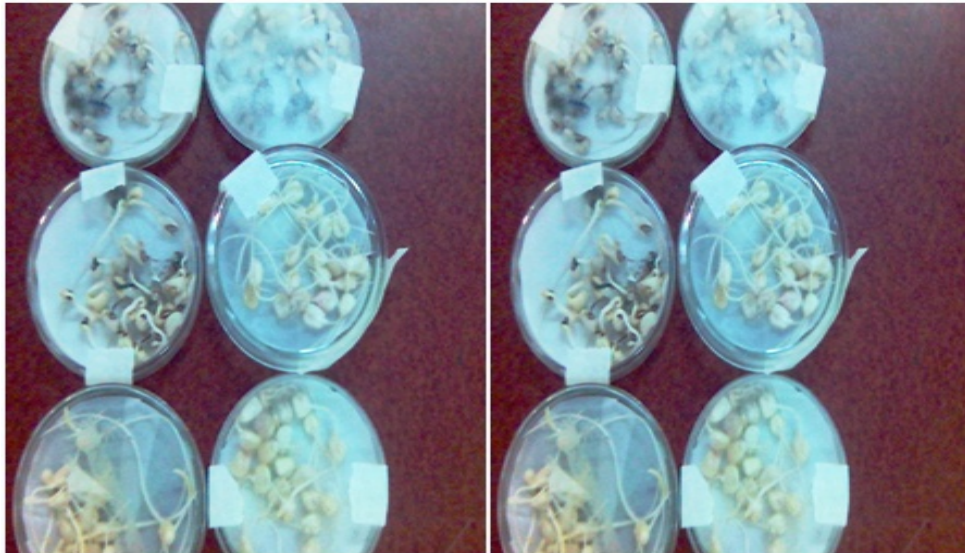


Fig. 6 Germinated seeds in the Petri-dish

Table 3: Percentage of Seeds that Germinated following Radiation at Different Doses.

Treatment dose (Gy)	cowpea seeds	Maize seeds
Initial germination	96	92
Control	37	33
40	38	42
80	50	57
150	50	57
200	57	52
300	52	53
500	58	57
L.s.d	11.67	14.22

L.s.d at $p < 0.05$

DISCUSSION

The idea of using gamma radiation as a quarantine treatment for agricultural products is more than 70 years old (Koidsumi, 1930), and a considerable amount of research has been done in this area in the last 50 years. However, the practical applications were delayed for years due to

scientific and technical reasons (Hallman, 2001). Further developments in the last decade, particularly those related to the phasing out of Methyl bromine (MB) revived interest in this technique, and this method has been recognized as an acceptable alternative to chemical fumigation (Burditt, 1994).

The results of this study show that the radiosensitivity of both *Sitophilus zeamais* and *Callosobruchus maculatus* to gamma irradiation at doses 40Gy, 80Gy, 150Gy, 200Gy, 300Gy and 500Gy. Consequently, the lack of adult emergence is used as a criterion for effectiveness for irradiation treatment. Dose 40Gy had no effect on the mortality of both *Sitophilus zeamais* and *Callosobruchus maculatus*; at this dose, mortality was almost identical to that in the untreated control. Exposure of both insects to doses 150Gy and greater did not result in a single adult emergence of adults. However, it turns out that *Sitophilus zeamais* would need a dose of 200Gy to kill about 98% of adults within 21 days (3 weeks). In addition, dose 300Gy and 500Gy had all *Sitophilus* dead and this confirms the work done by Tilton, E.W. *et al.*, 1973.

In addition, this research confirms the radiosensitivity studies on *Callosobruchus* that reported dose 500Gy to cause 100% mortality within 7-14days (Bhuiya *et al.*, 1991). The susceptibility of both insects at dose 300Gy and 500Gy confirm to the recommended dose proposed by the U.N. Food and Agricultural Organization, the World Health Organization, and the Codex Alimentarius Commission. The mortality of *S. zeamais* and *C. maculatus* increased with increasing dose.

Gamma irradiation has been reported to have no significant effect on seed viability at doses below 1kGy (Lan *et al.*, 1987). The decrease in viability of maize and cowpea seeds in the control and dose 40Gy were as a result of higher feeding by the insects which may have damaged the seed germ, resulting in poor seed germination and less viability. However doses above 80Gy recorded higher percentage seed germination because of the decrease in feeding by insects.

CONCLUSION

The experiment demonstrated that gamma irradiation can effectively eliminate the major pests' *Sitophilus zamias* and *Callosobruchus maculatus* in seed products. Dose 300Gy and 500Gy were much better for disinfecting both *Sitophilus zeamais* and *Callosobruchus maculatus* within 3 weeks after the irradiation. Irradiation is becoming popular as phytosanitary treatment worldwide due to its positive attributes to facilitate trade; fast and easy application, non-use of chemical and wide application compared to other phytosanitary treatments. Irradiation can very well become the phytosanitary treatment of the future.

According to the results obtained, it was found that gamma radiation promotes 100% control of *S. zeamais* and *C. maculatus* after exposure to a minimum of dose 300Gy and above. It can therefore be used as a quarantine treatment. However, some care must be taken during storage in order to avoid re-infestation. The results also showed that gamma irradiation has no significant effect on the viability of seeds. Seed germination tests done (Petri dish analysis) confirmed that for lower doses, irradiation has no significant effect on seed viability.

RECOMMENDATIONS

Future research should be done on the effect of gamma rays on germinated seeds in the field. In addition, more research should be done on the effects of higher doses that result in immediate death of insects on seed viability. Sufficient care and precaution, proper shielding and adequate monitoring by experts should be practiced to prevent any adverse effect on workers.

The combination of irradiation with other techniques, such as extreme temperatures, should be examined for those commodities and insects which show problems with single gamma irradiation treatment.

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