International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Effect of Foliar Application of Irradiated Chitosan on Okra

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Abstract: ***In recent decades, a greater knowledge of chitin chemistry, and the increased availability of chitin-containing waste materials from the seafood industry, has led to the testing and development of chitin-containing products for a wide variety of applications in the agricultural applications. A number of modes of action have been proposed for how chitin and its derivatives can improve crop yield with the help of gamma radiation. This study was designed to evaluate the potential uses of a natural biomaterial, irradiated chitosan, as plant growth promoter and anti-fungal agent. Chitin from prawn shells was extracted by using chemical methods, deproteinization with 6% NaOH at 80 °C for one hour and demineralization with 4% HCl at room temperature for one hour. The resultant chitin powders were divided into six groups: first package was non-irradiated and the others were packaged in PE bags and irradiated at doses of 5, 10, 15, 20 and 25 kGy, respectively. Then, non-irradiated and irradiated chitin samples were deacetylated in the aqueous sodium hydroxide solution (60%) at 100 °C for 60 min. Chitosan treated with gamma radiation at different doses, were used to evaluate the efficiency of irradiated chitosan on okra. The aim of this study was to increase the crop yield by using irradiated chitosan (50, 100, 150 ppm) spraying at 7 days interval. The growth attributes like total fruit weight per plant, plant height and number of fruits per plant were investigated. The results show that application of irradiated chitosan at 20 kGy and 100 ppm can improve nearly four times than control.***

Keywords: gamma radiation, prawn shells, irradiated chitosan, foliar application, okra

1. Introduction

In recent years, there is a trend in agriculture to limit the usage of chemical compounds and to encourage the use of biomaterials. Biomaterials are new promising materials that possess important properties like biodegradability or lack of toxicity. Radiation processing of biomaterials is an area of current research for development of new applications [1]. Different living organisms like prawns, crabs, brown algae etc. produce some biomaterials in their body to tolerate in adverse environmental conditions which have the plant growth promoting and anti-microbial capacity [2]. The advantages of using these biomaterials are that, they are naturally available, cheap and have no destructive effect on overall environment including plants and animals which may be occurred in case of application of chemical fertilizers and pesticides. In this experiment, irradiated chitosan extracted from prawn shells was used to increase okra productivity for our country, Myanmar.

Chitosan and its derivatives are examples of value-added materials. They are produced from chitin, which is a natural carbohydrate polymer found in the skeleton of crustaceans, such as prawn, crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton, including coral and jelly fishes. Insects, such as butterflies and lady bugs, also have chitin in their wings and the cell walls of yeast, mushrooms and other fungi also contain this substance. Chitin shown in Figure.1, a homopolymer of 2-acetamido-2-deoxy-D-glucopyranose (*N*-acetyl-D-glucosamine) residues linked by β (1-4) bonds, is a common constituent of insect exoskeletons, shells of crustaceans and fungal cell walls. Chitosan shown in Figure.2, is a polymer obtained from deacetylation of chitin, is a cationic polysaccharide with linear chain consisting of β (1-4) linkage 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D glucopyranose [3].

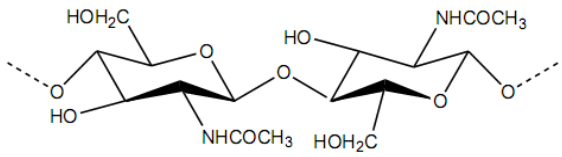


Figure 1: Structure of chitin

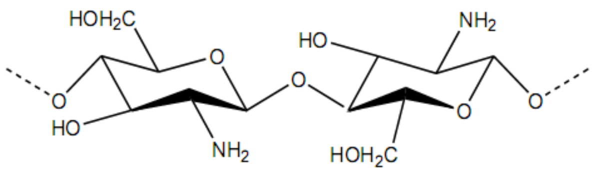


Figure 2: Structure of chitosan

Chitosan can be used in dietary supplements, water treatment, food preservation, agriculture, cosmetics, pulp & paper and medical application. There has been a large increase in chitosan research during the past decade. This is due to its biocompability, biodegradability, non-toxicity, and other unique properties such as film forming ability, chelation and adsorption properties and antimicrobial activity. The functional properties of chitosan are reported to be dependent on its molecular weight or viscosity. Due to its polycationic nature, chitosan can be used as flocculating agent and act as chelating agent and heavy metal trapper. Chitosan has antibacterial activity which can be prepared in the form of film or hydrogel to be used in burn and wound dressing and also for fabricating suturing threads.

The typical production of chitosan from crustacean shell generally consists of three basic steps: deproteinization, demineralization and deacetylation. Gamma radiation can degrade chitosan without producing obvious change in chemical structure of chitosan; moreover, it can usually be carried out at room temperature, and controlled easily. The resulting product can have high purity. On the other hand, the radiation sterilization leads to even better improvement of the biocompatibility of chitosan. Therefore, the study of degradation of chitosan by irradiation has raised the interest of scientists. However, the problem is that the irradiation of chitosan results in an initial depolymerisation beyond 25 kGy of dose.

Okra (*Hibiscus esculentus L.*) known as lady's finger is one of the most important vegetable in Myanmar. It is also popular home garden vegetable and a good source to fulfil the energy requirements of the body. It is also provides vitamin A, B, C, protein, amino acids, minerals and iodine (Hossain et al., 2006). This vegetable is quite palatable and linked equally by poor and rich.

Application of plant growth promoter (PGP) seems to be one of the important practices in view of convenience and cost. Recently, there has been global realization of the important role of PGPs in agriculture for better growth and yield of crops. Developed countries like Japan, China, Poland, South Korea etc. have long been using PGPs to increase crop yield. Very few efforts were done to study the effect of chitosan on plant growth, development and productivity, which is mainly attributed to stimulation of plants immunity against microorganisms (bacteria and fungi) (ChunYan et al., 2003; Patkowska et al., 2006; Sereih et al., 2007; No et al., 2007; Gornik et al., 2008). Recently, some researchers reported that chitosan enhanced plant growth and development (Khan et al., 2002; Chibu et al., 2003; Gornik et al., 2008). They reported that application of chitosan increased key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improved the transportation of nitrogen (N) in the functional leaves which enhanced plant growth and development [5]. Research works of irradiated chitosan on growth, yield attributes and fruit yield of okra is almost rare. Considering the above facts, the present research work was undertaken to study the effect of irradiated chitosan on growth, morphological features, yield attributes and yield in okra.

The main aim of this study is to produce irradiated chitosan for plant growth promoter in agricultural applications, to reduce chemical fertilizers and their side effects and to increase the crop yield by using irradiated chitosan for our country, Myanmar.

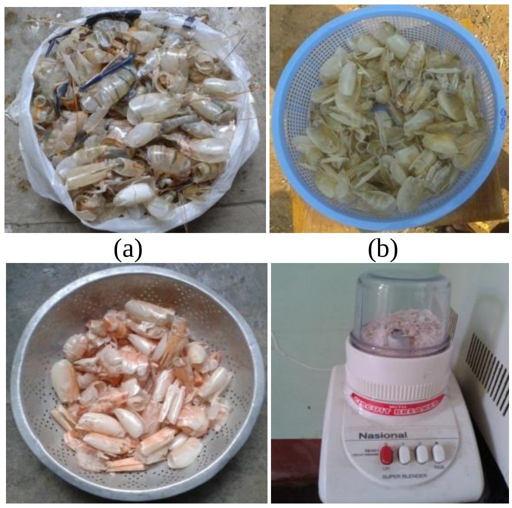
2. Materials and Method

2.1. Materials

Prawn shells were collected from local market, Mandalay region, Myanmar. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were used.

2.2. Preparation of sample

Prawn shells collected from local market were initially washed by tap water again and again to remove meat residues, dirty particles and other contaminants. They were dried on the sunshine about two days to a constant weight. Then, the dried prawn shells were ground with a blender to be fine powder and stored in a plastic bag. Figure.3 shows preparation of sample (a) raw material, (b) after washing, (c) after drying the sample and (d) grinding the sample.



(a) (b)
(b) (d)

Figure 3: Preparation of sample (a) raw material, (b) after washing, (c) after drying, (d) grinding the samples

2.3. Deproteinization

Chitin occurs naturally in association with protein (chitinoprotein) in crustacean shells. Prawn shells are usually ground and treated with dilute sodium hydroxide solution (6%) at elevated temperature (80 °C) for one hour to dissolve the protein present. To obtain uniformity in reaction, it is recommended to use relatively high ratios of solid to alkali solution of 1:10 with proper agitation. Then, the mixture is cooled and filtered and the deproteinized prawn shell powders are washed with distilled water up to neutrality and dried in oven. Figure.4 shows deproteinized prawn shell powders.

Figure 4: Deproteinized prawn shell powders

2.4. Demineralization

The most important minerals in prawn shells are calcium carbonate and calcium phosphate. Deproteinized prawn shell powders are demineralised with (4%) hydrochloric acid for 60 min at room temperature with a solid to solvent ratio of 1:10 (w/v) to remove calcium carbonate. Then, the mixture is filtered to separate solid and liquid phase. The solid phase is washed by distilled water until neutrality and dried in oven. Figure.5 shows chitin samples extracted from prawn shells.

Figure 5: Chitin samples extracted from prawn shells

2.5. Irradiation of chitin

The resultant chitin powders were divided into six groups: first package was non-irradiated and the other were packaged in PE bags and irradiated at doses of 5, 10, 15, 20 and 25 kGy, respectively, with dose rate 1.423 kGy per hour using Gamma Chamber (Cobalt-60, GC-5000) at Department of Atomic Energy (DAE). Figure.6 shows irradiation of chitin in Gamma Chamber.

Figure 6: Irradiation of Chitin by using Gamma Chamber

2.6. Preparation of irradiated chitosan

Preparation of chitosan was achieved by deacetylation of chitin with 60% sodium hydroxide solution at 100 °C for one hour. To obtain uniformity in reaction, it is recommended to use relatively high ratios of solid to alkali solution of 1:10 with proper agitation. Then, the mixture is cooled and filtered and the chitosan powders are washed with distilled water up to neutrality and dried in oven. Figure.7 shows deacetylation of chitin and Figure.8 shows non-irradiated and irradiated at 5, 10, 15, 20 and 25 kGy dose of chitosan powders.

(a) (b)

Figure 7: Deacetylation of chitin (a) deacetylation with strong NaOH, (b) after deacetylation

Figure 8: Non-irradiated and irradiated at 5, 10, 15, 20, 25 kGy doses of chitosan

Extraction of chitin from prawn shells, deacetylation of irradiated chitin, preparation of 2%solution in 2% acetic acid and preparation of irradiated chitosan (50, 100, 150 ppm) solutions were done at laboratory of Department of Nuclear Engineering, Mandalay Technological University, Myanmar. Figure.9 shows preparation of irradiated chitosan solution.

Figure 9: Preparation of irradiated chitosan solution (a) dissolution of irradiated chitosan with 2% acetic acid (b) after dissolution (c) solution of irradiated chitosan (d) different ppm of irradiated chitosan solution

3. Application of Irradiated Chitosan on Okra

3.1 Site description

The experiment was carried out at the pot-yard of Department of Nuclear Engineering, Mandalay Technological University (MTU), Myanmar, during August to December 2016. The soil used for pot culture was collected from MTU experimental farm.

3.2 Plant material and experimental design

Firstly, the land was prepared properly with ploughing and laddering and the soil was thoroughly mixed with natural fertilizer and cow dung. The unit plot size was 4 ft x 2 ft. And then, okra seeds were implanted in the plot. After 30 days, okra plants were transplanted into each pot. The pot size was 12 inch height and 10 inch diameter. Fertilizer doses and application methods were same to each pot experiment. Figure.10 shows implanting of okra seeds. Figure.11 and Figure.12 show soil collection and transplanting the plants, respectively.

(a) (b)

Figure 10: Implanting of okra seeds (a) implanting, (b) okra plants

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Figure 11: Soil collection for pot culture

Figure 12: Transplanting the plants

3.3 Treatments

Non irradiated and irradiated chitosan at 5, 10, 15, 20, 25 kGy and three concentrations of non irradiated and irradiated chitosan 50, 100, 150 ppm were applied. The foliar application of non irradiated and irradiated chitosan was done with hand sprayer once a week after transplanting. Intercultural operations like irrigation, weeding, mulching and pest control of pot experiment were carried out when necessary. The effects of non irradiated and irradiated chitosan solutions on growth and the growth attributes like total fruit weight per plant, plant height and number of fruits per plant were investigated. Figure.13 shows foliar application of irradiated chitosan with hand sprayer.

Figure 13: Foliar application of irradiated chitosan with hand sprayer

4. Results and Discussion

4.1 Effect of irradiated chitosan on morphological parameters

The effects of different concentrations of irradiated chitosan on morphological characters such as plant height, fruit length and fruit diameter were shown in Table 1. Results showed that all morphological, yield attributes and fruit yield were greater in chitosan applied plants than control plant. The plant height of okra was slightly increased with increasing radiation dose until 20 kGy at 100 ppm. However, the okra plant height using irradiated chitosan with 50 ppm and 150 were random. Especially, the okra plant height using 50 ppm of 15 kGy irradiated chitosan and 150 ppm of 10 kGy irradiated chitosan were lower than that of the others. Among them, the okra plant height using 20 kGy irradiated chitosan at 100 ppm was the highest. The lowest value of the above parameters was recorded in control plant. These results indicate that application of irradiated chitosan had tremendous effect on growth and development in okra. They are consistent with El-Tantawy (2009), who reported that plant growth and development enhanced by the application of chitosan in tomato. Furthermore, Ke (2001) reported that application of carboxymethyl chitosan increased key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease), which enhanced plant growth and development in rice. Similar phenomenon may be happened in the present experiment. Figure.14 shows okra plants (a) control and (b) irradiated chitosan treatment. Figure.15 shows effect of irradiated chitosan on plant height of okra.

Figure 14: Okra plants (a) control, (b) irradiated chitosan treatment

Table 1: Effect of irradiated chitosan on morphological parameters

Radiation Dose (kGy)	Plant height (inch)			Fruit length (cm)			Fruit diameter (cm)		
	Concentration (ppm)			Concentration (ppm)			Concentration (ppm)		
	50	100	150	50	100	150	50	100	150
0	50	53.5	55	19	18	14	6.8	6.4	6.5
5	63	57.8	55.2	20	19.8	13.8	6.7	6.6	6.6
10	68.5	68	46	20.6	19.9	15.5	6.6	6.7	6.7
15	57.5	70	56	18.9	20	15.3	6.4	6.8	6.8
20	63	76	74	20.4	21.6	20.2	6.8	7.1	6.5
25	60	63	56	16.2	18.5	15.5	6.9	6.7	6.6
Control	41			14.2			5.7		

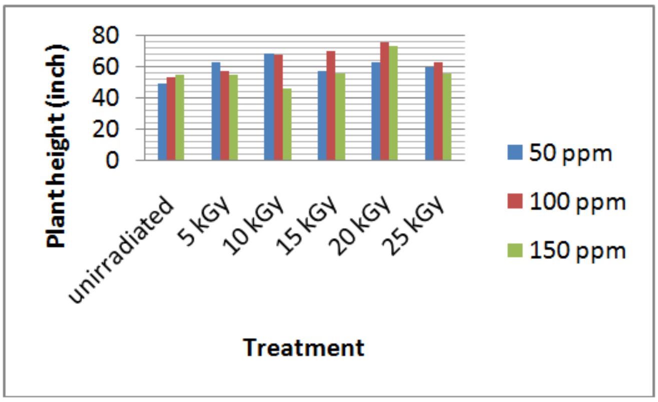

Figure 15: Effect of irradiated chitosan on plant height of okra

4.2 Effect of irradiated chitosan on yield attribute and fruit yield

There was significant variation in yield attributes and fruit yield of okra due to different levels of foliar application of chitosan and irradiated chitosan on okra plants (Table 2). Results revealed that number of fruits per plant, single fruit weight and total fruit weight were higher in chitosan applied plants than control. It was found that total fruit weight was significantly increased with increasing radiation dose of chitosan up to 20 kGy. However, foliar application of irradiated chitosan at 25 kGy slightly decreased total fruit weight of oka due to high gamma radiation dose of chitosan. The highest total fruit weight and total number of fruit were recorded in 20 kGy and 100 ppm chitosan treatments.

The fruit yield was increased at 100 ppm with increasing radiation dose until 20 kGy and slightly decreased at 25 kGy. Luan et al. had found that, the molecular weight of chitosan was decreased with the increase in the radiation dose and low molecular weight of chitosan had progressive impact on plant growth. Moreover, Mohammad Afza et al. also found that, growth promoting activity of chitosan is increased with increasing radiation dose, but at very height radiation doses, the growth promoting activity of irradiated chitosan was decreased. Previous studies have shown that a range of concentrations of radiation degraded polysaccharides depend upon the source and radiation dose (kGy) [2]. Although the fruit yield of okra at 50 ppm and 150 ppm were random, total number of fruit weight increased with increasing radiation dose. Again, the number of fruits per plant was increased in chitosan applied plants than control due to increase the plant height, resulting from increase in the fruit bearing nodes in okra. In contrast, the lowest fruit yield was recorded in control plant due to production of fewer fruits, smaller fruit size and total fruit weight (Table 3). Chibu et al. (2002) reported that application of chitosan increased plant growth and development thereby increased seed yield in rice and soybean. Similar results were also observed by Boonlertnirum et al. (2005) in rice and Rehim et al. (2009) in maize and bean. Figure.16 shows pot culture of okra (a) okra plants (b) weighting the okra fruits and (c) okra fruits. Figure.17 shows effect of irradiated chitosan on total fruit weight of okra. The effect of irradiated chitosan on single fruit weight of okra is shown in Figure.18. The effect of irradiated chitosan on number of fruit per plant is shown in Figure.19.

(a)

(b) (c)

Figure 16: Pot culture of okra (a) okra plants (b) weighting the okra fruits (c) okra fruits

Table 1: Effect of irradiated chitosan on yield attributes and fruit yield

Radiation Dose (kGy)	No of fruit per plant			Single fruit weight (g)			Total fruit weight (g)		
	Concentration (ppm)			Concentration (ppm)			Concentration (ppm)		
	50	100	150	50	100	150	50	100	150
0	15	17	17	20.3	18.7	18.3	305.13	318.2	311.3
5	14	18	19	22.9	18.8	17.7	321.3	338.71	337.22
10	16	19	23	25.3	21.66	17.8	405.97	411.58	410.74
15	20	21	24	21	21.9	18.7	421.15	461.47	448.47
20	24	27	25	23.4	22.81	22	561.62	615.87	552.75
25	24	24	26	20.1	22.4	19.6	483.15	538.02	509.17
Control	10			15.7			157.73		

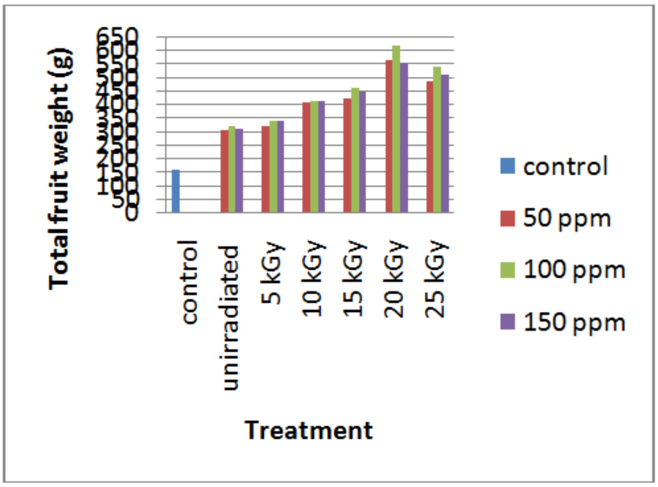

Figure 17: Effect of irradiated chitosan on total fruit weight of okra

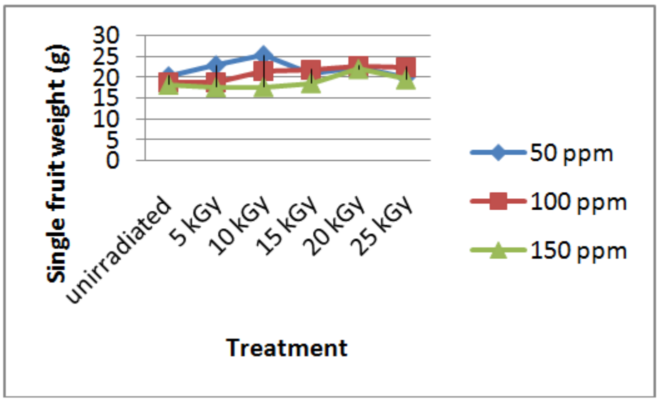

Figure 18: Effect of irradiated chitosan on single fruit weight of okra

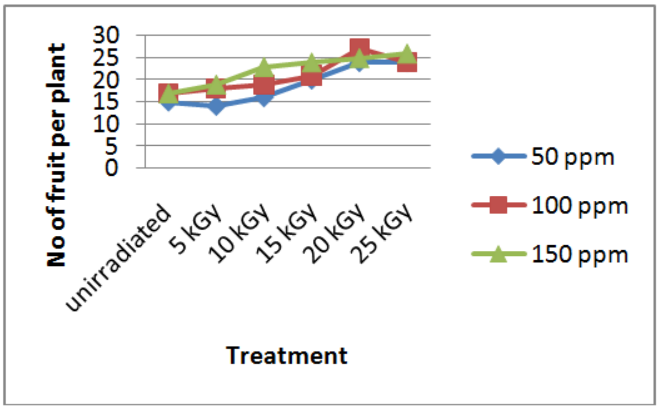

Figure 19: Effect of irradiated chitosan on number of fruit per plant

5. Conclusions

The study had an opportunity to look into potential uses of biomaterials as they are cheap, eco-friendly, non-harmful, and available in nature. Based on the present study, it can be concluded that, radiation processed biomaterials has progressive impact on okra plants in terms of fruit yield and total fruit weight. According to the result, okra plants showed different responses to biomaterials at different radiation doses and concentrations. From this study, it was found that, total fruit weight increased with increasing radiation dose until 20 kGy irradiated chitosan for all concentrations. The highest value of yield attributes and total fruit weight (615.87 g) was recorded in okra plant with foliar application of 20 kGy irradiated chitosan at 100 ppm. In contrast, the lowest yield attributes and total fruit weight (157.73 g) was recorded in control plant. Therefore, it can be said that, foliar application of irradiated chitosan can improve yield attributes and total fruit weight of okra nearly four times than control. However, more experiments should be conducted in different locations and seasons to draw a valid conclusion regarding the irradiated chitosan foliar application for fruit yield improvement of okra.

Acknowledgement

The authors want to acknowledge the Department of Atomic Energy for the permission and help for Gamma Chamber application and to express their gratitude to the colleagues from Department of Nuclear Engineering, Mandalay Technological University for their helpful facilities and suggestions.

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