Efficacy evaluation of irradiated carrageenan as plant growth promoter in Cowpea (*Vigna unguiculata* L.) Walp. and Bush Sitao (*Vigna unguiculata* (L.) Walp. *ssp sesquipedalis* (L.) Verdc. x *V. unguiculata* (L.) Walp. *ssp unguiculata*) production

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Abstract The efficacy of irradiated carrageenan as plant growth promoter in cowpea and bush sitao was determined using Mabunyi and PSB Bs3 varieties. The highest plant height in cowpea was obtained in RF + IC and  $\frac{1}{2}$  RF + IC treatments while for bush sitao, all RF- and IC-treated plants were taller than the control. For commercial crop production parameters, only weight and yield of fresh pods in cowpea showed significant differences among treatments. Cowpea pods were heavier in RF + IC and  $\frac{1}{2}$  RF + IC treated plants than in other treatments. Highest fresh yield in cowpea was obtained in  $\frac{1}{2}$  RF + IC treated plants. Seed diameter and 100-seed weight for cowpea and gross and net weight of harvested seeds and 100-seed weight for bush sitao were the seed production parameters that showed significant differences among treatments. Application of  $\frac{1}{2}$  RF + IC improved the gross and net weight of harvested seeds of bush sitao and 100-seed weight of both crops. Seed diameter of cowpea was highest in all treatments with IC. This study recommends the use of IC in cowpea and bush sitao crop and seed production in combination with the RF.

Keywords: Commercial crop production, Recommended fertilizer, Seed production

## Introduction

Some vegetable crops grown in the Philippines are legumes. Legumes offer necessary nutrients for humans, function as green manure and animal feed (McRae, 2017; Waltenburg, 2017). Cowpea (*Vigna unguiculata* L.) Walp. and bush sitao (*Vigna unguiculata* (L.) Walp. ssp *sesquipedalis* (L.) Verdc. x *V. unguiculata* (L.) Walp. ssp are two leguminous crops grown as subsistence and

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commercial crops. Cowpea originated and was domesticated in Southern and Central West Africa, and today it is widely adapted and grown worldwide because of its popularity as grains in processed food, vegetables (fresh leaves, peas and pods) and fodder (BPI, 2013a,b; Gomes *et al.*, 2019; Fatokun *et al.*, 2002). On the other hand, bush sitao originated in South-East Asia, possibly from Southern China, because of its large genetic diversity in the area. Both crops are inexpensive source of protein, are hardy and can be grown in combination with cereals or other crops in multiple cropping systems. In addition, these crops contribute to the sustainability of farming systems with their nitrogen fixation capacity and role in soil improvement and as ground cover (Fatokun *et al.*, 2002).

With these valuable features of cowpea and bush sitao, it is imperative to increase their productivity. Most farmers today use synthetic and inorganic fertilizers to improve crop yield (BPI, 2013a,b). But with the rising cost of synthetic fertilizers, it is but fitting to look for cheaper alternatives. There is an emerging fertilizer called irradiated carrageenan. Irradiated carrageenan which produces an oligosaccharide known as carrageenan plant growth promoter (CPGP) was developed by Philippine Nuclear Research Institute (PNRI) and registered as an inorganic fertilizer (PNRI, 2012). Irradiated carrageenan is obtained by exposing carrageenan, a polysaccharide obtained from red edible seaweeds, specifically carrageenophytes to gamma rays irradiation. Gammaray irradiation degrades the natural carrageenan into smaller oligomers with comparatively low molecular weight and have been used as plant growth promoters for several agricultural crops. Application of CPGR has been proven to stimulate plant growth and other processes like seed germination, shoot elongation, root growth, flower production, antimicrobial activity, amelioration of heavy metal stress, and synthesis of phytoalexins (Naeem et al., 2015).

Several studies have been undertaken by PNRI to determine the effect of irradiated carrageenan on the yield and other plant characteristics of rice (*Oryza sativa*) and legumes like mungbean (*Vigna radiata*) and peanut (*Arachis hypogaea*). In rice, foliar application of irradiated carrageenan at low concentrations increased yield by 15-30%. In another trial, data showed an increase of 65.4% in grain weight and 12% in panicle length when rice plants were sprayed with 20ml/L of CPGP in addition to three to six bags of chemical fertilizer per hectare (DOST, 2018). In the variety of mungbean, Kulabo, spraying of fresh irradiated carrageenan increased yield by 61.3% while a 104.7% increase was observed in plants sprayed with three-month old irradiated carrageenan. On-farm trials of mungbean using irradiated carrageenan in different parts of the Philippines showed an increase in yield of 33.4% (from 1,353 kg/ha to 1,805 kg/ha) in Pag-asa 19 variety and 20% to

86.9% in Pag-asa 7 variety compared to farmer's practice. In addition, mungbean sprayed with carrageenan PGP also produced more flowers and branches, longer pods, larger and heavier seeds, and extensive root system and nodulation (Gatan *et al.*, 2019). Meanwhile, field trial on peanuts using NSIC Pn 14 variety showed a 60% yield increase after foliar application of irradiated carrageenan over the farmers' practice (Business Mirror, 2018).

The experiment studied the commercial crop and seed production of cowpea and bush sitao treated with irradiated carrageenan. Specifically, the study aimed to identify the effects of irradiated carrageenan in combination with the recommended fertilizer on the time of flowering, yield and yield for commercial crop and seed production of cowpea and bush sitao.

## Materials and methods

The field experiment was done at the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños, Philippines. Seeds of cowpea and bush sitao were used in the study. For bush sitao, PSB-Bs3 was used. It is a variety of bush sitao with good quality pods that are light green in color and with a length of approximately 24.4 cm. Average yield is 8.96 tons per hectare. This variety is also resistant to pests and diseases. For cowpea, Mabunyi variety was used. It is a variety of cowpea that reaches maturity at 49 days after sowing (DAS). It has a dark green pod, 17 cm in length and weighing 4.0-6.0 grams. It is tolerant to shade and drought conditions.

The experimental design used was Randomized Complete Block Design in four replications. An area of 1,617 square meters was divided into four blocks, the first two blocks were for cowpea and the another two were for bush sitao. The blocks were subdivided into 10 plots with a size of 12 square meters per plot, and each plot has four rows measuring four meters each. Randomization is done by assigning the treatments in each replication, using the simple random sampling through draw lots.

For this study, combinations of irradiated carrageenan (IC) from the Philippine Nuclear Research Institute and Complete fertilizer (14-14-14) were applied. The treatment combinations used in this study was shown in Table 1. Complete fertilizer was applied once as basal fertilizer and IC was sprayed every week.

TREATMENT	IC <sup>*</sup> (%)	<b>RECOMMENDED FERTILIZER</b>
1	0	100% complete fertilizer
2	100	no fertilizer
3	100	100% complete fertilizer
4	100	50% complete fertilizer
5 (control)	0	no fertilizer
* IC ' 1' / 1		

**Table 1.** Treatment combinations used in the study recommended by NCPC-UPLB

\* IC- irradiated carrageenan

For treatments with recommended fertilizer (T1, T3 and T4), basal application of complete fertilizer was done, at rate of 96g per plot for T1 and T3, and a rate of 48g per plot for T4. For T2, T3 and T4, 160 ml of irradiated carrageenan diluted in 16 L of water and a rate of 192ml per plot were sprayed every week. On the other hand, the plants assigned to T5, no fertilizer and irradiated carrageenan were applied throughout the growing period.

Harvesting of bush sitao for commercial use was done 45-50 DAS, while in cowpea it was done during 60-90 DAS. The pods were hand-picked early in the morning or late in the afternoon to avoid weight loss. For seed production, the dry pods were harvested and placed in net bags dried under the sun for 2-4 days until brittle. After the seeds were removed from pods, the seeds placed under the sun for 4-5 days. After removing the seeds from the pods, further drying under the sun was done to attain the desired moisture content of the seeds.

#### Data gathered

The number of days from sowing (DAS) to 50% flowering of the plants was observed and recorded. The average fresh pod characteristics-length, diameter and weight were recorded from three pods of each five sample plants in a replicate. The fresh yield was obtained from pods harvested in a row per replicate divided by number of plants. At 45 DAS, plant height was recorded by measuring from ground level to the tip of the tallest leaf. For seed production, seed weight and number of seeds per pod were collected from ten sample pods in each five marked plants. The seed yield was obtained from total harvest in a row from each replicate. The seed diameter was determined from 25-seed samples while 100-seed weight data were gathered from seeds in each sample row in a replicate.

All data were statistically analyzed using Statistical Tool for Agricultural Research (STAR) version 2.0.1 2014 software and means were compared using Least Significant Difference (LSD) at 5% significance.

## Results

## Climate

Based on Corona Classification, the province of Laguna in the Philippines is under Type I climate, where two seasons are present. The dry season is from November to April, and the rest of the year is wet season with the maximum rain period from June to September. The average rainfall and temperature throughout the experiment period provided by National Agrometeorological Station in UPLB is shown in Figure 1. The highest average temperature was recorded in the last month of experiment (May) which is 34.4 °C. On the same month the highest rainfall was recorded, which coincided with the harvest time of cowpea and bush sitao.



Figure 1. The average rainfall (mm) and temperature (°C) throughout the experiment

## Days to 50% flowering

Result showed that the flowering time of cowpea was significantly affected by the IC treatment. Cowpea treated with 1/2 RF + IC exhibited 50% flowering on the 38<sup>th</sup> day (Table 2). Plants treated with RF only and control (no fertilizer) were the last ones to flower at 42 days.

On the other hand, flowering time of bush sitao treated with IC showed highly significant difference from plants without IC. Bush sitao plants under T3 exhibited the earliest 50% flowering on the  $38^{th}$  day, followed by plants under T2 and T4 on the  $39^{th}$  day, plants under T1 on the  $43^{rd}$  day, and last was the control (no fertilizer) on the  $46^{th}$  day.

	Number	of Days
Treatment	Cowpea	Bush sitao
T1-Recommended Fertilizer	42.0a	43.0a
T2-Irradiated Carrageenan	40.0ab	39.0b
T3- RF + IC	41.0a	38.0b
T4- 1/2 RF + IC	38.0b	39.0b
T5- Control	42.0a	46.0a
CV (%)	3.96	6.06
ANOVA	*	**

**Table 2.** Effects of irradiated carrageenan on 50% flowering time of cowpea and bush sitao

Means followed by same letter(s) are not significantly different, \*significant, \*\*highly significant, ns- not significant

#### Commercial crop production of cowpea

The treatments showed no significant differences on the pod length and pod diameter of cowpea. The longest pod was observed under T4 with 18.22 cm, and the shortest pod was under T1 with 16.00 cm. At the same time the highest pod diameter was seen under T4 with 8.58 and the lowest is 7.22mm from those plants under T5 (Table 3).

On the other hand, the pod weight and fresh yield showed significant differences among treatments. The pod weight from plants treated with 1/2 RF + IC was significantly different from untreated plants. In comparison, the plants without fertilizer were not significantly different with plants treated with IC and RF only. The highest pod weight was observed in plants under T4, followed by those plants under T3, T2, T1 and T5, with 22.15g, 20.90g, 20.70g and 18.15g, respectively. For the fresh yield, the crops under T4 was significantly differed on plants under T1, T2, and T5. The highest fresh yield was observed under T4 with an average of 221.59g, followed by those plants

under T3, T1, and T5, in contrast the plants under T2 had the lowest fresh yield, which was 120.67g (Table 3).

1 i catillent	()	()	(-)	(-)	
Treatment	I ou Length	I ou ulameter	i ou weight	r i esti yielu	
	Dod Longth	Pod diamotor	Dod woight	Fresh wield	
weight and f	resh yield of co	wpea			
		8	1 8,	1 ,	1
Table 3. Ef	fects of irradiat	ted carrageenan	on pod length,	pod diameter.	pod

Treatment	(cm)	(mm)	(g)	(g)
T1	16.00	7.60	20.70ab	131.47b
T2	18.12	7.92	20.90ab	120.67b
Т3	16.96	8.17	22.15a	170.59ab
T4	18.22	8.58	24.55a	221.59a
T5	16.73	7.22	18.15b	152.00b
CV(%)	12.04	11.95	11.80	21.68
ANOVA	ns	ns	*	*

Means followed by same letter(s) in the same column are not significantly different, \*significant, \*\*highly significant,

ns- not significant

Plant height is one of the parameters measured for crop production of cowpea. It showed significant differences among treatments. The plants treated with RF + IC were significantly different from plants applied with RF only and no fertilizer. The highest plant height of cowpea was observed in T4, 45 DAS, with an average of 37.06 cm, followed by T3, T2, T5, and T1 with a mean of 36.66cm, 34.35cm, 27.59cm, and 26.55cm, respectively (Figure 2).



**Figure 2.** Plant height of cowpea affected by irradiated carrageenan. \*Means followed in the same letter is not significantly different.

## Commercial crop production of bush sitao

The pod length, pod diameter, pod weight and fresh yield of bush sitao showed that there were no significant differences in using IC on plants for commercial crop production (Table 4).

**Table 4.** Effects of irradiated carrageenan on pod length, pod diameter, pod weight and fresh yield of bush sitao

Treatment	tment Pod Length (cm) Pod d (r		Pod weight (g)	Fresh yield (g)
T1	17.90	7.28	20.25	140.81
T2	18.65	7.68	21.35	107.28
T3	19.35	7.88	22.55	101.88
T4	20.17	8.40	23.40	182.37
T5	14.27	5.79	15.85	67.44
CV(%)	24.31	24.26	26.49	42.06
ANOVA	ns	ns	ns	ns

Means followed by same letter(s) in the same column are not significantly different, \*significant, \*\*highly significant,

ns- not significant

However, plant height of bush sitao showed significant differences. The plants in T4 had significant differences with plants without fertilizer. The highest plant height in bush sitao was observed at T4, 45 DAS, with an average of 43.71cm and followed by T3, T2, T1 and T5 with 42.72cm, 40.43cm, 38.87cm, and 33.52cm, respectively (Figure 3).



**Figure 3.** The plant height of bush sitao affected by irradiated carrageenan. \*Means followed in the same letter is not significantly different.

## Seed production of cowpea

The effects of IC on seed production traits of cowpea were summarized. The seed weight, number of seeds per pod, weight of seed-gross and seed-net had no significant differences among treatments (Table 5). On the other hand, seed diameter showed significant differences among treatments. The highest seed diameter was exhibited in T3 and T4 with 6.5mm, while the lowest seed diameter was from plants treated with RF only at 5.95 mm. The 100-seed weight showed a highly significant differences among treatments. The T4 had significant differences from T1, T2 and T5, where the highest 100-seed weight was exhibited at T4 with an average of 29.0g while the lowest was under T5 with an average of 20.75g.

**Table 5.** Effects of irradiated carrageenan on seed weight, number of seeds per pod, weight of harvested seed-gross and seed-net, seed diameter and 100-seed weight of cowpea

Treatment	Seed weight (g)	No. of seeds/pod	Weight of harvested seed-gross (g)	Weight of harvested seed-net (g)	Seed diameter (mm)	100-seed weight (g)
T1	85.00	11.00	271.50	247.00	5.95 b	24.75b
T2	74.75	9.25	288.75	221.25	6.32a	25.50b
T3	78.00	11.50	297.00	238.25	6.50a	23.50bc
T4	106.50	11.75	406.25	348.50	6.50a	<b>29.00</b> a
T5	80.00	11.00	220.00	165.00	6.20ab	20.75c
CV(%)	31.38	12.39	31.58	34.66	3.21	7.55
ANOVA	ns	ns	ns	ns	*	**

Means followed by same letter(s) in the same column are not significantly different, \*significant, \*\*highly significant,

ns- not significant

#### Seed production of bush sitao

The effects of irradiated carrageenan on seed production traits of bush sitao is shown in Table 6. Result showed that the seed weight, number of seeds per pod and seed diameter were not significantly different among treatments. On the other hand, the seed-net and seed-gross of bush sitao showed a significant difference among the treatments, where the T4 and T5 for the weight of harvested seed-net showed the highest and lowest data, with an average of 415.25g and 150.0g, respectively. The seed-gross showed that T4 was

significant different from T1 and T5, where the T4 plants exhibited the highest seed-gross weight with an average of 464.0g, followed by T2, T3, T1 and T5, with an average of 400.7g, 316.0g, 286.2g, and 219.0g, respectively. In contrast, the plants under T5 had the highest weight of damaged seeds, with an average of 69.0g.

bush sitao Weight of Weight of Seed Seed No. of harvested harvested 100-seed Treatment weight diameter seeds/pod seed-gross seed-net weight (g) (g)  $(\mathbf{mm})$ (g) (g) T1 77.25 13.50 286.25bc 243.75bc 6.42 22.75b T2 116.50 12.75 400.75ab 351.25ab 6.42 25.75a Т3 72.50 13.50 6.50 26.50a 316.00abc 246.75bc T4 137.25 14.25 464.00a 415.25a 6.51 27.25a T5 65.00 13.00 219.00c 150.00c 6.33 20.25c

**Table 6.** Effects of irradiated carrageenan on seed weight, no. of seeds per pod, weight of harvested seed gross and net, seed diameter and 100-seed weight of bush sitao

Means followed by same letter(s) in the same column are not significantly different, \*significant, \*\*highly significant,

31.20

36.80

\*

1.88

ns

5.45

\*\*

ns- not significant

CV(%)

ANOVA

38.87

ns

7.56

ns

#### Discussion

The days to 50% flowering was shortened by two to four days for cowpea and seven to eight days for bush sitao. In this study, cowpea attained 50% flowering time 38 DAS and 39 DAS for bush sitao. Cowpea usually flowers 60-90 DAS and bush sitao 45-50 DAS (BPI, 2013a, b). Based on this result, the use of IC promotes early flowering. These results were supported by the studies of Abad *et al.* (2018) on peanut and Bi *et al.* (2011) on chickpea, where the period of flowering of peanut with IC application was shortened by 14-21 days compared to control. In comparison, chickpea treated with carrageenan flowered earlier than the control. The carrageenan plant growth promoters contain macro and micro elements and plant hormones that helps to promote photosynthesis in plants. It has auxin, gibberellin and cytokinins that may promote early flowering (Gatan *et al.*, 2019).

In general, some commercial crop and seed production characteristics were improved with the application of IC, whether applied singly or in combination with RF. The results of this study were supported by various studies using IC as plant growth promoter. Better yields were obtained on crops like peanut (Abad *et al.*, 2018), snap bean (Abou El-Yazied *et al.*, 2012), chickpea (Bi *et al.*, 2011), and mungbean (DOST-PNRI, 2018). Plant height is one of the characteristics measured to evaluate the effect of IC on commercial crop production of cowpea and bush sitao. In both crops, the tallest plants were those treated with 1/2 RF + IC. In contrast, the shortest ones were those under the no fertilizer treatment. The use of IC might have enhanced photosynthesis, basal metabolism and cell cycle thereby stimulating plant growth as observed in a similar study by Castro *et al.* (2011) done in tobacco (*Nicotiana tabacum*).

One of the factors affecting crop production is pest infestation. It was observed that untreated plants were heavily infested with insect pests such as soybean looper (Chrysodeixis includens) and leaf miner (Liromyza sp.). Most of the plants that were not treated with either IC, RF or their combination were attacked by soybean looper. The presence of this insect in plants may cause defoliation causing decreased canopy photosynthesis, leaf abscission, delayed senescence, delayed crop maturity, and decreased nitrogen fixation, which may lead to a low crop yield. On the other hand, plants applied with RF and untreated plants were infested with leaf miners which are said to feed on the green inner portion of the leaves, leaving white zigzag lines (BPI, 2013a, b). Although these insect pests were controlled by chemical insecticide application, plant growth was still restricted, resulting to loss of vigor and low yield. Meanwhile, less pest infestation was observed in IC treated plants. Irradiated carrageenan tends to control insect pests by inducing plant defense responses against viruses, bacteria, fungi, viroids, and insects by the activity of different defense pathways like salicylate, jasmonate, and ethylene signaling pathways (Shukla et al., 2016).

This study recommends the use of irradiated carrageenan in cowpea and bush sitao commercial crop and seed production due to higher yield compared to the use of recommended fertilizer only. Among the different treatments evaluated,  $\frac{1}{2}$  RF + IC proved to be the most effective treatment.

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