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Preparation and Foliar Application of Oligochitosan - Nanosilica on the Enhancement of Soybean Seed Yield

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Abstract**—** *Oligochitosan with weight average molecular weight (Mw) of 5000 g/mol was prepared by gamma Co-60 radiation degradation of 4% chitosan solution containing 0.5% H_2O_2 at 21 kGy. Nanosilica with size of 10 – 30 nm was synthesized by calcination of acid treated rice husk at 700° C for 2 h. The mixture of 2% oligochitosan–2% nanosilica was prepared by dispersion of nanosilica in oligochitosan solution. Oligochitosan, nanosilica and their mixture were characterized by gel permeation chromatography (GPC), transmission electron microscopy (TEM), X-ray diffraction (XRD), energy dispersive x-ray spectroscopy (EDX), Ultraviolet-visible spectroscopy (UV-Vis), and Furrier transform infrared spectroscopy (FT-IR). Effect of foliar application of oligochitosan and oligochitosan–nanosilica on soybean seed yield was conducted in experimental field. Results indi-cated that soybean seed yield increased 10.5 and 17.0% for oligochitosan and oligochitosan–nanosilica, respect-tively for the control. Radiation degraded oligochitosan and its mixture with nanosilica can be potentially used for cultivation of soybean with enhanced seed yield.*

Keywords**— Oligochitosan, nanosilica, foliar, soybean, seed yield.***

I. INTRODUCTION

The excessive use of chemical fertilizer and pesticide in agriculture may lead to negative effects of toxic residues in food products causing toxin risk for consumers and in environment causing ecotoxicity and health hazard concerns. Recent trend in agriculture has been focused on organic and vertical farming not only addressing the rising concern for environmental issues but also accommodating the demands of food of increasing world population [1]. Organic farming is considered as a viable alternative in comparison to chemical based farming [2].

Chitosan and oligochitosan have attracted considerable interest due to their many unique biological activities such as antioxidant activity [3], antimicrobial activity [4], and antitumor activity [5]. These features, together with their biocompatibility, biodegradability, and nontoxicity make them as interesting biopolymers for application in medicine, cosmetic, biotechnology, food and agriculture, etc. Oligochitosan is effective at eliciting plant innate immunity against disease in plant such as tomato [6], grapevine [7], etc. Therefore, Yin *et al.* (2010) supposed oligochitosan as a plant vaccine that is similar with general animal vaccine [8]. Beside elicitation effect, oligochitosan also exhibits growth promotion effect for plant such as rice [9], soybean [10,11], etc. It is interesting to note that application of oligochitosan either by seed treatment [10] or through hyponex solution [11] increased seed yield of soybean from 15 to about 36%. In general, oligochitosan has better plant growth promotion and elicitation effect than chitosan [7–9 ,11].

Nanotechnology opens up a wide applicability in various fields like medicine, pharmaceutics, electronics and agriculture. Nanomaterials hold great promise of improved plant disease resistance, controlled release of agro-chemicals, enhanced plant growth, etc [12]. According to Taha (2016), nanomaterials can be used as a magical tool for enhancing growth and improvement of agricultural production [13]. Typically, treatment of tomato seed with nanosilica (SiO_2) of 8 g L^{-1} not only enhanced the characteristics of seed germination but also promoted seedling growth [14]. In addition, Suriyaprabha *et al.* (2012) reported that soil amendment with nanosilica of 15 kg ha^{-1} enhanced growth characteristics of maize particularly stem height, root length, leaf area and chlorophyll content [15]. They concluded that the application of nanoscale fertilizers was found to be superior to bulk silica as soil amendment. Recently,

Kiirika *et al.* (2013) reported for the first time the synergistic effect of mixture of chitosan–silica induced resistance in tomato against bacterial wilt caused by *Ralstonia solanacearum* [16]. To the best of our knowledge, no research on the effect of mixture of oligochitosan–nanosilica for plants has been reported yet. With the aim of contribution to promoting organic farming, in the present study, oligochitosan was prepared by gamma Co-60 irradiation degradation of chitosan in solution and nanosilica was prepared from rice husk. The effect of foliar application of oligochitosan and mixture of oligochitosan–nanosilica on the enhancement of soybean seed yield was investigated.

II. MATERIALS AND METHODS

2.1. Preparation of oligochitosan

Chitosan from shrimp shell with a degree of deacetylation (DDA%) of ~91.4%; the weight average molecular weight (Mw) of 44.5 $\times 10^3$ g/mol and the number average molecular weight (Mn) of 13.5 $\times 10^3$ g/mol was supplied by a factory in Vung Tau province, Vietnam. Oligochitosan was prepared by gamma Co-60 ray irradiation degradation method as described in our previous paper [17] with some modifications. Briefly, chitosan (4 g) was dissolved in 80 ml of 2% (w/v) lactic acid solution, then 1.5 ml of hydrogen peroxide (30% H_2O_2) and 18.5 ml water were added to prepare 4% chitosan (w/v) solution containing 0.5% H_2O_2 (w/v). The 4% chitosan (w/v) solution without H_2O_2 was also prepared by adding 20 ml water. Then, the prepared solutions were irradiated at room temperature and under atmospheric pressure on gamma SVST Co-60/B irradiator at the VINAGAMMA Center up to the dose of 21 kGy, with dose rate of 1.12 kGy/h measured by a dichromate dosimetry system [18]. The Mw and Mn of irradiated chitosan were measured by an Agilent 1100 gel permeation chromatography (GPC; Agilent Technologies, USA) with detector RI G1362A and the column ultrahydrogel models 250 and 500 from Waters (USA). The standards for calibration of the columns were pullulan. The eluent was aqueous solution 0.25 M CH_3COOH/0.25 M CH_3COONa with the flow rate of 1 ml min^{-1} and temperature at 30° C [17]. IR spectra were taken on an FT-IR 8400S spectrometer (Shimadzu, Japan) using KBr pellets. The degree of deacetylation (DDA%) was calculated based on FT-IR spectra according to the following equation [19]:

$$A_{1320}/A_{1420} = 0.3822 + 0.0313 \times (100 - DDA\%)$$

where A_{1320} and A_{1420} are absorbance of chitosan at 1320 and 1420 cm^{-1}, respectively.

2.2. Preparation of nanosilica

Raw rice husk was supplied by rice mills in the south of Vietnam. The nanosilica with particles size of 10 – 30 nm was prepared from rice husk according to the procedure described by Wang *et al.* (2011) with some modifications [20]. Briefly, raw rice husk was first rinsed with water to remove dusts, soluble substances, and other contaminants. It was then dried at 60° C in forced air oven (Yamato, DNF 410, Japan). 50 g of the dried rice husk was then treated with 500 ml of 0.5 N HCl at ambient temperature for 2 h by magnetic stirring. It was kept intact overnight. Then it was decanted and thoroughly washed with distilled water until the rinse became free from acid. The treated-rice husk was subsequently dried in forced air oven until to dry and ground into fine powder. Finally, the rice husk powder was incinerated at 700° C for 2 h inside a programmable furnace (Nabertherm GmbH, Germany) to obtain nanosilica. The silica content and the amount of metallic impurities in the sample were estimated by energy dispersive x-ray spectrometer (EDX), Horiba 7593-H. The X-ray diffraction (XRD) pattern of nanosilica was recorded on an X-ray diffractometer, D8 Advance A25, Brucker, Germany. The particle size of nanosilica was performed using transmission electron microscopy (TEM), model JEM1010, JEOL, Japan.

2.3. Preparation of oligochitosan–nanosilica

Oligochitosan with Mw ~5000 g/mol obtained from 4% chitosan/0.5% H_2O_2 solution irradiated at dose of 21 kGy was used for preparation of oligochitosan–nanosilica mixture. 10 g nanosilica was homogenized in 100 ml NaOH 1N for 1 h. Then, 250 ml of the prepared oligochitosan solution was mixed with homogenized nanosilica and water was added to the mixture to obtain final volume of 500 ml and mixture concentration of 2% oligochitosan–2% nanosilica. In order to increase the adsorption ability of oligochitosan on nanosilica, pH of the mixture was adjusted to ~7.5 [21]. The optical absorbance of samples was performed with an UV-Vis spectrophotometer model UV-2401PC, Shimadzu, Japan in the wavelength range 200 – 800 nm using the quartz cuvettes with a path length of 1 cm and using water as the blank sample. The particle size of nanosilica in the oligochitosan–nanosilica mixture was measured using TEM image, and FTIR spectrum of oligochitosan–nano-silica mixture was also recorded.

2.4. Experimental design, foliar spraying and crop management

The experiment was conducted in the experimental field of the Institute of Agricultural Sciences for Southern Vietnam in Dong Nai Province, Vietnam and was designed as a randomized complete block with three treatments. Each treatment consisted of three replications. The area of each replication was of 30 m^2 (5 m × 6 m). Three treatments included foliar spraying with water

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(control), oligochitosan and oligochitosan–nanosilica mixture. The concentration of oligochitosan and oligochitosan–nanosilica used for foliar spraying was of 50 mg/L and 50 mg/L – 50 mg/L, respectively. Foliar spaying was applied three times after seed sowing of 15, 22 and 30 days. Five plants were randomly selected to determine growth indexes particularly plant dry weight and plant height at flowering stage. In all three treatments, seeds were harvested when plants reached maturity. All data were statistically analyzed by analysis of variance (ANOVA) according to the experimental design and Least Significant Difference (LSD) at 5% probability level was utilized to compare the different means.

III. RESULTS AND DISCUSSION

3.1. Characteristics of radiation degraded chitosan

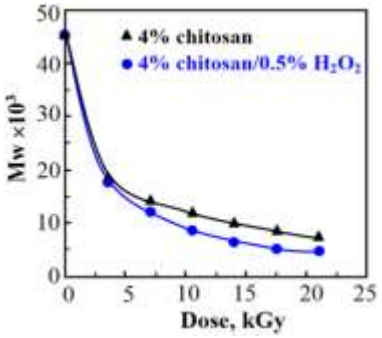

Fig.1: The weight average molecular weight (Mw) of chitosan versus dose.

The results in Fig. 1 indicated that the Mw of irradiated 4% chitosan/0.5% H_2O_2 solution were lower than that of irradiated 4% chitosan solution without containing H_2O_2, particularly Mw was of 5000 g/mol and 7800 g/mol at 21 kGy, respectively. It can be also observed in Fig. 1 that radiation degradation of chitosan in solution containing H_2O_2 is more effective in comparison with that of non-containing H_2O_2 due to synergistic effect, and the same results were also reported by the other studies [17,22]. This process has been put into large-scale production of oligochitosan (OC) with capacity of 500 L day^{-1} for field application as biotic plant elicitor and plant growth promoter for rice, sugarcane, ect. According to Das *et al.* (2015), the greatest challenge in the application of OC for plant protection lies in the development of efficient methods for large-scale production of OC [23]. Thus, the degradation method by gamma Co-60 ray irradiation can be applied on large-scale production of oligosaccharides including OC. The capacity of production could be easily raised by increasing intensity of Co-60 source.

For radiation degradation of chitosan, degradation extent was reported to depend on DDA [24] and initial molecular weight [25] as well as chitosan concentration in solution [17]. It was reported a general tendency that molecular weight distribution of OC obtained by radiation degradation of chitosan in solution is narrower in comparison with that of initial chitosan [17,26]. DDA of OC (Mw ~7800, Mn ~3400 g mol^{-1}) obtained from 4% chitosan solution irradiated at 21 kGy was of 88.3% that was slightly lower compared with initial chitosan (91.4%). The reason of deamination of chitosan by radiation degradation is still unclear. However, according to Mahmud *et al.* (2014) the oxidation reactions, which caused the cleavage of glycosidic linkages to reduce the molecular weight and also act to remove the amino groups of chitosan slightly under irradiation [27].

Table 1. Value of Mn, PI and DDA of irradiated chitosan from 4% chitosan solution and 4% chitosan/0.5% H_2O_2 solution with dose.

Dose, kGy	4% chitosan			4% chitosan – 0.5% H_2O_2		
	Mn ×10^3	PI*	DDA %	Mn ×10^3	PI*	DDA %
0	13.5	3.33	91.4	13.5	3.33	91.4
3.5	7.2	2.63	90.2	6.4	2.78	89.9
7.0	5.7	2.60	89.4	5.0	2.52	89.1
10.5	4.8	2.56	89.0	3.6	2.48	88.6
14.0	4.2	2.49	88.7	3.0	2.18	88.0
17.5	3.8	2.43	88.5	2.8	1.97	87.6
21.0	3.4	2.30	88.3	2.7	1.88	87.2

**PI (polydispersion index) = Mw/Mn*

The results in Table 1 indicated that the PI values were decreased with the increase of dose for both solutions. In addition, the obtained PI values also indicated that the lower the molecular weight of irradiated chitosan the narrower the molecular weight distribution. In other words, molecular weight distribution of OC is more homogenous than chitosan. The DDA% values (87 – 90%) for irradiated chitosan solution were slightly reduced in comparison with that of initial chitosan (~91%). Thus, it can be deduced that gamma Co-60 irradiation of 4% chitosan solution containing 0.5% H_2O_2 did not cause further decrease in DDA% compare to that of 4% chitosan solution without H_2O_2.

3.2. Characteristics of nanosilica and mixture of OC–nanosilica

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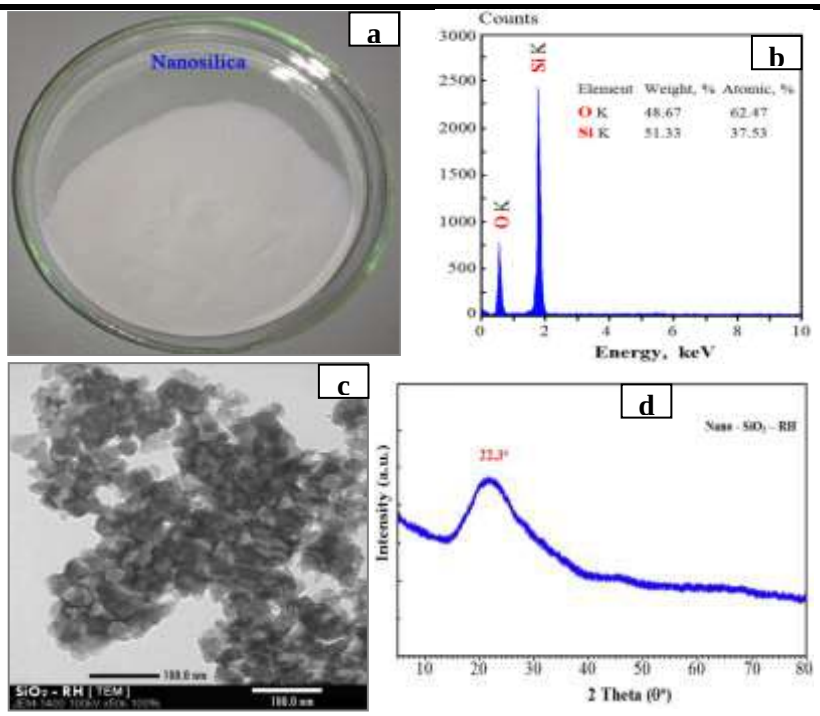

Fig.2: Photograph (a), EDX spectrum (b) TEM image (c) and XRD pattern (d) of nanosilica prepared from rice husk.

The size of as-prepared nanosilica in Fig. 2a was estimated from TEM image to be of 10 – 30 nm (Fig. 2c). The EDX spectrum (Fig. 2b) detected only two peaks for oxigen (O) at 0.525 keV and for silicon (Si) at 1.739 keV. The XRD pattern (Fig. 2d) appreared only one preak at 2θ ≈ 22.3º, which characterized the amorphous structure of nanosilica. Based on the EDX spectrum (Fig. 2b) and XRD pattern (Fig. 2d), nanosilica generated from acid treated rice husk was of high purity and amorphous structure [20].

Fig.3: Photograph of 2% OC solution (left), mixture of 2% OC–2% nanosilica (right), and TEM image of OC–nanosilica.

Photographs of OC solution and mixture of OC–nanosilica were shown in Fig. 3 (left). It was observed that the suspension of OC–nanosilica mixture was homogenous and stable fairly. The TEM image in Fig. 3 (right) indicated that the nanosilica morphology was almost maintained as the origin (Fig. 2c), however some small parts were aggregated that may be presumed due to interaction of nanosilica with OC. Nanosilica (SiO_2) may be changed to $Si(OH)_4$ due to slightly basic medium pH ~7.5 [21]. According to our observation, OC in solution, unlike chitosan, is not precipitated in basic medium at pH 7.5 – 8.5.

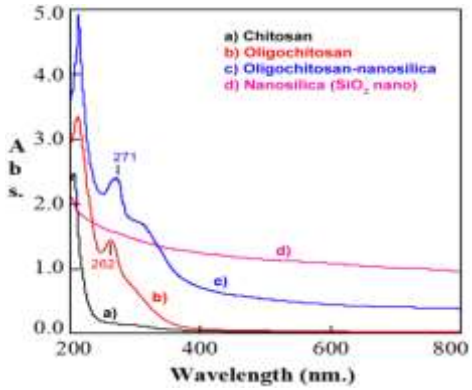

Fig.4: UV-Vis spectra of chitosan (a), OC (b), OC–nanosilica (c) and nanosilica (d).

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The UV-Vis spectra in Fig. 4 showed that the appearance of new peak at 262 nm for OC (Mw ~5000), which was not observed for initial chitosan. The UV-Vis spectrum of nanosilica had no absorption in the range of 200 – 800 nm. The UV-Vis spectrum of OC–nanosilica solution had a peak at 271 nm and a small shoulder around 320 nm. The absorbance band at 262 nm was assigned to C=O in carbonyl groups, which formed in OC molecules during irradiation [25]. In the range of 200 – 800 nm, the nanosilica had no absorption (Fig. 4d), the same result was also obtained by Lu *et al.* (2009) [28]. While the UV-Vis spectrum of OC–nanosilica exhibited a shift of the 262 nm peak of oligochitosan to 271 nm and a shoulder in the range between 300 and 350 nm. This phenomenon may be due to an interaction of OC with nanosilica in the dispersion solution.

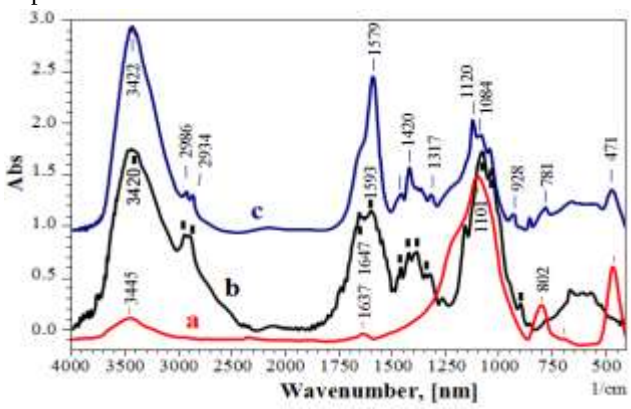

Fig.5: IR spectra of nanosilica (a), OC (b), and their mixture (c)

The FTIR spectroscopy was used to evaluate the interaction between OC and nanosilica. According to results reported our previous articles [17,22], the FTIR spectrum of resultant OC was almost not changed in comparison with that of initial chitosan, suggesting that the main chemical structures of chitosan still remained. The FTIR spectrum of OC (Fig. 5b), the characteristic peaks at 3462; 2850 – 3000; 1647; 1593; 1421; 1319; and 1031 – 1074 cm^{-1} assigned to the vibrations of –OH; C–H; amide I (C=O); amide II (N–H); –OH and C–H in –(CH_2OH); amide III (C–N) of –($NHCOCH_3$); and C–O–C bonds respectively were recorded [29]. The characteristic peaks of silica in Fig. 5a, particularly at 3444; 1637; 1103; 794; 491 cm^{-1} were attributed to stretching vibration of silanol groups (Si–OH); the H–O–H bending vibration of trapped water molecules in silica matrix; the asymmetric stretching; symmetric stretching; and bending vibration of O–Si–O linkages, respectively [30]. In the FTIR spectrum of the OC–nanosilca (Fig. 5c) presented the specific peaks of both OC and silica. Moreover, in this spectrum appeared some of new peaks at 927 cm^{-1} (vibration of silanol group) and the peaks at 1083, 781 cm^{-1} (assumed Si–O–C linkage), but concurrently the peak at 1647 cm^{-1} of OC was disappeared. In addition, the band 1593 cm^{-1} of $–NH_2$ bending vibrations in the spectrum of OC had a shift to 1579 cm^{-1} in the spectrum of OC–nanosilica. All changes in the spectrum of the mixture sample indicating that the interaction of OC and nanosilica in solution actually occurred.

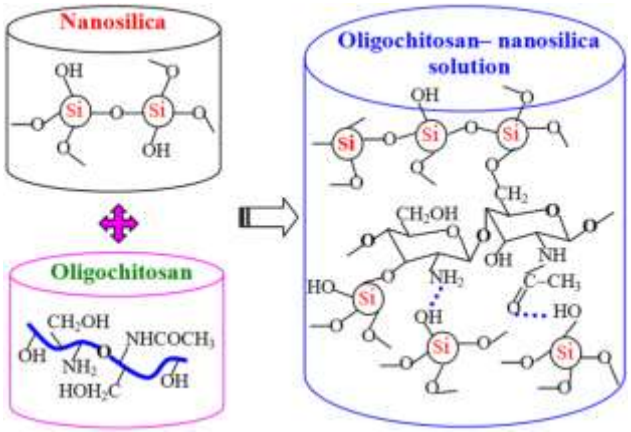

Fig.6: Schematic delineation for the interaction of OC with nanosilica in solution.

According to Al-Sagheer and Muslim (2010) argued that chitosan interacted with tetraethyl orthosilicate by formation of hydrogen bonds between amide groups of chitosan and silanol groups, covalent bonds of chitosan on silanol groups, and ionic bonds between chitosan amino groups and silanol groups of silica network [31]. In this work, however, the pH of OC–nanosilica solution was adjusted to ~7.5, so the ionic bonds between them were unlikely due to the $–NH_2$ groups non-protonated with pH > pKa ~6.3 and the silanol groups negative charge at pH higher than pI ~2 [32]. Consequently, we suggest interaction of OC with nanosilica in OC–nanosilica solution as in Fig. 6.

Table.2: Effect of OC and OC–nanosilica on plant height, dry weight and weight of 1000 soybean seeds.

Treatment	Plant height cm	Dry weight g/5 plants	1000 seed weight, g
Control (water)	45.1	100.1[a]	151.3
OC	48.5	129.7[b]	148.7
OC–nanosilica	48.6	137.3[b]	153.2
$LSD_{0.05}$	NS	28.7	NS

Mean values in each column with the same letter are not different at $P \leq 0.05$.

The results in Table 2 indicated that the treatment of OC and/or OC–nanosilica did not affect the height soybean plant and the weight of 1000 soybean seeds compared with the control. However, the dry weight of soybean plant was increased to 129.7 and 137.3 g/5 plants for OC and OC–nanosilica, respectively compared with the control (100.1 g/5 plants). These results clearly indi-cated

that OC and OC–nanosilica promoted the growth of soybean in term of dry weight of soybean plants.

Table.3: Effect of OC and OC–nanosilica on increase of seed yield and net profit for cultivation of soybean.

Treatment	Seed yield, ton/ha	Increase over control, %	Net profit over control, USD/ha
Control (water)	2.18[a]	-	-
OC	2.41[b]	10.5	120
OC–nanosilica	2.55[c]	17.0	220
$LSD_{0.05}$	0.12	-	-

Mean values in each column with the same letter are not different at $P \leq 0.05$.

The results in Table 3 presented the increase of seed yield of soybean of 10.5 and 17% for OC and OC–nanosilica, respectively compared with the control. Net profit was preliminarily calculated to be of 120 and 220 USD/ha for using OC and OC–nanosilica as plant growth promoter and seed yield enhancer for soybean cultivation based on the price of OC and OC–nanosilica and local labor expense. The results in Table 3 also indicated that nanosilica contributed significantly to increasing the seed yield of soybean together with OC. Although the weight of 1000 seeds was not significantly different among three treatments, but the seed yield even increased when treated with OC and OC–nanosilica. Moreover, further study of the effect of different concentration as well as synergistic effect of combined treatment of OC and nanosilica should be carried out. Khan *et al.* (2003) reported that application of chitin and chitosan oligomers to soybean leaf tissues caused increased activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase enzymes [33]. Results of the study by Luan *et al.* (2006) revealed that OC showed not only plant growth promotion effect but also enhancement of the activity of phytoalexin enzymes namely PAL and chitinase which help plants to prevent the infection of microbial diseases [11].

It is noteworthy that combined seed treatment and foliar application of chitosan increased total isoflavone content of mature soybean seeds by 16 to 93% compared to untreated plants [34]. They concluded that elicitors hold great promise as a way for increasing isoflavone content of mature soybean seeds. In addition, in certain conditions, the impacts of elicitors have on plants physiology and defense response may translate into yield increases, as observed by Luan *et al.* (2006) with 16% seed yield increase of soybean treated with OC [11]. Treatment of soybean seed with chitosan made also increase of seed yield but the concentration was of ten times higher in comparison with OC [10,35]. El-Sawy *et al.* (2010) also reported that OC with Mw of 5000 – 10.000 g/mol exhibited better effect on growth promotion and increase of seed yield of faba bean compared to that of chitosan with higher Mw [36]. Recently, Costales *et al.* (2016) reported that under field conditions, foliar application of both chitosan and OC enhances growth and nodulation of soybean plant [37]. However, it was surprising in their remarks that chitosan is more effective than OC. This hold contradictory result as above mentioned. More study works should be carried out to clarify the difference of the effect of chitosan and OC on plants.

IV. CONCLUSION

This study demonstrated that the method of gamma Co-60 ray irradiation degradation of chitosan in solution to prepare oligochitosan can be favorably applied on large-scale. Foliar application of oligochitosan or oligochitosan–nanosilica enhanced the plant growth and seed yield (10 – 17%) of soybean. Therefore, application of oligochitosan and/or oligochitosan–nanosilica may be recommended for soybean cultivation. However, more experiments on the effect of concentration as well as synergistic effect of combined treatment of oligochitosan and nanosilica should be carried out to draw a valid conclusion of foliar application of oligochitosan and oligochitosan–nanosilica for optimal improvement of soybean seed yield.

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