Carbohydrate Polymers 84 (2011) 751–755

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee

Nguyen Anh Dzung[a,*], Vo Thi Phuong Khanh[b], Tran Trung Dzung[c]

[a] Biological Plant Department, Faculty of Agriculture, Tay Nguyen University, 567 Le Duan Street, Buon Ma Thuot City, Viet Nam
[b] Biological Department, Faculty of Science and Technology, Tay Nguyen University, Viet Nam
[c] Department of Science Management & International Relation, Tay Nguyen University, 567- Le Duan Str., Buon Ma Thuot City, Viet Nam

ARTICLE INFO

Article history:
Received 1 October 2009
Received in revised form 9 May 2010
Accepted 29 July 2010
Available online 7 August 2010

Keywords:
Chitosan
Chitosan oligomer
Coffee
Chlorophyll
Drought resistance

ABSTRACT

Effects of chitosan and chitosan oligomer solutions on biophysical characteristics, growth, development and drought resistance of coffee have been investigated. The experiments which involved spraying chitosan and chitosan oligomer onto the leaves of coffee were conducted in a greenhouse and in the field. The concentration of chitosan and chitosan oligomer solution used was 0, 20, 40, 60 and 80 ppm. Obtained results showed that chitosan oligomer enhanced strongly the content of chlorophylls and carotenoid in the leaves of coffee seedlings up to 46.38–73.51% compared to the greenhouse control. Application of chitosan oligomers also increased mineral uptake of coffee and stimulated the growth of coffee seedlings. Spraying chitosan oligomers with concentration of 60 ppm increased the height of the coffee seedlings up to 33.51%, in the stem diameter up to 30.77% and the leaf in area by up to 60.53%. In addition application of chitosan oligomers reduced by 9.5–25.1% transpiration of the leaves at 60 and 120 min. Therefore the application of chitosan oligomer could be a good way of increasing the drought resistance of coffee seedlings.

Application of chitosan oligomer in field conditions increased content of total chlorophylls up to 15.36% compared to the control. Application of chitosan oligomers also enhanced mineral uptake of coffee by 9.49% N; 11.76% P; 0.98% K; 18.75% Mg; 3.77% Ca and decreased 15.25% the rate of fallen fruits compared to the control, contributed to increasing yield and developing sustainable production of coffee in Vietnam.

1. Introduction

Chitosan is a biopolymer of glucosamine residues, processed from seafood wastes such as shrimp, crab shell or cell wall of fungi (Rinaudo, 2006). Chitosan and its derivatives are non-toxic, biodegradable and friendly to environment. They have been recognized as a product to enhance crop production due to their bioactivities to plants: stimulating growth of plants and seed germination (Chandrkrachang, 2002; Li & Wu, 1998; Luan, Nagasawa, Tamada, & Nakanishi, 2006; Luan et al., 2005; Nge, Nitar, Chandrkrachang, & Steven, 2006; Reddy, Arul, Angers, & Couture, 1999; Sarathchandra, Jai, Amruthesh, & Shekar Shetty, 2004); coating for artificial seeds (Nhut et al., 2005); increasing content of chlorophylls, photosynthesis and chloroplast enlargement (Dzung & Thang, 2004; Dzung, 2005, 2007; Limpanavech et al., 2008); increasing nitrogen fixing nodes of species of *Leguminous* plants (Dzung & Thang, 2002, 2004; Lerouge, 1990); improving soil fertility and increasing mineral nutrient uptake of plant (Dzung, 2005, 2007) and reducing stress of plants (Bitelli, Flury, Campbell, & Nichols, 2001; Limpanavech et al., 2008; Tham et al., 2001). Chitosan and its derivatives also introduce antifungal, antibacterial antivirus activities and being applied as biofungicide for plants (Darvill, 1992; Rabea, Badawy, Steurbaut , & Steven, 2008; Dzung & Thang, 2004; Gou et al., 2008; Hadwiger et al., 2002); enhancing crop production and quality of agricultural products (Ramos-Garcia et al., 2009; Wongroung et al., 2002).

Chitosan and its derivatives can be used with various ways as foliar, coating seed, seedling root dipping; soil enrichment and supplement into plant tissue media. Effects of chitosan and chitosan oligomer on the growth and yield of rice, wheat, maize, black pepper, bean, cabbage, peanut, soybean, tomato, cotton, strawberry have been researched (Chandrkrachang, 2002; Chibu & Shibayama, 2000; Chmielewski et al., 2007; Dzung and Thang, 2002, 2004; Dzung, 2005, 2007; Dzung & Thuoc, 2006; Dzung, Khanh, & Ngoc, 2006; Li & Wu, 1998).

Vietnam is the second biggest coffee production and export country with area of over 500,000 ha and total production over one million tons annually. Coffee production plays an important role for social and economy of Central Highlands of Vietnam. In present,

* Corresponding author. Tel.: +84 905426524; fax: +84 500 3825185.
E-mail addresses: nadzung@dng.vnn.vn (N.A. Dzung), khanhvtp84@yahoo.com.vn (V.T.P. Khanh).

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doi:10.1016/j.carbpol.2010.07.066

main challenges for coffee production in Vietnam are lack of irrigated water and over use of chemical fertilizers. In this context, our research goal is to study effects of chitosan and chitosan oligomer on biophysical characteristics, growth, development and drought resistance of coffee.

2. Materials and methods

2.1. Chemicals and plants

Coffee was used for the research being *Coffea canephora var Robusta*.

Chitin was produced from shrimp shell by the method of Shimahara and Takiguchi (1988). Chitosan was produced by deacetylation of chitin in strong concentration of NaOH solution (50%, w/w) for 3 h at 110 °C. Degree of deacetylation of chitosan was approximately 80% determined by IR (Domzy & Robert, 1985). The viscosity molecular weight of the chitosan was about 750,000 determined by Mark–Houwink equation: the intrinsic viscosity $[\eta] = K_m \cdot M_v^a$, where K_m and a were indicated previously as 1.81×10^{-3} cm^3 g^{-1} and 0.93 (Robert & Domzy, 1982). Chitosan oligomers with average molecular weight being about 2 kDa (dp 8–16) was produced from 1% chitosan solution by cellulase degradation and fractionated by organic solvents (Muraki, Yaku, & Kojima, 1993).

Chitosan solution was prepared by dissolving 1 g of chitosan powder in 100 ml 0.5% acetic acid solution, kept it overnight and diluted by the addition of water.

2.2. Experiment of coffee seedlings in greenhouse

The experiment was conducted in the greenhouse of the Faculty of Agriculture, Tay Nguyen University. The experiment was performed with 5 plots and in triplicate and 30 the plants per plot. Coffee seedlings were planted in polyetylen bags (size 15 cm × 25 cm), consisting Ferrasols mixed with 5% degraded manure. After 1 month planting, 30 coffee seedlings (each plot) were sprayed with 0, 20, 40, 60 and 80 ppm of chitosan oligomer on two sides of the leaves and volume of 500 ml. During the time of the experiment, application of chitosan oligomer were used three times with period of 15 days. The irrigation control assured that water content in the soil of all plots were similar. Fertilizing regime and the micro-climate condition were identical (air temperature was 25 °C, humidity was 85% and light intensity was 20,000 lux).

The growth of coffee seedlings were determined by height of the plant, area of leaves and diameter of the stem. The height of the coffee seedlings were measured by ruler from ground to pinnacle of the plants. Diameter of the stem of coffee seedlings were measured by Panmer. Area of the leaf was determined by measuring length and diameter of the leaf by following equation:

$$S = Kab$$

where S is area of the leaf; a and b are length and diameter of the leaf. K is 0.66 (specific index of Robusta coffee leaf). All parameters of the growth of coffee seedlings were measured 5 plants for each plot.

2.3. Experiment of coffee in the field

Coffee plantation for field research was placed in Cu Bao commune, Krong Buk district, Dak Lak province, Vietnam and planted on Ferrasols in 1992.

The experiment was performed in the rainy season (from May to December). This is period of coffee bearing fruits. The experiment had 5 formulas of chitosan and chitosan oligomer (0, 20, 40, 60 and 80 ppm) and in triplicate; 15 plots were arranged with random complete block design (RCBD). The application volume of chitosan and chitosan oligomer solution was 5 l for 12 the plants of each plot. The coffee was sprayed with 3 times during the experiment with a period of 30 days. Fertilizing regime and the other cultivation condition were similar for all the experimental plots.

2.4. Plant analyses

After 1 month spaying chitosan and chitosan oligomer, the leaves were collected to analyze the content of mineral nutrients. The leaves were dried at 105 °C until their weight was unchanged and ground up for analysis.

Total nitrogen of the leaves was determined by Kjeldahl method. Total phosphorous was measured by the colorimetric molybdate blue method (Olsen & Sommers, 1982) following Kjeldahl digestion. Total potassium was measured by flame spectrophotometer. Total calcium and magnesium were measured by titration with Trilon B.

Content of chlorophyll and carotenoid was determined (Yoshida & Forno, 1976) as follows: Coffee leaves were cut in small pieces and immersed in 80% acetone solvent to extract pigments. The extraction solvent was diluted and measured with spectrophotometer at 663, 645 and 440.5 nm. The content of pigments were calculated by equation:

$$C_a = 0.0127 \cdot D_{663} - 0.00269 \cdot D_{645}$$

$$C_b\,(\text{mg g}^{-1}\text{ fresh leaf}) = 0.0299 \cdot D_{645} - 0.00468 \cdot D_{663}$$

$$C_{car}(\text{mg g}^{-1}\text{ fresh leaf}) = 0.004695 \cdot D_{440.5} - 0.000268\,(C_a + C_b).$$

where C_a, C_b (mg g^{-1} fresh leaf) are content of chlorophyll a and b; and C_{car} (mg g^{-1} fresh leaf) is content of carotenoid.

To determine transpiration six leaves of the six plants of each plot were collected and kept in greenhouse conditions. After 20, 40, 60, 80, 100 and 120 min, the leaves were weighed to measure their transpiration. The intensity of transpiration was determined as following:

$$I = (W_0 - W_t)\,S^{-1}\,t^{-1}$$

where I is intensity of transpiration (unit: mg cm^{-2} min^{-1}); S is area of the leaves (cm^2); W_0: the weight of the leaves after cutting; W_t: the weight of the leaves after t min in the greenhouse condition.

2.5. Method for measuring the growth of branches and the rate of fallen fruits

5 plants of each plot and 5 branches from each plant were selected at random. The length of branches and number of fruits before and after 3 months spraying with chitosan and chitosan oligomers were measured.

2.6. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and followed by Duncan's multiple range test with triplicate. P-Value ≤ 0.05 considered as significant.

3. Results and discussion

3.1. Effect of chitosan oligomer on content of chlorophylls in the coffee leaves

In plants, photosynthesis occurs primarily in leaf cells in organelles called chloroplast containing chlorophyll a, b and carotenoid. Chlorophylls and carotenoid have important roles in

Table 1
Effect of chitosan oligomer on content of chlorophylls and carotenoid in the leaves of coffee seedlings (in greenhouse) and production coffee (in the field). Data are means of triplicate. Different superscripts in the same row are significantly different between the treatments at 5% level according to Duncan' tests.

Plant	Pigments (mg g^{-1} fresh leaf)	0 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Coffee seedlings	Chlorophyll a	0.70^{c}	0.77^{c}	0.95^{b}	1.18^{a}	0.91^{b}
	Chlorophyll b	0.35^{c}	0.38^{c}	0.48^{b}	0.56^{a}	0.47^{b}
	Carotenoid	0.44^{c}	0.51^{c}	0.66^{b}	0.76^{b}	0.63^{b}
Production coffee	Chlorophyll a	1.60^{c}	1.66^{c}	1.86^{a}	1.87^{a}	1.73^{b}
	Chlorophyll b	0.61^{c}	0.62^{c}	0.74^{a}	0.75^{a}	0.65^{c}
	Carotenoid	0.78^{c}	0.81^{c}	0.92^{a}	0.93^{a}	0.85^{b}

Table 2
Effect of chitosan oligomer on mineral nutrients uptake of the coffee leaves in the field. Data are means of triplicate. Different superscripts in the same column are significantly different between the treatments at 5% level according to Duncan' tests.

Times of spraying	Concentration oligomer (ppm)	N%	P%	K%	Ca%	Mg%
1st	0	2.95^{b}	0.16	2.01	1.03	0.30^{b}
	40	3.16^{a}	0.18	2.02	1.06	0.30^{b}
	60	3.02^{a}	0.18	2.02	1.01	0.35^{a}
	80	3.12^{a}	0.18	2.03	1.05	0.34^{a}
2nd	0	2.96^{b}	0.17	2.05	1.03	0.30^{b}
	40	3.23^{a}	0.19	2.06	1.04	0.35^{a}
	60	3.34^{a}	0.19	2.08	1.13	0.39^{a}
	80	3.32^{a}	0.19	2.09	1.12	0.38^{a}
3rd	0	2.93^{b}	0.17	2.05	1.12	0.35^{a}
	40	3.16^{a}	0.18	2.07	1.16	0.38^{a}
	60	3.34^{a}	0.19	2.07	1.15	0.41^{a}
	80	3.33	0.18	2.06	1.15	0.42^{a}

photosynthesis such as the production of sugars and organic molecules by fixation of CO2 and water. It has been recognized that application of chitosan and chitosan oligomer on the leaves of the plants enhanced the content of chlorophylls and carotenoid. After 3 applications, the leaves of coffee seedlings were collected and analyzed. The experiment results (see Table 1) showed that chitosan oligomer enhanced content of chlorophyll a, b and carotenoid in the leaves. The content of chlorophyll a increased by 1.18 mg g^{-1} fresh leaf compared to the control of 0.70 mg g^{-1}. Concentration of chitosan oligomer of 60 ppm was optimal to increase content of chlorophyll of coffee seedlings in greenhouse and the production of coffee in the field.

In the greenhouse experiment, chitosan oligomers increased the content of chlorophylls up to 46.38–73.51% compared to the control. In the field experiment, the content of chlorophylls increased by only 15.36% (see Table 1). These results were demonstrated through the significant increasing of magnesium and total nitrogen in the leaves (Table 2), because they are very important elements in composition of chlorophylls. The same results were also showed on other plants. Dzung and Thang, 2004; Dzung, 2005 reported that chitosan oligomer with molecular weight of 2 kDa increased content of chlorophyll of soybean and peanut by 17.9% and 23.0% in the field experiment. Concentration of chitosan oligomers was suitable for increasing content of chlorophylls of soybean and peanut being 30 ppm as same as the optimal concentration for plant growth. Limpanavech et al. (2008) also used chitosan with Mw of 45 kDa, 90%DD at 1, 10, 50 and 100 ppm to spray on *Dendrobium* orchid plants. It was found that the chitosan increased significantly the chloroplast diameter of *Dendrobium* orchid at concentration of 10–50 ppm in young leaves and at 50 ppm in old leaves. Diameter of chloroplast was of 9.15 μm (control) and increased up to 12.16 μm at 10 ppm in young leaves and from 7.97 μm (control) up to 10.43 μm at 50 ppm in old leaves. Limpanavech also demonstrated that chitosan effected chloroplast gene expression, so the change in chloroplast size and enlargement of chloroplast may be one of the factors led to stimulate the growth of plants. Nitar, Chandrkrachang, and Stevens (2004) also reported that application of chitosan oligomer for rice helped the leaves of rice to be greener.

Uddin, Hashimoto, Shimizu, and Sakata (2004) reported that *Eustoma grandiflorum* treated with chitosan solution and monosaccharide enhanced bud growth and petal pigmentation. Treatment with chitosan promoted processes in developing the flower bud and enhanced the accumulation of anthocyanin in petals in vitro to increase the quality of the flowers.

3.2. Effect of chitosan oligomer on mineral nutrient uptake of coffee

Increasing content of chlorophyll led to the promotion of photosynthesis of coffee and the uptake mineral nutrients. Results in Table 2 showed that the chitosan oligomer increased accumulation of mineral nutrients in the leaf of production coffee. Application of chitosan oligomer increased mineral uptake of coffee such as: 9.49% N; 11.76% P; 0.98% K; 3.77% Ca. Particularly, the content of Mg increased up to 18.75%, leading to increasing of chlorophyll in the leaves (Table 1).

Table 3
Effect of chitosan oligomer on transpiration of the coffee leaves in the greenhouse. Data are means of triplicate.

Concentration of chitosan oligomer	Intensity of transpiration (mg cm^{-2} min^{-1})					
	20 min	40 min	60 min	80 min	100 min	120 min
Control	2.21	2.02	1.83	1.82	1.84	1.74
20 ppm	2.05	1.95	1.84	1.80	1.65	1.63
40 ppm	2.09	1.85	1.81	1.77	1.64	1.63
60 ppm	1.97	1.78	1.67	1.52	1.43	1.39
80 ppm	2.06	1.88	1.68	1.52	1.51	1.47

Table 4
Effect of chitosan oligomer on the growth of coffee seedlings in greenhouse. Data are means of three replicates. Different superscripts in the same row are significantly different between the treatments at 5% level according to Duncan' tests.

Data of growth	0 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Height of plant, cm	20.35[c]	21.91[c]	23.15[b]	27.17[a]	25.58[a]
Diameter of stem, cm	0.39[c]	0.41[c]	0.44[b]	0.51[a]	0.49[a]
Number of leaves per plant	12.10[c]	12.38[c]	12.82[b]	14.52[a]	14.04[a]
Area of leaves, cm^2 per plant	50.59[c]	57.31[c]	68.53[b]	81.21[a]	75.03[a]

Table 5
Effect of chitosan and chitosan oligomer on the growth of branches of coffee (after 3 months spraying). Unit: cm $month^{-1}$. Data are means of triplicate.

Concentration	0 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Chitosan	5.14	5.57	5.72	5.96	6.07
% increasing	0.00	8.38	11.28	15.95	18.09
Chitosan oligomer	5.34	5.52	5.96	6.37	6.09
% increasing	0.0	3.37	11.61	19.28	14.04

3.3. Effect of chitosan oligomer on transpiration of the leaves of coffee seedlings

Transpiration of coffee seedlings in the greenhouse applied chitosan oligomers was also tested. Results in Table 3 indicated that chitosan oligomers sprayed onto the leaves reduced transpiration of leaves of coffee seedlings in greenhouse from 1.83 mg cm^{-2} min^{-1} down to 1.67 mg cm^{-2} min^{-1} after 60 min at 60 ppm chitosan oligomer. After 120 min, the intensity of transpiration in 60 ppm chitosan oligomer decreased quickly, from 1.74 mg cm^{-2} min^{-1}(control) to 1.39 mg cm^{-2} min^{-1}. This result showed that application of chitosan oligomer increased drought resistance of coffee seedlings.

Application of chitosan reduced transpiration of plant by inducing closure of plant stomata (Bitelli et al., 2001). It was found that the foliar application reduced water consumption of pepper by 26–43% while maintaining biomass and yield of pepper in growth chamber as well as field condition. Reducing water use by chitosan foliar application is the best way helping plants to deal with drought and use economically water resources.

3.4. Effect of chitosan oligomer on the growth of coffee

It has been known that both bioactivities of chitosan and its derivatives as induction of defensive system of plants and plant growth stimulation play key roles in application of chitosan for agriculture. Table 4 showed that chitosan oligomers effected strongly the growth of coffee seedlings such as increasing height of the plants, diameter of stem, number of leaves and area of leaves. Results also recognized that suitable chitosan oligomer concentration for the growth of coffee seedlings was 60–80 ppm.

Growth and yield of coffee in the field depend on growth of branches. Effect of chitosan oligomer on growth of branches of coffee was shown in Table 5 as same as coffee seedlings, increased up from 3.37% to 19.28%. Chibu and Shibayama (2000) (2001) also showed that chitosan application at concentration of 0.5% was suitable for soybean and upland rice, but more effective concentration for lettuce and mini tomato was 0.1%. The leaf area of lettuce increased by 50–60% compared to the control after 3 times of foliar application at concentration of 0.1% and the leaf area of radish also increased up to 100% at concentration of 0.5%.

Degraded chitosan by irradiation of gamma rays supplemented in hydroponic solutions at concentration of 100 and 200 ppm stimulated strongly the growth of rice up to 24% and wheat up to 40% in comparison to the control (Tham et al., 2001).

3.5. Effect of chitosan oligomers on the rate of fallen fruits

Chitosan oligomers effected strongly the content of chlorophylls, enhanced accumulation of mineral nutrients in the leaf and growth of coffee that led to a reduction in the rate of fallen fruits and an increase in the coffee yield (Table 6). The results indicated that chitosan oligomer reduced the rate of fallen fruits from 26.20% (control) down to 19.40% at 60 ppm chitosan oligomer and from 21.20% (control) down to 18.65% at 60 ppm chitosan. The suitable concentration of chitosan oligomer and chitosan for growth and development of coffee was 60 ppm. When increasing concentration of chitosan and chitosan oligomers over the optimal concentration, chitosan and chitosan oligomers tend to decrease in the growth and development of plants. This is because the higher concentration of chitosan and chitosan oligomer increases closure of stomata of plants and reduces exchange of CO_2 gas of the leaf. This leads to a reduction in photosynthetic intensity of the plants.

Application of chitosan solution was strongly effective on the growth of pearl millet. Chitosan treatment increased the height of pearl millet from 81.6 cm (control) to 115.7 cm (treatment). Number of tillers, number of ear heads and 1000 seeds weight also increased significantly in the treated plants compared to the control. It was found that there was 19% increase in 1000 seeds weight and 50% in number of ear heads (Sarathchandra et al., 2004).

Wanichpongpan, Suriyachan, and Chandrkrachang (2001) also showed that stem length of Gerbera (*Gerbera jamesonii*) increased from 28.28 cm (control) to 30.68 cm (at 40 ppm chitosan); The leaf length was also increased from 7.39 cm (control) to 10.75 cm (at 40 ppm chitosan). But increasing the number of flowers per bush was only optimal at 60 ppm. At this concentration, the number of flowers increased from 2.84 unit (control) to 4.98 unit (at 60 ppm chitosan). Therefore, it appeared that 40 ppm chitosan was appro-

Table 6
Effect of chitosan and chitosan oligomer on the rate of fallen fruits. Data are means of triplicate. Different letters in the same row are significantly different between the treatments at 5% level according to Duncan' tests. Unit: %.

Concentration	0 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Chitosan	21.20[c]	20.44[c]	20.29[b]	18.65[a]	19.17[a]
Chitosan oligomer	26.20[c]	24.45[c]	20.73[a]	19.40[a]	20.19[a]

priate for growth of Gerbera, and 60 ppm chitosan was effective for development of Gerbera.

4. Conclusion

Chitosan oligomers effected significantly on the growth of coffee seedlings under greenhouse conditions and in the field by enhancing content of chlorophylls, carotenoid and mineral nutrient uptake; increasing area of leaves, height and stem diameter of plants. In addition, application of chitosan oligomer decreased intensity of transpiration of the leaves. Under field conditions, application of chitosan and chitosan oligomer increased mineral uptake and the growth of braches. The growth stimulation of chitosan oligomers led to a reduction in the rate of fallen fruit of coffee. The appropriate concentration of chitosan and chitosan oligomer for the growth and development of both coffee seedlings and production coffee in the field was of 60 ppm. When applying the same concentration, of chitosan oligomers had a greater effect on the growth and development of coffee than chitosan.

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