Chitosan as Soil Amendment Affects Lettuce Growth, Photochemical Efficiency, and Gas Exchange

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SUMMARY. Chitosan has become of interest as a crop biostimulant suitable for use in sustainable agriculture since it is biocompatible, biodegradable, environmentally friendly, and readily available in large quantity. Short-term (35 d after transplanting) effects of chitosan, applied as a soil amendment at 0%, 0.05%, 0.10%, 0.15%, 0.20%, or 0.30% (w/w), on lettuce (*Lactuca sativa*) growth, chlorophyll fluorescence, and gas exchange were evaluated in a growth chamber study. Chitosan at 0.05%, 0.10%, and 0.15% increased leaf area from 674 to 856, 847, and 856 cm², and leaf fresh weight from 28.6 to 39.4, 39.1, and 39.8 g, respectively. Only chitosan at 0.05% and 0.10% increased leaf dry weight from 3.42 to 4.37 and 4.35 g, respectively, while chitosan at 0.30% decreased leaf number, area, fresh and dry weight. Chitosan at 0.10%, 0.15%, 0.20%, and 0.30% increased leaf chlorophyll index from 29.8 to 34.4, 35.4, 37.5, and 41.4, respectively. Chitosan at 0.20% and 0.30% increased leaf maximum photochemical efficiency and photochemical yield, and chitosan at 0.10%, 0.15%, 0.20%, and 0.30% increased leaf electron transport rate. Leaf photosynthesis rate and stomatal conductance (g_s) increased from 9.3 to 12.7, 14.0, and 16.6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ carbon dioxide, and from 0.134 to 0.183, 0.196, and 0.231 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, under chitosan at 0.15%, 0.20%, and 0.30%, respectively. The results indicated that chitosan, at appropriate application rates, enhanced lettuce growth, and might have potential to be used for sustainable production of lettuce.

Chitosan is the deacetylated form of chitin, which is the second most abundant polysaccharide on the planet and the main component of fungal cell walls, insect exoskeletons, and crustacean shells (Gooday, 1990). It was initially reported as an elicitor of plant responses, since it induced phytoalexin (pisatin) production, and as a proteinase inhibitor in plants (Walker-Simmons et al., 1983). Since then, biochemical and molecular responses in plants exposed to chitosan have been investigated and they include increases in cytosolic calcium ion (Zuppini et al., 2003),

activation of mitogen-activated protein kinases (Yin et al., 2010), oxidative burst (Paulert et al., 2010), callose apposition (Kohle et al., 1985), increase in pathogenesis-related gene mRNA and protein synthesis (Loschke et al., 1983), phytoalexin accumulation and hypersensitive response (Hadwiger and Beckman, 1980), synthesis of jasmonic acid and abscisic acid, and accumulation of hydrogen peroxide (Iriti and Faoro, 2009; Lin et al., 2005). Chitosan has also been extensively studied as a plant protectant to reduce disease incidence and severity in many crops by inhibiting microbial growth and decreasing microbial membrane integrity (Maqbool et al., 2010; Palma-Guerrero et al., 2008; Prapagdee et al., 2007; Xu et al., 2007).

In addition, chitosan has become of interest as a crop biostimulant suitable for use in sustainable agriculture (Pichyangkura and Chadchawan, 2015; Sharp, 2013). Extensive application of synthetic chemicals, such as fertilizers, herbicides and pesticides, to increase crop productivity has been widely practiced to meet food demand around the world. However, they can cause considerable damage to the ecology of agricultural systems and reduce the nutritional quality of crops (Herrick, 2000; Kirschenmann, 2010). Chitosan is biocompatible, biodegradable, environmentally friendly, and readily available in large quantity. It has been reported to improve growth and production of many horticultural crops including vegetable, fruit, and ornamental crops, but in most of those experiments chitosan was foliar applied (El-Miniawy et al., 2013; Farouk and Amany, 2012; Pichyangkura and Chadchawan, 2015; Pirbalouti et al., 2017). Lettuce (*Lactuca sativa*) is one of the most important salad vegetables in the United States, and contains important phytochemicals, including vitamins, carotenoids, and other antioxidants (Humphries and Khachik, 2003; Nicolle et al., 2004). The objective of this study was to assess the effects of chitosan as a soil amendment on lettuce growth, chlorophyll fluorescence, and gas exchange.

Materials and methods

PLANT MATERIALS AND EXPERIMENTS. Two trials, each with four replications, were conducted in a growth chamber, maintained at day/night temperatures of 20/15 °C, and a photoperiod of 14 h with 700 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux (PPF). For each trial, 2 weeks after seeding, uniform lettuce seedlings (cv. Heart's Delight) were transplanted into 1 L plastic pots filled with 1.2 kg field soil (sandy loam) mixed with chitosan (Sigma-Aldrich, St. Louis, MO) at 0%, 0.05%, 0.10%, 0.15%,

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.0929	ft ²	m ²	10.7639
3.7854	gal	L	0.2642
6.4516	inch ²	cm ²	0.1550
0.4536	lb	kg	2.2046
28.3495	oz	g	0.0353
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

0.20%, or 0.30% (w/w), transferred to the growth chamber and watered to capacity with full-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). The four pots of lettuce for each treatment were watered twice weekly. A complete randomized design was used for each trial in this experiment. Each biological replicate contained one pot with one plant and each treatment included four replicate pots.

GROWTH AND PHYSIOLOGY MEASUREMENTS. For each trial, leaf chlorophyll index was measured at 35 d after transplanting on the four largest leaves of each plant with a chlorophyll index meter (SPAD-502; Konica Minolta Sensing, Tokyo, Japan). Leaf maximum photochemical efficiency (F_v/F_m), photochemical yield [$Y(II)$] and electron transport rate (ETR) were measured with a fluorometer (MINI-PAM-II; Heinz Walz, Effeltrich, Germany) on the four largest leaves of each plant. Leaf F_v/F_m was measured after leaves were adapted in darkness for 30 min. Leaf net photosynthetic rate [P_n ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ carbon dioxide (CO_2))], transpiration [Tr ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ water (H_2O))], and g_s [Cond ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)] were determined on the four largest leaves of each plant using a portable infrared gas analyzer (Li-6400; LI-COR, Lincoln, NE). The analyzer was set at a flow rate of $500\ \mu\text{mol}\cdot\text{s}^{-1}$, leaf temperature of $20 \pm 0.4\ ^\circ\text{C}$, relative humidity of $60\% \pm 5\%$, with a light emitting diode external light source providing a PPF density of $700\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Then plants were harvested to measure leaf number, area, shoot fresh and dry weight (FW and

DW). Leaf area was measured with a leaf area meter (CI-202 laser area meter; CID Bio-Science, Camas, WA). Sample DW was measured after drying at $65\ ^\circ\text{C}$ for 3 d.

STATISTICAL ANALYSIS. The interaction of the two trials was not significant, so data were pooled together. The mean values of each measured variable in lettuce growth, gas exchange, and chlorophyll fluorescence were separated by Duncan's multiple range test at the 0.05 level of probability using the JMP program (version 5; SAS Institute, Cary, NC) with general linear model. The regression analysis was performed on leaf FW, chlorophyll index, and P_n .

Results and discussion

Chitosan affected lettuce growth but the responses varied with growth parameters and application rates (Table 1). Chitosan at 0.15% increased leaf numbers from 12.4 to 13.6 leaves per plant. Leaf area per plant increased from $674\ \text{cm}^2$ to 856, 847, and $856\ \text{cm}^2$ with chitosan at 0.05%, 0.10%, and 0.15%, respectively. Chitosan at 0.05%, 0.10%, 0.15%, and 0.20% increased leaf FW from 28.6 to 39.4, 39.1, 39.8, and $36.2\ \text{g/plant}$, respectively. Only chitosan at 0.05% and 0.10% increased leaf DW from 3.42 to 4.37 and 4.35 g. All chitosan treatments reduced DW/FW ratio. Chitosan at 0.30% decreased leaf number from 12.4 to 10.9, area from 674 to $480\ \text{cm}^2$, FW from 28.6 to $21.1\ \text{g}$, and DW from 3.42 to $2.27\ \text{g}$. Leaf number, area, FW, and DW showed a quadratic function of application rates (Table 1). The quadratic equation for

FW was $y = -680.38x^2 + 172.35x + 29.672$ and R^2 of 0.6802 (Fig. 1). There was a significant linear effect on leaf chlorophyll index by application rates with an R^2 of 0.8509 and the equation was $y = 37.389x + 30.053$ (Fig. 1). Chitosan at 0.10%, 0.15%, 0.20%, and 0.30% increased leaf chlorophyll index from 29.8 to 34.4, 35.4, 37.5, and 41.4.

As a soil amendment, chitosan has been found to enhance plant height, canopy diameter, and leaf area of chili pepper [*Capsicum annuum* (Chookhongkha et al., 2012)], improve soybean (*Glycine max*) nodulation and seed yield (Ali et al., 1997), and increase plant height, DW, leaf number and area, and chlorophyll index of tomato (*Solanum lycopersicum*), rice (*Oryza sativa*), lettuce, and radish (*Raphanus raphanistrum* ssp. *sativus*) (Boonlertnirun et al., 2008; Chibu and Shibayama, 2003; Farouk et al., 2011). Soil-applied chitosan also significantly prompted seedling growth and induced early flowering of many ornamental crops (Pichyangkura and Chadchawan, 2015). Similarly, the present study shows that soil-applied chitosan increased lettuce leaf number, area, FW, DW, and chlorophyll index. The synergistic effects of many factors, such as suppression of plant diseases, insects, and nematodes, increased biomass and activities of beneficial microbes, high nitrogen and calcium content, improved physical structure of soil and nutrient availability, and direct plant growth stimulation, may have resulted from chitosan as a soil amendment.

Table 1. Lettuce leaf growth after 35 d grown on soil amended with different rates of chitosan. Two-week-old lettuce seedlings were transplanted in 1-L (0.26 gal) plastic pots with soil amended with different rates of chitosan and grown in a growth chamber for 35 d. Pots were irrigated to capacity with full-strength Hoagland's nutrient solution after transplanting and subsequently watered twice weekly.

Chitosan rate (%)	Leaves (no./plant)	Leaf area (cm^2) ^z	FW (g) ^y	DW (g) ^y	DW/FW (ratio)	Chlorophyll index
	mean $\pm$ SE					
0.00	$12.4 \pm 0.24\ \text{b}^*$	$674 \pm 27\ \text{b}$	$28.6 \pm 1.62\ \text{b}$	$3.42 \pm 0.20\ \text{b}$	$0.120 \pm 0.0012\ \text{a}$	$29.8 \pm 0.47\ \text{d}$
0.05	$13.0 \pm 0.32\ \text{ab}$	$856 \pm 18\ \text{a}$	$39.4 \pm 0.91\ \text{a}$	$4.37 \pm 0.12\ \text{a}$	$0.111 \pm 0.0014\ \text{b}$	$31.8 \pm 0.52\ \text{d}$
0.10	$12.8 \pm 0.37\ \text{ab}$	$847 \pm 17\ \text{a}$	$39.1 \pm 1.75\ \text{a}$	$4.35 \pm 0.26\ \text{a}$	$0.111 \pm 0.0017\ \text{b}$	$34.4 \pm 0.76\ \text{c}$
0.15	$13.6 \pm 0.24\ \text{a}$	$856 \pm 34\ \text{a}$	$39.8 \pm 2.39\ \text{a}$	$4.10 \pm 0.17\ \text{ab}$	$0.103 \pm 0.0024\ \text{c}$	$35.4 \pm 0.52\ \text{bc}$
0.20	$13.2 \pm 0.37\ \text{ab}$	$781 \pm 50\ \text{ab}$	$36.2 \pm 2.45\ \text{a}$	$3.69 \pm 0.25\ \text{ab}$	$0.102 \pm 0.0017\ \text{c}$	$37.5 \pm 0.81\ \text{b}$
0.30	$10.9 \pm 0.32\ \text{c}$	$480 \pm 81\ \text{c}$	$21.1 \pm 4.14\ \text{c}$	$2.27 \pm 0.49\ \text{c}$	$0.106 \pm 0.0023\ \text{bc}$	$41.4 \pm 1.38\ \text{a}$
Regression	0.006	0.001	0.011	<0.001	<0.001	<0.001
	$0.6144\ (\text{Q})^w$	$0.7187\ (\text{Q})$	$0.6802\ (\text{Q})$	$0.6479\ (\text{Q})$	$0.6846\ (\text{Q})$	$0.8509\ (\text{L})$

^z1 $\text{cm}^2 = 0.1550\ \text{inch}^2$.

^yFresh or dry weight; 1 g = 0.0353 oz.

^{*}Different letters indicate significant difference at $P \leq 0.05$ according to Duncan's multiple range test.

^wQuadratic or linear.

Fig. 1. The responses of lettuce leaf fresh weight, chlorophyll index and photosynthetic rate to chitosan application rate with quadratic or linear curve, regression equation and R^2 values. Two-week-old lettuce seedlings were transplanted in 1-L (0.26 gal) plastic pots with soil amended with different rates of chitosan and grown in a growth chamber for 35 d. Pots were irrigated to capacity with full-strength Hoagland's nutrient solution after transplanting and subsequently watered twice weekly; Pn = net photosynthetic rate, CO_2 = carbon dioxide, $1 \text{ cm}^2 = 0.1550 \text{ inch}^2$, $1 \text{ g} = 0.0353 \text{ oz}$.

Chitosan as a soil amendment has repeatedly been shown to have strong insecticidal activity, to reduce pathogenic nematode populations, and to control fungal and viral diseases in numerous crops (Aziz et al., 2006; Pospieszny et al., 1991; Rabea et al., 2005; Rodriguez-Kabana et al., 1984). In addition, a substantial body of evidence suggested that the addition of chitosan alters rhizosphere conditions to shift the microbial balance in favor of beneficial organisms and to the detriment of plant pathogens (Sharp, 2013). Chitosan can provide a carbon source for microbes in the soil, accelerate transformation of organic matter into inorganic matter, and assist roots in absorbing more nutrients from the soil (Bolto et al., 2004; Somashekar and Richard, 1996).

Chitosan, and all other chitin derivatives, have a high nitrogen content of 6% to 9% (Yen and Mau, 2007), comparable with other organic fertilizers such as dried blood and bone meal (White, 2006). Plants can access the nitrogen in chitin via microbial breakdown and the release of inorganic nitrogen, or by directly taking up monomers as organic nitrogen (Roberts and Jones, 2012; Spiegel et al., 1988). Chitosan can be used to add organic matter to soils without raising the carbon:nitrogen ratio. In addition to nitrogen, chitosan also contains substantial levels of calcium minerals, which provide structural rigidity to the exoskeletons of crustaceans (Boßelmann et al., 2007). Although chitosan contains nitrogen and calcium, its positive effects on crop growth and yield were not only due to its nutrients, since in some studies the nutrients in chitosan were equalized in the control plots treated with inorganic fertilizers. Ohta et al. (2004) and Spiegel et al. (1988) demonstrated that chitosan significantly prompted growth of seedlings of several ornamental plants and Chinese cabbage (*Brassica rapa* ssp. *pekinensis*), compared with standard mineral fertilizer.

The cationic properties of chitosan also make it suitable as a medium for supplying additional essential nutrients (Sharp, 2013). The functional hydroxyl and amino groups on deacetylated chitosan allow the formation of coordination compounds with ions of copper, zinc, iron, and others, but not with those of alkaline metals (e.g., potassium) or alkaline earth metals (e.g., calcium or magnesium) (Ramírez et al., 2010). This makes chitosan a sustainable alternative to synthetic chelation agents, such as ethylenediaminetetraacetic acid, that are routinely used to deliver iron and other nutrients to overcome their poor solubility in calcareous/neutral soils (Bohn et al., 2002). Due to its high molecular weight and porous structure, chitosan can form gels that absorb substantial volumes of water to increase soil water holding capacity (Jamnongkan and Kaewpirom, 2010; Tamura et al., 2006).

Plant growth stimulation by chitosan as a soil amendment might also result from its direct effect on plant nutrient status and metabolism, and photosynthesis. Soil-applied chitosan

increased the content of nitrogen, phosphorus, potassium, total sugars, and soluble proteins as well as total amino acids of radish (Farouk et al., 2011). Foliar application of chitosan was reported to increase leaf nitrate reductase activity in Indian spinach (*Basella alba*) and okra (*Abelmoschus esculentus*) (Mondal et al., 2011, 2012). Soil-applied chitosan has been reported to increase leaf chlorophyll content in many crops (Chibu and Shibayama, 2003; Farouk et al., 2011; Sheikh and Al-Malki, 2011). As a biostimulant, chitosan could also improve chlorophyll fluorescence and increase photosynthetic rate as discussed below.

In this study, application rates had a linear effect on leaf Fv/Fm, Y(II) and ETR with R^2 of 0.3427, 0.2219, and 0.7311, respectively (Table 2). Compared with control, chitosan at 0.20% and 0.30% increased leaf Fv/Fm from 0.84 to 0.87 and 0.87, and Y(II) from 0.359 to 0.503 and 0.497, respectively (Table 2). Chitosan at 0.10%, 0.15%, 0.20%, and 0.30% increased leaf ETR from 99 to 126, 126, 159, and 153 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. Leaf Pn, g_s , and Tr were also linearly affected by application rate with R^2 of 0.4984, 0.1595, and 0.1483, respectively (Table 2). The linear equation for Pn was $y = 24.603x + 8.435$ (Fig. 1). Leaf Pn and g_s increased from 9.3 to 12.7, 14.0, and 16.6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ CO_2 , and from 0.134 to 0.183, 0.196, and 0.231 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, under chitosan at 0.15%, 0.20%, and 0.30%, respectively (Table 2). Only chitosan at 0.30% increased leaf Tr from 3.56 to 5.12 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ H_2O . While much research focuses on crop growth, there are very limited reports on leaf chlorophyll fluorescence or gas exchange as affected by soil-applied chitosan. Previous studies indicated that foliar-applied chitosan increased photosynthetic rate in okra and coffee (*Coffea canephora*) (Mondal et al., 2012; Van et al., 2013). Other studies showed that foliar-applied chitosan reduced g_s in pepper and tomato (Bittelli et al., 2001; Lee et al., 1999). The present study suggests that soil-applied chitosan could stimulate crop growth through enhanced photosynthesis resulting from increased chlorophyll content and photochemical efficiency.

In summary, chitosan applied as a soil amendment at an appropriate

Table 2. Lettuce leaf chlorophyll fluorescence and gas exchange after 35 d grown on soil amended with different rates of chitosan. Two-week-old lettuce seedlings were transplanted in 1-L (0.26 gal) plastic pots with soil amended with different rates of chitosan and grown in a growth chamber for 35 d. Pots were irrigated to capacity with full-strength Hoagland's nutrient solution after transplanting and subsequently watered twice weekly.

Chitosan rate (%)	Fluorescence measurements [mean ± SE] ^z			Gas exchange [mean ± SE] ^y		
	Fv/Fm	Y(II)	ETR (μmol·m ⁻² ·s ⁻¹)	Pn [CO ₂ (μmol·m ⁻² ·s ⁻¹)]	g _s (mol·m ⁻² ·s ⁻¹)	Tr [H ₂ O (mmol·m ⁻² ·s ⁻¹)]
0.00	0.84 ± 0.007 b ^x	0.359 ± 0.0234 c	99 ± 2 d	9.3 ± 1.02 c	0.134 ± 0.015 c	3.56 ± 0.355 b
0.05	0.86 ± 0.005 ab	0.396 ± 0.0305 c	105 ± 6 cd	10.2 ± 0.83 c	0.132 ± 0.019 c	3.38 ± 0.290 b
0.10	0.85 ± 0.006 b	0.431 ± 0.0349 bc	126 ± 7 bc	9.9 ± 1.49 c	0.145 ± 0.0327 bc	3.83 ± 0.457 b
0.15	0.85 ± 0.007 ab	0.429 ± 0.0339 bc	126 ± 9 bc	12.7 ± 1.45 b	0.183 ± 0.0303 ab	3.95 ± 0.592 ab
0.20	0.87 ± 0.004 a	0.503 ± 0.0310 a	159 ± 9 a	14.0 ± 1.55 ab	0.196 ± 0.0219 ab	4.14 ± 0.536 ab
0.30	0.87 ± 0.006 a	0.497 ± 0.0496 ab	153 ± 7 ab	16.6 ± 1.82 a	0.231 ± 0.0257 a	5.12 ± 0.710 a
Regression	<0.001	<0.001	<0.001	<0.001	0.005	0.007
	0.3427 (L) ^w	0.2219 (L)	0.7311 (L)	0.4984 (L)	0.1595 (L)	0.1483 (L)

^zFv/Fm = maximum photochemical efficiency, Y(II) = photochemical yield, ETR = electron transport rate, 1 m² = 10.7639 ft².

^yPn = net photosynthetic rate, CO₂ = carbon dioxide, g_s = stomatal conductance, Tr = transpiration rate, H₂O = water.

^xDifferent letters indicate significant difference at *P* ≤ 0.05 according to Duncan's multiple range test.

^wLinear.

rate prompted increased lettuce production, significantly increased leaf number, area, FW, and DW. It also significantly enhanced leaf chlorophyll index and photochemical efficiency, leading to enhanced photosynthetic rate. Our study indicated that chitosan as a soil amendment enhanced lettuce growth and may have potential to be used for the sustainable production of lettuce. However, high rates of chitosan may negatively impact lettuce growth and development. Further investigation is needed to optimize application method, time, and rate for lettuce field production.

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