J. Pest Cont. and Environ. Sci. 17 (1/2): 77-101(2009)

Effect of a bioactive chitosan polymer with different molecular weights on seedling growth of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), corn (*Zea mays*) and lentil (*Lens culinaris*)

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ABSTRACT

The effects of chitosan molecular weight and its concentrations on growth vigour of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), corn (*Zea mays*) and lentil (*Lens culinaris*) sprouts were investigated. Plant seeds were germinated in 100, 250 and 500 mg/L of three molecular weights (3.60×10^5, 6.11×10^5 and 9.53×10^5 g/mol) chitosan for 8 days using a Neubauer technique. Traditional growth and potential vigour-associated parameters (protein, phenolics, chlorophyll and polyphenol oxidase) were investigated in details. Chitosan treatments significantly enhanced sprout growth to varying degrees over controls. Protein content in wheat and barley was gradually increased with an increase in the molecular weight and the concentration. Whereas, a chitosan of 3.60×10^5 g/mol was most significantly effective at 500 mg/L in corn and lentil. In addition, total phenolics were high accumulated and increased gradually in the tested plants with an increase in the molecular weight and the concentration. On the other hand, chlorophyll and polyphenol oxidase levels were significantly decreased.

Key words: Chitosan molecular weight; chlorophyll content; protein; phenolic content; polyphenol oxidase

INTRODUCTION

Improving overall growth and performance of agricultural crops is an important goal to improve the productivity. This is driven by the need to provide food for a steadily growing world population as well as the potential for commercial benefits (McDonald, 1994). Enhancement of seed vigour is a useful approach to meeting this goal. Seed vigour is defined as those seed properties

that determine the activity and performance of the seed during germination and seedling emergence (Perry, 1978). Traditional agronomic methods of seed vigour measurement include germination percentage, sprout length and weight. More recently, there is also evidence to support a link between certain biochemical characteristics and sprout vigour (Randhir and Shetty, 2003; Horii *et al.*, 2007).

Seed priming, the soaking of seeds in a solution prior to planting, is a known strategy to improve seedling establishment by increasing uniform seed germination (Bradford, 1986; Taylor and Harman, 1990). A number of commonly used as priming solutions are available, such as polyetheylene glycol, inorganic salts, fertilizer and plain water (Pill *et al.*, 1994). However, optimization of priming solutions is required for each crop species (Bradford, 1986; Taylor and Harman, 1990). At present, increasing interest is being devoted to the use of natural substances such as a bioactive chitosan polymer. Chitosan, a copolymer of glucosamine and *N*-acetyglucosamine units linked by 1,4-glucosidic bonds, obtained by *N*-deacetylation of chitin, is one of these compounds (No and Meyers, 1997). It has a number of interesting properties such as a good biodegradability and a low toxicity for mammalian cells (Muzzarelli *et al.*, 2001).

In agriculture, chitosan has been used in seeds, fruits and vegetables coating (Devlieghere *et al.*, 2004), to increase the plant product (Wanichpongpan *et al.*, 2001), to stimulate the immunity of plants (Hadwiger *et al.*, 2002) and to protect plants against microorganisms (Bhaskara Reddy *et al.*, 1999; Rabea *et al.*, 2003; Tripathi and Dubey, 2004; Badawy and Rabea, 2009). In the latter studies, a positive effect of chitosan was observed on the growth of roots, shoots and leaves of ornamental plants including *Gerbera spp* (Wanichpongpan *et al.*, 2001) and of several crop plants (Chibu and Shibayama, 2001; Nge *et al.*, 2006). Moreover, a numerous studies have revealed that chitosan treatment is effective in enhancement of the yield and quality of soybean sprouts (Lee *et al.*, 1999; No *et al.*, 2003).

In addition, its antimicrobial activity has received considerable attention due to problems associated with fungicidal and bactericidal agents (Rabea *et al.*, 2003; Badawy and Rabea, 2009). Moreover, plants are well adapted to cope

with a variety of attacking pathogens, and the plant's defense response against pathogens can be elicited by numerous external signals. One of the ways in which the plant's response can be manipulated genetically or by treatment with the oligosaccharide elicitors such as chitosan (Hadwiger *et al.,* 1989; Rabea *et al.,* 2003).

In the present study, we have hypothesized that the treatment of seeds of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), corn (*Zea mays*) and lentil (*Lens culinaris*) with different molecular weights of chitosan (3.60×10^5, 6.11×10^5 and 9.53×10^5 g/mol) may result in an increase in both biochemical and traditional agronomic indices of sprout vigour. Following treatment with various concentrations of chitosans, vigour-related growth and critical biochemical parameters such as chlorophyll content, total proteins, phenolic contents and polyphenol oxidase were determined after 8 days of growth period.

MATERIALS AND METHODS

Chemicals: Three acid-soluble chitosans (3.60×10^5, 6.11×10^5 and 9.53×10^5 g/mol molecular weight, calculated by determination of their intrinsic viscosities) were purchased from Sigma-Aldrich Chemical Co. (USA). Degrees of deacetylation were 85, 81 and 82 %, respectively. All chitosans were in a powder form and prepared from crab shell. Acetic acid, copper sulphate, disodium ethylenediamine tetra acetic acid (EDTA), ethanol, gallic acid, polyvinylpyrrolidone (PVP), potassium monohydrogen phosphate, potassium dihydrogen phosphate, pyrocatechol, sodium-potassium tartarate, Folin-Ciocalteu phenol reagent and Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich Chemical Co. (USA) and used without further purification.

Seeds of test plants and cultivation with chitosan treatments: Seeds of wheat (*Triticum aestivum* L. Poaceae), barley (*Hordeum vulgare* L. Poaceae), corn (*Zea mays var. amylacea* L. Poaceae) and lentil (*Lens culinaris* M. Fabaceae) were used for this study. Chitosan solutions were prepared in 0.05% aqueous acetic acid at concentrations of 100, 250 and 500 mg/L. Control treatments were an aqueous solution of acetic acid (0.05%, v/v) and distilled water. Three replicates of 10 seeds per species were prepared for each treatment

using glass Petri dishes (9cm) lined with Whatman No. 2 filter paper. Five milliliters of test solution were added to each Petri dish. The filled Petri dish was placed in the bottom of a 0.1-mm thick polyethylene bag (15×30 cm) which was expanded to contain air and then closed at the top with a rubber band according to the Neubauer technique as modified by Thomas and Cline (1985). This containment unit was placed in a controlled-environment growth chamber and subjected to 16 h of light and 8 h of darkness. This enclosed unit served both to isolate any toxic chemicals and to avoid the need for daily irrigation until germination or sprout measurements were made.

Seedling growth measurements: Germination percentage was calculated as the total number of seedlings that emerged versus the total number of seeds and was found to be higher than 96%. The average of shoot, root length (in cm) and shoot and root weight (in mg) of seedlings were measured after 8 days of germination.

Chlorophyll content assay: Chlorophyll *a*, *b* and total chlorophyll contents in seedling leaf tissue were measured by the method of Hipkins and Baker (1986). Fifty mg of leaves tissue was added to 3.0 mL of methanol and incubated in the dark for 2 h. Each sample was homogenized and centrifuged at 13,000 rpm for 10 min. Absorbance of the supernatant was measured at 650 nm and 665 nm by Unico 1200-Spectrophotometer. Absolute methanol was used as a blank. The calculation of chlorophyll *a*, chlorophyll *b* and total chlorophyll content was as follows:

$$\text{Chlorophyll } a\ (\mu g/mL) = 16.5\times A_{665}-8.3\times A_{650}$$
$$\text{Chlorophyll } b\ (\mu g/mL) = 33.8\times A_{650}-12.5\times A_{665}$$
$$\text{Total chlorophyll}\ (\mu g/mL) = 25.8\times A_{650}+4.0\times A_{665}$$

The chlorophyll contents were subsequently converted to µg of chlorophylls per gram of fresh weight (FW) tissue by the formula (µg chlorophyll/mL methanol) × 3 mL methanol/(g FW tissue).

Sample preparation: One-hundred mg of leaf tissue was ground in 2 mL of enzyme extraction buffer with glass beads by mortar and pestle chilled on ice. The extraction buffer consisted of 0.5% (w/v) PVP, 3 mM disodium EDTA, and 0.1 M potassium phosphate buffer, pH 7.5. The homogenate was

centrifuged at 13,000 rpm for 10 min at 4 °C and was used as the crude enzyme extract (Horii *et al.*, 2007).

Total protein assay: The Lowry *et al.*, (1951) method was used to determine the protein content in leaf samples. One-hundred µL of protein extract was added to 2 mL of alkaline copper reagent (48 ml of 2% (w/v) sodium carbonate in 0.1 N sodium hydroxide + 1 mL of 1% (w/v) sodium-potassium tartarate + 1 mL of 0.5% (w/v) copper sulphate) and immediately mixed well. After 10 min., 0.2 mL of Folin-Ciocalteu phenol reagent was added and the samples were thoroughly mixed then the absorbance of the developed blue color was measured at 600 nm using Unico 1200-Spectrophotometer. Protein content of the samples was calculated from the standard curve obtained using different concentrations of BSA.

Total soluble phenolics assay: Total phenolics in seedlings tissue were determined according to McCue *et al.*, (2000). Briefly, 50 mg of leaf tissues were taken after eight days of germination then was placed in 2.5 mL of 95% ethanol and frozen for 48-72 h. Samples were homogenized with a mortar and centrifuged at 10,000*g* for 10 min. One milliliter of the resulting supernatant was combined with 1 mL 95% ethanol, 5 mL distilled water and 0.5 mL of 50% Folin-Ciocalteu phenol reagent. After the incubation period 5 min at room temperature, 1 mL of 5% (w/v) sodium carbonate was added followed by brief vortexing to mix. The reaction mixture was incubated for 1 h in a dark-cupboard. After briefly vortexing, the absorbance was determined at 725 nm by spectrophotometer (Unico 1200-Spectrophotometer, USA). A standard curve was established using a gallic acid in 95% ethanol. Total phenolics content was standardized against gallic acid and absorbance values were converted to µg of phenolics per gram of fresh weight tissue. Each value reported is the average of three determinations assays of three separate samples for each replicate.

Polyphenol oxidase (PPO) assay: The activity of PPO (EC 1.10.3.2) was determined according to Zhi-qing *et al.*, (2008) by mixing of 1.5 mL of 0.2 mol/L pyrocatechol, 1.4 mL of 0.05 mol/L phosphate buffer (pH 6.8) and 0.1 mL enzyme extract, respectively. The mixture was incubated at 25°C for 25 min and the absorbance was measured at 420 nm by spectrophotometer (Unico

1200-Spectrophotometer, USA). The specific activity of PPO was calculated and expressed as O.D. mg^{-1}protein. 30 min^{-1}.

Statistical analysis: All the quantitative estimations of growth and biochemical parameters were based on three replicates and the values are expressed as mean ± standard error. The data were statistically analyzed separately for each experiment and were subjected to analysis of variance (ANOVA) using SPSS 12.0 software (Statistical Package for Social Sciences, USA). Mean separations were performed by Student-Newman-Keuls (SNK) test according to Snedecor and Cochran (1989) and differences at $P \leq 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Influence of chitosan treatments on seedling vigour of wheat, barley, corn and lentil: Initial vigour response was measured using traditional agronomic parameters including, shoot and root length as well as shoot and root weight. These parameters are all vital indices of sprout vigour. The effects of three molecular weights chitosan (3.60×10^5, 6.11×10^5 and 9.53×10^5 g/mol) at 100, 250 and 500 mg/L on the length and weight of wheat, barley, corn and lentil sprouts were investigated and presented in Tables 1-3. Chitosan treatments significantly enhanced the seedling vigour to varying degrees over the controls. In wheat, shoot length and weight was concentration and molecular weight dependant (Tables 1 and 2). The maximum shoot length and weight of wheat was obtained with a high molecular weight chitosan (9.53×10^5 g/mol) at 500 mg/L that was increased significantly (10.22 cm and 85.63 mg/plant, respectively) compared to the controls. In contrast, chitosans of 6.11×10^5 and 9.53×10^5 g/mol at 100 mg/L were the most significantly effective in root length and weight compared to the controls. Among three chitosan treatments, their high concentration (500 mg/L) significantly decreased the root length. The total weight of wheat seedling was significantly increased compared to the controls at concentrations of 100 and 250 mg/L for all three chitosan treatments (Table 2). The total weight (167.20 - 213.11 mg) of wheat sprout germinated in chitosan solutions was 5.76 - 34.81% higher than that of the control of 0.05% aqueous acetic acid (158.08 mg/sprout).The significant seedling growth of barley evidenced by the most increase in shoot length and weight was observed, reaching 13.55 cm and 107.17 mg, respectively after 8 days at 500 mg/L of a

high molecular weight chitosan (9.53×10^5 g/mol) (Table 1). Root length and weight of barley was also dependant on the concentration and molecular weight as previously mentioned in wheat. Barley seedling weight appeared to respond positively to the concentration during germination (Table 2), but only a 250 mg/L of 6.11×10^5 and 9.53×10^5 g/mol chitosans was significantly highest (180.33 and 178.04 mg/sprout, respectively). It can be noticed that the total weight of barley sprout treated with chitosans (142.29 - 180.33 mg/sprout) was 26.41 - 60.21% higher than that of the control of 0.05% aqueous acetic acid (112.56 mg/sprout).

The shoot length and weight of corn was affected negatively with an increase in the chitosan concentrations (Tables 1 and 3). Whereas, a concentration of 100 mg/L of 6.11×10^5 g/mol was the highest in activity of the shoot length and weight, 2.73 cm and 128 mg, respectively compared to the controls. In addition, a concentration of 250 mg/L of 9.53×10^5 g/mol was the highest activity in root length and weight, 11.43 cm and 112.44 mg, respectively compared to the controls. Seedling total weight was significantly affected by a 100 mg/L of 3.60×10^5 and 6.11×10^5 g/mol chitosans which account 230.28 and 234 mg/sprout, respectively (Table 3).

In corn sprout germinated with chitosans, the total weight (141-234 mg/sprout) was 0.19- 66.28% higher than that of the control of 0.05% aqueous acetic acid (140.73 mg/sprout) except that a chitosan low molecular weight with 500 mg/L caused a significant decreasing effect in the total weight (128.39 mg/sprout).

The results of the lentil, seedling growth parameters indicated that the low molecular weight chitosan (3.60×10^5 g/mol) was the most effective one in shoot length (Table 1) and weight (Table 3). The concentrations of 250 and 500 mg/L of 3.60×10^5 g/mol chitosan were the most significantly active compared to the controls and the other treatments. Whereas, a concentration of 100 mg/L of the medium molecular weight (6.11×10^5 g/mol) was the most effective one on the root length (8.92 cm) and on the root weight (59.22 mg). In contrast, a concentration (500 mg/L) of the high molecular weight (9.53×10^5 g/mol) was the lowest one (1.97 cm and 25.37 mg for root length and weight, respectively) among all chitosan treatments. As shown in Table 3, the total weight (64.34 - 85.06 mg/sprout) of the lentil sprout was 4.04-37.55% higher than that of the

Table 1. Effect of different molecular weights chitosan compounds on length of wheat, barely, corn and lentil sprouts.

Treatment (mg/L)	Wheat		Barley		Corn		Lentil	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
C1	$9.47^{abc}\pm0.22$	$10.89^{cd}\pm0.11$	$10.74^{c}\pm0.25$	$9.90^{bcd}\pm0.10$	$2.24^{abc}\pm0.11$	$8.86^{c}\pm0.19$	$2.63^{d}\pm0.12$	$4.11^{d}\pm0.11$
C2	$9.06^{c}\pm0.20$	$9.94^{de}\pm0.57$	$10.58^{c}\pm0.22$	$8.69^{d}\pm0.40$	$1.69^{d}\pm0.17$	$8.11^{c}\pm0.31$	$2.52^{d}\pm0.04$	$3.00^{e}\pm0.06$
100L	$9.32^{bc}\pm0.08$	$11.78^{abc}\pm0.11$	$12.37^{b}\pm0.20$	$9.68^{cd}\pm0.19$	$2.66^{ab}\pm0.17$	$9.84^{b}\pm0.22$	$3.53^{bc}\pm0.10$	$4.89^{c}\pm0.31$
250L	$9.58^{abc}\pm0.17$	$11.86^{abc}\pm0.07$	$12.62^{ab}\pm0.29$	$10.44^{abc}\pm0.24$	$2.33^{abc}\pm0.09$	$8.88^{c}\pm0.17$	$3.79^{b}\pm0.07$	$5.11^{c}\pm0.20$
500L	$9.64^{abc}\pm0.16$	$7.56^{f}\pm0.29$	$12.83^{ab}\pm0.29$	$9.58^{cd}\pm0.27$	$2.21^{bc}\pm0.03$	$4.63^{e}\pm0.09$	$4.75^{a}\pm0.08$	$5.19^{c}\pm0.24$
100M	$9.39^{abc}\pm0.06$	$12.86^{a}\pm0.16$	$12.38^{b}\pm0.20$	$10.47^{abc}\pm0.37$	$2.73^{a}\pm0.10$	$10.37^{b}\pm0.32$	$3.50^{bc}\pm0.10$	$8.92^{a}\pm0.14$
250M	$9.83^{abc}\pm0.17$	$11.96^{abc}\pm0.15$	$12.95^{ab}\pm0.15$	$11.33^{a}\pm0.22$	$2.48^{abc}\pm0.08$	$8.91^{c}\pm0.14$	$3.52^{bc}\pm0.27$	$5.37^{c}\pm0.09$
500M	$9.90^{ab}\pm0.21$	$10.06^{de}\pm0.24$	$13.36^{ab}\pm0.25$	$9.39^{cd}\pm0.31$	$2.27^{abc}\pm0.17$	$4.70^{e}\pm0.17$	$3.60^{bc}\pm0.19$	$3.09^{e}\pm0.41$
100H	$10.00^{ab}\pm0.10$	$12.44^{ab}\pm0.24$	$12.83^{ab}\pm0.10$	$11.10^{ab}\pm0.38$	$2.03^{c}\pm0.07$	$5.69^{d}\pm0.17$	$3.40^{bc}\pm0.14$	$6.07^{b}\pm0.34$
250H	$10.17^{ab}\pm0.33$	$11.39^{bc}\pm0.63$	$13.18^{ab}\pm0.32$	$11.41^{a}\pm0.54$	$2.38^{abc}\pm0.12$	$11.43^{a}\pm0.23$	$3.00^{cd}\pm0.19$	$2.71^{e}\pm0.15$
500H	$10.22^{a}\pm0.04$	$9.03^{e}\pm0.42$	$13.55^{a}\pm0.24$	$9.00^{d}\pm0.21$	$2.24^{abc}\pm0.05$	$4.43^{e}\pm0.30$	$2.83^{d}\pm0.19$	$1.97^{f}\pm0.19$

C1, control cultivated in water; C2, control cultivated in 0.05% (v/v) aqueous acetic acid. L = Low molecular weight (3.60×10^5 g/mol); M = Medium molecular weight (6.11×10^5 g/mol) and H = High molecular weight (9.53×10^5 g/mol) chitosan. Data are averages of three replicates ± SE. Values within a column bearing the same superscript are not significantly different ($P \leq 0.05$).

Table 2. Effect of different molecular weights chitosan compounds on weight of wheat and barley sprouts.

Treatment (mg/L)	Wheat			Barley		
	Shoot weight (mg/sprout)	Root weight (mg/sprout)	Total weight (mg/sprout)	Shoot weight (mg/sprout)	Root weight (mg/sprout)	Total weight (mg/sprout)
C1	79.13^{bcde}±2.07	97.78^{d}±1.54	176.91^{d}±2.42	88.67^{c}±0.51	42.81^{f}±0.80	131.47^{e}±0.49
C2	74.08^{e}±2.31	84.00^{e}±1.90	158.08^{f}±0.75	81.00^{d}±1.53	31.56^{g}±1.28	112.56^{f}±2.60
100L	76.77^{de}±0.45	116.00^{c}±2.00	192.77^{c}±1.93	98.78^{b}±0.97	43.51^{f}±1.91	142.29^{d}±1.11
250L	80.78^{abcd}±0.40	122.00^{bc}±3.21	202.78^{b}±3.39	99.67^{b}±1.45	44.50^{f}±1.32	144.17^{d}±1.48
500L	83.67^{abc}±1.65	83.53^{e}±0.29	167.20^{e}±1.36	103.67^{ab}±2.19	47.67^{ef}±1.20	151.33^{c}±2.33
100M	77.82^{cde}±0.99	127.67^{ab}±2.73	205.49^{ab}±2.10	99.00^{b}±1.15	58.33^{e}±0.88	157.33^{c}±1.20
250M	81.11^{abcd}±0.80	121.89^{bc}±3.02	203.00^{b}±3.48	102.00^{ab}±1.53	78.33^{a}±0.88	180.33^{a}±2.40
500M	84.55^{ab}±0.35	84.60^{e}±2.47	169.15^{de}±2.34	104.67^{ab}±2.67	49.00^{e}±1.53	153.67^{c}±3.48
100H	80.33^{abcd}±2.33	132.78^{a}±2.82	213.11^{a}±1.98	100.17^{b}±1.30	71.00^{c}±1.00	171.17^{b}±1.59
250H	82.29^{abcd}±0.71	129.11^{ab}±2.02	211.41^{ab}±2.16	103.38^{ab}±1.05	74.67^{b}±1.20	178.04^{a}±1.86
500H	85.63^{a}±0.32	86.45^{e}±1.90	172.08^{de}±2.21	107.17^{a}±1.01	44.00^{f}±1.00	151.17^{c}±0.44

C1, control cultivated in water; C2, control cultivated in 0.05% (v/v) aqueous acetic acid. L = Low molecular weight (3.60×10^5 g/mol); M = Medium molecular weight (6.11×10^5 g/mol) and H = High molecular weight (9.53×10^5 g/mol) chitosan. Data are averages of three replicates ± SE. Values within a column bearing the same superscript are not significantly different ($P \leq 0.05$).

Table 3. Effect of different molecular weights chitosan compounds on weight of corn and lentil sprouts.

Treatment (mg/L)	Corn			Lentil		
	Shoot weight (mg/sprout)	Root weight (mg/sprout)	Total weight (mg/sprout)	Shoot weight (mg/sprout)	Root weight (mg/sprout)	Total weight (mg/sprout)
C1	$99.42^{c}\pm1.40$	$82.40^{c}\pm1.47$	$181.82^{cd}\pm1.28$	$22.83^{ef}\pm0.25$	$43.04^{c}\pm0.53$	$65.87^{c}\pm0.76$
C2	$70.07^{f}\pm1.46$	$70.67^{e}\pm1.86$	$140.73^{f}\pm3.27$	$22.38^{f}\pm0.06$	$39.47^{c}\pm1.75$	$61.84^{c}\pm1.70$
100L	$127.50^{a}\pm1.61$	$102.78^{b}\pm1.13$	$230.28^{a}\pm2.64$	$26.73^{c}\pm0.12$	$51.40^{b}\pm0.50$	$78.13^{b}\pm0.56$
250L	$100.00^{e}\pm0.19$	$80.11^{cd}\pm0.91$	$180.11^{cd}\pm0.97$	$28.93^{b}\pm0.74$	$53.36^{b}\pm1.25$	$82.29^{ab}\pm1.25$
500L	$68.17^{f}\pm1.92$	$60.22^{f}\pm0.67$	$128.39^{g}\pm1.60$	$30.50^{a}\pm0.40$	$54.56^{b}\pm1.28$	$85.06^{a}\pm1.68$
100M	$128.00^{a}\pm1.07$	$106.00^{b}\pm1.15$	$234.00^{a}\pm3.03$	$23.89^{ef}\pm0.49$	$59.22^{a}\pm1.39$	$83.11^{a}\pm0.95$
250M	$101.53^{c}\pm0.96$	$83.78^{c}\pm2.21$	$185.31^{c}\pm1.33$	$24.80^{de}\pm0.64$	$53.44^{b}\pm0.99$	$78.24^{b}\pm0.62$
500M	$79.44^{e}\pm1.06$	$61.56^{f}\pm1.28$	$141.00^{f}\pm1.90$	$25.89^{cd}\pm0.29$	$31.57^{d}\pm0.88$	$57.46^{d}\pm0.98$
100H	$90.11^{d}\pm0.78$	$76.56^{d}\pm2.96$	$166.67^{e}\pm2.46$	$23.86^{ef}\pm0.52$	$54.82^{b}\pm0.53$	$78.68^{b}\pm1.03$
250H	$103.00^{c}\pm1.90$	$112.44^{a}\pm1.37$	$215.44^{b}\pm2.75$	$23.08^{ef}\pm0.13$	$41.27^{c}\pm1.79$	$64.34^{c}\pm1.91$
500H	$113.00^{b}\pm2.08$	$61.22^{f}\pm0.67$	$174.22^{d}\pm1.64$	$22.67^{f}\pm0.84$	$25.37^{e}\pm0.85$	$48.03^{e}\pm0.42$

C1, control cultivated in water; C2, control cultivated in 0.05% (v/v) aqueous acetic acid. L = Low molecular weight (3.60×10^5 g/mol); M = Medium molecular weight (6.11×10^5 g/mol) and H = High molecular weight (9.53×10^5 g/mol) chitosan. Data are averages of three replicates ± SE. Values within a column bearing the same superscript are not significantly different ($P \leq 0.05$).

control of 0.05% aqueous acetic acid (61.84 mg/sprout) except that a concentration of 500 mg/L with chitosan medium and high molecular weights caused a significant decreasing effect in the total weight (57.46 and 48.03 mg/sprout, respectively).

Chitosan was used as a seed treatment and subjected to seed germination and seedling vigour tests as a prerequisite to select concentrations desirable for seed treatment. The data that presented in this study are in agreement with earlier observations made by many scientists. For example, No *et al.*, (2003) established optimal soybean sprouts cultivation conditions of molecular weights, concentrations, solvents of chitosan, soaking times and soybeans/chitosan solution ratios. They observed that an increase in weight of soybean sprouts from 6.9 to 29.2% by chitosan treatment. Lee *et al.*, (1999) reported that the length of soybean (Korean *Iksan* cultivar) sprouts treated with chitosan was 25.4% longer than that of the control. The effect of shrimp and fungal chitosan on the growth and development of orchid plant meristemic tissue in culture was also investigated in liquid and on solid medium by Nge *et al.*, (2006). They found that the growth of meristem explants into protocorm-like bodies in liquid medium was accelerated up to 15 times in the presence of chitosan oligomer and the optimal concentration was 15 mg/L. They also added that a 1×10^3 g/mol shrimp oligomer was slightly more effective compared to 1×10^4 g/mol shrimp chitosan. Chitosan significantly enhanced growth factors of Gerbera plants in terms of the average values of flower-stem length, the number of growing leaves, including leaf width and length as well as the number of flowers per bush (Wanichpongpan *et al.*, 2001). However, the mechanism of the action of chitosan on plant growth remains unclear.

Influence of chitosan treatments on chlorophyll content in wheat, barley, corn and lentil: The effects of three different molecular weights of chitosan at 100, 250 and 500 mg/L on chlorophyll *a*, *b* and total chlorophyll content of wheat, barley, corn and lentil are shown in Figure 1. From the obtained result it can be noticed that a low concentration (100 mg/L) of the three chitosans caused an increase in the chlorophyll contents in wheat, whereas high concentrations (250 and 500 mg/L) led to a significant decrease in the chlorophyll contents compared to the controls (Figure 1). In addition, the higher molecular weight was the lower chlorophyll contents. In barely, corn and lentil

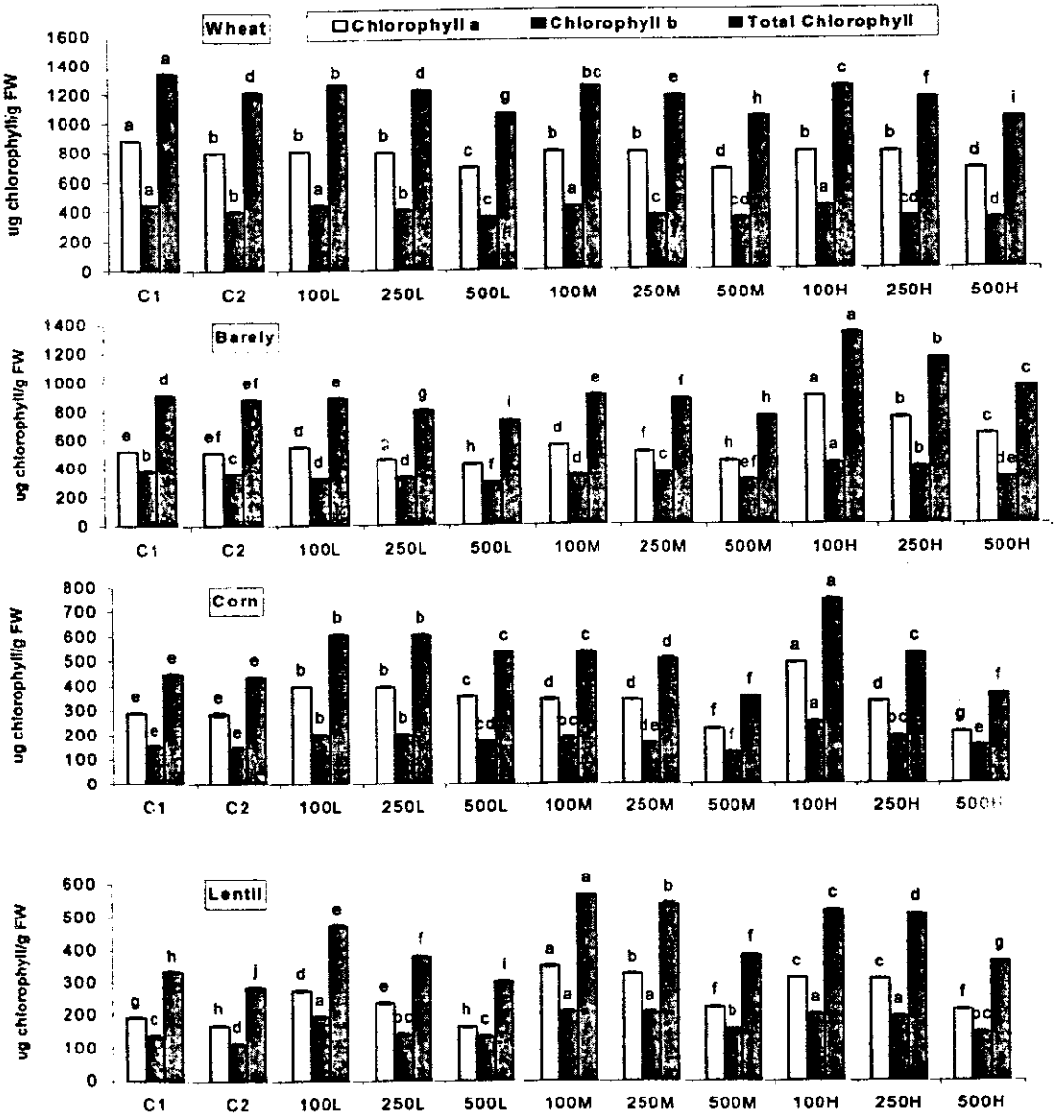

Figure 1. Chlorophyll content (ug/g fresh tissue) of wheat, barely, corn and lentil sprouts treated with different molecular weights chitosan compounds.C1, control cultivated in water; C2, control cultivated in 0.05% (v/v) aqueous acetic acid. L = Low molecular weight (3.60×10^5 g/mol); M = Medium molecular weight (6.11×10^5 g/mol) and H = High molecular weight (9.53×10^5 g/mol) chitosan. Data are averages of three replicates ± SE. Bars bearing the same letter are not significantly different ($P \leq 0.05$).

leaves, the chlorophyll *a*, *b* and the total chlorophyll contents were increased in all treatments compared to the controls and then dramatically decreased with the increasing in the concentration. Generally, it can be noticed that the chlorophyll contents in wheat and barley leaves were higher than that obtained in corn and lentil. However, the concentration of these pigments was more drastically decreased in wheat leaves with either increasing of the chitosan molecular weight or concentration than that caused in barley leaves.

Overall, the reduction in chlorophyll contents in wheat, barely, corn and lentil leaves after growth period may be attributed to the interference of magnesium metal in chlorophyll biosynthesis with high concentration of the chitosan molecule through the biosynthesis of photoactive protochlorophyll reductase enzyme complex and aminolevulinic acid (ALA) synthesis (Stobart *et al.*, 1985). In addition, the sulfhydryl groups of this enzyme complex, which provide chlorophyll biosynthesis, may be attributed to be blocked by the complexation of acid active thiol groups by amino group in chitosan molecule.

Influence of chitosan treatments on protein content in wheat, barley, corn and lentil: The protein content in leaves of wheat, barley, corn and lentil germinated in different molecular weights of chitosan at 100, 250 and 500 mg/L are shown in Table 4. The results indicated that the protein contents in wheat and barley was significantly increased with an increase in the molecular weight and chitosan concentration during the entire growth period versus controls. High values of protein content in both seedling wheat and barley (5.16 and 3.89 mg, respectively) were found at concentrations of 500 mg/L from high molecular weight of chitosan (9.53×10^5 g/mol). Whereas, a 500 mg/L of the chitosan low molecular weight (3.60×10^5 g/mol) was the most significantly effective in the protein contents of corn and lentil (9.62 and 5.03 mg protein/g fresh tissue, respectively). In the case of lentil, a significant decrease of protein content was found with an increase in the concentration of the high molecular weight chitosan (9.53×10^5 g/mol).

In general, the total protein content (4.29 - 5.16 mg protein/g fresh tissue) of wheat sprouts germinated in chitosans was 18.18 - 42.15% higher than that of the control of 0.05% aqueous acetic acid (3.63 mg protein/g fresh tissue). The total protein content (2.13 - 3.89 mg protein/g fresh tissue) of barley sprouts

treated with chitosans was 1.43 - 85.24% higher than that of the control of 0.05% aqueous acetic acid (2.10 mg protein/g fresh tissue).

Table 4. Protein content (mg/g fresh tissue) of seedling leaf tissues in wheat, barely, corn and lentil sprouts treated with different molecular weights chitosan compounds.

Treatment (mg/L)	mg protein/g fresh tissue			
	Wheat	Barely	Corn	Lentil
C1	$3.67^{f} \pm 0.09$	$2.35^{cd} \pm 0.04$	$7.88^{bc} \pm 0.06$	$3.90^{cde} \pm 0.04$
C2	$3.63^{f} \pm 0.03$	$2.10^{d} \pm 0.08$	$7.18^{cd} \pm 0.01$	$3.78^{de} \pm 0.05$
100L	$4.29^{e} \pm 0.07$	$2.13^{d} \pm 0.06$	$8.42^{b} \pm 0.21$	$3.99^{cd} \pm 0.10$
250L	$4.46^{cde} \pm 0.12$	$2.37^{cd} \pm 0.00$	$9.60^{a} \pm 0.12$	$4.47^{b} \pm 0.03$
500L	$4.71^{bc} \pm 0.05$	$3.52^{b} \pm 0.05$	$9.62^{a} \pm 0.16$	$5.03^{a} \pm 0.06$
100M	$4.34^{de} \pm 0.04$	$2.19^{d} \pm 0.07$	$6.79^{d} \pm 0.06$	$3.98^{cd} \pm 0.06$
250M	$4.56^{cde} \pm 0.08$	$2.44^{cd} \pm 0.20$	$8.28^{b} \pm 0.18$	$4.13^{c} \pm 0.09$
500M	$4.88^{b} \pm 0.02$	$3.80^{a} \pm 0.05$	$9.35^{a} \pm 0.65$	$4.47^{b} \pm 0.05$
100H	$4.51^{cde} \pm 0.06$	$2.59^{c} \pm 0.11$	$7.66^{bc} \pm 0.02$	$3.80^{de} \pm 0.05$
250H	$4.61^{cd} \pm 0.05$	$3.31^{b} \pm 0.07$	$8.12^{b} \pm 0.13$	$3.69^{ef} \pm 0.06$
500H	$5.16^{a} \pm 0.07$	$3.89^{a} \pm 0.12$	$9.29^{a} \pm 0.17$	$3.52^{f} \pm 0.04$

C1, control cultivated in water; C2, control cultivated in 0.05% (v/v) aqueous acetic acid. L = Low molecular weight (3.60×10^5 g/mol); M = Medium molecular weight (6.11×10^5 g/mol) and H = High molecular weight (9.53×10^5 g/mol) chitosan. Data are averages of three replicates ± SE. Values within a column bearing the same superscript are not significantly different ($P \leq 0.05$).

Whereas, the total protein content (7.66-9.62mg protein/g fresh tissue) of the corn sprouts germinated in chitosan solutions was 6.69 - 33.98% higher than that of the control of 0.05% aqueous acetic acid (7.18 mg protein/g fresh tissue), except that a 6.11×10^5 g/mol chitosan with 100 mg/L which caused a significant decrease in the protein content (6.79 mg protein/g fresh tissue). In later, the lentil sprouts germinated in chitosan solutions, the total protein (3.80 - 5.03 mg protein/g fresh tissue) was 0.53 - 33.07% higher than that of the control of 0.05% aqueous acetic acid (3.78 mg protein/g fresh tissue), except that concentrations of 250 and 500 mg/L with chitosan high molecular weight ((9.53×10^5 g/mol) which caused a significant decrease (3.69 and 3.52 mg protein/g fresh tissue, respectively) in total protein. These findings were corroborated well with the earlier findings of Badawy and Rabea (2009). They reported that the different molecular weights of chitosan (0.5×10^4, 3.7×10^4,

5.7×10^4 and 2.9×10^5 g/mol) at 500, 1000, 2000 and 4000 mg/L enhanced the total protein content in tomato fruit (*Solanum lycopersicum* L. var. *lycopersicum*) and a high value of protein was found with a chitosan of 5.7×10^4 g/mol at 4000 mg/L (11.08 and 7.36 mg protein/g fruit tissue at storage of 25 and 2°C, respectively).

Influence of chitosan treatments on total phenolic contents in wheat, barley, corn and lentil: The total soluble phenolics in leaf extracts of wheat, barley, corn and lentil germinated with different molecular weights chitosan at 100, 250 and 500 mg/L are shown in Table 5. The results showed that the overall response to chitosan treatments by each plant species was the same except with the corn sprouts. The content of the total phenolics in wheat, barley and lentil among all treatments was high gradually accumulated with an increase in the molecular weight and the concentration compared to the controls. Whereas, the phenolic content in corn was significantly increased (285.14 and 292.96 µg/g fresh tissue) with 250 and 500 mg/L, respectively of the high molecular weight chitosan (9.53×10^5 g/mol) than the others. In addition, a concentration of 500 mg/L of the chitosan low molecular weight (3.60×10^5 g/mol) was the following in a descending order. Moreover, there was no significant difference in the total phenolics content in corn with the chitosans at 100, 250 and 500 mg/L of the medium molecular weight (6.11×10^5 g/mol). This result was compatible with that obtained in the protein contents as previously mentioned.

From the results, the total phenolic content (80.91 - 96.64 µg/g fresh tissue) of wheat sprouts germinated in chitosan solutions was 1.39 - 21.10% higher than that of the control of 0.05% aqueous acetic acid (79.80 µg/g fresh tissue). The total phenolic content (41.32 - 59.16 µg/g fresh weight tissue) of barley sprouts was 1.75 - 45.68% higher than that of the control of 0.05% aqueous acetic acid (40.61 µg/g fresh tissue). Whereas, the total phenolic content in the corn sprouts (238.82 - 292.96 µg/g fresh tissue) was 0.03 - 22.70 % higher than that of the control of 0.05% aqueous acetic acid (238.76 µg/g fresh tissue). In lentil sprouts, the total phenolic content (46.47 - 59.36 µg/g fresh tissue) was 9.68 - 40.10% higher than that of the control of 0.05% aqueous acetic acid (42.37 µg/g fresh tissue).

Table 5. Total soluble phenolic content (µg/g fresh tissue) of seedling leaf tissues in wheat, barely, corn and lentil treated with different molecular weights chitosan compounds.

Treatment (mg/L)	µg phenolic content/g fresh tissue			
	Wheat	Barely	Corn	Lentil
C1	$82.23^{c} \pm 0.96$	$43.85^{cd} \pm 1.01$	$240.32^{c} \pm 5.43$	$49.27^{cde} \pm 0.41$
C2	$79.80^{c} \pm 2.41$	$40.61^{d} \pm 0.22$	$238.76^{c} \pm 2.78$	$42.37^{f} \pm 0.34$
100L	$85.38^{bc} \pm 2.40$	$41.32^{d} \pm 2.07$	$240.94^{c} \pm 1.12$	$46.47^{e} \pm 0.23$
250L	$86.17^{abc} \pm 1.95$	$45.46^{bcd} \pm 1.25$	$254.62^{bc} \pm 2.35$	$47.84^{de} \pm 1.01$
500L	$87.96^{abc} \pm 3.10$	$51.51^{abc} \pm 0.94$	$262.92^{b} \pm 4.11$	$50.34^{cd} \pm 1.11$
100M	$80.91^{c} \pm 2.44$	$49.01^{abcd} \pm 0.11$	$245.41^{bc} \pm 3.29$	$50.31^{cd} \pm 0.19$
250M	$89.43^{abc} \pm 0.90$	$53.81^{abc} \pm 0.85$	$250.32^{bc} \pm 8.11$	$51.35^{cd} \pm 0.79$
500M	$93.33^{ab} \pm 1.35$	$54.83^{ab} \pm 6.21$	$254.09^{bc} \pm 7.94$	$51.94^{c} \pm 1.13$
100H	$90.71^{abc} \pm 4.25$	$52.63^{abc} \pm 0.72$	$238.82^{c} \pm 0.73$	$55.45^{b} \pm 0.90$
250H	$94.07^{ab} \pm 2.49$	$55.45^{ab} \pm 3.15$	$285.14^{a} \pm 5.39$	$57.35^{ab} \pm 0.48$
500H	$96.64^{a} \pm 1.03$	$59.16^{a} \pm 0.78$	$292.96^{a} \pm 4.93$	$59.36^{a} \pm 1.58$

C1, control cultivated in water; C2, control cultivated in 0.05% (v/v) aqueous acetic acid. L = Low molecular weight (3.60×10^5 g/mol); M = Medium molecular weight (6.11×10^5 g/mol) and H = High molecular weight (9.53×10^5 g/mol) chitosan. Data are averages of three replicates ± SE. Values within a column bearing the same superscript are not significantly different ($P \leq 0.05$).

When we consider the amount of the phenolic content in the different plant species, another point deserves attention; it can be noticed that the highest phenolic content was recorded in the corn followed in a descending order by wheat, lentil and barley sprouts.

In addition to traditional sprout vigour parameters, the synthesis of secondary metabolites, such as phenolics, has proved to be useful in the evaluation of plant growth, stemming from the positive correlation between phenolic synthesis and sprout vigour response (Randhir and Shetty, 2003). In the plant cells, simple phenolics are believed to be scavengers of free radicals, protecting the cells from free radical damage. Phenolics are also involved in strengthening the plant cell walls during growth by polymerization into lignans and lignins (Lewis and Yamamoto, 1990; McCue *et al.*, 2000; Burguieres *et al.*, 2007). Moreover, plant phenolics have potential health benefits mainly due to their antioxidant properties such as reactive oxygen species scavenging and inhibition, electrophile scavenging, and metal chelation (Huang *et al.*, 1992).

Randhir and Shetty (2003) reported that a significant increase in total phenolic content of fava beans was to correlate with enhanced seedling height and weight.

In the present investigation seeds that had been germinated with chitosans showed enhancement of phenolic content among all plant species. This increase in the phenolic content was consistent with the increases in the chitosan molecular weight and concentration response of the seedlings. These results are in agreement with Benhamou and Thériault (1992). They reported that the production of phenolic compounds was induced in tomato plant treated with chitosan. Badawy and Rabea (2009) added that the total soluble phenolic compounds in chitosan-treated tomato fruit were significantly increased with an increase in the chitosan concentrations and a chitosan of 5.7×10^4 g/mol was the most active one. In addition, the production of phenolics, phytoalexins or related compounds in tomato plants, induced by chitosan, precedes or coincides with the action of hydrolytic enzymes of *F. oxysporum* f. sp. *radicis-lycopersici* (Benhamou and Thériault, 1992).

Influence of chitosan treatments on Polyphenol oxidase (PPO) activity in wheat, barley, corn and lentil: Polyphenol oxidase (PPO) activity in wheat, barley, corn and lentil germinated with different molecular weights of chitosan at 100, 250 and 500 mg/L are shown in Table 6. In control treatments, a high PPO activity was found, while the activity was decreased to low level with a specific activity of 0.55 OD mg^{-1} protein.30 min^{-1} (53.31% inhibition) in corn sprouts with a 500 mg/L of 9.53×10^5 g/mol chitosan. Generally, the results demonstrated that the PPO activity in all tested plant species was molecular weight and concentration dependant and its activity declined to the lowest value with a concentration of 500 mg/L (Table 6). In addition, a chitosan of 9.53×10^5 g/mol was significantly the most active one in inhibiting of the PPO activity. The reduction in PPO activity was most prominent in corn followed in a descending order by barley, wheat and lentil. It can be noticed that the PPO activity in all tested plant species showed some slight responsiveness to low concentration (100 mg/L) of the low molecular weight chitosan (3.60×10^5g/mol).

Table 6. Polyphenol oxidase (PPO) activity of seedling leaf tissues in barely, wheat, corn and lentil treated with different molecular weights chitosan compounds.

Treatment (mg/L)	PPO Specific activity (OD. mg^{-1} protein. 30 min^{-1})				PPO Inhibition Rate (%)			
	Wheat	Barely	Corn	Lentil	Wheat	Barely	Corn	Lentil
C1	$2.21^{a}\pm0.04$	$3.87^{a}\pm0.03$	$1.17^{a}\pm0.04$	$2.70^{a}\pm0.02$	$0.00^{de}\pm0.00$	$0.00^{f}\pm0.00$	$0.00^{h}\pm0.00$	$0.00^{g}\pm0.00$
C2	$2.20^{a}\pm0.01$	$3.82^{a}\pm0.05$	$1.12^{b}\pm0.01$	$2.67^{a}\pm0.01$	$0.55^{de}\pm0.30$	$1.33^{f}\pm1.17$	$4.37^{g}\pm0.65$	$1.00^{g}\pm0.41$
100L	$2.14^{a}\pm0.01$	$3.74^{a}\pm0.01$	$1.02^{c}\pm0.01$	$2.60^{ab}\pm0.02$	$3.39^{d}\pm0.49$	$3.43^{f}\pm0.21$	$12.59^{f}\pm0.53$	$3.55^{fg}\pm0.57$
250L	$1.88^{b}\pm0.01$	$3.56^{ab}\pm0.07$	$0.91^{d}\pm0.01$	$2.16^{e}\pm0.03$	$14.95^{c}\pm0.26$	$7.99^{ef}\pm1.93$	$22.65^{d}\pm0.91$	$19.85^{c}\pm0.96$
500L	$1.86^{b}\pm0.02$	$3.13^{cd}\pm0.12$	$0.84^{e}\pm0.00$	$2.12^{ef}\pm0.01$	$15.85^{c}\pm0.98$	$19.22^{cd}\pm3.01$	$27.82^{c}\pm0.33$	$21.38^{bc}\pm0.40$
100M	$2.13^{a}\pm0.01$	$3.56^{ab}\pm0.18$	$1.01^{c}\pm0.01$	$2.52^{bc}\pm0.05$	$3.47^{d}\pm0.57$	$8.10^{ef}\pm4.53$	$13.98^{ef}\pm1.00$	$6.51^{ef}\pm1.86$
250M	$1.81^{b}\pm0.01$	$3.32^{bc}\pm0.07$	$0.90^{d}\pm0.01$	$2.33^{d}\pm0.09$	$18.01^{c}\pm0.27$	$14.09^{de}\pm1.73$	$23.26^{d}\pm0.77$	$13.81^{d}\pm3.52$
500M	$1.73^{c}\pm0.01$	$2.37^{e}\pm0.14$	$0.79^{e}\pm0.00$	$1.72^{g}\pm0.02$	$21.54^{b}\pm0.38$	$38.82^{b}\pm3.54$	$32.43^{b}\pm0.16$	$36.18^{a}\pm0.65$
100H	$1.83^{b}\pm0.04$	$2.91^{d}\pm0.17$	$0.99^{c}\pm0.01$	$2.46^{c}\pm0.02$	$17.32^{c}\pm1.84$	$24.94^{c}\pm4.36$	$15.78^{e}\pm0.87$	$8.72^{e}\pm0.66$
250H	$1.73^{c}\pm0.03$	$2.30^{e}\pm0.07$	$0.81^{e}\pm0.01$	$2.02^{f}\pm0.04$	$21.66^{b}\pm1.14$	$40.51^{b}\pm1.73$	$31.10^{b}\pm1.23$	$25.29^{b}\pm1.47$
500H	$1.52^{d}\pm0.02$	$1.85^{f}\pm0.11$	$0.55^{f}\pm0.01$	$1.69^{g}\pm0.01$	$31.04^{a}\pm0.91$	$52.09^{a}\pm2.82$	$53.31^{a}\pm0.68$	$37.52^{a}\pm0.40$

C1, control cultivated in water; C2, control cultivated in 0.05% (v/v) aqueous acetic acid. L = Low molecular weight (3.60×10^{5} g/mol); M = Medium molecular weight (6.11×10^{5} g/mol) and H = High molecular weight (9.53×10^{5} g/mol) chitosan. Data are averages of three replicates ± SE. Values within a column bearing the same superscript are not significantly different ($P \leq 0.05$).

The PPO or tyrosinase is an enzyme with a dinuclear copper centre, which is able to insert oxygen in a position *ortho* to an existing hydroxyl group in an aromatic ring, followed by the oxidation of the diphenol to the corresponding quinone. The structure of the active site of the enzyme, in which copper is bound by six or seven histidine residues and a single cysteine residue is highly conserved. The enzyme seems to be of almost universal distribution in animals, plants, fungi and bacteria. Much is still unknown about its biological function, especially not only in plants, but also in fungi. Therefore, the PPO activity is an important biochemical parameter due to its ability to show whether the phenolics present are antioxidant in nature or are being used for lignification purposes (Mayer, 2006).

In the current study, PPO activity was decreased with the increase in the chitosan concentration and the molecular weight, correlating with the highest levels of the total phenolics (Tables 5 and 6). This may be attributable to the higher demand for oxygen during the seedling growth, requiring higher levels of antioxidant phenolics to protect the cells from potential oxidative damage (Randhir and Shetty, 2003; Burguieres *et al.*, 2007). Although chitosan can induce stress in plant tissues, treatment of suspension cultures of potato did not induce increased PPO activity (Dörnenburg and Knorr, 2001).

Although the mechanisms by which chitosan inhibits browning in fruit and vegetables are not clear but its inhibitory effect is probably a consequence of the ability of the positively charged polymer to adsorb suspended PPO, its substrates, or products. Moreover, its ability to remove the metal ions, such as copper and iron found at enzyme active sites by chelating mechanism with chitosan molecule can therefore render PPO enzyme inactive (McEvily *et al.*, 1992; Badawy and Rabea , 2009).

In general, induced defence reactions in plants are highly correlated with enzymatic responses. Several studies have demonstrated that chitosan is an exogenous elicitor of host defence responses, including accumulation of chitinases, β-1,3-glucanases and phenolic compounds, induction of lignification, synthesis of phytoalexins by the infected host tissue and inhibition of host tissue maceration enzymes (Bhaskara Reddy *et al.*, 1999).

CONCLUSION

The effects of different molecular weights chitosan on wheat, barley, corn and lentil sprouts germinated in 100, 250 and 500 mg/L were investigated in the present study. The major implications of the study are that chitosan linked enhancement of sprouts vigour response is closely linked to growth indices (length and weight of sprout) and biochemical parameters such as chlorophyll content, protein content, phenolic enhancement and polyphenol oxidase activity. The protein and total phenolic contents were gradually stimulated with the increasing of the chitosan molecular weights and their concentrations. This stimulation in protein and phenolic contents closely follows enhancement in growth indices. Therefore, this study suggests that priming seeds with a potential bioplomer chitosan has significant agronomic implications through the improved seed vigour as reflected in growth and the elicitation of biochemical responses. Moreover, the present study suggests a possibility to employ these elicitors for cultivation of some plant sprouts to enhance plant vigour and to improve overall plant productivity. However, to effectively utilize chitosan for such application, basic information is needed on how different molecular weights chitosans and various cultivation conditions affect growth and quality of plants.

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J. Pest Cont. and Environ. Sci. 17 (1/2): 77-101(2009)

تأثير البوليمر الحيوى كيتوزان ذو الأوزان الجزيئية المختلفة على نمو بادرات القمح، الشعير، الذرة والعدس

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تم دراسة تأثير الوزن الجزيئي للكيتوزان وكذلك التركيزات المختلفة منه على صفات النمو فى بادرات كل من القمح ،الشعير،الذرة والعدس. حيث تم إنبات البادرات فى تركيزات 100 ، 250 و500 ملجرام/لتر وذلك من الأوزان الجزيئية الثلاثه (3.6×10^5، 6.11×10^5 و 9.53×10^5 جم/مول) لمدة 8 أيام بإستخدام طريقة نيوبيير. تم دراسة صفات النموالتقليدى، العوامل المترتبطة به مثل تقدير البروتين، الفينولات ،الكلوروفيل، ونشاط إنزيم بولي فينول أكسيديز بالتفصيل. وأوضحت النتائج أن معاملات الكيتوزان حسنت نمو البادرات معنويا بدرجات متفاوته بالنسبة للكنترول. أيضا إتضح من النتائج أن المحتوى البروتينى فى كل من نبات القمح والشعير كان يزيد تدريجيا بزيادة كل من الوزن الجزيئي والتركيز. بينما أظهر مركب الكيتوزان ذو الوزن جزيئي 3.6×10^5 جم/مول أعلى تأثير معنوى عند تركيز 500 جم/لتر فى كل من الذرة ، العدس. بالإضافة لما سبق وجد أن الفينولات الكليه فى النباتات المختبرة حدث لها تراكم عالى بالتدرج بزيادة التركيز والوزن الجزيئي. وعلى الجانب الأخر حدث خفض معنوى فى مستوى كل من الكلوروفيل و إنزيم البولى فينول أوكسيديز.