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Exogenous application of chitosan and humic acid effects on plant growth and yield of pea (*Pisum sativum*)

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Abstract

Chitosan is an organic polymer obtained from de-acetylation of chitin and Humic acid contain acidic groups such as carboxyl and phenolic OH functional groups. Therefore, it provides organic macromolecules with an important role in the transport, bioavailability and solubility of heavy metals Foliar application of both shows positive impacts on the growth and yield of various important crops. A pot experiment was conducted to find out effect of foliar application of chitosan in combination with Humic acid on growth and yield of pea (*Pisum sativum*) at the University of Agriculture Peshawar during growing season of 2016. Chitosan an organic plant growth stimulant in combination with humic acid were tested as a foliar application for its effects on pea plant. Various combinations of Humic acid (0, 1, 2, 3 g L^{-1}) and Chitosan (0, 40, 60, 80 mg L^{-1}) were applied through foliar spray. Experiment was laid out in completely randomized design (CRD). Pea cultivar Meteor was used for research trail. Data were collected on plant growth parameters such as plant height, number of flowers, chlorophyll content and dry weight of plant and yield parameters such as number of pods, protein content in pods, pod length and weight. Plant growth and yield attributes were significantly affected by chitosan and humic acid in combination (CHI x HA). Results indicated that chitosan and humic acid significantly affected growth and yield attributes of pea. Maximum number of pods (94.33), pod length (13.50 cm) were recorded with 80mgL^{-1} CHI and 2gL^{-1} HA. It was concluded that chitosan and humic acid combination (80mgL^{-1} CHI and 2gL^{-1} HA) gave the best results in enhancing different growth and yield attributes significantly as compared with control treatment.

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Introduction

Pea (*Pisum sativum L.*) is an important vegetable crop largely cultivated around the Pakistan and all over the world. Peas are important for their nutritional values as it is constituent of high amount of protein, carbohydrates, vitamin A and B. Peas are source of minerals such as phosphorous, iron and zinc. It is a cheap source of protein and minerals especially important in developing countries where zinc deficiency is fifth major cause of diseases and even deaths (El-Hak *et al.*, 2012, Ibrahim and Ramadan, 2015). Many efforts have been previously made for increasing growth, yield and productivity of this important crop. Chitosan and Humic acid could be helpful in achieving this goal. Chitosan and Humic acid has been shown to increase plant growth, yield and improving physiological processes in plant (Farouk *et al.*, 2011). Humic acid contain acidic groups such as carboxyl and phenolic OH functional groups. Therefore, it provides organic macromolecules with an important role in the transport, bioavailability and solubility of heavy metals (Chen and Zhu, 2006). Humic acid is considered to increase the permeability of plant membranes and enhance the uptake of nutrients. Moreover, it is also considered to improve soil nitrogen uptake and encourage the uptake of potassium, calcium, magnesium and phosphorus, making these more mobile and available to plant root system (Kaya *et al.*, 2005). The stimulating effects of humic substances on plants growth have been reported, i.e., increase in dry weight of shoot, root growth, plant height and macronutrient uptake in oat plants has been observed (Rosa *et al.*, 2004), likewise phosphorus uptake and dry matter production was enhanced in corn (Andrade *et al.*, 2004), wild olives were shown to get stimulated in its growth along with leaf N and chlorophyll content (Murillo *et al.*, 2005). Chitosan is a natural, low toxic and inexpensive compound that is biodegradable and environment friendly with various applications in agriculture. It is obtained by the de-acetylation of chitin. In agriculture, chitosan is of keen interest now a days and used in seed, leaf, fruit and vegetable coatings, as fertilizer and in controlled agrochemical release, to increase plant productivity (Nwe *et al.*, 2004). The beneficial effect of humic acid (HA) and Chitosan (CHI) on plant growth may be attributed to the promoting effects on nutrients uptake and nutritional status especially nitrogen, potassium and phosphorous which are essential for plant growth. Chitosan is reported to have insecticidal and disease preventive actions, also it helps plants to stimulate stress tolerance (Malerba and Cerana, 2016). Keeping in view these points this research trial was designed to investigate the effect of chitosan and humic acid on growth and yield of pea plant in order to obtain high quality plants and yield.

Materials and methods

Experimental site and design

A pot experiment entitled "effect of chitosan and humic acid on growth and yield of *Pisum sativum*" was carried out at Horticulture Nursery of The University of Agriculture Peshawar during 2016-2017. Trial was planned in completely randomized design (CRD) with three replications. Black plastic pots of size 7x12 inch were used. Planting media containing 1:1 soil and farm yard manure was thoroughly mixed and pots were filled equally. Soil samples were taken and analyzed for chemical properties (table 1). Various combinations of chitosan (control, 40, 60, 80 mg L^{-1}) and humic acid (control, 1, 2, 3g L^{-1}) were under consideration.

Stage of foliar application

Three foliar application were done first at vegetative stage (2nd week) second at flowering stage (4th week) and third at pods formation (8th week) while control was sprayed with simple tap water. Pea cultivar Meteor seeds obtained from National Agriculture Research council (NARC) were used during this research trial. Data were collected on growth parameters i-e. Plant height (cm), number of flowers, plant dry weight (g) and chlorophyll content of leaves (SPAD). Chlorophyll content was recorded using spade meter. Also yield attributes such as number of pods, pod length (cm) and weight (g) and protein content in pods. Protein contents were determined by the method as described by AOAC (2000).

Statistical analysis

The recorded data were subjected to statistical analysis according to CRD factorial design using statistical package "Statistix8.1". Means were differentiated using least significant differences (LSD) test at 5% level of significance according to Steel and Torrie (1980).

Spray preparation

Chitosan obtained from Sigma Aldrich Company with 95 % degree of de-acetylation were dissolved in simple tap water with addition of small quantity of acetic acid. Humic acid crystals in the form of potassium humate were grinded into fine powder and dissolved in tap water. This solution was passed through mesh to remove large size particles.

Results and discussion

Chitosan (CHI) and humic acid (HA) interaction were found significant in improving plant growth (Table 2) and yield attributes (Table 3) of pea. Graphs showing interactive effect of chitosan and humic acid are presented in Fig. 1 while that of yield in Fig. 2. *Effect on plant growth attributes*

A linear increasing trend has been observed almost in all growth parameters. Increasing CHI with HA levels increased plant height, number of flowers, chlorophyll content and plant dry weight of pea plant. plant height showed highest values (61.50 cm) up-to 40 mgL^{-1} CHI and 2 gL^{-1} HA then exponential decrease was observed with increasing CHI and HA, while lower values for plant height (46.13 cm) were shown by treatments that received 60 mgL^{-1} and 3 gL^{-1} CHI and HA respectively (Fig. 1a). Maximum number of flower (102) and plant dry weight (32 g) was shown by 80 mgL^{-1} CHI and 3 gL^{-1} HA, while lower number of flowers (76) and plant dry weight (20 g) was shown by control 0 mgL^{-1} CHI and 0 gL^{-1} HA (Fig. 1b and 1d respectively). Chlorophyll content was found maximum (69.22) at 80 mgL^{-1} CHI and 3 gL^{-1} HA while lower values for chlorophyll content (41.69) was observed at 0 mgL^{-1} CHI and 0 gL^{-1} HA (Fig. 1c). Plant growth parameters showed a linear increasing trend with increasing chitosan and humic acid levels as compared to control.

Table 1. Showing different chemical and physical properties of soil used at time of sowing.

pH	6.5
Texture	Silt loam
N	29.5 ppm
P	13.3 ppm
K	231 ppm

This may be due to the protective effect of chitosan as it had shown insecticidal properties and helps in prevention of diseases caused by various agents such as bacterial and fungal, also chitosan induces stress tolerance (Malerba and Cerana, 2016). At the same time humic acid helps in nutrients uptake increase nutrients availability and works in membrane permeability as a results plant growth is enhanced.

Our results are confirmed by Mondal *et al.* (2013) who reported that foliar application of chitosan improved plant height, number of leaves, length, breadth and area of leaves in maize. Similar results were also reported by Algam *et al.* (2010) whoreported that chitosan improved plant growth in tomato crop when applied as a soil drench or seed treatment. Chitosanis also reported to significantly increase plant growth characteristics in chilli (Chookhongkha *et al.*, 2012) however Humic acid is reported to have stimulated plant growth in tomato (Adani *et al.*, 1998) and found to improve plant height in peas either alone or in combination with antioxidants(El-Hak *et al.*, 2012). Number of flowers are reported to be increased with humic acid foliar application (El-Nemr *et al.*, 2012). Unfavorable stressful conditions lead to flowers abortion and reduces number of flowers in lentil (Janmohammadi *et al.*, 2014).

Table 2. Pea plant growth traits as affected by foliar application of chitosan and humic acid.

Treatments	Traits			
	Plant height(cm)	Flowers plant^{-1}(No.)	Chlorophyll Content (spad)	Plant dry weight (g)
Chitosan(mg L^{-1})				
0 (Control)	50.51 b	84.17 b	52.52 b	22.25 d
40	54.72 a	85.42 b	61.00 a	24.67 c
60	52.35 ab	85.08 b	55.11 b	26.42 b
80	52.73 ab	91.58 a	54.15 b	29.42 a
LSD at 5%	2.88	3.68	5.87	1.08
Humic acid(g L^{-1})				
0 (Control)	51.92 b	80.17 d	48.12 c	23.92 c
1	52.34 b	84.00 c	53.14 bc	25.33 b
2	56.30 a	93.67 a	57.56 b	26.42 a
3	49.74b	88.42 b	63.96 a	27.08 a
LSD at 5%	2.88	3.68	5.87	1.08
Interaction				
CHI × HA	*Fig 1a	*Fig 1b	*Fig 1c	*Fig 1d
LSD at 5%	5.75	7.36	11.74	2.16

*Represent Significant differences at 5% level of significance using Least significant differences (LSD) test.
Mean values followed by different letter are significantly different at 5% level of significance.

Senesi and Loffredo (1994)found that pea plant dry weight was increased by exogenous spray of humic acid @ 100 mg+ herbicides. Our results are confirmed by Sani (2014) who found that humic acid could improve plant height of canola plant. Humic acid also improved plant growth in wheat (Tahir *et al.*, 2011).

Effect on yield attributes of pea

Table 3 pertaining means for pea yield components indicates that chitosan and humic acid significantly affected pea yield (number of pods plant^{-1}, length, weight and percent protein content of pods).

Table 3. Yield attributes of pea as affected by foliar application of chitosan and humic acid.

Treatments	Traits			
	Number of pods	Single Pod weight (g)	Pod Length (cm)	Protein in pods (%)
Chitosan (mg L^{-1})				
0 (Control)	75.25 c	6.18 d	9.85 c	39.60 c
40	78.08 b	6.49 c	9.26 d	41.13 c
60	80.25 b	7.21 b	10.58 b	44.46 b
80	86.08 a	7.88 a	12.41 a	50.20 a
LSD at 5%	2.43	0.08	0.56	1.61
Humic acid (g L^{-1})				
0 (Control)	78.50 b	6.72 d	10.74 a	41.46 c
1	77.00 b	6.83 c	9.85 b	43.60 b
2	82.00 a	6.98 b	10.60 a	46.37 a
3	82.17 a	7.24 a	10.92 a	43.97 b
LSD at 5%	2.43	0.08	0.56	1.61
Interaction				
CHI × HA	*Fig 2a	*Fig 2b	*Fig 2c	*Fig 2d
LSD at 5%	4.87	0.16	1.11	3.22

*Represent Significant differences at 5% level of significance using Least significant differences (LSD) test.
Mean values followed by different letter are significantly different at 5% level of significance.

Maximum number of pods (94.33) and pod length (13.50 cm) were recorded in plants sprayed with 80 mgL^{-1} CHI and 2 gL^{-1} HA while minimum number of pods (73) was shown by foliar application of 40 mgL^{-1} CHI and 1 gL^{-1}HA (Fig 2a and 2c respectively), also minimum pod length (7.6) was observed at 40 mgL^{-1} CHI and 2 gL^{-1} HA (Fig 2c). Maximum values for pod weight (8.2) were recorded at 80 mgL^{-1} CHI and 3 gL^{-1} HA (Fig 2b), and that of percent protein in pods (56.37%) were found at 80 mgL^{-1} CHA and 2 gL^{-1} HA (Fig 2d). While minimum pod weight (6 g) and percent protein in pods (37.53%) were shown in control plants (0 mgL^{-1} CHI, 0 gL^{-1} HA).

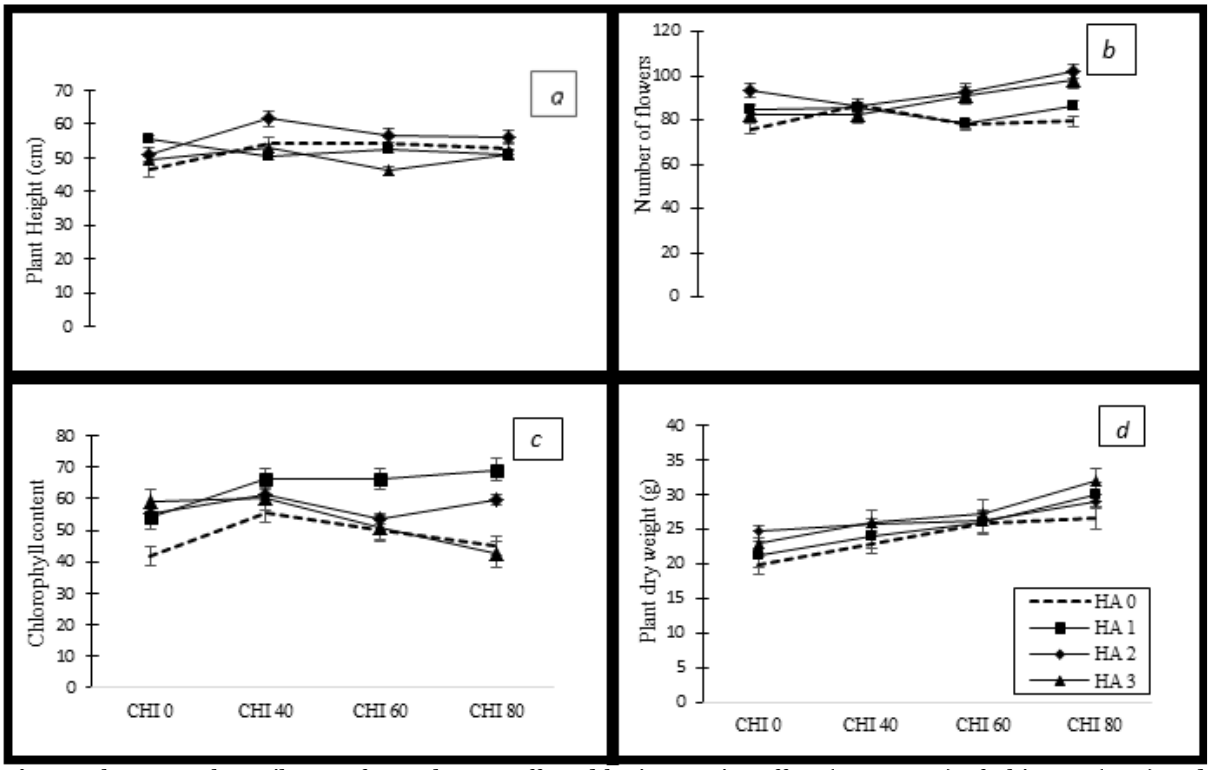

Fig. 1. Plant growth attributes of pea plant as affected by interactive effect (CHI × HA) of Chitosan (CHI) and Humic acid (HA). Fig. 1a= plant height, 1b=flowers $plant^{-1}$, 1c=Chlorophyll content, 1d=dry weight of plant.

The increment in yield parameter may be due to HA because they constitute a stable fraction of carbon, thus regulating the carbon cycle and release of nutrients, including nitrogen, phosphorus, and sulfur, which decreasing the need for inorganic fertilizer for plant growth. HA stimulate plant growth by the assimilation of major and minor elements, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production (Ulukan, 2008).

Chitosan has been reported to increase number of pods in feba bean (El-Ghamry *et al.*, 2009). Humic acid application at 1 g L^{-1} with irrigation water increased number of pods, yield per plant, and average pod fresh weight in pea (El-Hak *et al.*, 2012).

These results are also confirmed by Boehme *et al.* (2005)who found that plant yield parameters in cucumber was improved with application of humic acid. Number of flowers are subjected to stress condition. As chitosan improves plant tolerance to stress condition (Malerba and Cerana, 2016) that's why the flowers may be affected.Aisha *et al.* (2014) found that Humic acid improves total chlorophyll content in leaves of turnip and chitosan in chilli (Chookhongkha *et al.*, 2012).

These results are confirmed by the findings of El Nagar *et al.* (2013), who found that vegetative growth and yield of pea plants was significantly improved with foliar application of 1% chitosan.

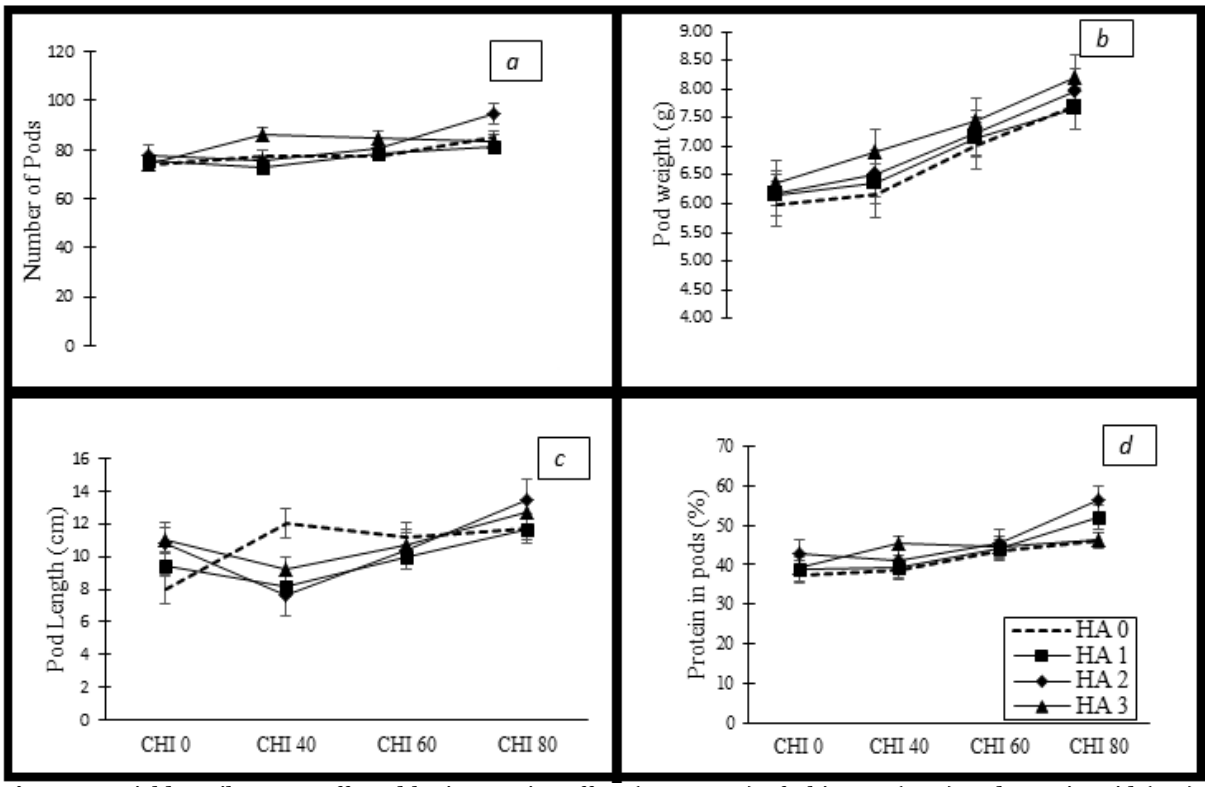

Fig. 2. Pea yield attributes as affected by interactive effect (CHI x HA) of Chitosan (CHI) and Humic acid (HA). Figure 2a=Number of pods, 2b pod weight, 2c=Pod length, 2d=percent protein in pods.

Conclusion

On the basis of this research we came to know that chitosan and humic acid combination had a good effect on vegetative growth as well as yield of pea plants specially the meteor variety. These compounds has potential in increasing plant height, dry matter, yield and yield components. Thus we suggest in this study that chitosan and humic acid both could be used to retain our yield and plant growth without damaging environment or soil health due to their organic origin. Likewise these compounds could be a safe alternative for synthetic fertilizers as well as insecticides that are harmful for environment and human health.

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