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Effect of sowing dates, natural bio-stimulants, and micro-nutrients on the growth and seed yield of dry bean (*Phaseolus vulgaris* L.) under semi-arid conditions

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Abstract

Two field experiments were conducted to study the effect of different sowing dates (October 1 and 15) during the 2021/2022 and 2022/2023 seasons on dry bean (*Phaseolus vulgaris* L.) variety (Nibrasica) and foliar spraying by natural bio-stimulants [chitosan (150 ppm) and humic acid (500 ppm)] as well as foliar spraying by some micro-nutrients [iron (200 ppm), manganese (100 ppm), and zinc(50 ppm)] under the conditions of Sohag governorate, Egypt. The experiments were carried out in a split split-plot design in three replicates. The main plots were allocated to sowing dates, while sub-plots were allocated to natural bio-stimulants and the sub-sub-plot was allocated to foliar spraying with micro-nutrients. Results show that the second sowing date (10/15) recorded the highest significance value for all vegetative and crop traits under study in both seasons. Late sowing surpassed early sowing in dry seed yield by (26.53% and 25.19%) in the first and second seasons, respectively. Spraying bean plants with chitosan and humic acid also led to a significant increase in all vegetative traits, as the chitosan spray treatment was superior to spraying with humic acid in plant height, number of branches per plant, number of pods per plant, and pod length. Spraying with chitosan led to an increase in 100 seeds weight by (29.59 and 30.45) and dry bean yield by (26.53% and 25.19%) compared to the comparison treatment. The results showed that foliar spraying with all the microelements resulted in a highly significant increase in the vegetative and yield characteristics of bean plants, and the foliar spraying treatment with iron distinguished itself from the rest of the elements. The results indicated that all interactions, whether binary or triple, were between the experimental factors, with the highest values recorded in favour of sowing on the second date (October 15). Overall, spraying chitosan (two times before and after flowering), and foliar spraying with iron after 20 and 40 days of planting lead to maximization dry bean production in the second sowing date (October 15).

Keywords: chitosan, dry bean, humic acid, Fe, Mn, Zn, yield components.

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1. Introduction

Dry bean (*Phaseolus vulgaris* L.) is widely cultivated in many countries with a total estimated harvested area of about 34.8 million hectares that produce about 27.5 million tons (FAO, 2022). In Egypt, it is cultivated for local market and exportation with a total estimated harvested area of about 88.12 thousand fed., that p about 144.8 thousand tons (World Bank, 2000). The exported amounted about 132 thousand tons, with an estimated value of about $115 million in 2020 (FAO, 2022). It cultivated in many region of Egypt. It is relatively sensitive plant to climate change. The optimum temperature range for optimum bean growth is 16-30 °C (Nonneck, 1989). Foliar application of chitosan as bio- stimulate and humic acid as one of the organic compounds enhances plant growth and consequently increased plant crops. Mondal *et al*., (2012). Results revealed that most of the morphological (plant height, leaf number plant^{-1}), growth and yield attributes (number of fruits plant^{-1} and fruit size) were increased with increasing concentration of chitosan until 25 ppm, resulted the highest fruit yield in okra (27.9% yield increased over the control). Shehata *et al*. (2012) showed that foliar application with yeast and chitosan significantly increased the vegetative growth, yield and its quality of cucumber. It can be concluded that foliar application with chitosan at rates of 4 mL recorded the best treatment to obtain the highest vegetative growth, yield and quality of cucumber plants. Moreover, Abu-Muriefah (2013) showed that foliar-application with chitosan at 200 mg L^{-1}, increased *Phaseolus vulgaris* plants growth, yield and its quality as well as physiological constituents in plant shoot under stressed or non-stressed conditions as compared to the control. Islam *et al.* (2016) indicated that foliar application of chitosan at 75, 50, 100 and 50 ppm enhanced plant growth and increased in total dry mass production of tomato, mungbean, maize and rice. Additionally, Fouda *et al*. (2022) found that single foliar spraying of faba bean with tryptophan 75 ppm, potassium silicate at 100 ppm, or chitosan at 750 ppm significantly increased all studied traits. Mahmoud *et al.* (2011) indicated that the combined application of soil application of HA (15 kg feddan^{-1}) + foliar spray of 0.1% HA recorded the highest mean plant height (74.9 cm), number of branches (3.22) and dry weight of shoot (89.7 g plant^{-1}). Gad El-Hak *et al.* (2012) recommended growing peas cv. Master with spraying 200 ppm salicylic acid and humic acid at the rate of 1 g L^{-1} to produce high quality fresh pods and seed yields. Micronutrients are essential mineral elements which considered very important in the development of plants among growth hormones, soluble elements, increased absorption and transport of nutrients, detoxification, increased transfer of sugar and amino acids in plant roots (Dimkpa *et al*., 2016). This study aims to investigate the impact of foliar application of chitosan and humic acid, as well as leaf feeding with select micronutrients, on the cultivation of dry bean crop (*Phaseolus vulgaris* L.) under semi-arid conditions in Egypt, with a focus on two different planting dates. Our hypothesis posits that these treatments, combined with adjustments according to sowing dates,

will positively influence the vegetative growth, yield components, and overall productivity of dry bean (*Phaseolus vulgaris* L.) cv. Nibrasica. Specifically, we anticipate that the application of natural biostimulants and micronutrients will enhance root development, improve nutrient uptake efficiency, and bolster plant resilience. Consequently, we expect to observe increased plant height, branch number, pod formation, pod length, 100-seed weight, and ultimately higher dry seed yield per unit area compared to untreated control groups.

2. Materials and methods

2.1 Experimental site and treatments description

Two field experiments were carried out during the Nili seasons of 2021/2022 and 2022/2023 at private Farm, in Shattora village, Tahta city, Sohag governorate, Egypt. The experiments were laid out in split-split plot design in three replicates. The main plots were assigned for sowing dates (1st October and 15th October), while sub-plots were designated for natural biostimulants (Control, Chitosan, and Humic acid), with micro-nutrients (Control, Fe, Mn, and Zn) placed in sub-sub-plots. The unit plot size was (3.5 × 3.0 m = 1/400 feddan). Sowing took place in the month of October of each season, with a difference of 15 days, meaning that the planting dates were began on the first and the date in the fifteenth of October. Natural bio-stimulants Chitosan (2-amino-2-deoxy-ˇ-d-glucosamine) namely Chito-Care® with a degree of de acetylation of 85% was used at a concentration of 150 ppm, while, soluble humic acid as potassium-humate (80% humic acid, 11–13% K_2O) was prepared to a concentration of 500 ppm. Natural bio-stimulants foliar spray compounds were sprayed twice before and after flowering. The sub-sub-plots were devoted to Fe, Mn and zinc foliar spray at a concentration of 200, 100 and 50 ppm, as nutrient sulfate, respectively. Micro-nutrients foliar spray treatments were applied two times (at 20 and 40 days after sowing). The control plants were sprayed with tap water. Bio-film as a wetting agent was applied at 0.5 mL liter^{-1} of the solution. The plants were sprayed until drop-off by using a hand-sprayer at afternoon. The physical and chemical analyses of experimental soil used during the two seasons (2020 and 2021) were demonstrated in Table (1). Agronomic practices were followed as usually done for the dry bean crop at Sohag region. Phosphorus and potassium fertilizers were applied prior to land preparation at the rate of 30 kg feddan^{-1} P_2O_5 and 24 kg feddan^{-1} K_2O in the form of super phosphate (15.5% P_2O_5) and potassium sulfate (48% K_2O), Nitrogen was applied at a rate of 20 kg feddan^{-1}, as amonium sulphate through its growth, respectively. Seeds of dry bean (Nebraska) were sown on the 28th of August and harvested on the 6th of November (85days) in both seasons, respectively. Seeds were inoculated with bio-fertilizer mixture of *Azotobacter chroococcum* and *Azospirillum ipoferum*. On the seeding day, 3-4 seeds were to be

placed in hills 30 cm apart along the dripper lines. After germination, number of plants was thinned to 2 plants per hill to achieve a full 100% stand at the beginning of the cultivation season. Floating irrigation was followed in both seasons. Some physical and chemical characteristics of the compost used are presented in Table (1).

Table (1): Physical and chemical properties of the experimental site soils.

Seasons	%			Texture	Electrical conductivity dS m^{-1}	pH (1:2.5)	$CaCO_3$ %
	Clay	Silt	Sand				
2021/22	6.20	24.20	69.60	Sandy Loam	1.81	8.36	5.36
2022/23	6.50	24.10	69.40	Sandy Loam	1.40	8.04	4.66

2.2 Data recorded

Four plants form each treatment in each replication was randomly selected and tagged for records on growth, yield components and total yield as well fruit quality parameters and some nutrient concentrations. All the following characters were determined after 75 days of transplant.

2.2.1 Effects on some vegetative growth properties

Plant height at harvest (cm) and number of branches were recorded.

2.2.2 Effects on some yield components properties

Number of pods and pod length were recorded.

2.2.3 Effects on some yield properties

100 seed weight (g) and dry seed yield (ton/feddan) were recorded.

2.3 Statistical analyses

All obtained data were subjected to statistical analysis of variance and treatment means were compared for significant differences using the LSD at p = 0.05. The MSTAT-C computer program was used to perform all the analysis of variance with the procedure outlined by Still and Torrie (1982).

3. Results and Discussion

3.1 Effects on some vegetative growth properties

3.1.1 Plant height at harvest (cm)

Data presented in Table (2) showed highly significant differences ($P<0.01$) were observed between bean plant height. The obtained results showed that the 2nd sowing date (15/10) recoded the maximum value of plant height (58.38 and 62.33 cm), while the 1st sowing date (1/10) recorded the minimum value (52.67 and 54.28 cm) in the 1st and 2nd seasons, respectively. Also, the 2nd sowing date (15/10) highly significantly increased plant height by (17.00% and 13.15%) over the 1st sowing date. The positively impact of late sowing on plant height might be related to climate change

on this date. In this respect, Elham Badr *et al.* (2013) showed significant increases in plant height among the studied sowing dates (31st October, 15th November and 30th November) of two faba bean cultivars (Sakha-1 and Giza- 461). Although, data show that main values of plant height significantly affected by spraying with bio-fertilizers. Spraying bean plants either with humic acid or chitosan significantly increased plant height compared with control treatments in both seasons. The highest plant height values were obtained (57.96 and 61.96 cm) due to the spraying with chitosan. While the minimum plant height values were recorded (49.33 and 53.02 cm) due to the control without bio-fertilizer. Spraying bean plants with chitosan highly significantly increased plant height by (49.33% and 16.86%) over the control treatment. The increase in plant height due to spraying chitosan could be attributed to the vital role of chitosan in increasing the key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improved the transportation of nitrogen (N) in the functional leaves which enhanced plant growth and development (Khan *et al*., 2002). Similar results were reported by Gornik *et al.* (2008) who reported that chitosan enhanced plant growth and development. Also, Fouda *et al.* (2022) stated that Chitosan spray (750 ppm) gave the tallest plants of faba bean. Concerning the effect of foliar application of micronutrients, results in Table (2) revealed that plant height was highly significantly increased by foliar application of all micronutrients in both seasons under the study. Foliar application of Fe gave the maximum plant height values (56.70 and 60.86 cm). Meanwhile, the minimum was (51.37 and 55.39 cm) in both seasons, respectively. The increase in plant height due to micronutrients foliar applications may be attributed to the fact that foliar application by Fe might be led to increasing the photosynthetic activity and the production of protein and carbohydrates in the plant (Mahmoud *et al*., 2022). Iron element is also used in the structure of cytochromes (Yilmaz *et al*., 2008). Abdel-Mawgoud *et al.* (2011) found that spraying micronutrients improve growth and production of green bean (*Phaseolus vulgaris* L.) cv. Pulista, under new reclaimed land conditions. Concerning the 2nd interaction effects (A × B), results in Table (2) indicated that the 2nd sowing date (D2: 15/10) and spraying bean plants by chitosan recorded the maximum plant height (62.68 and 66.41 cm) in both seasons, respectively. In relation to the interaction between sowing date and spraying by micronutrients (A × C), results in tables (2) indicated that the 2nd sowing date (D2: 15/10) and spraying bean plants by Fe recorded the maximum plant height (62.00 and 65.92 cm) in both seasons, respectively. Also, the demonstrated data shows that the interaction between spraying bio-stimulate and micronutrients (B × C), results in tables (2) indicated that spraying bean plants by chitosan and Fe recorded the maximum plant height (60.77 and

64.64 cm) in both seasons, respectively. The increase in bean plant height as result of all the 2nd interaction effects either under (A × B), (A × C), or (B × C) could be explained under the basis that interaction effects between bio-stimulants (chitosan or humic acid) and micronutrients foliar applications on plant growth might be due to the stimulation effect of hormones which used in the synthesis of proteins or might be due to the important role of growth hormones, soluble elements in increasing absorption and transport of nutrients, detoxification, increasing transfer of sugar and amino acids in plant roots, and enhancing induction resistance to environmental stresses (Fazeli-Nasab *et al.*, 2022). Regarding the third interaction effects between (A, B and C), results recorded that sowing bean seeds at the 2nd date (D2: 15/10) and spraying bean plants by chitosan at a concentration of 150 ppm twice before and after flowering) and foliar application of Fe at 200 ppm twice; at 20 and 40 days from sowing) recorded the highest plant height (65.33 and 69.13 cm) in both seasons, respectively.

Table (2): Effect of spraying bio-stimulate (humic acid and chitosan) and foliar spray by micronutrients (Fe, Mn and Zn) on plant height of dry bean (*Phaseolus vulgaris* L.) cv. Nebraska sown at different dates during 2021/2022 and 2022/2023 seasons.

Seasons		2021/2022					2022/2023				
Date	B.	C.	Fe	Zinc	Mn	Mean	C.	Fe	Zinc	Mn	Mean
A1 1/10	Control	44.80	48.00	46.20	45.00	46.00	47.93	51.87	50.13	49.23	49.79
	Humic	50.00	54.00	51.33	50.90	51.56	54.41	59.16	54.71	53.86	55.54
	Chitosan	51.00	56.20	53.33	52.40	53.23	55.87	60.15	57.91	56.11	57.51
Mean		45.00	57.33	55.33	53.00	52.67	52.74	57.06	54.25	53.07	54.28
A2 15/10	Control	45.00	57.33	55.33	53.00	52.67	49.21	61.43	58.20	56.19	56.26
	Humic	58.20	63.33	61.12	60.14	60.70	61.27	67.20	64.65	64.23	64.34
	Chitosan	62.00	65.33	62.40	61.00	62.68	66.31	69.13	65.67	64.51	66.41
Mean		55.07	62.00	59.62	58.05	58.68	58.93	65.92	62.84	61.64	62.33
B × C	Control	44.90	52.67	50.77	49.00	49.33	48.57	56.65	54.17	52.71	53.02
	Humic	54.10	58.67	56.23	55.52	56.13	57.84	63.18	59.68	59.05	59.94
	Chitosan	56.50	60.77	57.87	56.70	57.96	61.09	64.64	61.79	60.31	61.96
General mean		51.37	56.70	54.29	53.12		55.39	60.86	57.93	56.74	55.39

F test and LSD 0.05	F test	LSD 0.05	F test	LSD 0.05
Date (A)	**	-	**	-
Foliar (B)	**	1.09	**	1.17
A × B	**	0.891	**	0.974
C	**	1.11	**	1.31
A× C	**	1.23	**	1.42
B × C	**	1.87	**	1.91
A × B × C	**	1.95	**	2.03

3.1.2 Number of branches

Data presented in Table (3) showed that number of branches was significantly affected (P<0.01) by bio-stimulates, micro-nutrients foliar application, and sowing dates under Sohag governorate conditions during 2022/23 and 2023/24

seasons. The obtained results in Table (3) showed that the 2nd sowing season (D2: 15/1) significantly increased the number of branches compared to the 1st sowing date (D1: 1/10) in both seasons. Spraying chitosan recorded the maximum value of number of branches (13.5 and 14.65 cm), while the control treatment recorded the minimum value (9.69 and 10.44 cm) in the 1st and 2nd seasons, respectively. Many investigators reported the impact of chitosan in promoting growth of various crops; Lee *et al.* (2005) on soybean sprouts, Abd El Mawgoud *et al.* (2010) on strawberry and Ghoname *et al.* (2010) on sweet pepper plants. Similar results were obtained by Ibrahim and Ramadan (2015). Data presented in Table (3) showed significant ($P<0.05$) differences were observed between bio-stimulates on number of branches. Although, the highest values of number of branches (13.98 and 14.65 cm) were obtained due to the chitosan spraying. While the minimum number of branches values (8.06 and 8.89 cm) was due to the control (without bio-fertilizer) in both seasons, respectively. The increasing percentage of number of branches due to chitosan spraying reached (73.44% and 68.50%) in comparison to the un-sprayed plants in the 1st and 2nd seasons, respectively. This might be attributed to the stimulating effect of chitosan on plant growth and to the increase in the availability nutrients and uptake of water. This result is similar to that in sweet pepper (Ghoname *et al*., 2010) and cucumber and radish plants (Farouk *et al*., 2008; 2011). Sheikha and AL-Malki (2011) indicated that chitosan enhanced bean shoot and root length, fresh and dry weights of shoots, root and leaf area as well as the level of chlorophylls. Moreover, Fouda *et al.* (2022) indicated that number of branches of faba bean (*Vicia faba* L.) was significantly increased with the increasing chitosan spray to 750 ppm compared to control (spraying with tap water) under different irrigation regimes. Concerning the effect of foliar application by micronutrients, results in Table (3), revealed that number of branches were significantly affected by foliar application of micronutrients (Fe, Mn and Zn). Data shows clearly that Fe foliar application gave the maximum values of number of branches (12.69 and 13.54), while the minimum number of branches (9.89 and 11.03) were obtained due to the control treatment in both seasons, respectively. The increasing percentage of number of branches due to Fe spraying reached (15.33% and 22.75%) in comparison to the un-sprayed plants in the 1st and 2nd seasons, respectively. The impact of spraying micronutrients on Phaseolus bean plants' growth parameters were differ in their response to Fe or Mn or Zn foliar spray in the same stage, which reflect the important role of each nutrient in plant physiological processes at specific plant growth stages with specific nutrient concentration. Similar results were in agreement with Marschner (1995) and Silva *et al.* (2008). Concerning the 2nd interaction effects between (A × B), (A × C) and (B × C), results recorded in

Table (3) indicate that all interactions impact positively on number of branches in both seasons. Sowing Phaseolus bean seeds at the 2nd date (D2: 15/10) and sprayed by chitosan (A × B) recorded the maximum number of branches (16.17 and 17.37) in both seasons, respectively.

Table (3): Effect of spraying bio-stimulate (humic acid and chitosan) and foliar spray by micronutrients (Fe, Mn and Zn) on number of branches of dry bean (*Phaseolus vulgaris* L.) cv. Nebraska sown at different dates during 2021/2022 and 2022/2023 seasons.

Seasons		2021/2022					2022/2023				
Date	B.	C.	Fe	Zinc	Mn	Mean	C.	Fe	Zinc	Mn	Mean
A1 1/10	Control	4.60	7.60	7.00	6.20	6.35	5.12	8.21	7.67	6.87	6.97
	Humic	10.00	11.96	11.00	10.80	10.94	11.35	12.35	11.87	11.43	11.75
	Chitosan	11.80	12.67	11.66	11.00	11.78	12.91	13.41	12.24	11.81	12.59
Mean		8.80	10.74	9.89	9.33	9.69	9.79	11.32	10.59	10.04	10.44
A2 15/10	Control	6.20	11.86	11.00	10.00	9.77	7.21	12.56	12.11	11.33	10.80
	Humic	13.00	16.00	14.90	14.33	14.56	14.93	17.33	15.67	15.20	15.78
	Chitosan	14.80	18.00	15.67	16.20	16.17	15.91	19.61	16.75	17.20	17.37
Mean		11.33	15.29	13.86	13.51	13.50	12.68	16.50	14.84	14.58	14.65
B × C	Control	5.40	9.73	9.00	8.10	8.06	6.17	10.39	9.89	9.10	8.89
	Humic	11.50	13.98	12.95	12.57	12.75	13.14	14.84	13.77	13.32	13.77
	Chitosan	13.30	15.34	13.67	13.60	13.98	14.41	16.51	14.50	14.51	14.98
General mean		9.89	12.69	11.59	11.12		11.03	13.54	12.41	11.98	

F test and LSD 0.05	F test	LSD 0.05	F test	LSD 0.05
Date (A)	**	-	**	-
Foliar (B)	**	0.786	**	0.891
A × B	**	0.951	**	1.08
C	**	0.987	**	1.13
A × C	**	1.04	**	1.24
B × C	**	1.13	**	1.32
A × B × C	**	1.17	**	1.45

In relation to (A × C) interaction, sowing bean seeds at the 2nd date (D2: 15/10) and sprayed by Fe foliar application recorded the maximum number of branches (16.17 & 17.37) in both seasons, respectively. Spraying Phaseolus bean plants by bio-stimulate (chitosan) and Fe foliar application (B × C) gave the maximum number of branches (15.34 and 16.51) in both seasons, respectively. The increase in number of branches due to all interactions may be explanation that both biostimulator and micronutrients plays a very important role in the metabolism of the plant process by influencing the activity of growth enzymes as well as it is involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, and regulation of auxin synthesis and pollen formation. Regarding the third interaction effects between (A, B and C), results recorded that sowing Phaseolus bean seeds at the 2nd date (D2: 15/10) and spraying Phaseolus bean plants by chitosan at a concentration of 150 ppm twice before and after flowering and foliar application of Fe at 200 ppm twice; at 20

and 40 days from sowing recorded the highest no of branches of Phaseolus bean (18.00 and 13.41) in both seasons, respectively. Several experiments on the effects of concentration and frequency of chitosan application were conducted using various crops such as chilli, Chinese cabbage, celery and bitter cucumber (Chandrkrachang *et al*., 2003). Moreover, Lee *et al*. (2005) found that chitosan treatment increases the yield and marketability of soybean sprouts.

3.2 Effects on some yield components properties

3.2.1 Number of pods

The number of pods per plant can be considered as one of the most important components of seed yield because the pods contain seeds and participate in the growth and development of seeds in the early stages of seed filling through photosynthesis (Shirani-Rad *et al*., 2010). Average No. of pods of dry bean var. Nebraska (*Phaseolus vulgaris* L.) at harvest grown at Sohag governorate as affected by sowing dates, spraying bio-stimulates and foliar application of Fe, Mn, and Zn and their interaction effects in 2021/2022 and 2022/2023 seasons are presented in Table (4). Results show clearly that number of pods positively increased at the 2nd sowing date in comparison to the 1st one in both seasons. Sowing date on 15th October gave the highest number of pods (33.49 and 36.31) as compared with the 1st sowing date on the 1st of October (27.06 and 29.87) in both seasons, respectively. The increase percentage in number of pods reached (23.76% and 21.56%) due to the 2nd sowing date in both seasons, respectively. It is known that the sowing date varies from one region to another according to the climatic conditions in each country. Therefore, the planting dates for beans in Egypt differ from those in Ethiopia, which Getachew *et al*. (2014) gave results opposite to what was done in this research, it has found that there was significant effect ($P \leq 0.05$) of sowing date on the total marketable pod yield (kg ha^{-1}). Among the different sowing dates, green bean sowed on the 3rd of July resulted in the highest total marketable pod yield (kg ha^{-1}); whilst the lowest total marketable pod yield (kg ha^{-1}) was obtained from green bean sowed on the 17th of August. The highest total marketable pod yield was found for early sowing which might be due to the exposure of the green bean for long period of rainfall that resulted in higher vegetative growth that enhanced photo assimilate production in the pod. Islam *et al*. (2016) indicated that al the plants of tomato characters were greater in chitosan applied plants than control plant. Results revealed that the higher number of pods was produced from the 2nd sowing date. Results recorded in Table (4) show clearly that there was a significant difference among bio-stimulators on number of pods in both seasons. Spraying either humic acid or chitosan significantly increased number of pods in both seasons. Spraying chitosan gave the highest number of pods (35.09 and 40.36) as

compared with the control treatment in both seasons, respectively. The effects of chitosan might be explained under the basis that chitosan spraying increased chlorophylls and total carbohydrate contents as reported by Farouk *et al.* (2008; 2011; 2012) on cucumber, radish and cowpea, respectively. Results presented in Table (4) show clearly the effect of foliar application of micronutrients (Fe, Mn, and Zn) on number of pods was highly significant in both seasons. Hamouda *et al.* (2018) indicated that number of seeds per pod, weight of 100 seeds of dry bean (*Phaseolus vulgaris* L.) had showed strongly positive effects by foliar application of Zn. Foliar application of Fe recorded the maximum values of number of pods (32.96 and 47.81), meanwhile the minimum values of number of pods were recorded (27.75 and 40.36) in both seasons, respectively. The increased percentage of number of pods recorded (18.77% and 18.45%) over the control treatment in both seasons, respectively. The increase in number of pods due to Fe foliar application may be attributed to the primitive role of Fe on number of pods per plant which led to increasing growth and productivity. El-Foulyand Abou El-Nour (2021) reported that foliar feeding of nutrient can improve fertilizer use efficiency and reduce environmental pollution. These results might be explained that foliar feeding is a rabid technique in correcting nutritional disorders through applying such nutrients directly to the location of demand in which quickly absorption will be happened (Romheld, 1999). Results in Table (4) indicate that the interaction effect between sowing date and bio-stimulators (A × B) on number of pods were significant in both seasons, respectively. Sowing date on 15th October gave the highest number of pods (39.05 and 29.87) as compared with the 1st October in both seasons, respectively. Also, results show the significant effects of the 2nd interaction between sowing date and foliar application of Fe, Mn and Zn (A × C) on number of pods in both seasons, respectively. Data indicated that foliar application of Fe gave the maximum number of pods (37.40 and 41.15) as compared with all other interactions in both seasons, respectively. The 2nd interaction effect of spraying bean plants by Fe and chitosan foliar application (B × C) recorded maximum number of pods (38.14 and 47.81) as compared with all other interactions in both seasons, respectively. Abu-Muriefah (2013) reported that foliar-applied by chitosan, in particular 200 mg L^{-1}, increased plant growth, yield and its quality as well as physiological constituents in plant shoot under stressed or non-stressed conditions as compared to untreated plants. It is suggested that chitosan could be a promising material used to reduce the harmful effect of water stress on the growth and yield of *Phaseolus vulgaris* plants. It was found that foliar applications with chitosan resulted in higher vegetative growth and improvement in fruit quality of pepper,

radish, and cucumber (Farouk *et al*., 2008; Ghoname *et al*., 2010). Regarding the third interactions effects between (A, B and C), results recorded that sowing bean seeds at the 2nd date (D2: 15/10) and spraying bean plants by chitosan at a concentration of 150 ppm twice before and after flowering and foliar application of Fe at 200 ppm twice; at 20 and 40 days from sowing recorded the highest number of pods (42.60 and 37.56) in both seasons, respectively.

Table (4): Effect of spraying bio-stimulate (humic acid and chitosan) and foliar spray by micronutrients (Fe, Mn and Zn) on number of pods of dry bean (*Phaseolus vulgaris* L.) cv. Nebraska sown at different dates during 2021/2022 and 2022/2023 seasons.

Seasons		2021/2022					2022/2023				
Date	B.	C.	Fe	Zinc	Mn	Mean	C.	Fe	Zinc	Mn	Mean
A1 1/10	Control	20.60	25.80	23.33	21.67	22.85	29.87	37.56	32.65	29.20	29.87
	Humic	26.67	29.40	27.33	25.44	27.21	36.31	41.15	37.53	35.87	36.31
	Chitosan	29.33	33.67	31.33	30.20	31.13	40.36	47.81	43.91	41.53	40.36
Mean		25.53	29.62	27.33	25.77	27.06	29.87	37.56	32.65	29.20	29.87
A2 15/10	Control	24.40	32.00	28.33	26.80	27.88	36.31	41.15	37.53	35.87	36.31
	Humic	31.35	37.60	33.20	32.00	33.54	40.36	47.81	43.91	41.53	40.36
	Chitosan	36.40	42.60	39.60	37.61	39.05	29.87	37.56	32.65	29.20	29.87
Mean		30.72	37.40	33.71	32.14	33.49	36.31	41.15	37.53	35.87	36.31
B × C	Control	22.50	28.90	25.83	24.24	25.37	40.36	47.81	43.91	41.53	40.36
	Humic	29.01	33.50	30.27	28.72	30.37	29.87	37.56	32.65	29.20	29.87
	Chitosan	32.87	38.14	35.47	33.91	35.09	36.31	41.15	37.53	35.87	36.31
General mean		27.75	32.96	30.06	28.50		40.36	47.81	43.91	41.53	40.36
F test and LSD 0.05		F test		LSD 0.05			F test		LSD 0.05		
Date (A)		**		-			**		-		
Foliar (B)		**		1.45			**		1.65		
A × B		**		1.54			**		1.74		
C		**		2.19			**		2.39		
A × C		**		2.34			**		2.47		
B × C		**		2.47			**		2.62		
A × B × C		**		2.52			**		2.87		

3.2.2 Pod length

The pod length determines the number of seeds in each pod and thus affects the yield of the plant. (Falaknaz *et al*., 2015). Data demonstrated in Table (5) show that the main values of pod length (cm) were significantly affected by spraying bio-stimulates and foliar application of Fe, Mn, and Zn under two sowing dates and their interaction effects in 2021/2022 and 2022/2023 seasons. Obtained data indicated that the 2nd sowing date of bean seeds (D2) recorded the highest value of pod length (14.54 and 16.10 cm) at harvest in the 1st and 2nd seasons, respectively. On the other hand, the 1st sowing date (D1) gave the lowest values of pod length (12.80 and 13.81 cm) in both seasons, respectively. Also, main values of pod length in late sowing surpassed early sowing of bean plants by (13.59% and 16.58%) in the 1st and 2nd seasons, respectively. In another country,

Yoldas and Esiyok (2007) revealed that the longest pod was obtained from Melka-1 sowed on 18th of July, which was statistically similar with Melka-5 sowed on 2nd of August; whereas the smallest pod length was obtained from Melka-5 sowed on July 3rd. This result could be due to the fact that in early sowing plants gets more rainfall for longer growing periods that favoured higher yield, which might have compromised pod length. Data presented in Table (5) indicated that spraying bio-stimulants, either humic acid or chitosan significantly increased pod length in both seasons. The maximum values of pod length were obtained due to spraying bean plants by chitosan compared to the control treatment in both seasons. The increasing percentage due to spraying bean plants by chitosan reached (19.31% and 19.89%) in comparison to the control in the 1st and 2nd seasons, respectively. In this respect, Fawzy *et al.* (2023) indicated that chitosan gave the highest pod length of green bean (13.06 and 13.64) compared to various bio-stimulants in 2021 and 2022 seasons, respectively. Data presented in Table (5) indicated that pod length increased with Fe, Mn, and Zn foliar application. These results hold true in both seasons. The maximum pod length was recorded by Fe foliar application (15.03 and 16.39 cm) compared to the control in both seasons, respectively. Also, spraying Fe significantly increased pod length in comparison to the control by (19.57% and 19.54%) in both seasons, respectively. Falaknaz (2022) showed that foliar application has no significant effect on the pod length of Soybean (*Glycine max* L.), but according to the mean comparison, iron had the greatest effect with an amount of 4.76 cm. Analysis of data that recorded in Table (5) indicates that the interaction effect between sowing date and bio-stimulators (A × B) on pod length was significant in both seasons. Sowing date on 15th October gave the highest pod length (15.39 and 17.08) as compared with the 1st October in both seasons, respectively. Results recorded in Table (5) show the significant effects of the 2nd interaction between sowing date and foliar application of Fe, Mn and Zn (A × C) on pod length in both seasons. Data indicated that foliar application of Fe gave the maximum pod length (15.89 and 17.44) as compared with all other interactions in both seasons, respectively. The 2nd interaction effect of spraying bean plants by Fe and chitosan foliar application (B × C) recorded maximum number of pods (16.02 and 17.55) as compared with all other interactions in both seasons, respectively. Results presented in Table (5) illustrate also that the effect of interaction between sowing dates, bio-stimulates spraying and foliar application of micronutrients (Fe, Mn and Zn) gave highly significantly effect on number of pods in both seasons. Regarding the third interactions effects between (A, B and C), results recorded that sowing bean seeds at the 2nd date (D2: 15/10) and spraying bean plants by chitosan at a concentration of 150 ppm twice before and after flowering) and foliar application of Fe at

200 ppm twice; at 20 and 40 days from sowing) recorded the highest pod length (16.67 and 18.19 cm) in both seasons, respectively.

Table (5): Effect of spraying biofertilizers (humic acid and chitosan) and foliar spray by micronutrients (Fe, Mn and Zn) on pod length of dry bean (*Phaseolus vulgaris* L.) cv. Nebraska sown at different dates during 2021/2022 and 2022/2023 seasons.

Seasons		2021/2022					2022/2023				
Date	B.	C.	Fe	Zinc	Mn	Mean	C.	Fe	Zinc	Mn	Mean
A1 1/10	Control	9.46	13.33	11.67	10.86	11.33	10.36	14.13	12.43	11.67	12.15
	Humic	12.33	14.44	13.33	12.67	13.19	13.21	15.76	14.57	13.73	14.32
	Chitosan	13.17	15.37	13.67	13.33	13.89	14.26	16.91	14.53	14.21	14.98
Mean		11.65	14.38	12.89	12.29	12.80	12.61	15.60	13.84	13.20	13.81
A2 15/10	Control	12.40	14.67	13.33	12.40	13.20	13.37	16.20	14.87	13.93	14.59
	Humic	14.33	16.33	15.20	14.33	15.05	15.87	17.93	16.87	15.77	16.61
	Chitosan	14.67	16.67	15.20	15.00	15.39	16.31	18.19	17.16	16.67	17.08
Mean		13.80	15.89	14.58	13.91	14.54	15.18	17.44	16.30	15.46	16.10
B × C	Control	10.93	14.00	12.50	11.63	12.27	11.87	15.17	13.65	12.80	13.37
	Humic	13.33	15.39	14.27	13.50	14.12	14.54	16.85	15.72	14.75	15.46
	Chitosan	13.92	16.02	14.44	14.17	14.64	15.29	17.55	15.85	15.44	16.03
General mean		12.57	15.03	13.61	12.98		13.71	16.39	14.90	14.17	

F test and LSD 0.05	F test	LSD 0.05	F test	LSD 0.05
Date (A)	**	-	**	-
Foliar (B)	**	0.452	**	0.579
A × B	**	0.674	**	0.732
C	**	0.761	**	0.834
A × C	**	0.729	**	0.867
B × C	**	0.832	**	0.967
A × B × C	**	0.962	**	0.995

3.3 Effects on some yield properties

3.3.1 100 seed weight (g)

Data demonstrated in Table (6) show that main values of 100 seed weight was significantly affected by the different sowing dates under the study in both seasons, Sowing bean seeds later at (15/10) recorded the highest values of 100 seed weight which recorded (30.00 and 31.09 g) in the 1st and 2nd season, respectively. On the other hand, early sowing at (1/10) gave the lowest values of 100 seed weight (24.58 and 25.63 g) in both seasons, respectively. Although, Main values of 100 seed weight due to late sowing surpassed early sowing of bean seeds by (21.81% and 21.30%) in the 1st and 2nd seasons, respectively. These results were in agreement with İpekesen *et al.* (2022) who found that differences among sowing dates were significant for first pod height, number of branches per plant, pod width, plant weight, pods weight per plant, number of pods per plant, number of seeds per plant, seed yield per plant, biological yield and seed yield. Data presented in Table (6) indicated that humic acid and chitosan significantly increased 100 seed weight in comparison to the control treatment in the 1st and 2nd season, respectively. Also, data

indicated that the maximum main values of 100 seed weight were (29.59 and 30.45 g) in both seasons, respectively. Meanwhile, the minimum main values of 100 seed weight were (25.23 and 26.37) in both seasons, respectively. The increasing percentage in 100 seed weight due to chitosan spraying reached (17.28% and 15.47%) in comparison to the control in the 1st and 2nd season, respectively.

Table (6): Effect of spraying biofertilizers (humic acid and chitosan) and foliar spray by micronutrients (Fe, Mn and Zn) on 100 seed weight of dry bean (*Phaseolus vulgaris* L.) cv. Nebraska sown at different dates during 2021/2022 and 2022/2023 seasons.

Seasons		2021/2022					2022/2023				
Date	B.	C.	Fe	Zinc	Mn	Mean	C.	Fe	Zinc	Mn	Mean
A1 1/10	Control	18.23	26.93	23.52	21.91	22.65	19.31	27.94	24.67	22.86	23.70
	Humic	19.62	28.25	25.45	24.18	24.38	20.65	29.74	26.68	25.31	25.60
	Chitosan	21.89	30.76	28.07	26.16	26.72	22.34	31.34	29.1	27.67	27.61
Mean		19.91	28.65	25.68	24.08	24.58	20.77	29.67	26.82	25.28	25.63
A2 15/10	Control	24.86	31.76	28.62	26.02	27.82	25.94	32.94	29.64	27.64	29.04
	Humic	28.46	32.93	29.32	28.11	29.71	29.74	33.64	30.76	29.62	30.94
	Chitosan	31.16	34.71	32.81	31.18	32.47	31.76	35.64	33.5	32.27	33.29
Mean		28.16	33.13	30.25	28.44	30.00	29.15	34.07	31.30	29.84	31.09
B × C	Control	21.55	29.35	26.07	23.97	25.23	22.63	30.44	27.16	25.25	26.37
	Humic	24.04	30.59	27.39	26.15	27.04	25.20	31.69	28.72	27.47	28.27
	Chitosan	26.53	32.74	30.44	28.67	29.59	27.05	33.49	31.30	29.97	30.45
General mean		23.45	30.57	27.64	25.95		24.36	31.56	28.74	27.24	
F test and LSD 0.05		**		-			**		-		
Date (A)		**		0.765			**		0.787		
Foliar (B)		**		0.865			**		0.892		
A × B		**		0.895			**		0.921		
C		**		0.912			**		0.934		
A × C		**		0.937			**		0.958		
B × C		**		0.986			**		0.972		
A × B × C		**		-			**		-		

Fouda *et al*. (2022) indicated that 100-seed weight of faba bean (*Vicia faba* L.) was significantly increased with the increasing chitosan spray to 750 ppm compared to control (spraying with tap water) under different irrigation regimes. The results were in agreement with Islam *et al*. (2016) indicated that 1000-seed weight as well as seed yield plant^{-1} of mung bean was greater with chitosan applied plants than in control plants. Obtained data indicated that 100 seed weight values increased with spraying by Fe, Mn, and Zn micronutrients compared to control treatment in both seasons, respectively. The maximum 100 seed weight was recorded due to Fe spraying (30.57 and 31.56 g) compared to control treatment in both seasons, respectively. In this respect, Salehin *et al*. (2012) showed that the highest 100 seed weight 43.9 g, was obtained by zinc spraying of Phaseolus bean. Similar results were reported by Togay *et al*. (2004). Also, results show significant effects of the interaction between all the 2nd

interactions; (A and B), (B and C) and (B and C) on 100 seed weight. Analysis of data recorded also show that the maximum values of 100 seed weight (32.47 and 33.29 g) were obtained due to the interaction among sowing date and bio-stimulants (A × B) in both seasons, respectively. Late sowing and spraying bean plants by chitosan gave the maximum values of 100 seed weight in both seasons, respectively. Also, the maximum values of 100 seed weight (33.13 and 35.64 g) were obtained due to the interaction among sowing date and Fe spraying (A × C) in both seasons, respectively. Late sowing and spraying bean plants by Fe foliar application gave the maximum values of 100 seed weight in both seasons, respectively. Additionally, the maximum values of 100 seed weight (32.74 and 33.49 g) were obtained due to the interaction among chitosan spraying and Fe spraying (B × C) in both seasons, respectively. Concerning the third-order interactions, 100 seed weight was significantly affected by the interaction (A × B × C) in both seasons. Late sowing and spraying bean plants by chitosan and Fe foliar application gave the maximum values of 100 seed weight (34.71 and 35.64 g) in the 1st and 2nd season, respectively.

3.3.2 Dry seed yield (ton feddan^{-1})

The differences in dry seed yield among the two sowing dates have been found significant (Table 7). Average dry seed yield was significantly affected by the sowing dates in the 1st and 2nd seasons, late sowing gave the highest dry seed yield (0.596 and 0.636 ton feddan^{-1}) in the 1st and 2nd seasons, respectively. On the other hand, early sowing gave lowest dry seed yield (0.603 and 0.643 ton feddan^{-1}) in the 1st and 2nd seasons, respectively. Also, late sowing exceeds early sowing in dry seed yield by (26.53% and 25.19%) in the 1st and 2nd seasons, respectively. The variation in dry seed yield could be partially attributed to the good climatic conditions recorded throughout growing. Delaying the sowing date of dry bean from 1st to 15th October has a positive effect on the production of dry seed yield of. Phaseolus bean growth and nutrient uptake may be stimulated by higher temperature and improved light conditions during the vegetative growth phase (Marschner, 1995). Additionally, Basnet *et al.* (2022) revealed that the crop of French bean (*Phaseolus vulgaris* L.) sown on November 5th produced significantly higher grain yield (2.16 ton ha^{-1}) than October 21st (2.0 ton ha^{-1}) and November 20th (1.75 ton ha^{-1}) sowings. So, in order to minimize the impact of climate change and achieve higher productivity of French bean, November 5th can be considered as an optimum time of sowing in the humid sub-tropical condition of Chitwan of Nepal. Results presented in Table (7) show clearly that there was a significant effect of bio-fertilizers on dry seed yield in 2021/2022 and 2022/2023 seasons. Averages of dry seed yield were significantly affected by the type of bio-stimulants in the 1st and 2nd

seasons, respectively. Spraying chitosan gave the highest dry seed yield (0.603 and 0.643 ton feddan^{-1}) in the 1st and 2nd seasons, respectively. On the other hand, un-sprayed bean plants gave lowest dry seed yield (0.461 and 0.501 ton feddan^{-1}) in the 1st and 2nd seasons, respectively. Also, chitosan spraying exceeds un-sprayed (control) in dry seed yield by (30.88% and 28.34%) in the 1st and 2nd seasons, respectively. The increase in Phaseolus yield due to chitosan application may be due to its effects in stimulating physiological processes, improving vegetative growth (Abu-Muriefah, 2013). This finding was similar to the results obtained by Islam *et al.* (2016) revealed that seed yield plant^{-1} of mungbean was greater in chitosan applied plants than in control plants. The highest seed yield of mungbean (9.27 g plant^{-1}) was recorded with 50 ppm chitosan due to production of higher number of pods plant^{-1}. Results presented in Table (7) show clearly that there was a significant effect of foliar application by micronutrient on dry seed yield in 2021/2022 and 2022/2023 seasons. Foliar application by Fe gave the highest dry seed yield (0.631 and 0.669 ton feddan^{-1}) as compared with all other application in both seasons, respectively. On the other hand, control treatment recorded the lowest dry seed yield (0.412 and 0.446 ton feddan^{-1}) in the 1st and 2nd seasons, respectively. Spraying bean plants by Fe significantly increased dry seed yield by (53.15% and 50.00%) in both seasons, respectively. Similar results were obtained by Salehin *et al.* (2012) showed that the highest seed yield with 1996.1 kg ha^{-1}, was obtained by zinc spraying of Phaseolus bean. Similar results were reported by Togay *et al.* (2004). Heidarian et al., (2021) indicated that analysis of variance showed that the effect of micronutrients foliar application on grain yield of soybean (*Glycine max* (L) Merr.) was significant ($p<0.05$), but the effect of foliar spraying at different growth stages was not significant. Our results were in agreement with Bozorgi *et al.* (2011) in faba bean and Kobraee *et al.* (2011) in soybean. Results recorded in Table (7) indicate that the interaction between sowing dates and bio-stimulants (A × B) was significant on dry seed yield in both seasons. Late sowing and spraying bean plants by chitosan gave the highest dry seed yield (0.679 and 0.712 ton feddan^{-1}) in the 1st and 2nd season, respectively. Also, late sowing and spraying bean plants by Fe micronutrient (A × C) gave the highest dry seed yield (0.713 and0.758 ton feddan^{-1}) as compared with other interaction treatments in 2021/2022 and 2022/2023 seasons, respectively. Also, obtained results show that interaction between bio-stimulants and foliar application of micronutrient (B × C) was significant in both seasons. foliar application of bean plants by chitosan and 200 ppm Fe gave the highest dry seed yield (0.706 and 0.748 ton feddan^{-1}) as compared with other interaction treatments in 2021/2022 and 2022/2023 seasons, respectively. These results were in agreement with those obtained from

Lee *et al*. (2005) found that chitosan treatment increases the yield and marketability of soybean sprouts. Zakiullah *et al*. (2019), Khan *et al*. (2018), and Ragab *et al*. (2021) indicated that chitosan resulted in a large increase on pea seed yield and its components compared to control. Foliar application of chitosan was studied by many investigators on various crops: Shehata *et al*. (2012) on cucumber, Walker Ghoname *et al*. (2010) on sweet pepper plants, Abdel-Mawgoud *et al*. (2010) on strawberry, Farouk *et al*. (2021) on radish and Fawzy *et al*. (2012) on garlic plant. Results recorded indicate that the third interaction (A × B × C) between sowing dates, Bio-stimulants and foliar application of micronutrients was significant on dry seed yield in both seasons. Late sowing and sprayed bean plants by chitosan and Fe recorded the highest dry seed yield (0.762 and 0.798 ton feddan^{-1}) as compared with other interaction treatments in 2021/22 and 2022/23 seasons, respectively.

Table (7): Effect of spraying biofertilizers (humic acid and chitosan) and foliar spray by micronutrients (Fe, Mn and Zn) on dry seed yield (ton feddan^{-1}) of dry bean (*Phaseolus vulgaris* L.) cv. Nebraska sown at different dates during 2021/2022 and 2022/2023 seasons.

Seasons		2021/2022					2022/2023				
Date	B.	C.	Fe	Zinc	Mn	Mean	C.	Fe	Zinc	Mn	Mean
A1 1/10	Control	0.327	0.492	0.436	0.387	0.411	0.357	0.521	0.484	0.434	0.449
	Humic	0.361	0.568	0.513	0.462	0.476	0.392	0.587	0.537	0.486	0.501
	Chitosan	0.386	0.649	0.579	0.491	0.526	0.410	0.697	0.622	0.566	0.574
Mean		0.358	0.570	0.509	0.447	0.471	0.386	0.602	0.548	0.495	0.508
A2 15/10	Control	0.424	0.675	0.485	0.462	0.512	0.457	0.733	0.524	0.494	0.552
	Humic	0.457	0.701	0.672	0.565	0.599	0.489	0.743	0.709	0.631	0.643
	Chitosan	0.570	0.762	0.710	0.674	0.679	0.631	0.798	0.727	0.691	0.712
Mean		0.484	0.713	0.622	0.567	0.596	0.526	0.758	0.653	0.605	0.636
B × C	Control	0.376	0.584	0.461	0.425	0.461	0.407	0.627	0.504	0.464	0.501
	Humic	0.409	0.635	0.593	0.514	0.537	0.441	0.665	0.623	0.559	0.572
	Chitosan	0.478	0.706	0.645	0.583	0.603	0.521	0.748	0.675	0.629	0.643
General Mean		0.412	0.631	0.558	0.498		0.446	0.669	0.593	0.542	

F test and LSD 0.05	F test	LSD 0.05	F test	LSD 0.05
Date (A)	**	-	**	-
Foliar (B)	**	0.045	**	0.048
A × B	**	0.056	**	0.059
C	**	0.064	**	0.067
A × C	**	0.071	**	0.076
B × C	**	0.079	**	0.082
A × B × C	**	0.082	**	0.084

In summary, foliar spraying by 150 ppm chitosan (after and before flowering stage) and 200 ppm Fe (after 20 and 40 days from sowing) resulted in a significant increase in Phaseolus plant growth, yield and its components under Sohag governorate conditions, especially when Phaseolus seed sown at the 2nd sowing time (15/10).

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