Journal of Environmental Sciences (JES)
Faculty of Graduate Studies and Environmental Research, Ain Shams University

EXOGENOUS NANO-SELENIUM APPLICATION MITIGATES LOW-TEMPERATURE STRESS IN GREEN BEAN PLANTS

Latifa A. I. Attia [(1)]; Hany G. Metwally [(2)]; Al-Sayed A. Al-Sherbini [(3)] and Usama A. A. El-Behairy [(4)]

1) Arid land Agricultural research and services center, Ain shams university 2) Horticulture Department, Faculty of Agriculture, Ain Shams University 3) National Institute of Laser Sciences, Cairo University 4) Arid Land Agricultural Graduate studies and Research Institute, Ain Shams University

ABSTRACT

This research was conducted at the faculty of agriculture, Ain Shams university, Shubra El-kheima. The research aimed to test the effect of selenium (Se) and nano-selenium (NSe) on green bean to mitigate the cold temperature stress. Green bean seeds were sown late (mid-December) with low temperatures in open field (open) and under low tunnel (cover). Green bean plants were sprayed with selenium (Se) or nano-selenium (NSe) with four concentrations (0, 2, 4and 8 ppm). Cultivating green bean under low tunnel produced the highest vegetative and yield characteristics compared to open cultivation. Spraying plants with 8ppm NSe significantly increased the following parameters in the first season (2017) and second season (2018), respectively: plant height was (48.50 and 45.83 cm), number of leaves (7.17 and10), and nitrogen % in leaves (4.13 and 5.28), yield (2872 and 3029 gm) and reduced proline content in leaves (825 and754) compared to 0 ppm. Spraying green bean plants with NSe enhanced the following parameters; plant height (48.50 and 45.83 cm), number of leaves (48.50 and45.83), nitrogen % in leaves (6.13 ,and5.78),and yield (2479 and2749 gm).while, reduced proline content in leaves (1148 and 1122) compared to Se. Regarding concentration effect data showed that 8 ppm resulted the highest total yield (2747 and2949 gm), number of leaves (14.08 and 14.08), plant height(47.33 and 45.29 cm) while reduced proline content in leaves (922 and 834.5) compared to other concentrations. Lastly, 8 ppm NSe under cover obtained the highest total yield (3045 and 3178) compared to other treatments.

Key words: green beans, Selenium, Nano-selenium, Cold stress

Vol. (51); Iss. (12); No. (7); Dec. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

INTRODUCTION

Green bean (*Phaseolus vulgaris*) is one of the most important members of fabaceous family in Egypt for local consumption and exportation. Green bean is annual summer crop grown in open field along the year except cold times in winter conditions, wherever it could be produced under protected cultivation. According to (Enaam A.Mohamed *et al.,* 2018), green bean production under protected cultivation constitutes less than 20% of green bean cultivation area and achieves high returns. This is because of less production and less crop supply for local consumption moreover more demand for exportation. Climate change led to changes in seasonal pattern and biotic and abiotic factors as crop situation, status of yields and quality and pest and disease problems (Ayyogari *et al*., 2014)

Low temperatures act as a biotic stress factor that has a strong impact on the survival, growth, reproduction and distribution of plants. Low temperature affects germination, seedling growth, and overall crop growth and productivity (Yan *et al*., 2010)**.** Low temperature can affect membranes and their lipid composition thereby changing the water status of the cells/ plant. In addition, chilling may impair water absorption by the roots and water transport to the shoot (Aroca *et al.,* 2001). Antioxidant capacity increases during cold acclimation in several plants as an adaptive mechanism to low temperature (Foyer & Noctor, 2005).

Selenium (Se) is a trace element that can function as an essential nutrient for humans and animals or as an environmental toxicant (Nowak *et al.,* 2004). The boundary between the essential and toxic concentration is narrow and depends on its chemical form concentration, and other environmentally regulating variables. Stimulating effects to the additions of small amount of SE on plant growth and yield have been observed by several researchers (Terry *et al*., 2000).

Vol. (51); Iss. (12); No. (7); Dec. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

Numerous studies have shown that at low concentrations, SE exerts a beneficial effect on growth and stress tolerance of plants by enhancing their antioxidative capacity and thereby improve growth and yield (Kong et al., 2005)

Selenium is used to counteract various biotic stresses, such as salt stress (Djanaguiraman *et al.,* 2005; Hawrylak-Nowak, 2008), heavy metals, (Cartes *et al.,* 2010); (Mroczek-Zdyrska & Wójcik, 2012), and drought (Yao *et al.,* 2009). It exerts beneficial effects on plant growth and stress tolerance by enhancing their antioxidative capacity (Ríos *et al.,* 2009).

MATERIALS AND METHODS

The field experiment in this study was carried out during December for two successive growing seasons 2017 and 2018 at the Arid Land Agriculture Graduate Studies and Research Institute (ALARI), following faculty of agriculture, Ain Shams university, Shubra El-kheima. The aim of the research is to test the effect of nano-selenium on green bean to mitigate the cold temperature stress.

Green bean seeds *Phaseolus vulgaris Valentino* were obtained from Horticulture Research Institute Al-Giza. Green bean seeds were sown late (mid-December) in open field and under low tunnel. The green bean plants were sprayed with selenium (Se) or nano-selenium (NSe) with four concentrations (0, 2, 4, 8 ppm) in open field and under low tunnel.

Selenium (Se) and nano-Selenium (NSe) solutions were prepared from sodium selenate powder and diluted in distilled water to the target concentrations while the control was prepared with distilled water.

NSe were prepared according to the method described by (Bai *et al*., 2008). Briefly, Sodium Selenate Solution 7.5 mL of 0.23 M ascorbic acid and 5 mL of 2.4 M acetic acid solution were mixed with 0.25 mL of 0.51 M sodium selenite was slowly added into the mixture. It was noted, as a proof of the formation of the NSe, that the color of the solution was changed from colorless to orange red during the reaction process. Finally, the solution was diluted to 50 mL using distilled water to obtain a final concentration (2, 4 and 8 ppm).

NSe were characterized by morphology of the reductive spherical and size of 10–30 nm by High Resolution Transmission Electron Microscopy (HRTEM) JEOL (JEM-1010) Transmission Electron Microscopy 80KV at the regional center for mycology and biotechnology (RCMB) Al-Azhar University (Amin *et al*., 2021).

Morphological responses for the plants were assessed by recording Plant height using ruler and counting number of leaves.

Characterization of NSe:

Figure (1) shows the NSe under transmission electron microscope. Imaging under TEM showed that the NSe were in nano range.

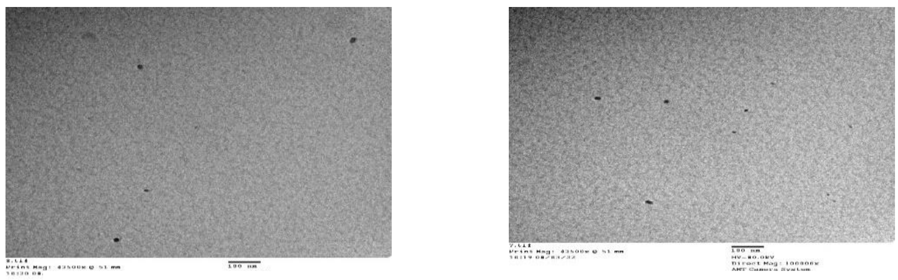

Figure (1) TEM imaging of nano-selenium (NSe)

Vol. (51); Iss. (12); No. (7); Dec. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

Macronutrients in leaves were measured in both seasons. Samples were prepared according to (chapman et al., 1961). Nitrogen % was determined using the Kjeldhal method (FAO, 1980). Potassium % was determined photometrically using flame photometer as the procedure described by (chapman et al., 1961). Phosphorus % was determined using spectrophotometer according to (Olsen et al., 1965) Proline was determined according to (Bates et al., 1973).

Yield is collected in four harvestings. The first harvesting was after 60 days from cultivation. The time interval between harvestings was one week.

The treatments were arranged using factorial compete randomized blocks design in 3 plots. Each plot size was (1.5 x 0.5 m). And each plot contain 12 plants with equal distance between each other.

Statistical analysis of the data according to (Snedecor & Cochran, 1973) Least significant difference (LSD = 0.05) was used to compare among the treatments.

RESULTS AND DISSCUSION

Data in Table (1) show the effect of spraying different concentrations of Se and NSe on green bean plant height under two cultivation method. Data show that cultivating green bean under cover increased plant height significantly in two successive seasons respectively (49.13 and46.92 cm) comparing with cultivating under open field condition (39.04 and 39.25 cm).

Regarding type of Se effect on green bean plant height, data shows that using NSe increased plant height significantly in two seasons respectively (44.96 and 46.92 cm) compared to use Se (39.04 and39.25 cm).

Concerning spraying different concentration of Se & NSe effect on green bean plant height, data shows that increasing Se concentration increased green bean plant height for 8 ppm results was (47.33 and 45.29) in two successive seasons. The differences were significant.

Concerning the interaction between cover and type of selenium, data shows that the highest plant height obtained using NSe under cover in two successive seasons (51.17 and48.00) followed by Se under cover (47.08 and 45.29) while the lowest plant height obtained using both Se in open field (39.33 39.3) & NSe cultivated in open field (38.75 and 38.42).

Data in Table (1) shows the effect of Se & NSe concentrations under different cultivation type on green plant height. The highest plant height obtained using 8 ppm under cover (53 and 50.67 cm) followed by other concentrations under cover while the lowest plant height obtained by open field cultivation control (33 and 33.67 cm).

On the other hand, data shows in the same table the interaction between type of Se and their concentrations. Data reveals that the highest plant height obtained using NSe with contractions 8 ppm (48.50 and 45.83 cm) followed by 4 ppm (47.83 and 43.33 cm). On the contrary, the lowest plant height obtained by control (40.50 and 39.50 cm).

Concerning the interaction among cover type & Se concentrations under different cultivation type, data revels that the highest green bean height in the two season respectively was obtained by plants sprayed with 8 ppm NSe under cover (56.00 and 52.33 cm), while the lowest plant height obtained by open field control (33.00 and 33.67).

(Shalaby et al., 2021) found that foliar application of NSe (25 mg L-1) clearly improved cucumber growth parameters in two seasons after 70 days from transplanting; plant height (286 and 355 cm), leaf area (79.65 and 96.40 dm2 per plant) compared to other anti-stressors; silicon

Vol. (51); Iss. (12); No. (7); Dec. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

(Si), H2O2 and control. (Sadak & Bakhoum, 2022) used different Se treatments and found that different Se concentrations (25 and 50 mg/L) caused significant increases in the studied growth criteria of Quinoa compared to the control plant. 50 mg/L Se was the most effective level as it caused the highest increases in the different growth parameters; shoot length (50.2 cm) and number of leaves (37.4)

Table (1): The effect of different type of Se and different concentrations of Se under different type of cultivation on green bean Plant height.

		Plant height									
		The first season "2018"					**The second season "2019"**				
Treatments		**conc.**					**conc.**				
		0	2	4	8	**Mean**	0	2	4	8	**Mean**
open	**Se**	33.00 h	41.00 fg	41.33 e-g	42.33 e-g	**39.33 C**	33.67 e	41.00 b-e	42.17 b-d	40.50 c-e	**39.33 B**
	NSe	33.00 h	38.00 g	43.00 ef	41.00 fg	**38.75 C**	33.67 e	40.00 de	40.67 c-e	39.33 de	**38.42 B**
Cover	**Se**	48.00 cd	45.67 c-e	44.67d-f	50.00 bc	**47.08 B**	45.33 a-d	47.00 a-d	40.00 de	49.00 sb	**45.33 A**
	NSe	48.00 cd	48.00 cd	52.67 ab	56.00 a	**51.17 A**	45.33 a-d	48.33 a-c	46.00 a-d	52.33 a	**48.00 A**
open		33.00 d́	39.50 ć	42.00 ć	41.67 ć	**39.04 B**	33.67 d́	40.50 c\`	41.42 c\`	39.92 c\`	**39.25 B**
Cover		48.00 b́	46.83 b́	48.67 b́	53.00 á	**49.13 A**	45.33 b́ć	47.67 áb́	43.00 b́c\`	50.67 á	**46.92 A**
Se		40.50^{c}	43.33^{bc}	42.83^{c}	46.17^{ab}	**43.17^{B}**	39.5^{b}	44.00^{ab}	41.08^{ab}	44.75^{ab}	**42.33^{A}**
NSe		40.50^{c}	43.00^{c}	47.83^{a}	48.50^{a}	**44.96^{A}**	39.49^{b}	44.17^{ab}	43.33^{ab}	45.83^{a}	**43.20^{A}**
Mean		**40.50 Cˆ**	**43.17 Bˆ**	**45.33 Aˆ**	**47.33 Aˆ**		**39.50 Bˆ**	**44.08 Aˆ**	**42.21 AˆBˆ**	**45.29 Aˆ**	

Se= selenium NSe= nano-selenium

Open= cultivation in open field Cover= cultivation under tunnels

Data in Table (2) shows the effect of spraying different concentrations of Se and NSe on number of leaves of green bean plant under two cultivation methods. Data shows that there were no significant differences regarding the effect of cultivation methods & type of Se and the interaction between cultivation methods and type of Se on no. of green bean leaves.

Vol. (51); Iss. (12); No. (7); Dec. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

Regarding the effect of Se & NSe concentrations on no. of green bean leaves, data shows that increasing concentration increased no. of leaves significantly. So, 8 ppm resulted the highest no. of leaves in the two respective seasons (14.08 and 14.08).

Concerning the interaction between different Se & NSe concentrations and cultivating method, data shows that the highest no. of green bean leaves in the two seasons respectivly bean leaves obtained using 8 ppm under in open field (15 and 14.33) followed by 8 ppm under cover (13.17 and13.83). The lowest no. of leaves obtained by open field control(7.33 and 9.67) and cover control(7.00 and 10.33).

lastly, the interaction among cover type & Se concentrations under different cultivation type, data shows that the highest no. of leaves obtained in the first season using Se 8 ppm (16.33) for plants cultivated in open field. And in the second season was Se 4ppm in open field (16.00). On contrary, the lowest no. of leaves obtained Se (0 ppm) under open filed(7.33 and 9.67) and cover(7 and 10.33).

(Sadak & Bakhoum, 2022) results showed that different Se treatments significantly increased Quinoa (Chenopodium quinoa) number of leaves under normal irrigation as well as under drought stressed conditions. Se 50mg/L resulted the heighst no. of leaves when used with different irregetion; I100 (42.7) and I60(32.1) compared to the other concentretions (25 and 0 mg/L).

Vol. (51); Iss. (12); No. (7); Dec. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

Table (2): The effect of different type of Se and different concentrations of Se under different type of cultivation on no. of leaves green bean.

Treatments		No. of leaves									
		The first season "2018"					**The second season "2019"**				
		conc.					**conc.**				
		0	2	4	8	**Mean**	0	2	4	8	**Mean**
open	**Se**	7.33c	7.67 c	12.67 b	16.33 a	**10.10 A**	9.67 bc	14.33 ab	16.00 a	14.67 ab	**13.67 A**
	NSe	7.33 c	7.67 c	13.33 ab	13.67 ab	**10.50A**	9.67 bc	14.67 ab	12.67 ab	14.00ab	**12.75 AB**
Cover	**Se**	7.00 c	13.33 ab	11.33 b	11.67 b	**10.83 A**	10.33 bc	12.33 ab	7.33 c	13.00 ab	**10.75 B**
	NSe	7.00 c	12.00b	13.00 b	14.67 ab	**11.67 A**	10.33 bc	11.67 a-c	13.00 ab	14.67 ab	**12.42 AB**
open		7.33 ć	7.67 ć	13.00 áb́	15.00 á	**10.75 Á**	9.67 b́	14.50 á	14.33 á	14.33 á	**13.20 Á**
Cover		7.00 ć	12.67 b́	12.17 b́	13.17 áb́	**11.25 Á**	10.33 b́	12.00 áb́	10.17 b́	13.83 á	**11.74 Á**
Se		7.17 a	10.50 b	12.00 ab	14.00 a	**10.92 A**	10.00 b	13.33 ab	11.67 ab	13.83 a	**12.20 A**
NSe		7.17 c	9.83 b	13.17 a	14.17 a	**11.08A**	10.00 b	13.17 ab	12.83 ab	14.33 a	**12.58 A**
Mean		**7.17 Dˆ**	**10.17 Cˆ**	**12.58 Bˆ**	**14.08 Aˆ**		**10.00 Bˆ**	**13.25 Aˆ**	**12.25 Bˆ**	**14.08 Aˆ**	

Se= selenium NSe= nano-selenium

Open= cultivation in open field Cover= cultivation under tunnels

Regarding the effect of spraying different concentrations of Se and NSe on N% in green bean leaves under two cultivation method. Data in Table (3) shows that there were no significant differences regarding the effect of type of Se and the interaction between cultivation methods and type of Se on % of N in green bean leaves. While on the second season, using Se under cover resulted the highest N% (6.24)

Concerning the effect of different cultivation method, first season data reveals that cultivation green bean under cover increased N% in the leaves (5.71) compared to open field

(5.49). While in second season, there were no significant effect between cover and open cultivation.

Regarding the effect of Se & NSe concentrations on N% in the leaves, data shows that increasing Se & NSe concentrations increased N% in the leaves significantly in both seasons.

Concerning the interaction between different Se & NSe concentrations and cultivating method, data reveals that spraying Se and NSe with all concentrations under both cultivation methods increased N% in green bean leaves significantly. The lowest N% in the leaves obtained using 0 ppm of Se & NSe in both cultivation methods.

Data in Table (3) also presents the interaction between Se type and their contractions on N% in the leaves. Data shows that using all Se & NSe concentrations increased N% in the leaves significantly compared to 0 ppm in both Se and NSe in two seasons respectively (4.13 and 5.28).

The interaction among cover type & Se concentrations under different cultivation type, data shows that the highest N% in the leaves obtained using Se 2 ppm for plants cultivated under cover followed by NSe 8 ppm under cover and Se 8ppm cultivated in the open field. On contrary, the lowest N% in the leaves obtained using Se & NSe 0 ppm under open field (4.10 and 5.73) and cover (4.16 and 4.83).

Table (3): The effect of different type of Se and different concentrations of Se under different type of cultivation on N% in green bean leaves.

		N %									
		The first season "2018"					**The second season "2019"**				
Treatments		**conc.**					**conc.**				
		0	2	4	8	**Mean**	0	2	4	8	**Mean**
open	**Se**	4.10 d	5.77 bc	5.73 bc	6.57 ab	**5.54 A**	5.73 b-e	5.57 c-e	5.50 c-e	5.00 de	**5.45 B**
	NSe	4.10 d	5.80 bc	6.10 a-c	5.67 bc	**5.42 A**	5.73 b-e	5.80 b-e	6.167 a-c	5.63 b-e	**5.83 AB**
cover	**Se**	4.167 d	7.20 a	6.76 ab	5.63 bc	**5.94 A**	4.83 e	6.33 a-c	7.10 a	6.70 ab	**6.24 A**
	NSe	4.17 d	5.13 cd	6.07 bc	6.59 ab	**5.49 A**	4.83 e	5.43 c-e	6.03 b-d	5.93 b-d	**5.56 B**
open		4.10 b́	5.78 á	5.92 á	6.12 á	**5.49 B**	5.73 b́c`	5.68 b́c`	5.83 b́c`	5.32 c`d́	**5.64 Á**
Cover		4.17 b́	6.1 á	6.41 á	6.11 á	**5.71 A**	4.83 d́	5.88 a-c`	6.57 á	6.32 áb	**5.90 Á**
Se		4.13 ^{c}	6.17 ^{a}	6.25 ^{ab}	6.10 ^{ab}	**5.66** ^{A}	5.28 ^{b}	5.95 ^{ab}	6.30 ^{a}	5.85 ^{ab}	**5.84** ^{A}
NSe		4.13 c	5.46 ^{b}	6.08 ^{ab}	6.13 ^{ab}	**5.45** ^{A}	5.28 ^{b}	5.62 ^{ab}	6.10 ^{a}	5.78 ^{ab}	**5.69** ^{A}
Mean		**4.13 Bˆ**	**5.98 Aˆ**	**6.17 Aˆ**	**6.11 Aˆ**		**5.28 Bˆ**	**5.78 Aˆ**	**6.20 Aˆ**	**5.82 Aˆ**	

Se= selenium NSe= nano-selenium

Open= cultivation in open field Cover= cultivation under tunnels

The effect of spraying different concentrations of Se and NSe on P% in green bean leaves under two cultivation methods illustrated in Table (4). Data shows that there were no significant differences regarding the effect of type of Se and Se & NSe concentrations and the interaction between Se type and their contractions on % of P in green plant leaves.

Concerning the effect of different cultivation method, data reveals that in the two seasons, cultivation green bean under cover (0.74 and 0.47) increased P% in the leaves compared to open field (0.40 and 0.26).

Concerning the interaction effect of Se type and cultivation methods on P% in the leaves, data shows that spraying Se under cover (0.48 and0.53) & NSe (0.48 and0.53) under cover increased P % in the leaves significantly in both seasons.

Concerning the interaction between different Se & NSe concentrations and cultivating method, data reveals that spraying Se and NSe with all concentrations under cover increased P % in green bean leaves significantly.

The interaction among cover type & Se concentrations under different cultivation type, data reveals that the highest P % in the leaves obtained using all Se & NSe concentrations for plants cultivated under cover compared to all Se & NSe cultivated in the open field. There were no significant differences among all Se types and their concentrations under cover.

Table (4): The effect of different type of Se and different concentrations of Se under different type of cultivation on P % in green bean leaves.

		P%									
		The first season "2018"					**The second season "2019"**				
Treatments		**conc.**					**conc.**				
		0	2	4	8	**Mean**	0	2	4	8	**Mean**
open	**Se**	0.40 b-d	0.40 b-d	0.37 cd	0.33 d	**0.37 C**	0.27 ef	0.23 f	0.23 f	0.23 f	**0.24 C**
	NSe	0.40 b-d	0.40 b-d	0.37 c-d	0.50 ab	**0.42 BC**	0.27 ef	0.27 ef	0.27 d-f	0.27 ef	**0.28 C**
Cover	**Se**	0.47 a-c	0.47 a-c	0.53 a	0.47 a-c	**0.48 A**	0.47 a-c	0.57 a	0.53 ab	0.53 ab	**0.53 A**
	Nse	0.47 a-c	0.47 a-c	0.47 a-c	0.40 b-d	**0.45 AB**	0.47 a-c	0.43 bc	0.40 cd	0.37 c-e	**0.42 B**
open		0.40 b́ć	0.40 b́ć	0.37 ć	0.42 b́ć	**0.40 B́**	0.27 b́	0.25 b́	0.27 b́	0.25 b́	**0.26 B́**
Cover		0.47 áb́	0.47 áb́	0.50 á	0.43 á-ć	**0.47 Á**	0.47 á	0.50 á	0.47 á	0.45 á	**0.47 Á**
Se		0.43 ^{a}	0.43 ^{a}	0.45 ^{a}	0.40 ^{a}	**0.43 ^{A}**	0.37 ^{a}	0.40 ^{a}	0.40 ^{a}	0.38 ^{a}	**0.38 ^{A}**
NSe		0.43 ^{a}	0.43 ^{a}	0.42 ^{a}	0.45 a	**0.43 ^{A}**	0.37 ^{a}	0.35 ^{a}	0.35 ^{a}	0.32 ^{a}	**0.35 ^{B}**
Mean		**0.4 Aˆ**	**0.43 Aˆ**	**0.43 Aˆ**	**0.42 Aˆ**		**0.37 Aˆ**	**0.38 Aˆ**	**0.37 Aˆ**	**0.35 Aˆ**	

Se= selenium NSe= nano-selenium
Open= cultivation in open field Cover= cultivation under tunnels

Vol. (51); Iss. (12); No. (7); Dec. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

The effect of spraying different concentrations of Se and NSe on K% in green bean leaves under two cultivation method shows in Table (5). Data reveals that there were no significant differences regarding the effect of method of cultivation & type of Se & Se concentration and the interaction between Se type and their contractions on % of K in green plant leaves.

Concerning the effect of the interaction between cultivation methods and type of Se on K% in green bean leaves, data shows that in the first season, the highest K% obtained by NSe followed by NSe combined plants cultivated under cover (3.97). While in the second season, there were no significant difference between the different interactions.

Concerning the interaction between different Se & NSe concentrations and cultivating method, data shows that spraying Se with all concentrations combined with plant cultivated under cover increased K% in green bean leaves significantly comparing with all Se concentrations under open field.

The interaction among cover type & Se concentrations under different cultivation type, data reveals that the were no significant differences among all treatments.

(Mattioli et al., 2019) found that Se supplementation affected mineral composition of olive leaves and presented higher levels of micronutrients; K% (2515.32 ± 152.48) and P% (1872.10 ± 80.65) compared to control. (Zahedi et al., 2019) found that, NSe significantly increased N% (1.23- and 1.22-fold), P % (1.36- and 1.44-fold), K % (1.08- and 1.11-fold) and said that; by increasing Se concentration in the leaves. It seems that physiological processes and biochemical activities, including enzyme activation, control the uptake of elements that defines the nutritional status of plants.

(Xu *et al.*, 2022) found that using of 50 mg/L SE caused the highest increases in carbohydrate, protein, and oil as well as, N, P and K contents compared to 25 mg/L of SE. In addition, 50 mg/L of Se had positive effect on the two different irrigation waters.

(Sardar et al., 2022) found that, Using NSe 10 mg L-1 increased P(mg g^{-1} DW) (7.61 ±0.38 mg L^{-1}) in *Coriandrum sativum* under normal growing conditions compared to control and other NSe concentrations(5 and15 mg L^{-1}).On the other hand, under cadmium stress (Cd =50 mg kg), data showed that using 15 resulted the heigest P (mg g^{-1} DW) (3.44 ± 0.1) and K(mg g^{-1} DW) (28.89± 0.08) compared to other NSe concentrations and Cd stress alone.

Table (5): The effect of different type of Se and different concentrations of Se under different type of cultivation on K % in green bean leaves.

		K%									
		The first season "2018"					**The second season "2019"**				
Treatments		**conc.**					**conc.**				
		0	2	4	8	**Mean**	0	2	4	8	**Mean**
open	**Se**	3.33 a	3.37 a	3.63 a	3.20 a	**3.38 B**	3.33 a	3.37 a	3.63 a	3.20 a	**3.38 A**
	NSe	3.33 a	3.47 a	3.47 a	3.10 a	**3.34 B**	3.33 a	3.47 a	3.47 a	3.10 a	**3.34 A**
cover	**Se**	4.23 a	3.53 a	3.97 a	3.73 a	**3.87 AB**	3.83 a	3.57 a	3.90 a	3.77 a	**3.77 A**
	NSe	4.20 a	3.50 a	4.20 a	3.97 a	**3.97 A**	3.83 a	3.37 a	4.17 a	3.37 a	**3.68 A**
open		3.33 b́ć	3.42 á-ć	3.55 á	3.17 ć	**3.36 Á**	3.33 áb́	3.42 áb́	3.55 áb́	3.15 b́	**3.36 Á**
Cover		4.22 á	3.52 á-ć	4.08 áb́	3.88 á-ć	**3.92 Á**	3.83 áb́	3.47 áb́	4.03 á	3.57 áb́	**3.73 Á**
Se		3.78 a	3.45 a	3.80 a	3.49 a	**3.62 A**	3.58 a	3.47 a	3.77 a	3.48 a	**3.58 A**
NSe		3.77 a	3.48 a	3.83 a	3.53 a	**3.65 A**	3.58 a	3.42 a	3.82 a	3.23 a	**3.51 A**
Mean		**3.77 Aˆ**	**3.47 Aˆ**	**3.82Aˆ**	**3.50 Aˆ**		**3.58 Aˆ**	**3.44 Aˆ**	**3.79 Aˆ**	**3.36 Aˆ**	

Se= selenium NSe= nano-selenium
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ONLINE ISSN 2636 - 3178

Data in Table (6) shows the effect of spraying different concentrations of Se and NSe on Proline concentration in green bean under two cultivation methods. Data shows that in two successive seasons respectively; cultivating green bean in open filed increased proline (1510 and 1463 ppm) in green bean significantly comparing with cultivating under cover (963.5 and 920 ppm).

Regarding type of Se effect on proline in green bean plant, data shows that in two seasons respectively, using Se (1325 and1260 ppm) increased proline concentration significantly compared to use NSe (1148 and 1122 ppm).

Concerning spraying different concentration of Se effect on proline concentration in green bean plant, data shows that increasing concentration reduced proline concentration in green bean significantly.

Concerning the interaction between type of cultivation and type of selenium, data shows that in two respective seasons the highest proline concentration obtained using Se in open field (1649 and 1593 ppm) followed by NSe under open field (1371 and 1333 ppm) condition while the lowest proline concentration obtained using NSe under cover (925 and 904 ppm).

Data in Table (6) shows the effect of Se concentrations under different cultivation type on proline concentration in green bean plant. In two respective seasons, The highest proline concentration obtained using open field control (2344 and 2306 ppm) treatment. while, the lowest proline concentration obtained using 8ppm under cover (800 and754 ppm).

On the other hand, data shows in the same table the interaction between type of Se and their concentrations. In two seasons respectively, Data reveals that the highest proline concentration obtained using by Se & NSe with concentration 0 ppm (1759 and 1719 ppm). On the contrary, the lowest proline concentration obtained using NSe with 8 ppm (825 and 834.5 ppm).

The interaction among cover type & Se concentrations under different cultivation type, data revels that the highest proline concentration in green bean obtained plants sprayed with Se & NSe 0 ppm. While the lowest proline concentration obtained NSe with concentration (4&8 ppm). Similar results obtained in the second season.

(Naidu et al., 1990) found that proline under cold stress for 5 days at 4oC increased (47.6) compared to control (0.9). and the total amino acid content of cold stressed wheat leaves increased 3.7 folds on dry wheat basis. Liu et al., 2013 found that an increase in proline content was observed upon exposure to cold stress. Compared with the control, the free proline content in seedling leaves under low temperature was obviously higher than that under room temperature. At 1∘C, the proline content increased with the prolongation of low temperature stress. At 10∘C, the proline content was increased in the first five days and reached the max of 601μg/g in the 5th day, about 6 times of control. Then proline content decreased to 404μg/g in the7th day, which was still much higher than control. At−10∘C, the plant accumulated more amounts of proline than at 1∘C. (Paleg et al., 1983) reported the same results in wheat seedlings. In developed tissues there are some biochemical responses to environmental changes in temperature, water deficit, flooding, and salinity.

According to (Xin & Browse, 1998) this phenomenon has been related to the protective role of this amino acid toward proteins and membrane structures. and was possibly associated with an augmented regulation of the activity of the corresponding biosynthetic enzymes (Fichman *et al.*, 2015; Visconti *et al*., 2019).

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ONLINE ISSN 2636 - 3178

(Bekheta et al., 2008) studied influence of using Se (5 ,10 and 20 ppm) on growth and metabolic activity of Gerbera jasmonii L. and found that, using 5 ppm Se reduced proline content (55.90) compared to control (86.56) and other Se concentrations. On the other hand, using 10 ppm Se resulted the highest proline content (129.45). and (Shalaby et al., 2021) found that using 25 ppm NSe increased proline content (23 mg g-1 FW) compared to control on cucumber (19 mg g-1 FW)

Although Se is an anti-oxidant. However, when absorbed in higher concentrations, Se could be harmful as it catalyzes the oxidation of thiols and simultaneously generates superoxide which means that it acts as a prooxidant (Adel Zayed et al., 1998)

Table (6): The effect of different type of Se and different concentrations of Se under different type of cultivation on proline in green bean leaves.

		Proline ppm									
		The first season "2018"					The second season "2019"				
Treatments		conc.					conc.				
		0	2	4	8	**Means**	0	2	4	8	**Means**
Open	**Se**	2344 a	1631 b	1450 c	1171 d	**1649 A**	2306 a	1585 b	1376 bc	1106 cd	**1593.25 A**
	NSe	2344 a	1157 d	1063 de	920 d-g	**1371 B**	2306 a	1145 cd	970 de	911 de	**1333 B**
Cover	**Se**	1174 d	1063 de	900 e-g	870 fg	**1002 C**	1126 cd	966 de	870 de	750 ef	**928 C**
	NSe	1174 d	990 d-f	807 g	730 gh	**925 D**	1126 cd	972 de	760 ef	758 ef	**904 C**
open		2344 á	1394 b́	1256.5 ć	1045.5 d́	**1510 Á**	2306 á	1365 b́	1173 b́ć	1008.5 ćd́	**1463 Á**
Cover		1174 ć	1026.5 d́	853.5 é	800 éf́	**963.5 B́**	1126 ć	969 ćd́	815 d́é	754 é	**920 B́**
Se		1759 a	1347 b	1175 c	1020.5 de	**1325 A**	1716 a	1275.5 b	1123 bc	928 cd	**1260 A**
NSe		1759 a	1073.5 cd	935 e	825 f	**1148 B**	1716 a	1058.5 cd	865 de	834.5 e	**1122 B**
Means		**1759 Aˆ**	**1210 Bˆ**	**1055 Cˆ**	**922 Dˆ**		**1716 Aˆ**	**1167 Bˆ**	**994 Cˆ**	**881 Dˆ**	

Se= selenium NSe= nano-selenium
Open= cultivation in open field Cover= cultivation under tunnels

Data in Table (7) illustrates the effect of spraying different concentrations of Se and NSe on green bean yield under two cultivation methods. In two seasons respectively, Data shows that cultivating green bean under cover (2545 and 2750 gm) increased green bean yield significantly comparing with cultivating in open field (2253 and 2600 gm). These results were in hegemony in both seasons.

Regarding type of Se effect on green bean yield, data shows that using NSe (2497 and 2749 gm) increased green bean yield significantly compared to using Se (2300 and 2601 gm)

Concerning spraying different concentration of Se effect on green bean yield, data shows that increasing concentration increased green bean yield significantly. So, 8 ppm showed the highest yield in the two successive seasons (2747 and2949 gm)

Concerning the interaction between type of cultivation and type of selenium on green bean yield, data shows that the highest yield obtained using NSe under cover (2685 and2848 gm) while the lowest green bean yield obtained using Se under open field conditions (2195 and2549 gm).

Data in Table (7) illustrates the effect of Se concentrations under different cultivation type on green bean yield. The highest yield obtained using 8 ppm cultivated under cover (2869 and 3025 gm) while the lowest yield obtained using 0ppm cultivated in open field (1961 and 2305 gm).

On the other hand, data shows in the same table the interaction between type of Se and their concentrations. Data reveals that the highest green bean yield obtained using NSe with concentration 8 ppm (2872 and3029 gm). On the contrary, the lowest yield using 0 ppm (2037 and2331 gm).

Finally, the interaction among cover type & Se concentrations under different cultivation type, data revels that the highest green bean yield obtained plants sprayed with NSe 8 ppm under cover (3045 and 3178 gm). While the lowest yield obtained using Se with concentration 0 ppm in open field (1961 and 2305 gm). Similar results obtained in the second season.

From the overall results, data shows that use NSe (8 ppm) increased vegetative growth (plant height, number of leaves) comparing with all treatments resulted from reducing the effect of cold stress showed by reducing proline production in the plant. The increase of vegetative growth increasing photosynthesis and produce more carbohydrates results of increasing green bean yield

(Shalaby et al., 2021) found that in two seasons, NSe 25ppm increased cucumber total yield (3.3 and3.57 kg/plant) compared to control (3.0 and 3.07 kg/plant). (Zahedi et al., 2019) used Se (1and 2 μM) and NSe (1and 2 μM) on pomegranate. And found that both Se types with different concentration increased pomagret fruit wegith significantly in the two successive seasons. In the first season the highest fruit weight obtained from Se 1 (305.67± 0.19). and in the second season from NSe 2 (322.00 ± 0.25) The use of Se also increased biomass and yield in garlic (Allium sat*ivum*)(Cheng et al., 2016; Poldma et al., 2013),

Table (7): The effect of different type of Se and different concentrations of Se under different type of cultivation on green bean yield.

Treatments		Yield (g)									
		The first season "2018"					The second season "2019"				
		conc.					conc.				
		0	2	4	8	**Mean**	0	2	4	8	**Mean**
Open	**Se**	1961 g	1972 g	2300 de	2550 c	**2195 D**	2305 f	2380 f	2647 e	2866b-d	**2549 C**
	NSe	1961 g	2250 e	2331 d	2700 b	**2310 C**	2305 f	2681 e	2737 de	2880 b-d	**2650 B**
Cover	**Se**	2113 f	2320 de	2497 c	2693 b	**2405 B**	2358 f	2627 e	2755 c-e	2872 b-d	**2653 B**
	NSe	2113 f	2733 b	2850 b	3045 a	**2685 A**	2358 f	2972 b	2887 bc	3178 a	**2848 A**
open		1961 f́	2111 é	2315.5 d́	2625 b́	**2253 B́**	2305 é	2530.5 d́	2692 ć	2873 b́	**2600 B́**
Cover		2113 é	2526.5 ć	2673.5 b́	2869 á	**2545 Á**	2358 é	2799.5 b́	2821 b́	3025 á	**2750 Á**
Se		2037 ^{g}	2146 ^{f}	2398 ^{e}	2621 ^{b}	**2300 ^{B}**	2331.5 ^{e}	2503.5 ^{d}	2701 ^{c}	2869 ^{b}	**2601 $^{B́}$**
NSe		2037 ^{g}	2491.5 ^{d}	2590 ^{c}	2872 ^{a}	**2497 ^{A}**	2331.5 ^{e}	2826.5 ^{b}	2812 ^{b}	3029 ^{a}	**2749 ^{Á}**
Mean		**2037 D^**	**2318 C^**	**2494.5 B^**	**2747 A^**		**2331.5 D^**	**2665 C^**	**2756 B^**	**2949 A^**	

Se= selenium NSe= nano-selenium
Open= cultivation in open field Cover= cultivation under tunnels

CONCLUSION

In conclusion, the results highlight the role of Se and NSe in regulation of cold stress and indicate that application of Se and NSe could protect green bean from the cold stress effect.

Foliar application of Se and NSe is an efficient option to improve green bean crop yield and quality. Using NSe under cover maximized the protection against low temperature. Using NSe with concentration (8) under cover resulted the highest green bean yield.

Vol. (51); Iss. (12); No. (7); Dec. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

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تطبيق النانو سيلينيوم الخارجي يخفف من الإجهاد الناتج عن درجات الحرارة المنخفضة في نباتات الفاصوليا الخضراء

لطيفة أحمد اسماعيل عطية [١]– هانى جمال متولى عبد الجواد [٢]– السيد عبد المجيد الشربيني [٣] أسامه أحمد علي البحيري [٤]

١) مركز خدمات وبحوث الزراعة في المناطق القاحلة، جامعة عين شمس، مصر ٢) قسم البساتين، كلية الزراعة، جامعة عين شمس، مصر ٣) المعهد القومى لعلوم الليزر، جامعة القاهرة، مصر ٤) معهد الدراسات العليا والبحوث الزراعية بالمناطق القاحلة، جامعة عين شمس

المستخلص

أجريت هذه التجربة في معهد الدراسات العليا والبحوث الزراعية في المناطق القاحلة (ALARI)، كلية الزراعة، جامعة عين شمس. خلال موسمين٢٠١٧ و٢٠١٨. أجريت التجربة لدراسة تحمل محصول الفاصوليا الخضراء الإجهاد البارد باستخدام السيلينيوم (Se) وجزيئات نانو السلينيوم (NSe) تحت نظامين من الزراعة المغطاة والمفتوحة، وتم اختيار موعد الزراعة ٢٠١٧/١٢/٢٠ و ٢٠١٨/١٢/٢٠ لأحداث تأثير البرودة على نبات الفاصوليا الخضراء. حيث ان موعد زراعه العروة الخريفية هو منتصف شهر سبتمبر. وتم استخدام ثلاث تركيزات من السلينيوم وجزيئات النانو سلينيوم(2,4,8 ppm). وأظهرت النتائج أن استخدام ٨ جزء في المليون من نانوسلينوم في الزراعة المغطاة أدت الطريقة إلى زيادة طول النبات وعدد الأوراق وكذلك كميه المحصول وأعطت اقل تركيز برولين في الاوراق وهذا مقارنتا بباقي التركيزات والكنترول. وعند مقارنه السليينيوم بالنانو سلينيوم نجد ان النانو سلينيوم ادي اي زياده طول النبات و عدد الاوراق و كميه المحصول وكذلك تركيز النتيروجين بالاوراق ولكنه قلل من تركيز البرولين بالاوراق. وان استخدام تركيز ٨ جزء من المليون من الناو سلينيوم تحت الاقبيه اعطي اعلي كميه محصول ممكنه مقارنتا بالمعاملات الاخرى.