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Nano NPK and Growth Regulator Promoting Changes in Growth and Mitotic Index of Pea Plants Under Salinity Stress

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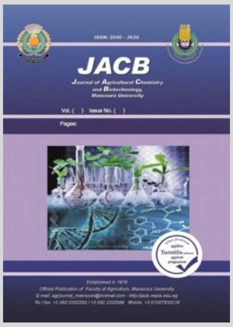
Sahar F. M. El-Hefnawy*

Biological and Environmental Science Department, Faculty of Home Economics, Al-Azhar University, Egypt



ABSTRACT

Salt stress considered one of the major environmental constraints to crop productivity worldwide. The present study was conducted to assess the ameliorative effects of potassium silicate (2% and 4%) and Nano NPK (50 and 100 ppm) exogenously applied such individually and combinations with salinity. NaCl (60, 120 mM) markedly inhibited the growth in pea (*Pisum sativum* L.) and increase chromosomal aberrations percentage. Thus, potassium silicate and Nano NPK markedly alleviated the salinity induced drastic effects on mitotic index, growth and productivity leading to reduce chromosomal aberrations. In addition, Nano NPK and potassium silicate increased the leaf area, shoot length and number of leaves. This study recommends the application of potassium silicate and Nano NPK as a foliar spray for improving pea growth irrigated with salty water and increases the growth and productivity. This work investigated the biological effects of Nano NPK 100 ppm and 2% potassium silicate *in vivo* to improve the mitotic index and reducing chromosomal aberrations under salinity stress on plants.

Keywords: Salinity, potassium silicate, Nano NPK, *Pisum sativum*, growth, mitotic index, chromosomal aberrations

INTRODUCTION

A numeral of abiotic stresses such as drought, salinity, and changes in weather temperature level are projected to occur more repeatedly, like cold, flooding, freezing, UV radiation, high and low light intensity, hypoxia heavy metal, and other soil toxicities have major effects on plant growth and crop yield worldwide (Takeda and Matsuoka, 2008). Salinity considered a general problem that harmfully affects 20 % of irrigated lands and decreases crop yields (Ghogdi *et al*., 2012; Hasanuzzaman *et al*., 2014; Helaly *et al*., 2016, Helaly *et al*., 2017; Elsheery *et al*., 2020a and Elsheery *et al*., 2020b). Several researchers have revealed that as a result of the increment in population there are effects to worry about necessary future global manufacture of food from crop plants because of soil corrosion, non-probable farming, global climate changes and soil declination (Lobell *et al*., 2008). Qadir *et al*. (2014) concluded that, NaCl stress results in the osmotic and oxidative stress, ion toxicity, reduction in cell divisions, the imbalances of nutritional and modifications in metabolic processes like respiration and photosynthesis also in inefficiency of membranes of plant cells (Sapre and Vakharia, 2017 and Elsheery *et al*., 2018).

Pea plants (*Pisum sativum* L.) are one of most necessary leguminous vegetable crops grown in winter season in Egypt. Pea can grow in diverse types of soils reaching from the light sandy loam to the heavy clay in texture. Pea plants are relatively sensitive to salinity (Regina and Katarzyna, 2011). Presence of excessive salts is a very serious problem for agricultural productivity (Munns, 2005). The application of foliar nutrition is imperative to osmoregulation and photosynthesis (Milford and Johnston, 2007). One of the mechanisms for improving plant tolerance to salinity is to apply potassium which seems to have beneficial effects. Potassium fertilization mitigates the adverse effects of salinity in plants by increasing translocation and maintaining water balance within plants (Greenwood and Karpinets, 1997). Hussein *et al.* (2012) established that spraying pepper plants with potassium increased the plant growth and production.

Potassium and silica are universal in worldly as well as aquatic environments are necessary nutrients in plants (Tarabih *et al.*, 2014) and animals (Shen *et al*., 2010). It was found that Silicon (Si) is an exceptional growth stimulating cause. Ahmed *et al.* (2007); Abbes *et al.* (2007) and Helaly *et al.* (2017) reported that silicon increase plant growth and encourage output in various crop plants. Also Si application fortified the biomass and plant height, as well as productivity under many stressed conditions. Also Si prompts growth by providing the extensibility to plant cells (Sommer *et al.* 2006). It was subsidizes extremely in proficient water utilization of plants by improving water potential, the rate of transpiration and photosynthesis under several abiotic stressed conditions (Liang *et al*., 2007 and Mateos *et al*., 2013). The performance of many crops could be upgraded by using numerous growth regulators in addition to the bio-nanoparticles as priming factor (Bhati-Kushwaha *et al*., 2013). Akram *et al.* (2009) concluded that foliar application of mineral nutrients (N, P, K) moderate the harmful effects of NaCl in numerous crops. The mode of action of these substances needs more explanation under different agroecosystem conditions. Moreover, studies are needed at several levels including the levels of molecular and subcellular in order to define the action of nanoparticles in preventing the harm effect of plant stress. In addition, Abou-Zeid and Moustafa (2014) established that nanoparticles have unique property as a result of their size, dispensation and morphology.

It was confirmed that remediation olive seedlings with Nano NPK fertilizers at exceed other treats recorded the highest value for the leaf area. To our knowledge, there is a

* **Corresponding author.**
E-mail address: saharzena@hotmail.com
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rare investigation about the cytological effects of Nano NPK and potassium silicate *in vivo* to improve the mitotic index and reduce the chromosomal aberrations under salinity stress on plants.

This study is an attempt to estimate the action of NPK nanoparticles compared with growth regulator on pea plants under salt stress, as well as examined the role of NPK nanoparticles on growth and cytological parameters in plant against NaCl stress.

MATERIALS AND METHODS

This study was carried out at the greenhouse of the Agriculture Botany Department, Faculty of Agriculture, Tanta University, Egypt during the winter of the growing season of 2019. The average of the daily temperature ranged between 10-27 °C and relative humidity between 65-70%. Pea variety used in this study was obtained from El korma company, Egypt to import seeds. Potassium silicate was obtained from El Gomhoria Chemical Company, Tanta Branch. Nano NPK consists of Potassium dihydrogen phosphate 15 g/L, Potassium chloride 17g/L, Trace elements mixture 1g/L, Magnesium sulfate 2.2g/L.

Hydroponic experiment

Pea seeds were washed and germinated in bags made of polyethylene (5 seeds per bag) filled with washed sand then the nutrient solution were add (half strength Hoagland's nutrient) according to Hoagland and Arnon (1950). The standard nutrient solution was adjusted to pH 6. Polyethylene bags placed in dishes containing 1/2 liter of Hoagland's solution (pH 6) in greenhouse with temperature ranged from 21-27^o during the day and 10-17^o during the night. Nutrient solutions were added every day and renewed every 3 days. Three replicates were used, each replicate with four polyethylene bags, every bag containing 5 plants were planted. Seedlings were exposed to two levels of salinized water 60 and 120 mM of NaCl added to the nutrient solution. Where the 4rd true leaf was emerged, foliar application with potassium silicate (2% and 4%) and Nano NPK (50 and 100 ppm) was carried out two times at mentioned above levels after 30 and 34 days from sowing. Fifteen pots left without foliar treatments, the first without salinity treatment (5 Rep), the second treated with 60 mM of NaCl (5 Rep), the third treated with 120 mM of NaCl (5 Rep). The plants were harvested after six days from foliar application and recorded the morphological parameters (El-Araby *et al*., 2020).

Morphological measurements

Recorded on seedling from each replicate for the following traits: leaf area, shoot length and number of leaves per plant were calculated using formula:

Leaf area/ plant with weight method (cm^2/plant) = B=L*S/ Z

Where, B (Green leafy area on one plant), S (Circle space for tablets on the plant), L (Total weight of the leaves), Z (Weight of tablets).

For shoot length from each treatment, shoots of 8 pea plants were separated from roots, washed using distilled water and dried carefully with tissue paper. Number of leaves per plant was counted (Zedan and Omar, 2019).

Cytological Parameters

For mitotic index, healthy seeds of *Pisum sativum* L. were germinated according to Darlington (1976). Cells were examined under a light microscope for calculated mitotic index. Numbers and types of abnormalities were examined of at least 2000 cells per treatment (about 700 cell/ replicate). Mitotic index (MI) and percentage of the abnormalities were calculated using the following formulas:

$$\text{Mitotic index (MI)} = \frac{\text{Total dividing cells}}{\text{Total dividing and non dividing cells}} \times 100$$

$$\text{Percentage of abnormal cells} = \frac{\text{Total abnormal cells}}{\text{Total dividing cells}} \times 100$$

RESULTS AND DISCUSSION

Growth parameters

Shoot length, number of leaves and leaf area per plant was affected by NaCl levels. Potassium silicate and Nano NPK foliar application improved plant tolerance to salt stress, as presented in Table 1. The data showed that all studied growth parameters were decreased under NaCl levels.

Table 1. Effect of potassium silicate and Nano NPK foliar application on growth parameters of field pea plants grown under salt stress levels.

Salinity level mM	Treatments	Concentration (ppm)	Leaf area (cm^2)	Shoot Length (cm^2)	Number of leaves
Control (0)	Control		127.74	26.75	6.00
	P.silicate	2%	132.65	29.88	7.00
		4%	130.07	27.49	6.00
	Nano NPK	50 ppm	128.74	27.16	6.00
		100 ppm	130.41	28.05	6.00
60 mM	Control		95.75	22.50	5.00
	P.silicate	2%	126.26	26.64	6.00
		4%	99.31	25.38	6.00
	Nano NPK	50 ppm	97.08	24.57	5.00
		100 ppm	107.27	26.00	6.00
120 mM	Control		49.27	17.18	5.00
	P.silicate	2%	70.63	24.75	5.00
		4%	60.38	19.32	5.00
	Nano NPK	50 ppm	57.87	18.31	5.00
		100 ppm	63.08	20.17	5.00
L.S.D. (0.05)					
Salinity			0.639**	0.405**	0.000**
Treat			0.825**	0.523**	0.000**
Salinity × Treat			1.429**	0.906**	0.000**

NaCl given through rooting medium significantly inhibited growth (shoot length, number of leaves and leaf area) in pea plants. However, the foliar application of potassium silicate (2% and 4%) and Nano NPK (50 and 100 ppm) individually or in combinations with NaCl was efficient in promoting growth under salinity conditions.

The high level of NaCl and potassium silicate (2%) gave the highest value of leaf area and shoot length which recorded 70.63 cm^2, 24.75 cm, respectively. The second highest value of leaf area and shoot length was with Nano NPK (100 ppm) which recorded 63.08 cm^2, 20.17 cm, respectively with respect to control which recorded 49.27 cm^2, 17.18 cm, respectively. But for number of leaves, potassium silicate, Nano NPK and control which gave the same value of number of leaves (5.00). Osman *et al*. (2017) concluded that exogenous application of silicon such as potassium silicate is a highly effective ameliorative approach to alleviate salinity induced hazardous effects in plants especially in pea, grown under a saline regime.

Potassium silicate encouraged the vegetative growth and mineral nutrient contents (Endris and Mohammed 2007 and Moussa and Shama 2019) such as nitrogen, phosphorus and potassium elements in several plants (Salim *et al*., 2014 and khalifa *et al*., 2017). Abbas and Fares, (2008) proved that potassium is very paramount for plants physiological functions like, the formation of sugars and starch, the synthesis of proteins, cell division, growth and fruit formation. Likewise, many researchers established that potassium has an important role in improving plant water status and reducing the toxic effects of Na element which reflects positively ultimately on the all growth parameters and the components of wheat yield (Tahir *et al*., 2011 and Hashemi *et al*., 2010). Si must be counted an essential element for plant growth because of the application promoted the biomass of plants, plant height and productivity under several stress conditions (Ahmed *et al*., 2007). Accordingly, Gomes *et al*., (2016); Patra *et al*., (2016) and Gil-Díaz *et al*., (2017) concluded that nanoparticles can enhance the capability of plants to nutrients uptake, then increase the fertility of soils, as well as crop production. Also, by using these Nano-materials, salt affected soils management as can be achieved (Patra *et al*., 2016). The data indicated that growth parameters of plants were improved by Nano NPK fertilizer treatment (100 ppm) if compared with the control. The results also indicated that nanoparticles have an enormous surface comparing with conventional form which plant metabolic processes were increased efficiency.

Cytological effects

As shown from the results summarized in Table (2) the mitotic index of root meristemic cells was statistically decreased at especially high concentrations as parallel to the increase of salt concentration as compared with control. However, under high level of NaCl stress (120 mM) potassium silicate (2%) gave the highest value of mitotic index which recorded (12.64%), the second highest value was with Nano NPK (100 ppm) which recorded (12.27%). The lowest value was with Nano NPK (50 ppm) which recorded (11.83%) if compared with the control which recorded 10.14%.

Table 2. Mitotic index of root meristemic cells germinated under salt stress.

Salinity level mM	Treatments		No. of examined Cells	Mitotic phase (%) Prophase	Metaphase	Anaphase	Telophase	Total no. of normal dividing Cells	Mitotic index (%)
Control(0)	Control		1072.67	37.33	26.33	21.67	17.00	101.00	9.79
	P.silicate	2%	1069.67	66.67	48.00	35.67	22.33	172.67	16.18
		4%	1041.67	33.33	38.00	27.67	16.67	115.67	11.11
	Nano NPK	50 ppm	1053.00	28.67	26.33	24.33	18.33	109.00	10.36
		100 ppm	1070.33	53.33	42.00	31.33	19.00	145.67	13.64
60 mM	Control		1054.00	20.00	24.00	19.67	19.67	82.67	9.79
	P.silicate	2%	1047.00	49.00	36.33	22.00	12.67	120.00	13.02
		4%	1176.33	36.00	26.67	16.00	9.00	87.67	9.05
	Nano NPK	50 ppm	1286.00	36.67	25.33	15.00	8.33	85.33	8.12
		100 ppm	1261.33	44.67	31.33	19.00	10.67	105.67	9.73
120 mM	Control		1103.67	16.67	15.67	15.67	12.00	60.00	10.14
	P.silicate	2%	1007.33	26.00	24.00	17.33	7.00	74.33	12.64
		4%	1027.67	25.00	21.00	14.00	6.67	66.67	12.27
	Nano NPK	50 ppm	1099.00	23.33	20.00	16.00	4.67	64.00	11.83
		100 ppm	1262.00	24.33	22.00	17.00	8.33	71.67	12.42
L.S.D. (0.05)									
Salinity			41.739**	1.870**	1.068**	0.851**	0.829**	2.167**	0.470**
Treat			53.885**	2.414**	1.379**	1.098**	1.070**	2.797**	0.607**
Salinity × Treat			93.332**	4.180**	2.388**	1.902**	1.853**	4.845**	1.051**

Si is considered as a growth triggers by providing vitality and extensibility to plant cells like the apical cells of the roots. So, Si engages in supports of a strong and deep root regulation (Keller *et al*., 2015). Thus, in this study high silicon concentrations reduced mitotic index. Also, there were limited studies available for potassium silicates effects on mitotic index rate in plants. NaCl stress affects the growth of plant through reduced water uptake and the imbalances of ions. It may be result in toxicity or deficiency symptoms as investigated by Naveed *et al*. (2020) who found that soil salinity reduced the growth, physiological, yield, and nutritional parameters of canola.

This study reports the effect of Nano NPK on vegetative and reproductive growth of salt-stressed on pea plants. So, it reduced plant height of plants salt-stressed compared to the control. All growth parameters were also alleviated by Nano-fertilizer application under all salinity levels (Sajyan *et al*., 2019).

Exogenous application of nanoparticles opposed the salt-induced adverse effects on plants (Pittol *et al*., 2017). Efficacy of foliar application is higher than that of soil application (Schmidhalter *et al*., 1999; Elsheery *et al*, 2020a and Elsheery *et al*., 2020c). Nano fertilizers have become a pioneer in agriculture researchers for the time being. Nanoparticles NPK used by foliar uptake were easily applied to leaf surfaces and entered the stomata via gas uptake. The required nutrient directly foliar spray on the leaves relatively quick absorption leading to the independence of root activity and soil water availability (Ling and Silberush, 2002). Foliar fertilization of plants significantly affected plant height of wheat under drought or salinity stress, it may be due to reduced cell dividing which affected on the rate of elongation (El-Dissoky, 2013).

The results presented in Table (3) indicated that salinity reduced mitotic index and increased the abnormalities%. Under the high concentrations of NaCl stress (120 mM), lowest value of abnormalities% were obtained (19.76%) in comparison to the control which recorded (25.09%). NaCl stress has a corrupt effect on DNA properties and the rate of division. Salt stress can cause prejudice to cells by breaking DNA strands in different locations, essentially cutting chromosomes into parts on barley (Mwando *et al*., 2020).

The results indicated that plants sprayed with potassium silicate solution significantly improved growth and mitotic index if compared to non-sprayed plants.

The reduction of plant growth under NaCl stress is organized with the fact that salinity induced accumulation of certain ions and decreased the external water potential of the cell (Kotagiri and Chaitanya, 2017). Furthermore, Fahad *et al*. (2016) illustrated that the reduction in plant growth may be due to the retardation in the activities of metabolic affected by the depression of water absorption and disturbance in the balance of water. Si induces many abiotic stresses such as radiation, nutrient imbalance, salinity, metal toxicity, drought, freezing, high and low temperature (Ma, 2004).

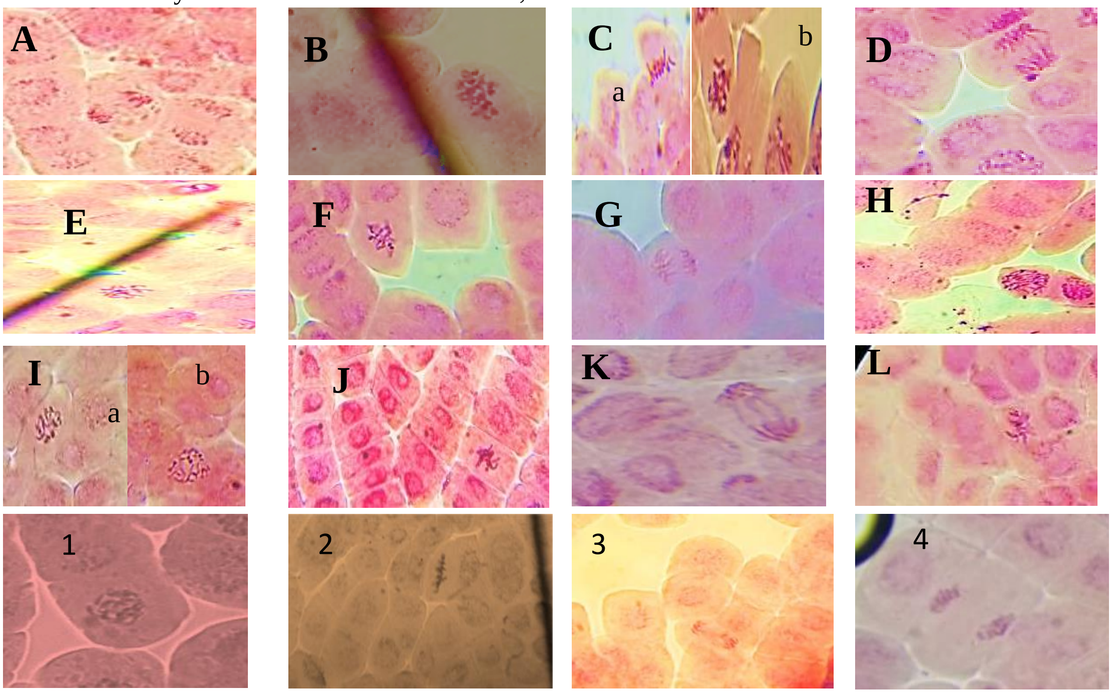

Figure 1. Some types of chromosomal abnormalities in meristematic cells of pea roots treated with Potassium silicate and Nano NPK.

A: Sticky Anaphase, B: C- Metaphase, C: (a and b) Vagrant chromosome, D: Chromosomal Laggard in Anaphase, E: Irregular prophase, F: Sticky Metaphase, G: Irregular Anaphase, H: Alignment anaphase, I: (a and b) Granulation, J: Overlapping chromosomes in Metaphase, K: Multiple bridge in anaphase, L: Disrupted Metaphase, 1: normal prophase, 2: normal metaphase, 3: normal anaphase, 4: normal telophase.

Table 3. Effect of nanoparticles on the frequency of chromosomal aberrations in *Pissum sativum* L. root tips.

Salinity level mM	Treatments		No. of examined cells	No. of dividing cells	No. of abnormal cells	Mitotic aberrations (%)									Abnormalities %
						Granulation %	C-metaphase %	Laggard%	Break%	Stickiness%	Vagrant%	Irregular%	Alignment %	Bridge%	
Control (0)	Control		1072.67	101.00	4.67	0.00	0.67	0.67	0.67	0.67	0.67	0.33	0.33	0.33	3.65
	P.silicate	2%	1069.67	172.67	1.00	0.00	0.33	0.33	0.00	0.00	0.33	0.00	0.33	0.00	0.78
		4%	1041.67	115.67	1.67	0.00	0.67	0.00	0.00	0.00	0.33	0.00	0.33	0.67	1.42
	Nano NPK	50 ppm	1053.00	109.00	1.67	0.00	0.33	0.00	0.00	0.33	0.67	0.00	0.00	0.33	1.58
		100 ppm	1070.33	145.67	1.33	0.00	0.33	0.00	0.00	0.00	0.67	0.00	0.33	0.33	1.36
60 mM	Control		1054.00	82.67	22.33	2.33	3.33	4.00	3.33	3.67	3.00	1.67	0.00	2.00	19.65
	P.silicate	2%	1047.00	120.00	16.00	1.33	1.50	3.00	1.33	2.33	1.50	3.33	0.33	2.67	11.79
		4%	1176.33	87.67	17.67	1.33	3.00	1.67	2.00	2.67	1.67	2.67	1.67	2.67	16.75
	Nano NPK	50 ppm	1286.00	85.33	18.00	0.00	2.50	2.33	1.67	2.33	3.00	2.67	2.67	4.33	17.24
		100 ppm	1261.33	105.67	16.00	1.00	1.33	2.00	1.00	1.33	3.00	2.67	0.00	3.67	13.08
120 mM	Control		1103.67	60.00	33.00	5.33	7.33	4.00	5.67	4.00	1.33	3.00	2.67	2.33	25.09
	P.silicate	2%	1007.33	74.33	20.33	2.67	4.00	1.00	1.33	4.00	2.33	2.67	4.33	2.33	15.97
		4%	1027.67	66.67	24.00	3.67	4.33	1.67	2.00	4.33	2.67	3.00	4.33	2.33	19.06
	Nano NPK	50 ppm	1099.00	64.00	25.67	3.00	5.67	3.67	2.67	3.33	2.00	3.00	2.67	2.33	19.76
		100 ppm	1262.00	71.67	23.33	3.33	4.67	1.67	2.00	5.00	2.67	2.67	4.33	1.33	18.02
L.S.D. (0.05)															
Salinity			41.739**	2.167**	1.441**	0.609**	0.564**	0.584**	0.497**	0.726**	0.872**	0.700**	0.464**	0.758**	1.284**
Treat			53.885**	2.797**	1.861**	0.786**	0.728**	0.754**	0.641**	0.938	1.126	0.903	0.598*	0.979	1.658**
Salinity × Treat			93.332**	4.845**	3.223*	1.361	1.261*	1.306*	1.111**	1.624	1.950	1.564	1.037**	1.695	2.872

Abd El-Gawad *et al.* (2016) found that encouraging potassium on enzymes activity stimulate the translocation of assimilates and protein synthesis. Sangakkara *et al.* (2000) demonstrated that the increase in plant growth due to the role of potassium in biochemical pathways of the cells enlarge the photosynthetic rates, CO_2 assimilation and supports carbon movements. On the other hand, potassium has a major role for number of physiological functions, carbohydrate translocation in plants, regulation of water and gas exchange.

Si enhanced the effects of salinity on the growth of plants across decreasing tissue Na^+ contents, maintaining the membrane solidity of root cells as evidenced by reduced lipid peroxidation, increased reactive oxygen species scavenging capacity and decreased lignification.

Hashemi *et al*. (2010) showed that silicon application alleviated the harmful effects of NaCl on the growth of canola plants by decreasing Na content in plant tissue, keeping the membrane solidity of cells as evidenced by reduced lipid peroxidation and increased reactive oxygen species scavenging. It was established that potassium (Khalid and Ahmed, 2017), Si (Hajiboland *et al*., 2017) and NPK adapted the profile of phenolics and the metabolising enzymes activity at the three developmental stages. As mentioned above, potassium silicate and Nano NPK revealed successful performance in ameliorating of the detrimental effect of salinity at all concentrations studied. It is known that potassium (Hasanuzzaman *et al*., 2018), Si (Ahmed and Raheem, 2011) and NPK (Shaheen *et al*., 2018) stimulate enzymatic activities in germinated seed tissues of many plant species under normal conditions. Kim *et al*. (2014) found that silicate components did not induce chromosomal aberrations in bone. Kwon *et al*. (2014) described that silica dioxide did not induce any aberrations of chromosomal structural (Vodenkova *et al*., 2015) on mice when treated with Zearalenone. However, the mechanism of Si Nano-toxicity is still unknown well.

CONCLUSION

In conclusion it has evident that the mechanisms by which salinity inhibit growth are very complex and controversial. Furthermore, previous mechanisms may substantially according to several factors such as the plant species, the developmental stage of the plant, the strength and duration of the salt treatment.

In this study and in future studies, the fulfillment of the effects of potassium silicate and Nano NPK on direct or indirect effective on the activity of the mitotic cycle and chromosomal abnormalities will share in understanding the mechanism type of salt stress tolerance in various plants.

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Nano NPK ومنظم النمو يعززان التغيرات في النمو ومؤشر الانقسام الميتوزي لنبات البازلاء (*Pisum sativum* L.) تحت إجهاد الملوحة

سحر فتحي محمد الحفناوي*

كلية الاقتصاد المنزلي – جامعة الأزهر – طنطا

يعتبر الإجهاد الملحي من أحد القيود البيئية الرئيسية لإنتاجية المحاصيل في جميع أنحاء العالم. وقد أجريت الدراسة الحالية لتقييم التأثيرات المحسنة لاستخدام مركب سيليكات البوتاسيوم المطبق خارجياً (2٪ و 4٪) وNano NPK (50 و 100 جزء في المليون) بشكل فردي والتوليفات مع الملوحة. وقد أدى كلوريد الصوديوم (60 ، 120 مل مول) إلى تثبيط نمو البازلاء (*Pisum sativum* L.) وزيادة نسبة الانحرافات الكروموسومية. ولذلك خففت سيليكات البوتاسيوم و Nano NPK بشكل ملحوظ من التأثيرات الشديدة الناتجة عن الملوحة على مؤشر الانقسام والنمو والإنتاجية وتقليل الانحراف الكروموسومي. بالإضافة إلى ذلك أدى Nano NPK وسيليكات البوتاسيوم إلى زيادة مساحة الورقة وطول الساق وعدد الأوراق وتوصي هذه الدراسة بتطبيق سيليكات البوتاسيوم و Nano NPK كرذاذ ورقي لتحسين نمو نبات البازلاء المروية بالمياه المالحة وزيادة نموها وإنتاجيتها. النتائج الحالية هي إحدى الدراسات النادرة لدراسة الآثار البيولوجية لـ Nano NPK 100 جزء فى المليون و2% بوتاسيوم سليكات في الحقل لتحسين مؤشر الانقسام وتقليل الانحراف الكروموسومي تحت تاثيرالملوحة على النباتات.