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ORIGINAL ARTICLES

Effect of Nitrogen, Phosphorus and Biofertilizers on Quinoa Plant

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ABSTRACT

Field experiments were carried out in Janaklees Farm, Ministry of Agriculture, Nobaria, Egypt; during the two successive growing seasons of 2010/2011 and 2011/2012. Quinoa plants (*Chenopodium quinoa* Willd.) were fertilized with ammonium nitrate (34 %N) at 0, 50, 100, 150 kg/fed. with combination of nitrobin (as a biofertilizer) or calcium super phosphate (15.5%P_2O_5) at 0, 50, 100, 150 kg/fed. with combination of phosphorin (as a biofertilizer) or used the same source and levels of chemical nitrogen and phosphorus together in combination with nitrobin and phosphorin (as a biofertilizers) to study the effect of interaction between biofertilizers and different levels of mineral fertilizers on growth, yield characters, chemical composition and anatomical structure of quinoa plant. The obtained results could be summarized as follows: The highest values of all growth and yield characters in the first and second season were recorded at treatment of 100 Kg ammonium nitrate/fed. in combination with nitrobin, 50Kg calcium super phosphate/fed. in combination with phosphorin and 100 Kg ammonium nitrate and 100Kg calcium super phosphate per fed. in combination with biofertilizers (nitrobin and phophorin) compared with other treatments or control too. Applied nitrogen and phosphorus (bio-and chemical fertilization) increased crude protein and mineral elements (phosphorus, potassium and calcium) in seeds. The increase in stem diameter of quinoa plant due to application of 100 Kg ammonium nitrate/fed. in combination with nitrobin, 50 Kg calcium super phosphate/fed. with phosphorin and 100 Kg ammonium nitrate plus 100Kg calcium super phosphate/fed. with nitrobin and phosphorin could be attributed to the prominent increase in all included tissues (the thickness of epidermis, cortex, vascular tissues and pith). Likewise, biofertilizers increased thickness of both midvein and lamina of leaf of quinoa plant. The increase in lamina thickness was accompanied with increments in thickness of palisade and spongy tissues. Also, the main vascular bundle of the midvein was increased in size as a result of treatment with biofertilizers.

Key words: *Chenopoduim quinoa*, Nitrogen, Phosphorus, Biofertilizers, Nitrobin, Phosphorin, Growth, Yield, Chemical composition, Anatomy.

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is one of the most important economic crops belongs to the family Chenopodiaceae. It is able to grow under conditions normally inhospitable to other cereals. These conditions include low rainfall, high altitude, sub-freezing or high temperatures (Ahamed *et al*., 1998). In comparison to other cereal, it has a higher protein content, better amino acid composition, minerals, and vitamin values and also has a high oil content meet or exceed the requirements of human (De Bruin, 1964 and White *et al*., 1978). The Food and Agriculture Organization (FAO, 1990) observed that quinoa seeds have high quality proteins and higher levels of energy, calcium, phosphorus, iron, fibre and B-vitamins than barley, oats, rice, corn or wheat (Koziol, 1992). In comparison to most cereals, quinoa seeds have a higher nutritional value (Matiacevich *et al*., 2006). Quinoa is a good source of essential amino acids such as lysine and methionine. Quinoa contains relatively high quantities of vitamins (thiamine, vitamin C) and minerals (Jancurová *et al.,* 2009). Quinoa is a highly nutritious food crop, with an outstanding protein quality and a high content of a range of vitamins and essential minerals (Shams, 2010). Quinoa has enormous potential in the food industry being gluten-free and highly nutritious (Doweidar *et al.,* 2011)**.** This is of great importance for the nutritional value of pseudocereals, because a high content of dietary fibre has positive effects on the reduction of the cancer risk. In general, quinoa contained higher total mineral contents than the other cereals such as rye and wheat (FAO, 2011). The evidence suggests wholegrain cereal foods and cereal fibre rich foods may protect against colorectal cancers, gastric cancers and possibly also breast, endometrial and prostate cancers (Valencia-Chamorro, 2003).

Nowadays, it has become necessary to search for untraditional fertilizers as substitutes for chemical nitrogen and phosphorus ones. Phosphorus nutrition is doubly critical because the total supply of phosphorus in most soils is low and is not readily available for the plant use. Remarkable effects of untraditional fertilizers,

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especially the biofertilizers have been reported on growth and yield of plants. Imam and Badawy (1978) found that treating seeds with *Azotobacter chrococcum* increased plant growth and yield and produced compounds detrimental to pathogens or that act as plant growth regulators. Nitrobin (nitrogen biofertilizer) was the most effective treatment in stimulating the elongation of stem, increasing both the number of leaves and branches per plant, as well as the dry weight of leaves. It could also be recognizes that the seed yield/plant showed the same response to nitrobin (Soliman, 1997; Naguib *et al*., 1998; Abd El-Kawy, 1999 and Dessouky, 2002).

Inderjit and Dakshini (1997) reported that inoculation with a cyanobacterial inoculum altered certain chemical characteristics of the test soils: pH; electrical conductivity; organic matter; organic N; total phenolics and exchangeable actions such as Cu, Zn, Na, K, Mg and Ca. It was found that the promotion was increased as the level of inculum increased. Chunchun *et al.* (1998) found that the a symbiotic nitrogen fixing bacteria (*Azospirillum*) could be used to replace some of the nitrogen fertilizer requirement on a wide range of maize field crop. Cocking (2003) indicated that nitrogen-fixing bacteria is able to enter into roots from the rhizosphere, particularly at the base of emerging lateral roots, between epidermal cells and through root hairs. *Azorhizobium caulinodans* is known to enter the root system of cereal and Arabidopsis, by intercellular invasion between epidermal cells and to internally colonize the plant intercellularly, including the xylem. This raises the possibility that xylem colonization might provide a non-nodular niche for endosymbiotic nitrogen fixation in maize. Mohamed and Medani (2005) found that *Azotobacter* and *Azospirillum* play a key role in nitrogen nutrition of cereals and produce plant growth hormones IAA, GA and cytokinine which enhance germination efficiency and stimulate rooting.

Accordingly, the present investigation is an attempt to bring to light more information about the influence of chemical and biofertilizers on growth and yield characters, as well as chemical and anatomical characteristics of quinoa plant.

Materials And Methods

The present investigation was carried out at Janaklees Farm, Ministry of Agriculture, Nobaria, Egypt; during the two successive growing seasons of 2010/2011 and 2011/2012 in order to study the effect of minerals and biofertilizers on morphological, chemical, and anatomical characters as well as on productivity of Quinoa plant.

Seeds of *Chenopoduim quinoa* Willd. were obtained through Non-Governmental National Organization from Denimark whereas, biofertilizers were secured from Agricultural Balance Institute (G.O.A.E.F.), Agricultural Research Center, Giza, Egypt.

Seeds of quinoa were sown in plots (10x6m).The plot contained 10 rows, 60 cm apart and the hills were spaced at 20 cm distance. Lit of seeds were sown in each hill, and the stand was later thinned to one plant per hill. Land preparations, agricultural operations followed the normal practices of crops cultivation in the sandy soils. Ammonium nitrate and Potassium sulfate (K_2SO_4 48%) were applied when plants aged 45 days from sowing date but Calcium supper phosphate was added with planting.

Control received all the proper agricultural procedures for quinoa production according to the estimated recommendations mentioned in the bulletin of the Denimark National Organization 2008 (ammonium nitrate 150 Kg/fed., calcium super phosphate 150 Kg/fed. and potassium sulfate 50 Kg/fed.).

-The treatments were as follows:

1- Ammonium nitrate (34%N) at the rate of 0, 50, 100 and 150 Kg/fed. in combination with 300 g/fed. nitrobein (*Azospirillium brasilense*) in addition to 150Kg calcium supper phosphate and 50Kg potassium sulfate.

2- Calcium supper phosphate (P_2O_5), 15.5%P, at the rate of 0, 50, 100 and 150 Kg/fed. in combination with 400 g/fed. phosphorin (*Bacillus megatheruim* var. *phosphaticum*) in addition to 150Kg ammonium nitrate and 50 Kg potassium sulfate.

3- Interaction among minerals and biofertilizres.

The experiment was made in a randomized complete block design with three replicates. A random sample of four plants was assigned for investigation in each plot; *i. e*., a total number of 12 plants was fixed for each treatment. The plants were labelled at the middle region of the plot. Data were recorded on individual plants with respect to morphological characters at the age of 12 weeks. For yield characters at harvest time (around mid-April in both seasons) another sample was assigned for this purpose. The procedure of recording the various data was carried out in the following manner:

A- Morphological characters:

1- Average plant height (cm).
2- Average number of branches / plant.
3- Average number of leaves / plant.

4- Average number of inflorescence / plant.
5- Average dry weight g / plant.

B- Yield characters:

1- Weight of seeds (g) / plant (Yield of seeds/plant).
2- Specific weight of seeds (g), using ten random samples from each of the three replicates, each comprised of 1000 seeds.
3- Yield of seeds (Kg) per feddan.

C- Biochemical studies:

Chemical analysis of seeds (seed quality) was performed at harvest time on seeds obtained from untreated and treated plants of *Chenopodium quinoa* Willd. in the second season. Percentages of crude protein and mineral elements were determined as follows:

1- Determination of crude protein:

Total nitrogen content was determined using the modified micro-Kjeldhahl method described by Pregl (1945). Nitrogen content of seeds was multiplied by 6.25 to calculate the crude protein content (Anon., 1990).

2- Determination of mineral elements:

The method of mineral elements determination described by Jackson (1967). Quinoa seed samples were taken to determine phosphorus, potassium and calcium.

D- Anatomical studies:

It was intended to carry out a comparative microscopically examination on plant material which showed the most prominent response of plant growth to investigated treatments. Specimens of median internode of the main stem and its corresponding leaf were taken throughout the first season of 2010/2011 at the age of two months. Specimens were killed and fixed for at least 48 hrs. in F.A.A. (10ml formalin, 5ml glacial acetic acid and 85ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56ºC, sectioned to a thickness of 20 microns, double stained with crystal violet-erythrosine, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were read to detect histological manifestations of noticeable responses resulted from minerals and biofertilizers compared to the control.

Statistical analysis:

Data on morphological and yield characters as well as on seed quality were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The least significant difference (L.S.D.) for each character was calculated at 0.05 level of probability.

Results And Discussion

Morphological characters:

Data on morphological characters of *Chenopodium quinoa* Willd. as affected by applying different levels of inorganic nitrogen and phosphorus in combination with biofertilizer nitrobin and/or phosphorin in two growing seasons are presented in Table (1).

1- Effect of nitrogen source on growth characters:

It was observed that application of 100 and 150 Kg ammonium nitrate/fed. combined with nitrobin gave the maximum significant effect in all studied growth characters (plant height, number of branches, number of leaves, number of inflorescence and dry weight /plant). Such increases by raising nitrogen fertilizer rates attested to the fact that nitrogen fertilization stimulates vegetative growth.

As to the effect of combination between 50 Kg ammonium nitrate/fed. and nitrobin, it was noted that such treatment showed insignificant differences in growth characters compared with control. But used nitrobin alone

decreased significantly all vegetative growth characters except that of plant height where the difference was not significant compared to the control (150 ammonium nitrate and calcium super phosphate Kg/fed.). Many workers recorded increase in growth characters of plants inoculated with biofertilizers,*e.g*. El-Moselhy and Zahran (2003) on barley, Virendra and Ahlawat (2004) on maize, Ogut *et al.* (2005) on bean and wheat as well as Gomaa (2008) on maize.

In this connection, Kineber *et al.* (1991) reported that nitrogen application enhance vegetative growth as well as the metabolism process in the plant and icrease in dry matter accumulation. Malik *et al*. (1993) found that N2-fixing bacteria produce plant growth hormones such as indole acetic acid, gibberellins and cytokinins. Soliman and Monem (1995) reported that combined inculation with *Asozpirillum brasilense* and *Azotobacter chroococcum* and application of chemical fertilizers saved about 60% of recommended mineral nitrogen application. Esaad *et al.* (1997) reported that biofertilization could compensate about 30-40% of the recommended nitrogen. Shams (2011)

Table 1: Morphological characters of *Chenopodium quinoa* Willd. as affected by different levels of nitrogen and phosphorus with biofertilizers in two growing seasons (2010/2011 and 2011/2012).

Treatments	Plant height (cm)		Number of branches / plant		Number of leaves / plant		Number of inflorescence / plant		Dry weight (g/plant)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
150 kg /fed. full dose from NPK (Control)	31.6	37.6	24.0	26.3	109.3	112.6	21.3	26.7	22.8	28.6
150 kg ammonium nitrate /fed. + nitrobin	39.6	40.3	27.3	29.6	126.6	123.5	22.0	32.6	38.6	40.3
100 kg ammonium nitrate /fed.+ nitrobin	56.6	60.6	34.7	37.7	121.3	127.2	34.7	36.7	69.9	73.3
50 kg ammonium nitrate /fed. + nitrobin	47.7	51.3	23.0	25.3	111.6	115.7	24.0	28.3	25.7	29.3
0 kg ammonium nitrate /fed. + nitrobin	36.7	40.3	19.3	21.6	49.7	65.7	16.3	20.3	14.2	18.7
150 kg calcium super phosphate /fed. + phosphorin	42.6	47.7	25.3	25.0	114.3	106.0	26.3	32.0	22.7	29.2
100 kg calcium super phosphate /fed. + phosphorin	49.7	56.3	26.0	28.6	100.7	113.3	25.7	33.7	23.8	27.7
50 kg calcium super phosphate /fed. + phosphorin	56.6	59.7	27.0	28.3	143.6	159.6	29.0	30.6	31.3	35.6
0 kg calcium super phosphate /fed. + phosphorein	47.3	52.3	25.7	23.3	90.0	99.7	22.7	27.4	23.3	26.3
150 kg ammonium nitrate and calcium super phosphate /fed. + nitrobein+ phosphorein	62.7	58.3	28.3	30.2	120.0	123.2	28.3	32.8	33.5	39.2
100 kg ammonium nitrate and calcium super phosphate /fed. + nitrobein + phosphorein	54.6	58.7	28.0	29.7	165.0	173.7	25.3	38.7	36.9	41.3
50 kg ammonium nitrate and calcium super phosphate /fed. + nitrobein + phosphorein	53.3	56.6	26.3	29.0	100.7	116.6	24.3	26.3	31.5	36.7
0 kg ammonium nitrate and calcium super phosphate /fed. + nitrobein + phosphorein	45.0	46.3	25.3	24.6	99.0	100.6	24.3	26.3	23.3	33.7
L.S.D (0.05)	9.2	8.5	2.7	2.9	9.6	8.1	2.7	3.5	5.3	6.1

revealed that fertilizing quinoa with nitrogen at level of 360Kg N/ha resulted in maximum growth characters.

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2- Effect of phosphorus source on vegetative growth:

It is clear from Table (1) that application of phosphorus levels (100 and 150Kg calcium super phosphate/fed.) to plants inoculated with phosphorin in the two successive seasons resulted in significant increase in the plant height and number of inflorescences /plant. Whereas number of branches, number of leaves and dry weight per plant were not affected compared to the control (150 Kg from ammonium nitrate and super phosphate/fed.).

Decreasing the inorganic phosphorus level to 50 Kg calcium super phosphate/fed. with phosphorin showed high significant increase in most of growth characters (plant height, number of leaves, number of inflorescence and dry weight /plant) except that of number of branches where the difference proved insignificant.

Phosphorin alone increased plant height but decreased number of leaves, whereas number of branches, number of inflorescences and dry weight /plant were not significantly affected.

In this connection, Martin (1982) mentioned that Azotobacters do synthesize stimulatory compounds such as gibberellins, cytokinins and indole acetic acid, which stimulate the plant cell expansion. Marshner and Cakmak (1986) stated that phosphorus plays an important role in many enzyme reactions depending on phosphorylation and energy conservation and transfer for a wide range of biochemical process. Dessouky (2002) remarked that, plant height, number of leaves, number of branches, as well as dry weight of Borago plant increased with phosphorin treatment than dressing calcium super phosphate at 200 Kg/feddan.

This result may be attributed to the major role of ATP in activating most processes in plant metabolism. In addition, the production of biologically active substances by the bacteria was the principal factor responsible for plant growth promotion.

3- Effect of nitrogen and phosphorus source on vegetative growth:

Data presented in Table (1) indicate that two levels of inorganic nitrogen and phosphorus (100 and 150 Kg ammonium nitrate and super phosphate/fed.) in combination with biofertilizers (nitrobin and phophorin) showed high significant increase in all vegetative growth characters (plant height, number of branches, number of leaves, number of inflorescences and dry weight/ plant) compared with control in the two successive seasons.

As to the effect of combination between biofertilizers (nitrobin and phosphorin) and 50Kg ammonium nitrate and calcium super phosphate/fed., it is revealed that such treatment promoted plant height and dry weight/plant, whereas number of branches, number of leaves and number of inflorescences/plant were not significantly affected compared with control plants.

It is noticed that, when used combination between biofertilizers (nitrobin and phosphorin) alone without any inorganic nitrogen or phosphorus led to increase in plant height only but most vegetative growth characters (number of branches, number of inflorescences and dry weight/plant) were not significantly affected, whereas number of leaves/plant showed significant decrease compared with control.

It could be stated that the highest values of the above mentioned criteria were estimated when nitrogen was used at 100Kg ammonium nitrate/fed. in combination with nitrobin, 50 Kg calcium super phosphate combined with phosphorin and 100 Kg ammonium nitrate and calcium super phosphate combined with nitrobin and phosphorin in the two studied seasons.

In this connection, Rai and Gour (1982) reported that inoculating seeds with some biofertilizers, *i.e.,* nitrobin and phosphorin plays an important role in helping nitrogen fixation in the soil. In addition, the utilization of biofertilizers increases the availability and absorption of nitrogen and phosphorus. Mohamed (2000) mentioned that the maximum increase in growth obtained by the treatment of 100% mineral fertilizer when combined with biofertilizers followed by the 50% mineral fertilizer treatment compared with untreated plants or the treatment of 100% mineral fertilizer alone. Salem *et al.* (2006) noticed that the inoculated seeds with nitrobin or phosphorien had positive effect on the studied growth characters. Mahfouz and Sharaf-Eldin (2007) mentioned that application of biofertilizer, which was a mixture of *Azotobacter chroococcum*, *Azospirillum liboferum*, and *Bacillus megatherium* applied with chemical fertilizers (only 50% of the recommended dosage of NPK) increased vegetative growth of fennel plant (plant height, number of branches, and herb fresh and dry weight per plant) compared to chemical fertilizer treatments only. Ahmed and Abo-baker (2010) revealed that biofertilization treatments of *Azospirillum* + *Bacillus* plus 100% chemical fertilizers produced the highest values in all sunflower growth and yield parameters compared with the control (full dose of chemical fertilization alone). The results also indicated that biofertilization, beside its ability to improve the nutrient supply in the soil, also increases the efficiency of added chemical fertilization.

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II- Yield characters:

The mean values of yield characters of *Chenopodium quinoa* Willd. as affected by nitrogen and/or phosphorus in combination with biofertilizers (nitrobin and/or phosphorin) in two seasons are given in Table (2).

1- Yield of seeds /plant:

Results in Table (2) revealed that treated quinoa plants with high level of nitrogen (150 Kg ammonium nitrate/fed.) as well as with nitrobin alone induced significant decreases in seed yield/plant in both studied seasons compared to control. Whereas, the two levels of 100 and 50 Kg ammonium nitrate/fed. in combination with nitrobin showed non-significant effect compared with control in two successive seasons. These results agree with Sa-nguansak (2004) who noticed that seed yield per unit/area decreased with increasing nitrogen fertilizer rates. Shams (2011) mentioned that the maximum nitrogen use efficiency values were obtained when quinoa received only 90 N/ha.

Increases in seed yield were significant when plants received phosphorus fertilizer at levels of 100 and 150 Kg calcium super phosphate/fed. in combination with phosphorin, but applied phosphorin alone or phosphorin with 50Kg calcium super phosphate/fed. showed no significant effect compared with control in this respect.

The low and high levels of minerals fertilizers (0 and 150 Kg ammonium nitrate and calcium super phosphate/fed.) in combination with two types of biofertilizars (nitrobin and phosphorin) showed no significant effect on seed yield, whereas maximum values of seed yield per plant of quinoa were obtained with application of 50 and 100 kg ammonium nitrate and calcium super phosphate /fed. with nitrobin and phosphorin in the two successive seasons. The above mentioned results are in harmony with those obtained by Sa-nguansak (2004), Schulte *et al.* (2005) and Salem *et al.* (2006).

In this connection, Shams (2011) revealed that fertilizing quinoa with 360 Kg N/ ha. resulted in maximum seed yield/plant in two successive seasons. Ahmed and Abo-baker (2010) reported that biofertilization treatments of *Azospirillum* + *Bacillus* plus 100% chemical fertilizers of the recommended dose produced the highest values in all growth and yield parameters of wheat plant compared with the control (full dose of chemical fertilization alone).

2- Specific weight of seeds (average weight of 1000 seeds):

Results in Table (2) revealed that there were significant increases in average weight of 1000 seeds with treatments of the two levels of nitrogen 50 and 100 Kg ammonium nitrate/fed. with nitrobin, whereas 0 or 150 Kg ammonium nitrate/fed. with nitrobin showed no significant effect compared with control in both seasons.

The increase in average weight of 1000 seeds recorded when phosphorus applied from 0 up to 150 Kg calcium super phosphate /fed. with phosphorin over the control in the two successive seasons. It is noticed that, used levels of 50 or 100 Kg calcium super phosphate /fed. with phosphorin recorded the highest values from average weight of 1000 seeds.

Nitrogen and phosphorus fertilizers application with biofertilizers (nitrobin and phosphorin) increased the average weight of 1000 seeds but levels of 0 or 150 Kg ammonium nitrate and calcium super phosphate /fed. with biofertilizers showed no significant effect. Mahfouz and Sharaf-Eldin (2007) mentioned that the highest yield per plant of wheat was observed with the treatment of biofertilizer (mixture of *Azotobacter chroococcum*, *Azospirillum liboferum*, and *Bacillus megatherium*) plus a half dose of chemical fertilizers (only 50% of nitrogen and phosphorus).

3- Seed yield/ fed.:

Data presented in Table (2), clearly show that inoculated quinoa seeds with nitrobin, in the presence of 50 Kg ammonium nitrate/fed. gave the highest seed yield 960 and 870 Kg/fed. in the first and second season; respectively. While the lowest values were obtained when applied 0 or 150 Kg ammonium nitrate/fed. with nitrobin but nitrobin with 100 Kg ammonium nitrate/fed. was not significant in the two studied seasons. In this concern, Berti *et al.* (2000) mentioned that maximum yields of quinoa were obtained when application of 225 kg N/ha. Likewise, Elkheir and Osman (2011) found that, inoculation with rhizobium significantly increased pigeon pea seed yield and 100-seed weight.

Inoculated quinoa seeds with phosphorin in the presence of all tested levels of phosphorus, showed significant increase in the yield (Kg/fed.). The level of 100 Kg/fed. calcium super phosphate/fed. with phosphorin recorded high value of yield (1380 and 1532 Kg/fed. in the first and second season; respectively).

The application of 0, 50 and 100 Kg ammonium nitrate and calcium super phosphate/fed. with both biofertilizers led to increase seed yield/fed. as compared with untreated plants (control) in both seasons. Treated

with 50 Kg ammonium nitrate and calcium super phosphate/fed. with biofertilizers recorded high significant values of yield (1344 and 1368 Kg/fed. in the first and second season; respectively). These results could be explained by the increasing vegetative growth and increasing translocation of metabolites from source to sink, due to nitrogen application (Mohamed, 2003).

Table 2: Yield characters of *Chenopodium quinoa* Willd. as affected by different levels of nitrogen and phosphorus with biofertilizers in two growing seasons (2010/2011 and 2011/2012).

Treatments	Yield of seeds (g)/plant		Weight of 1000 seeds (g)		Yield of seeds (Kg) / fed.	
	First Season	Second Season	First Season	Second Season	First Season	Second Season
150 kg full dose NPK /fed. (Control)	29.6	25.3	3.2	3.3	934	852
150 kg ammonium nitrate /fed. + nitrobin	20.0	21.2	3.3	3.5	760	685
100 kg ammonium nitrate /fed. + nitrobin	29.4	25.3	4.7	4.9	916	812
50 kg ammonium nitrate /fed. + nitrobin	29.5	27.5	4.9	4.9	960	870
0 kg ammonium nitrate /fed. + nitrobin	21.2	16.8	3.3	3.4	748	672
150 kg calcium super phosphate /fed.+phosphorin	33.2	38.6	4.3	4.5	1025	936
100 kg calcium super phosphate /fed.+phosphorin	33.0	38.3	4.6	4.5	1380	1532
50 kg calcium super phosphate /fed. + phosphorin	29.1	27.5	4.8	4.9	1284	1100
0 kg calcium super phosphate /fed. + phosphorin	28.8	25.4	3.7	4.4	1144	1136
150 kg ammonium nitrate and calcium super phosphate /fed. + Nitrobin + phosphorin	28.0	23.3	3.2	3.5	807	794
100 kg ammonium nitrate and calcium super phosphate /fed. + Nitrobin + phosphorin	31.0	31.5	4.3	4.7	1240	1100
50 kg ammonium nitrate and calcium super phosphate /fed. +Nitrobin + phosphorin	33.6	39.2	4.9	4.9	1344	1368
0 kg ammonium nitrate and calcium super phosphate /fed. +Nitrobin + phosphorin	28.3	23.9	3.1	3.2	852	756
L.S.D. (0.05)	2.6	3.1	0.3	0.8	225	213

Whereas treatment with 150 Kg ammonium nitrate and calcium super phosphate/fed. mix with biofertilizers showed no effect.

IV- Seed quality:

Chemical analysis was performed on mature dried seeds, at harvest time of the second season of *Chenopodium quinoa* willd. as affected by different levels of mineral nitrogen and/or phosphorus in combination with biofertilizers (nitrobin and/or phosphorin). For each treatment, chemical analysis was done to determine the percentage of crude protein and mineral elements.

The percentages of these fractions in seeds of treated and untreated plants of *Chenopodium quinoa* willd. are given in Table (3).

- Crude protein:

Data shown in Table (3) revealed that crude protein percentage in seeds of quinoa plants generally increased as a result of using different levels of nitrogen and / or phosphorus in combination with biofertilizers. The highest values were obtained from the plants which received high levels of 100 and 150 Kg ammonium nitrate and / or calcium super phosphate/fed. with nitrobin and / or phosphorin compared with control plants. Nitrogen application can be used for the nutritional improvement in human diet by increasing and maintaining protein and essential amino acid contents.

In this connection, David *et al.* (1998) pointed that nitrogen applications significantly increased protein content of quinoa. The author found that protein percentage increased by 0.1% per kg of ammonium nitrate applied and found that nitrogen applications have significantly increased protein content of quinoa. Berti *et al.* (2000) mentioned that protein yield was linearly correlated with increasing nitrogen application. Hevia *et al.* (2001) found that the average protein content in quinoa seeds was highest when applied 225 kg nitrogen/ha. Sa-nguansak (2004) reported that nitrogen supply was the dominant factor on the protein accumulation in the

quinoa seed. Varisi *et al.* (2008) suggested that the high concentration of lysine which was observed in quinoa seeds is possibly due to a combined effect of increased lysine synthesis and accumulation in the soluble form and/or as protein lysine.

- Mineral elements:

The data shown in Table (3) indicate that nitrogen fertilization treatments increased the mineral elements content of phosphorus, potassium and calcium in the seeds of treated plants. The highest values were recorded for high level 150 Kg ammonium nitrate /fed. in combination with nitrobin, 150 Kg calcium super phosphate/fed. in combination with phosphorin and 150 Kg ammonium

Table 3: Percentages of crude protein and mineral elements in mature dried seeds of *Chenopodium quinoa* willd. as affected by biofertilizers application with different levels of inorganic fertilizers in the second season (2011/2012).

Treatments	Biochemical analysis			
	Crude protein %	Phosphorus (g/Kg)	Potassium (g/Kg)	Calcium (mg/Kg)
150 kg full dose NPK /fed. (Control)	12.2	4.2	10.1	850.0
150 kg ammonium nitrate/fed. + nitrobein	15.6	4.9	13.5	925.0
100 kg ammonium nitrate /fed. + nitrobein	14.4	4.9	13.0	922.0
50 kg ammonium nitrate /fed. + nitrobein	13.4	4.6	12.3	855.0
0 kg ammonium nitrate /fed. + nitrobein	12.0	3.7	10.0	851.0
150 kg calcium super phosphate/fed.+ phosphorein	14.5	6.6	12.6	932.0
100 kg calcium super phosphate /fed.+ phosphorein	14.7	6.3	12.4	925.0
50 kg calcium super phosphate /fed. + phosphorein	14.4	6.3	11.5	915.0
0 kg calcium super phosphate /fed. + phosphorein	14.0	5.5	11.2	856.0
150 kg ammonium nitrate and calcium super phosphate/fed.+nitrobein + phosphorein	16.0	6.8	13.7	963.0
100 kg ammonium nitrate and calcium super phosphate /fed. +nitrobein + phosphorein	15.8	6.5	13.5	931.0
50 kg ammonium nitrate and calcium super phosphate /fed. +nitrobein + phosphorein	13.7	6.2	12.3	867.0
0 kg ammonium nitrate and calcium super phosphate /fed. +nitrobein + phosphorein	13.5	6.0	11.3	846.0
L.S.D. (0.05)	0.9 %	0.4	0.4	11.3

nitrate and calcium super phosphate /fed. in combination with two types of biofertilizers compared with control.

In this connection, De Bruin (1964) and White *et al.* (1978) found that protein percentages in the plant of quinoa were increased by phosphorus supply; quinoa had a high content of calcium, phosphorus, and iron and was low in sodium.

V- Anatomical studies:

1- Anatomy of the stem:

Histological characters of the tenth internode which resembled the median internode of the main stem of *Chenopodium quinoa* Willd. as affected by different levels of nitrogen and / or phosphorus with biofertilizers and those of control are given in Table (4). Likewise, microphotographs illustrating these treatments are shown in Figure (1).

It is obvious from Table (4) and Figure (1) that application 100 Kg ammonium nitrate /fed. or 50 Kg calcium super phosphate/fed. and 100 Kg ammonium nitrate and calcium super phosphate/fed. in combination with biofertilizers (nitrobin or/and phosphorin) increased the diameter of the stem at its median portion (at the tenth internode) of quinoa plant compared with control. The increase in diameter of the stem could be attributed to the prominent increase in all included tissues. The thickness of epidermis, cortex, fibers, vascular tissues and parenchymatous area of the pith more than those of the control. It is clear that the prominent increase which was observed in the thickness of vascular tissues of the stem of *Chenopodium quinoa* Willd. as affected by different levels of nitrogen and /or phosphorus with biofertilizers could be attributed mainly to the increase in thickness of phloem tissue and of xylem tissue more than those of the control. Moreover, vessel diameter in stem of treated plant was also increased over the control.

The present findings are generally in agreement with those obtained by El-Shaarawi *et al*. (2004); Muhammad *et al*. (2005) and Gomaa (2008). They recorded favourable anatomical changes in stem anatomy due to the effect of biofertilizers.

In this connection, Salem *et al*. (2006) mentioned that nitrogen is an essential element for plant growth to build up protoplasm and proteins, which induce cell division and meristemic activity and furtherly increase growth plant.

2- Anatomy of the leaf:

Microscopical counts and measurements of certain histological characters in transverse sections through the blade of the median leaf of the tenth internode on the stem of *Chenopodium quinoa* Willd. as affected by different levels of nitrogen or/and phosphorus with biofertilizers are presented in Table (5). Likewise, microphotographs illustrating these treatments are shown in Figure (2).

It is realized from Table (5) and Figure (2) that the different levels of nitrogen and /or

Table 4: Measurements in microns of certain histological characters in transverse sections through the stem of *Chenopodium quinoa* Willd. as affected by mineral elements in combination with biofertilizers

Characters	Treatments			
	Control (150 Kg full dose of NPK/fed.)	100 kg ammonium nitrate/fed. + nitrobin	50 kg calcium super phosphate /fed. + phosphorin	100 kg ammonium nitrate calcium super phosphate /fed. + nitrobin + phosphorin
Stem diameter	2594.2	3539.2	3468.6	3965.4
Epidermis thickness	26.9	31.9	29.7	31.0
Cortex thickness	218.5	309.5	296.9	300.7
Vascular tissue thickness	485.8	632.0	593.5	630.6
Phloem tissue thickness	139.7	159.5	158.8	183.5
Xylem tissue thickness	210.2	400.6	304.7	470.3
Vessel diameter	32.6	40.6	37.2	46.2
Pith thickness	2345.4	3146.9	3064.9	3629.0

Table 5: Counts and measurements in micron of certain histological features in transverse sections through the leaf of *Chenopodium quinoa* Willd. as affected by mineral elements in combination with biofertilizers.

Characters	Treatments			
	Control (150 Kg full dose of NPK/fed.)	100 kg ammonium nitrate/fed. + nitrobin	50 kg calcium super phosphate /fed. + phosphorin	100 kg ammonium nitrate and calcium super phosphate /fed. + nitrobin + phosphorin
Thickness of midvein	752.4	868.0	786.3	992.5
Thickness of lamina	268.2	377.3	369.7	381.0
Thickness of palisade tissue	123.9	169.0	166.7	183.3
Thickness of spongy tissue	106.8	155.8	148.6	162.2
Dimensions of midvein bundle:				
- Length	172.3	223.8	185.0	210.0
- Width	169.5	292.9	240.6	274.7
Number of vessels/ midvein bundles	27.9	54.6	35.4	46.3
Vessel diameter	18.6	26.1	19.7	23.5

phosphorus with biofertilizers increased thickness of both midvein and lamina of leaf of Chenopodium quinoa Willd. compared to control. It is noted that the increase in lamina thickness related to increments in thickness of palisade and spongy tissues compared with the control. The main vascular bundles of the midvein increased in size as a result of applying biofertilizers. The increment was mainly due to the increase in length and width more than the control. Also, average number of vessels per midvein bundle was increased over the control.

In this connection, El-Shaarawi *et al.* (2004) stated that histological examination of leaf cross sections revealed favourable anatomical effects for phosphorin treatments to mung bean plant leaves. Such favourable effects resulted in increasing leaf thickness, upper and lower epidermal layers, mesophyll tissue and dimensions of both main and smaller leaf vascular bundles. Likewise, Muhammad *et al.* (2005) revealed that the bacterial inoculation (*Cyanobacterial* strains) led to some enhancement of various growth parameters in wheat plant.

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Recommendations:

From these results, it might noticed that the growth characters, seed yield and seeds quality of quinoa plant grown in Egypt, can be improved by the application of some effective, safe and low cost treatments, *i.e.,* biofertilizers (nitrobin and/or phosphorin), so that we can use 100 kg ammonium nitrate /fed. plus nitrobin or 50kg calcium super phosphate /fed. plus phosphorin or 100 kg from each ammonium nitrate and calcium super phosphate /fed. plus two types of biofertilizers. These levels to more safety agriculture and economic too. Using of biofertilizers can minimize the total amount of the mineral fertilizer and its harmful effect on the environment.

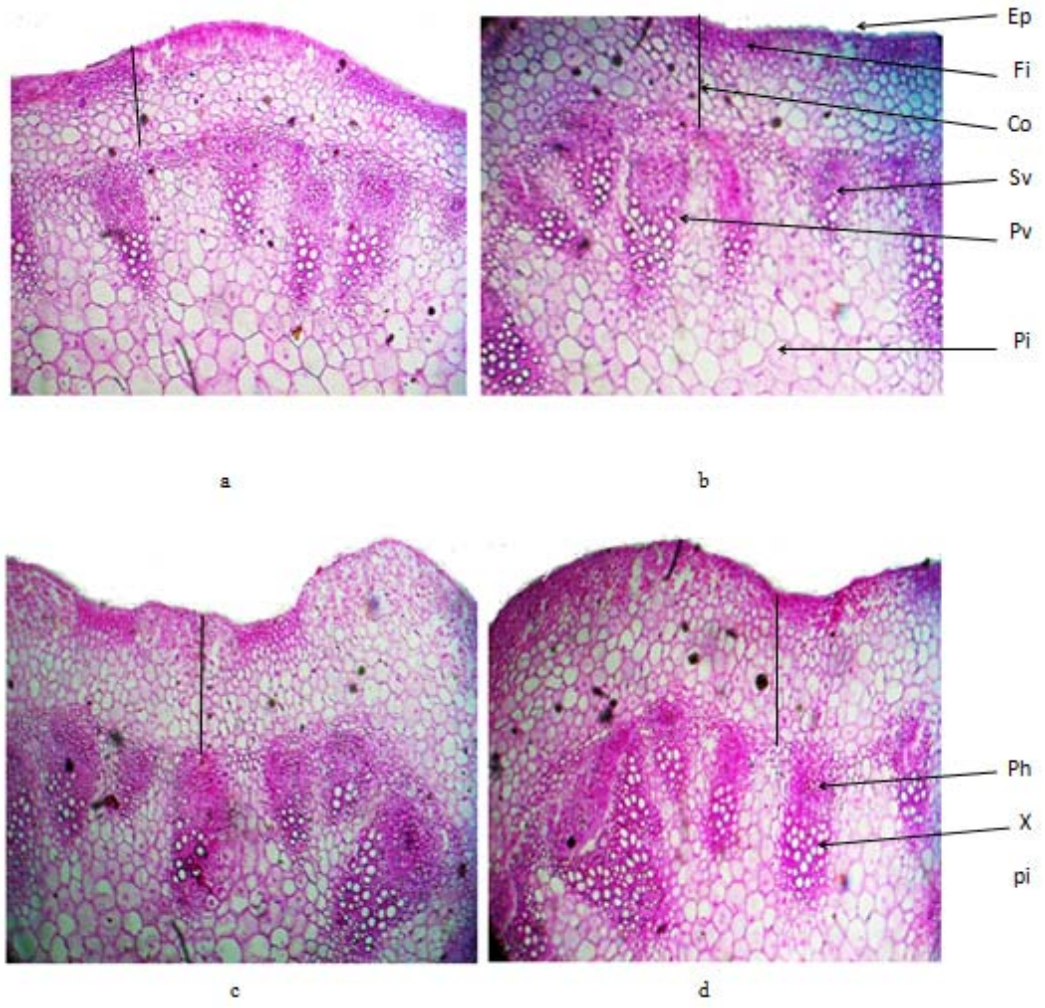

Fig. 1: Transverse sections through the tenth internode of the main stem of *Chenopodium quinoa* as affected by nitrogen and / or phosphorus in combination with bio fertilizers. (X 40)

a- From untreated plant 150 Kg NPK/fed. full dose (control).

b- From plant treated with 100 Kg ammonium nitrate/fed. with nitrobin

c- From plant treated with 50 Kg calcium super phosphate/fed. with phosphorin.

d- From plant treated with 100 Kg ammonium nitrate and calcium super phosphate/fed. with nitrobin and phosphorin.

Details: co, cortex; ep, epidermis; fi, fibres; ph, phloem; pi, pith; x, xylem; pv, primary vascular; sv, secondary vascular; and sx, secondary xylem.

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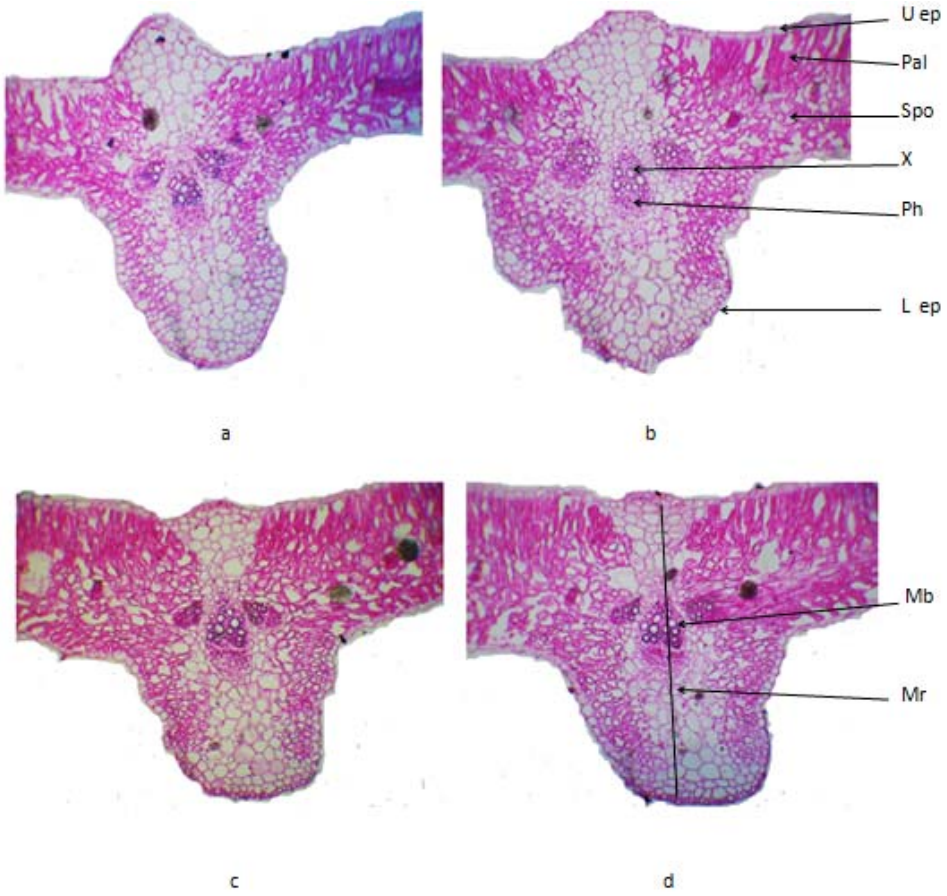

Fig. 2: Transverse sections through the blade of the tenth leaf developed on the main stem of *Chenopodium quinoa* Willd. as affected by nitrogen and / or phosphors in combination with bio fertilizers. (X 100)

a- From untreated plant 150 Kg NPK/fed. full dose (control).

b- From plant treated with 100 Kg ammonium nitrate/fed. with nitrobin

c- From plant treated with 50 Kg calcium super phosphate/fed. with phosphorin.

d- From plant treated with 100 Kg ammonium nitrate and calcium super phosphate/fed. with nitrobin and phosphorin.

Details: l ep, lower epidermis; mb, midvein bundle; mr, midrib region; pal, palisade tissue; ph, phloem; spo, spongy tissue; u ep, upper epidermis and x, xylem.

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