Response of Flax Plant (*Linum usitatissimum* L.) to Treatments with Mineral and Bio-Fertilizers from Nitrogen and Phosphorus

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Abstract: Field experiments were carried out at Sakha Agricultural Research Station, Kafr-El-Sheikh, Egypt during the two growing winter seasons of 2007/2008 and 2008/2009 in order to study the effect of different levels of mineral fertilizers from nitrogen and phosphorus (25, 50 and 100% of the recommended dose) alone or in combination with a mixture of biofertilizers containing nitrogen fixers (nitrobein) and phosphate dissolving bacteria (phosphorein) on morphological characters and yield of flax plant cv. Sakha 1 from seeds, oil, straw and fibers. Moreover, anatomy of the main stem was also investigated. The obtained results indicated that increasing level of the used mineral fertilizers induced significant increases in all investigated morphological and yield characters except that of number of seeds per capsule and seed oil percentage which showed no significant effect in this respect. The rate of promotion increased gradually as the rate of mineral fertilizers increased up to 100% of the recommended dose. It is clear that raising the level of the used mineral fertilizers from 25 to 100% of the recommended dose induced significant increases of 48.7, 46.6, 55.0, 14.1, 37.3, 19.1, 68.6, 45.4, 56.8, 44.5, 43.5, 42.3, 38.7 and 47.5% for plant height, technical length, length of fruiting zone, stem diameter, number of capsules / plant, weight of 1000 seeds, seed yield / plant, seed yield / feddan, seed oil yield / feddan, straw yield / plant , straw yield / feddan, fiber yield / plant, fiber yield / feddan and fiber length of flax plant cv. Sakha 1; respectively. Data also revealed that flax plants obtained from biofertilized seeds and grown in soil inoculated with biofertilizers (nitrobein + phosphorein) showed significant increases in all investigated morphological characters and in most of yield characters when compared with control plants which were obtained from uninoculated seeds and grown in uninoculated soil. The increments in the mentioned characters as a result of biofertilization treatment were 17.8, 17.6, 22.2, 6.2, 15.6, 8.5, 26.8, 20.5, 25.1, 17.3, 15.4, 15.9, 14.3 and 18.5% for plant height, technical length, length of fruiting zone, stem diameter, number of capsules / plant, weight of 1000 seeds, seed yield/plant, seed yield/feddan, seed oil yield/feddan , straw yield/plant, straw yield/feddan, fiber yield/plant, fiber yield/feddan and fiber length of flax plant cv. Sakha 1; respectively. The interaction between the used levels of mineral fertilizers and biofertilizers proved significant effect for the above mentioned characters. It is noted that the promotion induced by raising the level of the used mineral fertilizers was equal to that induced by biofertilizers treatment which substituted half of the recommended dose from the used NP and this decreased the environmental pollution caused by repeated application of mineral fertilizers. The effect of the used mineral and biofertilizers on anatomical structure of the main stem of flax plant cv. Sakha 1 was also investigated. [Journal of American Science. 2010;6(10):207-217]. (ISSN: 1545-1003).

Key words: Mineral Fertilizers, Bio-fertilizers, Flax, *Linum usitatissimum* L., Morphology, Productivity, Stem Anatomy.

1. Introduction

Flax (*Linum usitatissimum* L.) is a member of the genus *Linum* in the family Linaceae. It is an erect annual herb plant growing to about 1.2 m tall, with slender stems. The leaves are glaucous green, slender lanceolate, sessile, 20-40 mm long and 3 mm broad. The blue or white flowers are arranged in erect terminal panicles. The fruit is a globose capsule with shiny, flattened, brown seeds with a short blunt beak (Bunney, 1992).

Flax is now unknown in the wild but originally it may have been a native of Asia. It has been cultivated since at least 5000 BC, probably first by the ancient Mesopotamians and later by the Egyptians who wrapped their mummies in linen cloth. The Romans spread flax cultivation to Northern Europe and now the plant is grown all over the world for the oil extracted from the seeds and for its fibres, which are made into linen and other cloths. Various parts of the plant have been used to make fabric, dye, paper, medicines, fishing nets, hair gels, and soap. It is also grown as an ornamental plant in gardens.

The seeds are widely used medicinally. Their constituents include 30-40% of a fatty oil (linseed oil) with esters of linoleic acid, linolenic acid, stearic acid and oleic acid; also mucilage, proteins, a cyanogenic glycoside (linamarin) and enzymes. Whole of crushed, the seeds are a reliable means of relieving constipation. Externally, crushed seeds mixed to a paste with water are used to make hot poultices to relieve pain and to heal septic wounds, skin rashes and ulcers. The extracted oil is used in the pharmaceutical industry to make liniments for burns and rheumatic pain. The oil is also important in the manufacture of paints, soap and printer's ink.

In Egypt, flax is cultivated as a dual purpose (seeds for oil and stems for fibre). The cultivated area through the last 20 years was decreased from 60.000 to 30.000 feddan due to the great competition of other economic winter crops resulting in a gap between production and consumption. Therefore, it is necessary to increase flax productivity per unit area which could be achieved by using high yielding cultivars and improving the agricultural treatments (Hussein, 2007 and Ibrahim, 2009). One of these treatments is the use of mineral fertilizers which are important factors for vigorous growth and consequently higher yield of different plant species. However, repeated application of mineral fertilizers caused environmental pollution. Recently, under Egyptian conditions a great attention is being devoted to reduce the high rates of mineral fertilizers, the cost of production and environmental pollution via reducing doses of nitrogenous and phosphorus fertilizers by using biofertilized farming system (Ahmed *et al.,* 2005; El-Gazzar, 2006 and Hussein, 2007).

Biological fertilization of non-legume crops by N_2 – fixing bacteria had a great importance in recent years. The effect of inoculation had marked influence on the growth of plant, which was reflected to increase yield. This increase might be due to the effect of N, which was produced by bacteria species, in addition of some growth regulators like IAA and GA_3 which stimulated growth. Some bacteria called Plant Growth Promoting Rhizobacteria (PGPR), stimulate plant growth (Kapulnik, 1991 and Kloepper *et al.,* 1991). The stimulatory effects of microorganisms may result from either direct or indirect action. Direct effects include production of phytohormones (Noel *et al.,* 1996), enhancement of availability of some minerals (Kapulnik, 1991), liberation of phosphates and micronutrients, nonsymbiotic nitrogen fixation and stimulation of disease-resistance mechanisms (Lazarovits and Nowak, 1997). Indirect effects arise from (PGPR) altering the root environment and ecology (Glick, 1995). For example, acting as biocontrol agents and reducing diseases, liberation of antibiotic substances that kill noxious bacteria (Lazarovits and Nowak, 1997). Moreover, it was found that the application of phosphate dissolving bacteria as a biofertilizer resulted in a reduction of soil pH which increased the solubility of some nutrients such as P, Fe, Zn, Mn and Cu which in turn increased nutrient uptake by plants (Saber and Kabesh, 1990).

Thus, the present work is designed to study the effect of seed inoculation of flax with a mixture of biofertilizers containing nitrogen fixers (Nitrobein) and phosphate dissolving bacteria (Phosphorein) under different rates of mineral fertilizers from N and P on morphological characters and yield from straw, fibres and seeds per plant and per feddan as well as on seed oil percentage and stem anatomy of flax plant cv. Sakha 1.

2. Materials and Methods

The present investigation was carried out at Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt during the two growing winter seasons of 2007/2008 and 2008/2009 in order to study the effect of inoculation of flax seeds cv. Sakha 1 with a mixture of biofertilizers containing nitrogen fixers (nitrobein) and phosphate dissolving bacteria (phosphorien) under different levels of mineral fertilizers from nitrogen and phosphorus (NP) on morphological characters and yield of flax plant cv. Sakha 1 from straw, fiber and seeds as well as on their related characters. Moreover, stem anatomy and seed oil percentage were under consideration.

Source of seeds and the used biofertilizers:

Seeds of flax cv. Sakha 1 (a dual purpose field crop for oil and fiber productions) were procured from Fiber Crops Research Section, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Whereas, the biofertilizers nitrobein and phosphorein were obtained from General Organization for Agricultural Equalization Fund (GOAEF), Agricultural Research Center, Giza, Egypt. Nitrobein is the commercial name of nitrogen fixing bacteria containing *Azotobacter sp.* and *Azospirillum sp.* While, phosphorein was the commercial name of phosphate dissolving bacteria containing *Bacillus megatherium* var. *phosphaticum.* Both biofertilizers were added at the rate of 10 g/kg flax seeds.

Procedure of the experiment:

Flax seeds of approximately similar size were washed and immersed in the adhesive material Arabic gum to make their surface sticky before inoculation with specific bacteria of both used biofertilizers. Then, the seeds were allowed to dry before inoculation. Thereafter, seeds were inoculated with a mixture of the used biofertilizers in equal quantities (10 g/kg flax seeds for each biofertilizer) and mixed with finely sieved sterilized peat and vermiculite. (Allen, 1971).

Mineral fertilizers of NP were added at the rates of 25, 50 and 100% from that recommended by the Egyptian Ministry of Agriculture. For nitrogen fertilizer, the recommended dose was 100 kg urea (46% N)/ feddan. Whereas, the recommended dose for phosphorus fertilizer was 100 kg calcium superphosphate (15.5% P_2O_5)/feddan. For all treatments, basic dose of 50 kg potassium sulphate (48% K_2O) / feddan was added.

Phosphorus fertilizer was added as one part before sowing during the preparation of land. Whereas, nitrogen and potassium fertilizers were divided into two equal portions. The first portion was added before the first irrigation (21 days from sowing date), and the second one was added one month later. Sowing date was 15th November in the first season and it was 19th November in the second one. Seeds were drilled at the rate of 60 kg seeds/feddan.

Physical and chemical analysis for soil of the experimental sites in each growing season were done before sowing according to Jackson (1967). The preceding crop was maize (*Zea mays* L.) in both seasons. The soil type was clay and physical and chemical properties are presented in Table (1).

Table (1). Physical and chemical properties of the experimental soil in the two growing seasons

Soil properties	First season	Second season
Clay %	39.9	41.0
Silt %	36.7	34.8
Sand %	23.2	24.2
Soil textural class	Clay	Clay
pH	8.06	8.15
Total soluble salts %	0.28	0.31
Calcium carbonate %	3.52	4.11
Organic matter %	1.22	1.31
Total nitrogen %	1.03	0.80
Available N ppm	45.63	39.21
Available P ppm	12.00	11.20
Available K ppm	415.00	440.00

In each growing season, the experiment was made in a split plot design with three replicates. The replicate contained three main plots, each assigned for one level of mineral fertilizers. Each main plot was divided into two sub plots, one sown with flax seeds inoculated with the used biofertilizers and the other sub plot was sown with flax seeds not inoculated with biofertilizers. Thus, the three levels of mineral fertilizers (25, 50 and 100% of the recommended dose from N and P) beside the two levels of biofertilizers required that the experimental land of each replicate be divided into six subplots, each contained one treatment. The sub-plot area was 6 m^2 (2×3 m) and consisted of 10 rows, 20 cm apart. After hand weeding, soil was also inoculated with the chosen biofertilizers just before the first irrigation (three weeks from planting) at the rate of 600 g from each used biofertilizer / feddan. For this purpose, the applied biofertilizers were mixed well with adequate amount of fine dust (20 kg fine dust / feddan) and such mixture was applied between rows of flax seedlings directly before the first irrigation which was corresponding with the first application of nitrogen and potassium fertilizers. All other cultural practices were carried out as recommended.

Recording of data:

At maturity, 150 days from sowing date, 10 guarded plants were taken randomly from each sub-plot (totaling 30 plants for each treatment) for recording the following morphological and yield characters per plant in addition to straw, fiber and seed yields/feddan (each estimated according to yield/ 1m^2 of each sub-plot. The recorded characters included:

1- Plant height (cm); from the cotyledonary node till the top of the plant.
2- Ultimate diameter of the main stem (mm); the diameter was measured with a caliper at its median portion.
3- Technical length of the main stem (cm); from the cotyledonary node till the beginning of apical branching zone of the main stem.
4- Length (cm) of apical branching zone of the main stem (fruiting zone).
5- Number of capsules per plant.
6- Number of seeds per capsule; recorded on 50 random capsules from each sub-plot.
7- Specific seed weight (average weight of 1000 seeds in grams); recorded on 10 random samples from each sub-plot.
8- Seed yield (g) per plant.
9- Seed yield (kg) per feddan; estimated according to seed yield per 1 m^2 of each sub-plot.
10- Seed oil percentage; determined according to the methods described by Anonymous (1990).

11- Oil yield (kg/feddan); calculated by multiplying seed oil percentage ×seed yield per feddan.
12- Straw yield (g) per plant; weight of air dried straw per plant in grams.
13- Straw yield (ton) per feddan; estimated according to weight of air dried straw yield per 1 m^2 of each sub-plot.

After harvesting and removing the capsules from plants of each sub-plot, retting process took place at Fiber Crops Research Section, Sakha Agricultural Research Station. Straw of each sub-plot was arranged in bundles and put in retting basins and soaked in water for about 12 hours. After soaking, the water was changed to leach out all the soluble materials. Retting period was about one week in summer season. The degree of water temperature during retting process ranged from 28 to 30^{o}C and the acidity was pH 6-7. The retted straw was washed with water and finally dried in air. Thus, the fibers were easily extracted from above the woody part of the stem. The following fiber characters were studied:

14- Fiber yield (g) per plant; weight of air dried fibers per plant in grams.
15- Fiber yield (kg) per feddan; estimated according to fiber yield per 1 m^2 of each sub-plot.
16- Fiber length (cm); fiber ribbons of 10 plants from each sub-plot were extracted individually and mean length of each ribbon was measured.

Statistical analysis:

Appropriate statistical analysis according to Snedecor and Cochran (1982) was done. The data were statistically analyzed for each season and the homogeneity of experimental error, in both seasons, was tested. Then, the combined analysis of the two seasons was done.

Anatomical studies:

For anatomical investigation, specimens of selected treatments were taken during the second season of 2008/2009 from the middle part of the main stem at the age of 105 days from sowing date. Specimens were killed and fixed for at least 48 hr. in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series, embedded in paraffin wax of 56^{o}C melting point, sectioned to a thickness of 20 microns, double stained with safranin/light green. Cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were examined to detected histological manifestations of the chosen treatments and photomicrographed.

3. Results and Discussion

I- Morphological characters:

The effects of different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or in combination with a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria on some morphological characters of flax plant cv. Sakha 1 aged 150 days (harvest time) are presented in Table (2). The investigated morphological characters included plant height, technical length, length of fruiting zone and diameter of the main stem.

It is clear from Table (2) that increasing level of the used mineral fertilizers induced significant increases in all morphological characters under investigation. The rate of promotion increased gradually as the rate of mineral fertilizers increased up to 100% of the recommended dose, which gave the highest values of the studied morphological characters. It is obvious that raising the level of mineral fertilizers from 25 to 100% of the recommended dose induced significant increases of 48.7, 46.6, 55.0 and 14.1% for plant height, technical length, length of fruiting zone and diameter of the main stem; respectively.

The present findings are in agreement with those reported by Mostafa *et al.* (1998), El-Gazzar (2000), Awad *et al.*, (2001), El-Shimy *et al.* (2001), Mostafa *et al.* (2003), El-Gazzar (2006), Salem *et al.*, (2006), El-Sweify *et al.* (2007) and Hussein (2007).

As to the effect of biofertilizers, results in Table (2) indicate that flax plants obtained from biofertilized seeds and grown in inoculated soil showed significant increase in all investigated morphological characters when compared with flax plants obtained from uninoculated seeds and grown in non-inoculated soil. The beneficial effect of inoculation with nitrogen fixers containing *Azotobacter sp.* and *Azospirillum sp.* as well as with phosphate dissolving bacteria containing *Bacillus sp.* was mainly in improving the fixation of atmospheric N, increasing the release of P in the soil, which is reflected in increasing P activity and the growth promoting substances produced by them. Those may lead to the activation of cell division and cell enlargement and finally increasing the growth parameters (Patil, 1985). The increments in morphological characters of flax plant due to biofertilization treatment were 17.8, 17.6, 22.2 and 6.2% for plant height, technical length, length of fruiting zone and diameter of the main stem; respectively.

Table (2). Mean values of certain morphological characters of flax plant cv. Sakha 1, at harvest time, as affected by different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or in combination with a mixture of biofertilizers (nitrobein+phosphorein) containing nitrogen fixers and phosphate dissolving bacteria (Average of the two seasons, 2007/2008 and 2008/2009 combined)

Treatments		Characters			
Mineral fertilizers (NP)	**Biofertilizers (nitrobein + phosphorein)**	**Plant height (cm)**	**Technical length (cm)**	**Length of fruiting zone (cm)**	**Main stem diameter (mm)**
25% of recommended dose	-	66.8	54.9	11.5	1.83
	+	81.4	68.6	14.3	1.97
	Mean	**74.1**	**61.8**	**12.9**	**1.90**
50 % of recommended dose	-	83.7	69.5	14.7	1.99
	+	106.3	86.6	18.9	2.16
	Mean	**95.0**	**78.1**	**16.8**	**2.08**
100% of recommended dose	-	105.9	87.4	18.6	2.14
	+	114.5	93.7	21.3	2.23
	Mean	**110.2**	**90.6**	**20.0**	**2.19**
Means of seed and soil inoculation with biofertilizers	-	85.5	70.6	14.9	1.99
	+	100.7	83.0	18.2	2.12
L.S.D. (0.05) for:					
Mineral fertilizers (A)		8.15	6.91	2.14	0.099
Biofertilizers (B)		6.59	5.77	1.81	0.078
Interaction (A×B)		11.17	9.68	2.77	0.129

- = Without biofertilizers.
\+ = With biofertilizers.

The present results are in accordance with those reported by El-Gazzar (2000), El-Shimy *et al.* (2001), El-Azzouni and El-Banna (2002), Mostafa *et al.* (2003), Salem *et al.* (2006) and Hussein (2007).

The interaction between the used levels of mineral fertilizers and biofertilizers proved significant effect for all investigated morphpological characters. It is noted that increasing level of the used mineral fertilizers from NP or using a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria without raising the level of mineral fertilizers induced significant increase in all of the studied morphological characters. Worthy to note that the promotion induced by raising the level of the used mineral fertilizers was almost equal to that induced by biofertilizers treatment which, in general, substituted half of the recommended dose from the used NP(Table, 2) and this decreased the environmental pollution caused by repeated application of mineral fertilizers.

II- Seed yield and its related characters:

It is realized from Table (3) that increasing level of the used mineral fertilizers induced significant increase in number of capsules/plant, weight of 1000 seeds, seed yield/plant, seed yield/feddan and seed oil yield/feddan of flax plant cv. Sakha 1, and the rate of promotion increased significantly as the rate of mineral fertilizers increased up to 100% of the recommended dose which gave the highest values of the mentioned characters. By contrast, number of seeds/capsule and seed oil percentage of flax plant cv. Sakha 1 showed no statistical effect in this respect. Worthy to note that, raising the level of the used mineral fertilizers from 25 to 100% of the recommended dose induced significant increases of 37.3%, 19.1, 68.6, 45.4 and 56.8% for number of capsules/plant, weight of 1000 seeds, seed yield/plant, seed yield/feddan and seed oil yield/feddan; respectively.

Table (3). Mean values of seed yield and its related characters of flax plant cv. Sakha 1, at harvest time, as affected by different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or in combination with a mixture of biofertilizers (nitrobein+phosphorein) containing nitrogen fixers and phosphate dissolving bacteria (Average of the two seasons, 2007/2008 and 2008/2009 combined)

Treatments		Characters						
Mineral fertilizers (NP)	**Biofertilizers (nitrobein + phosphorein)**	**No. of capsules / plant**	**No. of seeds / capsule**	**Weight of 1000 seeds (g)**	**Seed yield (g) / plant**	**Seed yield (kg)/feddan**	**Seed oil percentage**	**Seed oil yield (kg) / feddan**
25% of recommended dose	-	10.33	7.53	6.85	0.5329	354.18	35.3	125.03
	+	12.69	7.81	7.69	0.7621	491.71	39.1	192.26
	Mean	**11.51**	**7.67**	**7.27**	**0.6475**	**422.95**	**37.2**	**158.65**
50 % of recommended dose	-	12.88	7.75	7.72	0.7705	483.93	38.7	187.28
	+	15.22	8.03	8.51	1.0399	595.22	40.8	242.85
	Mean	**14.05**	**7.89**	**8.12**	**0.9052**	**539.58**	**39.8**	**215.07**
100% of recommended dose	-	15.17	7.98	8.49	1.0281	593.17	40.6	240.83
	+	16.43	7.96	8.83	1.1550	637.06	40.3	256.74
	Mean	**15.80**	**7.97**	**8.66**	**1.0916**	**615.12**	**40.5**	**248.79**
Means of seed and soil inoculation with biofertilizers	-	12.79	7.75	7.69	0.7772	477.09	38.2	184.38
	+	14.78	7.93	8.34	0.9857	574.66	40.1	230.62
L.S.D. (0.05) for:								
Mineral fertilizers (A)		1.73	N.S.	0.538	0.0833	31.25	N.S.	19.84
Biofertilizers (B)		1.15	N.S.	0.344	0.0662	27.28	N.S.	15.29
Interaction (A×B)		2.16	N.S.	0.669	0.1085	40.96	N.S.	27.72

- = Without biofertilizers.
\+ = With biofertilizers

The present results are generally in accordance with those recorded by Mostafa *et al.* (2003), Hussein (2007) and Ibrahim (2009) using different levels of nitrogen fertilizer as El-Gazzar (2006) using different levels of phosphorus fertilizer. They stated that, increasing level of the used mineral fertilizer up to the recommended dose increased seed yield of flax plant and its related characters especially number of capsules/plant and seed index (weight of 1000 seeds) and consequently seed yield per plant and per feddan or hectare as well as oil yield per unit area. In this connection, Salem *et al.* (2006) found that increasing the level of nitrogen fertilizer from 30 to 60 kg N/feddan gave the highest value for number of seeds/capsule, seed yield/plant and seed yield/feddan of two flax cultivars, being partially in agreement with the present findings.

Concerning the effect of biofertilizers, data in Table (3) clearly show that flax plants obtained from biofertilized seeds and grown in soil inoculated with biofertilizers had significant increase in number of capsules/plant, weight of 1000 seeds, seed yield /plant, seed yield /feddan and seed oil yield /feddan when compared with flax plants obtained from uninoculated seeds and grown in non-inoculated soil (control plants).

However, biofertilization treatment did not affect number of seeds/capsule and seed oil percentage. The increments in seed yield and its related characters due to biofertilization treatment were 15.6, 8.5, 26.8, 20.5 and 25.1% for number of capsules/plant, weight of 1000 seeds, seed yield/plant, seed yield/feddan and seed oil yield/feddan; respectively. The present findings are generally in agreement with those reported by El-Gazzar (2000), El-Shimy *et al.* (2001), El-Azzouni and El-Banna (2002), Mostafa *et al.* (2003) and Hussein (2007). However, El-Azzouni and El-Banna (2002) found that number of seeds/capsule of flax plant was increased by biofertilization treatment, being in contrast with the present findings.

The interaction between the used levels of mineral fertilizers and biofertilizers proved significant effect for all investigated characters except that of number of seeds/capsule and seed oil percentage. Worthy to mention that the rate of promotion induced by raising the level of the used mineral fertilizers was almost equal to that induced by biofertilization treatment. It is clear that the treatment of 100% from the recommended dose of NP did not statistically differ than that of 50% of the recommended dose of NP plus biofertilizers in their effect. This means that using a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria substitute half of the recommended dose from the used mineral fertilizers of NP.

III- Straw and fiber yields and fiber length:

Mean values of straw and fiber yields per plant and per feddan as well as fiber quality, which is represented by fiber length, of flax plant cv. Sakha 1 are given in Table (4).

Data presented in Table (4) clearly show that increasing level of the used mineral fertilizers from nitrogen and phosphorus induced significant increase in straw yield per plant and per feddan, fiber yield per plant and per feddan and fiber length of flax cultivar under investigation. Worthy to mention that the rate of promotion increased gradually as the rate of mineral fertilizers increased up to 100% of the recommended dose, which gave the highest values of the previously mentioned characters for straw and fiber yields. In this concern, raising level of the used mineral fertilizers from 25 to 100% of the recommended dose induced significant increases of 44.5, 43.5, 42.3, 38.7 and 47.5% for straw yield/plant, straw yield/feddan, fiber yield/plant, fiber yield/feddan and fiber length; respectively.

Similar results were also reported by El-Gazzar (2000), El-Shimy *et al.* (2001), Mostafa *et al.* (2003), Salem *et al.* (2006), El-Sweify *et al.* (2007) and Hussein (2007).

As to the effect of biofertilizers, results presented in Table (4) reveal that flax plants obtained from biofertilized seeds and grown in soil inoculated with N-fixers and phosphate dissolving bacteria showed increments in their yields from straw and fibers as well as in fiber length when compared with control plants, of flax cv. Sakha 1, obtained from non inoculated seeds and grown in non inoculated soil. The increments in straw and fiber yield as a result to biofertilization treatment were 17.3, 15.4, 15.9, 14.3 and 18.5% for straw yield /plant, straw yield/feddan, fiber yield/plant, fiber yield/feddan and fiber length; respectively.

The present findings are in accordance with those recorded by El-Gazzar (2000), El-Shimy *et al.* (2001), El-Azzouni and El-Banna (2002), Mostafa *et al.* (2003) and Hussein (2007).

Table (4): Mean values of straw and fiber yields as well as fiber length of flax plant cv. Sakha 1, as affected by different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or in combination with a mixture of biofertilizers (nitrobein+phosphorein) containing nitrogen fixers and phosphate dissolving bacteria (Average of the two seasons, 2007/2008 and 2008/2009 combined).

Treatments		Characters				
Mineral fertilizers (NP)	**Biofertilizers (nitrobein + phosphorein)**	**Straw yield (g)/plant**	**Straw yield (ton)/ feddan**	**Fiber yield (g)/plant**	**Fiber yield (kg)/feddan**	**Fiber length (cm)**
25% of recommended dose	-	1.485	0.936	0.306	198.79	56.1
	+	1.887	1.182	0.379	246.15	69.6
	Mean	**1.686**	**1.059**	**0.343**	**222.47**	**62.9**
50 % of recommended dose	-	1.894	1.195	0.385	248.33	70.9
	+	2.377	1.498	0.486	299.81	90.1
	Mean	**2.136**	**1.347**	**0.436**	**274.07**	**80.5**
100% of recommended dose	-	2.380	1.514	0.482	303.67	89.2
	+	2.493	1.525	0.494	311.95	96.4
	Mean	**2.437**	**1.520**	**0.488**	**307.81**	**92.8**
Means of seed and soil inoculation with biofertilizers	-	1.920	1.215	0.391	250.26	72.1
	+	2.252	1.402	0.453	285.97	85.4
L.S.D. (0.05) for:						
Mineral fertilizers (A)		0.147	0.119	0.036	23.98	6.75
Biofertilizers (B)		0.112	0.088	0.027	18.51	5.58
Interaction (A×B)		0.201	0.164	0.051	32.79	9.42

- = Without biofertilizers.
+ = With biofertilizers

The interaction between the used levels of mineral fertilizers and biofertilizers proved significant effect. It is realized that the treatment of 100% of the recommended dose from NP did not statistically differ from that of 50% of the recommended dose from NP plus biofertilizers in their effect on all studied characters. Such result indicate that using a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria substitute half of the recommended dose from the used mineral fertilizers of NP required for flax cultivation.

In this respect, Mikhailouskaya (2006) stated that seed inoculation of flax by N_2-fixing and growth promoting bacteria *Azospirillum brasilense* B-4485 was equivalent to the introduction of 15 kg/ha of N that provided the possibility of partial flax N requirement supply, being in good agreement with the present findings.

IV- Stem anatomy:

From the aforementioned results of morphological characters of flax plant cv. Sakha 1 as affected by mineral and biofertilizers, it could be stated that the treatment of 100% of the recommended dose from the used mineral fertilizers (control treatment) did not statistically differ from that of 50% of the recommended dose from the used mineral fertilizers plus biofertilizers in their effects. Therefore, the anatomical structure of the main stem of such treatments was under consideration.

Counts and measurements of certain histological characters in transverse sections through the median portion of the main stem of flax plant cv. Sakha 1 as affected by mineral and biofertilizers from nitrogen and phosphorus are given in Table (5). Likewise, microphotographs illustrating the effects of these treatments are shown in Figure (1).

It is obvious that flax plants received 50% of the recommended dose from the used mineral fertilizers showed a prominent decrease in diameter of the main stem by 11.0% less than the control. The decrease in stem diameter, which was observed in plants that received half of the recommended dose from nitrogen and phosphorus could be attributed to the prominent decrease in most of the included tissues. The decrements less than the control were, 45.0, 16.1, 23.1, 39.2 and 42.4 % for thickness of fiberous region, number of fiberous bundles/cross section, average number of fiberous cells/bundle, thickness of secondary phloem and thickness of xylem tissue; respectively. However, a slight increase of 6.1, 6.7 and 9.0% over the control was observed for thickness of epidermis, thickness of cortex and diameter of the pith; respectively.

Table (5): Counts and measurements in micron of certain histological features in transverse section through the median portion of the main stem of flax plant cv. Sakha 1, at the age of 105 days from sowing date, as affected by mineral and biofertilizers from nitrogen and phosphorus (Means of three sections from three specimens)

Histological characters	Treatments				
	100% of recommended dose from NP (Control)	50% of recommended dose from NP	± % to control	50% of recommended dose from NP + biofertilizers treatment	± % to control
Diameter of the section	2218.4	1973.5	- 11.0	2242.7	+ 1.1
Thickness of epidermis	27.9	29.6	+ 6.1	28.4	+ 1.8
Thickness of cortex	41.6	44.4	+ 6.7	43.2	+ 3.9
Thickness of fiberous region	69.4	38.2	- 45.0	57.9	- 16.6
Number of fiberous bundles/cross section	31	26	- 16.1	33	+ 6.5
Average number of fiberous cells / bundle	13	10	- 23.1	11	- 15.4
Thickness of secondary phloem	121.5	73.9	- 39.2	126.8	+ 4.4
Thickness of xylem tissue	247.1	142.3	- 42.4	255.3	+ 3.3
Diameter of the pith	1212.9	1321.9	+ 9.0	1222.8	+ 0.8

Results also indicated that flax plants obtained from biofertilized seeds, with nitrobein and phosphorein, and received 50% of the recommended dose from the used mineral fertilizers and grown in biofertilized soil showed a slight increase in diameter of the main stem by 1.1% more than stem diameter of control plants which received 100% of the recommended dose from the used mineral fertilizers. The negligible increase which was observed in stem diameter could be attributed to the slight increases induced in most of included tissues by 1.8, 3.9, 6.5, 4.4, 3.3 and 0.8% over the control for thickness of epidermis, thickness of cortex, number of fiberous bundles/cross section, thickness of secondary phloem, thickness of xylem tissue and diameter of the pith; respectively. However, a decrement of 16.6 and 15.4% below the control was observed for thickness of fiberous region and average number of fiberous cells/bundle; respectively

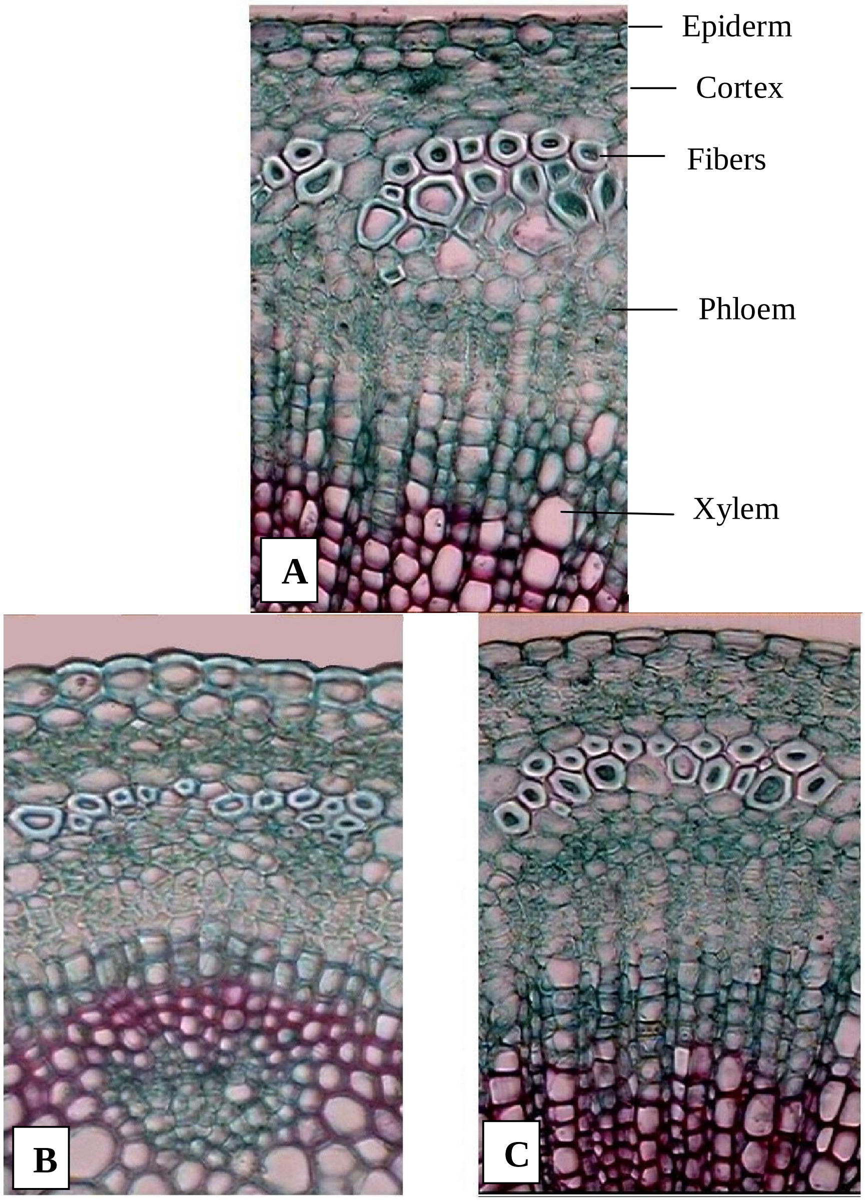

Fig.(1): Transverse sections through the median portion of the main stem of flax plant cv. Sakha 1, at the age of 105 days from sowing date, as affected by mineral and biofertilizers from nitrogen and phosphorus. (X 280)

- **A- From plant received 100% from recommended dose of mineral fertilizers (control plant).**
- **B- From plant received 50% from recommended dose of mineral fertilizers.**
- **C- From plant received 50% from recommended dose of mineral fertilizers + biofertilizers treatment.**

In this concern, Mostafa *et al.* (2003) studied the effect of different levels from nitrogen fertilizer alone or in combination with biofertilizers from phosphorein and nitrobein on stem anatomy of flax plant cv. Sakha1. The investigated characters were area of cross section, cortex, fiberous zone, phloem, xylem and pith. They stated that increasing nitrogen level from 20 up to 50 kg N/feddan induced significant increments for all anatomical characters except pith area which showed significant decrease in this respects. Also, biofertilizers treatment from nitrobein and phosphorein increased significantly all studied anatomical characters except xylem area which reduced significantly, being partially in harmony with the present findings.

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