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Effect of Mineral and Bio-Fertilizers on Vegetative Growth, Mineral Status, Seed Yield, Tropane Alkaloids and Leaf Anatomy of Thornapple Plant (*Datura stramonium* L.)

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ABSTRACT

Field experiments were conducted at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two growing seasons of 2014 and 2015 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 (namely, *Azotobacter chrococcum* and *Azospirillum brasilense*) and phosphate dissolving bacteria (namely, *Bacillus megaterium* var. *phosphoticum*) on vegetative growth characters (plant height, number of leaves/plant, total leaf area/plant, fresh weight of leaves/plant and dry weight of leaves/plant), seed yield/plant, tropane alkaloids (hyoscyamine and hyoscine), mineral contents in leaves and leaf anatomy of Thornapple plant (*Datura stramonium* L.). For all treatments, basic dose of 50 kg potassium sulphate (48% K_2O)/fed. was added. Such search hopes to substitute part of the mineral fertilizers by biofertilizers treatment which in turn could reduce environmental pollution caused by repeated application of mineral fertilizers. The obtained results revealed that increasing level of the used mineral fertilizers induced significant increases in all of the studied morphological characters and seed yield as well as in tropane alkaloids and induced favourable changes in anatomical structure of leaves of Thornapple plants. Likewise, it is clear that biofertilizers treatment enhanced all investigated characters and 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 and this decreased the environmental pollution caused by repeated application of mineral fertilizers.

Key words: Thornapple, *Datura stramonium* L., Mineral fertilizers, Bio-fertilizers, Growth, Seed yield, Mineral status, Tropane alkaloids, Leaf anatomy

Introduction

Medicinal plants are important economic products which represent significant sources of economic revenue and foreign exchange and are among the most important agricultural export products. Recently, an increasing interest in the cultivation and production of medicinal plants has been noticed in Egypt in order to cover the increasing demand of the local industries as well as for export purposes.

Datura stramonium L. (Thornapple, Jimsonweed, Stramonium) is one of the most important medicinal plants belongs to the family Solanaceae. Thornapple is a native of North America but it has been introduced elsewhere (Bunney, 1992). It is widely distributed in the Western Region of the Middle East and Sudan and grow well under environmental conditions of Egypt (Salama, 2006). The leaves and seeds are used medicinally. Their constituents include tropane alkaloids (0.4 percent), principally hyoscyamine, atropine and scopolamine, and traces of an essential oil. These substances give Thornapple antispasmodic and hallucinogenic actions, they inhibit glandular secretions and dilate the airways. In medicine today, Thornapple is mostly used in tinctures and proprietary preparations to treat asthma, bronchitis and Parkinson's disease. It is occasionally prescribed in the form of cigarettes. Worthy to note that all parts of the plant are extremely poisonous, especially the seeds. Thus, Thornapple is a dangerous plant; it should never be collected and used for self-medication (Bunney, 1992 and Robin *et al*., 1992).

It is well known that mineral fertilizers 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 greet 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. Biological fertilization of non-

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legume crops by N_2-fixing bacteria had a great importance in recent years (Anonymous, 2013). The effect of inoculation had marked influence on the growth of plant, which reflect to increase yield. This increase might 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; 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 (Gaur, 2010), liberation of phosphates and micronutrients, nonsymbiotic nitrogen fixation and stimulation of disease-resistance mechanisms (Deepali and Gangwar, 2010; Youssef and Eissa, 2014)). 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; Anonymous, 2010). Certain species of microorganism are widely used which have unique properties to provide natural products, and serve as a good substitute of chemical fertilizers (Deepali and Gangwar, 2010). *Azosprillum*, in addition to N fixation ability, improve root growth by produce growth stimulants and subsequent increase in water and nutrient uptake rate, that raising yield (Tilk *et al*., 2005).

Therefore, the present work is designed to study the effect of seed inoculation of Thornapple with a mixture of nitrogen fixers (namely, *Azotobacter sp*. and *Azospirillum sp*.) and phosphate dissolving bacteria (namely, *Bacillus sp*.) and different rates with mineral fertilizers on vegetative and reproductive growth, mineral content as well as on leaf anatomy and yield quality of such important medicinal plant species. Moreover, the use of biofertilizers is an attempt to substitute part of the mineral fertilizers which in turn could reduce environmental pollution caused by repeated application of mineral fertilizers.

Materials and Methods

Experiments and treatments

The present investigation was carried out at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two growing seasons of 2014 and 2015 in order to study the effect of inoculation of Thornapple seeds (*Dature stramonium* L.) with a mixture of biofertilizers containing nitrogen fixers (namely, *Azotobacter chrococcum* and *Azospirillum brasilense*) and phosphate dissolving bacteria (namely, *Bacillus megaterium* var. phosphoticum) under different levels of mineral fertilizers of nitrogen and phosphorus (NP) on vegetative growth characters, yield, chemical constituents and leaf anatomy of Thornapple plant.

Seeds of Thornapple were procured from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, University of Cairo, Giza, Egypt. Whereas, the mixture of biofertilizers was obtained from General Organization for Agricultural Equalization Fund (G. O. A. E. F.), Agricultural Research Center, Ministry of Agriculture, Egypt.

Thornapple 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 biofertilizers. Then, the seeds were allowed to dry before inoculation. Thereafter, seeds were inoculated with a mixture of biofertilzers in equal quantities 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 120 kg urea (46% N) and 120 kg ammonium sulphate (20.5% N)/fed. Whereas, the recommended dose for phosphorus fertilizer was 100 kg superphosphate (15.5% P_2O_5)/fed. In addition to 20 m^3 organic fertilizer / feddan was recommended. For all treatments, basic dose of 50 kg potassium sulphate (48% K_2O)/fed. was added. (1 feddan = 0.4047 hectare)

Phosphorus fertilizer and organic fertilizer were added as one part before sowing during the preparation of land. Whereas, nitrogen and potassium fertilizers were divided into two equal portions. The first one was added after three weeks from the sowing and the second was added after a month from the first one. Sowing date was 25th February in the first season and it was 13th March in the second one.

Physical and chemical analysis for soil of the experimental sites in each growing season were done before sowing according to Jackson (1967). The soil type was loamy clay and physical and chemical properties are presented in Table (1).

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 seeds inoculated with biofertilizers and the other sub plot was sown with 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 sub plots, each contained one treatment. The sub plot consisted of 7 rows, 4 m long, 60 cm apart and the spacing between hills was 30 cm. After three weeks from planting, seedlings were thinned out to one plant per hill. Soil was also inoculated with the chosen biofertilizers just before the first irrigation (three weeks from planting). All other cultural practices were carried out as recommended.

Table 1: Physical and chemical properties of the experimental soil in the two growing seasons

Soil properties	First season (2014)	Second season (2015)
Coarse sand %	1.7	1.5
Fine sand %	32.5	34.2
Silt %	37.2	39.4
Clay %	28.6	24.9
Soil texture	Loam clay	Loam clay
pH	7.6	7.4
Organic matter %	1.82	1.73
Available N ppm	48.0	43.0
Available P ppm	7.9	7.2
Available K ppm	438.0	419.0

Recording of vegetative growth data

The following morphological characters were recorded on 15 plants for each treatment (5 plants from each of the three replicates) at the age of three months from sowing date.

1-Plant height (cm).
2-Number of leaves developed / plant.
3-Total leaf area (cm^2) / plant, measured by means of LI-3000 A portable area meter.
4-Fresh weight of leaves (g) / plant.
5-Dry weight of leaves (g) / plant.

Chemical determinations

Specimens from leaves represents all treatments were taken from plants grown in the second season of 2015 at the age of 3 months for determination of tropane alkaloids according to Milan *et al*. (1990).

A known weight (0.2 gm) of dried leaves from plants grown in the second season of 2015 at the age of 3 months was digested in H_2SO_4 (concentrated), H_2O_2 (5:1) for chemical analysis of minerals: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sodium (Na), iron (Fe), manganese (Mn) , zinc (Zn) and copper (Cu) according to A.O.A.C. (1995). The concentration of the N, P, K, Ca, Fe, Na elements were expressed in mg/g DW, whereas the elements Zn, Mn and Cu were expressed in ppm.

Anatomical studies

For anatomical investigations, specimens of selected treatments were taken during the second season of 2014 from the middle part of the first leaf developed on the first terminal branch, at the age of 75 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 56ºC melting point, sectioned to a thickness of 20 microns, double stained with the crystal violet erythrosine. Cleared in xylene and mounted in Canada balsam (Willey, 1971). Sections were examined to detect histological manifestations of the chosen treatments and photomicrographed.

Yield traits measurements

Another 15 plants for each treatment were assigned (labeled) for recording of yield characters as follows:

1- Number of harvested fruits / plant. Mature fruits were collected every week till the end of the growing season.
2- Yield of seeds (g) / plant.
3- Weight of 1000 seeds (g); represents specific weight of the seeds.

Statistical analysis

The obtained data of morphological and yield characters as well as of tropane alkaloids and mineral content were subjected to appropriate statistical analysis according to Snedecor and Cochran (1982).

Results and Discussion

Morphological characters of vegetative growth

Data presented in Table (2) show the effect of different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or combined with a mixture of biofertilizers containing nitrogen fixers (*Azotobacter sp.* and *Azospirillum sp.*) and phosphate dissolving bacteria (*Bacillus sp.*) on morphological characters of Thornapple plant. All treatments received 100% of the recommended dose of potassium fertilizer. The investigated morphological characters, at the age of 90 days in both studied seasons of 2014 and 2015, included plant height (cm), number of developed leaves per plant, total leaf area (cm^2) per plant, fresh weight of leaves (g) per plant and dry weight of leaves (g) per plant.

It is realized from Table (2) that increasing level of the used mineral fertilizers induced significant increases in all of the studied morphological characters of vegetative growth in both studied seasons. It is clear that the rate of promotion increased gradually as the rate of mineral fertilizers increased up to 100% of the recommended dose (Figure, 1), which gave the highest values of vegetative growth of Thornapple plant. It is noted that raising the level of mineral fertilizers from 25 to 100% of the recommended dose induced significant increases of 52.41 and 43.94% for plant height, 52.32 and 54.60% for number of developed leaves per plant, 55.66 and 53.96% for total leaf area per plant, 59.01 and 61.60% for fresh weight of leaves per plant and 52.90 and 54.23% for dry weight of leaves per plant of Thornapple in the first and second season; respectively.

It is well known that, nitrogen, phosphorus and potassium are essential macro-elements for higher plants and have major functions in the growth and metabolism of plants which directly affect plant productivity. Thus, the positive effect of mineral fertilizers on growth characters of Thornapple plants may be due to the role of nitrogen in protoplasm formation and all proteins; *e.g*., amino acids, nucleic acids, many enzymes and energy transfer materials ADP and ATP (El-Gendy and Selim, 2014). Also, the role of phosphorus as a major nutrient element, where phosphorus compounds are of absolute necessity for all living organisms, nucleoproteins constituting the essential substances of the cell and for cell division and development of meristematic tissues (El-Gendy and Selim, 2014). Moreover, potassium is important for plant growth and is involved in every metabolic process, including carbohydrates metabolism, protein biosynthesis, assimilate translocation, conformation of enzymes and stomatal movement (El-Gendy and Selim, 2014). These effects reflected on vigorous vegetative growth such as, plant height, number of leaves per plant, total leaf area per plant and fresh and dry weights of leaves per plant.

Similar results were recorded by Shetty *et al*. (1990) using NPK on *Datura stramonium* L. and by Kewala and Bisht (1996) using nitrogen on different *Hyoscyamous* species. These results are also in harmony with those obtained by Hassan *et al*. (2006) on moghat plants as well as by El-Nagdy *et al*. (2010) on flax plants and by Boghdady *et al*. (2012) on sesame plants. Dordas and Sioulas (2009) suggested that nitrogen affects accumulation of dry matter in different parts of plants.

As for the effect of biofertilizers, results in Table (2) clearly show that Thornapple plants obtained from biofertilized seeds and grown in inoculated soil showed significant increases in all investigated morphological characters of vegetative growth in both studied seasons when compared with Thornapple plants obtained from uninoculated seeds. 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 Thornapple plant due to biofertilization treatment were 18.61 and 17.23% for plant height, 23.71 and 16.73% for number of leaves per plant, 24.54 and 16.15% for total leaf area per plant, 27.56 and 21.83% for fresh weight of leaves per plant and 25.21 and 19.17% for dry weight of leaves per plant in the first and second season; respectively. The present results are in accordance with those reported by Hassan *et al*. (2006) on moghat, El-Nagdy *et al*. (2010) on flax and Boghdady *et al*. (2012) on sesame. They stated that biofertilizers treatment stimulated vegetative growth and increased dry matter production in the previous studied plant species, being in harmony with the present findings. Simultaneously use of bacteria causes root development and better uptake of water and nutrients and is effective on vegetative growth and plant height (Biari *et al*., 2008). Dhanasekar and Dhandapani (2012) indicated that *Azotobacter*, *Azospirillum*, *Phosphobacter* and *Rhizobacter* strains can provide significant amount of nitrogen to *Helianthus annus* and to increase the plant height, number of leaves, stem diameter, percentage of seed filling and seed dry weight. Choudhury and Kennedy (2004) in rice, found that addition of *Azotobacter*, *Azospirillum* and *Rhizobium* promotes the physiology and improves the root morphology. It was found that *Azospirillum* inoculation can change the root morphology and producing plant growth regulating substances (Bashan *et al*., 2004 and Sahoo *et al*., 2014), increases the number of lateral roots and enhances root hairs formation to provide more root surface area to absorb sufficient nutrients (Mehdipour-Moghaddam *et al*., 2012) and improves the water status of plant and aids the nutrient profile in the advancement of plant growth and development (Ilyas *et al*., 2012).

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Fig. 1: Effect of different levels of mineral fertilizers from nitrogen and phosphorus (NP) on growth habit of Thornapple plant (*Datura stramonium* L.), age of eight weeks.
A- Plant received 25% from the recommended dose of the used mineral fertilizers.
B- Plant received 50% from the recommended dose of the used mineral fertilizers.
C- Plant received 100% from the recommended dose of the used mineral fertilizers.

Table 2: Certain morphological characters of vegetative growth of *Datura stramonium* L. (Thornapple), at the age of 3 months, as affected by different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or combined with a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria in the two growing seasons of 2014 and 2015

Treatments		Morphological characters									
Mineral fertilizers (NP)	Biofertilizers	Plant height (cm)		Number of leaves / plant		Total leaf area (cm^2)/plant		Fresh weight of leaves (g) / plant		Dry weight of leaves (g) / plant	
		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
25% of recommended dose	-	64.3	71.2	76.2	77.1	2739.2	2781.6	83.11	88.42	17.634	16.953
	+	76.9	87.1	104.6	92.5	3814.8	3398.6	117.28	113.98	24.192	21.629
	Mean	70.6	79.2	90.4	84.8	3277.0	3090.1	100.20	101.20	20.913	19.291
50 % of recommended dose	-	78.5	85.8	101.8	99.3	3754.4	3607.9	113.64	115.39	23.353	22.186
	+	104.2	106.4	132.4	121.7	4885.2	4314.5	153.97	149.45	30.256	26.728
	Mean	91.4	96.1	117.1	110.5	4319.8	3961.2	133.81	132.42	26.805	24.457
100 % of recommended dose	-	103.8	109.3	130.6	124.9	4816.1	4536.8	148.94	154.27	29.787	27.934
	+	111.4	118.7	144.8	137.3	5385.7	4978.2	169.72	172.81	34.163	31.572
	Mean	107.6	114.0	137.7	131.1	5100.9	4757.5	159.33	163.54	31.975	29.753
Means of seed inoculation with biofertilizers	-	82.2	88.8	102.9	100.4	3769.9	3642.1	115.23	119.36	23.591	22.358
	+	97.5	104.1	127.3	117.2	4695.2	4230.4	146.99	145.41	29.537	26.643
L.S.D. (0.05) for:											
Mineral fertilizers (A)		7.26	8.77	9.57	8.35	328.7	308.3	11.46	12.27	2.423	2.378
Biofertilizers (B)		5.91	6.68	7.82	7.01	255.2	239.9	9.34	10.15	1.946	1.895
Interaction (A x B)		9.68	11.24	12.71	10.89	429.6	394.7	14.86	16.29	3.197	3.116

\- = Seeds were not inoculated with biofertilizers.
\+ = Seeds were inoculated with biofertilizers just before sowing.

The interaction between the used levels of mineral fertilizers and biofertilizers proved significant effect in both studied seasons for all investigated morphological characters of vegetative growth of Thornapple plant. It is realized 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 vegetative growth characters under investigation in both studied seasons. It is evident 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 decrease the environmental pollution caused by repeated application of mineral fertilizers. In this respect, Maheshwari *et al*. (1988) on black henbane as well as Pareek *et al*. (1992) on opium poppy and henbane and Bhardwaj *et al*. (2014) who stated that the use of *Azotobacter* as biofertilizer reduced the N requirement for normal growth of the investigated crops, being in harmony with the present results. Mahfouz and Sharaf-Eldin, (2007) found that seed inoculation with *Azotobacter* and *Azospirillum* in the presence of chemical fertilizers resulted in improving growth of *Foeniculum vulgare* plant. Also, Hassan (2009) revealed that bacteria inoculation separately or combined with chemical fertilizers significantly improved growth characters of roselle plant compared to the control. Moreover, it is clear that the maximum increase in morphological characters of vegetative growth was recorded when raising the level of mineral fertilizers in the presence of biofertilizers treatment. In this respect, Helal *et al*. (2011) indicated that applying biofertilizer in combination with chemical N fertilizer increased the growth of dill plant compared to the untreated control and the greatest vegetative growth was recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer.

From the above mentioned results, it could be stated that inoculation of Thornapple seeds with a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria under different levels of mineral fertilizers from NP increased significantly all investigated characters of vegetative growth of Thornapple plant in both studied seasons. These results are in agreement with those recorded by Hassan *et al*. (2006) on moghat and El-Nagdy *et al*. (2010) on flax as well as Boghdady *et al*. (2012) on sesame. The enhancement effect of the used biofertilizers on certain morphological characters of vegetative growth of Thornapple plant, found here in, could be attributed generally to many factors such as (a) its ability to release plant promoting substances, mainly IAA, GA_3 and cytokinin-like substances which might be stimulated plant growth (Reynders and Vlassak, 1982 and Abd El-Fattah *et al*., 2013), (b) synthesis of some vitamins; *e.g*., B_{12} (Okon and Gonzalez, 1984 and Revillas *et al*., 2000), (c) increasing amino acids content (Schank *et al*., 1981), (d) increasing the water and mineral uptake from the soil (Sarig *et al*., 1984 and Ilyas *et al.*, 2012) and that could be ascribed to increases in root surface area, root hairs and root elongation as affected by *Azotobacter* as mentioned by Sundaravelu and Muthukrishinan (1993); Mehdipour-Moghaddam *et al*. (2012) and Sahoo *et al*. (2014), (e) increasing the ability to convert N_2 to NH_4 and thus make it available to plant and (f) enhancing the production of biologically active fungistatical substances which may change the microflora in the rhizosphere and affect the balance between harmful and beneficial organisms (Apte and Shende, 1981and Bhattacharyya and Jha, 2012).

Yield characters

The yield characters of Thornapple plant as affected by different levels of mineral fertilizers from nitrogen and phosphorus alone or combined with a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria in two growing seasons were investigated. Studied traits which are presented in Table (3) include: number of harvested fruits per plant, yield of seeds (g) per plant and specific weight of seeds (weight of 1000 seeds in grams).

Results given in Table (3) clearly show that increasing level of the used mineral fertilizers induced significant increase in number of harvested fruits per plant and in yield of seeds per plant in both studied seasons, 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 number of fruits and seed yield per Thornapple plant. By contrast, specific weight of Thornapple seeds, weight of 1000-seeds, showed no statistical effect in this respect in both studied seasons. Worthy to note that, raising the level of the used mineral fertilizers from 25 to 100% of the recommended dose induced significant increase of 54.35 and 47.26% for number of harvested fruits per plant and 61.53 and 48.95% for yield of seeds per plant in the first and second season; respectively. Similar results were recorded by Shetty *et al*. (1990) on *Datura stramonium* L., Moustafa *et al*. (1988) on tomato plants, Ahmed and Tanki (1991) and Olsen *et al*. (1993) on chilli plant, Schon *et al*. (1994) on pepper plant and Kewalanand *et al*. (2001) and Helal *et al*. (2011) on dill plant.

Regarding the effect of biofertilizers, data in Table (3) reveal that Thornapple plants obtained from biofertilized seeds and grown in soil inoculated with biofertilizers showed significant increase in number of harvested fruits and seed yield per plant in both studied seasons when compared with Thornapple plants obtained from uninoculated seeds and grown in uninoculated soil. However, biofertilization treatment did not affect specific weight of Thornapple seeds in both studied seasons. The increments in yield characters of Thornapple plant due to biofertilization treatment were 17.98 and 19.91% for number of harvested fruits per plant and 18.84 and 20.86% for yield of seeds per plant in the first and second season; respectively.

The interaction between the used levels of mineral fertilizers and biofertilizers proved a significant effect in both studied seasons for number of harvested fruits and yield of seeds per plant, whereas the specific weight of seeds showed no statistical effect in this respect. It is worthy to note 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 evident that the treatment of 100% of 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. In this respect, some investigators found that *Azospirillum* inoculation saved about 50% of the recommended N rate in low available nitrogen soil. So, using *Azospirillum* + half dose of nitrogen produced fruits similar to that obtained from using full dose of nitrogen (Subbiah, 1990 on tomato; Krishnaraj and Sreenivasa, 1992 on pepper and Ganeshe *et al*., 1998 on okra and Yadav *et al*., 2011 on corn). Yadav *et al*. (2011) reported that biological fertilizers, especially when combined with mineral fertilizers have many effects on crop yield and productivity. Also, they suggest that corn seed inoculation with *Azosprillum*, cause an increase in grain yield related to the control plot. Mahfouz and Sharaf-Eldin (2007) found that seed inoculation with *Azotobacter* and *Azospirillum* in the presence of chemical fertilizers resulted in improving yield of *Foeniculum vulgare* plant. Hassan (2009) found that bacteria inoculation separately or combined with chemical fertilizers significantly increased sepal yield of roselle plant compared to the control. Helal *et al*. (2011) indicated that applying bio-fertilizer plus two third of recommended dose of nitrogen fertilizer gave the highest yield of dill plant compared to the untreated control.

The beneficial effect of biofertilizers on yield is attributed to the vigorous growth of biofertilized plants and to the amount of metabolites synthesized by these plants which reflected to increase in number of harvested fruits and consequently increase in number and yield of seeds per plant. Such beneficial effect of biofertilizers on yield could be also attributed to the role of biofertilizers in absorbing nutrients especially P, Fe, Zn, Mn and Cu as shown in our results which plays an important role in activation of the metabolic processes. In addition to increasing the amounts of N-fixation by *Azotobacter* and *Azospirillum* (Mohamed, 2000 and Sahoo *et al*., 2013).

Table 3: Yield characters of *Datura stramonium* L. (Thornapple) as affected by different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or combined with a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria in the two growing seasons of 2014 and 2015.

Treatments		Yield characters					
Mineral fertilizers (NP)	Biofertilizers	No. of harvested fruits / plant		Yield of seeds (g) / plant		weight of 1000 seeds (g)	
		2014	2015	2014	2015	2014	2015
25% of recommended dose	-	21.1	18.3	75.12	67.05	6.21	6.18
	+	24.8	21.9	84.52	79.24	6.41	6.35
	Mean	23.0	20.1	79.82	73.15	6.31	3.27
50 % of recommended dose	-	25.3	22.1	86.91	78.59	6.32	6.24
	+	32.5	27.5	117.26	102.77	6.59	6.61
	Mean	28.9	24.8	102.09	90.68	6.46	6.43
100 % of recommended dose	-	33.8	27.4	122.04	101.37	6.71	6.58
	+	37.2	31.8	135.82	116.55	6.73	6.69
	Mean	35.5	29.6	128.93	108.96	6.72	6.64
Means of seed inoculation with biofertilizers	-	26.7	22.6	94.69	82.34	6.41	6.33
	+	31.5	27.1	112.53	99.52	6.58	6.55
L.S.D. (0.05) for:							
Mineral fertilizers (A)		2.37	2.62	6.98	8.29	N.S.	N.S.
Biofertilizers (B)		1.93	2.08	5.65	6.86	N.S.	N.S.
Interaction (A x B)		3.22	3.49	9.17	11.03	N.S.	N.S.

- = *Seeds were not inoculated with biofertilizers.*
+ = *Seeds were inoculated with biofertilizers just before sowing.*

Mineral content

Data presented in Table (4) indicate that the concentrations of some macroelements (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca)) and some microelements (Iron (Fe), Zinc (Zn), Copper (Cu)) were significantly increased, but sodium (Na) was not significantly affected with increasing the level of the used mineral fertilizers from nitrogen and phosphorus. The highest values of N, P, K, Mg, Fe and Cu were detected in the plants that received 100 % nitrogen and phosphorus fertilizers, whereas the highest one of Ca and Mn detected at the level of 50% fertilizers.

Concerning the effect of biofertilizer on mineral concentration (Table, 4), it can be noticed that application treatment with biofertilizer containing nitrogen fixers and phosphate dissolving bacteria significantly increased the concentration of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu, but decreased Na concentration in leaves of the treated plants compared with the untreated plants. Similar results were reported by Elkoca *et al*. (2008) on chickpea; Cakmakci *et al*. (2007) on barley; Orhan *et al*. (2006) on raspberry; Esitken *et al*. (2003) on apricot and Güneş *et al*. (2009) on strawberry, who found that PGPR bacteria stimulated macro- and micro-nutrient uptake such as N, P, K, Ca, Mg, Fe, Mn, Zn, Cu.

As for the interaction of mineral fertilization (NP) combined with application of biofertilizer on minerals concentration, data recorded in Table (4) show that application of NP fertilizers combined with biofertilizers treatments improved the mineral status in leaves of Thornapple. The level of 50% recommended dose of NP combined with biofertilizer containing nitrogen fixers and phosphate dissolving bacteria gave the highest concentrations of N, Ca, Mg, Zn and Mn and the lowest one of Na compared with the other treatments. The highest values of P, K, Fe and Cu were obtained by the combination of the 100% recommended dose of NP with biofertilizer compared with the other treatments. In this respect, Said-Al Ahl (2005) showed that nitrogen fertilizer and/or bio-fertilizer treatment led to an increase in N, P, K contents in *Anethum graveolens*. Gendy *et al*. (2103) found that different sources of nitrogen or bio-fertilizers increased N, P, K compared to untreated plants. The interaction treatment of ammonium sulphate at 60 kg N/fed., + bio-fertilizer (biogein at 1 kg/fed., + nitrobein at 1 kg/fed.,) gave the best result in this concern. Also, Mahfouz and Sharaf-Eldin (2007) found that seed inoculation with *Azotobacter* and *Azospirillum* in the presence of chemical fertilizers resulted in improving NPK elements in the dried herb. In addition, Hassan (2009) found that NPK percentages were increased as a result of applying biofertilizers alone or combined with chemical fertilizers. Moreover, Helal *et al*. (2011) indicated that the highest values of NPK percentages in dill plants were recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer.

The increase or accumulation of minerals resulted from the application of biofertilizer may be attributed to secretion of growth regulators and increase the root size as a result of the increment in root length which accelerate the uptake of minerals and explain these increases (Nickell, 1982; Kapulnik, 1991; Ali and Selim, 1996; Burger *et al.,* 1997; Bashan *et al*., 2004 and Mehdipour-Moghaddam *et al*., 2012) .

Table 4: The concentrations of some macro- and microelements in leaves of *Datura stramonium* L. (Thornapple), at the age of 3 months, as affected by different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or combined with a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria in the growing season of 2015.

Treatments		Minerals concentrations									
Mineral fertilizers (NP)	Biofertilizers	N	P	K	Ca	Mg	Na	Fe	Zn	Mn	Cu
		Mg/g Dw							ppm		
25% of recommended dose	-	8.40	2.42	46.50	17.50	9.25	4.30	4.70	21.20	16.20	15.15
	+	14.00	2.90	46.50	20.00	10.50	3.40	5.80	24.25	16.43	16.17
	Mean	11.20	2.66	46.50	22.50	9.88	3.85	5.25	22.73	16.32	15.66
50 % of recommended dose	-	8.40	2.74	51.30	22.50	10.34	3.40	5.20	44.50	17.33	17.50
	+	16.80	4.03	55.10	32.50	13.30	3.08	5.00	56.55	28.52	19.20
	Mean	12.60	3.39	53.20	27.50	11.82	3.24	5.10	50.53	22.93	18.35
100 % of recommended dose	-	11.20	3.54	52.50	22.50	11.50	4.30	5.60	23.25	17.10	18.40
	+	14.00	5.15	56.70	27.50	12.50	3.08	5.60	25.25	19.20	23.00
	Mean	12.60	4.35	54.60	21.25	12.00	3.69	5.60	24.25	18.15	20.70
Means of seed inoculation with biofertilizers	-	9.43	2.90	50.10	20.83	10.36	4.00	5.17	29.65	16.88	17.02
	+	14.95	4.03	52.77	26.67	12.10	3.19	5.47	35.35	21.38	19.46
L.S.D (0.05) for:	Mineral fertilizers (A)	NS	0.449	0.444	0.471	0.162	0.115	0.268	0.453	0.034	1.005
	Biofertilizers (B)	2.510	1.035	0.414	1.242	0.178	0.447	0.048	0.502	0.071	1.010
	Interaction (A x B)	3.074	1.276	0.507	1.521	0.218	0.548	0.059	0.615	0.087	1.237

\- = *Seeds were not inoculated with biofertilizers.*
\+ = *Seeds were inoculated with biofertilizers just before sowing.*

Tropane alkaloids content

Data given in Table (5) clearly show that increasing level of the used mineral fertilizers from nitrogen and phosphorus induced a significant increase in tropane alkaloids content in leaves of Thornapple plant and the rate of promotion increased steadily as the rate of mineral fertilizers increased up to 100% of the recommended dose. Worthy to mention that raising the level of mineral fertilizers from 25 to 100% of the recommended dose induced significant increase of 40.3% in hyoscyamine (the main tropane alkaloid), 37.1% in hyoscine (the second one) and 39.7% in total alkaloids. In this respect, Maheshwari *et al*. (1989) stated that total alkaloids content increased in herbage of *hyoscyamus albus* with increasing levels from nitrogen and phosphorus up to 80 kg/ha for each of the used mineral fertilizers. Likewise, Shetty *et al*. (1990) studied the effect of NPK on herbage yield and alkaloidal content in *Datura stramonium* L. and found that plants treated with 90+30+50 kg/ha NPK exhibited the greatest alkaloidal content in leaves (0.8%). Also, Kewala and Bisht (1996) found that hyoscyamine content in leaves of different *Hyoscyamus* species increased with increasing rates of N (30, 60 and 90 kg N/ha). All, being in agreement with the present findings. On the other hand, Ruminska and Gamal (1978) observed that total alkaloids content in leaves of *Datura innoxia* was not affected by N treatments or by the percentage of total N in the plant, being in contradication with the present findings.

Regarding the effect of biofertilizers, data in Table (4) reveal that leaves of Thornapple plants obtained from inoculated seeds with a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria had more alkaloidal content than those obtained from uninoculated seeds. The increase in tropane alkaloids content due to biofertilizers treatment was 16.3% for hyoscyamine, 15.9% for hyoscine and 16.3% for total alkaloids. In this respect, Hassan (2012) on periwinkle (*Catharanthus roseus* L.) plants found a positive effect of biofertilization on enhancing Alkaloids percentage .

The interaction between the used levels of mineral fertilizers and biofertilizers proved a significant effect. It is evident that increasing level of the used mineral fertilizers from NP or using a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria induced significant increase in tropane alkaloids in leaves of Thornapple plant. The maximum alkaloidal content (17.86 mg/g D.W. of leaves for hyoscyamine, 4.33 mg/g D.W. of leaves for hyoscine and 22.19 mg/g D.W. of leaves for total alkaloids) was detected at the treatment of 100% mineral fertilizers combined with biofertilizers. The previous report of Maheshwari *et al.* (1988) indicated that the highest herbage and total alkaloids yields were obtained from plants of *Hyoscyamus niger* receiving *Azotobacter chroococcum* as biofertilizer + 40 kg N/ha and stated that the use of *Azotobacter* reduced the N requirement of *Hyoscyamus niger*, being generally in accordance with the present findings. Hassan *et al.* (2012) found that inoculating *Lupinus termis* L. plants with Rh + Bm + 50% mineral fertilizer followed by Rh + 50% registered the increased the Alkaloids percentage by 72.23 & 64.17 and by 69.70 & 61.21 % over than those obtained by control in the two successive seasons; respectively.

Table 5: Tropane alkaloids (mg/g dry weight) in leaves of *Datura stramonium* L. (Thornapple) plant, at the age of 3 months, as affected by different levels of mineral fertilizers from nitrogen and phosphorus (NP) alone or combined with a mixture of biofertilizers containing nitrogen fixers and phosphate dissolving bacteria in the second season of 2015 (Means of 3 independent samples, each represents one replicate)

Treatments		Tropane alkaloids (mg/g D. W.)		
Mineral fertilizers (NP)	Biofertilizers	Hyoscyamine	Hyoscine	Total
25% of recommended dose	-	11.21	2.75	13.96
	+	13.09	3.23	16.32
	Mean	12.15	2.99	15.14
50 % of recommended dose	-	13.16	3.21	16.37
	+	16.28	3.85	20.13
	Mean	14.72	3.53	18.25
100 % of recommended dose	-	16.24	3.87	20.11
	+	17.86	4.33	22.19
	Mean	17.05	4.10	21.15
Means of seed inoculation with biofertilizers	-	13.54	3.28	16.81
	+	15.74	3.80	19.55
L.S.D. (0.05) for:				
Mineral fertilizers (A)		1.072	0.309	1.348
Biofertilizers (B)		0.860	0.237	1.051
Interaction (A x B)		1.431	0.395	1.763

- = *Seeds were not inoculated with biofertilizers.*
+ = *Seeds were inoculated with biofertilizers just before sowing.*

Leaf anatomy

From the above mentioned results of morphological characters of vegetative growth of Thornapple plant as affected by mineral and biofertilizers, it could be stated that the treatment of 100% of the recommended dose of the used mineral fertilizers (control treatment) did not statistically differ from that of 50% of the recommended dose of the used mineral fertilizers plus biofertilizers in their effects. Therefore, the anatomical structure of leaves of such treatments was under consideration.

Microscopical measurements of certain histological characters in transverse sections through the blade of the first leaf developed on the first terminal branch of Thornapple plant as affected by mineral and biofertilizers are presented in Table (6). Also, microphotographs illustrating the effects of these treatments are shown in Figures (2 and 3).

It is obvious from Table (6) and Figures (2 and 3) that Thornapple plants received 50% of the recommended dose of mineral fertilizers from nitrogen and phosphorus (NP) showed a prominent reduction in thickness of both midvein and lamina of the examined leaf by 19.99 and 34.46% less than the control (plants received 100% of the recommended dose of the used mineral fertilizers); respectively. The thinner leaves induced by median level of the used mineral fertilizers could be attributed to the decrease obtained in thickness of palisade and spongy tissue as well as in dimensions of midvein bundle. The decrements below the control were 32.44, 43.14, 21.62 and 21.89% for thickness of palisade tissue, thickness of spongy tissue, length of midvein bundle and width of midvein bundle; respectively. Also, mean diameter of vessel was decreased by 8.94% less than vessel diameter in leaves of control plants.

Data also indicate that Thornapple plants obtained from biofertilized seeds with a mixture of nitrogen fixers and phosphate dissolving bacteria and received half of the recommended dose of mineral fertilizers from nitrogen and phosphorus (NP) did not, generally, show prominent changes in leaf structure compared to the control although a slight decrease in midvein thickness by 3.14% below the control and a slight increase of 2.72% in lamina thickness above the control were observed in this respect.

The obtained results are almost in harmony with those recorded by Hassan *et al*. (2006) about the effect of mineral and biofertilizers on the anatomical structure of moghat leaves as well as by Ramdan *et al*. (2003) on sugar beet and Boghdady *et al*. (2012) on sesame.

Table 6: Measurements in microns of certain histological characters in transverse sections through the blade of the first leaf developed on the first terminal branch of Thornapple plant (*Datura stramonium* L.), at the age of 10 weeks, as affected by mineral and biofertilizers (Means of three sections from three specimens).

Histological characters	Treatments				
	Control (100% of recommended dose of mineral fertilizers)	50% mineral fertilizers	± % to control	50% mineral fertilizers + biofertilizers treatment	± % to control
Thickness of midvein.	1581.3	1265.2	-19.99	1531.6	- 3.14
Thickness of lamina.	237.7	155.8	-34.46	244.2	+ 2.72
Thickness of palisade tissue.	115.9	78.3	-32.44	119.2	+ 2.85
Thickness of spongy tissue.	96.2	54.7	-43.14	97.5	+ 1.35
Dimensions of midvein bundle:					
Length	308.1	241.5	-21.62	309.4	+ 0.42
Width	639.9	499.8	-21.89	642.5	+ 0.41
Mean diameter of vessel.	24.6	22.4	-8.94	25.7	+ 4.47

Conclusion

It can be concluded that the biofertilizer with a mixture of nitrogen fixers (namely, *Azotobacter* sp. and *Azospirillum* sp.) and phosphate dissolving bacteria (namely, *Bacillus* sp.) in combination with mineral fertilizers not only gave the best vegetative and reproductive growth, mineral status, yield quality and leaf anatomy, but also gave the highest tropane alkaloids of such important medicinal plant species *i.e.* Thornapple under Egyptian conditions. Worthy to mention that the combination of 50% NP mineral fertilizer and biofertilzer gave the best results than that of or at least equal to the 100% NP mineral fertilizer alone. Application of such level of mineral fertilizer is more safety and economic, and can minimize the environmental pollution caused by repeated application of mineral fertilizers.

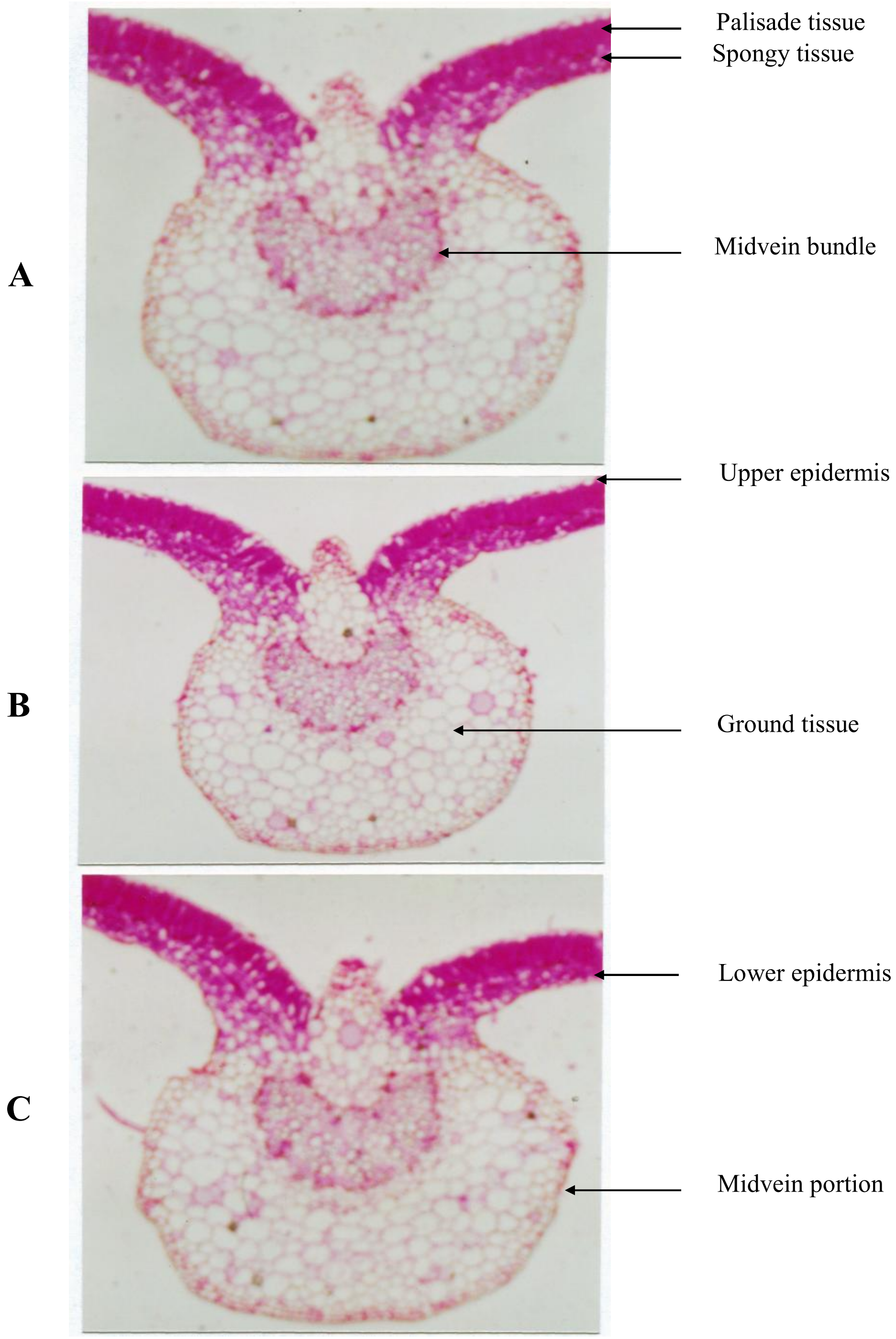

Fig. 2 : Transverse sections through the midvein portion of the leaf blade developed on the first terminal branch of Thornapple plant (*Datura stramonium* L.), at the age of ten weeks, as affected by mineral and bio-fertilizers. (X 52).

A- Control (100% form the recommended dose of mineral fertilizers).
B- 50% form the recommended dose of mineral fertilizers.
C- 50% form the recommended dose of mineral fertilizers + bio-fertilizers treatment.

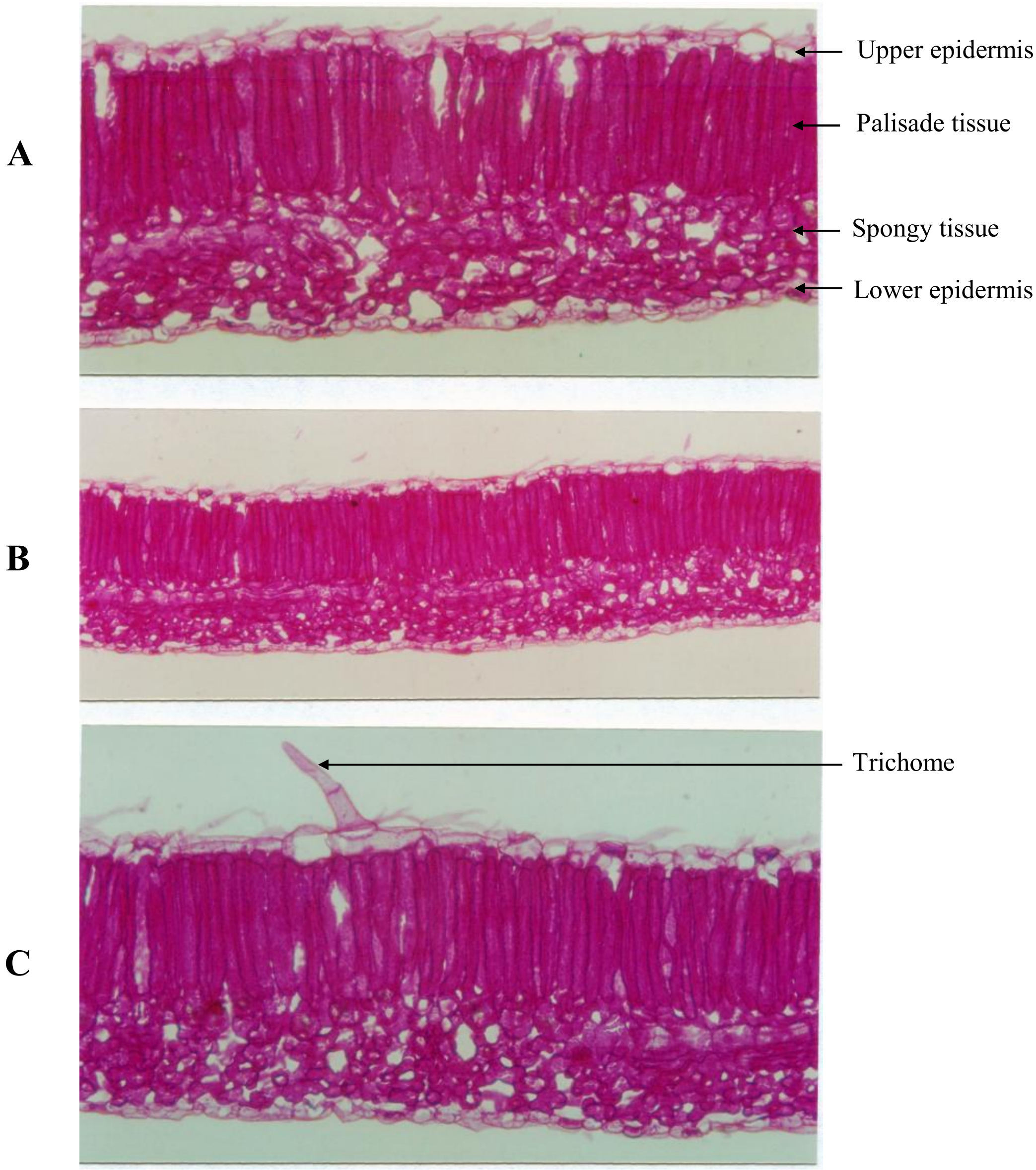

Fig. 3: Transverse sections through the marginal portion of the leaf blade developed on the first terminal branch of Thornapple plant (*Datura stramonium* L.), at the age of ten weeks, as affected by mineral and bio-fertilizers. (X 210**).**

A- Control (100% form the recommended dose of mineral fertilizers).
B- 50% form the recommended dose of mineral fertilizers.
C- 50% form the recommended dose of mineral fertilizers + bio-fertilizers treatment.

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