Plant Production (Group Lotus)

Effect of Plant Density and Harvest Time on Cotton Seed Quality. Field Studies on Acid-delinted Cotton Seed

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Introduction

Acid-delinted cotton seed is the highest improved seed quality approaches introduced in Egypt. In 1998, an experiment was carried out on the cultivar Giza 87 to investigate the effect of acid-delinted seed on the plant population and their influence on the productivity of cotton at Sakha, Kafr El-Sheikh, Egypt. The results discovered that the plant population could be reduced to 30-40 thousand plants per fed. without negative effects on yield from unit area in comparison to the traditional growing pattern, that recommend 60-70 $\times$ 10^3 plants per fed. Also, the yield of the first harvest was improved (Abdel-Hafezand Homeyer;1999). Moreover, further studies were carried out on the cultivar Giza 89 in another location (Abdel-Hafez;2000). The results showed that, there were not significant differences between 64,000 plants/fed and 40,000 plants/fed. So, growing 40,000 plants/fed is shown to be enough for getting the best yield/fed. This was achieved by 75 cm ridging distance and 25 cm among hills. This facilitates the culture practicing, save seed and reduce shading to encourage generative growth (fruiting) and reduce flower and capsule shedding. Also, it provides the best land use and input use efficiencies. Consequently increases seed cotton yield.
Such results are not strange, thus the breeding processes and plant improvement resulted in modern cultivars with different branching patterns and earliness. In other words the present varieties are different in one or another way from the old ones and need more suitable culture practices, e.g. in terms of planting patterns.
On the other hand, germination of cotton seed in the field and emergence of seedlings is very important factor, for encouraging the cotton growers. High quality certified cotton seed is the most important factor to reach excellent germination as well as seedling emergence, especially in the early sowing during March, under the Egyptian conditions. The quality of seed depends on several factors, e.g. seed maturity, seed purity, seed health and seed viability. Seed maturity and storage have effects on other important criterion, i.e. free fatty acid (FFA) content in its oil. The presence of high ratios of FFA in the oil of seeds adversely affects or inhibit the metabolism and germination of seed. Also, immature seeds have a much higher FFA content and the presence of immature seeds within the mature seeds could raise the FFA content.
Therefore, the present investigation was planed to study the effect of plant population and harvest time on seed quality. The obtained information will provide information to improve the quality of cottonseed in seed production process.

Material and Methods

An experiment was carried out in 1999 season at El-Magd village, Rahmania, El-Behira Governorate. The soil structure is mostly silt and the soil is highly fertile. The preceding crop was Berseem (two cuts) as early winter crop. The soil was operated and P_2O_5 fertilizer in form of monosuperphosphate 15.5% at the rate of 60 kg P_2O_5/fed incorporated in the soil before ridging and dividing. Also 50 kg Kalium sulfate (24 units and 18 units sulfur) was added. Three different ridge widths were used; 65 cm, 75 cm and 90 cm widths. Cotton was planted on one ridge-side in hills 20, 25 and 90 cm apart.

Planting date: April 1999:
Planting was done with special handy planter prepared for this experiment that allows only unique depth of 3 cm for the seeds.

The seeding rate:
3 different seed-numbers per hill were put. First, the control plots on ridges 65 cm wide had to ensure presence of 64×10^3 plants per fed and therefore, four seeds were put per hill and the seedlings were thinned to leave two plants per each hill. Second, the hills were spaced 25 cm on the 75cm wide ridges and spaced 30 cm on the 90 cm wide ridges. The number of seeds in each hill in the last two planting patterns; 75×25 cm and 90×30 cm; differed, from the control (65×20 cm). Herein, only three seeds were planted in each hill and not more than two plants were left per hill after emergence. Theoretically, the number of plants per feddan within the three planting patterns was 64×10^5 (60×20 cm $\times$ 2 plants), 40×10^3 (75×25 cm $\times$ 1-2 plants) and 30×10^3 (90×3 cm $\times$ 1-2 plants). The actual number of plants was almost 10% less than the former ones. The plants received the same normal growing culture practices.

Experimental design:
The treatments were arranged in a randomized complete block design (RCBD) with four replication. The plot size was $7 \times 9 m^2$ area in which the plot included the following number of ridges:
14 ridges for the planting pattern (65×20 cm),
12 ridges for the planting pattern (75×25 cm), and
10 ridges for the planting pattern (90×30 cm).

The two outer rows were left as guard rows and
12 central ridges were used for evaluation of the (65×20 cm) pattern,
3 replications were used for the determination of all the seed quality studies.

The following data were recorded on the seeds:

1- **Seed index first harvest**: Weight of 100 seeds in first harvest.
2- **Seed index second harvest:** Weight of 100 seeds in second harvest.
3- **Estimation of the mature/immature seed %:** An amount of 10 kg seeds from each population and harvest time were used to estimate the percentage of mature/immature seeds. The seeds were exposed to a seed blower. The airflow rate was adjusted to remove almost all the immature seeds and insect damaged seeds. However, some light mature seeds were also removed. The mature seeds were weighed

$$\text{Mature seed \%} = \frac{\text{Weight of mature seed g}}{\text{Weight of raw seed g}} \times 100$$

$$\text{Immature seed \%} = \frac{\text{Weight of immature seed g}}{\text{Weight of raw seed g}} \times 100$$

4- **Determination of free fatty acids (FAA) %:** The percent FFA was determined in the mature seeds after removing of the immature and insect damaged seeds obtained from the three plant populations and the two harvest times. The procedure of determination was carried out according to the quick method of the Association of official oil chemists as described by Dr. Bernhard Homeyer in the "Egyptian-German Acid Delinting for Cotton Seed Project". Bulletin. Three samples were taken from each oil stock to determine the FFA percent.

5- **Germination %:** Germination test of mature seeds was carried out in lab under controlled conditions. The germination test was carried out in sandy soil and in eight replications at 30°C. The seedlings were counted after five days were classified in normal seedlings and abnormal ones. Also, the not germinating seeds were assorted in dead/disease-infected seeds and not germinating seeds. Finally, the following traits were calculated.

$$\text{Germination \%} = \frac{\text{Mean No. of normal seedlings}}{\text{No. of sown seeds}} \times 100$$

$$\text{Abnormal seedling \%} = \frac{\text{Mean No. of abnormal seedlings}}{\text{No. of sown seeds}} \times 100$$

$$\text{Dead seed (infected)\%} = \frac{\text{Mean No. of dead seeds}}{\text{No. of sown seeds}} \times 100$$

$$\text{Not germinating seeds \%} = \frac{\text{Mean No. of not germinating seeds}}{\text{No. of sown seeds}} \times 100$$

Finally, the data were computed and the means were compared according to Duncan's Multiple Range Test. In some cases the data were transformed before subjecting it to statistical analyses.

Results

1- Seed Index:

The seed index data (Table1) showed that the plant populations used herein did not effect the seed index strongly. But, this trait was strongly affected by the harvest time. The seeds produced from the first harvest were higher in weight than that of the second harvest. This means, that second harvest produce low quality seeds, that are not suitable for cultivation. The differences in seed index between the two harvests were highly significant.

Table (1): Mean seed index (g) of Giza 89 cotton planted with acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H)		Means (p)	Difference
		1st	2nd		
65/20 (control)	2	9.058 a	11.265 a	10.162 a	-2.208**
75/25	1-2	8.912 a	11.308 a	10.110 a	-2.396**
90/30	1-2	9.187 a	11.261 a	10.224 a	-2.074**
Mean		**9.052**	**11.278**	**10.165**	**-2.226****

** = Significant at 1% level.
ns = not significant.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Comparison	S.E.D.	LSD (5%)	LSD (1%)
2-H*P means	0.258	0.550	0.761
2-p means	0.149	0.318	0.439

2- Lint percentage:

The lint percentage of the first harvest was highly significantly lower than that of the second harvest (Table 2). This is mainly caused due to the high seed index in the first harvest compared to that of the second one. However, these differences were only significant in both 65/20 cm (the control treatment) and 75/25 cm plant population patterns. But, the difference was highly significant in the 90/30 cm plant population pattern that produced high seed index besides its higher yields than the control in the first harvest.

Table (2): Mean lint percentage of Giza 89 cotton planted by acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H)		Means (p)	Difference
		1st	2nd		
65/20 (control)	2	0.362 a	0.396 a	0.379 a	-0.034 *
75/25	1-2	0.370 a	0.404 a	0.387 a	-0.034 *
90/30	1-2	0.358 a	0.399 a	0.378 a	-0.041 **
Mean		**0.363**	**0.400**	**0.381**	**-0.036 ****

** = Significant at 1% level.
ns = not significant.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Comparison	S.E.D.	LSD (5%)	LSD (1%)
2-H*P means	0.013	0.029	0.039
2-H means	0.008	0.016	0.023

3- Mature seed %:

The mean percentage of mature seeds (Table 3) was less in the second harvest by 20.88% than that of the first harvest. Such reduction reached 25.06, 16.44 and 21.35% at the 65/20, 75/25 and 90/30 cm^2 plant population patterns, respectively. Also, the mature seed percentage was influenced with the plant population density/pattern x harvest time interaction. Thus the high population of 64,000 plant/fed produced the least percentage mature seeds in the second harvest. On the other hand, the 75/25 cm^2 planting pattern/population produced the highest mature seed percentage at each of first and second harvest compared with the other populations and their correspondent harvest time.

Table (3): Mature seed percent as affected by the interaction of harvest time × plant population: means for mature seed % of Giza 89 cotton planted by acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H)		Means (p)	Difference
		1st	2nd		
65/20 (control)	2	77.917 c	58.394 c	68.155	19.524**
75/25	1-2	81.412 a	68.030 a	74.721	13.383**
90/30	1-2	80.357 b	63.204 b	71.781	17.153**
Mean		**79.896**	**63.209**	**71.552**	**16.686**

** = Significant at 1% level.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

4- Immature seed %:

Opposite to the mature seed %, the immature seed percentage (Table 4) was higher by 80.67 in the seeds derived from the second harvest than that obtained in the first harvest. In respect to the plant population, different relative values of mature seeds and immature seeds were produced in the first and second harvests. The highest plant population (64,400 plants/ fed) produced the highest relative immature seeds compared to the 40,000 and 30,000 plants/fed. The lightest plant population of 30,000 plants/fed produced more immature than the intermediate plant population of 40,000 plants/fed.

Table (4): Immature seed percent as affected by the interaction of harvest time × plant population: means for mature seed % of Giza 89 cotton planted by acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H)		Means (p)	Difference
		1st	2nd		
65/20 (control)	2	22.562 a	40.963 a	31.762	-18.402**
75/25	1-2	18.555 c	31.533 c	25.044	-12.977**
90/30	1-2	19.396 b	36.830 b	28.113	-17.434**
Mean		**20.171**	**36.442**	**28.306**	**-16.271**

** = Significant at 1% level.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

5- Free fatty acids%:

The percentage of free fatty acids in cotton seed oil of Egyptian cotton *Gossypium barbadense* seemed to be higher than that in the upland cotton *Gossypium hirsutum*. It was ranged from 2.98 to 4.88% in our experiment Table 5. The percentage of free fatty acids differed in cottonseed oil, depending on the source of the seeds. It was higher in seeds of second harvest (second pick) than that derived from the first harvest. The difference was very high and reached 76.35% increase free fatty acids in the second harvest seed. Also, such increase was existent irrespective of the plant population density ("Pflanzendichte") in field; 70.54% at 65 × 20 cm^2, 103.82% at 75 × 25cm^2 and 35.55 at 90 × 30 cm^2.

Table (5): Free fatty acid % in cotton seed oil as affected by interaction between harvest time and plant population: means for mature seed % of Giza 89 cotton planted by acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H) 1st	Harvest (H) 2nd	Means (p)	Difference
65/20 (control)	2	2.787 b	4.753 a	3.770 b	-1.966**
75/25	1-2	2.254 b	4.599 a	3.426 b	-2.345**
90/30	1-2	3.907 a	5.296 a	4.602 a	-1.389**
Mean		**2.983**	**4.883**	**3.933**	**-1.900****

** = Significant at 1% level.
In a column, means followed by a common letter are not significantly different at the 5% level by DMR

6- Germination %:

When the mature seeds produced from the first harvest were subjected to germination test, in comparison to the mature seeds produced from the second harvest, the data in table 6 showed highly significant differences among the means of the two seeds. Seeds from the first harvest which represent two thirds of the total seed yield, as presented in yield data, showed highly significantly higher germination percentage than that of the second harvest. The difference was 4.332% at 64,000 plants/fed 0.338% at 40,000 plants/fed and 13.705% at 30,000 plants/fed.

Table (6): Germination % as affected by interaction of harvest time x Plant population: means for mature seed % of Giza 89 cotton planted by acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H) 1st	Harvest (H) 2nd	Means (p)	Difference
65/20 (control)	2	83.704 ab	79.373 a	81.538	4.332**
75/25	1-2	82.007 b	81.669 a	81.838	0.338 ns
90/30	1-2	85.373 a	71.668 b	78.520	13.705**
Mean		**83.695**	**77.570**	**80.632**	**6.135**

** = significant at 1% level. ns = not significant.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

7- Number of abnormal seedlings % :

There was no significant difference. In other words, there were no significant effects for plant population or harvest time on the number of abnormal seedlings in germination tests (Table 7).

Table (7): Abnormal seedlings % as affected by interaction of harvest time × plant population: means for mature seed % of Giza 89 cotton planted by acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H) 1st	Harvest (H) 2nd	Means (p)	Difference
65/20 (control)	2	5.9 a	4.2 b	5.0 a	1.7 ns
75/25	1-2	7.0 a	5.7 ab	6.3 a	1.3 ns
90/30	1-2	5.3 a	7.7 a	6.5 a	-2.3 ns
Mean		**6.1**	**5.8**	**5.9**	**0.2 ns**

Ns = not significant.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

8- Dead seeds % and No. of non-germinating seeds:

Dead seeds exhibited disease infection instead of germinating. The mean number of dead seeds was not significantly effected by plant population. However, it was shown significantly influenced (increased) in seeds derived from the second harvest compared to the first one. Also, it was influenced by the interaction between plant population density and harvest time. Yet, the first harvest at 64,000 and 40,000 plants population densities obtained relatively significant higher relative dead seeds than the second harvest, but the opposite was obtained at the 30,000 plant population density. Moreover, the second harvest seeds showed highly significantly higher relative dead seeds than that of the first harvest (Table 8).

Table (8): Dead seeds % as affected by interaction of harvest time × plant population: means for mature seed % of Giza 89 cotton planted by acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H)		Means (p)	Difference
		1st	2nd		
65/20 (control)	2	4.655 ab	6.610 ab	5.632	-1.955 ns
75/25	1-2	6.667 a	4.580 b	5.624	2.086 ns
90/30	1-2	2.645 b	8.292 a	5.468	-5.647**
Mean		**4.655**	**6.494**	**5.575**	**-1.839**

** = significant at 1% level. Ns = not significant.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Also, data in Table 9 show that the relative number of non-germinating seeds was significantly or highly significantly higher for seeds derived from second harvest that derived from first harvest.

Table (9): Non-germinating seeds % as affected by interaction of harvest time × plant population: means for mature seed % of Giza 89 cotton planted by acid-delinted seed in 1999.

Planting pattern cm	Plants/hill	Harvest (H)		Means (p)	Difference
		1st	2nd		
65/20 (control)	2	4.624 a	7.327 a	5.975 a	-2.703*
75/25	1-2	4.250 a	7.584 a	5.917 a	-3.334*
90/30	1-2	2.645 a	8.625 a	5.635 a	-5.980**
Mean		**3.839**	**7.845**	5.842	**-4.005****

** = significant at 1% level. * = significant at 5% level.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Discussion

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In comparison between seeds produced from the first and the second pick ("Pflücken"), seed index of seeds from the first pick was higher than that derived from the second pick. This means that seeds of the first pick are vigorous than these of the second one. Such difference was increased as the plant population was reduced. Consequently, the seeds from the first pick especially, that produced under lower plant population should be preferred for planting.

The percent of mature seed was strongly higher in seeds of first pick compared to the second one. This findings, demonstrates the importance of reserving the seeds of the

first pick for seed production as they are the better quality seeds. Also, this idea can save a lot of time and costs in seed processing to separate the immature seeds from the raw seeds. On the other side, the second pick produces higher percentages of immature seeds than the first pick, and needs more time and costs in seed processing, transportation, packages, handling, storage etc., and lastly produce less amount of mature seeds for certification. Furthermore, seeds of the second pick proved to contain higher amounts of free fatty acids in their oil, compared to the seeds derived from the first pick.

The seeds of the second pick contained 76.35% more FFA in cotton seed oil, than that oil from the seed derived from the first pick. The presence of such sizeable increase in FFA is very effective in deterioration of the viability of the seed obtained from the second pick.

Therefore, it is wise to use seeds derived from the first pick alone to be processed and certified to grow cotton. This findings is strongly supported by the fact that our results clearly demonstrated that the germination percent of seeds from the first pick was highly significantly higher than that of seeds derived from the second pick. Moreover, the last seeds derived from the second pick contained more dead and disease infected seeds as well as non-germinating seeds than the that from the first pick. Finally, it will be possible to cultivate the unit area *feddan* with *less than twenty kg* of the acid-delinted seed of first pick.

Conclusion

Seeds produced from the first harvest have less content of free fatty acids and can have longer viability life in storage.

Seeds produced from first harvest have higher seed index, better germination ratios and are preferred to produce better quality seed with less total costs.

On the other hand, seeds from second harvest have less germination percentage, contain higher free fatty acids and could hardly keep good viability during storage. Therefore, it should be avoided as source of seed.

Seeds derived from the first pick are representing up to 2/3 of the seed yield per unit area and would be enough to produce high quality seed. Some financial promotion, for seed producing cotton growers could encourage them pick the fast yield at proper time.

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Response of Datura Innoxia Mill Plants to Jasmonic Acid Application

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Abstract

Two experiments were carried out during the seasons of 1994/1995 and 1995/1996 to study the effect of Jasmonic acid* at three concentrations; i.e., 200, 400 and 800 ppm, besides the distilled water as a control on the growth, chlorophyll content, the levels of endogenous hormones and alkaloid contents as well as yield of Datura innoxia Mill plants. The obtained results indicated that JA application at different concentrations significantly decreased plant heights and increased the number of both leaves and branches as well as dry weights of the different organs of Datura plants. Plants treated with JA showed a decrease in transpiration rate and total Chl a + b, with an increment of the JA concentration.

Results also showed that alkaloid content of the seeds was higher than that the different organs of Datura innoxia. Seeds yield and alkaloids as well as oil content in the seeds were significantly increased by JA treatments. The effect was more pronounced by JA at 800 ppm during the two seasons. On the other hand, JA treatments decreased growth promoters and increased growth inhibitors with raising of JA concentration.

Introduction

Datura innoxia Mill is one of the most important solanaceous plants, which is considered as a main source for producing tropane alkaloids needed for the pharmaceutical industries. In the last few years, great attention was drawn to improve the yield and its quality of Datura. Application of wide variety of both naturally occurring and synthetic chemical growth regulators have been extensively used in order to ascertain their beneficial effects upon the growth and development of plants. A number of synthetic growth retardants have been discovered and proved to be of considerable importance in agriculture (Yamane *et al.*, 1990; Yamane *et al.*, 1981; Sembdner and Klose, 1985; Vick and Zimmermann, 1986).

Jasmonic acid (JA) and its methyl ester (JA-Me) are endogenous physiologically active compounds with a phytohormone-like action and they are widely distributed in higher plants (Ueda *et al.*, 1981; Sembdner and Klose, 1985 and Parthier, 1990). They cause growth inhibition (Miersch *et al.*, 1986 and Popova *et al.*, 1988).

According to these contradictory opinions, we have carried out this investigation in order to determine the optimum JA levels, which may realize an increment in the obtained drug and alkaloid yield from Datura innoxia Mill plants, under our experimental and environmental conditions.

Materials and Methods

Two field experiments were carried out at the Experimental Farm of the Faculty of Agriculture at Shibin El-Kom, Minufiya University during two successive seasons of 1994/1995 and 1995/1996 for studying the effect of Jasmonic acid (JA) on the growth, phytosynthetic pigments, photohormone concentrations drug yield, and alkaloids content of the different plant organs of Datura innoxia Mill plants.

The seeds from the local plants were planted in seed pans at the 1st of October 1994 and 1995 in the nursery. The seedlings of 2-3 pair of leaves were transplanted at the mid of December in the seasons of 1994/1995 and 1995/1996 in plots of 2 x 4 m at 40 cms. apart between the plants. During the soil preparation, chemical fertilizers as calcium superphosphate (15% P_2O_5) and potassium sulphate (50% K_2O) were added to the soil at rates of 130 and 85 Kg/fed. respectively. Concerning, the nitrogen fertilizer, the plants received urea (46% N) at rate of 85 Kg/fed. in three equal side dressings on 10^{th} of January, February and March in the two seasons. All other cultural practices were performed as usual. The study was conducted in a randomized complete block design with three replicates.

Jasmonic acid (JA) was applied as a foliar application at the rates of 200, 400 and 800 ppm. Distilled water was used as a control plants. Plants were sprayed twice, at 35 days from transplanting and 7 days later by means of a hand atomizer until run-off. Tween 20 was used a wetting agent at a concentration of 0.5%.

At the full flowering stage, plant height, number of both leaves and branches and dry weights of leaves, stems, flowers and roots were measured. The different plant organs were dried at 70°C and grinded as a preparation of chemical analysis. The chlorophyll, and transpiration rate were taken, at the full flowering stage.

1) Chlorophyll a, Chl b and carotenoids were determined spectrophoto-metrically as described by Wettstein (1957).
2) The transpiration rate (g water/cm²/h) was determined according to Kreeb (1990).
3) The total alkaloid percentage in the dried different plant organs was determined according to the method described by Karawya *et al.* (1975).

Determination of endogenous growth hormones:

Extraction of endogenous growth hormones was carried out according to method of Shindy and Smith (1975), 30 grams fresh weight of the leaves at full flowering stage were used for the determination of auxin and their inhibitors, gibberellins and cytokinins. Plant material was extracted three times with 80% cold methanol. The combined alcohol extracts were evaporated under reduced pressure and the aqueous residue was partially purified by partition with ethyl acetate. The acidic ethyl acetate fraction was then collected and dried under vacuum at 37°C to dryness to determine auxin and their inhibitors and gibberellins whereas, the alkaline fraction was used to determine cytokinins. Separation was carried out by paper chromatography using a solvent composed of isopropanol : ammonia : water (10:1:1). Bioassay techniques were followed using wheat coleoptile straight assay (Bently and Housley, 1954) for auxins and their inhibitors, lettuce hypocotyl assay (Frankland and Wareing, 1960) for gibberellins and cucumb cotyledons assay (Fletcher *et al.*, 1982) for cytokinins. The results of phytohormones were statistically analyzed according to Tukey (1953).

At harvest, the seed yield, oil and alkaloid yield were recorded. The oil percentage in Datura seed was determined using Soxhlet continuous extraction apparatus according to A.O.A.C (1980). All data were subjected to statistical analysis of variance (Snedecor and Cochran, 1969).

Results and Discussion

1 - Growth analysis:

Data in Table (1) indicated clearly that, plant heights significantly decreased with increasing Jasmonic acid concentrations. the heighest value in this respect was obtained by 800 ppm at which plant heights were decreased by about 13% and 15% in the first and second seasons, respectively, as compared with untreated plants. It was clear from the same Table that, all levels of JA caused a significant increase in the number of both leaves and branches per plant, drug weights of different parts of Datura innoxia Mill plants (Table 2). The highest value in this respect was obtained by 800 ppm of JA. These results were true in both 1994/1995 and 1995/1996 seasons.

The dwarfing effect of Jasmonic acid may be due to the influences of JA on preventing cell elongation and/or stopping cell division, act as antigibberellin (Fig. 2). In this regard, several investigators reported that JA shortened the heights of many plants species (Dathe *et al.*, 1981; Sembdner and Gross, 1986; Parthier 1990; Gendy and Schilling, 1990; Gendy and Selim, 1994)

Table (1): Effect of Jasmonic acid on growth parameters of Datura plants during 1994/95 and 1995/96 seasons.

JA levels (ppm)	Plant height cm/plant	No. of leaves/plant	No. of branches/plant
1994/95 season			
Control	90.2	89.2	15.2
200	87.4	95.4	17.6
400	80.6	96.8	18.3
800	78.4	80.2	15.0
L.S.D 5%	5.2	18.1	4.2
1995/96 season			
Control	86.4	84.8	14.2
200	82.1	89.9	16.3
400	80.2	92.4	19.4
800	73.4	81.4	14.8
L.S.D 5%	4.8	16.4	3.4

Table (2): Effect of Jasmonic acid on the drug yield in gms/plant of Datura innoxia Mill plants during the seasons of 1994/95 and 1995/96.

JA levels (ppm)	Leaves	Stems	Flowers	Total herb	Roots
1994/85 season					
Control	80.2	45.5	9.4	135.1	15.8
200	82.4	48.2	9.8	140.4	18.2
400	86.9	50.4	10.2	147.5	19.6
800	88.1	52.8	10.3	151.2	19.8
L.S.D 5%	4.2	4.2	0.8	7.8	2.4
1995/96 season					
Control	79.2	46.2	9.2	134.6	14.2
200	80.4	49.4	9.6	139.4	15.4
400	83.2	50.8	10.0	144.0	18.4
800	84.6	53.7	10.2	148.5	19.6
L.S.D 5%	3.1	4.6	0.6	6.6	2.6

The increase in drug weights of treated plants (Table 2) might be attributed to the positive effect of JA on number of both leaves and branches and dry matter deposition. This increase also might be due to the enhancement at JA on CO2 fixation and/or the increase in the anabolic metabolism (Sembdner and Parthier, 1993).

2 - Transpiration rate:

Data recorded in Table (3) showed clearly that all levels of JA caused a significant decrease in the transpiration rate. The most effective treatments to decrease the transpiration rate was at the highest levels of JA. This decrease may be attributed to the stomata closure by JA (Satler and Thimann, 1981; Horton, 1991; Gendy and Selim, 1994). Jasmonic acid activities seem to be similar to abscisic acid on this character (Sembdner and Parthier, 1993).

3 - Photosynthetic pigments:

Data in Table (3) indicated clearly that all JA levels decreased Chl a, Chl b and total Chl (a+b) as well as carotenoids in both seasons. On the contrary, the carotenoids significantly increased with raising the JA levels. The lowest value of total Chl a+b resulted from plants sprayed at the highest levels of JA.

The decrease in photosynthetic pigments as a result of JA treatments was reported in other investigations by Parthier *et al.* (1987a), Ueda and Kato (1982), Parthier (1990), Gendy and Schilling (1990), Gendy and Selim (1994). The role of JA to inhibition the chlorophyll content was attributed to the capability of JA to reduce of chloroplast development during leaf growth and to promote the rate of leaf senescence as well as to stimulate the degradation of chlorophyll (Sembdner and Parthier, 1993). The reduction in Chl content may be due to several factors: 1) inhibition of endogenous hormonal activity, 2) suppression of rRNA incorporation into plastid nucleic acid and its synthesis, 3) inhibition of GA-dependent DNA biosynthesis which decrease protein content necessary for Chl biosynthesis, and/or 4) increasing chlorophyllase synthesis (Parthier, 1991).

4 - Phytohormones concentration:

Data presented in Table (4) exhibit a marked decrease in IAA, GA and Cytokinin concentration of treated leaves at all JA levels as compared with untreated leaves. On the contrary, the ABA concentration was unsignificantly increased. Similar findings were reported by Ueda *et al.* (1981), Ueda and Kato (1982), who found that the application of JA decreased the cytokinin concentration. In this connection, Gendy and Selim (1994) reported that the gibberellin concentration in faba bean was decreased after leaf treatment with JA.

Table (3): Effect of Jasmonic acid on chlorophyll concentration and transpiration rate in leaves of Datura plants (1994/1995 and 1995/1996 season).

JA levels (ppm)	Chlorophyll concentration (mg/g.f.wt) Chl a	Chl b	Chl a + b	Transpiration rate (mg/cm2/h)	Carotenoid
1994/1995 season					
Control	6.32	2.18	8.50	2.77	5.8
200	6.12	2.12	8.24	2.80	5.0
400	5.80	2.08	7.88	2.90	4.2
800	5.42	2.00	7.42	2.98	3.2
L.S.D 5%	0.85	0.12	0.89	0.21	1.0
1995/1996 season					
Control	5.12	2.00	7.12	2.30	4.9
200	5.00	1.90	6.90	2.45	4.6
400	4.82	1.82	6.64	2.54	3.9
800	4.34	1.68	6.02	2.62	3.2
L.S.D 5%	0.67	0.16	0.94	0.32	1.2

Table (4): Effect of JA on endogenus phytohormones concentration (ng/g/fwt) of datura leaves.

JA levels (ppm)	IAA	GA	Cytokin	ABA
1994/95 season				
Control	58.0	25.0	95.0	72.0
200	50.0	20.0	90.0	72.5
400	48.0	17.0	84.0	72.8
800	45.0	14.0	80.0	73.0
L.S.D 5%	6.2	4.5	4.8	NS
1995/96 season				
Control	59.0	28.0	96.0	74.0
200	51.0	22.0	91.0	74.6
400	49.0	19.0	83.0	74.8
800	46.0	15.0	80.0	75.0
L.S.D 5%	7.1	5.6	4.9	NS

5 - Total alkaloids:

a) alkaloid percentage:

The reported data in Table (5) show clearly that JA application on Datura innoxia Mill plants caused a slightly decrease in the alkaloid concentration in the dried leaves, stems, and roots than the untreated plants, whereas, the total alkaloid percentage in the flowers showed an increase tendency than untreated plants in both seasons.

The decrease in the alkaloid percentages as a result of JA application in the leaves, stems and roots of Datura innoxia Mill plants could be due to the dilution effect of alkaloid concentrations in previous mentioned organs as a result of the increment of drug yield. Similar results were obtained by Mostafa *et al.* (1984) on Datura plants.

b) alkaloid content:

The presented data in Table (5) indicate that JA application on Datura innoxia Mill plants at its different concentrations increased the alkaloid yield/plant in flowers than the control plants. The best results in this respect was obtained by spraying the plants with 400 and 800 ppm of JA in the two experimental seasons.

It seems from these results (Tables 3 and 5) that JA application was more effective in increasing alkaloids yield of Datura flowers as compared to untreated plants. These results are in agreement with the findings of Parthier (1991).

The beneficial effects of growth regulators on the biosynthesis of alkaloids and other secondary metabolites in many medicinal plants were also reported by Moskova-Simenova *et al.* (1987); Moftah and Attia (1992). The increase in N uptake and amino nitrogen content resulted from these growth regulators and reported by Abdou (1987) might be reasons for stimulating alkaloid biosynthesis.

6 - The yield:

As shown in Table (6) seeds yield was affected positively and significantly by Jasmonic acid application. The highest seed yield was obtained at the moderate rate of JA. The highest increase in seed yield over the control reached about 63% and 56% in the first and second seasons, respectively.

It can be also noticed from Table (6) that foliar spray of JA to Datura plants leads generally to an increase in oil and alkaloid percentages and yields in the seeds as compared with untreated plants. Seed content of alkaloid reached the maximum value when plants sprayed at 400 ppm of JA.

From the aforementioned discussion it could be concluded that JA application modify Datura plants growth. The modifications are characterized by significantly shorter plants. Moreover, the seed yield and alkaloid yield were increased.

Table (5): Effect of Jasmonic acid on the alkaloids content of the different Datura plant organs during the seasons of 1994/95 and 1995/96.

JA levels (ppm)	Leaves		Stems		Flowers		Total herb		Roots	
	%	g/plant	%	g/plant	%	g/plant	%	g/plant	%	g/plant
1994/95 season										
Control	0.86	0.69	0.56	0.25	0.89	0.08	0.79	0.59	0.40	0.06
200	0.74	0.61	0.52	0.25	0.96	0.09	0.70	0.56	0.31	0.06
400	0.72	0.63	0.45	0.23	1.20	0.12	0.62	0.57	0.24	0.05
800	0.70	0.62	0.36	0.19	1.42	0.15	0.60	0.42	0.20	0.04
L.S.D5%	-	0.06	-	0.02	-	0.02	-	0.08	-	0.02
1995/96 season										
Control	0.58	0.54	0.48	0.22	0.72	0.07	0.61	0.43	0.31	0.04
200	0.60	0.48	0.40	0.20	0.84	0.08	0.58	0.49	0.28	0.04
400	0.52	0.43	0.36	0.18	0.99	0.10	0.50	0.47	0.21	0.04
800	0.48	0.41	0.30	0.16	1.20	0.12	0.48	0.33	0.18	0.04
L.S.D5%	-	0.09	-	0.04	-	0.04	-	0.09	-	N.S.

Table (6): Effect of Jasmonic acid on seed yield, seed oil and seed alkaloids yields of Datura plants.

JA levels (ppm)	Seed yield		Seed oil		Seed alkaloid
	g/plant	%	g/plant	%	g/plant
1994/95 season					
Control	50.1	14.6	7.31	1.40	0.70
200	55.3	15.2	8.41	2.10	1.16
400	64.2	16.8	10.79	3.30	2.12
800	60.4	16.0	9.66	2.80	1.69
L.S.D 5%	2.5	1.2	1.45	1.12	0.62
1995/96 season					
Control	48.2	14.2	6.84	1.30	0.63
200	52.4	15.0	7.86	2.20	1.15
400	66.8	16.2	10.82	3.28	2.19
800	61.6	15.8	9.73	2.60	1.60
L.S.D 5%	2.9	1.4	1.66	1.20	0.75

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Response of Sweet Pepper to some Growth Regulator 1-Photosynthetic Pigments and Anatomical Structure

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Abstract

Pots experiments were carried out at the farm Kafr El-Sheikh, Faculty of Agriculture during the two successive summer seasons of 1992 and 1993. The main objective of this study was to recognize the responses of sweet pepper (*Capsicum annuum* L.) California wonder variety to foliar applications of several concentrations of some growth regulators, i.e., kinetin cycocel (ccc), ethrel and morphactin (CME) beside the control (water spray) on photosynthetic pigments and anatomical characteristics. The results are summarized as follows:

Physiological studies: All growth regulators increased concentrations of photosynthetic pigments of leaf.

Anatomical studies: These four growth regulators affected significantly structure of root, stem and leaf of sweet pepper plants.

Introduction

Family Solanaceae is one of the most important economic families which includes about 85 genera and more than 2200 species, many of its plants are grown as vegetable crops such as tomato, pepper, potatoes, eggplant etc. One of the most important genera is Capsicum of which the under investigation in one of its edible species.

California Wonder (*Capsicum annuum* L.) a nonpungent cultivar, is widely produced in Egypt during summer season for local market as it needs a relatively long growing season of high temperature.

Consumed fresh pepper in one of the best means for supplementing ascorbic acid (Vitamin C), whereas it is considered one of the higher nutritional value crops, particularly in vitamins. It is a favourite vegetable throughout the year and it is used as fresh, cooking, pickle food and may be stuffed with rice and minced meat cooked.

During the past 10 years, the use of plant growth regulators has increased more than other agricultural chemical groups (Davis and Curry, 1991). Growth regulators are used on a wide variety of ornamental crops to improve rooting, increase shoot formation, shorten internodes, and induce flowering. The effect of some of theses compounds on pests has been investigated on many crops, (Osbrne and Chase, 1990). The application of the plant growth regulators might improve the growth of plants and fruit set leading to increasing fruit yield and fruit quality. Therefore, certain experiments were carried out to study the effect of some growth regulators, i.e., kinetin, cycocel (CCC), ethrel and morphactin (CME) on morphological, physiological and anatomical characteristics as well as yield and its components besides fruit quality of sweet pepper (*Capsicum annuum* L.) California Wonder variety.

Materials and Methods

The present experiments were carried out during the late summer of 1992 and 1993 seasons in clay pots 30 cm inner diameter in the glasshouse of the Department of Agricultural Botany, Faculty of Agriculture, Kafr El-Sheikh Tanta University. The aim of this work was to study the effects of 4 growth regulators with different concentrations on morphological, physiological and anatomical characteristics of sweet pepper *Capsicum annuum* L., var California wonder.

Photosynthetic pigments:

Leaf pigments, i.e. chlorophyll a, b and cartenoids of the fifth leaf from the shoot tip were determined at 30 and 60 days after transplanting date i.e., 15 and 45 days after application of the plant growth regulators (Wettstein, 1957).

Anatomical studies:

The effect of growth regulators on the anatomical structure of leaves (lamina), stems and roots was studied after 15 days after application. Specimens 1 cm long were taken from the fifth internode and the fifth leaf including the midrib. Concerning the roots 1 cm samples from the subapical part of secondary root tip were cut. Sections were stained then cleared in xylol and mounted in Canada balsam and prepared for microscopic examination (Ghamrawi and Zaher, 1953).

Results and Discussion

Photosynthetic pigments:

Data presented in Tables (1 and 2) show that kinetin at all levels significantly increased photosynthetic pigments concentrations (Chl. a, Chl. b, Chl. a + b and carotenoids) at two sampling dates (15 and 45 days after application) in both seasons. The increase in carotenoids was not significant at 25 and 50 mg/L at the second sampling date in the first season. In fact, the increase in photosynthetic pigments occurred due to a corresponding increase in Lamina thickness. In addition, cytokinin increase a number of chloroplast in young leaves by increasing both intensity of cell growth phytohormones and the activity of cytoplasm ribosomes, thus chlorophyll synthesis is stimulated (Borzenkova and Mokronosov, 1976).

Table (1): Effect of some growth regulators on photosynthetic pigments concentrations of sweet pepper leaves (*Capsicum annuum* L. var. California wonder) during 1992 season.

Growth regulators (mg/L)		Pigments concentrations (mg/dm^2)							
		15 days after application				45 days after application			
		Chl. a	Chl. b	Ch. (a+b)	Carote-noids	Chl. a	Chl. b	Ch. (a+b)	Carote-noids
Control		1.20	0.89	2.09	0.81	1.88	0.80	2.68	1.59
Kinetin	25	1.95	1.49	3.44	1.40	3.32	2.20	5.52	1.84
	50	2.00	1.79	3.79	1.60	3.50	2.42	5.82	1.67
	100	2.66	2.36	5.02	1.90	3.80	2.07	5.87	2.00
CCC	500	1.50	1.64	3.14	1.20	2.23	1.13	3.46	1.85
	1000	1.69	1.31	3.00	1.44	2.33	1.30	3.63	1.67
	2000	1.93	1.31	3.24	1.44	2.53	1.30	3.83	1.84
Ethrel	100	2.06	1.79	3.86	1.25	3.18	2.09	5.30	2.10
	200	1.74	1.30	3.04	1.26	2.69	1.84	4.52	2.05
	400	1.85	1.46	3.31	1.20	3.00	2.30	5.27	1.95
CME	2.5	3.15	2.89	6.04	2.47	3.13	2.28	5.41	2.24
	5	2.18	1.42	3.60	1.71	3.04	2.23	5.27	2.13
	10	2.39	2.05	4.43	1.99	2.75	2.06	4.81	2.32
L.S.D.	5%	0.20	0.39	0.51	0.38	0.37	0.31	0.65	0.32

Table (2): Effect of some growth regulators on photosynthetic pigments concentrations of sweet pepper leaves (*Capsicum annuum* L. var. California wonder) during 1993 season.

Growth regulators (mg/L)		Pigments concentrations (mg/dm^2)							
		15 days after application				45 days after application			
		Chl. a	Chl. b	Ch. (a+b)	Carote-noids	Chl. a	Chl. b	Ch. (a+b)	Carotenoids
Control		1.26	0.80	2.12	1.05	1.79	0.90	2.69	1.45
Kinetin	25	2.10	1.64	3.37	1.59	2.70	1.88	4.58	2.01
	50	2.11	1.31	3.42	1.73	2.70	1.97	4.67	1.94
	100	2.22	1.99	4.21	2.02	2.78	2.08	4.86	1.85
CCC	500	1.80	1.23	3.03	1.60	2.95	1.89	4.84	1.76
	1000	1.81	1.23	3.04	1.62	2.08	1.87	3.95	1.76
	2000	1.81	1.55	3.36	1.60	2.21	1.91	4.12	1.80
Ethrel	100	1.92	1.50	3.42	1.73	3.15	2.44	5.59	2.00
	200	1.90	1.23	3.13	1.88	2.40	1.81	4.21	2.13
	400	1.90	1.55	3.45	1.59	2.70	1.89	4.59	1.91
CME	2.5	3.15	2.38	5.53	2.71	3.70	2.11	5.81	2.21
	5	2.59	2.29	4.88	1.36	3.32	2.35	5.67	2.19
	10	2.23	2.01	4.24	2.02	3.23	2.11	5.34	2.13
L.S.D.	5%	0.34	0.42	0.64	0.32	0.60	0.40	0.56	0.23

The plants treated with CCC at 500, 1000 and 2000 mg/L significantly increased photosynthetic pigments (Chl. a, Chl. b, Chl. a + b and carotenoids concentrations) at two sampling dates (15 and 45 days after application) during both seasons, except in case of carotenoids at the second sampling date during the first season. It is concluded that the favourable effects of cycocel on the concentration of photosynthetic pigments of pepper plant leaves may be due to the assumption that CCC retards chlorophyll that breakdown via inhibition of chlorophyllase enzyme (Gaber *et al*., 1983).

Plants treated with ethrel at all concentrations used significantly increased Chl. a, Chl. b, Chl. a + b and carotenoids concentrations at all sampling dates during both seasons. The produced increase in photosynthetic pigments by ethrel may be due to the increase in Lamina thickness observed in this work. Furthermore the increase in photosynthetic pigments due to ethrel application may be attributed to an increase in the biosynthesis of these pigments and their enzymes activity (El Zawily *et al.,* 1984) on onion.

CME treatments significantly increased Chl. a, Chl. b, Chl. a + b and carotenoids at all sampling dates during both seasons. The highest increase in photosynthetic pigments was recorded at 2.5 mg/L followed by 5 and 10 mg/L, respectively at the second sampling dates during both seasons as well as the first sample at the first season. As regards the favourable effect of CME on the concentration of photosynthetic pigments may be due to the increase in Lamina thickness and leaf area observed in this work. In addition, the effect of morphactin may be interpreted also by a delay in senescence of the leaves and retarding breakdown of chlorophyll. This is in agreement with the opinion of Schneider (1970).

Anatomical studies:

The root structure:

Data in Table (3) show that kinetin decreased the diameter of root by decreasing thickness of cortex and the diameter of vascular cylinder which may be due to decreasing the size of the individual cells as previously mentioned by Sakr and El-Kady (1981) on bean. On the other hand, diameter of xylem vessels was increased due to that kinetin induces cell enlargement (Devlin and Witham, 1983).

Cycocel at all concentrations increased the diameter of root by increasing thickness of cortex tissues, diameter of vascular cylinder and xylem vessels inside the vascular. The secondary roots were increased by all levels of cycocel. Similar results were obtained by Mahmoud (1987).

The ethrel at all concentrations increased both thickness of cortex tissues, diameter of root xylem vessels and secondary roots. The work of Boovarah (1974) shows that ethrel induced radial enlargement of been cells. The diameter of vascular cylinder was not affected with ethrel at 100 and 200 mg/L, but 400 mg/L ethrel very slightly decreased the diameter of vascular cylinder which may be due to that the effect of ethrel on vascular tissue and pith was normal as control.

Table (3): Effect of some growth regulators on the root structure of sweet pepper (*Capsicum annum* L. var. California wonder) in 1993 season.

Growth regulators (mg/L)		Diameter of root (u)	Thickness of cortex tissues (u)	Diameter of	
				Vascular cylinder (u)	Xylem vessels (u)
Control		1348	392	564	24
Kinetin	25	997	287	423	30
	50	1004	283	438	28
	100	1005	266	473	30
CCC	500	1534	444	464	46
	1000	1675	466	743	42
	2000	1738	493	752	52
Ethrel	100	1460	450	560	30
	200	1464	452	560	29
	400	1526	496	534	25
CME	2.5	1069	293	483	35
	5	1039	278	483	29
	10	997	276	445	28

All concentrations of morphactin decreased diameter of root, thickness of cortex tissue and diameter of vascular cylinder. For another point of view, morphactin increased diameter of xylem vessels and organized secondary root. From these results, it could be concluded that induction of cell divisions was stimulated. So the number of primordia may increase but the organization of primordia seems to be often histologically disturbed and thus the extension growth of the root may not take place normally or retarded (Schneider *et al*., 1969).

The stem structure:

Data in Table (4) show that kinetin decreased diameter of stem, thickness of cortex layer, vascular cylinder, xylem vessels and thickness of xylem tissues. However, the number of vascular bundles was increased. These results confirm those of Sakr and El-Kady (1981) on *Vicia faba* L.

Table (4): Effect of some growth regulators on the stem structure of sweet pepper (*Capdsicum annuum* L. var. California wonder) in 1993.

Growth regulators (mg/L)		Diameter of stem (u)	Diameter of epidermal cell (u)	Thickness of cortex layer (u)			Vascular cylinder			
				Collenchyma tissues	Parenchyma tissue	Total	Diameter of vascular cylinder (u)	No. of Vascular bundles	Xylem tissue	
									Thickness (u)	Diameter vessel (u)
Control		2088	16	150	134	284	1488	5	440	28
Kinetin	25	2046	17	182	98	280	1452	9	420	22
	50	197	17	163	105	268	1400	8	420	22
	100	2008	17	162	104	266	1442	9	403	21
CCC	500	2252	24	222	110	332	1540	10	513	42
	1000	2458	20	222	118	340	1738	11	550	40
	2000	3160	30	230	119	349	2402	11	564	42
Ethrel	100	2460	24	202	142	344	1724	11	502	38
	200	2030	24	194	88	282	1700	9	482	36
	400	2020	24	183	103	286	1400	9	480	34
CME	2.5	3248	32	240	130	370	2444	10	624	36
	5	2852	32	240	132	372	2044	8	602	36
	10	3160	30	240	131	371	2358	9	602	32

Diameter of stem was increased with increasing concentration of cycocel. This effect may be due to increasing the diameter of epidermal cells and thickness of cortex layer. Diameter of vascular cylinder, diameter/vessel and thickness of xylem tissues as well as number of vascular bundles in vascular cylinder were also increased. Similar results were obtained by Fouad *et al*. (1979), Mahmoud (1987) on pepper and tomato.

Ethrel increased the diameter of stem, diameter of epidermal cells and thickness of cortex layer. Diameter of vascular cylinder, diameter/vessel, thickness of xylem tissues and number of vascular bundles were increased. The highest increase was obtained with 100 mg/L. These results confirm those of Pooviah and Leopod (1974) on bean who reported that ethrel treatment caused extensive radial enlargement of cells rather than cell division in vascular region.

CME increased diameter of stem by increasing diameter of epidermal cells and thickness of cortex layer. Also diameter of vascular cylinder, thickness of xylem tissue and diameter/vessels were increased. The lowest concentration of CME gave the highest number of vascular bundles. Similar results were obtained by El-Masry *et al*. (1994) on *Orobanche crenata* and El-Nady (1994) on eggplant who concluded that morphactin increased diameter of stem.

The leaf structure:

Data in Table (5) show that kinetin caused thickening of the leaf blade with large diameter of epidermal cells and thickness of the mesophyll tissue as well as elongation of palisade cells and size of spongy tissue. These effects tended to increase as kinetin concentration increased due to that kinetin promoted enlargement and elongation of cells (Devlina and Witham, 1983).

Cycocel treatments caused large mesophyll cells by increasing size of palisade cells and spongy tissue. Diameter of epidermal cells tended to wide as cycocel concentrations increased. Similar results were recorded by Bandarenko and Ledovshii (1976), El-Kassas (1992) and El-Nady (1994) on pepper, cucumber and eggplant, respectively who reported that cycocel caused thickening of leaves.

Table (5): Effect of some growth regulators on the leaf structure of sweet pepper (*Capsicum annuum* L. var. California wonder) in 1993 season.

Growth regulators (mg/L)		Diameter of epidermal cell (u)	Thickness of mesophyll tissue (u)		Vascular bundles		
			Palisade	Spongy	No. of vascular/midrib	Thickness of xylem	Diameter/ vessel (u)
Control		15	40	78	10.5	132	10
Kinetin	25	24	70	86	13.5	133	14
	50	24	74	90	13.0	132	14
	100	24	73	96	14.5	133	15
CCC	500	20	52	92	13.0	136	14
	1000	24	60	92	13.5	139	16
	2000	24	62	100	13.0	142	20
Ethrel	100	24	60	84	12.2	142	18
	200	24	56	82	12.6	133	18
	400	24	50	82	12.6	132	10
CME	2.5	20	46	112	9.5	109	16
	5	18	45	100	9.0	92	16
	10	18	45	108	9.5	94	12

Ethrel caused thickening of the leaf blade by increasing diameter of epidermal cell. Thickness of mesophyll tissue tended to increase as a result of elongation of palisade cells and size of spongy tissue.

CME treatments caused larger mesophyll cells as larger size of spongy tissue is due to induced enlargement and increased number of cells by cell division and decreased air spaces, but thickness of palisade cells and diameter of epidermals cell were slightly increased under CME treatment similar results were obtained by Mahmoud (1987) on tomato who reported that CEL produced abnormal tomato leaflets and large cells of mesophyll.

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Synergic Effect of Herbicides Formulation on Energy Compounds and Myokinase Activity of Albino Rats Organs

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Abstract

The present work evaluate the effects of single and multi doses of glyphosate and fluazifop-butyl as herbicides in pure and formulated forms on the energetic compounds and myokinase activity in the organs of albino rats. The results indicated the following remarks:

The single dose showed an increase in ATP content in all investigated rat organs by both herbicides ingestion. However, the increase induced by formulated herbicides was more clear than those of pure ones. In contrast, the levels of ADP and AMP were decreased vigorously under the herbicides ingestion. The decrease caused by formulated herbicides was higher than that of the pure ones. Herbicides exhibited a remarkable inhibition in myokinase activity in all examined rat organs relative to control. The inhibition caused by formulated herbicides was more obvious than those of pure herbicides.

The results showed that, the effects of multi doses of herbicides were similar to the influence of the single dose in all items such as ATP, ADP, AMP contents and myokinase activity. However, these effects were more than that of single dose. The influence of the formulated herbicides was more obvious (toxic) than the pure ones .

Introduction

To establish any toxicological data acute toxicity tests are considered the base line or preliminary studies for chronic toxicity tests. In this respect, glyphosate and fluazifop-butyl are commonly used in Egypt. It should be emphasized that no or very little data are available in the literature about this influence of pure and formulated herbicides on different living organisms and human health.

In developing countries, the use of pesticides has become so important that their use is inextricably linked with improvement of human welfare (Osibajo, 1989). Pesticides are usually applied in their formulated form where the active ingredient is combined with organic solvents, emulsifying and wetting agents, which affect the pesticides penetration. The formulations way cause synergism or antagonism to the toxicity of the active ingredient (El-Sebae, 1985). Recently, the W.H.O. (1991) emphasized that the final toxic classification of any pesticide in intended to be by its formulation. Indeed, literature background revealed that a few reports dealt with the metabolic changes and with other side effects of formulated herbicides as compared with pure one. Abou-Zeid et al., (1993) observed that formulated malathion was more toxic to

rats than that of the pure technical ingredient, and blood serum profile was changed by formulated malathion more than that by pure one with dermal treatment.
In this respect, Abdel-Rahim et al (1994e) and Knopp and Glass (1997) reported that organs tissue contents of adenosine-5-phosphate (ATP) were increased, whereas the contents of adenosine-5-diphosphate (ADP) and adenosine-5-monophosphate (AMP) were decreased and accompanied by inhibiting the activity of myokinase either under the effect of pure or formulated herbicides of all tested organs, relative to control.

In the present study, pure herbicides and formulated ones in single and multi doses were administered separately in dose of 1/20 from LD_{50} every 48 hours to adult albino rats. The effect of these doses on the energetic compounds ATP, ADP, AMP, and myokinase were investigated.

Materials and Methods

Herbicides:
Two herbicides were used; the first one is known as Glyphosate (N-(phosphonomethyl)glycine); belongs to organophosphorous family, non-selective systemic herbicide, acts on various enzyme systems, thus interfering with the formation of amino acids and other important endogenous chemicals. Second one is known as Fluazifop-butyle (butyl(RS)-2-(4-(5-trifluoromethyl-12-pyridyloxy)phenoxy)propionate); belongs to phenoxy, trifluoromethyl and pyridine family; selective systemic herbicide, acts by interfering with ATP production (Agrochemicals Handbook, 1987). The herbicides were obtained from Katou Jonker, Denmark, and was provided from the central Agricultural Pesticide Laboratory, ARC, Ministry of Agriculture, Dokki, Egypt.

Experimental Animals:
A total of 45 adult male albino rats weighing 100-120 g were used in the present study and raised in the animal house of the Biochemistry Department, Faculty of Agriculture; Cairo University. The albino rats were kept under normal laboratory conditions for two weeks before the comencing of the experiments. The animal were allowed free access of water and fed on a uniformally basal diet for a period of three months and were orally administrated herbicides at one single dose of 1/4 from LD_{50} and multi doses of 1/20 from LD_{50} every two days as follows:
Group(1) control, untreated.
Group (2) rats administrated pure glyphosate at a dose of 165 mg/Kg b.w.
Group (3) rats administrated formulated glyphosate at a dose of 330 mg/Kg b.w.
Group(4) rats administrated pure fluazifop-butyl at 280 mg/Kg b.w.
Group (5) rats administrated formulated fluazifop-butyl at 560 mg/Kg body weight.

The animals were killed by decapitation after 2 days of the last induction, then liver, brain, spleen, kidney and hearts were dissected out.

Chemical Analysis:
A spectrophotometric method was used for the determination of ATP, ADP and AMP in the rat organs homogenates using a Pye Unicam spectrophotometer (Model Sp 1800), where ATP was determined according to Lamprecht and Trauschold (1962) and ADP and AMP were determined as described by Adams (1962). The activity of myokinase in the rat organs homogenates was also spectrophotometrically measured as reported by Bergmeyer (1974).

Statistical Analysis:
The obtained data were statistically analyzed using the method of Kalton (1967). The probability was determined by reference to (t) distribution table probability at 0.05 and used as a point of significance according to Bailay (1955).

Results

A- Single Dose:

A-1- Herbicidal Effect on ATP, ADP, and AMP Contents :
After two days of ATP, ADP and AMP were determined in different rat organ tissues. The results are shown in **Tables 1, 2 and 3**.

A significant decrease in the ADP and AMP contents in all investigated organs by herbicides induction was noticed. On the other hand, ATP showed an opposite trend where an increase in ATP level was encountered under herbicides application (**Table 1**). The ATP levels were increased by 20.00%, 26.67%, 13.33% and 22.67% for brain; 13.64%, 18.18%, 10.91% and 19.09% for liver; 12.50%, 25.00%, 17.50% and 17.50% for spleen; 15.00%, 50.00%, 15.00% and 50.00% for kidneys and 9.80%, 17.65%, 13.73 and 17.65% for heart relative to control under the ingestion of pure and formulated glyphosate and fluazifop-butyl, respectively.

On the other hand, the ADP levels (**Table 2**) were decreased by 21.54%, 36.92%, 23.08% and 35.38% for brain; 33.33%, 46.67%, 26.67% and 53.33% for liver; 13.64%, 22.73%, 18.18% and 22.73% for spleen; 37.50%, 43.75%, 31.25% and 50.00% for kidneys and 15.00%, 20.00%, 20.00% and 25.00% for heart than control under the effect of pure and formulated glyphosate and fluazifop-butyl, respectively.

In connection with AMP (**Table 3**), its levels were reduced than control by 35.48%, 41.94%, 32.26% and 38.71% for brain; 17.81%, 30.14%, 21.92% and 30.14% for liver; 14.29%, 22.86%, 17.14% and 25.71% for spleen; 13.64%, 22.73%, 18.18% and 22.73% for kidneys and 25.00%, 45.00%, 30.00% and 40.00% for heart, respectively after the administration of pure and formulated glyphosate and fluazifop-butyl.

A-2- Herbicidal Effect on Myokinase Activity :
The myokinase activity were determined in the various organ tissues after herbicides administration to rats and the results are shown in **Table 4**. The results indicated that herbicides caused a remarkable decrease in myokinase activity of all organs tissues. The decreased values were 31.91%, 40.43%, 27.66% and 40.43% for brain; 33.33%, 46.67%, 31.11% and 37.78% for liver; 20.00%, 37.14%, 17.14% and 34.29% for spleen, 35.00%, 45.00%, 30.00% and 30.00% for kidneys and 20.00%, 25.00%, 22.50% and 25.00% for heart relative to control respectively under the effect of pure and formulated glyphosate and fluazifop-butyl.

Table (1): Effect of herbicides single dose on ATP level in different organs of albino rats.

Organ		Control	Glyphosate		Fluazifop-butyl	
			Pure	Formulated	Pure	Formulated
Brain	*	7.5 ± 0.8 a	9.0 ± 0.8 b	9.5 ± 0.8 b	8.5 ± 0.6 b	9.2 ± 1.0 bc
	%	100.00	120.00	126.67	113.33	122.67
Liver	*	11.0 ± 1.0 a	12.5 ± 1.01 b	13.0 ± 1.1 b	12.2 ± 1.2 b	13.1 ± 1.2 bc
	%	100.00	113.64	118.18	110.91	119.09
Spleen	*	4.0 ± 0.3 a	4.5 ± 0.5 b	5.0 ± 0.4 bc	4.7± 0.5 b	4.7 ± 0.5 b
	%	100.00	112.50	125.00	117.50	117.50
Kidneys	*	2.0 ± 0.2 a	2.3 ± 0.3 b	3.0 ± 0.2 bc	2.3 ± 0.2 b	3.0 ± 0.3 bc
	%	100.00	115.00	150.00	115.00	150.00
Heart	*	5.1 ± 0.5 a	5.6 ± 0.6 b	6.0 ± 0.5 b	5.8 ± 0.6 b	6.0 ± 0.6 b
	%	100.00	109.80	117.65	113.73	117.65

The numbers in the row followed by the same letter are not significantly different at P = 0.05 for each parameter. * = μM/g tissue

Table (2): Effect of herbicides single dose on ADP level in different organs of albino rats.

Organ		Control	Glyphosate		Fluazifop-butyl	
			Pure	Formulated	Pure	Formulated
Brain	*	0.65 ± 0.07 a	0.51 ± 0.06 b	0.41 ± 0.04 b	0.50 ± 0.04 b	0.42 ± 0.04 bc
	%	100.00	78.46	63.08	76.92	64.62
Liver	*	0.15 ± 0.01 a	0.10 ± 1.01 b	0.08 ± 0.01 bc	0.11 ± 0.10 b	0.07 ± 0.01 bc
	%	100.00	66.67	53.33	73.33	46.67
Spleen	*	0.22 ± 0.02 a	0.19 ± 0.02 b	0.17 ± 0.02 b	0.18 ± 0.01b	0.17 ± 0.02 b
	%	100.00	86.36	77.27	81.82	77.27
Kidneys	*	0.16 ± 0.02 a	0.10 ± 0.01 b	0.09 ± 0.01 bc	0.11 ± 0.01 b	0.08 ± 0.01 bc
	%	100.00	62.50	56.25	68.78	50.00
Heart	*	0.20 ± 0.02 a	0.17 ± 0.02 b	0.16 ± 0.01 b	0.16 ± 0.02 b	0.15 ± 0.01 b
	%	100.00	85.00	80.00	80.00	75.00

The numbers in the row followed by the same letter are not significantly different at P = 0.05 for each parameter. * = μM/g tissue

Table (3): Effect of herbicides single dose on AMP level in different organs of albino rats.

Organ		Control	Glyphosate		Fluazifop-butyl	
			Pure	Formulated	Pure	Formulated
Brain	*	0.31 ± 0.03 a	0.20 ± 0.02 b	0.18 ± 0.02 b	0.21 ± 0.01 b	0.19 ± 0.02b
	%	100.00	64.52	58.86	67.74	61.29
Liver	*	0.73 ± 0.07 a	0.60 ± 0.0 b	0.51 ± 0.05 bc	0.57 ± 0.06 b	0.51 ± 0.05 bc
	%	100.00	82.19	69.86	78.08	69.86
Spleen	*	0.35 ± 0.03 a	0.30 ± 0.02 b	0.27 ± 0.03 b	0.29 ± 0.03 b	0.26 ± 0.02 bc
	%	100.00	86.71	77.14	82.86	74.29
Kidneys	*	0.22 ± 0.01 a	0.19 ± 0.02 b	0.17 ± 0.02 bc	0.18 ± 0.01 b	0.17 ± 0.02 b
	%	100.00	86.36	77.27	81.82	77.27
Heart	*	0.40 ± 0.03 a	0.30 ± 0.03 b	0.22 ± 0.02 bc	0.28 ± 0.03 b	0.24 ± 0.02 bc
	%	100.00	75.00	55.00	70.00	60.00

The numbers in the row followed by the same letter are not significantly different at P = 0.05 for each parameter. * = μM/g tissue

Table (4): Effect of herbicides single dose on Myokinase level in different organs of albino rats.

Organ		Control	Glyphosate		Fluazifop-butyl	
			Pure	Formulated	Pure	Formulated
Brain	*	0.47 ± 0.05 a	0.32 ± 0.03 b	0.28 ± 0.03 bc	0.34 ± 0.04 b	0.28 ± 0.02 bc
	%	100.00	68.09	59.57	72.37	59.57
Liver	*	0.45 ± 0.05 a	0.30 ± 0.02 b	0.24 ± 0.02 bc	0.31 ± 0.03 b	0.28 ± 0.03b
	%	100.00	66.67	53.33	68.89	62.22
Spleen	*	0.35 ± 0.04	0.28 ± 0.03 b	0.22 ± 0.02 bc	0.29 ± 0.03 b	0.23 ± 0.02 bc
	%	100.00	80.00	62.86	82.86	65.71
Kidneys	*	0.20 ± 0.01 a	0.13 ± 0.01b	0.11 ± 0.01 bc	0.14 ± 0.01 b	0.14 ± 0.01 b
	%	100.00	65.00	55.00	70.00	70.00
Heart	*	0.40 ± 0.03 a	0.32 ± 0.03 b	0.30 ± 0.03 b	0.31 ± 0.03 b	0.30 ± 0.03 b
	%	100.00	80.00	75.00	77.50	75.00

The numbers in the row followed by the same letter are not significantly different at P = 0.05 for each parameter.
* = μM/mg protein.

B- Multi doses:

B-1- Herbicidal Effect on the Levels ATP, ADP and AMP:

In this respect, the levels of ATP, ADP and AMP in different organs were determined and the data were presented in **Tables 5, 6 and 7**. The results illustrated that the ATP content **(Table 5)** of herbicides inducted rat organs was higher than that of control. The levels were 33.33%, 46.67%, 33.33%, and 33.33% for brain; 18.18%, 27.27%, 13.64% and 27.27% for liver; 17.95%, 28.21%, 15.38 and 28.21% for spleen; 20.00%, 25.00%, 25.00% and 25.00% for kidneys, and 14.00%, 20.00%, 16.00% and 20.00% for heart relative to control under the effect of pure and formulated glyphosate and fluazifop-butyl, respectively. Generally speaking, the formulated and pure herbicides under study possessed the same effect on ATP level of rats.

ADP and AMP contents were decreased in all rat organs than control after the herbicides induction period. The ADP decreased values than control **(Table 6)** were 24.24%, 31.82%, 22.73% and 28.79% for brain; 37.50%, 37.50%, 43.75% and 31.25% for liver; 17.39%, 30.43%, 13.04% and 21.74% for spleen; 33.33%, 53.33%, 40.00% and 46.67% for kidneys, and 19.05%, 28.57%, 19.05% and 28.57% for heart relative to control under the effect of pure and formulated glyphosate and fluazifop-butyl, respectively.

On the other hand, the decreased values than control of AMP **(Table 7)** were 30.00%, 40.00%, 36.67% and 36.67% for brain; 20.27%, 32.43%, 17.57% and 25.68% for liver; 14.29%, 22.86%, 11.43% and 20.00% for spleen; 17.39%, 30.43%, 13.04% and 30.43% for kidneys, and 29.27%, 51.22%, 24.39% and 46.34% for heart relative to control. Here again, the pure and formulated herbicides caused the same effect on the levels of energy compounds in most cases.

B-2- Herbicidal Effect on Myokinase Activity:

After the herbicides induction, myokinase activity was determined in brain, liver, spleen, kidneys and heart; and the results are shown in **Table (8)**. A remarkable decrease was observed in myokinase activity in all investigated organs by the herbicides induction. The decreased values than control were 37.50%, 43.75%, 27.08% and 37.50% for brain; 36.96%, 41.30%, 30.43% and 36.96% for liver; 22.22%, 41.67%, 13.89% and 30.56% for spleen; 38.10%, 52.38%, 39.10% and 47.62% for kidneys, and 25.00%, 25.00%, 20.00% and 25.00% for heart relative to control under the effect

of pure and formulated glyphosate and fluazifop-butyl, respectively. Once again, the pure and formulated herbicides under study possessed the same effect on myokinase activity in most cases.

Table (5): Effect of herbicides single doses on ATP level in different organs of albino rats.

Organ		Control	Glyphosate		Fluazifop-butyl	
			Pure	Formulated	Pure	Formulated
Brain	*	7.5 ± 0.6 a	10.0 ± 1.0 b	11.0 ± 1.1 bc	9.5 ± 0.9 b	1.0 ± 7.5 b
	%	100.00	133.335	146.67	126.67	133.33
Liver	*	11.0 ± 1.0 a	13.0 ± 0.9 b	14.0 ± 1.3 b	12.5 ± 1.2 b	14.0 ± 1.3 bc
	%	100.00	118.18	127.27	113.64	127.27
Spleen	*	3.9 ± 0.4 a	4.6 ± 0.5 b	5.0 ± 0.4 bc	4.5 ± 0.4 b	5.0 ± 0.4 bc
	%	100.00	117.95	128.21	115.38	128.21
Kidneys	*	2.0 ± 0.2 a	2.4 ± 0.2 b	2.0 ± 0.3 b	2.5 ± 0.1 b	2.5 ± 0.2 b
	%	100.00	120.00	125.00	125.00	125.00
Heart	*	5.0 ± 0.5 a	5.7 ± 0.6 b	6.0 ± 0.5 b	5.8 ± 0.5 b	6.0 ± 0.5 b
	%	100.00	114.00	120.00	116.00	120.00

The numbers in the row followed by the same letter are not significantly different at P = 0.05 for each parameter.
* = μM/g tissue.

Table (6): Effect of herbicides multi doses on ADP level in different organs of albino rats.

Organ		Control	Glyphosate		Fluazifop-butyl	
			Pure	Formulated	Pure	Formulated
Brain	*	0.66 ± 0.06 a	0.50 ± 0.04 b	0.54 ± 0.05 b	0.51 ± 0.04 b	0.47 ± 0.05 b
	%	100.00	75.765	68.18	77.27	71.21
Liver	*	0.16 ± 0.01 a	0.10 ± 0.01 b	0.10 ± 0.01 b	0.09 ± 0..01 b	0.11 ± 0.01 b
	%	100.00	62.50	62.50	56.25	68.75
Spleen	*	0.23 ± 0.02 a	0.19 ± 0.02 b	0.16 ± 0.01 bc	0.20 ± 0.02 b	0.18 ± 0.02 b
	%	100.00	82.61	69.57	86.96	68.75
Kidneys	*	0.15 ± 0.01 a	0.10 ± 0.01 b	0.07 ± 0.01 bc	0.09 ± 0.01 b	0.18 ± 0.02 b
	%	100.00	66.67	46.67	60.00	78.26
Heart	*	0.21 ± 0.02 a	0.17 ± 0.02 b	0.15 ± 0.01 b	0.17 ± 0.01 b	0.08 ± 0.01 b
	%	100.00	80.95	71.43	80.95	71.43

The numbers in the row followed by the same letter are not significantly different at P = 0.05 for each parameter.
* = μM/g tissue

Table (7): Effect of herbicides multi doses on AMP level in different organs of albino rats.

Organ		Control	Glyphosate		Fluazifop-butyl	
			Pure	Formulated	Pure	Formulated
Brain	*	0.30 ± 0.02 a	0.21 ± 0.02 b	0.18 ± 0.08 bc	0.19 ± 0.001 b	1.19 ± 0.01 b
	%	100.00	70.05	60.00	63.33	63.33
Liver	*	0.74 ± 0.07 a	0.59 ± 0.06 b	0.50 ± 0.05 bc	0.61 ± 0.05 b	0.55 ± 0.05 b
	%	100.00	79.73	67.57	82.43	74.32
Spleen	*	0.35 ± 0.04 a	0.30 ± 0.02 b	0.27 ± 0.02 b	0.31 ± 0.02 b	0.28 ± 0.02 b
	%	100.00	85.71	77.41	88.57	80.00
Kidneys	*	0.23 ± 0.02 a	0.19 ± 0.02 b	0.16 ± 0.01 bc	0.20 ± 0.02 b	0.16 ± 0.01 bc
	%	100.00	82.61	69.57	86.96	69.57
Heart	*	0.41 ± 0.04 a	0.29 ± 0.03 b	0.20 ± 0.02 bc	0.31 ± 0.03 b	0.22 ± 0.02 bc
	%	100.00	70.73	84.78	75.61	53.66

The numbers in the row followed by the same letter are not significantly different at P = 0.05 for each parameter.
* = μM/g tissue

Table (8): Effect of herbicides multi doses on Myokinase level in different organs of albino rats.

Organ		Control	Glyphosate		Fluazifop-butyl	
			Pure	Formulated	Pure	Formulated
Brain	*	0.48 ± 0.05 a	0.30 ±0.03 b	0.27 ± 0.03 b	0.35 ± 0.03 b	0.30 ± 0.02 bc
	%	100.00	62.505	56.25	72.92	62.50
Liver	*	0.46 ± 0.04 a	0.29 ± 0.03 b	0.27 ± 0.03 b	0.32 ± 0.03 b	0.29 ± 0.03 b
	%	100.00	63.04	58.70	69.57	63.04
Spleen	*	0.36 ± 0.04 a	0.28 ± 0.02 b	0.21 ± 0.02 bc	0.31 ± 0.03 b	0.25 ± 0.02 bc
	%	100.00	77.78	58.33	86.11	69.44
Kidneys	*	0.21 ± 0.02 a	0.13 ± 0.01 b	0.10 ± 0.01 bc	0.13 ± 0.01 b	0.11 ± 0.01 b
	%	100.00	61.90	47.62	61.90	52.38
Heart	*	0.40 ± 0.03 a	0.30 ± 0.03 b	0.30 ± 0.02 b	0.32 ± 0.03 b	0.30 ± 0.02 b
	%	100.00	75.00	75.00	80.00	75.00

The numbers in the row followed by the same letter are not significantly different at P = 0.05 for each parameter.
* = μM/mg protein

Discussion

A- Herbicidal Effect on The Energy Compounds :

Adenosine-5-triphosphate (ATP), adenosine-5-diphosphate (ADP) and adenosine-5-phosphate (AMP) were determined in different organ tissues of inducted rats. A remarkable decrease was observed in ADP and AMP contents. In contrast, ATP content was increased in all investigated organs by herbicides ingestion.
The significant effects of the investigated herbicides on the different organs are summarized in **Table (9).**
These results might be related either to the high rate of ATP synthesis or the energy liberated during the metabolic processes through trapping inorganic phosphate with AMP and ADP to form ATP. These findings were in parallel with the results of Abdel-Rahim et al., (1994e) and Knopp and Glass (1997).
The results of ATP, ADP and AMP led to suggest that at any circumstances associated with diminished availability of the prime dietary source of energy, namely carbohydrate, will accentuate utilization of fatty acids for this purpose. In this respect, the stimulation of glycolytic metabolism (forms pyruvic acids and then acetyl CoA) led to accumulate ATP and creatinine stores (Lehninger, 1982 and Abdel-Rahim et al, 1994b). ATP is rapidly utilized in protein biosynthesis through converting to cAMP which stimulated by the adenylate cyclase (Adams et al, 1993).

B- Herbicidal Effect on Myokinase Activity:

After herbicides ingestion the myokinase activity was determined in different organs tissues of rats and a remarkable decrease in the myokinase activity was noticed in all tissues relative to control.
In general, the increase in ATP level after herbicides ingestion was mainly due to the effect of herbicides on the respiratory system. The maintenance of tissues energy is likely accomplished through increase the glycolysis process. Accordingly, an inhibition in LDH activity caused an increase of energy utilization for tissue processes (Abdel-Rahim et al, 1994b).

The tight coupling of oxidation to phosphorylation, provided a means by which the role of oxidation of food stuffs by respiratory oxygen. The utilization of ATP to drive the divers energy requiring processes of the cells automatically increased the available supply of ADP and inorganic phosphate, which in turn become available to react in the

coupling mechanism and permit respiration to proceed. In the herbicides ingestion condition, the oxidative phosphorylation was stimulated due to the respiration oxygen and the increase of ATP formation (Lehninger, 1982).

Myokinase catalyzes the conversion of 2 molecules of ADP to one molecule of each of ATP and AMP through the following reaction:

2 ADP ----------> ATP + AMP

This reaction was observed after the complete utilization of ATP. The higher level of ADP than AMP might be due to the utilization of high amounts of ATP in metabolic processes and was converted to ADP (Lehninger, 1982).
The results of the present work emphasized that an urgent rules are needed for global regulation to impose legislation and guidelines for registration and handling of agro-chemical including pesticides which have direct access to the food chain, ought to include methodologies and laboratory facilities for their implementation in many of the developing countries.

Table (9): The influence of Glyphosate and Fluazifop[-butyl on rat organs parameters.

Parameters	Glyphosate		Fluazifop[-butyl	
	Pure	Formulated	Pure	Formulated
Heart parameters				
ATP level	+	++	+	+
ADP	-	-	-	-
ADP	-	--	-	--
Myokinase	-	-	-	-
Kidneys parameters				
ATP level	+	++	+	++
ADP	-	--	-	--
ADP	-	--	-	-
Myokinase	-	--	-	-
Spleen parameters				
ATP level	+	++	+	+
ADP	-	-	-	-
ADP	-	--	-	--
Myokinase	-	-	-	--
Brain parameters				
ATP level	+	++	+	++
ADP	-	-	-	--
ADP	-	-	-	-
Myokinase	-	--	-	--
Liver parameters				
ATP level	+	+	+	++
ADP	-	--	-	--
ADP	-	--	-	-
Myokinase	-	-	-	-

The symbols (+, -) and (++, --) indicates significant and highly significant increase or decrease, respectively relative to control rats group.

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Improved Wheat Production During some Agricultural Practices and Reducing Environmental Pollution

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Abstract

Two field experiments were conducted at extension farm, in El-Mansoura Center, Dakahlia district, Egypt, during 1996/97 and 1997/98 seasons to study the effect of different fertilization treatments; times of foliar spraying of super Grow of some wheat cultivars on the chemical composition of grains and straw for reducing pollution. The trails were arranged in a strip split plot design with four replications. The main findings could be summarized as follows:

The recommended NPK fertilization recorded highest concentrations of cadmium, lead, zinc, iron, nitrate, nitrite in grains and concentrations of nitrate and nitrite in straw compared with other fertilization treatment in both seasons. However, the lowest concentrations produced from biofertilization (syrialin + phosphorin + organic fertilizers) compared with other treatment over both seasons.

Foliar application of super Grow at tillering + elongation and or at tillering + heading stages significantly increased the concentration of cadmium, lead, zinc, iron, nitrate, nitrite in grains as well as concentrations of nitrate and nitrite in straw compared with other time of applications over both seasons. However, the lowest concentration of cadmium, lead, iron, zinc, nitrate, nitrite in grains as well as concentrations of nitrate and nitrite in straw produced from foliar application at tillering stage (40 days from sowing) over both seasons.

The three wheat cultivars did not differ in cadmium, lead, nitrate, nitrite concentration in grains and nitrate, nitrite in straw over both seasons. However, the maximum cadmium and lead concentration in grains were produced from the Gemmiza 3 cultivar (0.120 and 0.114 ppm) followed by Sakha 69 cultivar (0.116 and 0.106 ppm) and Sids 8 cultivar (0.117 and 0.109 ppm) over the two seasons. Highest zinc and lead concentrations in grains was obtained from Sakha 69 cultivar followed by Gemmiza 3 and Sids 8 cultivars.

Maximum concentration of cadmium in grains was obtained from adding the recommended NPK fertilization with spraying Super Grow at tillering + heading stages (40 + 80 days from sowing) over both seasons. The highest concentration in grain from adding the recommended NPK fertilization with spraying at tillering + elongation stages (40 and 60 days from sowing) and/or at tillering + heading stages (40 + 80 days from sowing) over both seasons. The minimum concentration of zinc and iron in grain was produced from spraying Super Grow at tillering + elongation stages (40 and 60 days from sowing) and sown Sakha 69 and/or Gemmiza 3 cultivars over both seasons.

Generally, it can be concluded that adding biofertilization of syrialin + phosphorin + organic fertilizer, spraying Super Grow at tillering + elongation stages (40 and 60 days from sowing) and sown sids 8 or Gemmiza 3 cultivars was the most effective treatment for raising wheat productivity and reducing mineral fertilization as well as reducing pollution under environmental conditions of Dakahlia Governorate.

Introduction

Wheat (*Triticum aestivum*, L.) is considered one of the main cereal crops in the world as well as in Egypt and Libya. The importance of wheat as a major food source for man in many countries has increased consistently in the last decade. Increasing wheat productivity is a national target in Egypt and Libya to fill the gap between wheat consumption and production.

The total cultivated wheat area with wheat in Egypt was about million hectars (2.5 million faddans) producing was about 6.3 million tons (42 million ardabs) in 1999 season with an average of 6.76 ton/hectares (18.94 ardab/faddan), (Gomaa, 1999). Meanwhile, the total cultivated area with wheat in Libya was about 30 thousand hectares (12.6 thousand faddans) in 1999 season with a national average of about 5.41 ton/hectares (15.15 ardab /faddan)*.

Environmental pollution, especially by increasing chemical fertilization is one of the most effective factors in the destruction of the biosphere components. Among all chemical contaminants nitrate, nitrite and heavy metal particularly in soil and subsequently plants are considered potential hazardous contaminants in the biosphere to human health. The utilization of chemical fertilization on agricultural land introduces harmful substances into soil. When absorbed by wheat plants these contaminants enter the food chain affecting both animal and human.

The nitrates being in water and food have serious effects, as they cause diseases for children and ruminant animals called “Methemoglobinemia” that caused a high ratio of mortality for children and animals, while the adult can endure nitrates being in water. These diseases are caused when the child or animal drink water full of high ratio of nitrates or take food full of a high ratio more than 10 parts of million and, in this case the nitrates are to be reduced in the bowels to nitrates that are to be absorbed in the blood current, then combined with hemoglobin transforming it to methemoglobin, and blood comes to be in capable of carrying oxygen during respiration. Of the bad effects that are possible for nitrification process is composing of nitrosamines compounds and these consist by combining of nitrite (whether consisted by ammonia oxidation or nitrates reduction) with some products of insecticides decay. It has become evident that these compounds cause cancer and cells mutation (W.H.O., 1984). According to Bergstrom and Brink (1986), it is reasonable to expect that the loss of nitrate by leaching occurs more readily than ammonium especially in coarse-texture soils. Ammonium may be retained in soils as an exchangeable ion on clay surface or it may from relatively stable complexes with some organic substances. Ibrahim (1990) found that the concentration of NH_4-N and NO_3-N in the drainage water from silty clay soils is much higher than from clay soil. Tahoun *et al.* (1993) did a quantitative estimate of nitrogen losses from Egyptian soils. Fields with tile drain facilities were monitored for nitrogen inputs and outputs. They found that leaching losses of nitrogen from soils under corn ranged between 7 and 49 kg/fad. Also, Sveda *et al.* (1992) found that ammonium fertilizers and urea may undergo loss by volatization soon after application but, denitrification and leaching loss may occur later when the NH_4-N has been oxidized to NO_3-N.

Plant absorb nitrogen from soil in the nitrate form or ammonium or both depending on the availability of each. As early as El-Baisary *et al.* (1982) studied the effect of N applied as calcium ammonium nitrate or urea to wheat plant. They stated that the amount of nitrogen accumulated in grains was less with calcium ammonium

nitrate than with urea. In addition, the amount of nitrogen in straw greatly affected by nitrogen application without significant differences among nitrogen forms. In another study, Mabler *et al.* (1994) found that grain yield of wheat and nitrogen use efficiency were insignificant affected when NH_4NO_3 or urea were used.

Toxic metals derived from soil parent materials usually constitute by far the major group in soils. Hassan (1997) cited from literature that cadmium pollution of the environment has been rapidly increasing in recent decades as a result of rising consumption of cd by industry. Unlike pb and cu which have been utilized for centuries, cd had only been widely used this century. More than half of the cd ever used in industry was produced in the last 25 years. Hutton (1982), also mentioned that sources of soil contamination by cd are the mining and smelting of pb and zn, atmospheric and soil pollution. He has also added that phosphate fertilizers are an important example of cd impurity and their continual use has led to significant increases in the cd, zn, fe, content of many agricultural soils.

The objectives of this investigation was to study the utilization of some agricultural practices to improve wheat productivity for the three evaluated wheat cultivars, Sakha 69, Sids 8 and Gemmiza 3 through different fertilization treatments, time of foliar nutrients application and their interaction. Minimizing the environmental pollution with the mineral fertilizers, in particular nitrogenous ones is considered among the study targets.

Materials and Methods

Two field experiments were conducted in Mansoura Center, Dakahlia Governorate, during 1996/97 and 1997/98 seasons. This investigation was aimed to study the effect of different fertilization treatments

1- without
2- recommended NPK (70 kg N, 23 kg P_2O_5 and 25 kg K_2O/fad)
3- 40 m^3 farmyard (FYM) manure/fed
4- inoculation grains of syrialin (400) + phosphorin (600) + 40 m^3 organic fertilizer/fed. 5- inoculation grains of syrialin (600) + phosphorin (600) + 40 m^3 organic fertilizer/fed and
5- syrialin 800 gm /fad + phosphorin 800 gm /fad + organic fertilizer at rate 40 m^3 /fad.

Times of foliar nutrition of Super Grow nutrient at tillering (40 days from sowing), at elongation (60 days from sowing), at heading (80 days from sowing), at tillering + elongation stages, at tillering + heading stages on growth, yield and yield components of the three wheat cultivars i.e. Sakha 69, Sids 8 and Gemmiza 3.

A strip split plot design with four replications was used. The horizontal plots were devoted as above mentioned six fertilization treatments. The vertical plots were allocated with the five times of foliar application of Super Grow nutrients as above mentioned. The sub plots were occupied by the chosen three wheat cultivars, namely Sakha 69, Sids 8 and Gemmiza 3. The sub plots area was 3.0 x 3.5 m (10.5 m²) i.e. 1/400 fad. The recommended of nitrogen fertilization in the form of urea (46.5 % N) was used at a rate of 70 kg N /fad and applied in two equal portions with the first watering and before the second watering. Calcium super phosphate at a rate of 150 kg/fad (15.5 % P_2O_5) and potassium sulphate at a rate of 50 kg K_2O/fad (50 % K_2O)

were added during land preparation. Bacterial inoculation of wheat grains was done immediately before sowing irrigation. Bio-fertilizer included Azotobacter, Azosprillum and Bacillus bacteria and obtained from A.R.C. Ministry of Agriculture. Organic fertilizer as farmyard manure was taken from dairy farm, Agric. Experiments Station Fac. of Agric. Mansoura Univ. and it contents showed in Table 1. Foliar application of Super Grow 20-20-20 at a rate of 50 gm/300 liter water was used in this study. Super Grow contains of 20 % of total nitrogen, 20 % available phosphoric acid (P_2O_5), 20 % soluble potash (K_2O), 0.15 % Fe, 0.05 % Mn, 0.05 % Cu, 0.005 % Mo, 0.2 % S, 0.15 % Zn, 0.05 % Mg, 0.05 % Ca and 0.02 % B. Grains of wheat cultivars were obtained from Wheat Breeding Section, A.R.C. The experimental soil was loamy clay texture, the mechanical and chemical analysis of experimental soil are presented in Table 2. Water samples were collected from the different drains passing through the area and used in irrigating the soils at different periods. Also, water samples were taken from normal canal (Dommitta branch) to represent Nile water, the chemical composition of Nile water from normal canal (Dommitte branch) are presented in Table 3.

Table 1: Chemical analysis of the Farmyard manure in the two seasons.

PH	Organic carbon %	Total nitrogen %	C/N ratio %	Total phosphorus %	Total potassium %
7.21	19.35	1.46	13.1	0.26	1.41

Table 2: Mechanical and chemical analysis of experimental soil in both seasons.

	Mechanical analysis						
seasons	Coarse sand %	Fine sand %	Silt %	Clay %	Organic matter %	pH	Total nitrogen %
1996/97	5.49	19.80	36.29	38.42	1.88	7.80	0.122
1997/98	6.59	18.80	40.41	34.20	1.81	7.75	0.117

In both seasons, wheat was preceded by maize. Grains of wheat cultivars were sown on mid November at a rate of 70 kg/fad in both seasons. At harvest, ten guarded plants of one square meter of each sub plots were taken at random to estimate the following characters:

1- Heavy metals (cadmium, lead, zinc and iron) was estimated in a digestive solution ($HClO_4$ - H_2SO_4 - HNO_3) by an atomic absorption spectrophotometer method according A.O.A.C. (1980).
2- Nitrates in the plant: As described by Singh (1988), 0.1 gm of finely ground sample with 50 ml of 2 % acetic acid in a conical flask was rotary shaken for 20 minutes and filtered. Nitrate was determined in the filtrate according to Bremner (1965).

Table 3: Chemical composition of Nile water from normal canal (Dommitte branch) in 1996/97 and 1997/98 seasons.

Variables	Seasons	
	1996/97	1997/98
pH	7.55	7.53
Ec ds/m	0.48	0.47
Soluble anions and cations (mg/L):		
CO_3 (ppm)	---	---
HCO^-_3 (ppm)	3.10	3.09
Cl^- (ppm)	1.42	1.39
SO^{--}_4 (ppm)	0.28	0.22
Ca^{++} (ppm)	1.79	1.77
Mg^{++} (ppm)	1.22	1.21
Na^+ (ppm)	1.55	1.50
K^+ (ppm)	0.24	0.22
Fe (ppm)	0.400	0.300
Mn (ppm)	0.080	0.070
Zn (ppm)	0.070	0.090
Cu (ppm)	0.010	0.020
Co (ppm)	0.050	0.040
Ni (ppm)	0.010	0.010
Cd (ppm)	0.001	0.002
Pb (ppm)	0.050	0.051

Data of the two seasons were subjected to the proper statistical analysis of the technique of analysis of variance of strip split plot design as mentioned by Gomez and Gomez (1984). Treatment means were compared using New Least Significant Differences (N.L.S.D.) test at 5 % and 1 % level of probability.

Results and Discussion

A- Fertilization treatments effects:

Data presented in Tables 4 and 5 show that fertilization treatments significantly affected the concentrations of cadmium, lead, zinc, iron, nitrate, nitrite in grains and nitrate, nitrite in straw over both seasons. Adding the treatments of recommended NPK fertilization as mineral fertilization produced highest concentration of cadmium, lead, zinc, iron, nitrate, nitrite in grains and nitrate, nitrite in straw compared with other treatments over both seasons, which were 0.204, 0.177, 40.50, 38.35, 4.140, 0.198 ppm in the grains and 3.168, 0.136 ppm in the straw, respectively. In addition, the lowest concentrations produce from without fertilization treatment followed by bio-fertilization treatments

B- Time of foliar nutrition effects:

Super Grow foliar application at different stages significantly affected concentrations of cadmium, lead, zinc, iron, nitrate, nitrite in grains and nitrate, nitrite in straw as presented in Tables 4 and 5. Foliar application of Super Grow at tillering + elongation and/or at tillering + heading stages maximized concentrations of cadmium, lead, iron, nitrite in grains and nitrate, nitrite in straw compared with other times of spraying in both seasons. Meanwhile, foliar spraying of Super Grow at tillering + elongation stages significantly maximize concentrations of zinc and nitrate in grains compared with other time of spraying in both seasons. In addition, the lowest concentration produce from foliar spraying at tillering stage.

C- Cultivar performance:

Data presented in Tables 4 and 5 show that tested cultivars significantly differed in concentrations of zinc and iron in both seasons. Maximum concentration of iron in grains were obtained from sown Gemmiza 3 cultivar followed by Sakha 69 and Sids 8 cultivar came in the last rank, which were 29.39, 29.08 and 28.80 ppm over both seasons. Meanwhile, the highest concentration of zinc in grains were obtained from sown Sakha 69 cultivar followed by Gemmiza 3 and Sids 8 cultivars came in the last rank, which were 28.25, 28.11 and 27.64 ppm over both seasons. Over both seasons, concentrations of cadmium, lead, nitrate, nitrite in grains and nitrate, nitrite in straw of wheat cultivars was not significantly affected in wheat cultivars, but Gemmiza 3 cultivar tended to be the highest as shown in Tables 4 and 5.

D- Significant interaction effects:

The interaction between fertilization treatments and times of foliar nutrient application significantly affected concentrations of Cadmium and lead in both seasons as shown in Table 6. Maximum concentration of cadmium was obtained from adding recommended NPK fertilization and foliar application at tillering + heading stages over both seasons compared with other treatments. Meanwhile, the highest concentration of lead was obtained from adding recommended NPK fertilization and foliar application at tillering + elongation and/or at tillering + heading stages, which were 0.205 and 0.207 ppm with insignificant differences over both seasons. The lowest concentrations of cadmium and lead in grains were produced from the interaction between without fertilization treatment and foliar application at all stages with insignificant differences over both seasons.

The interaction between times of foliar application and some wheat cultivars significantly affected concentration of zinc and iron in both seasons as presented in Table 7. Maximum concentration of zinc and iron were produced from foliar application os super grow at tillering + elongation and/or at tillering + heading stages and sown Sakha 69 or Gemmiza 3 cultivars in both seasons, which were 29.73, 30.17 and 31.32, 31.37 ppm and/or 29.35, 29.38 and 31.35, 31.76 ppm respectively. All interactions had insignificant effects on concentration of nitrate, nitrite in grains and nitrate, nitrite in straw over both seasons indicating that each both the three tested factors acted separately.

Table 4: Means of cadmium, lead, zinc and iron concentrations in grains (ppm) as affected by fertilization treatments, time of foliar nutrition of some wheat cultivars over both seasons.

Characters / Treatments	Concentrations in grains (ppm)			
	Cadmium	Lead	Zinc	Iron
A: Fertilization treatments				
Without fertilization	0.060	0.051	18.80	19.46
NPK fertilizer (Recom.)	0.204	0.177	40.50	38.35
Organic fertilizer	0.115	0.111	29.79	28.63
S 400+P 400+O40	0.108	0.105	27.79	28.96
S 600+P 600+O40	0.107	0.106	25.53	29.58
S 800+P 800+O40	0.111	0.107	25.59	29.56
F-Test	*	*	**	*
N-LSD at 5 %	0.003	0.002	0.43	0.37
N-LSD at 1 %	---	---	0.58	---
B: Time foliar nutrition				
At tillering stage	0.098	0.091	24.56	25.58
At elongation stage	0.118	0.109	28.13	27.06
At heading stage	0.121	0.112	28.55	30.07
At tillering + elongation	0.125	0.119	29.61	31.33
At tillering + heading	0.126	0.119	29.16	31.40
F-Test	*	*	**	*
N-LSD at 5 %	0.001	0.004	0.25	0.25
N-LSD at 1 %	---	---	0.34	---
C: Cultivars				
Sakha 69	0.116	0.106	28.25	29.08
Sida 8	0.117	0.109	27.64	28.80
Gemmiza 3	0.120	0.114	28.11	29.39
F-Test	N.S	N.S	**	**
N-LSD at 5 %	---	---	0.21	0.20
N-LSD at 1 %	---	---	0.27	0.26

S= Syrialin , P= Phosphorin and O= Organic fertilizer

Table 5: Means of nitrate, nitrite concentration in grains and straw (ppm) as affected by fertilization treatments, time of foliar nutrition of some wheat cultivars over both seasons.

Characters / Treatments	Concentrations in grains		Concentrations in straw	
	Nitrate (ppm)	Nitrite (ppm)	Nitrate (ppm)	Nitrite (ppm)
A: Fertilization treatments				
Without fertilization	2.122	0.102	1.021	0.044
NPK fertilizer (Recom.)	4.140	0.198	3.168	0.136
Organic fertilizer	3.242	0.129	2.424	0.109
S 400+P 400+O40	3.046	0.135	2.342	0.102
S 600+P 600+O40	2.837	0.128	2.329	0.098
S 800+P 800+O40	2.955	0.127	2.331	0.106
F-Test	*	*	**	*
N-LSD at 5 %	0.008	0.003	0.014	0.003
N-LSD at 1 %	---	---	0.019	---
B: Time foliar nutrition				
At tillering stage	2.551	0.125	2.087	0.083
At elongation stage	2.908	0.136	2.267	0.096
At heading stage	3.246	0.139	2.309	0.104
At tillering + elongation	3.306	0.141	2.342	0.106
At tillering + heading	3.274	0.142	2.340	0.106
F-Test	**	*	*	*
N-LSD at 5 %	0.008	0.002	0.007	0.003
N-LSD at 1 %	0.010	---	---	---
C: Cultivars				
Sakha 69	3.058	0.143	2.277	0.100
Sida 8	3.043	0.134	2.255	0.099
Gemmiza 3	3.069	0.133	2.275	0.098
F-Test	N.S	N.S	N.S	N.S
N-LSD at 5 %	---	---	---	---
N-LSD at 1 %	---	---	---	---

S= Syrialin , P= Phosphorin and O= Orgaic fertilizer

Table 6: Means of cadmium and lead concentrations in grains (ppm) as affected by the interaction between fertilization treatments and time of foliar nutrients over both seasons.

Characters / Treatments		Concentrations in grains (PPm)	
		Cadmuim	Lead
Without fertilization	At tillering stage	0.058	0.050
	At elongation stage	0.058	0.049
	At heading stage	0.060	0.051
	At tillering +elongation	0.062	0.055
	At tillering + heading	0.062	0.051
NPK fertilizer (Recom.)	At tillering stage	0.158	0.133
	At elongation stage	0.200	0.175
	At heading stage	0.212	0.167
	At tillering +elongation	0.225	0.205
	At tillering + heading	0.226	0.207
Organic fertilizer	At tillering stage	0.109	0.103
	At elongation stage	0.117	0.110
	At heading stage	0.118	0.117
	At tillering +elongation	0.117	0.112
	At tillering + heading	0.114	0.115
Syrialin 400 + phosphorin 400 + organic 40	At tillering stage	0.082	0.083
	At elongation stage	0.113	0.105
	At heading stage	0.111	0.107
	At tillering+elongation	0.117	0.115
	At tillering + heading	0.116	0.115
Syrialin 600 + phosphorin 600 + organic 40	At tillering stage	0.082	0.085
	At elongation stage	0.111	0.107
	At heading stage	0.111	0.112
	At tillering +elongation	0.112	0.113
	At tillering + heading	0.119	0.113
Syrialin 800 + phosphorin 800 + organic 40	At tillering stage	0.099	0.090
	At elongation stage	0.110	0.106
	At heading stage	0.112	0.115
	At tillering+elongation	0.119	0.111
	At tillering + heading	0.117	0.111
F-Test		**	**
N-LSD at 5 %		0.007	0.006
N-LSD at 1 %		0.009	0.008

Table 7: Means of zinc and iron concentrations in grains (ppm) as affected by the interaction between time of foliar nutrients and wheat cultivars over both seasons.

Characters / Treatments		Concentrations in grains (PPm)	
		Cadmuim	Lead
At tillering stage	Sakha 69	25.13	25.98
	Sids 8	24.31	25.45
	Gemmiza 3	24.23	25.32
At elongation stage	Sakha 69	28.70	26.67
	Sids 8	27.64	26.48
	Gemmiza 3	28.05	28.02
At heading stage	Sakha 69	28.36	30.07
	Sids 8	28.54	29.66
	Gemmiza 3	28.74	30.48
At tillering+elongation	Sakha 69	29.73	31.32
	Sids 8	28.94	31.29
	Gemmiza 3	30.17	31.37
At tillering + heading	Sakha 69	29.35	31.35
	Sids 8	28.76	31.10
	Gemmiza 3	29.38	31.76
F-Test		**	**
N-LSD at 5 %		0.51	0.49
N-LSD at 1 %		0.67	0.65

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