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5. SUMMARY AND CONCLUSION

The present study aimed to investigate the response of three sunflower (*Helianthus annus,* L.) genotypes (Vidoc, Euroflour and Miak) to some biofertilization treatments and nitrogen fertilizer levels in pot and field experiments. Experiments were carried out at the Experimental Farm, Faculty of Agriculture, Mansoura University.

1. Pot Experiment:

The pot experiment was carried out in the greenhouse for 21 days at the Department of plant pathology, Faculty of Agriculture, Mansoura University during the summer of season 2000 to evaluate the response of three sunflower genotypes to some biofertilization treatments under sterilized soil and unsterilized soil conditions.

A factorial experiment in complete randomized design with four replicates was used. The combination of the three studied factors resulted 72 pots in this experiment. The first factor was occupied with the following two different soil conditions:

1- Sterilized soil.

2- Unsterilized soil.

While the second factor was devoted to the following three sunflower genotypes:

1- Vidoc: French hybrid.

2- Euroflour: French hybrid.

3- Miak: Russian open pollinated cultivar.

The third factor was assigned to the following three biofertilization treatments:

1- Uninoculation (without biofertilization).

2- Inoculation with the mixture of A.b (No. 40) and A.l (Br-17).

3- Inoculation with the mixture of B.p (B-32) and B.p (CF-43).

Each pot used in this experiment was (15 cm diameter) and filled with 2.5 kg (2 clay :1 sand) sterilized or unsterilized soil. The following parameters were determined:

1.1. Averages shoot length (cm)

1.2. Averages shoot fresh weight (g/plant)

1.3. Averages shoot dry weight (g/plant)

1.4. Averages root length (cm)

1.5. Averages root fresh weight (g/plant)

1.6. Averages root dry weight (g/plant)

The most important results obtained from this preliminary experiment can be summarized as follows:

A. Soil conditions effects:

Concerning plant growth parameters under sterilized and unsterilized soil, the results indicated that, shoot length, shoot fresh weight and shoot dry weight as well as root length, root fresh weight and root dry weight significantly increased and the highest values of all characters were achieved from unsterilized soil compared to sterilized soil.

B. Genotypes performance:

1- The three tested sunflower genotypes performance were markedly varied in most of estimated characteristics. Miak genotype markedly exceeded both of Vidoc and Euroflour genotypes in shoot length, shoot fresh weight, shoot dry weight and root dry weight. While Miak and Vidoc genotypes surpassed Euroflour genotype and there was no significant difference between Miak and Vidoc genotypes in root length.

2- Vidoc genotype surpassed Miak and Euroflour genotypes by 6.3 and 42.1% in root fresh weight respectively. Miak genotype ranked second while, Euroflour genotype ranked last.

C. Biofertilization treatments effects:

Inoculation with the bacterial strains (as a biofertilization) significantly affected shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight and root dry weight. Inoculation with A.b&A.l significantly produced the highest values of all the previous characters compared with uninoculated (control treatment) and inoculation with B.p treatment ranked second after A.b&A.l treatment. Whilst, the difference between A.b&A.l and B.p treatments did not reach 5% level of significance in shoot dry weight and root dry weight.

D. Interaction effects:

1- The interaction effect between different soil conditions and sunflower genotypes was highly significant on shoot length, shoot fresh weight and shoot dry weight in the pot experiment under greenhouse conditions. It can be concluded that, the best results were obtained from planting Miak genotype in the unsterilized soil conditions.

2- With regarding to the interaction between different soil conditions and biofertilization treatments, it was exerted highly significant effects on shoot dry weight, root length and root dry weight. The highest values of shoot dry weight were resulted from inoculation with B.p treatment under unsterilized soil. There was no significant difference between inoculation with B.p and inoculation with A.b&A.l under unsterilized soil conditions. Also, the maximum values of root length and root dry weight were produced from inoculation with A.b&A.l followed by inoculation with B.p treatment under sterilized and unsterilized soil conditions. On the other side, the lowest values were obtained from uninoculation under sterilized and unsterilized soil conditions.

3- Concerning the interaction between sunflower genotypes and biofertilization treatments has a significant effect on shoot fresh weight, shoot dry weight root length and root fresh weight. The highest results of shoot fresh and dry weights were recorded from planting Miak genotype and inoculated seeds with A.b&A.l. Vise versa, the lowest values of shoot fresh and dry weights were obtained from planting Euroflour genotype and uninoculated seeds

(control treatment). Also, the maximum root length and root fresh weight were produced from Vidoc and Miak genotypes and there was no markedly difference between Vidoc and Miak genotypes with inoculation by A.b&A.l treatment. Meanwhile, the lowest values of root length and root fresh weight were resulted from planting Vidoc genotype and uninoculated seed (control treatment).

Conclusion

1- It could be resulted that sunflower plants respond positively to natural organisms within the soil. It is clear that great attention should be made to use the inoculants.

2- It could be predict that inoculation of sunflower seed by bacterial strains have great effect to improve sunflower root growth. The application will be more useful in increasing growth and seed yield of sunflower under field conditions.

3- It could be predict that great response of sunflower seed when inoculated with A.b& A.l under field conditions.

4- The results indicated to the role of the bacterial strains of A.b& A.l in enhancing and promoting the growth and development of sunflower genotypes. This early studies reveal to the possibility of using this promising inoculants under field condition.

2. Field Experiments:

The field experiments aimed to study the effect of biofertilization and nitrogen fertilizer levels on growth, yield and yield components as well as quality of sunflower genotypes (*Helianthus annus,* L.) Vidoc, Euroflour and Miak. Also, analytical electrophoresis of protein seeds. Three field experiments were carried out at the Experimental Station, Faculty of Agriculture, Mansoura University during the three successive growing seasons of 2000, 2001 and 2002.

A strip-split plot design with three replicates was used. The horizontal strips were randomly assigned to the following the three same sunflower genotypes which used in the pot experiment.

While, the main vertical strips were occupied with the following three biofertilization treatments:

1- Uninoculation (without biofertilization).

2- Inoculation with A.b (No. 40) and A.l (Br-17).

3- Inoculation with B.p (B-32) and B.p (CF-43).

The sub vertical plots were devoted to the following three nitrogen fertilizer levels:

1- 15 kg N/fad.

2- 22.5 kg N/fad.

3- 30.0 kg N/fad.

Each experimental unit consisted of four ridges, 60 cm width and 3.5 meters long, occupying an area of 8.4 square meters (i.e. 1/500 feddan). The following parameters were determined:

2.1. Microbiological measurements:

1- Total bacterial count (CFU x 10^6/g dry soil)

2- Dehydrogenase activity (μg TPF/g dry soil)

2.1. Growth parameters and attributes:

1- Dry matter accumulation (g/plant)

2- Leaf area index (LAI)

3- Crop growth rate (CGR)

4- Relative growth rate (RGR)

5- Net assimilation rate (NAR)

6- Plant height (cm)

7- Stem diameter (cm)

8- Number of leaves/plant.

2.3. Yield and its components:

1- Head diameter (cm).

2- 1000-seed weight (g).

3- Seed yield (g /plant).

4- Seed oil %

5- Seed crude protein %

6- Seed yield (kg /fad)

7- Oil yield (kg /fad)

8- Protein yield (kg /fad)

2.4. Chemical composition of sunflower seeds:

1- Nitrogen percentage

2- Phosphorus percentage

3- Potassium percentage

2.5. Analysis of oil:

1- Acid value:

2- Iodine number:

3- Peroxide value:

2.6. SDS-protein electrophoresis:

Electrophoresis analysis of seed storage proteins is assumed to provide information concerning the type and biosynthesis of different protein fractions. The most important results obtained from this dissertation can be summarized as follows:

A. Sunflower genotypes performance:

1- Regarding microbiological measurements, the results showed that, total count of bacteria and dehydrogenase activity in rhizospher plant (at 45 and 65 days from sowing in all seasons) significantly differed among the three tested sunflower genotypes at age 45 and 65 days from planting, except total bacteria count at 45 days from planting in the second season only. Euroflour genotype surpassed in total bacteria count over other genotypes in the first sample in all seasons, while Miak genotype surpassed in total bacteria count over other genotypes in the second sample in the first, second and third seasons, respectively. Also, Euroflour genotype in DHA in its rhizospher was superior over other genotypes at age 45 and 65 days from planting.

2- Concerning plant growth parameters and attributes, the results indicated that dry matter accumulation at 45 days from planting in the first and second seasons and at 65 days from planting in all seasons, leaf area index at 45 and 65 days after cultivation time in all seasons, CGR, RGR and NAR over all seasons, plant height, stem diameter and number of leaves /plant in the three seasons were significantly differed by genotypes performance. While dry mater accumulation at 45 days from planting and NAR in the third season only were not significantly affected by genotypes performance. It can be noticed that, the highest dry matter values at all samples dates, leaf area

index at 65 days from planting, CGR, RGR, NAR (in the first and second seasons), plant height, stem diameter and number of leaves/plant were resulted from Miak genotype over all seasons. While Euroflour genotype produced the highest values of leaf area index (at 45 days from sowing in all seasons) and NAR in the third season only.

3- Yield and yield components characters i.e. head diameter (in the first and second seasons), 1000-seed weight (in the first and third seasons), seed yield/plant (in the first and second seasons), seed oil and crude protein percentages (in all seasons) as well as seed yield/fad (in the first and second seasons), oil yield/fad (in all seasons) and protein yield/fad (in the first season only) exerted a significant effect due to sunflower genotypes performance. The best genotype was Euroflour which produced the greatest values of yield and its components over all seasons, except crude protein percentage. Whereas, Miak genotype recorded the maximum values of crude protein percentage.

4- With reference to the composition of sunflower seeds such as nitrogen content (%) and potassium content (%) were significantly differed as a result of variation of sunflower genotypes performance in all seasons. Vice versa, phosphorus content (%) was not significantly affect by genotypes performance over all seasons. Euroflour genotype produced the highest values of P% (0.651) and K% (2.851) over all seasons. While, Miak genotype recorded the highest values of nitrogen content in seeds (%) which were 3.38, 3.37 and 3.42% in the first, second and third seasons, respectively. On the other hand, the lowest values of this characters were given by Vidoc genotype.

5- Acid value and iodine number (in the second and third seasons) as well as peroxide value (in all seasons) were significantly affected by genotypes performance. While genotypes performance had no significant effect on acid value and iodine number in the first season only. Euroflour was the best genotype in quality of oil characters which produced the lowest values of acid value and peroxide value and resulted the highest values of iodine number.

B. Biofertilization treatment effects:

1- With regard to the effect of inoculation with the bacterial strains on microbiological characters at two samples after 45 and 65 days from sowing, it was significant. Total bacterial count and DHA in plant rhizospher was markedly increased and achieved maximum values in treatment of inoculation with A.b&A.L compared with the uninoculated seeds (control treatment) in the three seasons (30.7, 35.3 and 41.8 x 10^6 CFU/g dry soil) and (24.0, 26.2 and 29.1 x 10^6 CFU/g dry soil) as well as DHA were (150.3, 156.1 and 154.5 µg TPF/g dry soil) and (108.1, 108.2 and 110.8 µg TPF/g dry soil) at 45 and 65 days from planting in three seasons, respectively.

2- The results indicated that dry matter accumulation/plant and leaf area index (at 45 and 65 days from sowing), as well as, plant height, stem diameter and number of leaves/plant significantly affected by biofertilization treatments in all seasons, except number of leaves/plant which was not significantly affect by biofertilization treatments in the third seasons only. CGR, RGR and NAR were insignificantly affected due to the different biofertilization treatments in all growing seasons. The best treatment was inoculation with A.b&A.L which produced the greatest values of the previous characters except NAR compared with the other treatments.

3- Concerning the effect of biofertilization treatments on yield and its components, the results indicated that yield and its components were significantly increased by different biofertilization treatments over all seasons compared with the control (untreated seed). Inoculation with mixed culture of A.b&A.L produced the highest values of yield and yield components except seed oil percentage which was decreased with inoculation by A.b&A.L. Also, the results showed that the highest seed oil % was recorded from control treatment (uninoculation). On the other hand, the lowest values of yield and its components were resulted from the control treatment (uninoculation).

4- Chemical composition of sunflower seeds such as nitrogen content,

phosphorous content and potassium content were significantly affected by biofertilization treatments in all seasons except nitrogen content (%) in the second season. Therefore, the highest means of N, P and K% were obtained from inoculation with A.b&A.L where results of N content were (3.36, 3.23 and 3.23%), P (0.636, 0.648 and 0.708%) and K (2.632, 2.737 and 3.104%) in the first, second and third seasons, respectively. Whilst, the lowest values of the previous characters were resulted from control treatment (without biofertilization).

5- With regard to the analysis of oil i.e. acid value, iodine number and peroxide value which were significantly affected due to biofertilization treatments in all seasons except iodine number which was not significantly affect by biofertilization treatments in the first and second seasons. Inoculation with A.b&A.l decreased acid value (in all seasons) and peroxide value (in the second and third seasons). The results were (0.653, 0.631 and 0.572) and (6.72 and 7.14). It can be concluded that the best treatment to improve oil quality was inoculation with A.b&A.l. More, the highest iodine number was recorded from inoculation with A.b&A.l.

C. Effect of nitrogen fertilizer:

1- All microbiological characters under study were proved to be significantly increased as a result of nitrogen fertilizer levels at two samples after at 45 and 65 days from planting in all seasons. Raising nitrogen fertilizer levels from 15.0 to 22.5 and 30.0 kg N/fad significantly increased total count of bacteria and dehydrogenase activity at all samples dates in all seasons. Increasing nitrogen fertilizer level up to 30.0 kg N/fad resulted the highest means of all microbiological characters which mentioned above, on the other side, total count of bacteria and DHA were resulted the lowest ones at two samples with adding 15.0 kg N/fad.

2- Growth parameter and attributes (dry matter accumulation/plant, LAI, CGR, plant height, stem diameter and number of leaves/plant were highly significantly increased with increment of nitrogen fertilizer level from 15.0 to 30.0 kg N/fad over all seasons of this experimentation. While RGR and

NAR were significantly decreased with raising nitrogen fertilizer level from 15.0 to 30.0 kg N/fad over all seasons, except RGR which was not significantly affected by nitrogen fertilizer levels in the first season only. Fertilizing sunflower plant with 30.0 kg N/fad gave the highest values of dry matter/plant, LAI at the two samples dates as well as CGR, plant height, stem diameter and number of leaves/plant. Whilst, increasing nitrogen fertilizer level up to 30.0 kg N/fad produced the lowest values of RGR and NAR over all seasons.

3- Increasing nitrogen fertilizer rates from 15.0 through 30.0 kg N/fad significantly increased head diameter, 1000-seed weight, seed yield/plant and seed yield/fad as well as oil yield/fad, protein percentage and protein yield/fad in all seasons. However, it significantly reduced seed oil percentage. Maximum yield and its components were produced with the addition of 30.0 kg N/fad. However, maximum seed oil percentage was produced with adding 15 kg N/fad.

4- The increase in nitrogen fertilizer rates from 15.0 through 30.0 kg N/fad significantly increased nitrogen percentage, phosphorous percentage and potassium percentage in seeds over all seasons.

5- Application of 30.0 kg N/fad produced the lowest values of acid value and peroxide value in all seasons. Vice versa, maximum iodine value was produced with adding 30.0 kg N/fad and there was insignificant difference between 22.5 and 30.0 kg N/fad in acid value and iodine value.

D. Interaction effects:

1- The interaction between sunflower genotypes and nitrogen fertilizer levels had a significant effect on dry matter accumulation/plant (45 and 65 days from planting), LAI (at 45 and 65 days from planting), CGR, RGR and NAR as well as plant height and stem diameter. The maximum values of these characters were noticed with Miak genotype when fertilized with 30.0 kg N/fad. Also, the interaction between sunflower genotypes and biofertilization treatments had a significant effect on LAI at 45 and 65 days from planting in all seasons. The highest values of LAI at 45 days from

sowing resulted from planting Euroflour genotype when inoculated seed with A.b&A.l, the results were 3.33, 3.85 and 3.70 in the first, second and third seasons, respectively. While, the maximum values of LAI at 65 days from cultivation were recorded from Miak genotype and inoculated seed with A.b&A.l, which were 6.38, 6.42 and 6.44 in the first, second and third seasons, respectively. Moreover, the interaction between biofertilization treatments and nitrogen fertilizer levels had a significant effect on dry matter accumulation/plant and LAI at 45 and 65 days from planting as well as plant height over all seasons. The highest values of dry matter accumulation (g/plant) and the highest values of LAI at 45 and 65 days from sowing as well as the tallest plants were obtained from inoculation with A.b&A.l and fertilized plants with 30.0 kg N/fad over all seasons.

2- With regard to the interaction between sunflower genotypes and biofertilization treatments, it had a significant effect on head diameter, seed yield/plant, crude protein percentage as well as seed and oil yields/fad. The highest values of head diameter and seed yield/plant as well as seed and oil yields/fad were resulted from planting Euroflour genotype and inoculation with A.b&A.l. Whilst, the highest values of crude protein percentage was obtained from planting Miak genotype and inoculation with A.b&A.l or B.p whereas there was not significant differences between inoculation with A.b&A.l and B.p strains. Also, the interaction between sunflower genotypes and nitrogen fertilizer levels had a significant effect on seed yield/plant as well as seed and oil yields/fad in the first season only. The maximum values of seed yield/plant, seed yield/fad and oil yield/fad were noticed with Euroflour genotype and addition of 30.0 kg N/fad. The results were 50.2 g/plant, 1756.9 kg /fad and 754.4 kg /fad in the first season only. In addition, the interaction between biofertilization treatments and nitrogen fertilizer levels had a significant effect on crude protein percentage in the second season. The highest percent of crude protein was resulted from inoculation with mixed cultures of A.b&A.l and adding 30.0 kg N/fad which were 22.3% in the second season only.

3- Concerning the interaction between sunflower genotypes performance and biofertilization treatments, it had a significant effect on protein yield/fad,

nitrogen percentage and potassium percentage. The highest values of protein yield/fad and potassium percentage were recorded from Euroflour genotype and inoculation with A.b&A.l, the results were (357.2 and 375.6 kg /fad) and (2.74 and 2.87%) in the first and second seasons, respectively. Meanwhile, the highest value of nitrogen percentage was noticed from Miak genotype and inoculation with A.b&A.l strains which were 3.42% in the second season only. More, the interaction between biofertilization treatments and nitrogen fertilizer levels had a significant effect on protein yield/fad. Maximum values of protein yield/fad was resulted from Euroflour genotype when fertilized with 30.0 kg N/fad, the results were 395.1 and 398.4 kg /fad in the first and second seasons, respectively. The interaction between biofertilization treatments and nitrogen fertilizer levels had a significant effect on protein yield/fad, nitrogen content in seeds and peroxide value. The maximum values of protein yield/fad, nitrogen content were obtained from inoculation with A.b&A.l and addition of 30.0 kg N/fad which were (429.3 kg/fad) and (3.57%) in the second season only. On the other hand, the lowest values of these characters were resulted from uninoculated seeds and adding 15.0 kg N/fad. While, the highest peroxide value was recorded from the control treatment and adding 15.0 kg N/fad. Also, the lowest values of peroxide value was obtained from inoculation with B.p strains and application of 30.0 kg N/fad which were 6.47, 6.28 and 6.46 mq/kg oil in the first, second and third seasons, respectively.

4- With concerning to SDS- PAGE electrophoresis in protein seeds of sunflower genotypes under biofertilization treatments uninoculation seeds and inoculation with different bacterial strains. The molecular weight of protein bands was gradually decreased from first number of band up to the latest band.

5- In Vidoc genotype the molecular sizes of bands were ranged between 148.16 and 79.59 kDa. The maximum number of bands (15) was achieved at the level 30.0 kg N/fad. The inoculation with strain A.b&A.l and adding 15.0 kg N/ fad resulted in 15 bands. While, the inoculation with strain B.p and adding 30.0 kg N/fad led to 13 bands. These results indicated that the inoculation with A.b&A.l with 15.0 kg N/fad and nitrogen fertilizer with

30.0 kg N/fad only are the best treatments for increasing seed storage protein. However, the cost of treatment and freedom of environmental pollution should be considered.

6- In Euroflour genotype, the results indicated that the treatments with uninoculation with 15.0 kg N/fad, inoculation by A.b&A.l with 22.5 kg N/fad and inoculation by B.p with 15.0 kg N/fad have the same effect on storage seed protein (16 bands). Also, the molecular sizes of bands were ranged between 125.93 and 8.6 kDa.

7- With regard to Miak genotype, the results showed that the molecular sizes of bands were ranged between 219.3 and 18.49 kDa. The number of bands was ranged from 14-17 bands which not necessarily present in all treatments. The results showed that no difference between the three treatments of nitrogen fertilization and the three treatments of inoculation with strain A.b&A.l. Also, the results indicated that the inoculation is better than nitrogen fertilizer only and the inoculation by B.p with 22.5 kg N/fad is the best treatment.

8- Generally, we can conclude that the genotypic variation was the main factor affected the utilization of the different treatments whether with uninoculation or inoculation treatments.

Conclusion

From the previous results, it can be concluded that planting inoculated seeds with *Azospirillum brasilense* and *Azospirillum lipoferum* of the genotype Euroflour and adding 30.0 kg N/fad will achieve high productivity and good quality of sunflower under Mansoura district, Dakahlia Governorate.