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Influence Of Foliar Application Of Bio-Regulators On Yield And Quality Of Egyptian Clover Seed

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Abstract: A field experiment was conducted at Sids Agricultural Research Station, ARC, during 2018/19 and 2019/20 winter seasons. This study amid to detect the effect of treatment of bio-regulators at flower initiation phase to estimate more flowering, enhance seed yield production and quality of Egyptian clover var. Giza 6 (*Trifolium alexandrinum*, L.). Eight treatments were applied i.e., T_1: Control (untreated), T_2: Naphthalene acetic acid (20 mg L^{-1}), T_3: Naphthalene acetic acid (30 mg L^{-1}),T_4: Salicylic acid (40 mg L^{-1}), T_5: Salicylic acid (60 mg L^{-1}), T_6: Potassium nitrate (1%), T_7: Potassium nitrate (2%) and T_8: 50 kg fed^{-1} potassium sulphate (soil application) as a farmer treatment. The study was conducted using the design of complete randomized block with three replications. The plants were foliar sprayed three times with bio-regulators at weekly intervals starting from the beginning of flowering. Four cuttings of green fodder, the first one was taken after about 55 days after planting and the following cuts were taken after about 30 days after the previous cut. After the fourth cut, the plants are allowed to grow without cutting to produce the seed. Current data indicated that foliar spraying of bio-regulators of all treatments under study increased significantly seed yield and its quality of Egyptian clover as compared to the control (untreated). Foliar treatment of salicylic acid at 40mgL^{-1} and potassium nitrate at 2% showed significantly higher values of heads m^{-2}, seeds $head^{-1}$, 1000-seed weight, seed yield and seed quality traits which were significantly higher than other foliar application treatments. Naphthalene acetic acid (20 mg L^{-1}) appeared lower impact on seed yield and seed quality, but was significantly higher than untreated plants.

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1. Introduction

Egyptian clover (*Trifolium alexandrinum*, L.) is the main forage crop grown in Egypt during the winter season. Seed production of Egyptian clover is mostly depressed due to the lack of interest of farmers to seed production which led to decreased seed production. As farmers continue to take green forage cuts until mid-April, which leads to low leaf retention and poor flowering, and this results in reduced seed production. Dominate hot and drought conditions during flowering and the formation of Egyptian clover seeds lead to a reduction of good quality seeds. The yield of seed can be improved by optimum use of cuttings and the foliar spray with growth bio-regulators. In addition, the adoption of enhanced agricultural procedures and foliar application of plant growth regulators may improve the yield of seeds (**Meena et al., 2017).**

Abd Al-Naby and Sakr (2012) reported that high temperature and low relative humidity during the breeding period lead to pollen viability, and thus it is one of the main factors responsible for the shortage in Egyptian clover seed production. Also, **Bakheit et al., (2012)** found that seed production of Egyptian clover also affected by the environmental conditions like temperature and relative humidity epically at the seed formation stage. In addition, **Mohammed and Tarpley (2011)** reported that high temperature especially during flowering stimulates respiration and reduces net photosynthesis. Likewise, the physiological loss of the pollinated flowers and the increased abortion of embryos in Egyptian clover lead to a shortage of the resulting seeds. **(Iannucci, 2001)**. **Abd El-Naby and Sakr, (2012)** demonstrated that temperature ranged from (28 ◦C to 32 ◦C), a relative humidity between 45% to 55%, little difference in day and night temperatures and sufficient sunshine hours may be the major environmental factors in Egypt for producing highest seed yield of Egyptian clover. Abiotic stresses such as heat stress in field crops can be controlled by foliar application of bio-stimulators like salicylic acid (C6H4 (OH) COOH) and potassium nitrate (KNO3), as they are able to increase heat tolerance in the long term **(Wahid et al., 2007),** and supports to enhance flowering and pod formation in clovers **(Patil et al., 2005; Hayat et al., 2009; Zhang et al., 2009)**.

Plant growth regulators can be defined as organic compounds or nutrients, these materials may be natural occurring or synthetic compounds and are exogenously treated directly to plants to change their metabolic processes or structure in a way that is beneficial to enhance yield, increasing quality or facilitate harvesting **(Meena et al., 2017)**. Salicylic acid plays many physiological functions in plants involving thermo genesis, flower formation, nutrient absorption, stomatal opening and closure processes, inhibit the production of ethylene, improving the photosynthetic machinery, and hence enhance in seed yield **(Hayat et al., 2009; Mohammed and Tarpley, 2011).** Osmoprotectants such as potassium nitrate (KNO3) play an essential role in plant abiotic stresses recovery through their imact on water absorption, root growth, preserve the turgour pressure and Thus, the plant cells help to restore their normal functions **(Bonomelli and Ruiz, 2010)**. Also, to increase yield of alfalfa (*Medicago sativa*) and Vigna radiate, naphthalene acetic acid (NAA) is bio-regulator known to delay the abscission of flowers and controls the fall of flowers and pods **(Zhang et al., 2009; Patil et al., 2005).**

The foliar treatment of bio-stimulants at early phase of flowering is known to stimulate more flowering as well as seed formation and enhance the seed production. Therefore the current experiment was intended to estimate the impact of various concentrations of salicylic acid (as ethylene production inhibitors), potassium nitrate (as an osmoprotectants), NAA (as a growth hormone) and potassium sulphate (farmer application) on seed yield and quality of Egyptian clover in Egypt.

2. Materials and Methods

I- Field work

The present study was conducted on the experimental farm of Sids Agricultural Research Station, ARC, during two successive seasons (2018/19 and 2019/20) to study the effect of treatment of bio-regulators at flower beginning phase to stimulate further flowering and enhance seed yield production and quality of Egyptian clover var. Giza 6 (*Trifolium alexandrinum*, L.). All recommended cultural practices by the Forage Crops Research Dept. Field Crops Res. Inst., ARC, for the old land had been followed during the two seasons. The physical and chemical characters of samples representing the experiment soil were studied, soil samples were collected from a layer of 20 cm thickness, then air dried and sifted through a 2 mm sieve using the methods reported by **Piper (1950)** and **Jackson (1973),** and cleared in Table (1).

The experiment was sown on 30 October in the first and the second seasons, respectively. Seeds were drilled in plots 2 X 3 m (1/700 fed.) during the two seasons, seeding rate was 20 kg fed^{-1} (30 g plot^{-1}) were equally distributed within each plot.

The following foliar bio-regulators treatments were tested:

(T_1) Control treatment (untreated).
(T_2) Naphthalene acetic acid (NAA) at 20 mg L^{-1}.
(T_3) Naphthalene acetic acid (NAA) at 30 mg L^{-1}.
(T_4) Salicylic acid at 40 mg L^{-1}.
(T_5) Salicylic acid at 60 mg L^{-1}.
(T_6) Potassium nitrate (KNO_3) at 1%.
(T_7) Potassium nitrate (KNO3) at 2%.
(T_8) 50 kg fed^{-1} potassium sulphate (soil application) as a farmer treatment.

The study was conducted using the design of complete randomized block with three replications. The plants were foliar sprayed three times with bio-regulators at weekly intervals starting from the beginning of flowering.

Four cuttings of green fodder were taken after about 55 days after planting and the following cuts were taken after about 30 days after the previous cut. After the fourth cut, the plants are allowed to grow without cutting to produce the seed and the seed crop was collected when the seeds in pods turned yellow.

Studied characters:

Forage yield and its components:

- Plant height (cm).
- Leaf /stem ratio.
- Number of tillers plant^{-1}.

- Fresh forage yield (ton fed^{-1}): Plants of the whole plot were hand cutted and weighed in kg $plot^{-1}$ and transformed to fresh forage yield (ton fed^{-1}).
- Dry forage yield (ton fed^{-1}): Samples of 100 gm were dried at 105 C^0 to fixed weight and dry matter percentages were recorded.
- Dry forage yield = Fresh forage yield X Dry matter percentage.

Table 1. Some physical and chemical properties of the experimental soil in 2018/19 and 2019/20 growing seasons.

Property	Value	
	Season 2018/19	Season 2019/20
Particle size distribution :		
Crosse sand %	5.90	5.83
Sand %	22.18	23.37
Silt %	30.52	29.57
Clay %	41.70	40.68
Texture grade	Clay	Clay
$CaCO_3$ (%)	6.14	6.10
pH	7.81	7.80
E.C (dS m^{-1})	3.50	3.52
Soluble cations and anions (meq L^{-1}):		
Ca^{++}	8.22	8.10
Mg^{++}	5.88	5.05
Na^+	18.34	18.86
K^+	0.85	0.83
$CO^=_3$	0.00	0.00
HCO^-_3	2.28	2.81
Cl^-	12.33	13.12
$SO^=_4$	17.68	17.39
Organic matter (%)	0.75	0.73
Total soluble N (mg kg^{-1})	44.12	44.20
Available-P (mg kg^{-1})	4.89	4.65
Available-K (mg kg^{-1})	179.50	173.16
DTPA-extractable (mg kg^{-1}):		
Fe	2.88	2.12
Mn	1.77	1.41
Zn	0.69	0.73
Cu	0.63	0.58

Seed yield and its components:

Number of heads m^{-2}, number of seeds $head^{-1}$, 1000-seed weight (gm), seed yield (kg fed^{-1}).

Seed quality characters:

Laboratory experiments were carried out at Seed Technology Research Department Lab, Field Crops Research Institute, ARC, Giza, to assess seed quality as affected by experimental treatments in the field experiment**.**

Vigor test:

Germination percentage (%):

Germination (%) was determined according to the methods outlined in procedures for seed testing **(ISTA, 1999)**. Three counts of 50 seeds each from each treatment in three replications were grown folded filter papers and then incubated in an incubator at 20°C for one week.

Shoot and radical length of seedling (cm):

Ten seedlings were randomly selected and recorded in cm as described by **Krishnasamy and Seshu (1990)**.

Fresh and dry weight of seedling (g):

After measured shoot and radical length the seedlings were weighed to get fresh weight and then put into paper packet separately and dried in the preheated oven, and their weights were recored at 70 °C for 12 hours.

Germination index:

Germination index was calculated using the following equation according to **Copeland (1976)**:

$$\text{Germination index} = \frac{\text{Number of seeds germinated (1}^{st}\text{ count)}}{\text{Number of days to first count}} + \frac{\text{Number of seeds germinated (last count)}}{\text{Number of days to last count}}$$

Seedling vigor index:

Seedling vigor index was detected as described by **Reddy and Khan (2001)** equation**:**

Seedling vigor index (1) = Seedling length (cm) X Germination percentage

Seedling vigor index (2) = Seedling dry weight (g) X Germination percentage

Chemical analysis:

The oven dried plant materials were wet digested using a mixture of pure perchloric acid ($HClO_4$) and sulfuric acid (H_2SO_4) at a ratio of 1:1, as cleared by **Jackson (1973)**. Nitrogen was estimated using the micro-Kjeldahel procedure, phosphorus was detected Spectrophotometrically using ammonium molybdate and stannus chloride reagents, and potassium was also detected Flamephotometerically **(Page et al., 1982)**. Seed crude protein percentage was calculated by multiplying N% by 6.25 according to **A.O.A.C (2000)**. Micronutrients concentration (Fe, Zn, Mn and Cu) was detected by using the Atomic Absorption Spectrophotometer according to **Cottenie et al. (1982)**. Carbohydrate percentage in seeds was assayed according to the methods described by **A.O.A.C. (2000)**.

Statistical analysis:

The obtained results were statistically analyzed by methods reported by **Steel et al. (1997)** using the computer program PLABSTAT **Utz (2004)**. Means were compared by least significant differences (LSD) at 5% level. Bartlett's test was done to test the homogeneity of error variances. The test was non-significant for all traits, thus combined analysis for the two seasons was formed for all studied traits.

3. Results and Discussion

Means of plant height, leaf/stem ratio, tillers plant^{-1}, fresh and dry forage yields as affected by various foliar application on Egyptian clover at the first and second season and their combined are presented in Table (2). Data of the combined analysis showed that the foliar application treatments were applied at weekly interval starting from flowering initiation, so there are no significant differences between means of green and dry forage yield and its components were observed.

Seed yield and its components

Heads number (m^{-2}):

Data of the combined analysis showed that application of all the bio-regulators significantly affected heads number (m^{-2}), it was significantly increased as compared to the untreated plants (Table 2). The highest heads m^{-2} were estimated with treatment T_4 (salicylic acid at 40 mg L^{-1}), it was 16.6% higher than the control which was statistically being significantly better than the other bio-regulators. Enhance in heads m^{-2} was recorded by 12.2% and 14.9% with the foliar application of T_6 (1% KNO3) and T_7 (2% KNO3), respectively. Salicylic acid is known to be concomitant with high tolerance to high temperature in plants therefore decreasing physiological loss of pollinated flowers and improving photosynthetic efficiency in several plants (**Mohammed and Tarpley, 2011**), which enhanced Egyptian clover to detained higher number of heads per plant.

Significant increase in heads m^{-2} (7.0- 4.0%) with the application of T_3 (NAA at 30 mg L^{-1}) and T_2 (NAA at 20 mg L^{-1}), respectively were estimated than untreated plants and might be resulted by decreased flower and unripe pod descent, it prevents the formation of the separation zone that results in the formation of more heads and is kept on the plants. **Zhang et al. (2009)** reported that foliar spray of NAA (20–40 mg L^{-1}) on alfalfa significantly increased seed yield over control. **Kumar et al. (2013)** reported similar trend in Egyptian clover, they found that application of all bio-regulators increased head density than the untreated plants and the highest heads m^{-2} were determined with salicylic acid at 50 mg L^{-1}. On the other hand, application of potassium sulphate T_8 (50 kg fed^{-1}) as soil treatment led to increase in head number m^{-2} by 8.6% than the control treatment.

Seeds head^{-1}:

Results of the combined analysis showed that application of all foliar treatment treatments recorded significantly maximum values of seeds head^{-1} than the control treatment (Table 2). It's clear that foliar application of T_4 (salicylic acid at 40 mg L^{-1}) and T_7 (potassium nitrate 2%) gave 36.6% and 34.6% higher value of seeds head^{-1} than control treatment, respectively. These results are in accordance with those reported by **Sharma and Kaur (2003)** who showed that foliar treatment of 50 mg L^{-1} salicylic acid caused significantly enhance seeds pod^{-1} of soybean than the untreated plants. Formation of high number of seeds might be a cause for enhance in seeds head^{-1} with foliar treatment of salicylic acid and KNO3. Also, Salicylic acid can save cell membranes and preserve their structure, and recovery the toxic and harmful impacts of reactive oxygen species. So, increase uptake and movement of minerals element from the soil to the plants cell **(Dicknson et al., 1991; Hayat et al., 2009)**.

Spraying NAA at 20 mg L^{-1} (T_2) also helped in increase seed head^{-1} by 4.2% over the control treatment. These data are in agreement with those obtained by **Zhang et al. (2009) and Patil et al. (2005)** who reported that significant enhance in yield attributing characters such as flowers and pods plant^{-1} because of better transport of photo assimilates over untreated with foliar application of NAA in alfalfa and mungbean. Also, Application of potassium sulphate at 50 kg fed^{-1} (T_8) as soil treatment led to increase number of seed head^{-1} by 14.8% than the control treatment.

Weight of 1000-seed:

Data in (Table 2) showed the combined analysis of 1000-seed weight, it was significantly increased with all foliar application treatments of the bio-stimulants in comparison to the untreated plants, exclude the naphthalene acetic acid at 20 mg L^{-1} (T_2). Salicylic acid at 40 mg L^{-1} (T_4) gave the highest 1000-seed weight, with enhance of 12.1% as compared to the untreated plants.

The explanation of this results was reported by **Mohammed and Tarpley (2011)** who found that, this increase in 1000-seed weight may be due to the enhance in translocation of photosynthates to the seed under foliar treatment of salicylic acid, might be causing the improvment the 1000-seed weight.

Treatment with KNO3 at 1% (T_6) and KNO3 at 2% (T_7) recorded 7.6–9.0% raising the 1000-seed weight and was showed to be significantly in comparison with the untreated plants. It is may be due to the effect of spraying KNO3 on leaves of plant caused improve in chlorophyll content, enzyme activity, enhance synthesis and translocation of photosynthates, and raise water absorption and root permeability and might be causes for improving 1000- seed weight. **Bardhan et al. (2007)** found that foliar spraying of KNO3 (100 mg L^{-1} and 200 mg L^{-1}) at 40 and 60 days after planting in chickpea (*Cicer arietinum*) gave significant increase in seed weight than untreated plants.

Application of NAA at 20 mg L^{-1} (T_2) and NAA at 30 mg L^{-1} (T_3) recorded 2.8% and 4.2% increase in 1000-seed weight superior to control treatment, respectively. These data are in agreement with those reported by **Patil et al. (2005)** who reported that higher seed weight of mungbean with the treatment of NAA (25 mg L^{-1}) as foliar spray over untreated plants. Also, soil application of potassium sulphate at 50 kg fed^{-1} (T_8) recorded 6.2% increase in 1000-seed weight superior to the control treatment.

Seed yield (kg fed^{-1}):

Data presented in (Table 2) showed the combined analysis of seed yield. The highest seed yield was observed by using salicylic acid as compared to other treatments. The highest values were reported with the foliar treatment of salicylic acid at 40 mg L^{-1} (T4) which was 22.1% higher than untreated plants. The increase in seed yield with foliar spray of salicylic acid at 40 mg L^{-1} caused the higher number of heads m^{-2}, seeds $head^{-1}$ and seed weight (Table 2). These results were in agreement with **Hayat et al., (2009); Mohammed and Tarpley, (2011) and Kumar et al., (2013)** they found that salicylic acid plays diverse physiological roles in plants and Hence increase in seed yield. While, application of salicylic acid at 60 mg L^{-1} (T5) was 11.4% higher than the control treatment. These results are agreed with those found by **Amin et al. (2007)** who mentioned that increasing the concentration of salicylic acid to 100 mg L^{-1} significantly reduced the seed yield likely due to detrimental effect on the photosynthetic activity of plant.

Spraying of KNO3 at 1% (T_6) and KNO3 at 2% (T_7) concentrations gave the respective 13.8% and 15.4 % higher seed yield than control treatment. Also the enhancement in seed yield with the treatment of KNO3 is paralleled with the obtained improvement in heads m^{-2}, seeds $head^{-1}$ and1000-seed weight (Table 2). Same increase by foliar spray with KNO3 in seed yield was observed by **Bardhan et al., (2007).** Foliar spray with NAA at 20 mg L^{-1} (T_2) and NAA at 30 mg L^{-1} (T_3) recorded 3.1% and 6.4 % higher seed yield as compared with untreated plants because of the enhance in yield contributing characters (Table 2). Seed yield improving up to 22.1% in mungbean and harvest index by 10% in alfalfa had been recorded by **(Patil et al., 2005 and Zhang et al., 2009)** with the foliar spraying of NAA at 30 and 40 days after sowing in mungbean and at flower bud formation phase in alfalfa.

From the recorded results it is noted that NAA at 20 mg L^{-1} was the least effective in improving seed yield of Egyptian clover among all tested treatments, and it found to be significantly better than untreated control.

Soil application of potassium sulphate at 50 kg fed^{-1} (T_8) recorded 7.7% increase in seed yield over that of the control treatment

Year's effect:

Results in Table (3) show that crop characters and seed yield of Egyptian clover in the two years (results collected across foliar applications). Plant height and leaf/stem ratio did not significantly differ among the two years. Significantly higher number of heads m^{-2} was reported in the first season (2018/19) was 2.5 % higher than in the second one (2019/20). Number of seeds $head^{-1}$ was 1.6% higher in 2018–19 than in 2019–20. Weight of 1000-seed was significantly higher by 0.66% in 2019–20 compared to that in 2018–19. Seed yield was about 3% higher in 2018–19 than in 2019–20 due to higher number of heads m^{-2} and seeds $head^{-1}$ (Table 3).

Table 2. Effect of different foliar application on plant height (cm), leaf/stem ratio, tillers plant^{-1}, fresh forage yield (ton fed^{-1}), dry forage yield (ton fed^{-1}), heads number (m^{-2}), seeds head^{-1}, 1000-seed weight (gm) and seed yield (kg fed^{-1}) of Egyptian clover.

Treatment	Plant height (cm)	Leaf/ Stem Ratio	Tillers plant^{-1}	Fresh forage yield (ton fed^{-1})	Dry forage yield (ton fed^{-1})	Heads (m^{-2})	Seeds head^{-1}	1000-seed weight	Seed yield (kg fed^{-1})
	First season 2018/2019								
T_1	77.01	0.46	6.53	53.63	6.25	325.21	38.54	2.83	397.56
T_2	75.50	0.44	6.74	58.46	6.34	334.35	40.59	2.94	415.55
T_3	76.50	0.45	6.92	57.44	6.30	342.28	42.85	2.96	428.44
T_4	77.00	0.46	7.47	57.38	6.36	374.73	54.63	3.25	498.66
T_5	77.25	0.43	7.06	56.43	6.21	356.52	47.66	3.08	448.00
T_6	77.42	0.43	7.11	55.68	6.08	361.61	51.33	3.11	456.22
T_7	76.42	0.44	7.32	56.99	6.25	369.81	53.22	3.15	456.89
T_8	76.75	0.44	7.01	54.92	6.13	351.65	45.18	3.08	429.81
LSD 0.05	2.62ns	0.04ns	0.51ns	3.36ns	0.39ns	7.34	2.34	0.11	8.19
	Second season 2019/2020								
Treatment	**Plant height (cm)**	**Leaf/ Stem Ratio**	**Tillers plant^{-1}**	**Fresh forage yield (ton fed^{-1})**	**Dry forage yield (ton fed^{-1})**	**Heads (m^{-2})**	**Seeds head^{-1}**	**1000-seed weight**	**Seed yield (kg fed^{-1})**
T_1	77.21	0.47	6.25	54.00	6.58	311.33	39.61	2.94	393.33
T_2	79.00	0.44	6.38	54.50	6.79	327.64	40.87	3.00	400.00
T_3	78.67	0.44	6.79	54.00	6.62	338.81	42.65	3.05	413.34
T_4	78.67	0.46	7.41	54.88	6.91	367.58	52.14	3.23	466.67
T_5	78.42	0.45	7.02	54.11	6.76	348.52	45.33	3.06	433.33
T_6	78.67	0.46	7.16	56.34	7.16	352.64	50.89	3.11	443.96
T_7	78.58	0.44	7.25	53.66	6.57	361.39	51.95	3.14	455.56
T_8	78.00	0.45	6.91	54.83	6.85	339.92	44.52	3.06	422.22
LSD 0.05	1.17 ns	0.12ns	0.74 ns	2.28 ns	0.43 ns	5.63	1.98	0.17	7.86
	Combined								
Treatment	**Plant height (cm)**	**Leaf/ Stem Ratio**	**Tillers plant^{-1}**	**Fresh forage yield (ton fed^{-1})**	**Dry forage yield (ton fed^{-1})**	**Heads (m^{-2})**	**Seeds head^{-1}**	**1000-seed weight**	**Seed yield (kg fed^{-1})**
T_1	77.11	0.47	6.39	53.82	6.42	318.27	39.08	2.89	395.45
T_2	77.25	0.44	6.56	56.48	6.58	331.00	40.73	2.97	407.78
T_3	77.59	0.45	6.86	55.72	6.47	340.55	42.75	3.01	420.89
T_4	77.84	0.46	7.44	56.13	6.65	371.16	53.39	3.24	482.67
T_5	77.84	0.44	7.04	55.27	6.49	352.52	46.50	3.07	440.67
T_6	78.05	0.45	7.14	56.01	6.62	357.13	51.11	3.11	450.09
T_7	77.50	0.44	7.29	55.33	6.42	365.60	52.59	3.15	456.23
T_8	77.38	0.45	6.96	54.88	6.50	345.79	44.85	3.07	426.02
LSD 0.05	1.54 ns	0.07 Ns	0.41 ns	19.00 ns	1.65 ns	4.47	1.33	0.11	10.45

T_1= Control (untreated), T_2= Naphthalene acetic acid (20 mg L^{-1}), T_3= Naphthalene acetic acid (30 mg L^{-1}), T_4= Salicylic acid (40 mg L^{-1}), T_5= Salicylic acid (60 mg L^{-1}), T_6= Potassium nitrate (1%), T_7= Potassium nitrate (2%), T_8= Potassium sulphate (50 kg fed^{-1}).

Table 3. Crop characters and seed yield of Egyptian clover in different two years (Results collected across foliar applications).

Year	Plant height (cm)	Leaf/ Stem ratio	Tillers $plant^{-1}$	Fresh forage yield (ton fed^{-1})	Dry forage yield (ton fed^{-1})	Heads (m^{-2})	Seeds $head^{-1}$	1000-seed weight	Seed yield (kg fed^{-1})
First season 2018/2019	76.73	0.44	7.02	56.37	6.24	352.02	46.75	3.05	441.39
Second season 2019/2020	78.40	0.45	6.90	54.54	6.78	343.48	46.00	3.07	428.55
LSD 0.05	2.14ns	0.02ns	0.64ns	1.71	0.47	6.34	0.28	0.01	5.37

Seed quality:

Results in Table (4) showed that all the bio-stimulants caused significant effect on all the traits of seedling characters (Table 4). Salicylic acid at 40 mg L^{-1} (T_4) cleared highest enhance in seed germination percentage (24.1%), germination index (70.2%) shoot length (69.0%), radical length (32.2%), seedling fresh weight (63.6 %), seedling dry weight (300.0 %), seedling vigor index 1 (109.8%) and seedling vigor index 2 (397.8%) followed by KNO3 at 2% (T_7) and KNO3 at 1% (T_6) as compared to the control. NAA at 20 mg L^{-1} (T_2) was the least effective in improving seed quality. From this results it can be noted that salicylic acid at 40 mg L^{-1} (T_4) reported the highest improve in all seed quality traits, this due to the protective and growth promoting effects of salicylic acid because of production of healthy and plump seeds, which detected an improve in germination % and better seedling growth. **Gutierrez-Coronado et al., (1998)** also found that foliar application of salicylic acid significantly increased the growth of shoots and roots in either greenhouse or field condition. In addition, **Meena et al., (2017)** reported that salicylic acid led to production of healthy seeds which resulted in better seedling growth. On the other hand, the increase in concentration of salicylic acid to 60 mg L^{-1} reduced the seed quality traits of Egyptian clover as compared to 40 mg L^{-1}.

Table 4. Seedling characters of Egyptian clover as affected by different foliar application treatments (mean of two seasons).

Treatment	Germination (%)	Germination Index	Shoot length (cm)	Radical length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Seedling vigor index (1)	Seedling vigor index (2)
T_1	69.67	6.27	3.13	2.61	0.33	0.02	218.07	1.39
T_2	70.00	6.47	3.19	2.79	0.37	0.02	223.30	1.40
T_3	70.33	7.26	4.25	2.95	0.40	0.02	298.90	1.41
T_4	86.48	10.67	5.29	3.45	0.54	0.08	457.48	6.92
T_5	77.00	8.93	4.52	3.10	0.43	0.03	348.04	2.31
T_6	77.00	9.37	4.60	3.13	0.44	0.04	354.20	3.08
T_7	81.00	10.47	4.88	3.23	0.48	0.06	395.28	4.86
T_8	71.33	7.83	4.33	3.09	0.43	0.03	308.86	2.14
Mean	75.35	8.41	4.27	3.04	0.43	0.04	321.55	2.78
LSD 0.05	1.37	0.68	0.78	0.62	0.10	0.001	5.78	0.14

$T_{1=}$ Control (untreated), T_2= Naphthalene acetic acid (20 mg L^{-1}), T_3= Naphthalene acetic acid (30 mg L^{-1}),T_4= Salicylic acid (40 mg L^{-1}), T_5= Salicylic acid (60 mg L^{-1}), $T_{6=}$ Potassium nitrate (1%), T_7= Potassium nitrate (2%), $T_{8=}$ Potassium sulphate (50 kg fed^{-1})

Results in Table (5) and Figs. (1-4) showed seed quality of Egyptian clover as affected by different foliar application treatments, results indicated that N, P, K, crude protein, carbohydrate percentage and microelements (Fe, Zn, Mn, and Cu) contents were significantly affected by different foliar application treatments. Salicylic acid at 40 mg L^{-1} (T_4) gave the highest values of all characters followed by Potassium nitrate at 2% (T_7).

Salicylic acid at 40 mg L^{-1} (T_4) cleared highly improve in seed nitrogen percentage (11.1%), phosphor percentage (12.5%), potassium percentage (52.6%), Crude protein percentage (11.1%), Carbohydrate percentage (4.2 %), Fe (ppm) (22.4%), Zn (ppm) (18.2%), Mn (ppm)

(31.1%) and Cu (ppm) (100.3%) followed by Potassium nitrate at 2% (T_7) and Potassium nitrate at 1% (T_6) as compared to the control. The superiority effect of salicylic acid may be due to its role in ion uptake and transport **Khan et al., (2003).**

Table 5. Seed quality of Egyptian clover as affected by different foliar application treatments (mean of two seasons).

Treatment	N (%)	P (%)	K (%)	Crude protein (%)	Carbohydrate (%)
T_1	2.44	0.32	1.16	15.25	71.27
T_2	2.55	0.33	1.26	15.94	72.10
T_3	2.56	0.33	1.38	16.00	72.47
T_4	2.71	0.36	1.77	16.94	74.27
T_5	2.63	0.34	1.54	16.44	73.07
T_6	2.65	0.34	1.55	16.56	73.27
T_7	2.68	0.35	1.64	16.75	73.40
T_8	2.57	0.33	1.46	16.06	72.50
Mean	2.60	0.34	1.47	16.24	72.79
LSD 0.05	0.10	0.04	0.27	0.40	0.21

T_1= Control (untreated), T_2= Naphthalene acetic acid (20 mg L^{-1}), T_3= Naphthalene acetic acid (30 mg L^{-1}), T_4= Salicylic acid (40 mg L^{-1}), T_5= Salicylic acid (60 mg L^{-1}), T_6= Potassium nitrate (1%), T_7= Potassium nitrate (2%), T_8= Potassium sulphate (50 kg fed^{-1}).

Seed content of some microelements (Fe, Zn, Mn, and Cu) contents of seed of Egyptian clover as affected by different foliar application treatments (mean of two seasons).

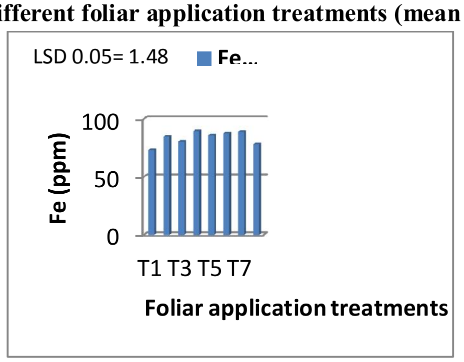

Fig. 1. Effect of foliar application treatments on Fe (ppm)

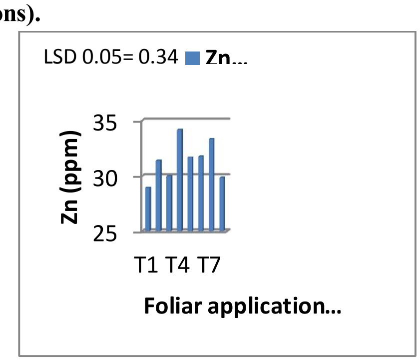

Fig. 2. Effect of foliar application treatments on Zn (ppm)

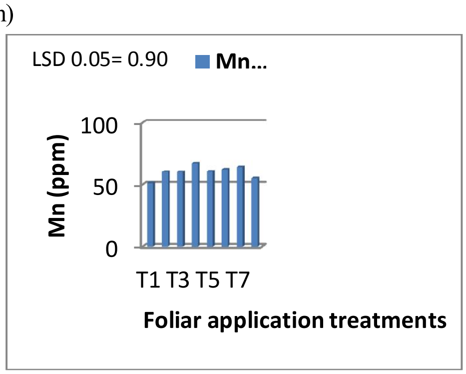

Fig. 3. Effect of foliar application treatments on Mn (ppm)

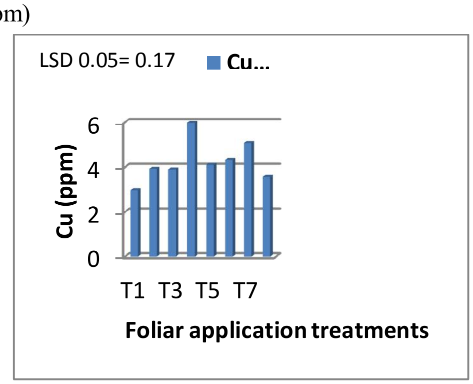

Fig. 4. Effect of foliar application treatments on Cu (ppm)

T_1= Control (untreated), T_2= Naphthalene acetic acid (20 mg L^{-1}), T_3= Naphthalene acetic acid (30 mg L^{-1}),T_4= Salicylic acid (40 mg L^{-1}), T_5= Salicylic acid (60 mg L^{-1}), T_6= Potassium nitrate (1%), T_7= Potassium nitrate (2%), T_8= Potassium sulphate (50 kg fed^{-1})

Conclusion

Foliar application of bio-regulators especially salicylic acid at 40 mg L^{-1} or potassium nitrate 2% when applied at three foliar sprays weekly interval starting from flower initiation, significantly increased seed yield and quality in Egyptian clover. Salicylic acid is more effective compared to potassium nitrate. It could be found that foliar application of salicylic acid caused increase in seed yield production of Egyptian clover which increase forage yield and it is in favor of the production of livestock in Egypt.

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