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Effect of different nitrogen forms and bio-treatments on the growth and seed yield of downy safflower (*Carthamus lanatus*)

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Abstract: A field experiment was carried out to investigate the effect of different nitrogen forms and some biotreatments (*Trichoderma viride*, vermicompost and arbuscular mycorrhiza fungi) alone or in combination on vegetative growth, seed yield and some chemical traits of downy safflower (*Carthamus lanatus* L.). Nitrogen was supplied as ammonium sulfate, ammonium nitrate and urea at the rates (5, 3 and 2 g/plant, respectively). Bio treatments included *Trichoderma viride*, vermicompost and arbuscular mycorrhiza fungi. The results showed that all nitrogen forms significantly increased the plant growth and yield, pigments content, and total carbohydrates in leaves and seeds, as well as N, P and K%, total phenols and oil content in seeds. All bio treatments significantly increased the tested parameters compared to control. The integration of ammonium sulfate with *T. viride* was the most effective treatment since determined the highest increases of the tested traits. Results showed that for enhancing downy safflower plant growth, and nutritional values of seed, the combined treatment of *T. viride* at 5 ml/plant and ammonium sulfate at 5 g/plant is recommended.

1. Introduction

Carthamus lanatus L. (also called downy safflower, woolly distaff thistle or saffron thistle) is an erect spiny biennial plant native of the Mediterranean region. It is closely related to safflower, which is in the same genus. Downy safflower is reported to be sudorific (sweat inducing), fever-reducing and anthelmintic (Hellwig, 2004; DiTomaso *et al*., 2017; Adel El-Gazzar *et al*., 2019), Previous studies revealed its importance due to different components of diverse chemical nature such as flavonoids, sesquiterpenes glycosides, lipids, aromatic acids, sterols, triterpenes , volatiles alkaloids, tannins and saponins (Abu El-Khair, 2020).

Plant nutrition is one of the most essential factors which increase plant production. Nitrogen (N) is the most recognized in plant as it is present in the structure of the protein molecule and plays a vital role in syn-

thesis of plant compositions via the action of various enzymes activities and protein synthesis (Taiz and Zeiger, 2002). Nitrogen has an important role in plant metabolism that impacts quantitative and qualitative plant production by enhancing the growth and stimulating the essential processes which leads to increase the active substances. Ammonium sulfate (AS), ammonium nitrate (AN) and urea are the main forms of inorganic N fertilizers and are extensively utilized in modern agriculture. AS application was better than AN and urea for increasing vegetative growth yield parameters, chlorophyll content, NPK % in seeds and seed oil % of sunflower and jojoba (El Mantawy, 2017; El Sayed, 2020; Hegab *et al*., 2021).

Trichoderma is a genus of saprotrophic fungi and a widespread component of the soil rhizosphere; it has been reported to enhance plant growth and to control many of plant diseases (Colla *et al*., 2014). One of the well-known stimulatory effects of *Trichoderma* on plants is the ability to dissolve phosphate through acidification, chelation or redox activity to improving the utilization by plants (Mansour *et al*., 2021). The benefits of *Trichoderma* species in stimulating plant growth can be realized via various mechanisms including boost nutrient uptake, solubilization, sequestration of inorganic nutrients and enhancement of root hair development (Harman, 2006; Lorito *et al*., 2010). *Trichoderma* spp. promote plant hormone synthesis that improve root growth and root hair formation which lead to more efficient use of nitrogen, phosphorus, potassium and micronutrient (Mastouri *et al*., 2010). Moreover, *Trichoderma* is able to produce metabolites with hormonal activities such as indole-3-acetic acid (Contreras-Cornejo *et al*., 2011). The positive impact of *T. viride* inoculation on physiological and biochemical features of plants has been reported by many authors. The fungus is able to improve growth and yield parameter (Ghoneem *et al*., 2019), promote photosynthetic pigments (Kumar *et al*., 2015; Ghoneem *et al*., 2019), enhance nutrient status in leaves and roots (Metwally, 2020), increase essential oil and total phenol content (Shaikh *et al*., 2019; Hassanin *et al*., 2020; Sanei and Razavi, 2018; Ghoneem *et al*., 2019) and promote peroxidase activity.

Vermicompost is an organic product that is obtained from biodegradation and stabilization of organic waste via the interaction between earthworms and microorganisms, lead to break up organic matter residues into fine particles (Ndegwa and Thompson, 2001; Campitelli and Ceppi, 2008). It has a favorable effect on the physical and chemical structure of soil as well as plant growth (Bachmana and Metzger, 2008). Additionally, it induces and boosts the absorption of nutrients by plants and favors a biological control of bacterial and fungal plant pathogens (Rivera and Wright, 2009). It has high microbial and enzymatic activity and contains large amounts of plant growth regulators like auxins, gibberellins cytokinins, macronutrients and micronutrients (Atiyeh *et al*., 2002). The favorable effect of vermicompost application on the growth and yield of many plants has been reported by previous studies (Adamipour *et al*., 2019; Levinsh, 2020; Abd El-Hamed *et al*., 2021).

Arbuscular mycorrhizal fungi (AMF) are soil fungi which are prevalent in most agricultural ecosystems to associate with more than 80% of plant species (Wang and Qiu, 2006). Previous studies have shown that plant inoculation with AMF improves growth, seeds yield, promotes photosynthetic pigments and carbohydrates content, enhances accumulation of macro- and micronutrients in leaves (Amiri *et al*., 2017; Gashgaril *et al.,* 2020; Mohamed, 2020), as well as increases the nutritional values of seeds like proteins and oil percentage (Ashour *et al*., 2021).

Although the beneficial roles of nitrogen and bio treatments on medicinal and aromatic crops and their valuable effect on improving growth and production, there are no sufficient available data about their effectiveness on the growth and yield of downy safflower plants. Therefore, this research is aimed to evaluate the influence of different nitrogen forms (ammonium sulfate, ammonium nitrate and urea) and some biotreatments (*T. viride*, vermicompost or arbuscular mycorrhiza fungi) on vegetative growth, seed yield and some chemical parameters of downy safflower (*Carthamus lanatus*) plant.

2. Materials and Methods

The field experiment was conducted at the Experimental area of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza Governorate during the two successive seasons of 2019/2020 and 2020/2021. The latitude, longitude and altitude of the experimental site was 0°01'92.70" N, 31°20'68.08" E and 22 m above sea level, respectively.

Experimental procedure

Seeds of *Carthamus lanatus* plants were acquired

from experimental farm of Faculty of Pharmacy, Cairo University. On 1st November (of the two consecutive years), seeds were sown in a seedling trays (50 x 90 cm diameter) at saran house with 42% shading, 28/18°C (day/night) temperature, 14 h light conditions, and 30-35% relative humidity. After 30 days from seeds sowing, uniform seedlings, with an average height of 18-20 cm, were transplanted in the experimental open field in plots (3×3 m), with a distance of 50 cm among rows, 70 cm between plants. Some physical and chemical properties of the experimental soil (average value of the two seasons) were determined according to Jackson (1973), and the results are presented in Table 1.

Nitrogen fertilization included ammonium sulfate (21 %N and 23-24%S) at 5 g/plant, ammonium nitrate (33 %) at 3 g/plant and urea (46% N) at 2 g/plant. Nitrogen forms were applied as two separate doses. The first addition was before transplanting and the second was before flowering.

Plants treated with nitrogen forms were also inoculated with *T. viride*, vermicompost and arbuscular mycorrhiza fungi (Amf), the control plants were not treated. *T. viride*, were obtained from Pest Rearing Department, Central Agricultural Pesticides Laboratory, Agricultural Research Centre, Dokki, Giza, Egypt. 10^9cfu/ml conidial suspension of *T. viride* was diluted in 5 liters of water so as to prepare solution strength of $2X10^5$cfu/ml. For each seedling, 100 ml of solution was used which accounted $2X10^7$cfu of Trichoderma per seedlings. 100 ml of the solution was used to drench the soil per seedlings (Mastouri *et al*., 2010; Chirino-Valle *et al*., 2016).

Table 1 - Physical and chemical properties of experimental soil (mean of two seasons)

Soil characteristics	Data
Physicical characteristics	
Soil Texture	Clay
Clay (%)	43.30
Coarse sand (%)	4.20
Fine sand (%)	21.70
Silt (%)	30.80
Field capacity (V %)	67.85
Chemical characteristics	
Macro-nutrients (%)	
N	94.19
P	21.29
K	59.64
Organic matter (%)	1.76
$CaCO_3$ (%)	1.54
Electrical conductivity (dS/m)	1.54
Cation exchange capacity (meq/100 g)	40.22
pH	7.27

$CaCO_3$= calcium carbonate, pH= soil acidity.

Vermicompost was acquired from Central Laboratory for Agricultural Climate (CLAC), Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. It was applied at 5 g/seedling. Chemical analyses of vermicompost used in this work (average value of the two seasons) are shown in Table 2.

Amf inoculum contained roots, hyphae, spores colonized by *Glomus mosseae* NRC31 and *Glomus fasciculatum* NRC15 obtained from Agricultural Microbiology Department, National Research Center, Dokki, Giza, Egypt. Inoculum material consisted of 275 spores g^{-1} (the infectivity 10^4 propagola). AMF inoculation treatments were carried out by injecting 5 g/seedling of the inoculum.

The three bio treatments were applied as two doses, the first addition was after 3 weeks from transplanting (21th December in both seasons, respectively) and the second was after 2 months from transplanting at branching start (21th February in two seasons, respectively). Irrigation, manual weeding, pest and diseases control were done when needed.

The layout of the experiment was factorial 4x4 in randomized complete blocks design with 16 treatments. The first factor was 4 nitrogen forms (including the control). The second factor was 4 biotreatments (including the control) with 3 replicates, each replicate consisting of 32 plants (2 plants from each treatment).

Vegetative growth and yield parameters measurement

Vegetative growth parameters were registered after 120 days from transplanting (On 1st April). Two

Table 2 - Chemical analysis of vermicompost used in this work (mean of two seasons)

Properties	pH	EC (dS/m)	Organic matter (%)	N (%)	P (%)	K (%)	Fe ppm	Zn ppm	Mn ppm
Vermicompost	8.41	6.6	42.9	1.65	1.14	1.69	166	109	96

samples of plants were taken and used to measure growth parameters including plant height (cm), number of branches/plant, stem diameter (cm, at 5 cm above the soil surface), fresh and dry weights of leaves, stems and roots as well as leaf area (cm^2). At the harvesting stage (on 1st to15th May) yield parameter were measured: number of flower heads/plant, weight of flower heads/plant, weight of seeds/plant and weight of 100 seeds (gr).

The seed content of total carbohydrates, (N, P and K), total phenols and oil were also determined.

Chemical analysis

The chemical analysis were performed at the end of each season (on 1st to15th May).

Chlorophyll and carotenoid contents. Chlorophyll pigments including Chl a, Chl b and carotenoid contents (mg g^{-1}) were determined according to Lichtenthaler and Buschmann (2005), leaves extracted by suspending them in 5 ml of 95% aqueous acetone at 60° C then the total volume completed to 10 ml with 95% aqueous aceton. The aqueous acetone supernatant was then taken for spectrophotometric measurement. A blank of acetone was taken at wavelengths of 663, 645 and 452.5 nm respectively, and data were then calculated using the following equations:

Chlorophyll a (mg g^{-1}) = 0.0127 A663 – 0.00269A645
Chlorophyll b (mg g^{-1})= 0.0029A663 – 0.00468A645
Carotenoids (mg g^{-1}) = 4.2 E 452.5 – 0.0264

Total carbohydrates. Total carbohydrates content in leaves and seeds (percentage of dry matter) was determined in dried samples according to Dubois *et al*. (1956). A known weight (0.1 g) of the dried samples was completely hydrolyzed with 10 ml sulphuric acid (67%) in a test tube on a boiling water bath for one hour. The solution was decolorized and the filtrate was diluted to 100 ml with distilled water. A known volume (1 ml) of the extract was taken in a test tube, to which 1 ml phenol solution (5%) was added, followed by 5 ml of concentrated sulphuric acid. The optical density of the resulting color was measured at 490 µm, using a spectrophotometer, against a blank reagent. The standard curve of glucose was used to calculate the total carbohydrates concentration in the extract.

N, P and K content of seeds. Half gram of dried seeds samples was digested using tertiary acid mixture ($HClO_4$ + HNO_3 + $H2SO_4$) and the extract was analyzed to determine concentrations of N, P and K (as percentage of dry seeds) according to Estefan *et al*. (2013). Nitrogen concentration was determined by using the micro-Kjeldahl method. Phosphorus was determined calorimetrically by using the chlorostannous molybdophosphoric blue colour method in sulphuric acid. Potassium was determined by using the flame photometer apparatus (CORNING M 410, Germany).

Total phenolics content. Total phelolics content was determined in the seeds extract by using the Folin Ciocalteau's reagent colorimetric method and results are expressed as milligram of gallic acid equivalent per gram of seeds dry weight extract (mg GAE/g DW) (John *et al*., 2014). Briefly, 1 mL of seed extract was mixed with 2.5 mL of 10% (w/v) Folin-Ciocalteu reagent. After 5 min, 2.0 mL of Na_2CO_3 (75%) was subsequently added to the mixture and incubated at 50°C for 10 min with intermittent agitation. Afterwards, the sample was cooled and the absorbance was measured utilizing a UV Spectrophotometer (Shimazu, UV-1800) at 765 nm against a blank without extract. The outcome data were expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

Seed oil (%). The oil content of seeds was determined according to AOAC (1995) using soxhlet apparatus using petroleum ether as a solvent. The clean air dried seeds were separately crushed in a Willey mill, then extracted in Soxhlet apparatus, samples of 10 g of seeds were moved into Soxhlet apparatus in 100 ml of N-hexane and the extraction period extended to 6 hours (30-36 syphon cycle approx.). The N-hexane extract was dried over anhydrous sodium sulfate, then filtered and the oil was obtained by distillation under vacuum. oil % was calculated according to the equation:

oil % = extracted oil weight (g)/seeds sample weight (g)/× 100.

Statistical analysis

Results of the two field trials performed in two different growth seasons were combined in order to obtain an average value for each parameter. The means of all results were subjected to Two-Ways analysis of variance (ANOVA) in randomized complete blocks design. Means of data were compared by using Duncan's multiple range tests at P = 5% (Snedecor and Cochran, 1989).

3. Results and Discussion

Vegetative growth parameters

The data in Table 3, 4 and figure 1 revealed that

Table 3 - Mean square for the effect of nitrogen forms and biotreatments and their interaction on vegetative growth, yield parameters of *Carthamus lanatus*

Traits	Source of variation				
	Treatment			Error	CV
	Biotreatments (A)	Nitrogen sources (B)	(A × B)		
Plant height (cm)	4102.63 ***	3164.686 ***	133.843 ***	2.236	0.926
No. of branches/plant	123.894 ***	140.852 ***	18.727 ***	0.785	6.276
Stem diameter (cm)	0.779 ***	0.888 ***	0.058 ***	0.011	8.215
Fresh weight of leaves (g/plant)	3539.035 ***	2397.09 ***	484.993 ***	3.234	1.495
Dry weight of leaves (g/plant)	345.389 ***	253.726 ***	47.235 ***	1.86	3.728
Fresh weight of stems (g/plant)	56027.069 ***	16413.722 ***	1476.819 ***	9.154	1.383
Dry weight of stems (g/plant)	3390.971 ***	989.016 ***	74.53 ***	0.902	1.799
Fresh weight of roots (g/plant)	1451.436 ***	1788.839 ***	476.746 ***	5.688	4.887
Dry weight of roots (g/plant)	117.628 ***	128.286 ***	25.247 ***	0.611	6.305
Leaf area (cm^2)	870.237 ***	1304.444 ***	115.687 ***	0.734	2.09
No. of flower heads/ plant	453.436 ***	382.616 ***	46.209 ***	1.077	6.178
weight of flower heads/ plant	388.412 ***	606.905 ***	45.35 ***	1.783	3.706
weight of seeds/ plant	964.929 ***	1057.951 ***	102.45 ***	0.736	3.534
weight of 100 seeds/ plant	0.549 ***	0.799 ***	0.072 ***	0.002	1.993

*, **, *** significant at P≤0.05, P≤0.01, P≤0.001 respectively, (n=3).

Table 4 - Plant height, No. of branches/plant, Stem diameter, Fresh and dry weights of leavesof *Carthamus lanatus* as affected by the interaction between nitrogen forms and biotreatments (mean of two seasons)

Biotreatments (A)	Nitrogen forms (B)	Plant height (cm)	No. of branches/plant	Stem diameter (cm)	Fresh weight of leaves (g/plant)	Dry weight of leaves (g/plant)
Control	Control	124.33±0.60 k	7.00±0.58 g	0.70±0.05 e	86.50±2.18 i	26.07±0.81 h
	AS	140.83±1.17 gh	11.33±0.33e	1.15±0.06 c	101.83±0.67 g	30.97±0.48 fg
	AN	130.83±0.44 j	9.33±0.44 f	0.82±0.06 dc	96.83±1.17 h	28.20±0.9 h
	N-urea	139.67±1.59 hi	11.50±0.29 e	1.17±0.03 c	107.50±0.76 f	34.03±0.12 e
T. viride	Control	148.67±0.88 f	11.00±0.29 e	0.93±0.07 d	109.17±1.01 f	34.07±0.62 e
	AS	189.00±0.76 a	23.17±0.17 a	1.87±0.03 a	178.33±1.42 a	54.57±1.4 a
	AN	175.50±1.50 d	15.50±0.29 c	1.53±0.09 b	136.17±2.6 c	41.42±0.86 c
	N-urea	179.50±0.29 c	17.83±0.44 b	1.53±0.06 b	136.67±1.86 c	41.63±0.78 bc
Vermicompost	Control	137.83±1.17 i	9.17±0.33 f	1.30±0.06 c	110.17±0.44 f	30.63±1.12 g
	AS	184.17±0.60 b	13.33±0.73 d	1.62±0.09 b	142.67±1.09 b	43.65±0.71 b
	AN	175.83±1.09 d	15.33±0.93 c	1.52±0.01 b	107.5±1.61 f	32.98±0.61 ef
	N-urea	179.67±0.88 c	16.50±0.87 bc	1.65±0.01 b	128.17±0.83 d	38.37±0.59 d
Amf	Control	142.67±1.17 g	11.17±0.33 e	0.82±0.03 de	108.33±0.88 f	32.70±0.73 e-g
	AS	187.00±0.76 a	21.83±0.93 a	1.52±0.07 b	121.17±0.67 e	36.48±0.81 d
	AN	169.50±1.04 e	15.17±0.44 c	1.15±0.01 c	118.67±0.44 e	38.02±0.46 d
	N-urea	179.50±0.76 c	15.33±0.33 c	1.57±0.02 b	134.67±0.83 c	41.55±0.42 bc

Amf= Arbuscular mycorrhiza fungi, AS= ammonium sulfate, AN= ammonium nitrate. Data represent the mean value ±S.E. the mean of three replicates. Means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test.

under the same level of N forms, application of *T. viride*, vermicompost or Amf treatments resulted in significant increase of tested vegetative growth parameters (*viz*., plant height, number of branches/plant, stem diameter, fresh and dry weights of leaves, stems and roots and leaf area) compared to control. Among the tested treatments, application of *T. viride* appeared to be the most effective treatment since

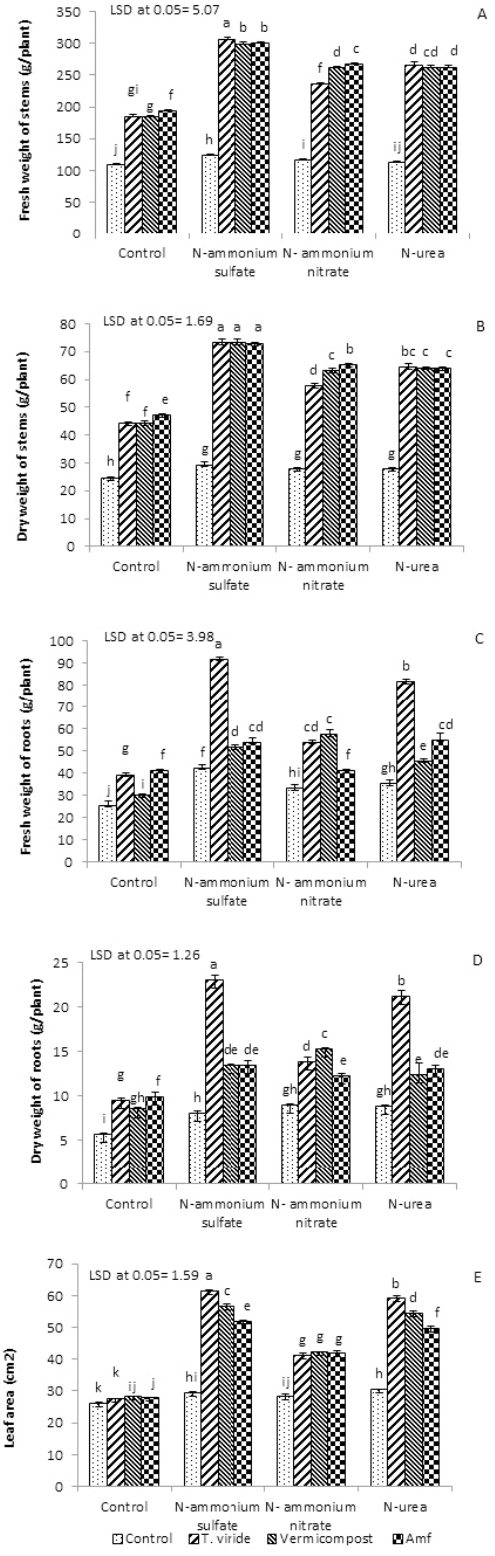

Fig. 1 - Fresh weight of stems (g/plant) (A), dry weight of stems (g/plant) (B), fresh weight of roots (C), dry weight of roots (D), leaf area (E) of *Carthamus lanatus* as affected by the interaction between nitrogen forms and bio treatments (mean of two seasons). Column with different letters indicate a significant difference at 5% level. Data represent the mean values ± SE the mean of three replicates.

registered the highest values. These results are in line with those findings of prior authors (Lakshman and Ghodke, 2018; Shaikh and Mokat, 2018; Ghoneem *et al*., 2019; Guo *et al*., 2020; Hassanin *et al*., 2020; El-Dabaa *et al*., 2021), who reported increases in vegetative growth parameters due to *T. viride* inoculation.

The useful effect of *T. viride* on vegetative growth parameters may be related to participation of such microorganisms in biotransformation of cellulose, increasing cell reproduction, nitrogen mineralization and phosphorus solubilization. They also increase the volume of roots which in turn, increases absorption of water and nutrients, consequently increasing both growth and yield of the crops (Nepali *et al*., 2020).

The data in Table 4 and figure 1 also revealed that under the same rate of bio treatment (*T. viride*, vermicompost or Amf) treating the plants with different nitrogen forms resulted in significant increase in vegetative growth parameters compared to control and among the tested nitrogen forms, with the application of ammonium sulfate leading to superior growth compared with ammonium nitrate or urea. The increases in vegetative growth parameters due to ammonium sulfate treatments are in agreement with the findings of several studies on different plants including *Cynara cardunculus* (Sarhan *et al*., 2014), *Urtica pilulifera* (Wahba *et al*., 2014), *Nigella sativa* (Khalid and Shedeed, 2015), Sunflower (El Mantawy, 2017; El Sayed, 2020), *Thymus vulgaris* (Basal *et al*., 2019) and Jojoba (Hegab *et al*., 2021).

The positive effect of ammonium sulfate may be attributed to the role played by the acidic component that decrease the values of soil pH and thus simplify the uptake of nutrients by the plant roots (Fouda, 2017).

Yield parameters

Results of figure 2 indicated that within each level of N forms, in most cases, application *T. viride*, vermicompost or Amf treatments caused a significant increase in yield parameters (namely, No. of flower heads/plant, weight of flower heads/plant, weight of seeds/plant, weight of 100 seeds) compared to control. *T. viride* treatment appeared to be the most effective one since recorded the highest values. Increases in yield parameters due to *T. viride* treatments are matched well with those of previous studies on different crops including *Coriandrum sativum* (Khan and Parveen, 2018), *Triticum aestivum* (Mahato *et al*., 2018), *Cuminum cyminum* (Ghoneem *et al*., 2019).

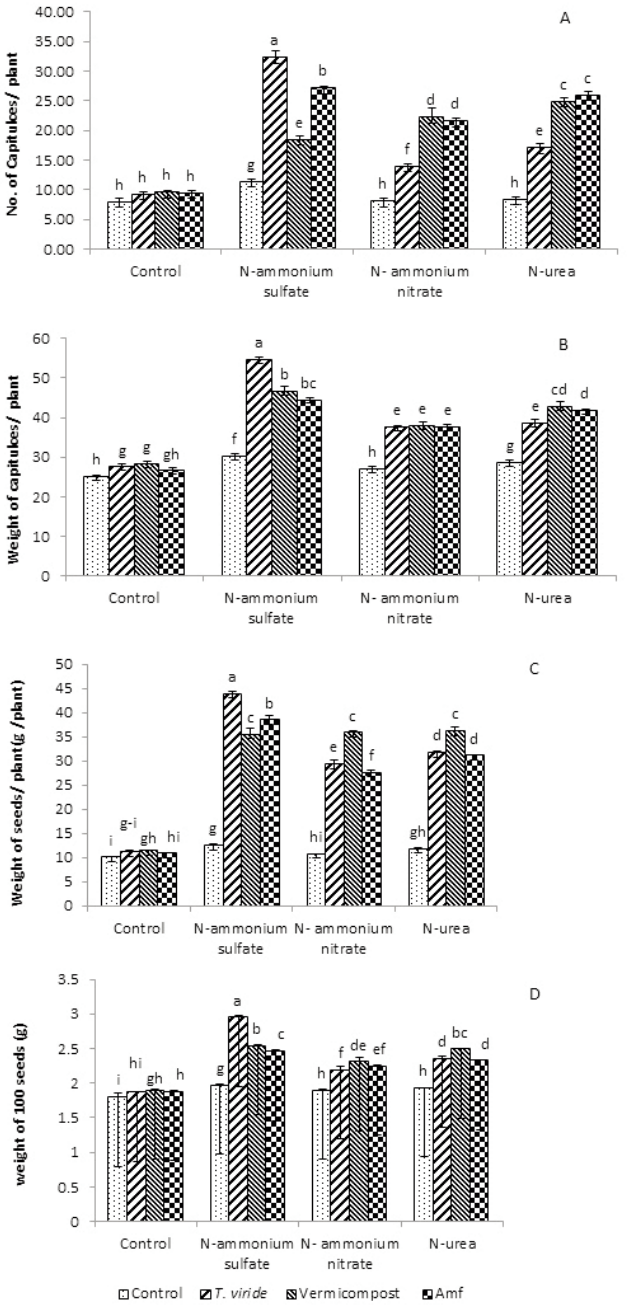

Fig. 2 - Evolution of total phenolic content in season 2018 (mg gallic acid/100 g of fresh fruit) of four selected farms (131, 272, 351, 432) and their average trend. Bars represent standard error of the mean (±SEM). Values followed by the same letter in every sampling point are not significantly different from each other. Mean separation by LSD test (P≤0.05).

Data in figure 2 also exhibited that within each rate of *T. viride*, vermicompost or Amf, the plants treated with nitrogen forms had significantly higher values of yield parameters, in most cases, than those of control. Among the tested nitrogen sources, the most effective one was ammonium sulfate, for which the highest mean value was found. The increases in yield parameters due to ammonium sulfate treatments are the same as the results of Sarhan *et al*., 2014 on *Cynara cardunculus*, Wahba *et al*. (2014) on *Urtica pilulifera*, Khalid and Shedeed (2015) on *Nigella sativa*, El Mantawy (2017); El Sayed (2020) on *Helianthus annuus*, Prinsloo and Plooy (2017) on *Sutherlandia frutescens*, Hegab *et al*. (2021) on *Simmondsia chinensis*.

Contents of pigments and total carbohydrates in leaves

As shown in Table 5 and 6 within each rate of N forms, in most cases, application of *T. viride*, vermicompost or Amf rates resulted in significant increase in the mean values of pigments content (chlorophyll a, b and carotenoids) and total carbohydrates in leaves compared to control. The highest mean values were found for the plants treated with *T. viride*. Such increase in pigments content or total carbohydrates in leaves due to *T. viride* inoculation is in accordance with those obtained by previous reports on *Salvia officinalis* (Kumar *et al*., 2015), *Cuminum cyminum* (Ghoneem *et al*., 2019), *Allium cepa* (Metwally, 2020).

Chlorophyll is used by plants for light-trapping and energy transduction during the anabolic process of photosynthesis. A higher content of photosynthetic pigments can be correlated to the augmentation in carbohydrates content of leaves.

Results of Table 6 also pointed out that within biotreatments (*T. viride*, vermicompost or Amf), in most cases, the mean values of pigments content and total carbohydrates in leaves of the plants treated with various nitrogen forms were significantly higher than the control. Nitrogen in the form of ammonium sulfate was superior to the other two nitrogen sources for enhancing the value of the parameters considered. The results are analogy with that recorded by earlier research (Sarhan *et al*., 2014; Wahba *et al*., 2014; El Mantawy, 2017), they reported increase in pigments content or total carbohydrates in leaves due to application of ammonium sulfate.

The superior effect of ammonium sulfate in increasing pigments contents may be related to sulfur element that is a constituent of succinyl Co-A which involved in chlorophyll synthesis in leaves and its activation at cellular level enhances photosynthesis that eventually boost vegetative growth.

Table 5 - Mean Square for the effect of nitrogen forms and bio treatments and their interaction on some chemical constituents of *Carthamus lanatus*

Traits	Source of variation				
	Treatment			Error	CV
	bio treatments (A)	Nitrogen sources (B)	(A × B)		
Chlorophylls A content (mg/g f.w)	14.545 ***	9.932 ***	0.801 ***	0.002	2.18
Chlorophylls B content (mg/g f.w)	8.327 ***	71.103 ***	3.727 ***	0.112	2.932
Carotenoids content (mg/g f.w)	0.288 *	0.268 *	0.148 **	0.073	7.57
Total carbohydrates [%] in leaves	35.699 ***	55.094 ***	2.029 *	0.771	4.30
Total carbohydrates [%] in seeds	22.491 ***	47.094 ***	2.901 *	1.188	4.68
N% in seeds	1.682 **	1.761 **	0.102 *	0.158	17.71
P% in seeds	0.036 ***	0.029 ***	0.004 **	0.002	9.91
K% in seeds	0.078 ***	0.161 ***	0.01 *	0.004	4.58
Total phenols [%] in seeds	0.568 ***	0.795 ***	0.068 ***	0.003	1.79
Oil [%] in seeds	29.47 ***	48.638 **	0.423 *	1.498	4.63

*, **, *** significant at P≤0.05, P≤0.01, P≤0.001 respectively, (n=3).

Table 6 - Pigments, total carbohydrates in leaves as affected by the interaction between nitrogen forms and bio treatments (mean of two seasons)

Bio treatments (A)	Nitrogen forms (B)	Chlorophylls A content (mg/g f.w.)	Chlorophylls B content (mg/g f.w.)	Carotenoids content (mg/g f.w.)	Total carbohydrates [%] in leaves
Control	Control	3.68±0.03 k	7.04±0.12 i	2.64±0.57 b	15.44±0.59 g
	AS	5.06±0.01 i	13.74±0.07 c	3.55±0.01 a	19.88±1.15 d-f
	AN	4.09±0.11 j	11.06±0.33 f	3.58±0.01 a	16.22±0.58 g
	N-urea	5.88±0.04 g	11.27±0.1 ef	3.58±0.01 a	20.18±0.52 c-e
T. viride	Control	5.88±0.04 g	7.28±0.05 i	3.60±0.03 a	18.90±0.58 ef
	AS	8.24±0.09 a	15.26±0.14 a	3.88±0.06 a	24.37±1.15 a
	AN	6.11±0.09 f	12.13±0.34 d	3.63±0.02 a	21.32±0.57 b-e
	N-urea	6.74±0.02 d	12.26±0.21 d	3.65±0.02 a	23.02±1.73 ab
Vermicompost	Control	6.62±0.07 de	8.95±0.02 h	3.57±0.08 a	17.28±1.15 fg
	AS	7.8±0.14 b	13.78±0.04 bc	3.59±0.11 a	22.58±1.21 a-c
	AN	5.92±0.11 fg	11.79±0.02 de	3.63±0.09 a	21.21±0.64 bc-e
	N-urea	7.83±0.10 b	14.31±0.06 b	3.57±0.07 a	22.42±0.59 a-d
Amf	Control	5.50±0.02 h	8.78±0.35 h	3.55±0.06 a	18.90±0.58 ef
	AS	7.30±0.05 c	11.11±0.05 f	3.64±0.13 a	21.55±0.55 bcd
	AN	6.45±0.12 e	10.11±0.31 g	3.70±0.04 a	20.67±0.57 b-e
	N-urea	7.95±0.03 b	13.37±0.13 c	3.62±0.03 a	22.83±1.17 ab

Amf= Arbuscular mycorrhiza fungi, AS= ammonium sulfate, AN= ammonium nitrate. Data represent the mean value ±S.E. the mean of three replicates. Means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test.

Moreover, it is known the function of sulfur in the synthesis of proteins, oils, vitamins, and flavored compounds in plants since, it is a constituent of the three amino acids methionine (21% S), cysteine (26% S) and cystine (27% S), that are the building blocks of protein (El Mantawy, 2017).

Contents of total carbohydrates, N, P and K in seeds

It is clear from data reported in figure 3 that within each rate of N forms, in most cases, application of *T. viride*, vermicompost or Amf caused significant increase in total carbohydrates, macronutrients (N, P and K %) in seeds compared to control. Among the

tested treatment *T. viride* appeared to be the most effective one. In this regard, Kumar *et al*. (2015) on *Salvia officinalis* stated that the plants inoculated with *T. viride* had higher phosphorus content in shoot and root as compared with control. Also, Metwally (2020) on *Allium cepa* declared that the plants inoculated with *T. viride* improved total carbohydrates and N, P or K% in plant organs.

Data in figure 3 also exhibited that within *T. viride*, vermicompost or Amf treatmens, the plants treated with nitrogen forms had significantly higher values of total carbohydrates, N, P and K in their seeds than the control. Among the tested nitrogen forms ammonium sulfate was superior in its effect than the other two nitrogen sources. Such results confirmed the reports of prior works (Wahba *et al*., 2014; Khalid and Shedeed, 2015; El Sayed, 2020; Hegab *et al*., 2021) who showed increase in total carbohydrates or N, P and K% in seeds as result of ammonium sulfate application.

The increased content of total carbohydrate in seeds may be related to the increase in chlorophyll content of plants, corresponding to improved photosynthesis efficiency (Khalid and Shedeed, 2015).

Total phenols in seeds

Data in figure 4 A displayed that within each rate of N forms, in most cases, application of *T. viride*, vermicompost or Amf treatments caused a significant increase in total phenols in seeds compared to control. The highest mean values were found to be associated with the plants treated with vermicompost. Similar results were reported by Abd El-Hamed *et al*. (2021) who found that vermicompost caused increase in total phenols in leaves of *Dracocephalum moldavica*.

Within each treatment of *T. viride*, vermicompost or Amf, the plants fertilized with different rates of nitrogen forms had significantly higher values of total phenols in seeds than those of control. Nitrogen as ammonium sulfate was superior to the other nitrogen forms in augmentation total phenols in seeds. These results confirmed the reports of earlier researches (Munene *et al*., 2017; Petropoulos *et al*., 2018; Prinsi *et al*., 2020; Machado *et al*., 2022) that reported increase in total phenols in plant organs due to ammonium sulfate treatments.

Seed oil (%)

It is evident from data figure 4 (B) that within each rate of N forms application of *T. viride*, vermicompost

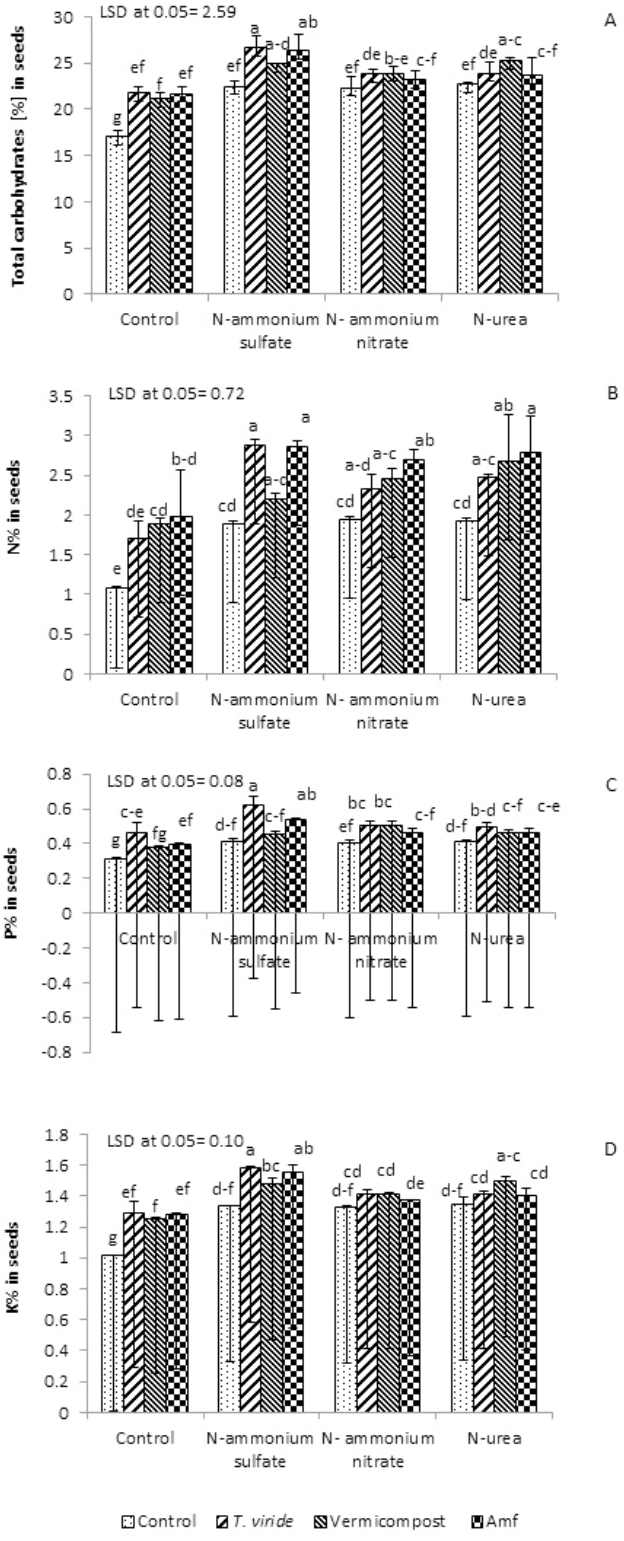

Fig. 3 - Evolution of superficial SS index of four selected farms (131, 272, 351, 432) and their average trend during storage in 2018. Bars represent standard error of the mean (±SEM). Values followed by the same letter in every sampling point after harvest are not significantly different from each other. Mean separation by LSD test (P≤0.05).

or Amf rates caused a significant increase in oil percentage in seeds compared to control. Among the tested treatments, *T. viride* appeared to be the most effective one since recorded the highest values. The positive effect of *T. viride* treatments for enhancing oil % in seeds is similar to those obtained by previous reports (Shaikh and Mokat, 2018; Guo *et al*., 2020; Hassanin *et al*., 2020).

Improved the quantity of oil percentage may be due to *T. viride* regulating the genes that encode the enzymes involved in essential oil metabolism through a potential MAPK-mediated signaling pathways (Guo *et al*., 2020).

Results in figure 4 also indicate that, within each treatment of *T. viride*, vermicompost or Amf, oil percentage in seeds of plants treated with different nitrogen forms were significantly higher than those of control. Ammonium sulfate was slightly better in its effect than the other two nitrogen forms. These

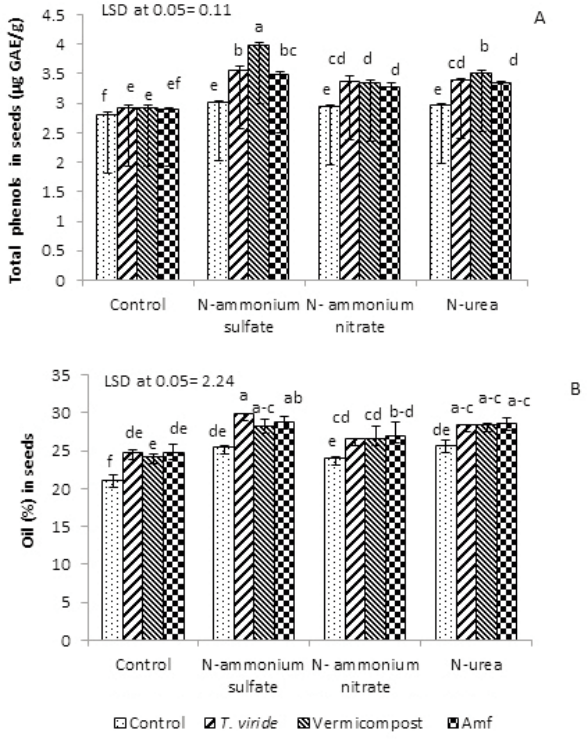

Fig. 4 - Evolution of antioxidant capacity (mg ascorbic acid/100 g of fresh fruit) and SS index after 4 months of cold storage (T2) in seasons 2019 of four farmers (131, 272, 351, 432) and their average trend. Bars represent standard error of the mean (±SEM). Values followed by the same letter between four producers are not significantly different from each other considering DPPH values at T0 and T1 or SS index during storage. Mean separation by LSD test (P ≤0.05).

results are in agreement with those reported by previous researches on different plants including *Cynara cardunculus* (Sarhan *et al*., 2014), *Helianthus annuus* (El Mantawy, 2017) and *Simmondsia chinensis* (Hegab *et al*., 2021), they indicated that ammonium sulfate treatments caused increase in oil percentage in seeds.

The increments in oil content due to ammonium sulfate application may be attributed to its promoting role in the formation of amino acids methionine (21% S) and cysteine (27% S); synthesis of proteins and oil content of seeds. Also, sulfur is an important element for oil crops which a constituent of acetyl Co-A, which converted into malonyl Co-A to synthesis of fatty acid (El Mantawy, 2017).

4. Conclusions

Summing up the results, it can be concluded that for enhancing growth, nutritional values of seeds, the interacted treatment of *T. viride* inoculation at 5ml /plant and ammonium sulfate at 5 g/plant is recommended for downy safflower plants.

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