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Chitosan And Chitosan Nanoparticle Effect On Growth, Productivity And Some Biochemical Aspects Of Lupinustermis L Plant Under Drought Conditions

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Abstract

Chitosan is a natural biopolymer formed from chitins that considered as aneffectivebioregulator and elicitor in agriculture. Therefore, this investigation was done to study the effect of chitosan and chitosan nanoparticles (0 and 50 mg/l) on alleviating the adverse effect of drought stress on *Lupine termis*L plant. Lowering water irrigation requirement from 100%to 60%,significantly reduced different growth criteria, photosynthetic pigments, indole acetic acid (IAA), yield and itscomponents in addition to carbohydrate, protein and oil contents of the yielded seeds of lupine plant. On the other hand, drought stress significantly increasedtotal soluble sugars (TSS), proline and free amino acids of lupine plants, in addition to phenolic and flavonoids contents of the yielded seeds. On the other hand, pre sowing treatment with (50 mg/l) Chito or Chito NPs could increase significantly growth parameters, yield quantity and quality of lupine plant viaimprovingphotosynthetic pigments, IAA, TSS, proline, free amino acid contentscomparing with their corresponding controls.However,Chito NPs was more effective than Chito.Finally it could be concluded that, the promotive role of chitosan in improving tolerance of lupine to droughtstress and improve growth and yield through improving different studied biochemical processes.

Keywords: Chitosan, Chitosan nanoparticles, Drought, Flavonoids, Lupine plant, Osmoprotectants, Phenolics, Growth and Yield.

1. Introduction

A biotic stress or adverse environmental factors(such as salinity, drought and heat stresses) affect different aspects of plant life from germination to maturity stages[1, 2, 3].Water stress is a main environmental stress and a major unpredictable constraint, with reduced effects on plantyield all over the world.Drought inducesseveral devastating effects on plants via disturbing variousphysiological and biochemical processesas carbon assimilationrate, reduced turgor, induced oxidative stress, and variations in leaf gas exchange, thuscausing reductions in plant productivity [4, 5&6].Plant response to water stress is complicated and depends ongrowth stage of plant, genetic variability, duration and severity ofstress[7].Water stress also affects leaf growth, enzymes activity, ion balance, and consequentlyreduce crop production [8 &5].To induce stress tolerance, plants develop several mechanisms, including increasing non enzymatic antioxidants compounds and improving enzymatic antioxidant activities. Osmoregulation is another biochemicalmechanisminducedby the decreases in cellular water potential and stabilizing physiological processes neededfor plant growth[9]. The main roles of these compounds are improving osmoregulation,protect structure of different biomolecules & membranes and scavenge free radicals over accumulated [9]

Lupine (*Lupinustermis*L.) is one of the oldest plants cultivated under a wide range of environments. The nutritional quality of lupine seeds is similar to soybean seed and superior to other legumes seeds[10]. Its seeds have 33-40% protein with relatively high contents of essential amino acids profile and 5-13% oil contents [11].Cultivation of lupine plant as other legumes could improve soil fertility andpermeability as well asenhancing water

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storagebecause it have the ability to fix atmospheric nitrogen in soil[12]. Thusit cancultivated well in sandy soils and used as additive to such soil in the form of green manure.

One of these natural growth promotors is chitosan, it is a biopolymer ofcarbohydrate family composed from glucose ring with free amino group [13]. Recently, chitosan could be used in agriculture as plant growth promoter[14].Moreover, it is low toxic and cheap material.Earlier investigations were done to study the effect of chitosan on plant growth, development and productivity. Chitosan improved growth of wheat plants under salinity stress[15].

Nanotechnology is a promising technology inmany fields including agriculture[16]. It changes material at the nuclear, atomicor macromolecular level to make objects on thenanometre scale with new characters in view of theirsmall size[17].Chitosan nanoparticles (nano chitosan) are one of the engineered nanomaterials and natural materials with excellent physicochemical properties, like surface area, size, cationic nature [18] as well as, they are environmentally friendly and bioactive molecule [19]. Chitosan nanoparticles are easily absorbed by leaves, transported to stems thatenhancing the absorption of active molecules and improved growth and yield of several crop plants [20].

Thus, the aim of this work was to compare between the physiological role of pre sowing treatment of either chitosan or chitosan nanoparticles on improving growth and yield of *lupines termis*L plant under drought stress conditions.

Experimental

Experimental procedure: Two field experiments were conducted at the Experimental Station of the National Research Centre (Research and Production Station, Nubaria region, Behira Governorate, Egypt) at the winter season of 2018/2019 and 2019/2020to study the effect of pre sowing treatment of chitosan or chitosan NPs on *Lupinustermis*L. plant grown under drought stress.The experiments were carried out under sandy soil conditions thephysical and chemical proprieties of the soil are presented in Table(1) according to [21]**.**

Chitosan and chitosan NPs solution preparation:

1 g of the chitosan or chitosan NPs was dissolved in 1% acetic acid. Then, 100 ml distilled water was added to the solution under constant stirring until it was completely dissolved. Next, the solution was alkalized to pH 6 with 1 M NaOH solution [22]. Finally, 50 mg/l dose of the chitosan and chitosan NPs were prepared for the test.

Lupines(*Lupinustermis* L. cv. Giza 2) seeds were secured from Agricultural Research Centre, Egypt, cleaned and soaked for 12 h in the 50 mg/l of either chitosan or chitosan NPs for 12 hours, expressed as Chit0 and Chit1 for chitosan and ChitNp0 and ChitNp1then left to dry in open air.

The soaked seeds were inoculated with nitrogen fixing bacteria (Rhizobia) and sown on 14th and 17thNovember 2018 and 2019, respectively. The experimental design was split – plot design with four replications.The main plotswere devoted to the drought stress treatments,two irrigation regimes were applied, (100WIR as regular irrigationreferred as D0 and 60% WIR as drought stress referred as D1). Each irrigation treatment had valve and flow-meter to control water application,Total irrigation water (m3 /fed./season) was calculated from the meteorological data of the Central Laboratory for Agricultural Climate (CLAC) depending on Penman method [26]. The seasonal irrigation water applied of 100% WIR in the experimental location was found to be 1584 and 1592 m^3 /fed. in both seasons respectively. Irrigation was carried out using drip irrigation system where water was added every 7 days by applying the specified WIR, while differentsoaking treatments of chitosan, Chitosan(50 mg/l), Chitosan nanoparticles (50 mg/l) in addition to control treatment (tap water) were randomly occupied the sub-plots. Plot area was 10.5 m^2 (3.0 m x 3.5 m) and consisted of four rows 60 cm apart and the distance between hills along the row 25 cm apart. Calcium super-phosphate (15.5% P_2O_5) at rate of 30 kg/fed was applied to the soil prior sowing. While, nitrogen fertilizer was applied at the rate 120 units of N/fed in form of ammonium nitrate (33.5%N) and was divided in four equal portions. The dosage was added before the irrigation.

Plant samples were taken after 75 days from sowing for determination of growth characters as shoot length (cm), leaves number/plant, fresh and dry weight (g/plant). Plant samples were taken for chemical analysis. Chemical analysis of fresh samples was photosynthetic pigments andindole acetic acid (IAA). After drying of plant samples, total soluble sugars (TSS), proline and free amino acids were determined. At harvest time, ten guarded plants were taken out at random from the middle two ridges of each plot to determine the mean values of yield

and its related parameters, i.e., number of pods/plant, number of seeds/pod, number of seeds/plant, ,dry weight of pods and seeds/plant, 100 seed weight and seeds yield (kg / feddan). Some chemical analysis of the yielded seeds as total carbohydrates%, protein%, oil%, phenolic and flavonoids contents**.**

Table (1): Mechanical, chemical and nutritional analysis of the experimental soil. Mechanical analysis

Mechanical analysis	Sand		Silt 20-0µ %	Clay < 2µ %	Soil texture
	Course 2000-200µ%	Fine 200-20µ %			
	47.46	36.19	12.86	4.28	Sandy

Chemical analysis

Chemical analysis.	pH 1:2.5	EC dSm^{-1}	$CaCO_3$ %	OM %	Soluble cations (meq/l)				Soluble anions (meq/l)			
					Na^+	K^+	Mg^+	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
	8.25	0.11	0.9	0.9	0.7	0.02	0.1	0.3	0.0	0.2	0.8	0.12

Nutritional analysis

Nutritional analysis.	Available nutrients						
	Macro element (ppm)			Micro element (ppm)			
	N	P	K	Zn	Fe	Mn	Cu
	12.9	3.6	52.9	0.12	1.98	0.46	0.06

Measurements

Photosynthetic pigments:Total chlorophyll a and b and carotenoids contents in fresh leaves of lupines plant were estimated using the method of Lichtenthaler and Buschmann[23]. The fresh tissue was ground in a mortar and pestles using 80% acetone. The optical density (OD) of the solution was recorded at 662 and 645 nm (for chlorophyll a and b, respectively) and 470 nm (for carotenoids) using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in mg/g FW.

Indole acetic acid content: A known weight of the fresh samples was taken and extracted with 85% cold methanol (v/v) for three times at 0ºC. The combined extracts were collected and made up to a known volume with cold methanol. Then take 1ml of the methanolic extract and 4ml of PDAB reagent (para-dimethylamino benzoic acid 1g dissolve in 50 ml HCl, 50 ml of ethanol 95%) and left for 60 min in 30-400C. The developing colour was spectophotometrically measuredat wave length of 530 nm[24].

Total soluble sugars (TSS): Total soluble carbohydrates (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates[25]. TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using SpekolSpectrocololourimeterVEB Carl Zeiss[26].

Free amino acids: Free amino acid and proline contents were extracted accordingto the method[27]. Free amino acid was determined with the method[28]. Further, 1.0 ml acetate buffer (pH 5.4) and 1.0 ml chromogenic agent were added to 1.0 ml free amino acid extraction. The mixture was heated in boiling water bath for 15 min. After cooled in tap water, 3 ml ethanol (60% v/v) was added. The absorbance at 570 nm was then monitored using SpekolSpectrocololourimeter VEB Carl Zeiss.

Proline: Proline was assayed according to the method described [29].2ml of proline extract, 2ml of acid ninhydrin and 2ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using SpekolSpectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

Total carbohydrate: Determination of total carbohydrates was carried out according [30]. A known mass (0.2-0.5 g) of dried tissue was placed in a test tube, and then 10 ml of sulphuric acid (1N) was added. The tube was sealed and placed overnight in an oven at 100ºC. The solution was then filtered into a measuring flask (100ml) and completed to the mark with distilled water. The total sugars were determined Colorimeterically as follows: An aliquot of 1ml of sugar solution was transferred into test tube and treated with 1ml of 5% aqueous phenol solution followed by 5.0 ml of concentrated sulphuric acid. The tubes were thoroughly shaken for ten minutes then placed

in a water bath at 23-30ºC for 20 minutes. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer model UV 1201.

Protein:Total protein concentration of the supernatant was determined according to the method described by [31]with bovine serum albumin as a standard. An amount of 2 gm of samples was grinded inmortar with 5ml of phosphate buffer (pH 7.6) and was then transformed to the centrifuge tubes. The homogenatewas centrifuged at 8000 rpm for 20 minutes. The supernatant of different samples were put in separate tubes.The volume of all of the samples in tubes were then made equal by adding phosphate buffer solution and theextraction were stored in the refrigerator at 40c for further analysis. After extraction, 30µl of different sampleswere taken out in separate tubes and were mixed with 70µl of distilled water separately. In all of these separatesample tubes 2.9 ml of CoosmassicBrillaint Blue solution was then added and mixed thoroughly. The Totalvolume now was 3ml in each tube. All these tubes were incubated for 5 minutes at room temperature andabsorbance at 600 nm was recorded against the reagent blank. A standard curve of Absorbance (600 nm) versusConcentration (µg) of protein was calculated.

Oil contents: The oil of lupine seeds were extractedaccording to [32], the powdered seeds is shakenovernight with isopropanol: chloroform (1:1). The solvent were evaporated under reduced pressure of CO_2 atmosphere. The lipid residue is taken up in a chloroform: methanol (2:1 v/v) and given a folch wash, thedissolved total oils were purified by washing with 1% aqueous saline solution. The aqueous phases were washedwith chloroform that was combined with the pure oil solution. Chloroform was evaporated and the total pure oilwas weighed.

Total phenol content: The extract was extracted as IAA extraction, and then 0.5 ml of the extraction was added to 0.5 ml Folin, shaked and allowed to stand for 3 min. Then one ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60min. The optical density was determined at wave length of 725 nm using spectrophotometer as describedby [33].

Flavonoids contents: Flavonoid content of crude extract was deter-mined by the aluminium chloride colorimetric method [34]. In brief, 50 µL of crude extract (1 mg/mL ethanol) were made up to 1 mL with methanol, mixed with 4 mLof distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight.

Statistical Analysis

The data obtained were statistically analyzed using MSTAT-C statistical software. The differences between treatment means were compared by Duncan's Multiple Range Test (DMRT) at 5% probability level according to [35].

Results

Changes in growth indices:Table (2) show the effect of chitosan and chitosanNpspre sowing treatments with 50 mg/l concentration on growth indices of lupine plants grown under normal (regular irrigation) or drought stress(60% WIR) conditions. Results showed that, shoot length, leaves number/plant, fresh and dry weight of shoot of drought stressed plants decreased significantly (from 24.0 to 16.0 cm, 16.7 to 11.00, 12.62 to 7.93 g and 3.18 to 1.33 g , respectively) compared with those plants irrigated normally (100 IR). On the other hand, soaking lupines seeds with either Chit or ChitNp(50 mg/l concentration) increased significantly the above mentioned growth parameters of lupine plant as compared with controls treatment. Data also show that, chitosan nanoparticles was more effective than chitosan on increasing the studied growth parameters. The percentages of increases under normal water irrigation by chitoNPs reached to 29.17%, 68..00%, 51.45 and 18.93% in shoot length, leaves number/plant, fresh weight and dry weight, respectively compared with 22.22%, 38%, 41.96%, 3.39% and 20.17% in plants treated with Chito. Meanwhile the increases in drought stressed plants reached to 57.78%, 66.67%, 42.01 and 45.36% of plants treated with Chito NPs compared with 42.22%, 51.51%, 26.67% and 9.77% of plants treated with normal Chito.

Table (2): Effect of chitosan (Chito) and chitosan nanoparticles (Chito NPs) soaking treatment (50 mg/l) on growth criteria of lupines plants under drought stress conditions (D0 &D1). Data are means of two seasons

Irrigation requirement (WIR)	Treatment	Shoot length (cm)	No of leaves/plant	Fresh wt of shoot/plant (g)	Dry wt of shoot/plant (g)
D0	Control	24.0^{c}±0.58	16.7^{d}d±0.33	12.62^{cd}±0.54	3.18^{cd}±0.16
	Chito	29.3^{ab}±0.88	23.0^{b}±0.58	17.91^{b}±0.44	3.49^{b}±0.17
	Chito NPs	31.0^{a}±1.15	28.0^{a}±0.57	19.11^{a}±0.29	3.79^{a}±0.17
D1	Control	16.0^{e}±0.58	11.0^{e}±0.58	7.93^{d}±0.31	1.33^{g}±0.16
	Chito	21.3^{d}±0.88	16.7^{d}±0.67	10.05^{e}±0.35	2.46^{ef}±0.07
	Chito NPs	23.7^{c}±0.67	18.3^{c}±0.67	11.26^{d}±0.29	2.93^{de}±0.10

Mean values (n=3) in each column followed by a different letter are significantly different at ≤ 0.05 by Duncan"s multiple range test.

Changes in photosynthetic pigments: Droughtstress (60% WIR) significantly decreased photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) in Lupine leaves as compared with control plants (those plants irrigated normally) (Table 3). The percentage of reductions were 18.1%, 23.3%, 10.9% and 19.1% of *Chl* a, *Chl* b, carotenoids and total pigments respectively. On the other hand, external treatments of Chito orChitoNPscaused significant increases in photosynthetic pigments in unstressed plants as well as water stressed plants relative to their untreated control plants. Chito NPs foliar treatment gave the highest increases in all components of photosynthetic pigments (Table 3). Chito NPs increased *Chl* a, *Chl* b, carotenoids and total pigments by 11.7%, 10.5%, 23.5 and 12.6% under unstressed conditions. Meanwhile, the ratios were 20.1%, 26.9%, 15.3% and 21.8% under water stressed conditions relative to their corresponding controls.

Table (3): Effect of chitosan (Chito) and chitosan nanoparticles (Chito NPs) soaking treatment (50 mg/l) on photosynthetic pigments (mg/g fresh wt) contents of lupines plants under drought stress conditions (D0 & D1)

Drought	Treatment	*Chl* a	*Chl* b	Carot	Total
D0	Control	1.07^{b}±0.035	0.72^{c}±0.002	0.23^{cd}±0.003	2.03^{c}±0.030
	Chito	1.18^{ab}±0.005	0.76^{b}±0.003	0.25^{b}±0.002	2.21^{ab}±0.012
	Chito NPs	1.20^{a}±0.002	0.79^{a}±0.005	0.28^{a}±0.001	2.29^{a}±0.006
D1	Control	0.88^{d}±0.013	0.55^{f}±0.004	0.21^{e}±0.002	1.64^{e}±0.020
	Chito	0.92^{c}±0.005	0.67^{e}±0.007	0.23^{cd}±0.002	1.83^{d}±.015
	Chito NPs	1.05^{b}±0.026	0.70^{d}±0.003	0.24^{c}±0.003	1.99^{cd}±0.029

Mean values (n=3) in each column followed by a different letter are significantly different at ≤ 0.05 by Duncan"s multiple range test.

Changes in indole acetic acid: Table (4) show the changes in endogenous indole acetic acid (IAA) of *Lupine termis* plants treated with chitosan and chitosan nanoparticle and grown under normal and drought stressed conditions. Data clearly show, drought stress caused significant decrease (by 36.1%) in endogenous IAA content as compared with those plants irrigated normally (100 WIR). Meanwhile, soaking lupine plants with 50 mg/l of either chitosan or chitosan nanoparticle caused significant increases in IAA contents compared with untreated control plants either in plants irrigated normally or drought stressed plants. Data clearly show that Chito NPs was more effective than Chito.

Changes in total soluble sugars, proline and free amino acids: Total soluble sugars (TSS) contents of *Lupine termis* plants was significantly decreased in plants subjected to water deficit stress, the percentage of decrease was 2.3% relative to control plant (those plants irrigated normally)(Table 4). Meanwhile subjecting lupine plants to drought stress caused significant increases in proline and free amino acids contents On the other hand, different applied treatments (Chito or Chito NPs) significantly increased TSS, proline and free amino acids contents in lupine plant either at normal or drought stress conditions. Chitosan NPs was more effective than Chitosan treatment Table (4).

Table (4): Effect of chitosan (Chito) and chitosan nanoparticles (Chito NPs) soaking treatment (50 mg/l) on IAA (µ g /100 g fresh wt), TSS, Proline and Free amino acids (mg/100 g dry wt) contents of lupines plants under drought stress conditions (D0 & D1).

Drought	Treatment	IAA	TSS	Proline	Free amino acids
D0	Control	18.97^{e}±0.182	2254^{d}±11.26	35.16^{e}±3.24	235.34^{f}±12.6
	Chito	24.98^{c}±0.193	2624^{c}±34.35	69.65^{c}±4.12	252.15^{e}±13.2
	Chito NPs	36.25^{a}±0.346	2826^{b}±22.52	70.83^{c}±7.35	263.5^{d}±16.4
D1	Control	12.11^{f}±0.309	2203^{b}±10.39	49.18^{d}±3.45	271.65^{c}±13.6
	Chito	20.11^{d}±0.139	2297^{a}±13.57	74.65^{b}±6.95	288.15^{b}±11.6
	Chito NPs	27.15^{b}±0.867	2331^{a}±15.29	84.12^{a}±7.95	306.95^{a}±14.5

Mean values (n=3) in each column followed by a different letter are significantly different at ≤ 0.05 by Duncan"s multiple range test.

Changes in yield and yield components: Data presented in Table (5) showed the effect of Chito or Chito NPs soaking treatments with 50 mg/l concentration on yield and yield components of lupine plant grown under normal and drought stress conditions. Data clearly show that, drought stress decreased significantly yield and yield components of lupine plantsas compared with those plants irrigated normally. The percentages of decreases were 17.65% in pods number/plant, 25.0% in seeds number/pod, 41.13% in seeds number/plant, 30.79% and 33.79% in weight of pods and seeds /plant,39.74% in 100 seeds weight and 31.17% in seeds yield kg/fed. Meanwhile, soaking lupines seeds with either Chit or ChitNps each with 50 mg/l concentration increased significantly the above mentioned yield and yield components of lupine plant as compared with their corresponding untreated controls. Data in Table (5) also show that, chitosan nanoparticles was more effective than chitosan in increasing the above mentioned parameters, The percentages of increases in pods number/plant, seeds number/pod, in seeds number/plant, weight of pods and seeds /plant, 100 seeds weight and seeds yield (ton /fed) of lupine plants at normal irrigation in response to Chit Nps were 70.59%, 41.67%, 67.65%, 61.98%, 21.42%, 19.29%and 44.96% compared with 57.07%, 44.33%, 123.31%, 104.54, 37.97, 55.96% and 30.72%of lupine plants treated with Chit. While under drought stress, the percentages of increases were 58.82%, 16.67%, 57.35%, 25.84%, 15.13%, 10.92% and 32.42% in response to Chit Npscompared with 28.57%, 11.11%, 82.71%, 30.89%, 13.71% and 39.67%, 32.42%with Chit.

Table (5): Effect of chitosan and chitosan nanoparticles soaking treatment (0.0 & 50 mg/l) on yield and yield components of lupines plants under drought stress conditions (D0 & D1). Data are means of two seasons.

Drought	Treatment	No of pods/plant	No of seeds/pod	No of seeds/plant	Weight of pod/plant (g)	Weight of seeds/plant (g)	Weight of 100 seeds (g)	Seed yield (kg/fed)
D0	Control	8.7^{de}±0.57	3.0^{d}±0.33	22.7^{de}±1.35	8.9±0.16	5.6^{c}±0.57	22.7^{c}±0.55	438.2^{c}±12.35
	Chito	12.0^{ab}±0.57	3.7^{b}±0.57	35.7^{b}±1.49	11.2^{bc}±0.47	6.7^{ab}±0.38	25.2^{b}±0.58	572.8^{b}±10.42
	Chito NPs	15.7^{a}±0.33	3.7^{a}±0.33	38.0^{a}±1.34	14.4^{a}±0.44	6.8^{a}±0.47	27.1^{a}±2.28	635.4^{a}±15.62
D1	Control	6.7e±0.33	2.0^{f}±0.00	13.3^{f}±1.20	6.2^{f}±1.24	3.7^{f}±0.57	13.7^{f}f±.27	351.6e±10.84
	Chito	8.0^{d}±0.33	2.3^{e}±0.00	24.3^{d}±1.25	8.6^{e}±0.74	4.3^{e}±0.44	19.1^{e}±0.0	415.2d±16.63
	Chito NPs	9.^{c}±0.33	3.3^{c}±0.33	29.7^{c}±0.67	12.6^{b}±0.45	5.1^{cd}±0.44	21.5^{cd}±0.27	465.6^{d}±$^{18.45}$

Mean values (n=3) in each column. Means followed by a different letter are significantly different at ≤ 0.05 by Duncan"s multiple range test.

Changes in nutritional value of the yielded seeds: Table (6) shows the effect of treatment of lupine plant with either Chito or Chito NPs grown under normal and drought stress conditions on the nutritional value of the yielded seeds. Data clearly show that, drought stress caused significant decreases in total carbohydrate (CHO) percentage, protein percentage and oil percentage relative to control plant (those plant grown under normal irrigation). In the meantime drought stress increased significantly phenolic and flavonoids content as compared with control plant (those plant grown under normal irrigation). Different chitosan treatments either Chito or Chito NPs significantly increased the above mentioned parameters at both irrigation levels as compared with their corresponding controls (Table 6). Chito NPs was more effective treatment.

Table (6): Effect of chitosan and chitosan nanoparticles soaking treatment (50 mg/l) on nutritional value of the yielded seeds of lupines plants under drought stress conditions (D0 & D1). Data are means of two seasons

					Phenolics	Flavonoids
Drought	Treatment	CHO%	Protein%	Oil%	mg/100 g	
D0	Control	36.83^{bc}±2.32	35.08^{b}±1.65	8.85^{c}±0.65	17.53^{f}±0.98	0.68^{d}±0.02
	Chito	37.53^{b}±2.48	36.78^{ab}±1.98	9.35^{ab}±0.85	19.38^{e}±1.16	0.76^{c}±0.03
	Chito NPs	38.42^{a}±2.14	36.95^{a}±2.15	9.75^{a}±0.67	21.75^{d}±1.08	0.88^{b}±0.01
D1	Control	34.25^{d}±3.15	33.85^{cd}±1.05	7.55^{e}±0.35	22.93^{c}±1.34	0.77^{c}±0.01
	Chito	35.19^{cd}±3.48	34.02^{c}±2.41	8.15^{d}±0.47	26.25^{b}±1.25	0.88^{b}±0.02
	Chito NPs	35.95^{c}±3.15	35.03^{b}±2.68	8.92^{c}±0.95	31.41^{a}±1.65	0.97^{a}±0.02

Mean values (n=3) in each column followed by a different letter are significantly different at ≤ 0.05 by Duncan"s multiple range test.

Discussion

Water deficit or drought is one of the main environmental stresses factor responsible of decreasing plant growth and consequently reductions in crop production [36,37]. In the present investigation, amarked significant reduced effect of drought stress on growth criteria of lupineplant were obtained (Table 2). Similar results were obtained earlier confirmed these results [38, 39]on moringa. These reductions might be resulted from disorders caused by drought stresson physiological and biochemical processes as different plant growth regulators contents, photosynthetic assimilation activities, and activities of key enzymes responsible of the different vital metabolicprocesses [40].Regarding to chitosan treatment, seed priming with 50 mg/l chitosan or chitosan nanoparticles resulted in increased growth criteria. Similar results were also reported by [41] they stated that chitosan improved plant growth of tomato crop when applied as a soil drench or seed treatment. Chitosan is also reported to significantly increase plant growth characteristics in chili plant [42]. Moreover, Mondal [43] added that, foliar application of chitosan improved plant height, number of leaves, length, breadth and area of leaves of mungbean plant. Moreover, Khan [44] confirmed this stimulatory effect of chitosan on pea plant growth. Chitosan is considered as one of growth regulators and as a signal molecules in addition to its role as a high effective biomolecule[45]. Moreover, this promotive role resulted by enhancing activities of enzymes of nitrogen metabolism as well as, improving the translocation of nitrogen in the leaves thus increased growth and development [46]. With respect to chitosan nanoparticle, in agreement with obtained data alleviate the adverse effect of salinity stress on Phaseolusseedlings [47]. Using nanoparticles as nutrients to plant cells at the needed time help in enhancing the release of nitrogen and phosphorus fertilizer and their uptake by plant, thus decreasing nutrient loss[48].

In the present investigation, the decreases in photosynthetic pigments contents resulted by water stress (Table 3) might be resulted bychloroplast lipids oxidation and variations in pigments and protein molecules structures[49]or resulted via chlorophyll degradation by proteolytic enzymes as chlorophyllase, deterioration in chloroplast molecule and finally stomatal closure [50]. Previous results on different plant species[38, 51&52, 53]confirmed the reduced effect of water stress. On the other hand, the obtained results showed that, Chito or Chito NPs increased photosynthetic pigments. These increases could be due to improving cytokinins contents that stimulated chlorophylls synthesis or to the increased

availability of amino compounds released from chitosan[54].Farouk and Amany [55] stated that, chlorophylls and carbohydrates of cowpea plant were reduced under water stress; whereas, foliar application of chitosan, significantly increased these parameters. Moreover, Pereira [56] found that chitosan treatment increased photosynthetic pigments of *Phaseolus vulgaris*. In another report, [51] foliar treatment of chitosan increased photosynthetic pigments of barley plant. These results might be due to the increases of nitrogen and magnesium contents in the leaves because nitrogen and magnesium are the most important elements in the chemical composition of chlorophylls [57 & 58]. Regarding to the enhancing role of chitosan nanoparticles as Chito NPs might act as an efficient photocatalyst by improving the photosynthetic complexes and nitrogen metabolism [59]. Furthermore, the improving effect of chitosan NPs on photosynthetic pigments might be resulted via the enhancing of cytokinins, that stimulated chlorophyll bisynthesisor to the greater availability of amino compounds released from chitosan [54].

Drought stress decreased significantly IAA contents of lupine leaves (Table 3). In agreement with the obtained results, Khater[60] stated that subjecting cowpea plant to drought stress decreased IAA contents. Generally,the reduction of different phytohormones among them IAA caused by drought stress might be attributed the decrease of enzyme activity which participates in phytohormone synthesis or and increases in enzymes participate in its degradation[61]. Moreover, different treatments could increase IAA contents of lupine plants. Muthukrishnan[62] confirmed the promoting role of chitosan on IAA contents of chickpea plant. These increase might be due to the induced effect of Chito on auxin-related gene expression, accelerated IAA biosynthesis and transport and reduced IAA oxidase activity increases [63].

Moreover, Table (3) show that, water deficit reduced TSS meanwhile increased proline and free amino acids contents of lupine plants. The decreases in TSS contents under various environmental stresses has already been reported in different plant species [9, 40, 64].This decrease might be attributed to the reduced effect of stress on photosynthesis and/or the induced effect on partial utilization of carbohydrates into other metabolic pathways[64]. Moreover, [40] stated that sugars are the most effective solutes used in osmotic adjustment in plants under osmotic stress.Another common response in plant to drought stressis the increased content of osmoprotectamtssuch as proline[65].Proline increased is a main compound responsible of hydration of biopolymers, surviving as a readily utilizable energy source and serving as a nitrogen source compound during periods of inhibited growth[66]. Anincreased level in prolinein cowpeaupine plant which actas an indicator of its high drought tolerance[40].It is play adaptive roles in plant stress tolerance[67]. Proline is not only act as an osmolyte but it also, play an important roles in protecting subcellular structures (cell membrane and proteins) as well as it act as reactive oxygen species scavengerall of these help plant to alleviate stress[68]. Free amino acids was proven earlier to play a pivotal role in plant cytoplasmic osmotic adjustment [39].AbdElhamid [39] stated that proline and free amino acids increased by drought stress in plant.With respect to osmoprotectant compounds, total soluble sugars increased in chitosan treated plants. Our obtained data on the increased contents of proline and free amino acids are confirmed on thyme plants under drought stress [69].

Regarding to yield and its components, in the present investigation, the reductions in yield and yield components of lupine plant might be through reductions in growth (Table 2) and photosynthetic pigments (Table 3) contents thus reduced the output of photosynthesis[5] and diminished activities of calvin cycle enzymes[70]. [53]found that, drought stress decreased yield of flax plant. subjecting lupines plant to drought stress,decreased carbohydrates, protein and oil contents of lupine seeds. These decreases might be due to reduction in photosynthetic pigments and decreased activities of calvin cycle enzymes [70]. Ali [71] confirmed the obtained results in chemical composition of maize yielded seeds underlow water stress. Ali &Alqurainy [72]stated that the maincellular constituents susceptible to damage by free radicals increased level caused by drought stress are lipids of cell membranes, proteins, carbohydrates and nucleic acids. Carbohydrate changes are of particular importance because of their direct relationship with different physiological processes as photosynthesis, translocation, and respiration. The reduction in the oil content under drought stress could be due to oxidation of some of the polyunsaturated fatty acids [73].Seed priming with 50 mg/l chitosan or chitosan nanoparticles

resulted in increased yield and yield components of lupine plant. Similar results were also reported by [41] they stated that chitosan improved yield of tomato crop when applied as a soil drench or seed treatment. Chitosan is also reported to significantly increase plant productivity characteristics in chili [41]. Moreover, Khan [44] confirmed this stimulatory effect of chitosan on pea yield. Chitosan treatment induces overexpression of genes involved in photosynthesis, changes in programming of protein metabolism with an enhancement of various storage proteins and hormone metabolism [74]. Moreover, chitosan nanoparticles alleviate the adverse effect of salinity stress on bean seedlings [47]. The role of chitosan NPs in improving the adverse effect of water stress could be due to an increase in stomatal conductance and net photosynthetic CO_2-fixation activity under drought stress [75]. Also, this compound is able to increase leaf resistance to water vapor loss, thus improving plant water use and increasing biomass or yield [76]. Moreover, chitosan induced ABA activity, which plays a key role in the regulation of stomatal aperture and reduced the rate of transpiration when the plant is going through stress phase [77].

Conclusion:

From the current field study it was concluded that using either chitosan or chitosan nanoparticle with 50 mg/l concentration is beneficial to alleviate the reduction effects of drought stress in sandy soil under a wide range of field conditions. The results of this investigation highlight the role of chitosan or chitosan nanoparticle in improving*Lupinustermis*growth and yield under sandy soil conditions through enhancing various biochemical and physiological processes to minimize the hazardous effects of drought as abiotic stress.

References

1. Xu J., Yuan Y., Xu Y., Zhang G., Guo X., Wu F., Wang Q., Rong T., Pan G., Cao M., et al., Identification of candidate genes for drought tolerance by whole-genome resequencing in maize. *BMC Plant Biol.*, 14, 83 (2014). https://bmcplantbiol.biomedcentral.com/articles/10.1186/1471-2229-14-83
2. Negrão S., Schmöckel S. M., TesterM.,. Evaluating physiological responses of plants to salinity stress. *Ann. Bot*. 2017, 119, 1–11 (2017). https://dx.doi.org/10.1093%2Faob%2Fmcw191.
3. Nadeem M., Li J., Yahya M., Wang M., Ali A., Cheng A., Wang X., Ma C., Grain legumes and fear of salt stress: Focus on mechanisms and management strategies. *Int. J. Mol. Sci*., 20 (4), 799 (2019). https://doi.org/10.3390/ijms20040799.
4. Golldack D., Li C., Mohan H., Probst N., Tolerance to drought and salt stress in plants: Unraveling the signaling networks. *Front. Plant Sci*. 2014, 5, 151 (2014). https://doi.org/10.3389/fpls.2014.00151.
5. Anjum S.A., Ashraf U., Zohaib A., TanveerM.,Naeem M., Ali I., Tabassum T., Nazir U., Growth and developmental responses of crop plants under drought stress: A review. *Zemdirbyste-Agriculture*. 104, 267–276 (2017).DOI 10.13080/z-a.2017.104.034
6. Hussain M., Farooq S., Hasan W., Ul-allah S., Tanveer M., Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. Agric. *Water Manag. J.*, 201, 152–166 (2018). DOI: 10.1016/j.agwat.2018.01.028
7. Todaka D, Zhao Y., Yoshida T., Kudo M., dokoroS, Mizoi J., Kodaira K., Temporal and spatial changes in gene expression, metabolite accumulation and phytohormone content in rice seedlings grown under drought stress conditions. *Plant J.* 90: 61–78(2017). doi: 10.1111/tpj.13468. Epub 2017 Feb 11.
8. Zhu J. K., Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol,* 53, 247–273 (2002). doi: 10.1146/annurev.arplant.53.091401.143329.
9. Ashraf M., AkramN. A., Al-QurainyF., FooladM. R., Chapter five - drought tolerance: roles of organic osmolytes, growth regulators, and mineral nutrients.*Advances in Agronomy*. Vol 111,249-296 (2011). https://doi.org/10.1016/B978-0-12-387689-8.00002-3
10. Raza S., Jrnsgard B., Screening of white lupine accessions for morphological and yield traits. *African Crop Science Journal;* 13(2): 135-141 (2005). https://doi.org/10.4314/acsj.v13i2.27906
11. Písaříková B., Zralý Z., Nutritional value of lupine in the diets for pigs (a review). *Acta Vet. Brno;* 78: 399-409

(2009).https://doi.org/10.2754/avb200978030399

12. Wolko B., Clements J. C., Naganowska B., Nelson M. N., Yang H., *Lupinus*. In: Kole, C. (Ed.), Wild Crop Relatives: Genomic and Breeding Resources. Legume Crops and Forages. Springer, Berlin,: 153-206 (2011). http://dx.doi.org/10.1007/978-3-642-14387-8_9
13. Falk M., Smith D. G., Mclachland J., Mclnnes A. G., Studies on chitin 9-(1-14)-Linked 2-acetamido-2-deoxy-D-glucan) Fibres of the Diatom thalassiosirafluviatilis Husted. Can.j. chem. 44, 2269-81 (1966). https://cdnsciencepub.com/doi/abs/10.1139/v66-342
14. Mahdavi B., Rahimi A., Seed priming with chitosan improves the germination and growth performance of ajowan (*Carumcopticum*) under salt stress. *Eurasia J Biosci*7: 69-76 (2013).https://doi.org/10.5053/EJOBIOS.2013.7.0.9
15. Ma L., Li Y., Yu C., Wang Y., Li X., Li N., Chen Q., Bu N., Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress. *Protoplasma*249: 393-399 (2011). https://doi.org/10.1007/s00709-011-0290-5
16. Dimetry Z. N.,HanyM. H., Role of nanotechnology in agriculture with special reference to pest control. *Inter. J. of Pharm Tech Res.* 9(10): 121-144 (2016). https://www.sphinxsai.com/2016/ph_vol9_no10/abstracts/A(121-144)V9N10PT.pdf
17. Leiderer P., DekorsyT.. Interactions of nanoparticles and surfaces Tag der m ÄundlichenPrÄufung: 25. April (2008). URL: http://www. ub. unikonstanz. de/kops/volltexte/2008/5387.
18. Chandra S., Chakraborty N., Dasgupta A., Sarkar J., Panda K., Acharya K., Chitosan nanoparticles: A positive modulator of innate immune responses in plants. *Scientific Reports*, 5, 13 pages (2015), doi: 10.1038/srep15195.
19. Agnihotri S. A., Mallikarjuna N. N., Aminabhavi T. M., Recent advances on chitosan based micro- and nanoparticles in drug delivery. *Journal of Controlled Release,* 100, 5-28 (2004). https://doi.org/10.1016/j.jconrel.2004.08.010
20. Malerba M., Cerana R., Chitosan effects on plant systems. *International Journal of Molecular Sciences*, 23;17(7): 996(2016). doi: 10.3390/ijms17070996.
21. Carter M. R.,GregorichE. G., Soil Sampling and Methods of Analysis. Univ. Canadian Society of Soil Science by Taylor & Francis Group, LLC(2006). https://www.aweimagazine.com/
22. Nguyen Van S., Dinh Minh H., Nguyen Anh D., Study on chitosan nanoparticles on biophysical characteristics and growth of *Robusta coffee* in green house. *Biocatalysis and Agricultural Biotechnology*; 2(4):289-94 (2013). http://dx.doi.org/10.1016/j.bcab.2013.06.001
23. Lichtenthaler H. K., BuschmannC., Chlorophylls and carotenoids: measurement and characterization by UV–VIS spectroscopy. In: Wrolstad RE, Acree TE, An H, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Sporns P (eds) Current protocols in food analytical chemistry (CPFA). Wiley, New York, pp F4.3.1–F4.3.8 (2001).https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1315275.
24. Gusmiaty M.,Restu A.,Payangan R. Y., Production of IAA (*Indole Acetic Acid*) of the rhizosphere fungus in the Suren community forest stand IOP Conf. Series: Earth and Environmental Science 343, 012058 IOP Publishing (2019)doi:10.1088/1755-1315/343/1/012058
25. Homme P. M, Gonzalez B., Billard J., Carbohydrate content, frutane and sucrose enzyme activities in roots, stubble and leaves of rye grass (*Loliumperenne*L.) as affected by sources / link modification after cutting. *J. Plant Physiol.*, 140: 282-291 (1992).http://dx.doi.org/10.1016/S0176-1617(11)81080-1
26. Chow P. S,Landhausser S. M., A method for routine measurements of total sugar and starch contentin woody plant tissues.*Tree Physiology* 24, 1129 –1136 (2004). https://academic.oup.com/treephys/article-pdf/24/10/1129/4641775/24-10-1129.pdf
27. Kalsoom U., Bennett I. J., Boyce M.C.,A Review of Extraction and Analysis: Methods for Studying Osmoregulants in Plants Kalsoom et al., *J Chromatogr Sep Tech,* 7:1 (2016)DOI: 10.4172/2157-7064.1000315
28. Tamayo P., Pedrol N., Free Proline Quantification, In book: Handbook of Plant EcophysiologyTechniquesChapter: 22 Publisher: Kluwer Academic Publishers, Dordrecht, The Netherlands Editors:

Reigosa MJ (2001) DOI: 10.1007/0-306-48057-3_22

29. Verslues P. E.,Quantification of water stress-induced osmotic adjustment and proline accumulation for Arabidopsis thaliana molecular genetic studies. *Methods MolBiol*, .639:301-15(2010). doi: 10.1007/978-1-60761-702-0_19.
30. Albalasmeh A. A., BerheA A., GhezzeheiT. A.,A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry, *Carbohydrate Polymers,* 97(2): 253-261 (2013). https://doi.org/10.1016/j.carbpol.2013.04.072
31. Pedrol N., TamayoP. R., Protein content quantification by Bradford method. In book: Handbook of Plant EcophysiologyTechniquesChapter: 19, Publisher: Kluwer Academic Publishers, Dordrecht, The NetherlandsEditors: Reigosa MJ, (2001)DOI: 10.1007/0-306-48057-3_19
32. Das M., Das, S.K., Suthar, S.H.,Composition of seed and characteristics of oil from Karingda. *International Journal of Food Science and Technology*. 37:893 – 896(2002).https://doi.org/10.1046/j.1365-2621.2002.00638.x
33. Gonzalez M., Guzman B., Rudkyk R., Romano E., Molina M.. A.,Spectrophotometric Determination of Phenolic Compounds in Propolis Lat. Am. J. Pharm. **22** (3): 243-8 (2003).http://www.latamjpharm.org/trabajos
34. Chang C., Yang M., Wen H., Chern J., Estimation of total flavonoid content in propolis by two complementary colorimetricmethods, J. Food Drug Anal. 10: 178–182 (2002). http://www.sciepub.com/reference/209455
35. Rudolf J., Freund, William J. Wilson and Donn L. Mohr., Statistical Methods, Academic Press, third edition(2010)https://www.sciencedirect.com/book/9780123749703/statistical-methods
36. Ashraf M., Athar H. R.,HarrisR. J. C., Kwon T. R., Some prospective strategies for improving crop salt tolerance. AdvAgron 97:45–110 (2008). https://pureportal.coventry.ac.uk/en/publications/
37. Kaya C., Ashraf M., Sonmez O., TunaA L., Polat T., Aydemir S., Exogenous application of thiamin promotes growth and antioxidative defense system at initial phases of development in salt-stressed plants of two maize cultivars differing in salinity tolerance. ActaPhysiol Plant, 37:1741(2015). DOI 10.1007/s11738-014-1741-3.
38. Ezzo M, AbdElhamid E. M., SadakMervat Sh., AbdallaA M.,. Improving drought tolerance of moringa plants by using trehalose foliar treatments. Bioscience R. 5(4):4203-4214(2018).
39. AbdElhamid, E. M. A., SadakMervat Sh., EzzoM. I., AbdallaA M., Impact of glycine betaine on drought tolerance of *Moringaoleifera*plant grown under sandy soil. Asian J. Plant Sci., 20: 578-589 (2021). DOI: 10.3923/ajps.2021.578.589
40. Ashraf M., Iram A., Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. *Flora,* 200, 535–546 (2005). https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.503.2036&rep=rep1&type=pdf
41. Algam S., Xie G., Li B., Yu S., Su T., Larsen J., Effects of Paenibacillus strains and chitosan on plant growth promotion and control of Ralstonia wilt in tomato. Journal of Plant Pathology 92, 593-600 (2010). https://www.jstor.org/stable/41998847
42. Chookhongkha Y. N., Miyagawa S., Jirakiattikul Y., Photchanachai S., Chili growth and seed productivity as affected by chitosan. Proceedings of the International Conference on Agriculture Technology and Food Sciences, Manila, Philippines. Manila, Philippines (2012).
43. Mondal M., Malek M., Puteh A., Ismail M., Foliar application of chitosan on growth and yield attributes of mungbean (*Vignaradiata (L.) Wilczek). Bangladesh J. of Botany* 42, 179-183(2013). https://doi.org/10.3329/bjb.v42i1.15910
44. Khan R., Manzoor N., Zia A., Ahmad I., Ullah A., Shah S. M., Naeem M., Ali Sh., Khan I. H., Zia D., Malik Sh., Exogenous application of chitosan and humic acid effects on plant growth and yield of pea (*Pisumsativum*). *Inter J of Biosci*. 12(5): 43-50 (2018). http://www.innspub.net
45. Górnik K., Mieczysław G., Romanowska – Duda B., The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. *J. of Fruit and Ornamental Plant Res.*16: 333-

343(2008).http://www.inhort.pl/files/journal_pdf/journal_2008/full30%202008.pdf
46. Sultana S., Islam M., Khatun M. St., Hassain M., Huque R., Effect of foliar application of oligo-chitosan on growth, yield and quality of tomato and eggplant. *Asian J of Agricultural Res,* 11 (2): 36-42 (2017). DOI: 10.3923/ajar.2017.36.42.
47. Zayed M. M, ElkafafiS. H., ZedanA. M. G., Dawoud Sh., Effect of nano chitosan on growth, physiological and biochemical parameters of *Phaseolusvulgaris* under salt stress *J. Plant Production, Mansoura Univ*., 8 (5): 577 – 585(2017).
48. Morales-Díaz A. B., Ortega-Ortíz H., Juárez-Maldonado A., Cadenas-Pliego G., González-Morales S., Benavides-Mendoza A., Application of nanoelements in plant nutrition and its impact in ecosystems. *Adv. Nat. Sci.: Nanosci. Nanotechnol*., 8(1): 013001, pp. 13(2017). https://iopscience.iop.org/article/10.1088/2043-6254/8/1/013001
49. Marcinska I., Czyczylo-Mysza I., Skrzypek, M Filek E., Grzesiak S., et al, Impact of osmotic stress on physiological and biochemical characteristics in drought – susceptible and drought-resistant wheat genotypes. *Acta Physiol. Plant.*, 35: 451-461(2013). https://www.cabdirect.org/cabdirect/abstract/20133059014
50. Jomo M., Netondo W., Musyimi M., Drought inhibition of chlorophyll content among seven *Amaranthuss*species. *Int. J. Adv. Res. Sci. Eng. Technol*., 3: 1362-1371 (2016). http://www.ijarset.com/upload/2016/february/2_IJARSET_JOMOOSCAR.pdf
51. Behboudi F, TahmasebiSarvestani Z., ZamanKassaee M., ModaresSanaviS. A. M., Sorooshzadeh A., Ahmadi S. B.,. Evaluation of chitosan nanoparticles effects on yield and yield components of barley (*Hordeumvulgare*L.) under late season drought stress. *J. Water Environ. Nanotechnol*., 3(1): 22-39 (2018). DOI: 10.22090/jwent.2018.01.003.
52. Bakry AB, MervatShSadak, MF El-Karamany,MM Tawfik MM, 2019. Sustainable production of two wheat cultivars under water stress conditions. Plant Archives 19(2): 2307-2315.
53. SadakMervatSh, AB Bakry, MH Taha, 2019. Physiological role of trehalose on growth, some biochemical aspects and yield of two flax varieties grown under drought stress. *Plant Archives*. 19(2): 215-225.
54. Chibu H., Shibayama H., Effects of chitosan applications on the growth of several crops. P. 235-239. In: Uragami T., K. Kurita, T. Fukamizo (eds) Chitin and chitosan in life science. Yamaguchi, Japan (2001).
55. Farouk S., Amany A. R., Improving growth and yield of cowpea by foliar application of chitosan under water stress. *Egyptian Journal of Biology.*;14(1):14-6(2012).DOI: 10.4314/ejb.v14i1.2.
56. Pereira A. S., SilvaP. M., OliveraJ. L., OliveraH. C., FracetoL. F., Chitosan nanoparticles as carrier systems for the plant growth hormone gibbereillic acid. Collodis and Surfaces B: *Bioenterfaces*150: 141-152 (2017). doi: 10.1016/j.colsurfb.2016.11.027. Epub 2016 Nov 23.
57. Dzung N. A., PhuongV. T., DzungT. T., Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. *Carbohydr. Polym.*84: 751-755 (2011).DOI: 10.1016/j.carbpol.2010.07.066 AGR: IND600878274
58. Mahmoud S. H., SalamaD. M., Abd El-AzizM. E.,.Effect of chitosan and chitosan nanoparticles on growth, productivity and chemical quality of green snap bean. *Bioscience Res*., 15(4):4307-4321(2018).
59. Choudhary R. C., KumaraswamyR. V., Kumari S., Sharma S. S., Pal A., Raliya A., Biswas P., Saharan V., Cu-chitosan nanoparticle boost defense responses and plant growth in maize (*Zea mays* L.). *Sci. Rep*., 7(1): 9754 (2017). https://www.nature.com/articles/s41598-017-08571-0
60. Khater M. A., Dawood M. G., SadakMervat Sh., ShalabyM. A. F., El- Awadi M. E., El-Din K. G.,. Enhancement the performance of cowpea plants grown under drought conditions via trehaloseapplication.,*Middle East Journal of Agriculture Research*., 7 (3): 782-800(2018).
61. Vaseva-Gemisheva I., Lee D., KaranovE.. Response of *Pisumsativum*cytokinin oxidase /dehydrogenase expression and specific activity to drought stress and herbicide treatments. *Plant Growth Regul.*, 46: 199-208(2005).
62. Muthukrishnan S., Murugan I., Selvaraj M., Chitosan nanoparticles loadedwith thiamine

stimulate growth and enhances protection against wilt disease in Chickpea. *CarbohydPolym.* (2019)https:// doi. org/ 10. 1016/j. carbpol. 2019. 02. 037

63. Li. R., He J., Xie H., Wang W., Bose S. K., Sun Y., Hu J., Yin H.,. Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticumaestivum* L.), *Inter J of Biol Macromolecules* 126: 91–100 (2019). . doi: 10.1016/j.ijbiomac.2018.12.118. Epub.
64. Rady M. M., SadakMervat Sh., El-BassiounyH. M. S., Abd El-MonemA. A., Alleviation the adverse effects of salinity stress in sunflower cultivars using nicotinamide and α-tocopherol. *Australian Journal of Basic and Applied Sciences,* 5(10): 342-355(2011).
65. Moradshahi A., Eskandari S. B., Kholdebarin B., Some physiological responses of canola *(Brassica napus*L.*)* to water deficit stress under laboratory conditions, Iranian J of Sci& Technology, Transaction A, 28(A1):43-50 (2004).
66. Kala S., Godaram A. K., Effect of moisture stress on leaf total proteins, proline and free amino acid content in commercial cultivars of *Ziziphusmauritiana.* Journal of Scientific Research, 55, 65-69 (2011). https://www.bhu.ac.in/journal/Issues/JournalofScientifcResearchVol55/7.%20Shashi%20Kala-Effect%20of%20moisture.pdf
67. Mafakheri A., Siosemardeh A., Bahramnejad B., Straik P. C., Sohrabi E., Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust. J. Crop Sci. 4, 580–585 (2010). https://edepot.wur.nl/159961
68. Makhdum M. I., Shababuddin M., Effects of different doses of glycine betaine and time of spray application on yield of cotton (*Gossypiumhirsutum* L). J. Res. Sci. 17: 241-245 (2006).https://www.semanticscholar.org/paper/-Makhdum/270519b87822cb32ae60d24ee2cd7b9ff2e6c445/figure/1
69. Bistgani Z. E., SiadatS. A., Bakhshandeh A., Pirbalouti A. G., Hashemic M., Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of Thymus daenensisCelak. Crop J. 5, 407–415 (2017). DOI: 10.1016/j.cj.2017.04.003
70. Ashraf M., Shahbaz M., Ali Q.,. Drought-induced modulation in growth and mineral nutrients in canola (*Brassica napus* L.). Pak. J. Bot., 45: 93-98 (2013). https://www.pakbs.org/pjbot/PDFs/45(1)/11.pdf
71. Ali Q., Ashraf M., Anwar F., Seed composition and seed oil antioxidant activity of maize under water stress. J. Am. Oil Chem. Soc., 87:1179-1187 (2010). https://aocs.onlinelibrary.wiley.com/doi/10.1007/s11746-010-1599-5
72. Ali A., AlqurainyF., Activities of antioxidants in plants under environmental stress. In: Motohashi N (ed.), The lutein-prevention and treatment for diseases. Trans-world Research Network, India PP.187- 256 (2006).https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.473.1412&rep=rep1&type=pdf
73. Singh S., Sinha S., Accumulation of metals and its effects in *Brassica juncea*(L.) Czern. (cv. Rohini) grown on various amendments of tannery waste. Ecotoxicology and Environmental Safety, Orlando, 62: 118-127 (2005). doi: 10.1016/j.ecoenv.2004.12.026.
74. Landi L., De MiccolisAngelini R. M., Pollastro, E Feliziani S., Faretra F., Romanazzi G., Global transcriptome analysis and identification of differentially expressed genes in strawberry after preharvest application of Benzothiadiazole and chitosan. Frontiers in Plant Science. 2017; 8:235 (2017)https://doi.org/10.3389/fpls.2017.00235
75. Khan W. M., PrithivirajB., Smith D. L., Effect of FoliarApplication of Chitin and Chitosan Oligosaccharides on Photosynthesis of Maize and Soybean. Photosynthetica. 40(4):621-4 (2002). https://link.springer.com/article/10.1023/A:1024320606812
76. Tambussi E. A., Bort J., Araus J. L., Water use efficiency in C3 cereals under Mediterranean conditions: a review ofphysiological aspects. Ann Appl Biol. 2007;150(3):307-21 (2007).DOI: 10.1111/j.1744-7348.2007.00143.x AGR: IND43920414
77. Lim C. W., Baek W., Jung J., Kim J. H., Lee S. C., Function of ABA in stomatal defense against biotic and drought stresses. Int. J. Mol. Sci. 16, 15251–15270 (2015). [CrossRef] [PubMed]. doi: 10.3390/ijms160715251.