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Response of wheat to oxytetracycline during vegetative stage

Samia Haroun, Rasha Eid, Ahmed M. Mansour and Amr M. Mowafy

Botany Department, Faculty of Science, Mansoura University

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Abstract: This study was conducted to evaluate the role of two oxytetracycline levels (100 and 200 ppm) on growth and metabolism of *Triticum aestivum* (cultivar Masr1) at the vegetative stage (65 days from sowing).The results revealed that the determined growth parameters (length of shoot and root as well as fresh and dry weights and water percentage of shoot and root), pigments content (chl a, chl b and carotenoids), carbohydrates content (glucose, sucrose, total soluble sugars and starch), nitrogen fractions and protein level increased significantly in response to oxytetracycline treatments especially in case of 200 ppm. Plant nitrogen and potassium contents increased but that of phosphorous, as well as the antioxidant enzymes (polyphenoloxidase, peroxidase and catalase), decreased upon oxytetracycline treatments.

keywords: Wheat, Oxytetracycline, Growth Parameters, Metabolism

1.Introduction

Wheat is the foremost common diet in the world compared to other cereals especially in developing/poor countries **[46]**. According to **[18],** wheat (*Triticum aestivum* L.) is the staple food for over one-fifth of the human populace around the globe. The cultivated wheat area in Egypt is 8.8 MT (million tons), with a production of 6.58 tons per hectare, resulting in a total wheat productivity of about 8795,483 tons **[19]**. Due to the high consumption of bread, Egypt is the main importer of wheat in the world **[17]**. There's a huge gap between wheat production and consumption in Egypt which come to around 52.1% of the country requirements **[20]** and one of the conceivable ways to fill this gap is cultivation/breeding of wheat in recently reclaimed soils.

Antibiotics have been used since the 1950s to control certain bacterial diseases of high-value fruits, vegetables, and ornamental plants. Today, the most commonly antibiotics used for plants are oxytetracycline and streptomycin. In the USA, antibiotics applied to plants account for less than 0.5% of total antibiotic use **[35]**.

The oxytetracycline are broad action against microbes activity and including positive and negative Gram bacteria, rickettsiae, chlamydiae, mycoplasmas and protozoan parasites **[11** and **12]**.

Oxytetracycline is inexpensive antibiotics that have been applied extensively in the pro-phylaxis and therapy of human and animal infections and also at sub-therapeutic levels in animal feeding as growth stimulators **[10]**. Nowadays, oxytetracycline is ranked first among the antibiotics used in China **[9]**.

Oxytetracycline antibiotics are important subset of pharmaceuticals, which are designed to have biochemical and physiological impact and to be recalcitrant to metabolic degradation, in particular, are considered to pose a potential environmental threat **[13]**.

The active components of most anti-microbials on plants are poorly caught on. As protein amalgamation apparatus of plant plastids and mitochondria is comparable to that in microscopic organisms, protein synthesis inhibitors (tetracyclines, fluoroquinolones and macrolides) are anticipated to influence chloroplast and mitochondrial protein union, and such impacts at the level of transcription and interpretation, primarily in plastids are repress plastid replication **[7** and **31]** with negative impacts on plants morphology and photosynthesis **[34]**.

For all the afore-mentioned points, this investigation aims to asses the effect of oxytetracycline on growth and performance of healthy *Triticum aestivum* (cultivar Masr1)

growth at vegetative stage (Age: 65 days from sowing)

2. Materials and methods

Pure grains of *Triticum aestivum* cultivar Masr 1 were obtained from the Agricultural Research Center, Ministry of Agriculture, Sakha, Kafr El Sheikh, Egypt. The antibiotic; oxytertracycline (oxy) was obtained from pharmaceutical companies. All chemicals used in this investigation were of analytical grade. The used potassium nitrate and urea as

Fertilizers were obtained from Ministry of Agriculture Giza, Egypt**.**

Time course of experiment:

Homogenously-sized grains of *Triticum aestivum* cultivar Masr 1 was selected and surface sterilized by soaking in 0.01% $HgCl_2$ solution for 3 minutes. After washing with distilled water several times, the grains were divided into 3 equal groups each contains 100 grains which soaked for four hours as follow:

Group (1): soaked in dist. water to serve as a control.

Group (2): soaked in 100 ppm oxy.

Group (3): soaked in 200 ppm oxy.

Grains of the three groups were cultivated in the experimental field of the faculty of science, Mansoura University, Egypt at 11 November 2018. One hundred grains were sown in each line and irrigated as usual practice by putting equal amounts of water to each line when required. Plants were exposed to normal day night conditions.

Samples were taken; after 65 days from sowing, representing the vegetative stage and used for assessment of growth parameters (shoot length, number of leaves/plant, total leaves area/plant, shoot fresh & dry weights, shoot water content, root depth, root fresh & dry weights and root water content), as well as photosynthetic pigments, carbohydrates content, nitrogen content, total protein, elements and antioxidant enzymes.

Data were subjected to one way ANOVA using Costat (Cohort software, California, USA), followed by mean separation using the LSD at $P<0.05$

Plant analysis

Estimation of photosynthetic pigments: Leaf photosynthetic pigments concentration such as chlorophyll a, chlorophyll b and carotenoids were determined using spectrophotometer according the method described [**3]** for chlorophylls and carotenoids [**27]** as adopted by **[30]**.

Estimation of carbohydrates: The methods used in this investigation to extract the different carbohydrate fractions tested were basically attributed to **[50]. Glucose** was estimated by the O-toluidine method **[21]**. **Sucrose** content was determined using modification of the procedures **[44]**. **The total soluble sugars** were estimated according to **[23]**. Estimation of **polysaccharides (starch)** was that of **[51]. Total carbohydrates** were calculated as the totaling of the amount of total soluble sugars and polysaccharides of the same sample.

Estimation of nitrogenous constituents: The method of **[45]** was applied**. Ammonia-N** was estimated using Nessler's reagent by the method adopted by **[14]** and described by **[37].** The method used for estimation of **amide-N** was that recommended by **[37].** To estimate the **amino-N**, the method **[36]** was designed**. Protein** was determined spectrophotometrically according to **[6]**. **The total nitrogen** was determined by the conventional semi-micropropagation of Kjeldahl method of **[43]** and described by **[24].**

Estimation of total phosphorus: The method of **[32]**, as described by **[28]** and adopted by **[25]** was followed**.**

Estimation of total potassium: Wet ashing method, plant materials were dried in an oven at 80° C till constant weight. The dried matter was digested according to the method of **[8].**

Estimation of antioxidant enzymes activity: Fresh tissue (0.2 g) was homogenized and extracted according to **[1]**. **Peroxidase (POX)** activity was assayed as the increase in absorbance at 420 nm due to the formation of purpurogallin **[16]**. **Polyphenol oxidase (PPO)** activity was assayed as the increase in absorbance at 420 nm due to the formation of purpurogallin **[16]**. **Catalase (CAT)** activity was assayed by the method of **[48]** that modified by **[22]**.

3.Results and Discussion

Changes in growth parameters:

Data of table 1 show that length and dry weight of shoot increased non significantly and significantly, respectively by the two used oxy concentrations. Shoot water content was non-significantly affected by oxy treatments. Shoot fresh weight, number of leaves and total leaf area progressively increaed with the increase in oxy concentration .

Root length was non-significantly affected by oxy treatments; but fresh and dry weights of root were progresively increased with the increase in oxy concentration. , The decline in root water content was most evident at 100 ppm oxy (Table 2).

In this connection, results of **[50]** indicated that tetracycline at 0.5–10 mg L−1 could stimulate seed germination, cell mitotic division and growth; shoot height & seedling root of wheat seedlings. On the other hand, the activity components of most anti-microbials caused negative impacts on morphology and photosynthesis of plants **[4]**. Tetracycline in particular doxycycline has a major impact on plant and at high concentrations influence cell division **[47]**.

On the other hand, the enhancement of growth and the decline in the antioxidant enzymes suggest that treatments with oxytetracycline made the soil conditions more suitable for grain growth. In this connection, tetracycline has been found to cause phytotoxic impacts in numerous plant species reflected in enhancing the activity of antioxidant compounds **[51],** and repressed plant growth **[26; 33; 41; 42]**.**Changes in pigments content:**

Data in table 3 showed that, in wheat leaves chl a, carotenoids, total chlorophylls (a+b) and total pigments increased by oxy treatments; non significantly by 200 ppm and significantly by 100 ppm. Chl b and chl a / chl b ratio increased non significantly by the used oxy concentrations.

In this respect, penicillin affect photosynthetic electron transport rate, as well cephalosporin and tetracycline. Contents of photosynthetic pigments, chlorophylls and carotenoids were most strstrongly reduced in response to tetracycline **[38]**. They reported that, electron transport rate in photosynthesis was influenced by tetracycline. Contents of photosynthetic colors, chlorophylls and carotenoids, were most unequivocally decreased in treatments by tetracycline.

The antibiotics affect the division of plastids in lower plants, but not confirmed in higher plants [**7** and **29]**. A negative effect of penicillin lesson anti-microbial amoxicillin on electron transport of photosynthesis has been illustrated **[40]**.

Moreover, in this study, higher oxy concentration (200 ppm) gave the most response, in harmony, the magnitude of plant photosynthetic and pigment responses generally increased in response to increasing the antibiotics dose **[50]**.

Changes in carbohydrates content:

The tabulated data in table 4 cleared that, glucose,starch and total carbohydrates in wheat shoot increased significantly by the two used concentrations 100 and 200 ppm oxy. However total soluble sugars increased non significantly by these treatments. Moreover sucrose increased by oxy treatments; non significant by 100 ppm and significant by 200 ppm.

In this concern, on one hand, sucrose and starch are the two main compounds that provide the major substrates for respiration in many cells and are considered to dominate carbohydrate metabolism in higher plants **[49].** On the other hand, starch is the predominant storage polysaccharide in plants and its importance is confirmed by its wide distribution **[2]**. Although sucrose is the major immediate product of photosynthesis, about 30% of the CO_2 fixed by leaves in the light period is incorporated into starch **[15]**. The beneficial effect of soaking wheat grains in 100 ppm tetracycline can be related to enhancement of seed germination, cell mitotic division, and plant growth as a result of improved imbibition and stimulation of α-amylase synthesis **[33]**.

Changes in nitrogen content:

Nitrogen content in wheat plant in response to treatment with oxy as showed in table 5 cleared that, amide-N, amino-N and protein

content increased significantly by oxy

decrease below the control at 100 ppm oxy, followed by significant increase at 200 ppm.

In support to this investigation, compared with the antibiotic-free control, symbiotic nitrogen fixation increased signifiantly in soils mixed with manure containing, oxytetracycline. and the plant and soil parameters can not account for the observed increase in nitrogen fixation **[39]**.

Changes in N, P and K content:

Considering the determined NPK content of wheat shoot, the data in table 6 showed significant increments in K content and significant decrease in P contents by oxy treatments as compared to control values, the treatment with 200 ppm oxy was more effective than the treatment with 100 ppm.

In this respect, the increment of wheat shoot nutrients Ca, K, and N contents was significant with oxytetracycline and chlortetracycline but the remarkable variations of these antibiotics effects no showed until 10 days of wheat growth before harvest **[5]**.

Changes in antioxidant enzymes content:

Throughout vegetative stage of wheat plant, the results showed that, treatment with 100, 200 ppm oxy decreased catalase activity significantly and non significantly respectively. Peroxidase and polyphenol oxidase increased significantly by treatment with 100 ppm oxy and decreased significantly by 200 ppm, comparing to the control values (Table7).

In this concern, regularly, SOD, CAT, and POD activities showed a

treatments. Ammonia N exhibited significat

slight increase at the low levels (0.5–10 mg L−1) of tetracycline exposure compared with those in the control, whereas tetracycline at the high levels (25–300 mg L−1)

Could significantly ($P<0.05$ and 0.01) stimulate the three

enzymes activities **[50]**. On the other hand, phytotoxic impacts in numerous plant species by tetracycline have been reported and reflected in enhanced action of antioxidant chemicals [51]. Moreover the treatment of tetracycline in wheat (*Triticum aestivum*) can stimulate antioxidant enzymes activity at

high concentration (10–300 mg L−1) but at low concentrations (0.5–10 mg L−1) couldn't stimulate the antioxidant defense system in the dose-dependent manner, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) **[51]**.

In conclusion

Few investigations have been performed on adverse biological effects of the antibiotics on agricultural crops. The physiological and biochemical responses of plants to the toxicity induced by tetracycline remain to be compared and elucidated. From the aforementioned data it could be concluded that, the suitable level of oxytetracycline enhance growth and some metabolites of *Triticum aestivum* (cultivar Masr 1) and this suggest that the used treatments may be acclimatize the soil environment around the wheat grains

Table (1): Effect of oxy on shoot growth parameters of *Triticum astivum* (Masr1) during the vegatative stage (65 days after sowing) each value is the mean of ten replicates**.**

Treatment	Shootlength (cm)	Shootfresh weight (g)	Shootdry weight (g)	Shootwater (%)	Numberof leaves/ plant	Total leaf area / plant(cm^2)
Control	25.25^{b}	16.228^{b}	2.891^{c}	82.144^{ab}	8.10^{b}	12.95^{c}
100 ppm oxy	28.00^{ab}	24.399^{ab}	4.968^{ab*}	79.037^{b}	8.80^{ab}	13.20^{c}
200 ppm oxy	29.45^{ab}	31.253^{a*}	5.467^{a*}	82.557^{ab}	9.30^{a*}	16.63^{b*}

(*)= significant increase or decrease at 0.05 LSD.

Table (2): Effect of oxy on root growth parameters of *Triticum astivum* (Masr1) during the vegatative stage (65 days after sowing) each value is the mean of ten replicates

Treatment	Rootlengh(cm)	Rootfresh weight(g)	Rootdry weight (g)	Root water (%)
Control	8.66^{c}	1.350^{d}	0.204^{b}	84.185^{ab}
100 ppm oxy	9.35^{bc}	1.941^{d}	0.679^{a*}	63.031^{c*}
200 ppm oxy	10.94^{bc}	2.910^{bc*}	0.644^{a*}	76.905^{b}

(*)= significant increase or decrease at 0.05 LSD.

Table (3): Effect of oxy on pigments content (mg/g fresh weight) of *Triticum astivum* (Masr1) during the vegatative stage (65 days after sowing) each value is the mean of three replicates.

Treatment	Chla	Chlb	Carotnoids	Chl(a+b)	Chl(a/b)	Total pigments
Control	0.731^{c}	0.356^{a}	0.260^{c}	1.087^{c}	2.068^{a}	1.357^{c}
100 ppm oxy	0.823^{b*}	0.401^{a}	0.344^{b*}	1.224^{ab*}	2.102^{a}	1.567^{ab*}
200 ppm oxy	0.758^{c}	0.372^{a}	0.271^{c}	1.131^{bc}	2.068^{a}	1.402^{c}

(*)= significant increase or decrease at 0.05 LSD.

Table (4): Effect of oxy on carbohydrate content (mg/g dry weight) of *Triticum astivum* (Masr1) during the vegetative stage (65 days after sowing) each value is the mean of three replicates.

Treatment	Glucose	Sucrose	Total soluble sugars	Starch	Total carbohydrates
Control	21.60^{e}	33.33^{c}	90.89^{c}	155.06^{d}	245.94^{d}
100 ppm oxy	53.31^{b*}	35.46^{bc}	96.32^{abc}	220.16^{b*}	316.48^{b*}
200 ppm oxy	64.42^{a*}	45.44^{b*}	97.13^{abc}	284.25^{a*}	375.38^{a*}

(*)= significant increase or decrease at 0.05 LSD.

Table (5): Effect of different oxy concentrations on nitrogen content (mg/g dry weight) of *Triticum astivum* (Masr1) during vegatative stage (65 days after sowing) each value is the mean of three replicates.

Treatment	Ammonia-N	Amide-N	Amino-N	Total protein
Control	119.63^{c}	84.02^{c}	103.91^{c}	80.92^{c}
100 ppm oxy	95.89^{d*}	156.77^{b*}	176.15^{a*}	129.05^{ab*}
200 ppm oxy	160.12^{b*}	157.08^{ab*}	175.75^{a*}	106.94^{bc}

(*)= significant increase or decrease at 0.05 LSD.

Table (6): Effect of oxy on N, P & k content (g/100 g dry weight) of *Triticum astivum* (Masr1) during the vegatative stage (65 days after sowing) each value is the mean of three replicates**.**

Treatment	N	P	K
Control	$\mathbf{8.7^{e}}$	$\mathbf{4.2^{d}}$	$\mathbf{1.60^{f}}$
100 ppm oxy	$\mathbf{9.8^{d*}}$	$\mathbf{4.0^{e*}}$	$\mathbf{4.11^{b*}}$
200 ppm oxy	$\mathbf{10.5^{c*}}$	$\mathbf{3.6^{f*}}$	$\mathbf{3.06^{c*}}$

(*)= significant increase or decrease at 0.05 LSD.

Table (7): Effect of oxy on antioxidant enzymes activity of *Triticum astivum* (Masr1) during the vegatative stage (65 days after sowing) each value is the mean of three

***replicates*.**

Treatment	Catalase (µmoles H_2O_2 consumed/ min/mg protein)	Peroxidase (mg/g fresh weight/min)	Polyphenol oxidase (mg/g fresh weight /min)
Control	0.040^{ab}	115.30^{b}	17.33^{b}
100 ppm oxy	0.032^{c*}	119.10^{a*}	19.08^{a*}
200 ppm oxy	0.037^{b}	88.63^{c*}	9.87^{f*}

(*)= significant increase or decrease at 0.05 LSD

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