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### Drought Stress and Nitrogen Fertilization Affect Cereal Aphid Populations in Wheat Fields

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#### ABSTRACT

Water stress and nitrogen deficiency are two constraints that negatively affect growth and productivity of wheat plants. Moreover, the interaction between these two stresses could affect the susceptibility of wheat plants to insect attack. In order to examine the influence of drought stress and nitrogen fertilization on piercing-sucking insect pests, the population abundance of four cereal aphid species; English grain aphid *Stobion avenae*, wheat aphid *Schizaphis graminum*, Bird cherry-oat aphid *Rhopalosiphum padi*, and corn leaf aphid *Rhopalosiphum maidis* has been monitored in two water treatments (water stress and well-watered) and two nitrogen levels (120 and 180 kg N ha<sup>-1</sup>). Results revealed that *S. graminum* was the most dominant specie in all treatments, followed by *S. avenae*. The population of all aphids tends to increase in the second half of the season coinciding with the emergence of wheat spikes. Stressed-wheat plants attracted more aphids, especially when water deficit synchronizes with an increase in N fertilization. Aphids vigorously thrive better on stressed plants amended with high nitrogen level. Contrary, the lower infestation rate was observed in well-watered plots with lower nitrogen rate. Plant characteristics, such as days to heading, plant height, and chlorophyll content were significantly decreased in drought-stressed plants, while higher N rates increased such parameters. Grain yield and its components were reduced with drought stress and reduced nitrogen fertilization. The current study advocates avoiding severe drought concurrently with an increasing in nutritional augmentation which it might contribute in an increase in insect pest infestation and decreasing in wheat plant traits.

**Keywords:** Water deficiency, N fertilization, piercing-sucking insect pests, aphids, yield, chlorophyll content.



#### INTRODUCTION

Wheat is the most vastly grown crop all over the world (Akhtar *et al.* 2010). The cultivation of wheat has been started about 10,000 years ago with known incipient origin at the southeastern region of Turkey (Dubcovsky and Dvorak, 2007). Egypt is considered the world's largest wheat-importing country with annual increase reached 10,164,412 tons in 2017; about one million above the average for the last five years (FAO, 2019). The government is in a confrontation with the growing population, and efforts have been made to expand wheat-cultivated area. However, reports showed a decline in total cultivated area from 1.46 million hectares in 2015, followed by 1.41 million hectares in 2016, and 1.34 million hectares in 2017 (FAO, 2019).

The wheat crop is hold in defiance with a number of biotic and abiotic stresses from sowing to harvest. Drought is a major problem, which recently arises in Egypt due to neoteric structures in upper Nile river stream. Not only does drought directly affect plant physiological processes leading to growth impairment (Mewis *et al.* 2012), but also it modifies the metabolism of plant secondary metabolites and the apportionment of plant products (Khan *et al.* 2011), in addition to amending the levels of carbohydrates (Streeter *et al.* 2001), soluble

proteins (Chandra *et al.* 1998), and free amino acids (Ingram and Bartels, 1996).

Plants are the main provider of nutrients to insects. Therefore, mineral modulation in plants can adjudicate to change host plant palatability to insects' population. It may also affect insect survival, growth rate, oviposition, and reproduction (Altieri and Nicholls, 2003). Nitrogen fertilization indirectly affects the nutrient composition of the plant, and improves plant resistance to many insects. Slansky and Rodriguez (1987) stated that total nitrogen is a critical component for both plant and the insect. In a laboratory study when the nitrogen (N) fertilization level increased, the reproduction and the population growth rate of pea aphids *Acyrtosiphon pisum* (Moravvej and Hatefi, 2008) and the green peach aphid *Myzus persicae* (Jansson and Smilowitz, 1986) had been arise, however the highest anomaly level of nitrogen inhibits the population growth rate. Environmental stresses such as, drought and nitrogen deficiency can modulate crop associated herbivorous insects (Moravvej and Hatefi 2008; Mewis *et al.* 2012).

Wheat stunting was the most susceptible parameter under various combinations of water stresses, while the drastic effects of drought on wheat plants included the reduction in the number of tillers, leaves as well as leaf area, which eventually affect the total grain yield (Campbell *et al.*, 1992).

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Sustainable agricultural methods that control irrigation and fertilization practices would improve plant growth and chemical composition, which in turn affect herbivorous insects population. Cereal aphids are major sap-sucking insects hampering wheat production. The population of cereal aphids often fluctuated from one year to another with nearly 10-20 generations per year (Leslie *et al.* 2009). The aim of this study was to evaluate the response of wheat plants to water and nitrogen-deficiency stresses, and the impact on the abundance and diversity of associated aphid species populations.

## MATERIALS AND METHODS

### Experiment

Two field experiments were carried out at the experimental farm of the Faculty of Agriculture, Kafrelsheikh University, Kafr Elsheikh Governorate (31.1107° N, 30.9388° E), Egypt, during 2017/2018 and 2018/2019 seasons. The experiment was conducted in randomized complete blocks with split-plots and three replicates. Main plots were used for drought (D) [irrigated twice at sowing and stem elongation stages] and well-watered (WW) [irrigated four times at sowing, stem elongation, booting, and reproductive stages] treatments. Two N fertilization levels of 120 kg/ha<sup>-1</sup> (N1) and 180 kg/ha<sup>-1</sup> (N2) were allocated to the subplots. So, four treatments were presented as follow; drought and 120 kg N/ha<sup>-1</sup> (DN1), drought and 180 kg N/ha<sup>-1</sup> (DN2), well-watered and 120 kg N/ha<sup>-1</sup> (WWN1), and well-watered and 180 kg N/ha<sup>-1</sup> (WWN2). Wheat seeds (*Triticum aestivum* L. cv. Sids 13) were brought from Wheat Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Seeds were sown using a drilling method at seeding rate of 140 kg ha<sup>-1</sup> where each plot consisted of 20 rows, 3 m width, 3.5 m length, row spacing of 15 cm. Plots and blocks were separated by 1.5 m unplanted distances. The preceding cultivated crop was maize (*Zea mays* L.) in both seasons. When irrigation treatments were applied, all precautions were taken to separate treatments and prevent infiltration. Nitrogen fertilization in the form of urea (46.5% N), calcium superphosphate at a rate of 360 kg ha<sup>-1</sup> (15.5% P<sub>2</sub>O<sub>5</sub>), and potassium sulfate at a rate of 120 kg ha<sup>-1</sup> (48% K<sub>2</sub>O) were added during soil tillage.

### Aphid Populations

Four dominant aphid species in wheat field were selected in this study; English grain aphid *Sitobion avenae* Fabricius, Wheat aphid (greenbug) *Schizaphis graminum* Rondani, bird cherry-oat aphid *Rhopalosiphum padi* Linn, and corn leaf aphid *Rhopalosiphum maidis* Fitch. Infestation by aphids was recorded on weekly basis from January 20 to March 24 of 2018 and from January 22 to March 27 of 2019. To record aphids' populations, six wheat plants per replicate (*i.e.* 18 plant/plot) were randomly selected and aphids were directly counted on plant leaves. Later, individuals appeared on spike were also counted.

### Aphid's dominance percentage

Dominance percentage of each aphid species on wheat plants were determined according to the formula of Fasulati (1971), where dominance percentage = (t/T)×100; t = total number of each species during inspection period,

T = total number of all species counted during inspection date.

### Plant growth, yield, and yield characteristics

The number of days from sowing to heading was recorded right after the complete emergence of 50% of spikes from leaf sheath. Ten plants were randomly selected in each plot to measure plant height. Each plant was measured from the soil surface to the spike's tip (awns excluded) at harvest time. Total leaf chlorophyll content (SPAD index) was estimated using spad-502 chlorophyll meter (spad-502 plus, Konica Minolta, Kearney, NE, USA) during the flowering stage. Spike length, number of grains per spike, and the weight of 1000 grains were measured at maturity stage using 10 plants randomly selected from each plot. Grain yield was measured (kg/ha) per the entire plot.

### Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the MSTAT-C Statistical Software package (MSTAT software informer, version 5.5, 2012).

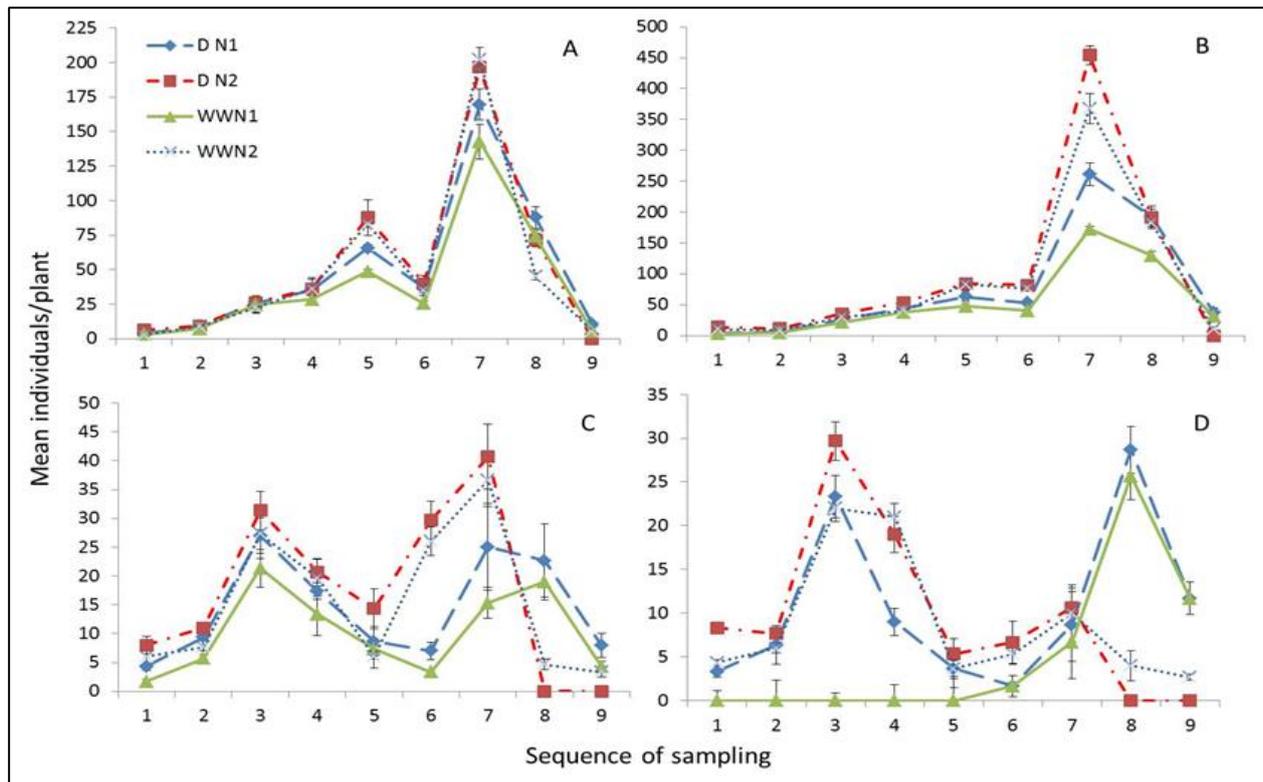
Least significant difference (LSD) was used to compare means at P≤5% (Snedecor and Cochran, 1990).

## RESULTS AND DISCUSSION

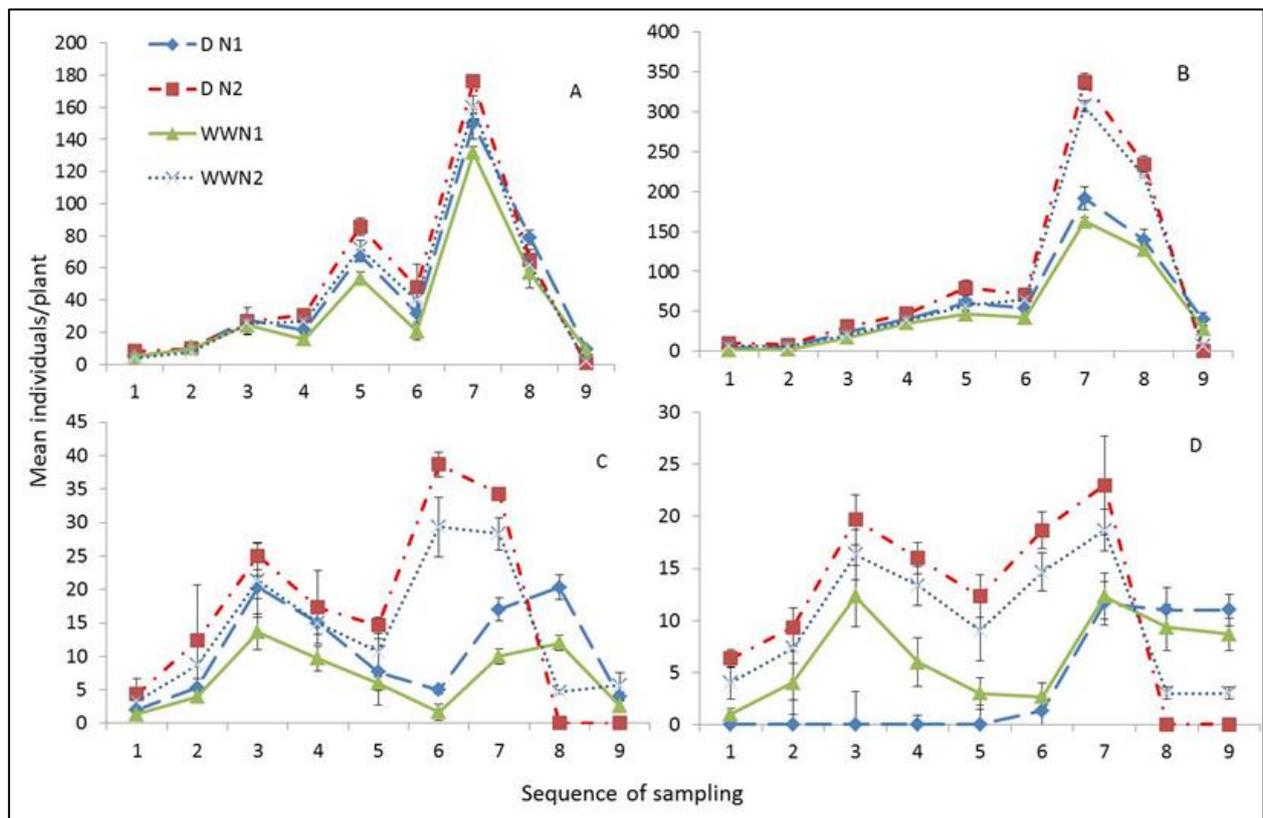
### Aphid populations

The number of English grain aphid and the wheat aphid were the most abundant, however their populations were low in the beginning of the season. The population of English grain aphid reached two peaks during the season. The first was at the fifth investigation date in February 20 and 23 during the first and the second seasons, respectively. The second peak was at the seventh inspection date in March 9 and 11 during the first and the second seasons, respectively (Fig. 1 A and 2 A). The wheat aphid attained only one peak in the seventh inspection date during both seasons (Fig. 1 B and 2 B). The other two species *R. padi* and *R. maidis* fluctuated with small populations. Generally, it was clearly observed that the numbers of aphids tend to increase in the second half of the season. Results also revealed that aphids populations were the highest with drought and high nitrogen level conditions, while the least populations were occurred with well-watered low nitrogen level conditions (Fig. 1 and 2).

Aphids' population varied with different levels of water and nitrogen fertilization (Fig. 3). The population of English grain aphid reached the highest value in DN2 plot with 1422 and 1351 individuals/plot during the first and the second seasons, respectively. However, these values were insignificantly different with those recorded in DN1 and WWN2 plots. The population of *S. avenae* in WWN1 plot recorded the lowest values ( $F = 5.92$ ,  $P = 0.019$  and  $F = 8.447$ ,  $P = 0.0073$  for the first and the second seasons, respectively) (Fig. 3 A). The population of wheat aphid *S. graminum* recorded the highest values compared to all other aphids' populations in all four plots ( $F = 37.24$ ,  $P = 0.0000$  and  $F = 143.76$ ,  $P = 0.0000$  for the first and the second seasons, respectively). Its population recorded the highest values in DN2 with 2774 and 2455 individuals/plot during the first and the second seasons, respectively. This was followed by 2424 and 2180 individuals/plot in WWN2 plot during the first and the second seasons, respectively. Among all treatments, the WWN1 plots were less favorable for the wheat aphid (Fig. 3 B).



**Fig. 1.** Mean weekly abundance of four aphid species in wheat fields treated with different water and nitrogen regimes during the first season (weekly basis from January 20 to March 24). A; *S. avenae*, B; *S. graminum*, C; *R. padi*, D; *R. maidis*, DN1; drought and 120 kg N/ha<sup>-1</sup>, DN2; drought and 180 kg N/ha<sup>-1</sup>, WWN1; well-watered and 120 kg N/ha<sup>-1</sup>, and WWN2; well-watered and 180 kg N/ha<sup>-1</sup>.



**Fig. 2.** Mean weekly abundance of four aphid species in wheat fields treated with different water and nitrogen regimes during the second season (weekly basis from January 22 to March 27). A; *S. avenae*, B; *S. graminum*, C; *R. padi*, D; *R. maidis*, DN1; drought and 120 kg N/ha<sup>-1</sup>, DN2; drought and 180 kg N/ha<sup>-1</sup>, WWN1; well-watered and 120 kg N/ha<sup>-1</sup>, and WWN2; well-watered and 180 kg N/ha<sup>-1</sup>.

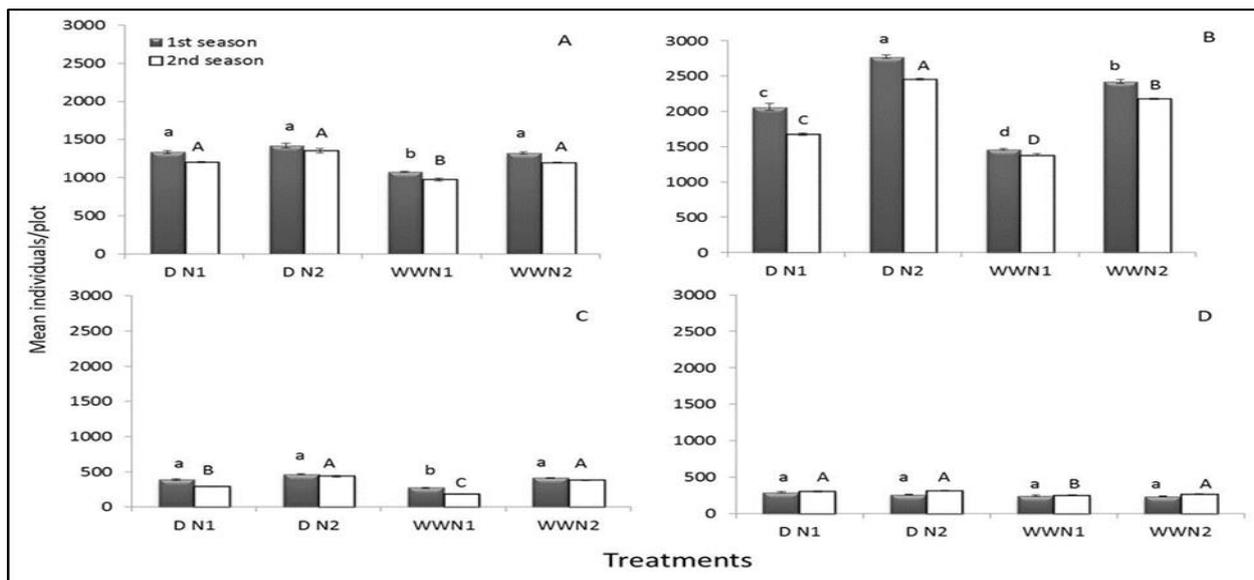


Fig. 3. Mean numbers of four aphids species/plot occurred in wheat plants grown under different water and nitrogen regimes during two seasons. A; *S. avenae*, B; *S. graminum*, C; *R. padi*, D; *R. maidis*, DN1; drought and 120 kg N/ha<sup>-1</sup>, DN2; drought and 180 kg N/ha<sup>-1</sup>, WWN1; well-watered and 120 kg N/ha<sup>-1</sup>, and WWN2; well-watered and 180 kg N/ha<sup>-1</sup>. Columns with same letters are insignificantly different at  $P \leq 5\%$ . Lower case letters are for the first season and capital letters are for the second season.

Results indicated that wheat aphid *S. graminum* was significantly ( $P = 0.0000$ ) the most dominant species in all four treatments. Its population recorded  $50.51 \pm 2.04$ ,  $56.32 \pm 2.95$ ,  $47.87 \pm 5.20$ , and  $55.12 \pm 3.70\%$  in DN1, DN2, WWN1, and WWN2 plots, respectively during the first season, while it was  $48.32 \pm 1.09$ ,  $53.86 \pm 3.31$ ,  $49.57 \pm 4.10$ ,

and  $54.13 \pm 1.20\%$  in DN1, DN2, WWN1, and WWN2 plots, respectively during the second season (Fig. 4 A, B, C and D, respectively). Contrary, corn aphid *R. maidis* was the least abundant pest in all four plots. In general, the population of all aphid species was higher in the first season compared to the second season.

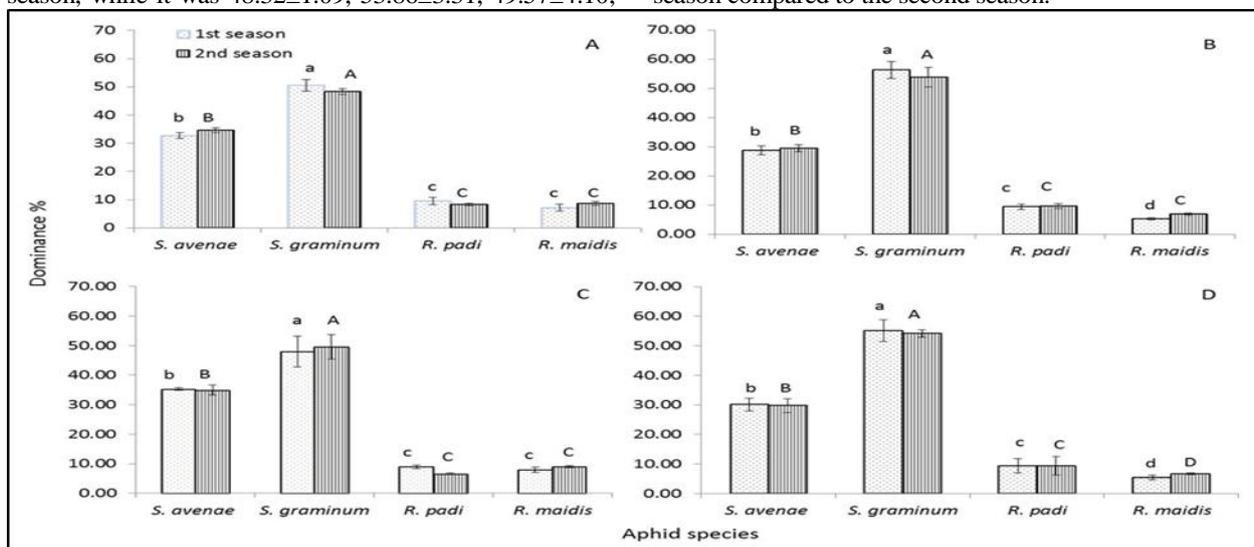


Fig. 4. Dominance percentage of aphid species in wheat field during 2017/2018 and 2018/2019, seasons. A; *S. avenae*, B; *S. graminum*, C; *R. padi*, D; *R. maidis*, DN1; drought and 120 kg N/ha<sup>-1</sup>, DN2; drought and 180 kg N/ha<sup>-1</sup>, WWN1; well-watered and 120 kg N/ha<sup>-1</sup>, and WWN2; well-watered and 180 kg N/ha<sup>-1</sup>. Columns with same letters are insignificantly different at  $P \leq 5\%$ . Lower case letters are for the first season and capital letters are for the second season.

Plants are always vulnerable to several biotic and abiotic stresses, which possibly have some consequences on the performance of associated herbivorous insects. Wheat fields are usually attacked by cereal aphid species. Some of these species are most abundant than others.

Many authors studied the fluctuation of cereal aphid in wheat fields, and most of them pointed out to the involvement of metrological factors in aphid population

fluctuation (Wains *et al.* 2010; Wang *et al.* 2015; Ahmad *et al.* 2016), while others reported the impact of nitrogen fertilization (Rostami *et al.* 2012; Wagan *et al.* 2015) and irrigation levels (Foote, 2017) on aphids' population. The current study revealed that aphids' population (nymph and adult) were significantly related to the level of nitrogen fertilization; high level resulted in maximum aphid population. Nitrogen directly affects plant growth (Rostami

*et al.* 2012) and the excessive dose of nitrogen could produce plants with prolific foliage, which might attract more insects (Sohail *et al.* 2007). Piercing-sucking insects *i.e.* aphids, mainly secure their nutritional needs from the phloem sap, which is rich in nutrients including nitrogen (Hölttä *et al.* 2013). Nowak and Komor (2010) found that increasing the levels of nitrogen fertilization increased the concentration of amino acid in phloem sap. The current work shed the light on the preference of aphids to nitrogen-rich plants with high reproduction rate.

Results also revealed that plots which received less water attracted more aphids. It is necessary to avoid severe drought because in this case it increased the infestation by aphid species. due to water deficit, the content of water in plant leaves could decreased, and when that reduction parallel occurred with an increasing in nutritional augmentation it might contribute in an increasing in plant nutritional value *e.g.* total soluble sugar, antioxidants, carbohydrates, and free amino acids per unit of surface area (Ramanulu *et al.*, 1999; Garg *et al.* 2001; Showler and Castro, 2010) consumed by arthropods. Plants which under stresses entailed to emit a variety of chemical cues (Ninkovic *et al.* 2019) that cues can be perceived by arthropods (Zhao and Kang, 2002). In the current study aphid population's abundance strongly correlated with plants in water deficiency plots. Aphids appeared to thrive better on plants suffering with drought and amended with more nitrogen. In contrast the lower infestation rate was observed on low nitrogen rate and normally irrigated.

**Plant growth, yield and yield characteristics**

Water stress significantly decreased plant height, chlorophyll content, and days to heading during both growing seasons (Table 1). The reduction of plant height

could be attributed to the reduction in cell turgor, along with the inhibition of cell division and expansion. (Abdelkhalek *et al.*, 2015; Abdrabbo *et al.*, 2016; Teama *et al.*, 2016; Attia *et al.*, 2018; Morsy *et al.*, 2018). The reduction in chlorophyll content under water stress could be due to the inhibition of photosynthesis process as a result of the acceleration in chlorophyll degradation (Wu *et al.* 2008; Hafez and Gharib, 2016).

Data in Table 1 showed that nitrogen fertilization levels significantly affected the number of days for heading, plant height, and chlorophyll content. Increasing N supply from 120 to 180 kg N ha<sup>-1</sup> significantly increased those parameters in both seasons. Makoto and Koike (2006) reported that increasing nitrogen levels leads to increase in wheat plant height and photosynthesis rate. Results also indicated that higher N supply could enhance plant height and chlorophyll content, but could not change the declining tendency under water stress. These results are in consistence with the previous findings of Lopes and Araus (2006) and Hafez and Gharib (2016).

Data presented in Table 1 indicated that grain yield and its traits were significantly reduced with the reduction of water supply compared to well-watered plants during both seasons. The reduction in these traits was might be due to the lower amount of water supply in root zone, which caused a depression in plant growth and photosynthesis activity, and hence reducing in dry matter production and grain yield. Similar results were reported by Dong *et al.* (2011), Attia *et al.* (2016), and Gangwar and Lodhi (2018).

**Table 1. Effect of water stress and two nitrogen levels on growth, yield, and yield characteristics of wheat plants in 2017/2018 and 2018/2019 seasons.**

Treatments	2017/2018						2018/2019							
	Days to heading	Plant height (cm)	Chlorophyll content (SPAD)	Number of spikes per m <sup>2</sup>	Spike length (cm)	Number of Grains per Spike	Days to heading	Plant height (cm)	Chlorophyll content (SPAD)	Number of spikes per m <sup>2</sup>	Spike length (cm)	Number of Grains per Spike		
Water treatments (W)														
Drought (D)	85.64 <sup>b</sup>	69.70 <sup>b</sup>	38.83 <sup>b</sup>	222.45 <sup>b</sup>	8.30 <sup>b</sup>	43.52 <sup>b</sup>	3021.83 <sup>b</sup>	86.08 <sup>b</sup>	66.82 <sup>b</sup>	40.85 <sup>b</sup>	219.33 <sup>b</sup>	8.09 <sup>b</sup>	42.25 <sup>b</sup>	3350.07 <sup>b</sup>
Well-watered (WW)	90.52 <sup>a</sup>	86.28 <sup>a</sup>	45.41 <sup>a</sup>	304.91 <sup>a</sup>	10.28 <sup>a</sup>	32.35 <sup>a</sup>	4781.12 <sup>a</sup>	91.83 <sup>a</sup>	83.73 <sup>a</sup>	46.283 <sup>a</sup>	299.83 <sup>a</sup>	11.05 <sup>a</sup>	31.37 <sup>a</sup>	4473.83 <sup>a</sup>
F-Test	**	**	**	**	**	**	**	**	**	**	**	**	**	**
N levels (kg ha <sup>-1</sup> )														
120 (N1)	86.17 <sup>b</sup>	73.60 <sup>b</sup>	40.33 <sup>b</sup>	249.48 <sup>b</sup>	8.17 <sup>b</sup>	36.158 <sup>b</sup>	3451.69 <sup>b</sup>	86.83 <sup>b</sup>	70.80 <sup>b</sup>	38.71 <sup>b</sup>	245.25 <sup>b</sup>	8.23 <sup>b</sup>	38.37 <sup>b</sup>	3149.52 <sup>b</sup>
180 (N2)	89.33 <sup>a</sup>	82.35 <sup>a</sup>	43.52 <sup>a</sup>	277.88 <sup>a</sup>	10.28 <sup>a</sup>	40.65 <sup>a</sup>	4351.26 <sup>a</sup>	90.08 <sup>a</sup>	80.73 <sup>a</sup>	42.75 <sup>a</sup>	274.18 <sup>a</sup>	9.96 <sup>a</sup>	35.25 <sup>a</sup>	4866.90 <sup>a</sup>
F-Test	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Interaction (W × N)	*	**	**	**	*	**	**	*	**	**	**	*	*	**

Means with the same letters in each column are insignificantly different; \*, \*\*: Significant at 0.05 level of probability.

Results have also indicated that high level of Nitrogen fertilization has significantly affected wheat grains yield and its traits in both seasons (Table 1). The role of nitrogen was may be due to improving plant growth and chlorophyll content, which reflected on yield and its components (Zewail, 2007; Abdelkhalek *et al.*, 2015; Attia *et al.*, 2018; and Gangwar and Lodhi, 2018). In addition, the combined effect of water stress and nitrogen levels was also effective on yield and yield characteristics during both seasons. Application of nitrogen resulted significantly improved grains characteristics either in water-stressed or

well-watered plants. However, the role of Nitrogen was more pronounced in non-stressed plants. These results confirm the previous findings on the effective role of nitrogen under non-stressed conditions, because water improve Nitrogen uptake by plant (Zeidan *et al.*, 2009; Abdelkhalek *et al.*, 2015; and Hafez and Gharib, 2016).

Agricultural methods can affect the susceptibility of plants to insects (Nowak and Komor, 2010; Mousa and Ueno, 2019). The ability of the plant to tolerate or repel an insect is limited to plant characteristics (Mitchell *et al.*, 2016), which are affected by various agricultural practices.

Little is known about how drought stress synchronize with nitrogen fertilization levels, and their effect on the palatability of wheat plants to the infestation of piercing-sucking insects, which eventually affect plant productivity and yield characteristics. The current study revealed that increasing N fertilization level from 120 to 180 kg/ha<sup>-1</sup> with full irrigation regime (*i.e.* four times at sowing, stem elongation, booting, and reproductive stages) improved plant growth, chlorophyll content, yield, and grain characteristics. This was associated with moderate infestation of aphids. The highest infestation percentage was noticed with water deficit and high N fertilization (180 kg/ha<sup>-1</sup>). Therefore, it is indispensable to avoid high levels of nitrogen fertilization during the periods of water deficit.

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### الإجهاد المائي ومستويات التسميد النيتروجيني وتأثيره على عشائر من الحبوب في حقول القمح

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الإجهاد المائي ونقص النيتروجين هما عائقان يؤثران سلباً على نمو وإنتاجية القمح. علاوة على ذلك، فإن التداخل بين هذين الإجهادين يمكن أن يؤثر على قابلية نباتات القمح للإصابة بالآفات الحشرية. من أجل دراسة تأثير الإجهاد المائي وكمية التسميد النيتروجيني على مستويات الإصابة بالآفات الحشرية التاقية الماصه، قمنا بدراسة الوفرة العددية لأربعة أنواع من الحبوب وهي *Rhopalosiphum padi*, *Schizaphis graminum*, *Sitobion avenae* و *Rhopalosiphum maidis* في إثنان من المعاملات المائية (جفاف، رى جيد) ومستويان من التسميد النيتروجيني (120 و 180 كجم/الهكتار). أظهرت النتائج أن أعداد *S. graminum* و *S. avenae* كانت الأكثر وفرة بين الأنواع قيد الدراسة. وكانت أعداد المن تميل إلى الزيادة في النصف الثاني من الموسم وذلك تزامناً مع ظهور السنابل. النباتات التي نمت في ظروف الجفاف جذبت أعداد أعلى من المن وبصفه خاصه عندما تزامن هذا الجفاف مع زيادة مستوى التسميد النيتروجيني. فقد لوحظ زيادة أعداد المن بصفه ملحوظه في القطاعات الجافه والتي تم تسميدها بالسماد النيتروجيني بمعدل 180 كجم/الهكتار. على النقيض فقد قلت أعداد المن معنوياً في القطاعات التي رويت بالمعدل الطبيعي للمياه وبمستوى نيتروجيني 120 كجم/الهكتار. الفتره المطلوبه لطرد السنابل، ارتفاع النبات و المحتوى من الكلورفيل قد إنخفض بشكل ملحوظ في القطاعات التي تعرضت للجفاف، في حين أدت الزيادة في إمداد النيتروجين من 120 إلى 180 كجم/الهكتار إلى زيادة في هذه الصفات. كما ان عملية تكوين الحبوب قد تأثرت بشكل كبير مع الانخفاض في امدادات المياه والتسميد النيتروجيني. لذلك فان هذه الدراسه توصي بتجنب زيادة التسميد النيتروجيني تزامناً مع فترات الجفاف أو النقص في معدلات الرى مما قد يساهم في زيادة انتشار الآفات الحشرية وانخفاض في خصائص نبات القمح.