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Irrigation Stress to Wheat at Sensitive Growth Stages: Tri-trophic Effects and Implications for Aphid Control

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ABSTRACT

Weather extremes can have profound impact on plant physiology. The altered physiology of stressed plant can also modify trophic interactions in that plant environment. Aphids are important pests of wheat crop causing direct or indirect injury to the crop. The pest is routinely managed through use of insecticides. Insecticides due to their toxic effects are mostly not desired for use on food crops. Thus, alternative approaches such as biological and cultural control are more desirable. This research explores irrigation stress impacts on wheat aphids, predators, and yield characteristics under field conditions. The wheat crop was stressed at any of the tillering, booting, heading or grain formation stages by skipping irrigation during entire length of each stage. Unstressed wheat enjoyed irrigation at all four stages. *Schizaphis graminum* (Rondani) was the most abundant aphid species and coccinellids, the most abundant predators. *S. graminum* was least on unstressed or tillering stressed wheatbut was the most on wheat stressed at booting, heading, and grain formation stages. Irrigation stress reduced chlorophyll contents (5-25%) and wheat yield (kg/ha) (6-31%) when compared with unstressed wheat. The irrigation stress changed coccinellid abundance and predator-prey ratio. Altered plant physiology and a weaken plant defense under irrigation stress attracted feeding by more aphids which resultantly reduced chlorophyll amount. The abundant prey attracted more predation in the stressed wheat. The aphids and predator's preference change under irrigation stress are clear in our findings. We also discuss tri-trophic effects and implications of current study toward wheat aphid control.

INTRODUCTION

Arthropod insect pests are a significant threat to agricultural production (Razaq *et al*., 2019b), and are responsible for an estimated crop loss of 15%, worldwide (Maxmen, 2013). Agricultural intensification practices have played vital role in changing pest population dynamics and behavior to cause damage to food crops, making them more challenging (Razaq *et al*., 2019a). For example, monoculture practice relies more on agriculture inputs like fertilizer and pesticides to manage food crops pests (Naeem *et al*., 2021; Shah *et al*., 2019, 2020). Extreme weather conditions such as drought are also major challenges, affecting normal trophic interactions and pest control in the ecosystem. Drought affected plant changed their metabolic processes such as reduced photosynthetic capacity or prolonged stomata closing to prevent the loss of available water (Pinheiro and Chaves, 2010). In addition, drought affected plants have reduced vigour and their carbon/nitrogen ratios are also altered (Rosenzweig *et al*., 2001), which could resultantly influence plant susceptibility or resistance traits (Holtzer *et al*., 1988; Meyer *et al*., 2006) against a variety of phytophagous arthropod pests (Huberty and Denno, 2004; Rouault *et al*., 2006). Therefore, agro-climatic stressors can make pest management a bigger challenge for pest managers on food crops (Noman *et al*., 2021; Tubiello *et al*., 2008).

Wheat, *Triticum aestivum* L. is an important cereal grain crop, providing 20% of the world's food calories (Hawkesford *et al*., 2013). It provides essential dietary elements, fibers, and phytochemicals, necessary for good consumer health (Shewry and Hey, 2015). At global level, about 77% of the wheat produced is consumed by the developing world (Ortiz *et al*., 2008). Being a staple diet, wheat shares 9.1% to the value added in agriculture and 1.7% of the GDP in Pakistan (GOP, 2018). The wheat production is challenged by many insect pests, particularly aphids. Aphids represent a complex of pest species that infest a range of agricultural and horticultural crops (Khan

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et al., 2012; Simon and Peccoud, 2018). They can infest both the above ground and the underground parts of the plants. Aphid species feed on phloem sap that is a rich source of nutrients with high sugar levels and free amino acids (Douglas, 2006). While feeding on phloem sap, aphids extract large amount of amino acids and sugars (Hawkins *et al*., 1985) and release excessive fluid as sugary exudates on leaf surface that subsequently invites sooty moulds and hinders photosynthetic and respirational functions. Also, aphids act as carrier of plant pathogens such as barley yellow dwarf virus (Douglas, 1993; Jakobs *et al*., 2019). *Schizaphis graminum* (Rondani), *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabricius) are major wheat yield reducers (Shah *et al*., 2017) that can cause huge crop losses of 61% (Kieckhefer and Gellner, 1992; Shah *et al*. 2017). Insecticides such as neonicotinoids are widely applied to manage aphid infestation since natural enemies alone are unable to provide sufficient pest control in wheat fields (Magalhaes *et al*., 2009; Shah *et al*., 2017). However, increasing awareness on concerns regarding residual contamination of insecticides in food crops discourages synthetic insecticides use for aphid management (Mahmood *et al*., 2016; Shah *et al*., 2019b). Non-chemical approaches such as biological control and cultural practices (sowing date, host plant resistance and plant spacing disrupting aphid population) are important pesticide alternatives (Akbar *et al*., 2017; Aslam *et al*., 2005; Schwarz and Frank, 2019).

Environmental stressor such as temperature can regulate aphid buildup and feeding dynamics, and can play a mediating role in insect pest management (Shah *et al*., 2017; Tofangsazi *et al*., 2012). Iirrigation (stressed or unstressed plants) is an important agronomic practices that may also change behavior of insect pests and may have implications toward pest control (Perfect, 1986). The effect from weather extremes such as drought, i.e., irrigation stress, have been investigated previously (Ahmed *et al*., 2017) focusing some of the phenological stages of wheat crop, like heading stage (Fereres *et al*., 1988) mainly under controlled condition, but rarely under field conditions and with regard to all phenological stages of wheat crop. The primary objective of the present study was to assess whether aphids and their predators show preference between stressed (any of the tillering, booting, heading and grain formation stages) and unstressed wheat. Furthermore, plant chlorophyll contents and biological yield components were also measured and compared between stressed and unstressed wheat.

MATERIALS AND METHODS

Field experimental setup

During the winter wheat growing season of 2018-19, a field experiment was conducted in the wheat crop field, planted in the agriculture research farm area of Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Punjab province of Pakistan (30° 11' 44" N/ 71° 28' 31" E.). Seeds used for the study were wheat variety 'Galaxy' and were sown on 31st December 2018 using a hand drawn drill. All the agronomic practices and recommendations by the local agriculture station were followed (Punjab, 2018).

Water stress treatments were applied by skipping irrigation at any of the following four wheat growth stages: Tillering, botting, heading and grain formation. Once the stress stage was over in the respective treatment plot, the irrigation was resumed in that stressed plot for the rest of wheat growth period. This procedure was similarly followed for all four stress treatments. These four are the recommended stages where wheat requires irrigation to attain optimum yield potential (Waraich *et al*., 2007). Alongside these, an additional treatment that enjoyed normal irrigation (no stress) was also included as check. The treatment plots were arranged in a randomized complete block design with 3 replicates. Each treatment plot measured 5m×3m. Adjustment treatment plots were separated by 1m row to avoid seepage effects. Aphids were the only pest species on wheat and no insecticides were applied for their management during the experiment.

Sampling

Pest sampling was carried out on a weekly basis from the initial pest appearance i.e., 26th February, 2019 until the time the crop matured and there was complete disappearance of aphid pests in all treated plots. At each sampling date, 10 plants from each treatment were selected at random and the numbers of aphids (mainly *Schizaphis graminum*) were estimated visually by counting from stem, leaves and ears of the selected plants. From the selected plants, predators (coccinellids, chrysopids, syrphids and spiders) were counted without disturbing plants, following Ibrar-ul-Hassan *et al*. (2004) and Shah *et al*. (2017).

Chlorophyll contents measurement and harvesting

Chlorophyll contents were measured from tillering until end of grain formation stage from ten wheat plants selected at random per each treatment. Three leaves from each of the upper, middle and lower stratum of a selected plant were used for measuring chlorophyll contents using a chlorophyll meter (SPAD-502) (Xiong *et al*., 2015).

The wheat crop was harvested from 1m ×1m from each treatment plot to estimate yield (kg/ha). Additionally, thirty wheat plants (tillers) were also harvested from each treatment and transported to the laboratory to measure plant height, shoot biomass, grains per spike and 1000 seed weight (Shah *et al*., 2017).

Statistical analysis

Wheat aphids and predators data were tested for normality using Shapiro-Wilk test. Non-normalized data were square root transformed before analysis but results in tables and figures are presented from untransformed data. Effect of treatments on aphid abundance was assessed using repeated measures analysis of variance (ANOVA), fitting treatment and sampling time as factors (Shah *et al*., 2017). The chlorophyll contents measured at various sampling dates were similarly analysed. Predator mean densities per plant were pooled across sampling dates to obtain seasonal totals. The seasonal counts of pest and predators were further used to calculate predator-prey ratio. The effects of water stress treatments on predators, predator-prey ratio, chlorophyll contents and wheat yield and its components were analyzed using ANOVA. Significant (P<0.05) group means were separated using least significance difference (LSD; P<0.05) test. All analysis was performed using SPSS, software package, version 21.

RESULTS

Insect species

Three species of aphids were found to feed on wheat crop, including *S. graminum*, *R. padi*, and *S. avenae*. *S. graminum* was the most abundant species ($F_{2,6}$=92.90, P<0.001, Fig. 1a), comprising about 90% of the total aphids, whereas other two species comprised only 10% of total aphids with the pest complex. The aphid data were pooled for the purpose of presenting results in next sections.

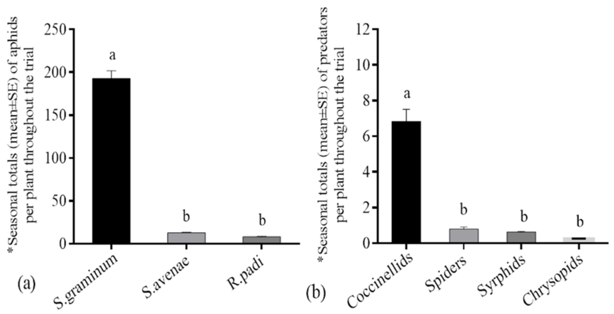

Fig. 1. Total counts of wheat aphids (a) and natural enemies (b). The counts are based on data collection during 2019 from a wheat-trial, involving different stressed and unstressed stages of wheat crop as stress treatments. The field used for data collection was located at the University Farm in Multan, Pakistan. Bars topped with the same letter are not significantly different (P>0.05; LSD test). *Total densities are the means of three replicates.

Natural enemy assemblages included coccinellids, mainly *Coccinella septempunctata* L., syrphids (*Ischiodon scutellaris* (Fabricius), chrysopids (*Chrysoperla carnea* (Stephens) and spiders (*Oxyopes javanus* Thorell). About 80% of the total predators were coccinellids ($F_{3,8}$=452.14, P<0.001, Fig. 1b) followed by syrphids, spiders and chrysopids that altogether comprised 20% of the total predators. The results of the predatory insects are discussed on pooled data across species.

First trophic level: wheat yield and characters

Plant height and shoot biomass was significantly highest in wheat that was maintained unstressed or stressed at tillering stage among various stress treatments (Table I). Grain per spike and thousand seed yield was similar among unstressed, tillering stressed, and booting stressed wheat and was higher than wheat stressed at heading and grain formation stages. Final wheat yield (kg/ha) differed markedly among stress treatments ($F_{4,10}$= 8.90, P=0.002; Fig. 2). Yield obtained from unstressed or tillering stressed wheat was the highest, whereas it was the lowest from wheat stressed at heading stage. The intermediate yield was from wheat stressed at heading or grain formation stages. The reduction percentage was 18.93% and 31.20%, respectively, for wheat stressed at heading and grain formation stages when compared with unstressed wheat.

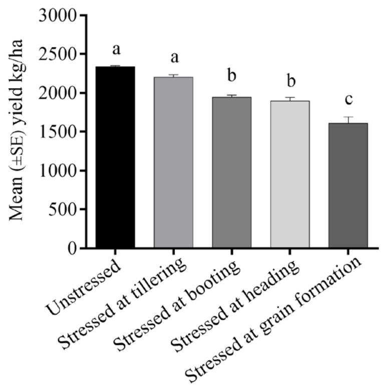

Fig. 2. Wheat yield kg/ha from irrigation-stressed and unstressed wheat crop in the field at University farm in Multan, Pakistan, in 2019. Bars topped by the same letter are not significantly different (P>0.05; LSD test).

The effect of each treatment and sampling date was found to be significant for relative amount of chlorophyll contents ($F_{24,60}$=3.73, P=0.002; Fig. 3a). The chlorophyll amount was the highest for unstressed wheat followed by tillering stressed wheat. Stressing wheat at booting, heading or grain formation stages significantly reduced chlorophyll amount ($F_{4,10}$=73.31, P<0.001; Fig. 3b). The reduction in chlorophyll contents was the maximum for stressed wheat at heading stage (i.e., 24.51%), lowest for stressed wheat at tillering stage (i.e., 5.27%), and intermediate for stressed wheat at grain formation (i.e., 14.86%) and booting stages (i.e., 11.70%).

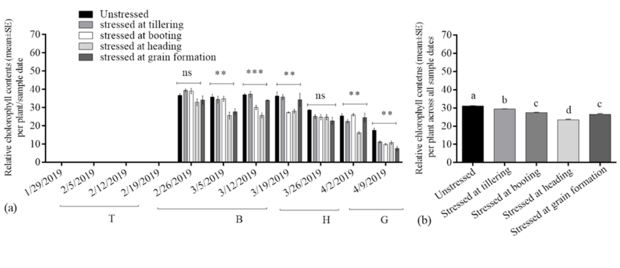

Fig. 3. Mean chlorophyll contents per week (a); and their amount per season (b) per leaf from irrigation-stressed and unstressed wheat crop in the field at University farm in Multan, Pakistan, in 2019. Bars topped by the same letter are not significantly different (P>0.05; LSD test). ns indicates non-significant difference. T: duration of tillering stage; B; duration of booting stage; H; duration of heading stage; G: duration of grain formation stage.

Second trophic level: Aphid dynamics

There was no colonization by wheat aphids in our trial through seedling until tillering stage (Fig. 4a). It was during the booting stage, when aphid colonization started on the wheat plants in the trial. Abundance of the aphids increased steadily, reached to peak numbers in the last week of March during the heading stage, following which, a steady declined started in the aphid populations. The sampling date by treatment interaction effect was significant ($F_{20,50}$=6.74, P<0.001; Fig. 4a), which means differential effects by treatments over the entire sampling duration.

When treatment effects were evaluated using seasonal averages, aphid densities were significant among treatments ($F_{4,10}$=33.76, P<0.001; Fig. 4b). Significantly fewer densities (P<0.001) were obtained from unstressed wheat and tillering stressed wheat but higher aphid densities were obtained from booting stressed wheat. Wheat stressed at heading or grain formation stages recorded intermediate aphid densities. In comparison to unstressed plant, percent aphid increase was 57.28%, 46.14% and 20.70%, respectively, for the wheat stressed at booting, heading and grain formation stages (Fig. 4b).

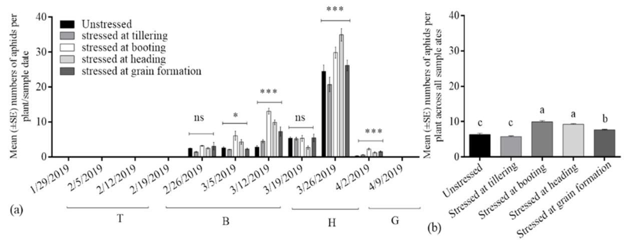

Fig. 4. Aphid abundance per week (a); and their seasonal counts (b) per plant from irrigation-stressed and unstressed wheat crop in the field at University farm in Multan, Pakistan, in 2019. * and *** indicates significant difference at P<0.05 and P<0.001; ns indicates non-significant difference. Bars topped by the same letters are not significantly different (P>0.05; LSD test). T: duration of tillering stage; B: duration of booting stage; H; duration of heading stage; G: duration of grain formation stage.

Third trophic level: Natural enemy abundance

Predatory insect species were found to be active in wheat fields from mid-March until first week of April in 2019. Irrigation treatments showed a significant impact on overall predator abundance in our trial ($F_{4,10}$=8.00, P=0.004; Fig. 5a). The predators were abundant in wheat stressed at booting stage and fewer in wheat stressed at tillering stage. Predator densities were lower and similar among the other stress treatments (i.e., heading and grain formation). There was significant effect of water stress on predator-prey ratio ($F_{4,10}$=13.18, P=0.001; Fig. 5b). The predator-ratio was the highest at heading stage, but the lowest and similar at other stages.

Table I. Effect of water stress on plant height, shoot biomass, grains/spike and seed weight (1000 seeds) of wheat crops infested with aphids in the field at university farm in wheat season 2018-19.

	Plant height (cm)	Shoot biomass (g)	Grains/spike	Thousand seed weight (g)
Unstressed plants	63.50±0.45a	2.62±0.06a	32.83±1.39a	38.38±0.99a
Plants stressed at tillering	61.83±0.82a	2.49±0.07a	30.70±0.86a	38.60±1.33a
Plants stressed at booting	55.40±1.28c	1.97±0.14b	30.30±1.25a	35.36±0.79ab
Plants stressed at heading	58.23±0.71b	2.03±0.12b	25.87±1.41b	34.69±0.43b
Plants stressed at grain formation	58.13±0.75b	2.09±0.14b	22.87±1.08c	33.52±1.41b
ANOVAs				
$F_{4,10}$	15.09	6.14	19.61	4.76
P	0.0009	0.0147	0.0003	0.0293

Means within columns followed by the different letter are significantly different (ANOVA with significant (P<0.05) treatment effects followed LSD test for mean comparison).

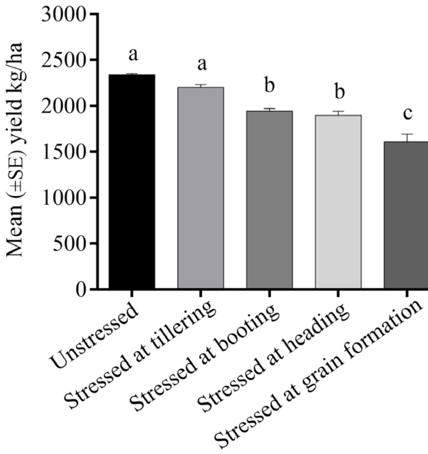

Fig. 5. Predator abundance (a) and predator-prey ratio (b) per plant from irrigation-stressed and unstressed wheat crop in the field at University farm in Multan, Pakistan, in 2019. Predator data were pooled across species. Bars topped by the same letter are not significantly different (*P*>0.05; LSD test).

DISCUSSION

The current study explored how irrigation stress impacts tri-trophic interactions on wheat crop. Water has a key role in the survival of plants as it helps in photosynthesis, necessary for plant to make food (Charmaine, 2018). The quality and quantity of food resource relies on the amount of water available to the plant (Farooq *et al*., 2009). Often herbivorous insects have chemoreceptors that detect plant water (Städler, 1984; Visser, 1986). It helps them to decide, which plant is nutritionally suitable for their growth and development (Awmack and Leather, 2002). The influence of water stress on feeding behavior of different aphid species have been investigated previously on different host plants including wheat, barley and canola (Liu *et al*., 2018; Tariq *et al*., 2012) using different water stress levels (high, medium and low) under controlled conditions (Fereres *et al*., 1988) and at heading stage typically for wheat crop. These reports showed no preference for stressed barley by *Rhopalosiphum maidis* (Fitch) and *Rhopalosiphum padi* (L.) (Hale *et al*., 2003; Oswald and Brewer, 1997), and for stressed canola by *Myzus persicae* (Sulzer) (Simpson *et al*., 2012) and *Lipaphis erysimi* (Kaltenbach) (King *et al*., 2006). On the other hand, *M. persicae* strong preference for stressed *Arabidopsis thaliana* (L.) was noted (Mewis *et al*., 2012). These reports suggest differential response of aphids to water stress on different host plants (Inbar *et al*., 2001) and supports the need for individual assessments for highly species-specific nature of trophic interactions, which also stimulated us to undertake present research.

We proved that aphid densities were greater in stressed plants than unstressed plants. Also, the study found that the aphids preferred wheat plants stressed at booting, heading and grain formation stages in comparison to wheat that was stressed at tillering stage. Other studies reported that aphids response to stress plants depend on the severity of the drought stress (Tariq *et al*., 2012). In severe water stress, aphids may avoid these plants, change to alate forms and move away to suitable hosts. This may be due to the fact that severely stressed plants become poor in nutrition due to unavailability of water. However, low and medium stress plants have more aphids due to better nutrient availability. In our trial, irrigation stress was applied for a particular stage but not for all stages. Although plants would have undergone some stress when irrigation was stopped, but resorting irrigation on other critical stages would have allowed plants to recover its water and resultantly affect the severity of drought. Under water stress, plants can conserve moisture by closing their stomata, which lessens plants transpiration but raises cell temperature (Martin-StPaul *et al*., 2017). Studies have shown that stressed plants with raised temperature are more preferable for aphids over unstressed plants with normal temperature because it favours the growth of aphids (Cahon *et al*., 2018), possibly explaining our results of increased number of aphids in water stressed plants.

It has been reported that plants can compensate stress effects by increasing their nitrogenous compounds (Chapin III, 1988). The supply of nitrogenous compounds like amino acid has direct influence on efficiency of phloem feeding herbivores (Khan and Port, 2008; Mattson Jr, 1980). In response to aphid feeding, plants lose their valuable amino acid and soluble sugar contents (Doorsher, 1988) and the extent of loss varies according to the species of aphid (Khattab, 2007; Singh and Sinhal, 2011). Aphid feeding can also cause a significant reduction in plant chlorophyll contents (Goławska *et al*., 2010; Sytykiewicz *et al*., 2013). In our findings, chlorophyll content reduction was higher in wheat, which was stressed at booting, heading and grain formation stages. As mentioned above, these stages had higher aphid infestations, which also reduced chlorophyll contents in these treatments, meaning an indirect role of short-duration water stress in reducing chlorophyll contents by attracting feeding by more aphids. Another plausible reason can be the effect of water stress itself at these stages. However, this conclusion shall remain provisional until the discrimination of the effect on chlorophyll contents either due to aphids or water stress. Further research will be directed towards determining the effect of water stress at growth stages of the wheat with and without aphids' infestation.

The management of wheat aphids primarily relies on natural enemies in wheat agroecosystem of Pakistan. Coccinellid, chrysopids, syrphids, spiders, and parasitoids are commonly found agents of biological control providing natural aphid suppression in wheat ecosystem (Shah *et al*., 2017). Intraguild predation (Mirande *et al*., 2015), temperature (Islam *et al*., 2020, 2021) and prey availability (Shah *et al*., 2017) are among the major factors affecting predation efficiencies or biological control. In our findings, wheat stressed at booting and grain formation stages had abundant predators, whereas predator abundance was lowest in tillering stressed wheat. In order to search their prey, predators do rely upon several vibratory, olfactory, and visionary cues from their host (Azandémè-Hounmalon *et al*., 2016; Ye *et al*., 2018). This suggests that wheat field with abundant prey resource are more likely to attract heavy predator abundance (Oelbermann and Scheu, 2009; Shah *et al*., 2017). The predator-prey ratio was the highest at heading stage. This suggests that stressing wheat at heading stage increases predator-prey ratio, whereas applying stress at other stages does not change predator-prey ratio when compared with unstressed wheat. This also means that irrigation stress is more likely to affect predation when applied at heading stage, which explains interactive effects of irrigation stress with wheat phenology. Through such interactive roles, water management practices can play their role in reducing aphid pressure; however, such interactions or their outcomes are likely to differ when stress is applied for a long duration, because prolonged stress can badly affect plant health and nutritional profile, which may cause shifts in prey and predator preferences, causing prey to find other plant hosts and predators to locate their prey (Gillespie and McGregor, 2000; Han *et al*.,, 2015).

In our findings, water stress at different growth stages of the wheat crop affected crop yield. Higher yield reduction was recorded from wheat that was stressed at grain formation stage followed by booting and heading stages. These stages had more aphid infestation, suffered more loss of chlorophyll content and produced low yield, indicating the high sensitivity of these stages toward water stress under the particular influence of aphid infestation (Diaz-Montano *et al*., 2014; Gitelson *et al*., 2006). Although many studies have shown that plant chlorophyll contents have positive effect on yield enhancement (Barutçular *et al*., 2016; Paknejad *et al*., 2007) but the production of high yield did not always ensure by high chlorophyll contents (Guler and Ozcelik, 2007; Wang *et al*., 1999). Wheat grain yield depends on many plant characteristics including tillering density, spike length, grains/spike and grains weight (Khan *et al*., 2010). In the present study, water stress at different growth stages caused variations in plant height, shoot biomass, grains per spike and weight of seeds. The reduction in weight of grains and total yield under water stress treatments can be attributed to reduced translocation of nutrients within the plants (Iqbal *et al*., 1999) that generated shrivelled grains due to accelerated maturity in response to reduced plant moisture.

CONCLUSIONS

Irrigation stress has a great tendency to induce aphids and predator's preference and affect tri-trophic interactions on physiologically stressed plants. It is important to note, the effects from irrigation stress can be stage-dependent, as not all stages led to similar results. Booting, heading and grain formation are highly irrigation-sensitive stages, where irrigation stress can be highly dangerous, as results of physiological consequences or weaken plant defence, attracting feeding by more aphids. Tillering stressed wheat can recover from later irrigations to counter aphid infestation to produce more chlorophyll content and high wheat yield. Among the stressed stages, heading stage could significantly improve predator-prey ratio, however, further studies are needed to unveil the underlying mechanism. Overall, irrigation is necessary for wheat and determines preference for aphids and their predators. A strategic water management practices in the wheat crop can reduce aphid pressure in wheat fields. Further research is needed to quantify yield losses due to the impact of aphid control and aphid infested wheat plants in stressed and well-irrigated wheat crop.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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