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Investigation of Induced Resistance in Wheat to *Sitobion avenae* (Hemiptera: Aphididae) under Greenhouse Conditions

R. Moradi[1], J. Shakarami[1*], and M. Mardani-Talaee[1]

ABSTRACT

English grain aphid, *Sitobion avenae* (Fabricius), is an important worldwide phloem-feeding pest of wheat due to direct sucking damage and transmission of viruses. Here, we tested the effects of six treatments on the fitness traits of *S. avenae* including: (1) Wheat with a 6-days aphid infestation, (2) Wheat sprayed with Biomin zinc, (3) Wheat seed treated with *Bacillus subtilis*, and (4 and 5) Combined treatments of Biomin zinc+pre-infestation and *B. subtilis*+pre-infestation as well as (6) A control treatment. Results revealed that there were significant differences among treatments concerning some allelochemical contents and aphid fitness traits. Treated with *B. subtilis*+pre-infestation and *B. subtilis* increased the total contents of phenol in the wheat leaves versus Biomin zinc+pre-infestation (183.63 mg g^{-1} FW). The net Reproductive rate (R_0) of *S. avenae* was significantly reduced by *B. subtilis*+pre-infestation (1.533 offspring per adult) compared to the control (6.887 offspring per adult). Treated with *B. subtilis*+pre-infestation (0.022 d^{-1}) significantly reduced the intrinsic rate of increase (r) of *S. avenae* compared with the control (0.105 d^{-1}). The lowest (0.024) and highest (0.058) Nymph Growth Index (NGI) of aphid were found on *B. subtilis*+ pre-infestation and the control, respectively. Hence, it was concluded that *B. subtilis*+pre-infestation in wheat plants can induce systemic resistance to *S. avenae*, which can be used in the IPM of this aphid.

Keywords: Age-stage, Aphid, *Bacillus subtilis*, Life table, Plant-insect interactions.

INTRODUCTION

Wheat (*Triticum aestivum* L.; Poaceae) is an essential cereal grown in many parts of the world including Iran. This crop is constrained by both destructive phloem-feeding insects and pathogens. One of the most important phloem-feeding constraints of wheat crop is the damage inflicted via English grain aphid, *Sitobion avenae* (Fabricius) (Hem.: Aphididae), which is a holocyclic aphid (Powell and Bale, 2004). The *S. avenae* not only causes direct damage via phloem-feeding but also can increase transmission of *Barley Yellow Dwarf Virus* (BYDV) that might result in significant crop losses (Thackray *et al.*, 2009). Presently, the control of phloem-feeding insects is mostly managed with synthetic insecticides; however, it causes the evolution of resistant populations through strong selective pressure (Bass *et al.*, 2015). In the recent decades, fertilizer and insecticide applications were an agronomical method to increase yield, but their residues in crops have become a great concern of users in the current years (Savary *et al.*, 2012). Some chemical fertilizers can have a positive influence on plant mineral nutrition, but it may result in increased population of phytophagous pests through enhancing the nutritional quality of the host plants (Lu *et al.*, 2007; Mardani-Talaee *et al.*, 2016, 2017). Therefore, the use of resistant cultivars and/or Induction of Resistance (IR) in the host plants are novel tactics for less emphasis on fertilizer and insecticide, in

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[1] Department of Plant Protection, College of Agriculture, Lorestan University, Khorramabad, Islamic Republic of Iran.
*Corresponding author; e-mail: shakarami.j@lu.ac.ir

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addition to an ecologically safe method for insects' control (Shen *et al.*, 2013).

Induced Systemic Resistance (ISR) is a resistant mechanism in plants that can enhance the defense system via mechanical, biological, and chemical factors to plant pathogens and insect herbivores (Walters *et al.*, 2013). The induction of plant defenses via insect feeding is also regulated through two key methods of action that consist of ISR and/or Systemic Acquired Resistance (SAR) pathways (Morkunas and Gabry´s, 2011). During plant-phloem-feeding- insect interactions, the most important defense systems to aphid are induced through phytohormones and molecular pathways (Morkunas and Gabry´s, 2011; Pieterse *et al.*, 2014; Giron *et al.*, 2013). Synthetic jasmonic acid (JA) is not directly toxic to herbivores (Kagale *et al.*, 2004); although the salicylic acid (SA) signaling can induce defenses in some plant species via phloem-feeding insects (Coppola *et al.*, 2013).

Plants are in constant interaction with potentially beneficial microorganisms such as Plant Growth-Promoting Rhizobacteria (PGPR), which are the vital components of the soil and can expand plant growth in different biotic activities of the soil ecosystem for nutrient turn over (Pieterse *et al.*, 2014; Verbon and Liberman, 2016). Furthermore, PGPRs can improve ISR and/or SAR in crop plants to phloem-feeding and chewing insects due to increasing JA- independent and JA-dependent genes (Zamioudis *et al.*, 2015; Verbon *et al.*, 2016).

Recently, the study of ISR has become an active field of research due to negative influence on some phytophagous insects (Mahfuza and Gordo, 2008; Huan– Huan *et al.*, 2012). The purpose of the present research was to evaluate the impact of biological treatment (Pre-infestation), chemical (Biomin zinc), and biological [Probio96 (*Bacillus subtilis* UTM96)] fertilizers either individually or in combination on some secondary metabolites of the wheat leaves and the effects on the fitness of *S. avenae* under greenhouse conditions. This study can help to find some solutions for pest control management.

MATERIALS AND METHODS

Plant and Aphid Cultures

Seeds of wheat cultivar Azar 2 were obtained from the Agricultural Research and Education Center in Khorramabad, Iran. The seeds were sown in 2 L pots (10 cm in diameter by 22 cm in height) filled with a suitable mixture of soil (2 parts of field soil and 1 part of sand). During 2018-2019, soil, as growing media, was collected from a fallow wheat field in Khorramabad plain (Lorestan Province, Iran) (6.00 mg kg^{-1} P, 0.160% K, 1.15% Ca; 0.087 % N, 0.018% Na, 0.084% Mg, 0% Zn, pH= 7.55, and Electrical Conductivity (EC)= 0.891 dS m^{-1}). Pots were protected by muslin (100 meshes) to prevent natural enemies attack and escape of aphids. The pots were arranged in a completely randomized design in a greenhouse set at 25 ±5°C, 60 ±5% RH, and 16:8 hours (L:D) conditions. Once the plants reached 4-6 leaf stage, they were used for the experiments. Irrigation of small pots related to the main experiment was done daily and larger pots (related to the aphid colony) were watered every 2 or 3 days, as needed.

A colony of *S. avenae*, was collected from a wheat field in Khorramabad in May 2018. The aphids were transferred to the potted plants grown in the greenhouse under the above-mentioned conditions. To maintain an aphid colony, the individuals were weekly transferred from infested plants to new young plants. After rearing the *S. avenae* for many generations on the wheat plant, they were used in the experiments.

Experiments and Treatments Application

All following experiments including induction of resistance, some secondary

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metabolites, fitness traits, and growth index were carried out for all the treatments and controls, and were replicated 50 times in a completely randomized design under greenhouse conditions including the following treatments:

1. Aphid Per-infestation Test: To determine the induced resistant by aphid apterous, adults (five aphids per plant) from the stock colony were transferred to central leaves of wheat plants. Aphids were removed from plants after being allowed to feed for 6 days. Then, the plants were kept aphid-free for 48 h. Afterward, one adult aphid was transferred to each plant for the experiments
2. Biomin Zinc Induced Resistance: The chemical (Biomin zinc) was obtained from the Bazargan Kala Company in Tehran, Iran. Resistance was artificially induced on wheat via foliar application of Biomin zinc spray (15%) with a concentration of 0.1 g L^{-1} of water at 4-6 expanded leaves stages. After 2 days, one adult aphid was transferred to each plant for the experiments.
3. *Bacillus subtilis* Strain UTM96 Induced Resistance: PGPR strain (bio-fertilizer of Probio96) for evaluation was obtained from the Biorun Company in Karaj, Iran. To determine the induced resistant via *B. subtilis* (Probio96), each seed of wheat was dipped into a concentration of l mL of *B. subtilis* before planting in plastic pots (8 cm in diameter by 7 cm in height) containing sterilized soil and sand. After that, the population of *B. subtilis* around 1×10^7 colony forming units mL^{-1} was grown into each pot after planting. Then, in the 4-6 leaf stage, one adult aphid was transferred to each plant for the experiments.
4. Biomin Zinc+Pre-Infestation Treatment: To determine the effects of Biomin zinc plus Pre-infestation period (6 days) treatments, all plants were sprayed with Biomin zinc (15%) at 4-6 leaves stage. Then, the pre-infestation period (6 days) was carried out under the above-mentioned conditions.
5. Probio 96+Pre-Infestation Treatment: To determine the effects of Probio96 plus pre-infestation period (6 days) treatments, all seed of plants were treated with *B. subtilis* strain UTM96 solution. Then, the pre-infestation period (6 days) was carried out under the above-mentioned conditions.
6. Control: Seeds of wheat grown in the collected field soil were used. Then, the treated and control plants were used for bioassays and/or extractions.

Plant Allelochemicals Bioassay in Wheat Leaves, *T. aestivum*

To investigate the secondary metabolite contents of the wheat leaves without aphid infestation (un-infested plants) with four replications per treatments, randomly selected leaves from different parts of the plant, treated at 4- 6 leaf stage, were used for flavonoid, anthocyanin and total phenolic analysis.

The concentration of flavonoid and anthocyanin in wheat leaves was measured based on the method of Kim *et al.* (2003) Briefly, 0.1 g of fresh wheat leaves from per treatments were homogenized in 3 mL of acidified ethanol solutuin (1:100 acid acetic: ethanol). Then, the samples were centrifuged at 12,000×g for 15 minutes. Afterword, the supernatants were passed through the Whatman filter paper (No.1), and the tubes were incubated for 5 min in a hot water bath (80°C) for measuring the flavonoids. Finally, 1 mL of the reaction mixture was poured into cells of spectrophotometer (Jenway™ 6705 Model) and absorbance was read at the wavelengths of 230, 300, and 330 nm. Also, a similar procedure was used to measure the anthocyanin, except for the homogenized amount of 0.2 g of wheat fresh samples in acidified Methanol solution (3 mL). Followed by centrifugation at 12,000×g for 15 minutes and then supernatants were passed from Whatman filter paper (No. 1). The reaction mixture was incubated in lightness for 24 hours at room temperature and afterward, absorbance was read at 550 nm.

According to the method of Slinkard and Singleton (1977), the amount of total

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phenolic components of the wheat plant was assayed via adding 10 mL of Methanol (80%) in 1 g of fresh samples of wheat leaves. The samples were passed through Whatman filter paper (No. 1) and the supernatants were centrifuged at 1,000×g for 5 minutes. Gallic acid as standard phenolic solutions (0, 20, 40, 60, 80, 100, 120, 180 and 200 mg mL^{-1}) were prepared before adding 100 µL of samples and 15 mL of Folin–Ciocalteau (1:10). Afterward, 14 mL sodium carbonate (7%) was also added and incubated for 5 minutes at 35℃. Finally, the standards and samples were measured with spectrophotometer at 765 nm wavelength.

Determination of the Fitness of *S. avenae*

To evaluate *S. avenae* fitness, 50 apterous adult aphids were randomly transferred to wheat leaves per treatment. Each adult aphid was restricted in a clip cage (8 cm diameter, 30 cm height, with a hole covered by a fine mesh net for ventilation) on leaf surface with suitable ventilation in the greenhouse conditions. The adult aphids were removed from the clip cages after 24 hours. Each plant received one newborn nymph that was confined to the first true leaf. The duration of nymph and adult stages was recorded at 24 hours intervals. After the appearance of adults, the duration of successive developmental stages, the beginning of reproduction, and adult fecundity were recorded daily, and the offspring were removed from each plant until the adult died.

The data were processed based on the theory of the age- stage and two-sex life table developed by Chi and Su (2006) and Chi (2018). The life expectancy (e_{xj}) is the length of time that an individual of age x and stage j is expected to live, and it was calculated as:

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{\beta} S'_{iy}$$

Where, S'_{iy} is the possibility that individuals of age x and stage j will survive to age i and stage y, and is calculated by assuming $S' = 1$.

The age-stage specific fecundity (f_{xj}) is the mean fecundity of individuals of age x and stage j and was calculated by dividing E_{xj} (total produced nymphs) by n_{xj} (individuals). Also, the formula for the age-specific survival rate (l_x) and the age-specific fecundity (m_x) are as follows:

$$f_{xj} = \frac{E_{xj}}{n_{xj}} \qquad l_x = \sum_{j=1}^{k} S_{xj} \qquad m_x = \frac{\sum_{j=1}^{k} S_{xj} f_{xj}}{\sum_{j=1}^{k} S_{xj}}$$

Where, k is the number of stages; m_x, the daily number of nymphs produced per adult aphids; S_{xj}, the probability that a newborn nymph will survival to age x and stage j and f_{xj}, the daily number of nymphs produced per adult aphid at age x.

The formula of the population parameters such as the Gross Reproductive Rate (GRR) and the net Reproductive rate (R_0) are as follows:

$$GRR = \sum_{X=\alpha}^{\beta} m_x \qquad R_0 = \sum_{x=0}^{\infty} l_x m_x$$

The intrinsic rate of increase (r) can be estimated with the iterative bisection method by Euler-Lotka Equation with age indexed from zero (Goodman, 1982) and the finite rate of increase (λ) were calculated as follows:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \qquad \lambda = e^r$$

The mean generation Time (T) is the time required for a population to increase to R_0 fold at stable age-stage distribution, and was calculated as:

$$T = \frac{LnR_0}{r}$$

The formula for the Nymphal Growth Index (NGI) is based on the equation of Setamou *et al.* (1999).

$$NGI = \frac{l_x}{T}$$

Where, l_x is the survival rate of the nymphal stage and T is the period of each nymphal stage.

Data Analysis

In the first step, data of secondary metabolites were tested using Kolmogorov-Smirnov for normality. Then, data were analyzed by one- way Analysis Of Variance (ANOVA) followed by comparison of the means with Turkey' HSD test, with a significant level at $P< 0.05$ using statistical software MINITAB 16.0. Also, data of life table were analyzed according to an age-stage, two- sex life table and mean comparisons were done through the paired bootstrap test based on CI of differences using statistical software TWO-SEX-MSChart (Chi, 2018). The method of bootstrap used 100,000 repetitions per treatment (Reddy and Chi, 2015).

RESULTS

Plant Allelochemicals

Root incubation with *B. subtilis* significantly increased the content of flavonoids in un-infested leaves compared to other treatments when the absorbance was read at 270, 300 and 330 nm (2.030, 1.848 and 1.962 mg g^{-1}, respectively). In the presence of Biomin zinc+pre- infestation (2.439 mg g^{-1}) and pre-infestation (1.409 mg/g) treatments the contents of anthocyanin increased and decreased; although there was no significant difference among the maximum and minimum contents of anthocyanin versus control. Treatment with *B. subtilis* + pre-infestation (298.20 mg g^{-1} FW) and *B. subtilis* (292.17 mg g^{-1} FW) increased the total contents of phenol in the wheat leaves versus Biomin zinc + Pre-infestation (183.63 mg g^{-1} FW). However, a significant difference was found between the treatments and the control (Table 1).

Lifespan of *S. avenae*

Application of Biomin zinc on wheat leaves reduced the nymphal period of *S. avenae* (14.69 days) compared to other treatments. The interaction between *B. subtilis*+pre- infestation treatment and wheat significantly decreased adult longevity of *S. avenae* (6.78 days) and enhanced the adult longevity of *S. avenae* in the untreated control (14.77 days) and *B. subtilis* (14.33 days) under greenhouse conditions (Table 2). The reproductive period was significantly lower in *S. avenae* fed on *B. subtilis*+pre-infestation and Biomin zinc+pre- infestation treatments (2.883 and 3.653 days, respectively) than the control and Biomin zinc (5.778 and 5.293 days, respectively). In the study, *B. subtilis*+pre-infestation-wheat-aphid interactions PGPR and feeding aphid significantly decreased Adult Pre-Reproductive Period (APRP) of *S. avenae* versus *B. subtilis* (1.53 days). Moreover, there were no significant differences among different treatments in the Total Pre-Reproductive Period (TPRP) of *S. avenae*. Treatment *B. subtilis*+pre-infestation (0.024) reduced Nymphal Growth Index (NGI) of aphid compared to untreated control (0.058) in the wheat plant (Table 2).

Fitness of *S. avenae*; Population Growth Parameters

Wheat treated with *B. subtilis*+pre-infestation and Biomin zinc+pre-infestation (5.85 and 5.71 offspring, respectively) significantly decreased the value of Gross Reproductive Rate (*GRR*) of aphid compared to Biomin zinc (14.44 offspring) application. The amount of net Reproductive rate (R_0) of aphid was significantly reduced by *B. subtilis*+pre-infestation treatment (1.533 offspring per adult) compared to

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Table 1. The mean (±SE) amount of secondary metabolites in un-infested leaves of wheat in different treatments under greenhouse conditions [25±5°C, 65±5% RH, and 16:8 hours (L:D)].

Treatments	Flavonoids (mg g^{-1})			Anthocyanin (mg g^{-1})	Total phenol (mg g^{-1} FW ^{a})
	270 nm	300 nm	330 nm	550 nm	765 nm
Control	0.773 ± 0.135^{b}	0.674 ± 0.149^{b}	0.894 ± 0.152^{b}	1.841 ± 0.281^{ab}	232.38 ± 7.37^{b}
Pre-infestation	0.712 ± 0.132^{b}	0.758 ± 0.134^{b}	1.0909 ± 0.056^{b}	1.409 ± 0.129^{b}	204.04 ± 6.42^{bc}
Biomin zinc	0.826 ± 0.360^{b}	0.689 ± 0.374^{b}	0.780 ± 0.280^{b}	2.129 ± 0.153^{ab}	236.75 ± 3.65^{b}
Bacillus subtilis UTM96	2.030 ± 0.248^{a}	1.848 ± 0.214^{a}	1.962 ± 0.209^{a}	2.182 ± 0.104^{ab}	292.17 ± 3.07^{a}
Biomin zinc+Pre-infestation	1.106 ± 0.184^{ab}	1.030 ± 0.094^{ab}	1.037 ± 0.084^{b}	2.439 ± 0.244^{a}	183.63 ± 2.17^{c}
B. subtilis +Pre-infestation	1.038 ± 0.215^{ab}	0.818 ± 0.172^{b}	1.288 ± 0.225^{ab}	1.795 ± 0.174^{ab}	298.20 ± 3.16^{a}
df	5, 23	5, 23	5, 23	5, 23	5, 23
F	4.69	4.59	5.20	3.57	33.66

[a] FW: Means Fresh Weight. (a-c) Means followed by different letters in each column are significantly different (HSD, $P< 0.01$).

Table 2. The mean (±SE) developmental time of life stages and some biological parameters of *Sitobion avenae* reared in different treatments under greenhouse conditions [25±5°C, 65±5% RH, and 16:8 hours (L:D)].[a]

Treatments	Developmental time	Adult longevity	Reproductive period	APRP	TPRP	NGI
Control	15.38 ± 0.81^{ab}	14.77 ± 0.99^{a}	5.778 ± 0.606^{a}	1.334 ± 0.185^{ab}	16.417 ± 0.880^{a}	0.058
Pre-infestation	16.64 ± 0.93^{a}	8.41 ± 1.29^{bc}	4.177 ± 1.064^{ab}	1.295 ± 0.203^{ab}	16.589 ± 1.090^{a}	0.030
Biomin zinc	14.69 ± 0.75^{b}	12.77 ± 1.56^{ab}	5.292 ± 0.878^{a}	1.334 ± 0.228^{ab}	15.500 ± 0.736^{a}	0.041
Bacillus subtilis UTM96	15.17 ± 0.93^{ab}	14.33 ± 1.97^{a}	4.412 ± 0.830^{ab}	1.530 ± 0.256^{a}	16.470 ± 1.013^{a}	0.026
Biomin zinc+Pre-infestation	16.26 ± 0.85^{a}	9.930 ± 1.11^{b}	3.653 ± 0.542^{b}	1.220 ± 0.152^{ab}	17.218 ± 0.954^{a}	0.036
B. subtilis+Pre-infestation	16.44 ± 0.96^{a}	6.780 ± 0.69^{c}	2.883 ± 0.407^{b}	1.000 ± 0.000^{b}	17.236 ± 0.998^{a}	0.024
df	5, 166	5, 144	5, 144	5, 128	5, 128	-
F	2903.612	157.704	55.998	14.762	0.527	-

[a] APRP: Adult Pre-Reproductive Period; TPRP: Total Pre-Reproductive Period, NGI= Nymph Growth Index. (a-b) The SEs were estimated by using 100000 bootstraps and compared by using paired bootstrap test based on CI of differences ($P< 0.05$).

Table 3. The mean (±SE) life table parameters of *Sitobion avenae* reared on different treatments under greenhouse conditions [25±5°C, 65±5% RH, and 16:8 hours (L:D)].^{a}

Treatments	Cohort size (N)	*GRR* (Offspring)	R_0 (Offspring per adult)	r (d^{-1})	λ (d^{-1})	T (d)	F (Offspring)
Control	39 (50)	10.42±1.618^{ab}	6.887±0.965 ^{a}	0.1045±0.011^{a}	1.1101±0.012^{a}	18.47±0.951^{a}	7.77±1.005 ^{a}
Pre-infestation	22 (50)	10.32±3.134^{ab}	2.591±0.850 ^{bc}	0.0476±0.017^{bc}	1.0488±0.018^{bc}	20.01±1.366^{a}	5.18±1.522 ^{ab}
Biomin zinc	26 (50)	14.44±5.710^{a}	4.069±1.005 ^{b}	0.0711±0.013^{ab}	1.0740±0.015^{ab}	19.74±1.638^{a}	6.88±1.472 ^{ab}
Bacillus subtilis UTM96	18 (50)	7.17±2.322^{ab}	2.489±0.767 ^{bc}	0.0466±0.016^{cb}	1.0477±0.017^{bc}	19.57±1.411^{a}	6.22±1.572 ^{ab}
Biomin zinc+ Pre-infestation	27 (50)	5.71±1.072^{b}	2.444±0.525 ^{bc}	0.0447±0.012^{cb}	1.0457±0.013^{bc}	20.03±1.097^{a}	4.07±0.726 ^{b}
B. subtilis + Pre-infestation	18 (50)	5.85±1.024^{b}	1.533±0.382 ^{c}	0.0220±0.013^{c}	1.0222±0.014^{c}	19.45±1.202^{a}	3.83±0.663 ^{b}
d.f.	-	5, 261	5, 261	5, 261	5, 261	5, 261	5, 144
F	-	57.219	268.592	188.042	193.865	7.479	49.391

^{a} *GRR*= The Gross Reproductive Rate; R_0= The Net Reproductive Rate; r= Intrinsic rate of increase; λ= Finite rate of increase; T= Mean generation Time, and F= Fecundity. (a-c) The SEs were estimated by using 100000 bootstraps and compared by using paired bootstrap test based on CI of differences . $P < 0.05$

control (6.887 offspring per adult). Treatment by *B. subtilis*+pre-infestation indicted that the intrinsic rate of increase (r) and finite rate of increase (λ) (0.022 and 1.022 d^{-1}, respectively) of aphid significantly decreased; but increased their values on control (0.105 and 1.110 d^{-1}, respectively). Also, there were no significant differences among various treatments in mean generation Time (T) of *S. avenae*. The Fecundity (F) of aphid reared on wheat-treated with *B. subtilis*+pre-infestation and Biomin zinc+pre-infestation (3.83 and 4.07 offspring, respectively) significantly decreased compared to control (7.77 offspring) (Table 3).

Fitness of *S. avenae*; Life Table Parameters

At the first day, the age- stage life expectancies (e_{xj}) of *S. avenae,* reared on the control, Biomin zinc, Biomin zinc+pre-infestation, pre-infestation, *B. subtilis,* and *B. subtilis*+pre- infestation-treated plants was recorded as 28.18, 21.59, 20.31, 19.23, 17.26, and 16.44 days, respectively (Figure 1). The e_{xj} amount of the adult stage at the first day was also observed on control (22.93 days), *B. subtilis* (20.21 days), Biomin zinc (19.82 days), pre-infestation (14.14 days), Biomin zinc+pre-infestation (12.34 days) and *B. subtilis*+pre-infestation (6.26 days), respectively. Hence, the *B. subtilis*+pre-infestation presence decreased the e_{xj} of phloem- feeding aphid versus control plants (Figure 1).

The interaction among different treatments and wheat had a different effect on the age-specific survival rates (l_x) of aphid (Figure 2). The highest and lowest number of the female age-stage specific fecundity (f_{x2}) of phloem-feeding aphid recorded 1.25 nymphs on the control plant at 10th day and 2.00 nymphs on *B. subtilis*+pre-infestation at 9th day, respectively (Figure 2). The maximum and minimum values of the age-specific fecundity of the total population (m_x) of aphid were found as 1.33 nymphs on Biomin

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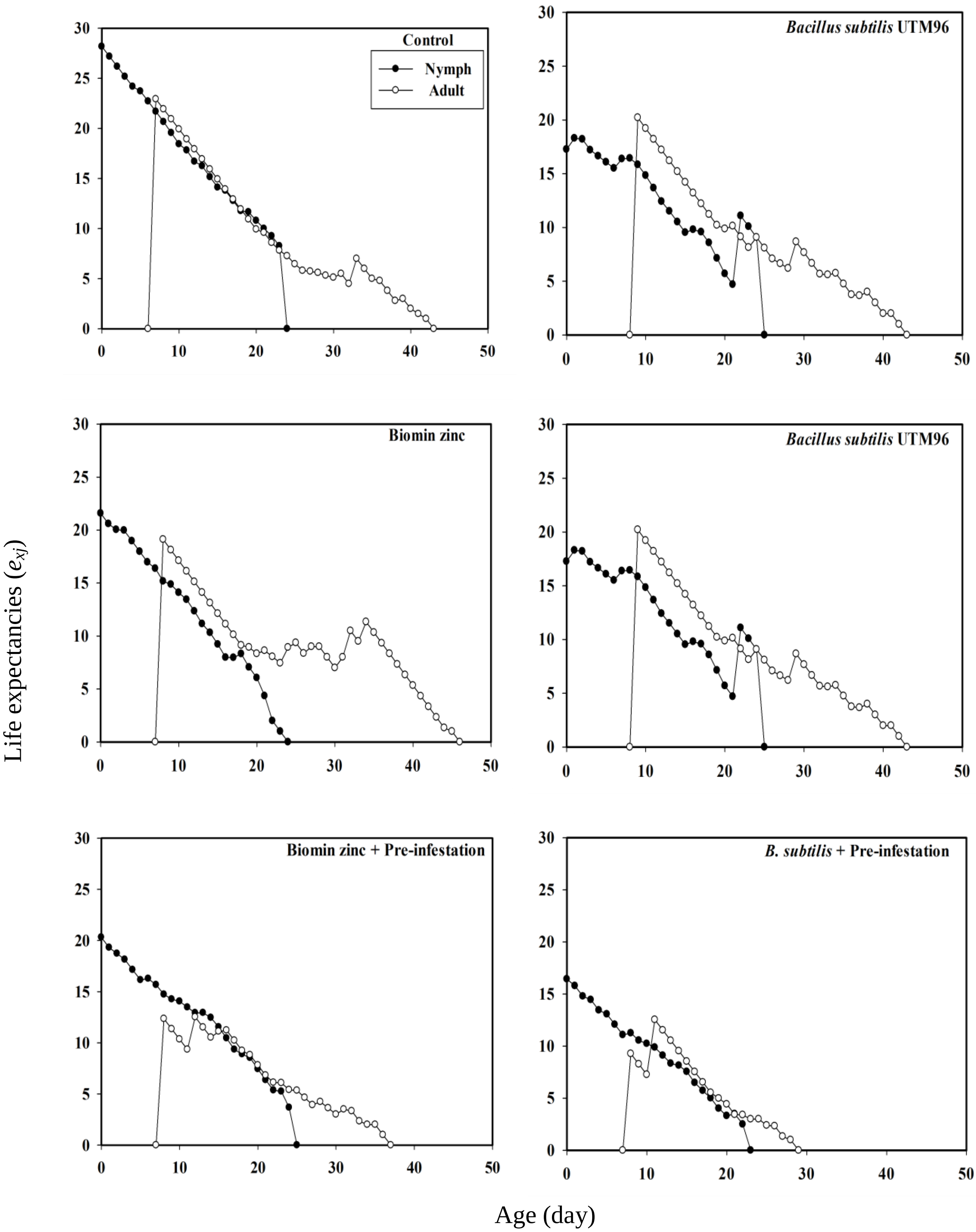

Figure 1. The age-stage life expectancies (e_{xj}) of *Sitobion avenae* reared in different treatments under greenhouse conditions [25±5°C, 65±5% RH, and 16:8 hours (L:D)].

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zinc at day 35 and 0.40 nymphs on Biomin zinc+pre-infestation at days 20 and 25, respectively (Figure 2).

Interaction among various treatments, wheat, and aphid had mainly effect on the age- specific net maternity (l_xm_x) values of *S. avenae*, of which the highest and lowest ones were recorded as 0.50 nymphs on control at day 22 and 0.15 nymphs on *B. subtilis*+pre- infestation at days 15, 18, and 22, respectively (Figure 3).

DISCUSSION

Currently, healthy and safe food free of poisonous residues is demanded by consumers. To avoid dangerous chemicals against herbivorous insects, Induced Resistance (IR) can be used to reduce the pest population and the resulting damage. Several kinds of research have proved that IR by various factors in many plant species has negative effects on the fitness of insect pests (Mahfuza and Gordo, 2008; Pieterse *et al.*, 2014; Zamioudis *et al.*, 2015; Verbon *et al.*, 2016). According to the results achieved in this research, various artificial inductions led to significant effects on Growth Index (GI) and life table traits of *S. avenae* and on some allelochemical components in the wheat leaves, which confirm the potential effects of plant quality in induced resistance to *S. avenae.*

Plants are frequently challenged by insects in their natural environments and have to develop diverse defense responses to protect themselves from pest (including aphids). Therefore, decreasing and/or increasing the fitness of phloem-feeding insects depends on the defense traits (for instance repellents, deterrents, anti- nutrients and digestive compounds) of their host plants (Lu *et al.*, 2014). Our results point out that the nymph developmental time of *S. avenae* fed on *T. aestivum*-treated with Biomin zinc decreased and their reproductive period time and APRP increased compared to the other treatments. Hence, the suitability of Biomin zinc for *S. avenae* can due to increased quantities of nutrients or reduced levels of defenses-related chemical compounds (such as flavonoids, anthocyanin, total phenol and etc.), These compounds which are synthe-sized via JA/Ethylene (ET) and SA path-ways in response to various environmental stresses for instance insects and pathogens attacks (Bourgaund *et al.*, 2010; Campos *et al.*, 2014), and preclude the fitness of phloem-feeding insects (Mardani-Talaee *et al.*, 2016).

The Growth Index (GI) represents the effects of nourishment quality on both survival rate and developmental time of herbivore insects (Setamou *et al.*, 1999). The value of aphid NGI decreased on *B. subtilis*+pre-infestation treatment versus the control. Wheat-mediated *B. subtilis*+pre-infestation reduces NGI and increases the mortality rate of phloem-feeding aphid *S. avenae* that can induce gene expression of transcription factors for activating flavonoids biosynthesis (Ali and McNear, 2014). Flavonoids are found in many plants as anti-feedant and/or pigments against herbivorous insects (Schoonhoven *et al.*, 2005). In this study, treated wheat seed with *B. subtilis* UTM96 increased the amount of flavonoids contents in leaves versus control; this can induce ISR to aphid due to production of apoplastic peroxidase activity, callose deposition, and reactive oxygen species (Niu *et al.*, 2011; Pieterse *et al.*, 2014; Rahman *et al.*, 2015). Thus, increased flavonoid content in treatment with PGPRs and feeding aphid decreased the NGI of the aphid due to enhanced defense mechanisms of host plants and showing ISR activity against aphid (Pineda *et al.*, 2010; de Oliveira Araujo, 2015).

The foliar spraying of the wheat plants by Biomin zinc increased *GRR* values of *S. avenae*, compared to other treatments. Zinc is a vital micronutrient for the function of various enzymes that is required for healthy growth and reproduction of plants, and plays an important role in synthesis of protein, lipids, and carbohydrates, maintaining integrity of the membrane structure and nucleic acid metabolism in plants (Spiegel-Roy and Goldschmidt, 2008). Therefore,

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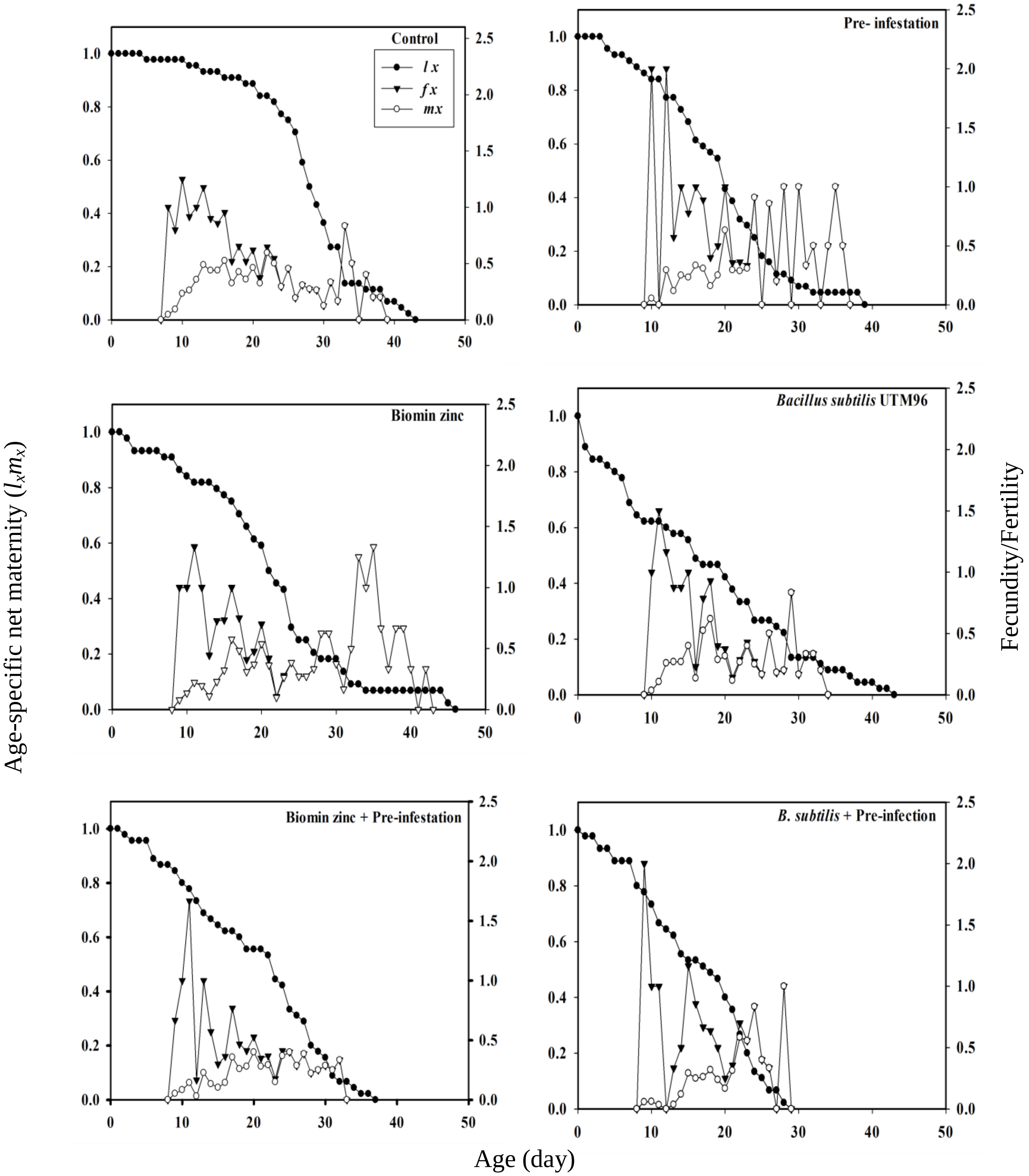

Figure 2. The age- specific survival rate (l_x), age-stage specific fecundity (f_x) and age-specific fecundity (m_x) of *Sitobion avenae* fed on different treatments under greenhouse conditions [25±5°C, 65±5% RH, and 16:8 hours (L:D)].

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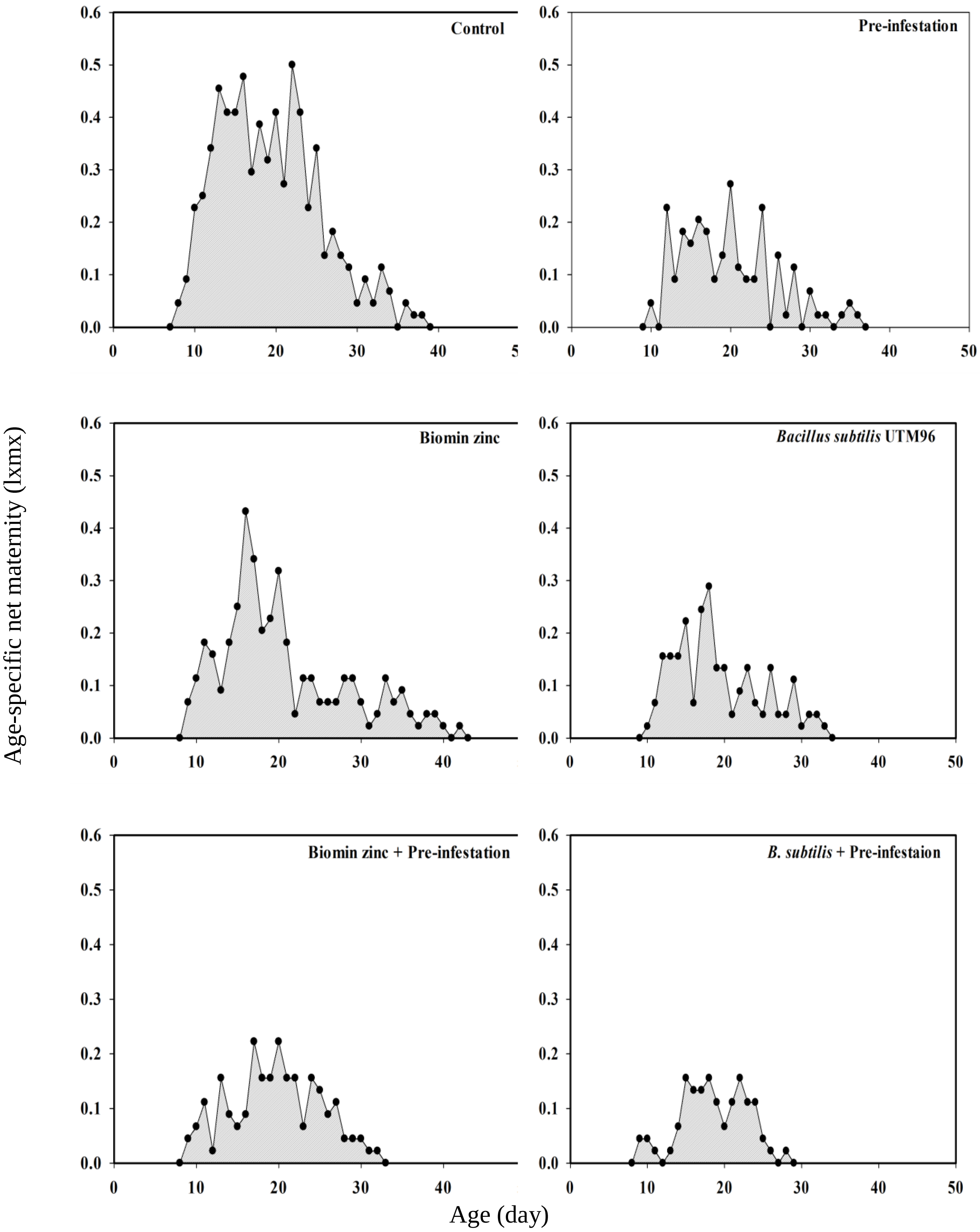

Figure 3. The age-specific net maternity (l_xm_x) of *Sitobion avenae* fed on different treatments under greenhouse conditions [25±5°C, 65±5% RH, and 16:8 hours (L:D)].

foliar application of Zinc to wheat plant may increase the nutritional quality of plants for aphid and as result in its increased *GRR* and m_x values.

B. subtilis+pre-infestation-wheat interactions decreased R_0, r, λ, e_{xj}, S_{xj} and l_xm_x values of *S. avenae*, that are generally due to the longer development time, higher morality of pre- adult stages, low fecundity, and a later peak in reproduction compared to the control. The amount of R_0 illustrates the ratio of population growth in each generation over the earlier generation that associates the physiological capability of a creature to its reproductive ability (Liu *et al.*, 2004). Also, r is a basic parameter to prognosticate the population growth rate of an insect, and is an appropriate parameter to calculate the performance of a herbivore insect on various plants (Southwood and Henderson, 2000). The total phenolic components in leaves of wheat in *B. subtilis* + pre-infestation treatment commences IR and reduces the r of *S. avenae* that is dependent on molecular pathways. A direct role of phenolic compounds is IR against aphid (Wójcicka, 2010; Alizamani *et al*., 2020; Pourya *et al*., 2020). However, evidence about the role of phenolic compounds in a plant is limited in conifer and unconvincing to herbivores (Mumm and Hilker, 2006).

Phenolic compounds are the major class of secondary metabolites in plant defense against herbivorous insects (War *et al.*, 2011). Treatment with *B. subtilis* + pre-infestation and *B. subtilis* can enhance amounts of total phenolic, which immediately procreate poisonous and/or HR in plants (Kiprovski *et al.*, 2016). Numerous studies showed that colonization of plant roots by PGPRs induces ISR enhancing the amount of phenolic compounds, Hydrogen peroxide (H_2O_2) production, cell death, and callose deposition with *S. avenae* infestation in plant that reduces consumption rates, and feeding performance of chewing- and phloem-feeding insects (Sharma *et al.*, 2009; Rani and Jyothsna, 2010; Ali and McNear, 2014). Therefore, insect feeding also induced oxidative stress that is the main component of plant defense to phloem-feeding insects (Lei *et al.*, 2014). In general, the higher amounts of phenolic, H_2O_2, and other oxidative products of ROS in wheat leaves can directly damage the midgut of *S. avenae* and have considerable negative effects on R_0 and r parameters of *S. avenae* (Bi and Felton, 1995; Lukasik *et al*., 2017).

Anthocyanin is the soluble compounds in plant cells that can repel destructive pests and attract beneficial natural enemies (War *et al*., 2012). In the present research, the level of anthocyanin contents in the un-infested leaves increased significantly on Biomin zinc+pre-infestation treatment vs. pre-infestation treatment. Hence, increased anthocyanin in Biomin zinc+pre-infestation treatment can both directly and indirectly affect the feeding performance of herbivorous insects.

In summary, our result demonstrated *B. subtilis*+pre-infestation as the effective host vis- à- vis other treatments to induce resistance of wheat against *S. avenae*. *B. subtilis* + pre- infestation can significantly decrease the population of *S. avenae* under greenhouse conditions, and hence, it is useful for ecological management of *S. avenae* in combination with other tactics. However, further studies must be performed in field studies.

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ارزیابی مقاومت القایی در گندم نسبت به شته ***Sitobion*** (Hemiptera: Aphididae) ***avenae*** در شرایط گلخانه‌ای

ر. مرادی، ج. شاکرمی، و م. مردانی طلایی

چکیده

شته سبز گندم، *Sitobion avenae* F. (Hem.: Aphididae)، یک آفت جهانی مهم برای گندم به علت خسارت مستقیم و انتقال ویروس می‌باشد. در مطالعه حاضر، تاثیر شش تیمار از جمله: (۱) گندم با یک دوره‌ی شش روزه آلودگی به آفت، (۲) گندم محلول‌پاشی شده با بیومین‌روی، (۳) بذور گندم تیمار شده با *Bacillus subtilis* UTM96، (۴ و ۵) تیمارهای تلفیقی بیومین‌روی + پیش-آلودگی و *B. subtilis* + پیش‌آلودگی، و (۶) همچنین یک تیمار شاهد بر ویژگی‌های زیستی *S. avenae*مورد آزمایش قرار گرفت. نتایج نشان داد که تفاوت معنی‌داری بین تیمارها از نظر برخی از محتویات متابولیت‌های ثانویه و ویژگی‌های زیستی شته وجود داشت. تیمارکردن با *B. subtilis* + پیش‌آلودگی و *B. subtilis* (۲۹۸/۲۰ و ۲۹۲/۱۷ میلی‌گرم بر میلی‌لیتر) میزان فنول کل در برگ‌های گندم را در مقایسه با بیومین‌روی + پیش‌آلودگی (۱۸۳/۶۳ میلی‌گرم بر میلی‌لیتر) افزایش داد. نرخ

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خالص تولیدمثل (R_0) *S. avenae* به طور معنی‌داری بوسیله تیمار *B. subtilis* + پیش‌آلودگی (۱/۵۳۳ نتاج به ازای هر فرد بالغ) در مقایسه با شاهد (۶/۸۸۷ نتاج به ازای هر فرد بالغ) کاهش یافت. تیمارکردن با *B. subtilis* + پیش‌آلودگی (۰/۰۲۲ بر روز) به طور معنی‌داری نرخ ذاتی افزایش جمعیت (r) *S. avenae* در مقایسه با شاهد (۰/۱۰۵ بر روز) کاهش داد. کم‌ترین (۰/۰۲۴) و بیش‌ترین (۰/۰۵۸) شاخص رشد پورگی (NGI) شته به ترتیب در تیمارهای *B. subtilis* + پیش‌آلودگی و شاهد به دست آمد. بنابراین، می‌توان نتیجه گرفت که *B. subtilis* + پیش‌آلودگی در گیاه گندم می‌تواند باعث القای مقاومت سیستمیک نسبت به *S. avenae* شود که می‌تواند در برنامه مدیریت تلفیقی (IPM) این شته استفاده شود.

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