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Research Article

Response to Magnetic Field-Induced Stress on the Demographics and anti-ROS Activity of Aphid *Macrosiphum rosae* L. (Hemiptera:Aphididae)

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Abstract

Magnetic field plays an essential role for many species, including the migratory pest aphid. Our previous study investigated the long-term exposure of static magnetic fields on the growth development and productivity of the aphid *Macrosiphum rosae*, however, it is necessary to expand the knowledge of short-term exposure on the insects for a wider spectrum of magnetic field radiation. To achieve this, aphid nymphs were exposed to four magnetic field of induction at 0.065T, 0.1T, 0.176T and 0.28T for 4min under laboratory conditions. The results showed that the short-term magnetic radiation significantly prelonged the four instar development while shortened the first, second and third instar period. 0.28 T radiations caused significantly difference in the parameters of TPOP, adult longevity and total longevity (3.2d, 7.07d, and 8.82d, compared with the control of 4.94d, 8.06d, and 10.23d, respectively). Population parameter of r was $0.057d^{-1}$ with 0.28 T compared with the control of $0.150\ d^{-1}$. The SOD, CAT and POD activity increased more than 30% in static magnetic fields compared with the controls. Our study presents a feasible evidence showing the growth development change as a representative disturbing symptom for short-term exposure to magnetic fields, and the static magnetic field applied being capable of modifying the fitness components and antioxidant defense in aphids.

Keywords: aphid, magnetic field, *Macrosiphum rosae*, growth development, antioxidant enzyme, insect

1. Introduction

The electric power transmission lines and transformers generate electromagnetic fields. Technical development was contributed to environmental contamination with artificial electromagnetic fields produced by electromagnetic waves, nuclear magnetic resonance, high-voltage power lines [1]. One of these environmental contamination constituents is the magnetic field and this radiation constituents exert biological pressure. Magnetic fields can be classified as static magnetic field (SMF) and dynamic magnetic field (DMF), based on whether the intensity and direction of magnetic field changes over time. According to their magnetic field strength, they are usually

categorized into weak (100nT-1T), moderate (1mT-1T), strong (1T-5T) and super magnetic fields (>5T). Most previous studies focused on aspects of long-term exposure of magnetic field on various living organisms (as recently reviewed in [2]). However, A few studies have reported effects from short-term exposure to magnetic field on biological organisms.

Additionally, multifarious and sometimes contradictory conclusions had been suggested in previous researches about the effects of the long-term exposure on organisms., For example, while some reported that *Drosophila melal1ogasler* exposed to 3.7 T magnetic field for 7 days did not appear severe inducing mutation [3]; others were observed obviously negative effects of *Drosophila melal1ogasler* exposed to 2.4 T magnetic field for 2 hr compared with sham-exposed controls [4], 9.0 T of static magnetic field exposure for 8 hr delayed the early development of Danio rerio (zebrafish) [5], development retard and gene expression aberration of 15 T exposure from uncleaved to 2-cell, 2-cell to blastula and blastula to neurula on Xenopus embryos [6], lifespan shorten of 8 T exposure for 1, 3 and 5 h on Caenorhabditis elegans [7], hatching rate delay of 9.4 and 14.1 T exposure for 70–163 h on mosquito eggs [8], viability reduce of 1.5 T exposure for 30 min on mouse fetuses [9] and so on. Moreover, the combination of short-term and long-term magnetobiology were contradictory, for instance, while some reported that magnetic field exposure examined not evidence on the development of *Xenopus laevis* (6.34 T for 6 and 18 h or 8 T for 20 h) [10] or mice (1.5 and 7 T, 75 min each day of gestation, or 4.7 T exposure from 7.5 to 9.5 day during the entire pregnancy) [11]; other studies reported obvious negative effects, including the cleavage plane alteration of 1.7-16.7 T exposure from fertilization to the third cleavage or cortical pigmentation of 9.4 T exposure on *Xenopus* eggs from 15 to 109 min [12], Therefore, short-term exposure of magnetic field studies on biological organisms is probably necessary to interpret these multifarious and contradictory conclusions, and will provide valuable information to expand the knowledge of the influence of the magnetic field for a wider spectrum of electromagnetic radiation, and above studies only observed a few aspects of growth development, a full and comprehensive view is still lacking as to the effects of MF on living systems.

Investigations of the influence of a wide range of SMF on genetics, fitness traits, antioxidant defense, and orientation in insects are numerous. Insects populations are significantly important for the environment and are also response to magnetic field to influence the mortality development, behavior and metabolism, enzyme reactions, membrane rigidity, metallothionein content, replication and transcription mechanism, replication and mutations , including honeybees, asps, mollusks, cockroach and monarch butterfly, and so on [2,13]. The most exact mechanism(s) regarding the SMF is that free radical reactions probably contributed to one of the effective mechanisms underlying the influences of magnetic exposure [14]. Because the most common free radicals productions are oxygen or nitrogen based with an unpaired electron, the authors interpreted that a complex structure likely involved in contrast to a magnetic field leading to the terms "reactive oxygen species," such as superoxide anion (O_2^-), hydroxyl radical (OH) and singlet oxygen (O_2^*), or "reactive nitrogen species," such as nitric oxide (NO) [15]. In the spin states of radicals, the theory predicts the applied MF is extremely tightly regulated by a magnetohydrodynamic effect on flow processes, the positive ion-negative ion pair are initially in a singlet state which can evolve into a triplet state via the hyperfine interaction mechanism, MF perturbs the interconversion of the singlet and triplet states to lead to an increase proportion of the triplet state, therefore, the free radical concentration and oxidative stress effects produced [16].

Oxidative stress leads to excessive amounts accumulation of free radicals, these radicals react with various biomolecules to damage the cellular compounds such as protein, carbohydrate, DNA, and lipid. Insects have involved a complex antioxidant mechanism to overcome the genotoxic effects of overproduction free radicals. Antioxidant defense mechanisms are impaired through exposure to a magnetic field that causes the overproduction of ROS, antioxidants may not be sufficient or free radical formation may increase to such an extent that it overpowers the defense capabilities of antioxidants. The antioxidant defense systems primarily constituted by the action of enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) [17]. The metalloenzyme of SOD catalyzes the dismutation of O_2^- into O_2 and stabile hydrogen peroxide (H_2O_2). In return, the antioxidant enzyme of CAT take H_2O_2 as targets and turns it to water and oxygen [18]. To study the short-term effects of SMF on growth development from multiple dimensions, we chose an entomology model organism, *Macrosiphum rosae* L. (Hemiptera: Aphididae), which has never been used in previous reports on this question. *M. rosae* is a kind of migratory pest distributed world widely. This species periodically causes serious damage on the inflorescences. The direct damage for plant was leading to discolored leaves, stunted growth and gall formation by *M. rosae* through phloem feeding with sap sucking mouthpart; the indirect damage were incurred through honeydew flowers excretion and leaves surface to mold grow resulting in the reduction of photosynthesis. Rose aphid also acted as a vector to transmit viral diseases such as cauliflower mosaic, cabbage black ring spot and pea mosaic [19]. Furthermore, aphid provided valuable information of the most applicable and very useful model systems, because it displays genetic and phenotypic variation in traits to response different level of environmental stressful conditions [20]. Currently, the aphid population suffers many harmful stressors influencing their development, such as light, temperature, humidity, wind, climate changes, and their dynamics [20]. Significant changes of certain gene arrangements at the level of individual chromosomes on rose plant are specific for the reproduction of rose population. Also, the similarity of metabolic signaling between mammals and *Macrosiphum rosae* allows the investigation of the effects of different ecological factors in prevention of genetic damage in humans [19]. Artificial magnetic fields probably contributed to the phenotypic variation and metabolic signaling. Yet, limited researches are reported about the artificial magnetic fields on *Macrosiphum rosae* of fundamental processes (growth, development) and antioxidant enzyme activity changes.

Our previous study only investigated the long-term exposure of SMFs on the growth development and productivity of the aphids [21], it is necessary to expanded the knowledge of the influence of the short-term exposure of magnetic field for a wider spectrum of electromagnetic radiation and to study the possible mechanisms of free radical reactions, thereby helping us to better understand the adaptive implications of the mechanisms in genotype×environment interactions. The present study tries to study the effects of short-time exposure of SMFs on the development of aphid, and furtherly measure the enzymatic activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), respectively. The developmental time, survival, and fecundity of *M. rosae* were analyzed using an age-stage, two-sex life table. Life tables have been regarded as powerful tools to analyze external factors such as ultraviolet radiations, temperatures and light on the viability, survivorship, fecundity, and intrinsic rate of insect populations. Several researches have reported the different methods using life tables, many of which have been become widespread among the ecological studies of insect populations, including insect mass rearing and harvesting, pest control timing and predation rates, host preference and fitness as well population

parameters and dynamics [22]. The traditional life table is only considered the female population, and did not ignore the male population, different developmental stages and individual differences. The age-stage, two-sex life table could eliminate the inherent errors of female-based life tables and incorporate population data from both sexes [23]. Additionally, variations in instar stages could precisely reflect in the survival and fecundity curves.

2. MATERIALS AND METHODS

2.1 Insects and Plants

The laboratory populations of *Macrosiphum rosae* of adult apterous viviparous parthenogenetically producing female rose aphids were initially collected from the rose field at Linfen, Shanxi province during March 2022 and reared separately following a simple mass-rearing method for five generations. The experiment nymphs born within 24h were maintained on potted rose plant at 23 ± 1°C and 65 ± 5% RH, under a 16- hr light: 8- hr dark photoperiod growth in the Entomology Research Laboratory of Department of Zoology, Shanxi normal university. In order to study biology, a stock culture of rose aphids was maintained on fresh tender apical portions of rose plants.

Rose plants were removed from the rose field at Linfen, Shanxi province during February 2022 and then single potted in rectangular plastic trays (320 × 220 × 50 mm) with nutrition soil in growth chamber (23 ± 1°C, L16:D8 photoperiod, 65 ± 5% relative humidity).

2.2 Experimental Arrangement

The laboratory magnetic fields were manufactured as described by He (2012) to generate the expected intensities of 0.065T, 0.1T, 0.176T and 0.28T [21]. The magnetic fields used to measure and standardize the induction by numerical control electromagnet magnetic field generation system (Beijing Cuihai Jiancheng Magneto electric Technology Co., LTD, working range: magnet model wD-50 / set, power model 2031 (generating current -5 A ~ +5 A)/set, magnetic field 0.0-0.3T, power 0.3KW), supplied by the Key Laboratory of Magnetic Molecules and Magnetic Information Materials of the Ministry of Education, College of Chemistry and Material science, Shanxi Normal University. The bioassay carried out by placing each concentration of *M. rosae* on the center of the magnet's surface. Parallel control experiment populations conducted with the laboratory samples were not being exposed.

2.3 Biological Experiments

The development time, survival, and reproduction of nymphs feeding singly on potted rose plant were investigated and compared. More than 10 female individuals were isolated from the aphid culture and maintained to obtain the nymph stages. After 24 h, newly-laid nymphs were exposed to 0.065 T, 0.1 T, 0.176T and 0.28 T for 4 min upon each concentration contained 30 individuals. Subsequently, the experiment nymphs were separated by a camel hairbrush and reared in potted rose plant individually. The survival and development times of each developmental stage were recorded daily. Oviposited nymphs were counted daily and discarded for parthenogenetic until adult died. Parameters such as survivorship, fecundity and oviposition period were recorded daily until the death of all individuals. These bioassays were conducted under laboratory conditions at the temperature and RH conditions described above.

2.4 Statistical Analysis

The life history traits and population parameters, including the total pre-oviposition period (TPOP), the age-stage-specific survival rate (s_{xj}), age-specific survival rate (lx), age-specific fecundity (mx), age-specific maternity (lxmx), age-stage life expectancy (e_{xj}), reproductive value (v_{xj}), the intrinsic rates of increase (r), finite rates of increase (λ), net reproductive rates (R0), and mean generation times (T) were conducted according to the approach of age-stage, two-sex life table by the TWOSEX-MSChart software [23,24]. The calculation formula is as follows:

$$lx = \sum_{j=1}^{k} s_{xj}$$

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{k} s_{iy}$$

$$mx = \sum_{j=1}^{k} s_{xj} f_{xj} / \sum_{j=1}^{k} s_{xj}$$

$$V_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{a} e^{-r(i+1)} \sum_{y=j}^{k} s_{xj} f_{xj}$$

$$R_0 = \sum_{x=0}^{\infty} lxmx$$

$$\sum_{x=0}^{\infty} e^{-r(x+1)} lxmx = 1$$

$$T = \frac{\ln R_0}{r}$$

$$\lambda = e^{r}$$

Population parameter variation and standard errors calculated following the 100,000 random re-sampling bootstrap techniques. The significant differences between population parameters analyzed separately by using paired bootstrap tests upon the confidence intervals of TWOSEX-MSChart software. All scientific graphs were plotted using SigmaPlot v.12.5software ((Systat Software Inc., San Jose, CA, USA, 2013).

2.5 Antioxidant Enzyme Activities Assay

The enzymatic activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were measured, since these groups of enzymes showed the characteristics of paramagnetic elements (Fe, Mn, and Cu) in the catalytic domain, and therefore the aphid exposed the magnetic field was affected during the experiment. 30 aphid individuals of each treatment were homogenized in a phosphate buffer (0.1 M, pH 7.0) at 0 °C using an electric mechanical homogenizer, and then the homogenate centrifuged at 8,000× g for 10 min at 4 °C. The supernatant collected and used for antioxidant enzyme activities assays of SOD (EC 1.15.1.1), POD (EC 1.11.1.7) and CAT (EC 1.11.1.6) with the equipment of spectrophotometer (SP-756P, Shanghai, China). The enzyme activities expressed as unitmg per fresh weight of aphid (μ mg^{-1} FW). The measurement of enzyme activity conducted at a controlled temperature of 25 °C. The SOD activity determined from the measurement of absorbance at 560 nm [25]. The aphid homogenate (100 μl) mixed with nitroblue terazolium (500μl, 0.4 mM) in phosphate buffer (0.2 M, pH 7.8) and xanthine solution (0.25 mM). The mixture incubated for 20min was used for the absorbance measurement at 560 nm (TeCAN Infinite 200 microplate reader). The POD activity measured the absorbance at 470 nm [26]. The aphid homogenate 100 μl mixed with phosphate buffer (0.1 M, pH 7.0), 20 μl distilled water and 0.2 M pyrogallol were incubated at 30 °C for 25 min. Then, the mixture was added to 50μl of 25% trichloroacetic acid (TCA) solution. The absorbance was measured with the TECAN Infinite 200 microplate reader at 470 nm. The CAT activity was assayed according to the methodologies proposed by Aebi (1984) with minor modifications [27]; 30 mM H_2O_2 solution was added to the aphid homogenate, and subsequently the absorbance measured at 240 nm with the spectrophotometer during the disappearance of H_2O_2. Three biological replicates established at each measurement.

3. RESULTS

3.1 Development and Survivorship

Establishment of aphid population on magnetic field radiation of 0.065T, 0.1T, 0.176T and 0.28T for 4 min has been observed from the aspects of the development time of each immature stage, total pre-oviposition period (TPOP), adult longevity and total life span (Table 1). There were significant differences in growth development and survivorship among the treatments. The mean developmental period of the first and second instar of *M. rosae* were strongly affected, and the

Table 1. Development time for the different instar stages, adult longevity, total pre-oviposition period (TPOP), and total longevity of 0.065T, 0.1T, 0.176T and 0.28T for 4 min exposure on *Macrosiphum rosae*.

Parameters	CK	0.065T	0.1T	0.176T	0.28T	P
First instar	2.04±0.231c	2.73±0.239a	2.26±0.237b	2.10±0.176c	1.50±0.158d	P < 0.0001
second instar	2.09±0.146b	2.30±0.227a	2.00±0.216b	2.00±0.205b	1.48±0.131c	P < 0.0001
Third instar	1.95±0.162a	1.73±0.182a	1.69±0.175a	1.82±0.196a	2.21±0.164a	P < 0.0001
Fourth instar	2.06±0.266a	2.08±0.211a	2.09±0.315a	1.92±0.193a	2.20±0.243a	P < 0.0001
Adult longevity	4.94±0.536a	4.46±0.685a	4.09±0.693ab	4.25±0.641a	3.20±0.368b	P < 0.0001
TPOP	8.06±0.281a	8.15±0.373a	8.82±0.296a	8.17±0.167a	7.07±0.408b	P < 0.0001
Total Longevity	10.23±0.794a	9.93±0.976a	9.90±0.1226a	10.00±0.849a	8.82±0.630b	P < 0.0001

Standard errors (SEs) were estimated using 100,000 bootstraps. Mean values followed by the same letters in rows are not significantly different among the different magnetic fields as assessed using the paired bootstrap test at the 5%significance level.

values were significantly highest at 0.065T with 2.73d and 2.30d, and then gradually decreased to 0.28T with 1.50d and 1.48d, compared the control two instar of 2.04d and 2.09d, respectively. However, diverged from this pattern, the third instar were significantly decreased to 1.69d and then gradually increased to 2.21d, compared the control group of 1.95d ($P < 0.0001$). The fourth instar was not statistically distinguishable from each other. Statistics presented in Table 1 showed that the development period of adult longevity were strongly fluctuated increased with the magnetic field. The TPOP were statistically increased to 8.82 d at 0.1T and then gradually decreased to 7.07d at 0.28T, contrast with the control of 8.06d. The total life span with a negative effect followed a nonlinear pattern than control females ($P < 0.0001$) (Table 1). Totally, 0.28 T radiations show statistical difference in the parameters of TPOP and adult longevity compared with other radiation, while other three radiations 0.065 T, 0.1T and 0.176T for 4min exposure show no significant difference.

3.2 Age- stage Survival Rate and Fecundity

The probability of the age- stage survival rate (s_{xj}) showed difference among the treatments, though a trend towards stressed females could have higher probability of survivorship than control females. The survivorship of the immature stages indicated nearly 36%, 39%, 61% and 50% of 0.065T, 0.1T, 0.176T and 0.28T compared with 58% of control groups. The female population exhibited similar survivorship trends of 38%, 44%, 50% and 48% of 0.065T, 0.1T, 0.176T and 0.28T compared with 62% of control groups (Figure 1).

Figure 3 shows age- specific survival rates (lx), age- specific fecundity (mx) and age-specific maternity (lxmx). The lx curve of *M. rosae* gradually decreased from age 10, 12, 9 and 14d until death at 0.065T, 0.1T, 0.176T and 0.28T. The mx curve described the start times and duration of the reproductive phase. The highest peak occurred at age 9.5, 10, 9 and 12d of 0.065T, 0.1T, 0.176T and 0.28T. Based on both lx and mx, the maximum lxmx value of 0.065T, 0.1T, 0.176T and 0.28T was recorded at age 10, 10, 8 and 8d, respectively, compared with the control peak with 13d (Figure 2).

Life expectancy (e_{xj}) shows the time length of an individual of age x and stage j expected to surviving. The life expectancy values of a newly-laid egg of 0.065T, 0.1T, 0.176T and 0.28T were 5, 6, 5 and 4 d, respectively, compared with the control

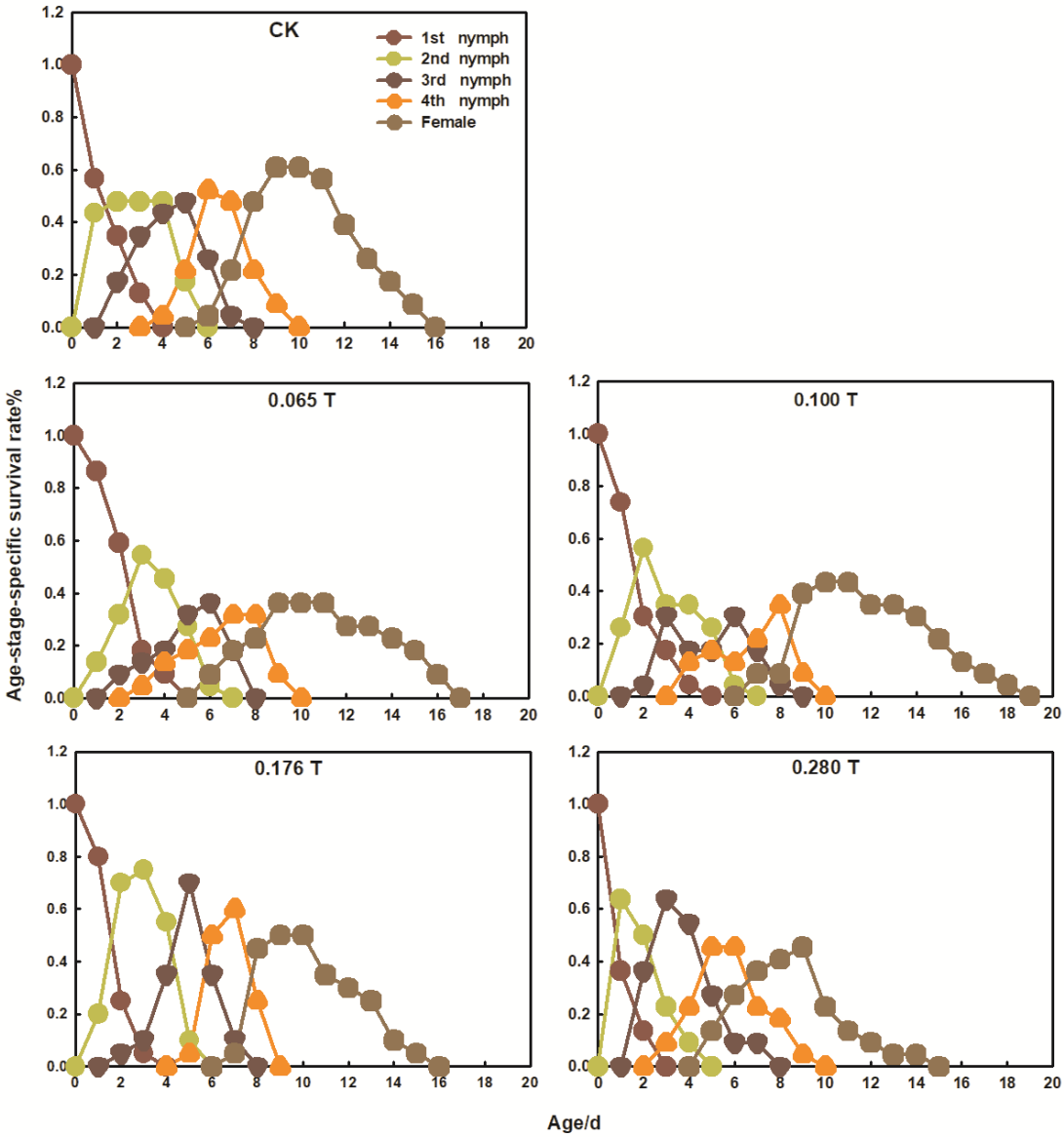

Figure 1. Age-stage specific survival rate (s_{xj}) of 0.065T, 0.1T, 0.176T and 0.28T for 4 min exposure on *Macrosiphum rosae.*

at 5d (Figure 3). This parameter was highest at 0.1T while was lowest at 0.28T (Figure 4).

The value of the reproductive value (v_{xj}) figures the expected contribution of an individual of age x and stage j to the future population. The reproductive parameter gradually increased with an increase in age and stage. The highest reproductive values of 0.065T, 0.1T, 0.176T and 0.28T were recorded at age 5, 6, 5 and 3d, compared with the control peak with 7d (Figure 4).

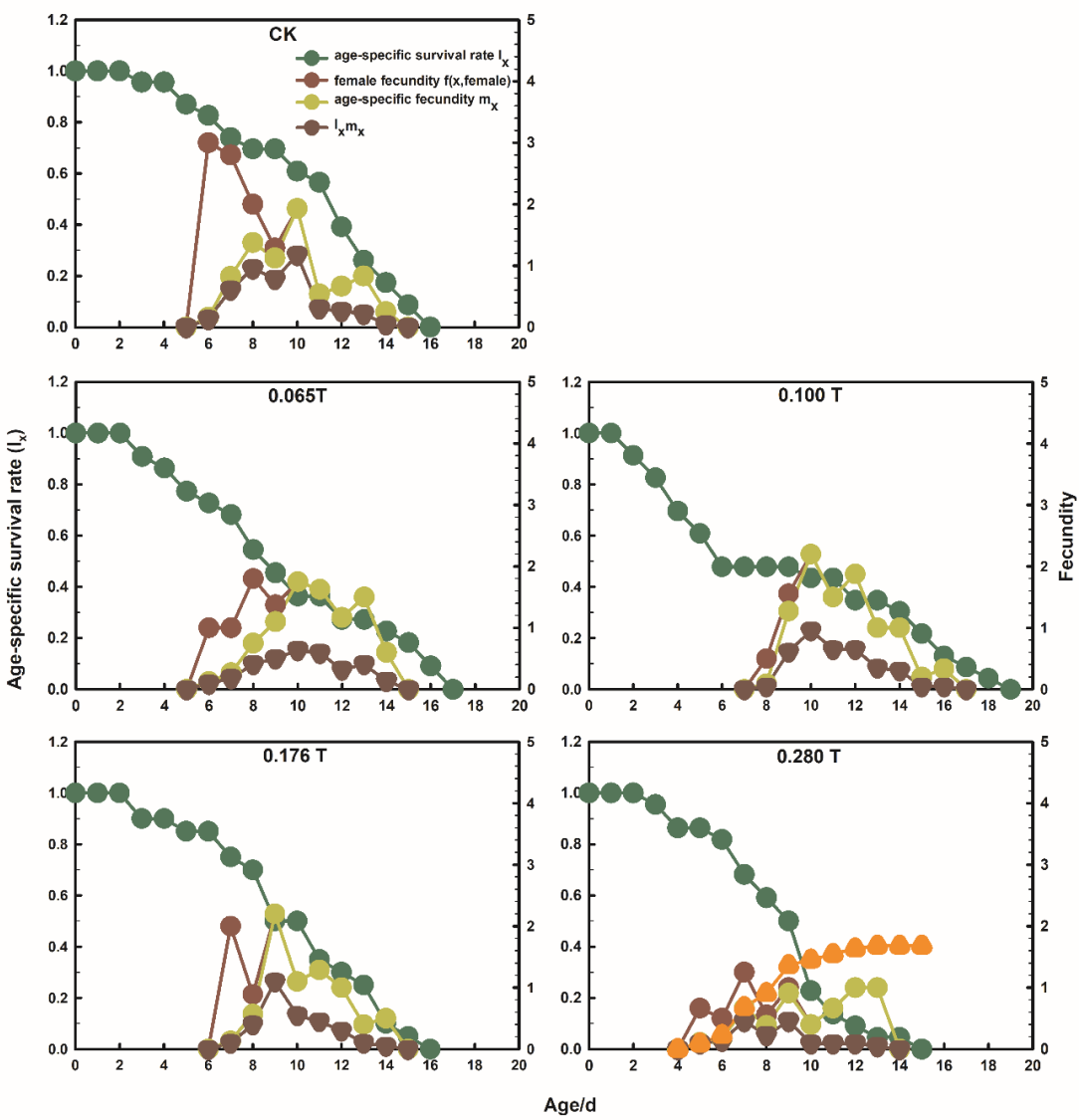

Figure 2. Age-specific survival rate (lx), age-specific fecundity (mx), and age-specific maternity (lxmx) of 0.065T, 0.1T, 0.176T and 0.28T for 4 min exposure on *Macrosiphum rosae.*

3.4 Population Parameters

We have taken aphid population dynamics data from the intrinsic rates of increase (r), finite rates of increase (λ), net reproductive rates (R0), and mean generation times (T) of 0.065T, 0.1T, 0.176T and 0.28T ensembles into Table 2. Our research indicates that the r of *M. rosae* decreased from 0.108d^{-1} at 0.065T to 0.057d^{-1} at 0.28T in comparison with the control of 0.150 d^{-1} without statistical significance, and 0.28 T radiation was statistically different from other experiment groups ($p < 0.0001$). In contrast, the T, λ and R0 increased

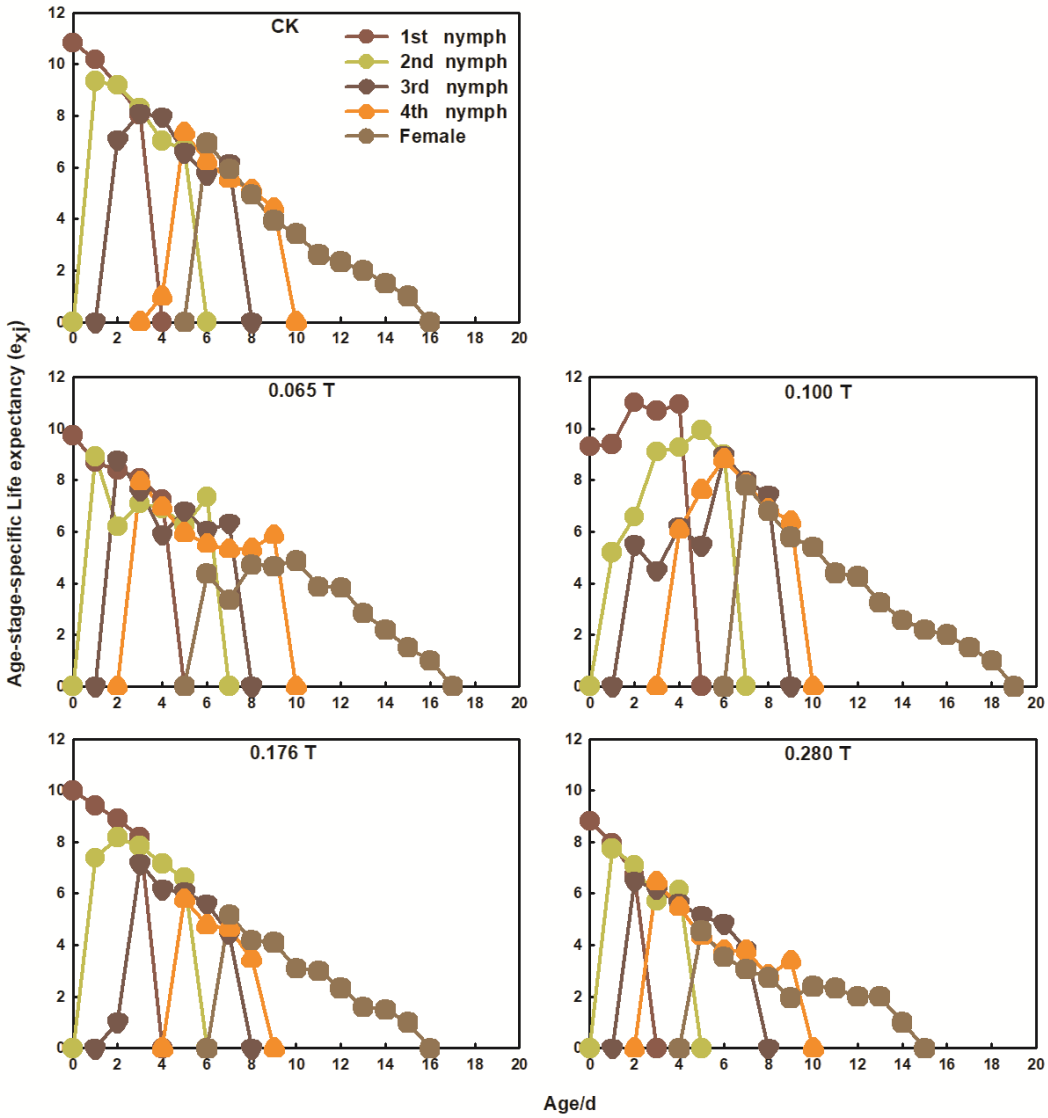

Figure 3. Age-stage specific life expectancies (e_{xj}) of 0.065T, 0.1T, 0.176T and 0.28T for 4 min exposure on *Macrosiphum rosae*.

to the highest at 0.1T with 11.95d^{-1}, 1.115d^{-1} and 3.65d^{-1} without statistical significance, subsequently statistically decreased to the lowest at 0.28T, with 11.95d^{-1}, 1.115d^{-1} and 3.65d^{-1}, these parameters show higher than the control groups of 10.02d^{-1}, 1.160d^{-1} and 4.48d^{-1}, respectively ($p < 0.0001$). 0.28 T radiations show statistical difference in the parameters of population parameters (R0, r, λ) compared with other radiations, while other three radiations 0.065 T, 0.1T and 0.176T for 4min exposure show no significant difference.

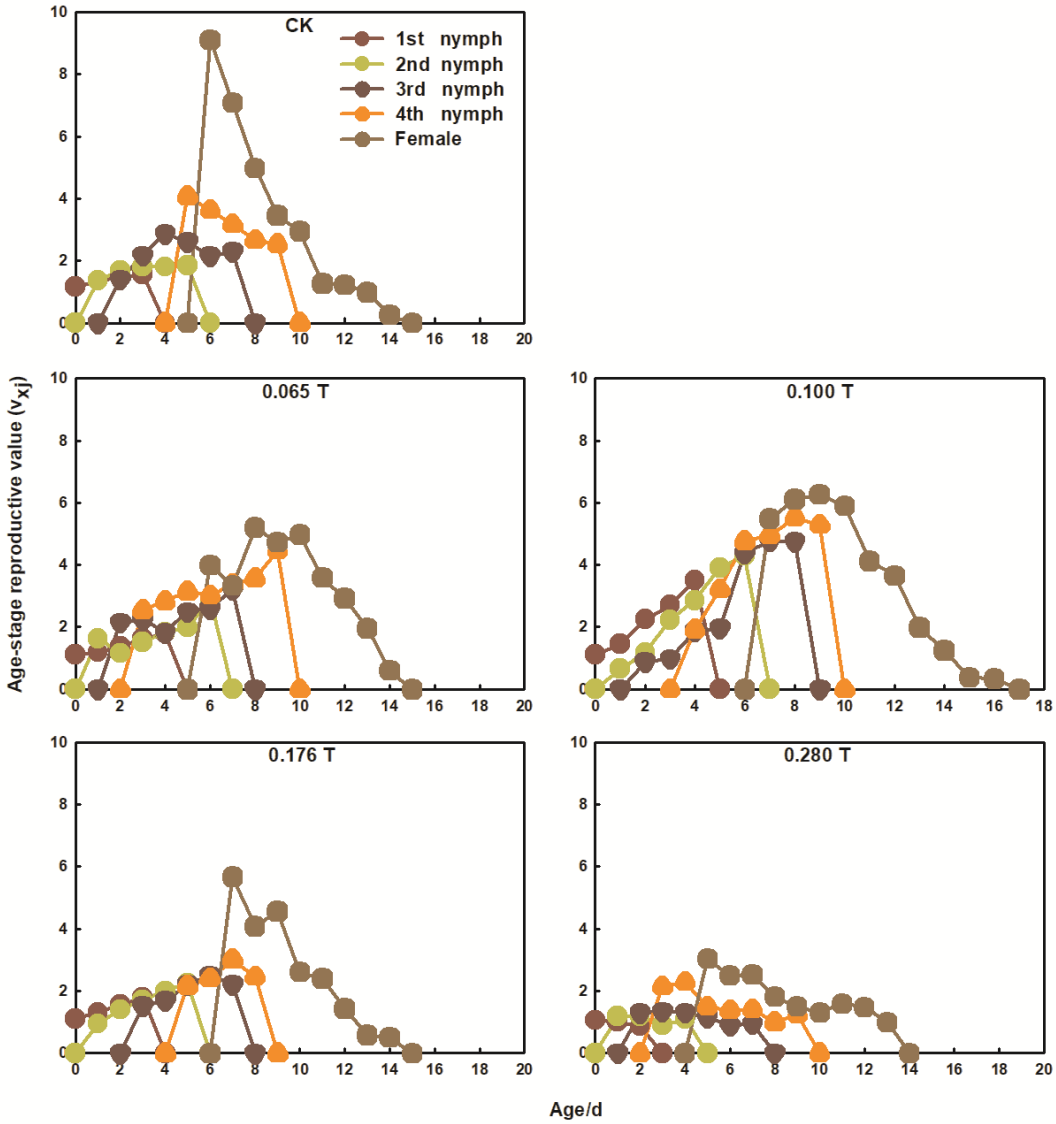

Figure 4. Age-stage-specific reproductive value (v_{xj}) a of 0.065T, 0.1T, 0.176T and 0.28T for 4 min exposure on *Macrosiphum rosae.*

3.5 Antioxidant Enzymatic Activity

Antioxidant enzymatic activity of aphid population was determined on magnetic field radiation of 0.065T, 0.1T, 0.176T and 0.28T for 4 min. Figure 5 depicts statistical metabolites of interest antioxidant enzymes activity of the superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), respectively. It is observed that the SMF significantly influences the enzymatic activity in the *M. rosae* since the SOD levels under four exposure increased up to 0.81U mg $^{-1}$ for 0.065 T, 1.22 U mg $^{-1}$ for 0.1 T, 0.63 U mg $^{-1}$ for

Table 2. Effects of 0.065T, 0.1T, 0.176T and 0.28T for 4 min exposure on population parameters of *Macrosiphum rosae.*

Parameters	ck	0.065T	0.1T	0.176T	0.28T	P
R0	4.48±0.91a	3.27±0.90a	3.65±1.07a	3.05±0.89a	1.68±0.38b	P < 0.0001
T	10.02±0.34c	11.03±0.48a	11.95±0.24a	10.67±0.29b	9.20±0.64d	P < 0.0001
r	0.150±0.020a	0.108±0.026a	0.106±0.027a	0.104±0.030a	0.057±0.025b	P < 0.0001
λ	1.160±0.025a	1.114±0.029a	1.115±0.030a	1.110±0.032a	1.060±0.027b	P < 0.0001

Standard errors (SEs) were estimated using the bootstrap technique with 100,000 re-samplings. Mean values followed by the same letters in rows are not significantly different between the two species as assessed using the paired bootstrap test at the 5% significance level, r means the intrinsic rates of increase, λ means finite rates of increase, R0 means net reproductive rates, and T means mean generation times.

0.176 T and 1.62 U mg^{-1} for 0.28 T, respectively, representing a 25%, 66%, 8% and 96% relative difference with respect to the control condition (Figure 5A, $p < 0.05$). Nevertheless, the enzymatic activity of POD is apparently reverted to an inhibitory effect under the four exposure mode since POD activity with respect to the control values reduced by 32% for 0.065 T, 75% for 0.1 T, 2% for 0.176 T and 94% for 0.28 T for the four magnet configurations, corresponding to 0.80 U mg^{-1}, 1.23 U mg^{-1}, 0.50 U $mg^{-1}$and 1.42 U mg^{-1} of enzymatic activity, respectively (Figure 5B, $p < 0.05$). In the case of *M. rosae* CAT increased up to 0.71U mg^{-1} for 0.065 T, 0.89 U mg^{-1} for 0.1 T, 0.50 U mg^{-1} for 0.176 T and 1.42 U mg^{-1} for 0.28 T, respectively under the exposure mode, representing 25%, 43%, 4% and 44% higher production compared to the control values (Figure 5C, $p < 0.05$). Totally, the 0.28 T radiation show the highest antioxidant enzymes activity of the superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), respectively.

4. Discussion

We found both consistencies and contradictions between ours and previous results. Previously, we reported hatching rate delay of instar stages for long-term (2hrs) exposure 0.065 T and 0.176 T in *S.avenae*[8], but we did not find adult longevity and reproduction statistical significance induced by magnetic exposure of 0.65 T, 0.1 T, 0.176 T except 0.28T radiation, which we previous reported significant difference on *S.avenae* and *M. persicae* nymphs by long-term (2hrs) exposure [8]. Multiple factors presumably take into account these variabilities. Firstly, the characteristics of magnetic field applied were different in gradient, orientation and homogeneousness. Magnetic field of 0.176 T of short-term exposure did not influence the adult development and productivity while significant in long-term exposure to *Sitobion avenae* of 0.176 T for 1hr [21]. However, 0.28 T magnetic induction cause retardation of growth development and induced malformations in nymphs. Secondly, different organisms probably respond variously to strong magnetic field account for their inherent characteristics. As mentioned above, 3.7 T strong magnetic fields described no significant difference in *Drosophila melanogaster* [3]. Thus, the magnetobiology on organisms are varied depending on the species category, the radiation parameters of filed threshold and time threshold The motion of the animal through the geomagnetic field induce voltage gradients with sign and magnitude depending on orientation, which

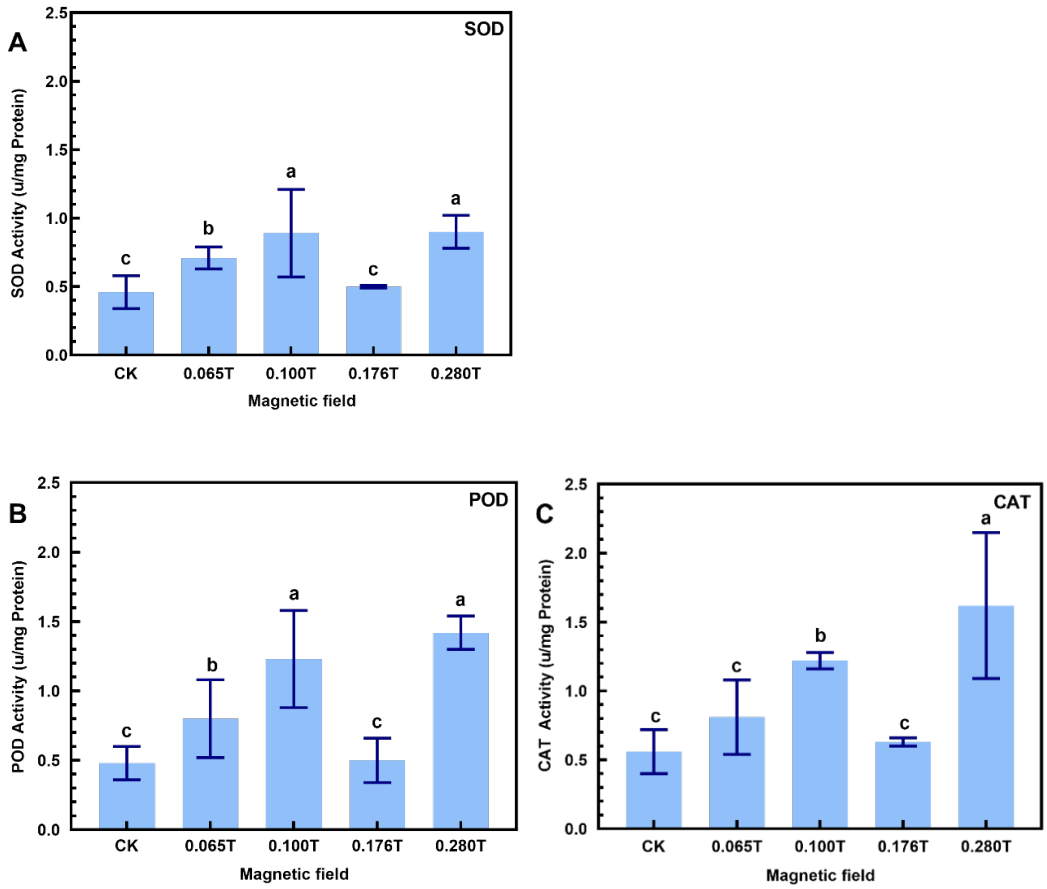

Figure 5. Measurements of the antioxidase enzymatic activities of superoxide dismutase (SOD) (A), peroxidase (POD) (B) and catalase (C) of 0.065T, 0.1T, 0.176T and 0.28T for 4 min exposure on *Macrosiphum rosae.*

are generally above sensitivity threshold of the animal. Generally, magnetobiology considered this relationship in terms of the magnets and time threshold. Time thresholds investigated the biological effects after a certain time of exposure on organisms; field threshold explain the magnetobiology effects of animals when field strength exceed the certain threshold value [29]. We obtained the remarkable effects of short-term MF combinations in agreement with the time and field thresholds. Magnetic field strength exceeded 1 T are strong magnetic fields (1T-5T). In our studies, 0.28 T radiations show statistical difference in the parameters of TPOP and adult longevity, population parameters (R0, r, λ) compared with other radiation, while no significant difference was found at 0.1T and 0.176. The developmental period of instar stages displays significant difference among the four magnetic fields. The short-term magnetic radiation is capable of inducing instar development. These findings were corresponding with the development documented in previous studies [2, 21].

Dynamics of the growth development is important for understanding the possible effects of different environmental factors. The present consequences are various for four magnetic field of 0.065 T, 0.1T, 0.176T and 0.28 T. Negative effects of instar development were observed in the four magnetic field while only 0.28 T radiations show statistical difference in the parameters of TPOP and adult longevity, population parameters (R0, r, λ) compared with other radiation. The previous literatures likewise provided various and contradictory suggestions about the impacts of magnetic field on development of living systems. 4.5mT increased mortality of eggs and diminished adult viability in *Drosophila melanogaster* [30], 3.7 T magnetic field did not observe obvious negative effects on the development of *Drosophila melal1ogasler* [3], 60mT SMF reduced the embryonic and post-embryonic development in *Drosophila melanogaster* [31], 9.4 T magnets disturbed development of Xenopus embryos [12], 375mT SMF caused the disturbance development and survival of the yellow mealworm pupae [32], 8 T radiation retarded lifespan of *Caenorhabditis elegans* [7], 50 mT SMF exposure showed no effect on the pupa-adult development dynamic of *Tenebrio* [33], 9.4 T and 14.7 T magnets delayed hatching rate of mosquito eggs [8] and so on.

All the differences above reminded us to be very wary of simply applying theory to explain our results. As for our viewpoint, both previous and our results contributed to the experimental implementation and research the effects of magnetic field on the negative effects of short-term and long-term exposure. Some of our results were different when we scrutinize the organisms and conditions. These multifarious results possibly described the variability and complexity of the effects of magnetic field. Indicating the capability of magnetic induction modified the processes underlying insect viability and thereby achieving the impacts on different life stages. Researches considering the influences of SMF on growth development and reproductivity of insects are multifarious and contradictory depending on species and the characteristics of magnetic field applied [8, 21, 33]. In insects, neuroendocrine system plays a significant role in transmissing information induced by magnetoperception system [34]. The neurosecretory neurons of insect release of neurohormones through the Ca^{2+} ions in the normal stages, therefore, we hypothesized that magnetic field induced the levels of intracellular Ca^{2+} expression to lead to the release of neurotransmitters [35], and consequently changed all vital processes including development and viability. Furthermore, the magnetic gradient mentioned by Zablotskii et al. indicated that high magnetic field intensities related with the probability of the ion-channel on/off change, magnetically induced cell responsiveness cell division and cell reprograming, and forced migration of membrane receptor proteins [36]. Some researchers regarded the most influential factors and mechanisms of magnetic field effects on a wide insight that the radical pair recombination and the diamagnetic anisotropy contributed to the biomolecules susceptibility, intracellular structural modifications, and enzymatic reactions [16].

In the cells of different organisms, magnetic field produces free radicals to probably induce oxidative stress [37]. Free radical production in the cellular environment primarily enhanced the antioxidant defense enzyme activity of SOD and CAT [38]. In our study, antioxidase enzymatic activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) increased more than 30% compared to control, at the four applied magnetic field of 0.065T, 0.1T, 0.176T and 0.28 T.. These results contributed to the specific characteristics of environmental factors. The living systems could produce large amounts of reactive oxygen species (ROS) generated as byproducts of normal aerobic metabolism during the influence of physical and chemical environmental stressors [40]. The insect generally responses to different environmental stressor depend on local adaptations in terms of physiology mechanism, including constitutive activity

of enzymatic and non-enzymatic antioxidative defense characteristics. The selection pressure of some environmental factors contributed to change allele frequencies within populations. The phenotypes with the best performances to dispose with environmental stress are favored. The magnetic exposure aphid has less stable environmental conditions compared with the control group. Differences in determination the constitutive level of antioxidant defense associated with natural ecological factors of insects have already been depicted. Our results considering SMF-induced changes in the antioxidant enzymes also agreed with research data indicating the capability of SMF modifying free radical production and antioxidant defense [15, 17, 41]. However, we must pointed out that only a few of these findings conduct with the impacts of antioxidant enzyme activity on strong magnetic fields (>1 T). Additionally, the characteristic of decreased or increased ROS production and enzyme activity, as well as absence of impacts largely depends on the magnetic induction, exposure duration and the examined species. Some studies propose that magnetic field as biostimulants promotes growth and oxygen production in *Scenedesmus obliquus* [42]. Moreover, an increase in growth stimulation of antioxidant defense in *Chlorella vulgaris* has been demonstrated to production of exopolysaccharides [43].

Magnetic field plays essential roles in the formation and modulation of ROS to cause changes in metabolism, activity, concentration, and life stage of free radicals [44].Toxic effects of ROS are well known such as second messengers, enzyme inactivation, lipid peroxidation of membrane-bound organelles, and protein degradation in many cellular processes [45]. Magnetic field probably caused oxidative stress and damage to the genetic material through elevated ROS production [37]. Cryptochrome (CRY)-based magnetoreception is capable of generating magnetically sensitive radical pair described in different insects [46]. Therefore, it we hypothesized that CRY-based magnetoreception is a potential mechanism responsible for the examined reactions of aphid toward the induced magnetic field. Additionally, magnetic field can produce damage on different genetic structures, including DNA, RNA, and other macromolecules through the ROS production [48]. Genotoxic impact of magnetic field has been depicted in different organisms including Drosophila [49]. Therefore, the observed results could be contributed to four magnetic field (0.065 T, 0.1 T, 0.176 T and 0.28 T) induced oxidative DNA damage.

The peculiarity of the inhibition of enzymatic activity also observed in *S. obliquus* and *Drosophila melanogaster*, where the amount of ROS production exposure magnetic field exceeds the threshold for the proper cells' antioxidant capacity compared with the control in *S. obliquus* and *Drosophila melanogaster* [23, 42]. The radicals contain an unpaired electron in an atomic orbital as a molecular species, and the radical probably remove an electron from a stable molecule to reach electrochemical stability. Correspondingly, oxygen and hydrogen atom constitute free radicals. Particularly, these unpaired electrons are capable of making molecular species paramagnetic and susceptible to magnetic fields. Then, the magnetic field present in biological systems probably affects ROS dynamics in the case. Particularly, the main organelles of animal generating ROS are mitochondria, to a lesser extent, peroxisomes as a consequence of oxidative energy metabolism and the flow of electrons exists in the mitochondrial and animal membranes. The highly reactive characteristics of ROS can oxidize essential macro-molecules. Cells possess an antioxidant defense system that neutralizes or metabolizes ROS, including antioxidant enzymes of catalase, superoxide dismutase, peroxidase, and others. Therefore, if defense system is overcome, the cellular model systems of oxide-reduction equilibrium shifts to a pro-oxidant situation, then the oxidative stress would contribute to oxidative damage occurs from aspects of nucleic acids and proteins [50]. Additionally, the contaminant of environmental factors enter into the cellular

systems can intensify the production of molecular species as described in algae [51].

In conclusion, these findings indicated that applied SMF is capable of modifying the fitness components and antioxidant defense in aphid originating from four populations, whereby more detailed examinations of the various molecular mechanisms of ion flow, DNA synthesis and interaction of normal cells with the neurotransmitters are required to define the exact mechanism(s) underlying this interaction. Stress responses are critical determinants of the survival capacity of specie under stressful conditions and in the presence of pollution. Therefore, using aphid as model system can be a way to monitor micro evolutionary changes in studying different influences of environmental factors.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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