

Effects of humic acid and plant growth-promoting rhizobacteria (PGPR) on induced resistance of canola to *Brevicoryne brassicae* L

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Abstract

The cabbage aphid, *Brevicoryne brassicae* L. (Hem: Aphididae), is an important pest of canola that can considerably limit profitable crop production either through direct feeding or via transmission of plant pathogenic viruses. One of the most effective approaches of pest control is the use of biostimulants. In this study, the effects of humic acid, plant growth-promoting rhizobacteria (PGPR), and integrated application of both compounds were investigated on life table parameters of *B. brassicae*, and the tolerance of canola to this pest. *B. brassicae* reared on plants treated with these compounds had the lower longevity, fecundity, and reproductive period compared with control treatment. The intrinsic rate of natural increase (r) and finite rate of increase (λ) were lowest on PGPR treatment ($0.181 \pm 0.004 \text{ day}^{-1}$ and $1.198 \pm 0.004 \text{ day}^{-1}$, respectively) and highest on control ($0.202 \pm 0.005 \text{ day}^{-1}$ and $1.224 \pm 0.006 \text{ day}^{-1}$, respectively). The net reproductive rate (R_0) under treatments of humic acid, PGPR and humic acid + PGPR was lower than control. There was no significant difference in generation time (T) of *B. brassicae* among the tested treatments. In the tolerance test, plants treated with PGPR alone or in integrated with humic acid had the highest tolerance against *B. brassicae*. The highest values of total phenol, flavonoids, and glucosinolates were observed in treatments of PGPR and humic acid + PGPR. Basing on the antibiosis and tolerance analyses in this study, we concluded that canola plants treated with PGPR are more resistant to *B. brassicae*. These findings could be useful for integrated pest management of *B. brassicae* in canola fields.

Keywords: cabbage aphid, reproduction, life table, tolerance, antibiosis

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Introduction

Canola, *Brassica napus* L. (family Brassicaceae) is an important oilseed crop, which originated in either the Mediterranean region or the northern and western parts of Europe (Tsunoda,

1980). The diamondback moth, flea beetles, swede midge, cabbage seedpod weevil, pollen beetle, bertha armyworm, sucking insects, and noctuid pests can considerably limit canola production worldwide (Reddy, 2017). The cabbage aphid, *Brevicoryne brassicae* L. (Hem: Aphididae) is one of the most serious pests of this crop that creates large colonies on the leaves, stems, and buds of this crop and causes the twisting of leaves by sucking the phloem sap directly. It also interrupts photosynthesis by honeydew secretion and creating an environment for the growth of black mold fungus. This aphid could damage oilseed brassicas at flowering and pod founding stages (Ellis *et al.*, 1998; Anwar & Shafique, 1999). The seed

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loss is likely to occur 9–77%. Aphids also cause an 11% reduction in seed oil content (Kelm & Gadomski, 1995). On the other hand, they cause indirect damage via the transmission of viral diseases in plants, including the yellow mosaic virus, cauliflower mosaic virus, cucumber mosaic virus, onion yellow dwarf virus and cabbage black ringspot virus (Blackman & Eastop, 2000). Chemical fertilizers may cause a population increase of herbivorous insects via modification of the nutritional quality of the host plant (Bentz *et al.*, 1995; Van Emden, 1995). Furthermore, chemical control by insecticides is the most effective and easiest way to control aphids (Verkerk *et al.*, 1998), but this method can lead to problems such as residues of pesticides in food and the environment, and pesticide resistance development in pests (Furk & Hines, 1993). Therefore, more attention should be paid to other environmentally-safe and effective control methods for this pest. The use of biostimulants can limit the pest population and result in reduced reliance on its management on synthetic insecticides (Zehnder *et al.*, 1997; Nardi *et al.*, 2015). A plant biostimulant is any microorganism or substance applied to plants or the rhizosphere with the aim to stimulate natural processes for the enhancement of nutrition efficiency, stress tolerance and/or crop quality, in spite of its nutritional content (du Jardin, 2015). One group of more widely used biostimulants is humic substances. Several studies have demonstrated humic substances can improve plant growth and physiology (Bottomley, 1914; Cacco & Dell'Agnola, 1984; Dell'Agnola & Nardi, 1987; Nardi *et al.*, 1988; Pizzeghello *et al.*, 2013), as well as plant defenses against biotic and abiotic stresses (Nardi *et al.*, 2015). Humic acid is a suspension derived from potassium-humates, which can be explored as a plant growth stimulant or soil conditioner for increasing natural resistance to plant diseases and pests (Scheuerell & Mahaffee, 2004; Abd-El-Kareem, 2007).

Bio fertilizers like plant growth-promoting rhizobacteria (PGPR) can be classified as microbial biostimulants (du Jardin, 2015). PGPR are able to mediate plant growth by diverse direct and indirect mechanisms (Glick, 1995). Some of the mechanisms usually observed are the enhancement of nutrient availability; biological nitrogen fixation; protection from diseases and pests by the production of antibiotics, siderophores, hydrogen cyanide (de Medeiros *et al.*, 2005; Keel & Maurhofer, 2009), production of plant hormones and increasing stress tolerance (Glick *et al.*, 1999).

The construction of life tables is appropriate to study the dynamics related to the population growth potential, also called demographic parameters (Carey, 1993; Southwood & Henderson, 2000). Since the intrinsic rate of natural increase is a reflection of many factors, such as fecundity, survival, and generation time, it adequately summarizes the physiological qualities of an animal in relation to its capacity for population growth. Therefore, it is an appropriate index to evaluate the performance of an insect on different host plants as well as the host plant's antibiosis resistance to herbivorous insects (Smith, 1989; Carey, 1993; Southwood & Henderson, 2000). Antibiosis (one of the basic modalities of host plant resistance) is a negative effect of the host plant on the biological parameters of pests (Hesler & Tharp, 2005). Tolerance is another way of the host-plant resistance to pests and is defined as the plant's ability to endure an insect population that can damage a more susceptible host plant (Hesler & Tharp, 2005). The application of humic substances and PGPR might lead to induced resistance of plants to some pests (Hosseini, 2014; Mohamadi *et al.*, 2017; Rashid & Chung, 2017).

Induced resistance is defined as an enhancement of the plant's defensive capacity against a broad spectrum of pathogens and pests, acquired after appropriate stimulation (Broadway *et al.*, 1998). Yildirim & Unay (2011) reported the resistance induction of tomato plants by humic substances such as fulvic acid in combination with calcium nitrate against *Liriomyza trifolii* (Burgess). Moreover, induced resistance by PGPR has been found in corn to corn earworm, *Helicoverpa zea* Hübner (Bong & Sikorowski, 1991); in cucumber against cucumber beetles, *Diabrotica undecimpunctata* Barber (Zehnder *et al.*, 1997); in cotton to cotton bollworm, *Helicoverpa armigera* Hübner (Qingwen *et al.*, 1998), and in bell pepper against the green peach aphid, *Myzus persicae* (Sulzer) (Mardani-Talae *et al.*, 2016; 2017).

Secondary plant chemicals, especially phenolic compounds are important resistance factors of plants, having unfavorable effects on insect growth and feeding behavior (Cipollini *et al.*, 2008). Furthermore, flavonoids are one of the largest phenolic compounds in plants that play a key role in plant defense against pests and diseases (Hondo *et al.*, 1992). Qingwen *et al.* (1998) reported polyphenol and terpenoid contents of cotton plants treated with *Pseudomonas gladioli* influenced the relative growth rate, consumption rate, and the digestibility of feed in *H. armigera*. Additionally, glucosinolates (GSLs) (a group of naturally-occurring thioglucosides) are the main secondary metabolites accumulated in Brassicaceae plants (Halkier & Gershenzon, 2006), and are essential in the nutrition of the cabbage aphid (Tjallingii, 1976). Many studies have demonstrated that GSL accumulation can be 'induced' by various factors, such as insect attack (Lammerink *et al.*, 1984; Birch *et al.*, 1990; Bennett & Wallsgrove, 1994). Moreover, the presence of higher contents of GSLs in Brassicaceae plants induces plant resistance. GSLs are significant factors impairing the nutritional quality of the plant family of Brassicaceae and restricting their use as high-quality protein animal feed (Jezek *et al.*, 1999).

Biostimulants can affect the amounts of defensive chemical components in plants against herbivorous insects. Therefore, the aim of this research was to evaluate the effects of humic acid as an organic fertilizer and PGPR as a bio-fertilizer on the contents of phenolic compounds and GSLs in canola leaves and the resultant effects on antibiosis and tolerance parameters of canola for *B. brassicae*, which could then be used in the integrated management of this pest.

Materials and methods

Plant collection

The seeds of tested canola plants (cv. Jerry) were procured from Research, Education, Agriculture, and Natural Resources of Kerman, Iran, and were grown individually in 20-cm-diameter pots filled with a mixture of soil, sand, and manure (2:1:1). The plants were reared in a greenhouse (20–30°C, 60 ± 5% RH and natural photoperiod).

Insect colony

The used aphids in the experiments were acquired from the aphid colony reared in the laboratory of the plant protection department of Shahid Bahonar University, Kerman, Iran, in September 2017 and transferred to the potted plants under the above-defined conditions. Aphids were reared on canola

plants (cv. Jerry) for four generations before starting the experiments.

Induction treatments

In this study, four different treatments were used on canola plants twice (two and six-leaf stages): (1) the use of an aqueous solution of distilled water for control; (2) addition of humic acid to the pots ($2.5 \text{ mg kg}^{-1}/\text{pot}^{-1}$) with irrigation water; (3) the spraying of Roshdafza (commercial product of Biorun company) as PGPR on plants (3 cc litre^{-1} water), which contain *Pseudomonas fluorescens* strain NBTRI68, *Azotobacter chroococcum* strain NBTAz2; and *Azospirillum brasilense* strain NBTazoof (each of these bacteria with the rate of $2 \times 10^7 \text{ CFU ml}^{-1}$) (4) integrated application of both treatments (humic acid + PGPR).

Canola plants were exposed to cabbage aphid 48 h after the second application of the treatments.

Life table study

To evaluate the effects of studied treatments on the life history parameters of the cabbage aphid, experiment was carried out using clip cages (6 cm diameter and 1.5 cm depth) established on leaves of potted canola plants ($N=5$ potted plant for each treatment) in a growth chamber ($20 \pm 1^\circ\text{C}$, $60 \pm 5\%$ RH and 16L: 8D).

48 h after the second application of treatments, adult apterous aphids individually were placed on the lower surface of a given leaf of the respective plant. Each clip cage was considered a replicate, and a total of 30 clip cages were established on canola plants in each treatment. After 24 h, aphid mother and all nymphs except one nymph were removed. Each cage was monitored daily until the maturity of the aphid to determine nymphal developmental time and survival rate of *B. brassicae* for each treatment. After maturity, daily observations were followed until each female aphid died. The numbers of nymphs produced per female aphid were recorded daily, and then nymphs were completely removed from the cages. The obtained data from this experiment was used for assessing the population growth parameters.

Tolerance experiment

This experiment was carried out with the four above-mentioned treatments (ten replications for canola plants without any infestation to cabbage aphid and ten replications for canola plants with artificial infestation). The plants reached the six-leaf stage at the onset of the experiment. In the ten later replications, each plant within a plastic cage was infested with five apterous adults of *B. brassicae*. Every day, the plants were checked, and the number of aphids per plant was regulated to five aphids. The experiment was terminated 21 days after infestation. Then, the percentage of reduction in growth parameters, including leaf area, chlorophyll content, shoot length (the height of plant from the surface of the soil), root length, fresh weight, and dry weight in the infested plants to non-infested plants for each treatment was calculated as:

$$RGP\% = \frac{GP_N - GP_I}{GP_N} \times 100$$

Where *RGP* % is the percentage reduction of the growth parameter in infested plants to non-infested plants, GP_N is the growth parameter in non-infested plants, and GP_I is the growth parameter in infested plants.

Determination of total phenolic compounds

The level of total phenolic compounds in leaves of canola plants treated with humic acid and PGPR was measured based on Ronald & Laima (1999) for which a 0.1 mg sample of the leaf was milled in 95% ethanol and permitted to extract for 24–72 h. Thereafter, to 1 ml of the sample, 1.5 ml of 95% ethanol was added and made up to a volume of 5 ml with distilled water. To this mixture, 0.5 ml of 50% Folin's reagent and 1 ml of 5% sodium carbonate was added and vortexed. The mixture was kept in the dark for 1 h. Then, the absorbance was measured at 725 nm using a spectrophotometer (Ronald & Laima, 1999). Plants samples treated with distilled water used as control treatment.

Determination of flavonoids

The leaves of canola (0.1 g) grown in studied treatments were weighed and placed in a porcelain mortar and pestle to be crushed. Then, 10 ml of acidified ethanol was slowly added to the contents of porcelain mortar. After grinding the plant samples, they were centrifuged at $8000 \times g$ for 10 min. The extract was passed through Whatman filter paper No.1 and it was put in a hot water bath (80°C) for 10 min for measuring the flavonoids. After cooling of the extracts, the absorbance was read using a spectrophotometer in the wavelengths of 270 nm (Krzek *et al.*, 1998).

Determination of total GSL content

The powder (0.1 g) of canola leaves under each treatment was transferred to a 10 ml glass tube with a lid. Then, using the method described by Ishida *et al.* (2011), GSL was extracted as follows. To the powder in glass tubes, 4.8 ml of 80% methanol kept at room temperature was added. After the addition of 0.2 ml of 5 mM sinigrin as an internal standard, the tubes were kept at 25°C for 30 min. and then shaken reciprocally for 30 min. in a shaker. The tubes were centrifuged at $1600 \times g$ for 10 min. The supernatant was used as a crude extract.

Palladium colorimetric analysis of the total GSL content

Colorimetric analysis of the total GSL content was performed by simplifying the method described by Møller *et al.* (1985). Purification with ion-exchange chromatography was omitted. To 0.2 ml of crude GSL extract, 0.3 ml of distilled water and 3 ml of 2 mM palladium chloride reagent, in which 3.54 mg PdCl_2 had been dissolved in 1.68 ml of concentrated hydrochloric acid and diluted to 1000 ml with distilled water, were added and mixed. After incubation at 25°C for 1 h, absorbance at 425 nm was measured using a spectrophotometer.

Data analysis

The raw life-history data of all individuals of *B. brassicae* were analyzed using the TWSEX-MSChart program (Chi, 2017) based on the age-stage, two-sex life table theory (Chi & Liu, 1985). The age-stage specific survival rate (s_{xj}) (where x is the age and j is the stage), age-stage specific fecundity (f_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), and age-specific maternity (l_{xmx}) were evaluated from the daily records of the survival and fecundity of all individuals in the cohort. Furthermore, the fertility life table

Table 1. Mean (\pm SE) nymph period, adult longevity, reproductive period and fecundity of *Brevicoryne brassicae* L. on canola plants treated with humic acid and PGPR.

Fertilizer treatments	Nymph period (days)	Adult longevity (days)	Reproductive period (days)	Numbers of progeny female
Control	10.40 \pm 0.26a	29.67 \pm 0.85a	24.39 \pm 0.79a	44.44 \pm 1.61a
Humic acid	10.33 \pm 0.20a	26.37 \pm 1.21ab	18.39 \pm 0.93b	31.56 \pm 1.80b
PGPR	10.60 \pm 0.20a	24.07 \pm 1.43b	17.46 \pm 0.98b	28.14 \pm 1.96c
Humic acid + PGPR	10.50 \pm 0.20a	24.30 \pm 1.30b	17.4 \pm 0.89b	28.40 \pm 1.73bc

Means within a column followed by different letters are significantly different according to the paired bootstrap test at 5% significance level.

parameters including the intrinsic rate of natural increase (r), net reproductive rate (R_0), mean generation time (T), finite rate of increase (λ), and doubling time (DT) were calculated.

The intrinsic rate of increase was estimated using the iterative bisection method from the Euler–Lotka formula with age indexed from 0 (Goodman, 1982):

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

The means and standard errors of life table parameters were determined using the bootstrap technique (Efron & Tibshirani, 1993; Huang & Chi, 2012) with 100,000 resampling. The bootstrap method is embedded in the computer program TWOSEX-MSChart. The paired bootstrap test was used to evaluate the differences between treatments.

Data of the tolerance experiment, determination of total phenolics, flavonoids and GSLs were assessed for normality with the Kolmogorov–Smirnov test and were analyzed using the one-way analysis. Arcsin square root transformation was applied for percentage data to handle the heterogeneity of variance prior to data analysis. Then, multiple comparisons were made using the Tukey test (SPSS, 2015).

Result and discussion

There was no significant difference in the nymph period of *B. brassicae* among treatments ($F = 8.49$; $df = 3, 116$; $P > 0.05$; table 1). However, tested treatments significantly influenced longevity ($F = 137.14$; $df = 3, 116$; $P < 0.05$), reproductive period ($F = 404.93$; $df = 3, 116$; $P < 0.05$), and fecundity of this aphid ($F = 545.14$; $df = 3, 116$; $P < 0.05$) (table 1). The longevity of adult females under PGPR and PGPR + humic acid treatments was significantly lower compared with control treatment (table 1). Moreover, no significant difference was found in adult longevity between humic acid and the other studied treatments. The reproductive period and fecundity of *B. brassicae* on plants treated with humic acid, PGPR, and PGPR + humic acid were significantly lower than those on plants without fertilizer treatment (control) (table 1). There are several reports on the significant effects of humic compounds and PGPR on the biological characteristics of other aphids such as *Aphis gossypii* Glover reared on cucumber (Fahimi *et al.*, 2014; Hosseini, 2014) and *M. persicae* reared on bell pepper (Mardani-Talaei *et al.*, 2017). In our research, the lowest number of nymphs produced per female was calculated for PGPR treatment but did not differ significantly from the PGPR + humic acid treatment (table 1). The lower fecundity of *B. brassicae* on bacterial treatments indicated a minor suitability of canola plants treated with PGPR than the others for the cabbage aphid. Moreover, Fahimi *et al.* (2014) reported that *Pseudomonas fluorescens* strains UTPF68 and PF169 could decrease the average progeny produced by

A. gossypii adults on cucumber. Our results showed that the adult pre-reproduction period (APRP) of each reproduced female was zero in the four tested treatments and all females that emerged started producing immediately. The APRP is calculated based on the adult stage. It assumes all females emerged at the same time. Therefore, no significant differences were observed in the APRP of *B. brassicae* among the tested treatments.

The age-stage specific survival rate (s_{xj}) represents the probability that a nymph of *B. brassicae* will survive to age x and stage j (fig. 1). The variable development rates among individuals in the cohort resulted in an overlapping of the stage-specific survivorship curves. The age-stage-specific survival rate (s_{xj}) curve showed a similar pattern in all the different fertilizer treatments. When all stages are pooled, the age-specific survival rate (l_x) gives a simplified overview of the survival history of the whole cohort (fig. 2). The l_x curve indicated that survival was approximately 100% in the nymph period, and then it declined slowly until the death of the last adult. This could be because of increased secondary metabolites in the plant and the composition of the epicuticular lipids (Eigenbrode & Espelie, 1995). Death of the last female under treatments of humic acid, PGPR, PGPR + humic acid, and control occurred at days of 47, 46, 41, and 47, respectively.

The highest f_{x2} peak in treatments of humic acid, PGPR, PGPR + humic acid, and control was 2.4, 2.2, 2.6, and 2.9, respectively. The age-specific fecundity (m_x) and the age-specific maternity (l_{xmx}) of *B. brassicae* are also shown in fig. 2. The highest peaks of m_x and l_{xmx} were recorded as 1.9 on humic acid (at 15 day), 1.6 on PGPR (at 19 day), 1.9 on PGPR + humic acid (at 18 day) and 2.5 on control (at 15 day).

The reproductive value (v_{xj}) gives the expected contribution of individuals of age x and stage j (fig. 3). At age 0, the reproductive values (v_{0j}), were the same as the finite rates on the four treatments, i.e. 1.211 day⁻¹ on humic acid, 1.198 day⁻¹ on PGPR, 1.201 day⁻¹ on PGPR + humic acid and 1.224 day⁻¹ on control (fig. 3). The values of v_{xj} peaks on these treatments increased to 9.452 day⁻¹, 8.896 day⁻¹, 9.30 day⁻¹, and 9.880 day⁻¹ after the emergence of female adults (fig. 3). It shows that individuals at the peak reproductive phases can contribute much more than a newborn nymph. The age-stage life expectancy (e_{xj}) (where x is the age and j is the stage) shows the expected lifespan for an individual of age x and stage j (fig. 4). The life expectancies of *B. brassicae* at age 0 (e_{0j}) were 36.7, 34.7, 34.8, and 40.1 days, on treatments of humic acid, PGPR, PGPR + humic acid and control, respectively, and, at the stage of aphid maturity, 27.7, 25.7, 25.8, and 32.1 days (fig. 4).

The population growth parameters are appropriate indexes to compare pest performance under different conditions in host plants (Carey, 1993; Southwood & Henderson, 2000). The net reproductive rate (R_0) and the intrinsic rate of population increase (r) are the two key demographic parameters used to evaluate the fitness of populations across diverse climatic

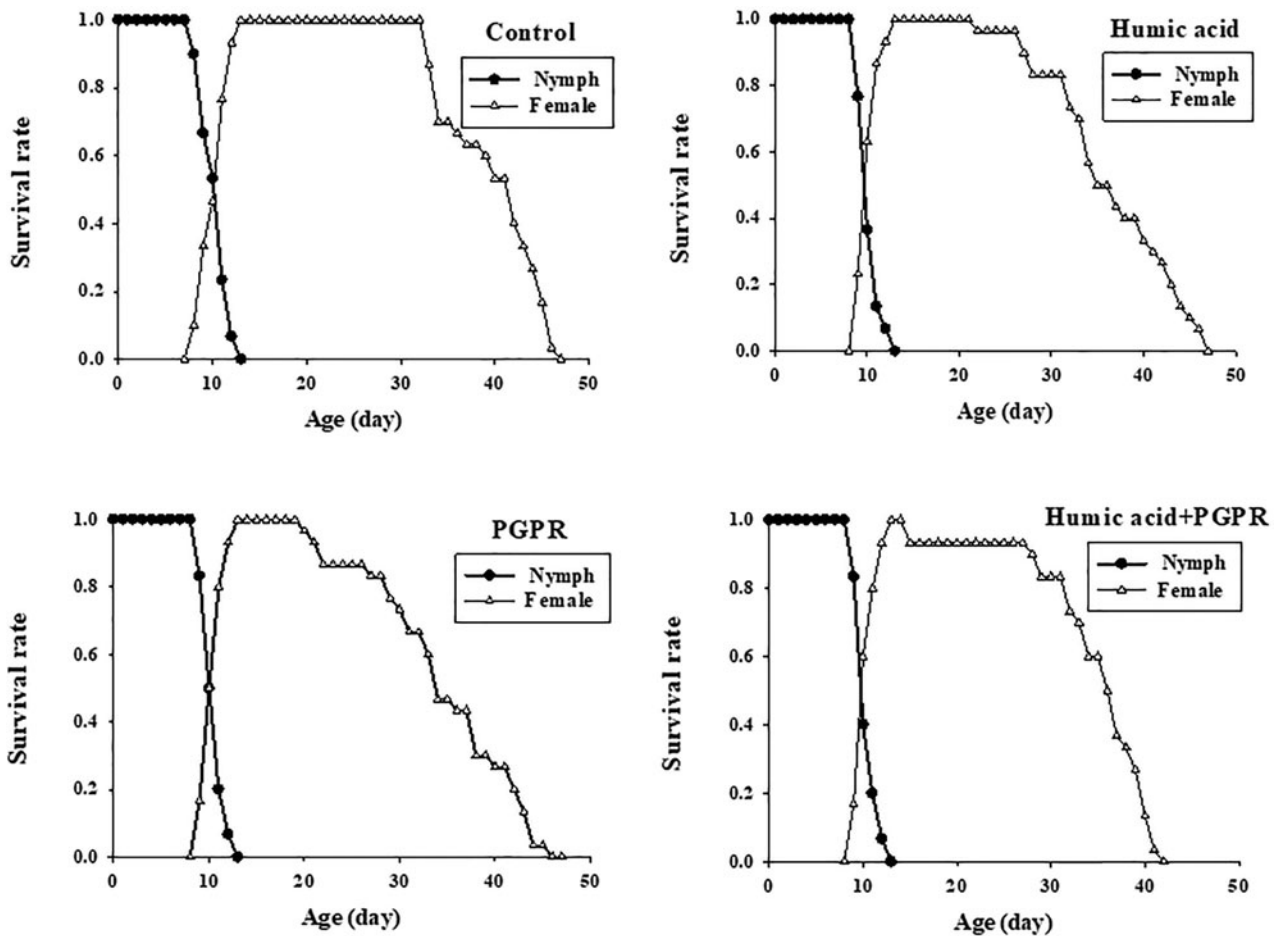


Fig. 1. Age-stage specific survival rate (s_{xj}) of *Brevicoryne brassicae* L. on canola plants treated with humic acid and PGPR.

and food-related conditions (Liu *et al.*, 2004). The value of the net reproductive rate (R_0) under fertilizer treatments of humic acid, PGPR, and PGPR + humic acid was significantly lower compared with no-fertilizer treatment (control) ($F = 545.14$; $df = 3, 116$; $P < 0.05$; table 2). In our study, this could be attributed to the lower fecundity of *B. brassicae* under the mentioned fertilizer treatments than control treatment. Furthermore, Mohamadi *et al.* (2017) reported that R_0 of *Tuta absoluta* (Meyrick) was significantly lower on tomato plants treated with PGPR (*Pseudomonas fluorescens*) and humic fertilizer than on untreated plants. It may be related to the promoted plant growth and induced systemic resistance. The lowest values of the intrinsic rate of increase (r) and finite rate of increase (λ) of *B. brassicae* were achieved in the PGPR treatment (0.181 and 1.198 day^{-1} , respectively) and the highest values of these parameters were observed in control (0.202 and 1.224 day^{-1} , respectively) ($F = 150.28$; $df = 3, 116$; $P < 0.05$ and $F = 149.74$; $df = 3, 116$; $P < 0.05$; respectively) (table 2). Mardani-Talaei *et al.* (2016) showed that r of *M. persicae* on bell pepper treated with *Bacillus subtilis* and *Glomus Intraradices* \times *Pseudomonas fluorescens* was significantly lower than on control (no treatment). In the current study, there was no significant difference in the generation time (T) of *B. brassicae* among the tested treatments ($F = 49.98$; $df = 3, 116$; $P > 0.05$; table 2). However, the DT was longest in treatments of PGPR and PGPR + humic

acid and shortest in control treatment ($F = 153.20$; $df = 3, 116$; $P < 0.05$; table 2).

Our results demonstrated that the population growth of *B. brassicae* under treatments containing PGPR was limited mostly by the shorter reproductive period and poor fecundity. Furthermore, the decreased performance of the cabbage aphid on plants treated with PGPR was related to the lower age-stage life expectancy (e_{xj}) of individuals used in the life table study in this treatment. In fact, PGPR led to the lowest intrinsic rate of increase, finite rate of increase, and the reproductive value (v_{xj}) of *B. brassicae* on canola plants, and hence, it appeared to be the most suitable induction treatment to reduce the population growth of this pest among the tested treatments. Therefore, it can be concluded that the PGPR cause antibiosis resistance in canola plants to cabbage aphid.

In this experiment, the analysis of growth parameters data showed significant differences in leaf area ($F = 6.76$; $df = 3, 36$; $P = 0.001$), chlorophyll content ($F = 5.49$; $df = 2, 98$; $P = 0.044$), shoot length ($F = 3.47$; $df = 3, 36$; $P = 0.026$), fresh weight ($F = 13.15$; $df = 3, 26$; $P < 0.0001$), and dry weight ($F = 14.77$; $df = 3, 36$; $P < 0.0001$). No significant differences were found in root length among the tested treatments ($F = 1.73$; $df = 3, 36$; $P = 0.179$; table 3).

In this study, the percentage reduction of the leaf area under PGPR and PGPR + humic acid treatments (6.05 and

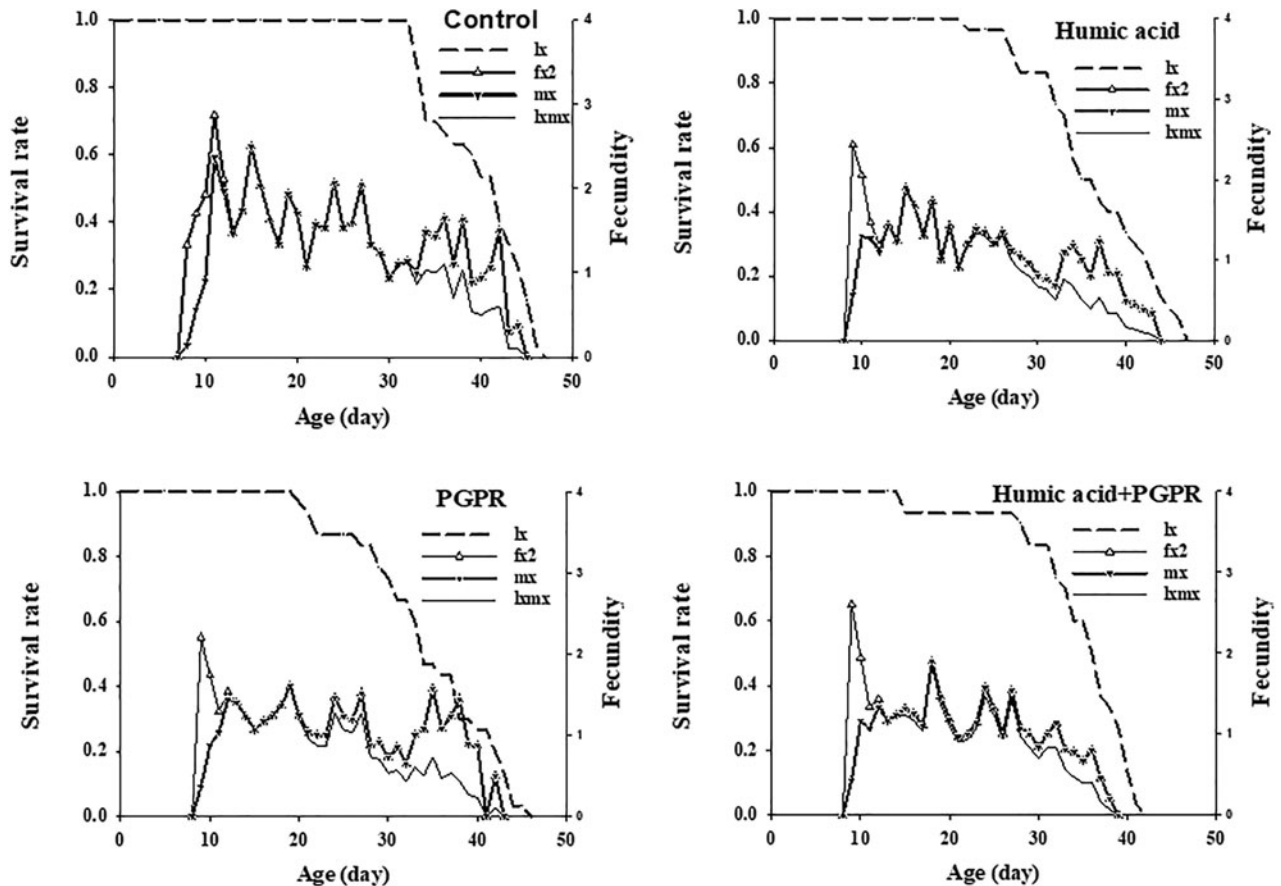


Fig. 2. Age-specific survival rate (l_x), female age-specific fecundity ($f_{x,2}$), age-specific fecundity (m_x), and age-specific maternity (l_{x,m_x}) of *Brevicoryne brassicae* L. on canola plants treated with humic acid and PGPR.

4.61, respectively) was calculated to be significantly lower compared with control treatment (22.51) (table 3). Furthermore, the percentage reduction of Chlorophyll content was lowest on PGPR + humic acid treatment (15.66) and the highest on control treatment (39.48) (table 3). The least and the maximum percentage reduction of shoot length was determined in treatments of PGPR (3.25) and control (17.47), respectively (table 3). In addition, the percentage reduction of fresh weight and dry weight was significantly lower in treatments of PGPR and PGPR + humic acid rather than humic acid and control (table 3).

Our research indicated that PGPR application resulted in a greater tolerance level of canola plants for *B. brassicae*. PGPR can accelerate plant growth by helping to escape from attacks by some pathogenic microorganisms and pests (Pineda *et al.*, 2010). Cakmakci *et al.* (2007) showed the considerable effects of PGPR on plant growth parameters such as shoot fresh weight, plant height, and leaf area in wheat and spinach. Cheng *et al.* (2007) reported inoculation of the *Pseudomonas putida* strain UW4, containing the ACC deaminase enzyme in the presence of salt, significantly improved canola growth. Generally, PGPR can increase plant growth through the production of hormones such as auxins, gibberellins, and cytokinins (Bhattacharyya & Jha, 2012). The positive effect of an integrated application of PGPR and humic acid on plant growth indices was evident in our study. Furthermore,

Ahmad *et al.* (2016) concluded that an integrated use of humic acid and PGPR was an effective approach to improve canola nourishment and yield. Some research has shown that humic acid application could increase the growth indices such as the dry weight, fresh weight, and shoot length of maize (Eyheraguibel *et al.*, 2008) and pepper (Gulser *et al.*, 2010). The effect of humic acid was dependent on the generation of reactive oxygen species (ROS) in the inducement of root growth and the spread of lateral root (Cordeiro *et al.*, 2011).

Our results showed that fertilizer treatments significantly influenced the amount of total GSL in leaves of canola ($F = 12.29$; $df = 3, 36$; $P < 0.001$). The level of total GSL was significantly higher in treatments of PGPR and PGPR + humic acid rather than humic acid and control (table 4). Earlier studies had shown a significant negative correlation between the GSL content of *Brassica* species and populations of aphids feeding on them such as *Lipaphis erysimi* (Kaltenbach) (Labana *et al.*, 1983; Malik *et al.*, 1983).

In the current study, the amount total phenolic compounds and flavonoids varied significantly among different fertilizer treatments ($F = 10.12$; $df = 3, 36$; $P < 0.001$ and $F = 5.64$; $df = 3, 16$; $P = 0.008$; respectively). The level of total phenolic compounds in leaves of canola was significantly higher under treatments of PGPR and PGPR + humic acid rather than humic acid and control (table 4). Moreover, the flavonoids in

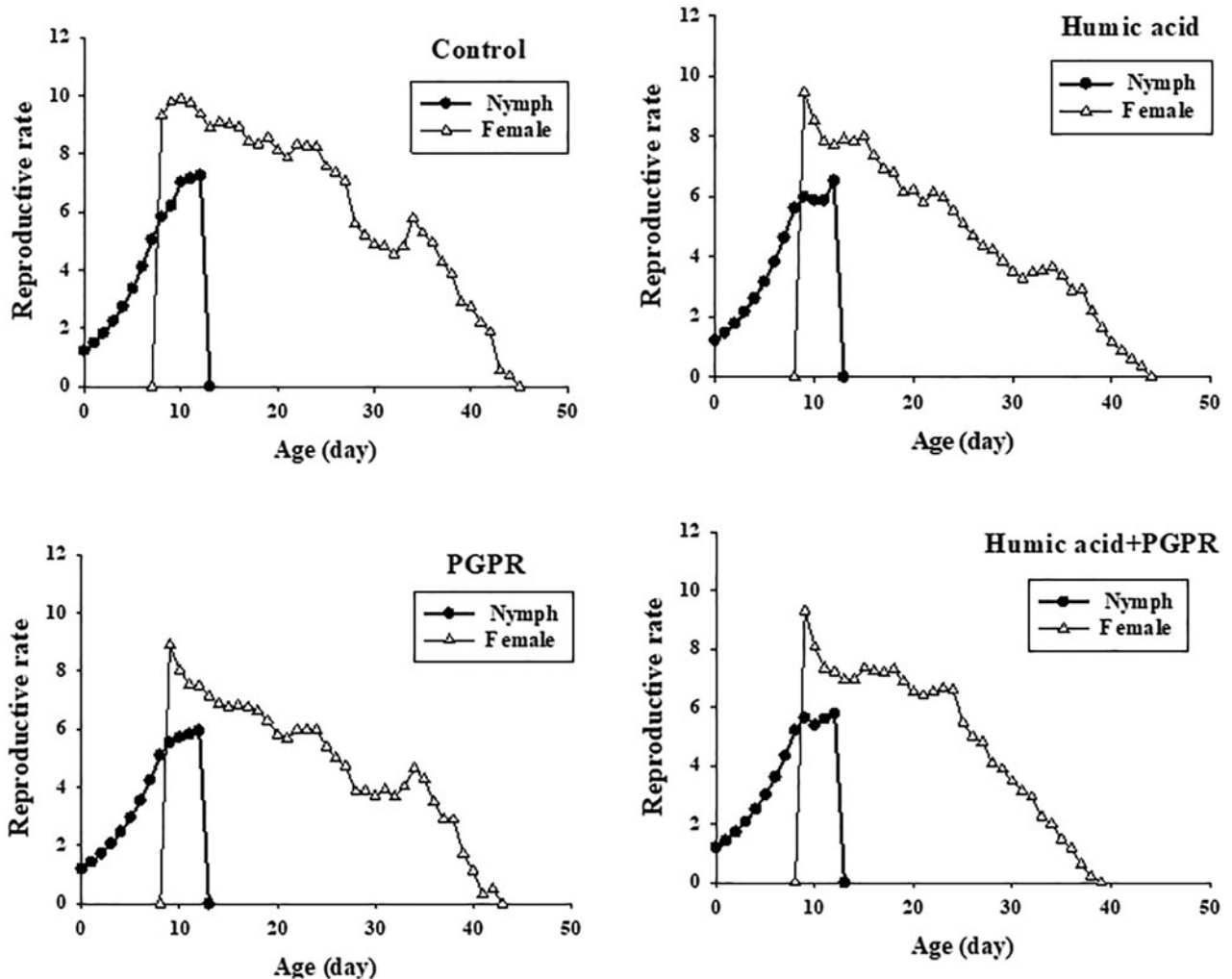


Fig. 3. Age-stage reproductive value (V_{xj}) of *Brevicoryne brassicae* L. on canola plants treated with humic acid and PGPR.

treatments of PGPR and PGPR + humic acid were significantly more than control (table 4). The amount of flavonoids in humic acid treatment did not differ significantly from the other tested treatments.

Chamam *et al.* (2013) demonstrated that phenolic compounds such as flavonoids and hydroxycinnamic derivatives were the main rice metabolites affected in response to *Azospirillum* bacteria. Also, foliar application of *Pseudomonas fluorescens* strain Pf1 could increase phenolic compounds of peanut plants (Meena *et al.*, 2000). The productions of secondary metabolites in plants are usually enhanced in response to environmental tensions such as herbivores and pathogens (Bourgaund *et al.*, 2010). Hence, phenolics are biologically effective secondary metabolites, negatively impacting development, on development, reproduction, and population growth parameters of the aphids (Wójcicka, 2010). Negative associations between the presence of phenolic compounds in plant species and aphid's invasion have been recorded for some aphid species (Havlíčková, 1995; Sandström *et al.*, 2000). Chassy *et al.* (2006) reported that humic acid increased flavonoids, total phenol, and ascorbic acid in organic tomatoes. Hanafy Ahmed *et al.* (2010) showed that the amount of sugars,

amino acids, proteins, and total phenol content in green bean plants increased after humic acid application. Furthermore, tomato plants treated with humic substances had a negative effect on populations of *Liriomyza trifolii* (Burgess) (Yildirim & Unay, 2011).

In our research, humic acid alone (without integrated with PGPR) was not significantly effective on the resistance of canola to cabbage aphid. This could be attributed to the level of consumption, source, and type of humic substances (Arancon *et al.*, 2006). Our results indicated that canola plants treated with PGPR having a high level of phenolic compounds were more resistant to the cabbage aphid. Mardani-Talaei *et al.* (2016) demonstrated that phenolics in leaves of bell pepper could decrease fecundity and the growth rate of the *M. persicae* population. Previous research suggested that the PGPR was able to induce resistance responses against insects by promoting plant growth and distinctive alterations in biochemical profiles and plant molecular mechanisms (Zehnder *et al.*, 1997; Rashid & Chung, 2017). Zebelo *et al.* (2016) reported that cotton root colonization by PGPR could induce systemic resistance to *S. exigua* due to increased plant hormones. In addition, induced systemic resistance has been found in cotton

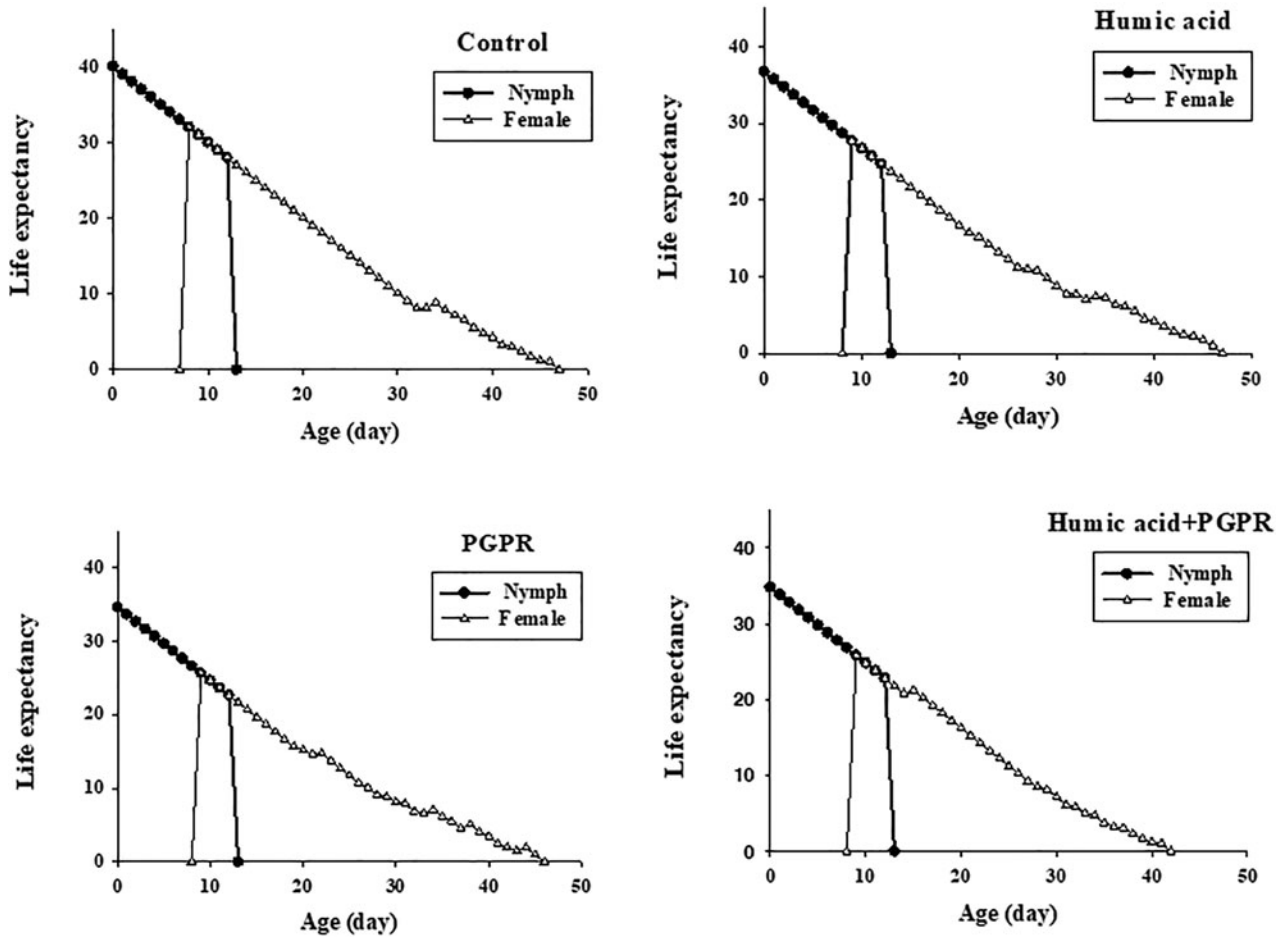


Fig. 4. Age-stage specific life expectancy (e_{xj}) of *Brevicoryne brassicae* L. on canola plants treated with humic acid and PGPR.

Table 2. Mean (\pm SE) life table parameters of *Brevicoryne brassicae* L. on canola plants treated with humic acid and PGPR.

Fertilizer treatments	R_0 (offspring)	r_m (day^{-1})	λ (day^{-1})	T (days)	DT (days)
Control	44.44 \pm 1.61a	0.202 \pm 0.005a	1.224 \pm 0.006a	18.77 \pm 0.42a	3.43 \pm 0.08b
Humic acid	31.56 \pm 1.79b	0.192 \pm 0.004ab	1.211 \pm 0.005ab	18.02 \pm 0.35a	3.52 \pm 0.10ab
PGPR	28.14 \pm 1.96b	0.181 \pm 0.004c	1.198 \pm 0.004c	18.45 \pm 0.27a	3.83 \pm 0.07a
Humic acid + PGPR	28.40 \pm 1.72b	0.183 \pm 0.004bc	1.201 \pm 0.005bc	18.26 \pm 0.23a	3.78 \pm 0.08a

R_0 , net reproductive rate; r_m , intrinsic rate of increase; λ , finite rate of increase; T , mean generation time; DT , doubling time. Means within a column followed by different letters are significantly different according to the paired bootstrap test at 5% significance level.

Table 3. The mean (\pm SE) percentage reduction of growth parameters in aphid-infested canola plants treated with humic acid and PGPR.

Fertilizer treatments	Leaf area (%)	Chlorophyll content (%)	Shoot length (%)	Root length (%)	Fresh weight (%)	Dry weight (%)
Control	22.51 \pm 3.56a	39.48 \pm 6.63a	17.47 \pm 3.91a	16.84 \pm 5.56a	32.57 \pm 2.48a	36.73 \pm 4.43a
Humic acid	10.13 \pm 1.88ab	26.99 \pm 4.47ab	13.69 \pm 2.79ab	8.60 \pm 2.29a	22.92 \pm 2.96a	29.34 \pm 2.25a
PGPR	6.05 \pm 1.95b	20.18 \pm 3.46ab	3.25 \pm 1.07b	5.38 \pm 0.85a	12.78 \pm 2.33b	9.15 \pm 1.93b
Humic acid + PGPR	4.61 \pm 1.62b	15.66 \pm 1.56b	5.76 \pm 1.14ab	4.56 \pm 1.30a	10.62 \pm 2.38b	7.18 \pm 2.40b
P	0.001	0.044	0.026	0.179	< 0.0001	< 0.0001

Means followed by a different letter within a column are significantly different (Tukey's HSD test; $P < 0.05$).

Table 4. The mean (\pm SE) amount of total Glucosinolate, total phenol, and flavonoids in aphid-infested leaves of canola plants treated with humic acid and PGPR.

Fertilizer treatments	Sample size (n)	Total glucosinolate ($\mu\text{mol g}^{-1}$)	Sample size (n)	Total phenol (mg g^{-1} fresh weight)	Sample size (n)	Flavonoids (Abs mg^{-1} dry weight)
Control	10	1.81 \pm 0.04b	10	45.42 \pm 5.91b	5	0.29 \pm 0.05b
Humic acid	10	1.72 \pm 0.05b	10	57.64 \pm 8.1b	5	0.37 \pm 0.02ab
PGPR	10	1.99 \pm 0.04a	10	80.52 \pm 3.62a	5	0.48 \pm 0.04a
Humic acid + PGPR	10	2.03 \pm 0.04a	10	85.21 \pm 5.18a	5	0.53 \pm 0.07a
P		<0.0001		<0.0001		0.008

Means followed by a different letter within a column are significantly different (Tukey's HSD test; $P < 0.05$).

plants against *H. armigera* (Rajendran *et al.*, 2007), and in tomato plants against whitefly (Hanafi *et al.*, 2007) due to PGPR application. The relative growth rate and the relative consumption rate of *H. armigera* larvae were reduced in cotton plants treated with *Pseudomonas gladioli* because of an increase in the content of polyphenol and terpenoids in cotton (Qingwen *et al.*, 1998). In fact, rhizobacteria can increase plant health and resistance to herbivore insects by triggering systemic defense responses (Rashid & Chung, 2017).

In summary, we concluded that canola plants treated by PGPR were the least suitable host for the *B. brassicae*. Therefore, PGPR could induce resistance in canola against the cabbage aphid. The research could provide valuable information for integrated management of *B. brassicae* in the canola fields. More attention should be paid to field experiments to obtain more accurate results with respect to the performance of this pest.

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