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Foliar and Soil Treatments of *Brassica napus* That Elicit Antibiosis in *Brevicoryne brassicae*

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Abstract: In screenhouses, foliar and soil applications of induced resistance (IR), nutrient deterrence (ND), and soil amendment (SAM) treatments to canola, *Brassica napus* L., reduced fitness in an aphid pest, *Brevicoryne brassicae* L. Effects of different combinations and doses of IR, ND, and SAM on aphids were compared with those of labeled doses of Carbosulfan and a control. Dose differences between treatments and the control were found for several measures of *B. brassicae* fitness, including Kaplan–Meier functions, reproduction time, effective fecundity (M_d), intrinsic rates of natural increase (r_m), relative growth rate (*RGR*), and generation time. Progeny, development/pre-reproductive period, and percent progeny nymph survival were also significantly different from the control. Carbosulfan was the most rapidly acting treatment; however, 1 mM salicylic acid (SA) produced statistically significant reductions in aphid fitness compared to the control, followed by 1 mM citric acid (CA), 0.5 mM SA, and 0.5 mM CA. Silicon (Si) at 50 kg/ha was the most effective ND approach. Ammonium sulfate (AS) only decreased fitness at 25 kg/ha. The SAM combinations of both elemental sulfur (ES) and bio-sulfur (BS) treatments with compost (Cp) also yielded statistically significant aphid fitness reductions. These results provide context for future exploration of IR, ND, and SAM approaches to improve canola yield and reduce aphid damage.

Keywords: biological interactions; bio-sulfur; citric acid; organic compost; plant resistance inducers; salicylic acid; silicon

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1. Introduction

Brassica napus L. (Brassicales, Brassicaceae) is the third most widely used oilseed crop in the world [1]. Aphids infest and damage this crop heavily [2], including the cabbage/mealy aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae), one of its major insect pests [2–4]. *Brevicoryne brassicae* can reduce crop yield up to 75%, either directly, due to sap-sucking, and/or indirectly due to virus transmission [4,5]. Similar to many aphids, *B. brassicae* has a high reproduction rate, with up to 15–20 generations in a single growing season [4] and has already developed resistance against various insecticides [6]. Alternative approaches to manage this and other resistant pests are under investigation, including eco-friendly approaches [7,8] with potential to ameliorate rising levels of insecticide resistance, while reducing environmental harm, and negative oncogenic, neurotoxic, and teratogenic effects to growers and consumers [9–12].

Furthermore, widespread use of carbamate, organophosphate, organochlorine, and pyrethroid insecticides for pest management has resulted in a loss of biodiversity and pollinators including honeybees and other pollinators of important crops [13,14]. Nontarget impacts of insecticides have been reported on natural bio-control agents [15]. Immediate insect knockdown is a capability of synthetic insecticides lacked by other management approaches [16]; however, it is not always necessary to kill a pest immediately to improve crop yield. Consequently, attention is beginning to focus on potential eco-friendly approaches that enable improved crop yields but cause less harm to nontarget insects [8,12].

Induced resistance (IR), nutrient deterrence (ND), and soil amendments (SAM) are relatively new, eco-friendly management tools for the control of aphids and other insect pests [17–19]. IR tools treat crop plants with doses of different inducers, e.g., salicylic acid (SA), jasmonic acid (JA), and citric acid (CA), that activate their inherent resistance mechanisms against biotic and abiotic stresses [20–23]. The SA generates 'systemic acquired resistance (SAR)' in plants against various insect pests, including aphids (*Aphis craccivora, Aphis gossypii*, *Brevicoryne brassicae, Sitobion avenae*) [18,24–26]. The SAR is induced in plants after the accumulation of 'pathogenesis-related proteins' [27]. Plant ultra-membranous receptors identify the pest attack by recognizing pest elicitors, using 'pattern-trigged immunity-PTI'. In addition, plant cellular network also recognizes the pest attack using 'effector-trigged immunity—ETI'. Both ETIs and PTIs are regulated by other complex molecular tools stimulated by physiological cascades [28], under different aphid attacks, e.g., *Aphis gossypii* on melon [18], *Brevicoryne brassicae* on canola [24], *Sitobion avenae* on wheat [25], and *Aphis craccivora* on faba beans [26]. Whereas CA has shown potential to mitigate abiotic stress [21,22]. Recent studies have shown effects of SA in activating insect resistance against various aphid species, e.g., *Aphis craccivora, Aphis gossypii*, *Brevicoryne brassicae,* and *Sitobion avenae* [18,24–26,29].

ND involves the application of silicon (Si) and ammonium sulfate (AS) to increase plant yield and resistance against imposed stress [19,30,31]. Silicon has been widely documented to have antibiosis against various aphids and other insect pests [19,30,32,33]. Likewise, AS has been found to enhance 'systemic acquired acclimation' in plants [31] by increasing the availability of sulfur that activates plant defense compounds (glucosinolates) [34–36].

SAM with various combinations of compost (Cp), elemental sulfur (ES), and/or bio-sulfur (BS) have been reported to improve crop growth and yield [17,36–38]. The SAM treatments must be used carefully for each combination of crop and insect pest, as it has been reported that *Brassica*-feeding specialists sometimes remain unharmed after exposure to sulfur treatments [39–41]. Additionally, SAM treatments that include nitrogen, phosphorus, and potassium have been reported to enhance rather than decrease fitness of planthoppers and other rice pests [42]. Soil amendment treatments have been reported to improve crop yield without toxicity against pollinators and other insects of conservation importance [37,38,43–46]. Several studies have found that sulfur has potential for antibiosis against chewing insects [39–41].

The study hypothesis is that various foliar and soil treatments on *B. napus* could affect fitness attributes of *B. brassicae*, which would support their potential use in insect pest management. The study assesses applications of different doses of IR, ND, and SAM treatments to reduce *B. brassicae* fitness and thereby improve *B. napus* yield. The fitness attributes measured herein include eight that have been defined and used in the applied ecology literature, e.g., [47–51]: (1) d, the time (in days) from birth until each treated aphid (female) produces its first nymph, i.e., the pre-reproductive period; (2) the time (in days) from birth until each nymph develops to adulthood, i.e., the development period; (3) the time (in days) when each treated aphid produces its last nymph, the reproduction time; (4) M_d, the number of nymphs produced by each treated female during a period equal to d, i.e., the effective fecundity (No. females/day); (5) $r_{dp,}$ the ratio of the development period to the pre-reproductive period; (6) $T_d = r_{dp}/0.738$, the mean generation time, (days); (7) $r_m = 0.74 \ln(M_d)/d$, the intrinsic rate of natural increase, i.e., No. offspring/day/No. Female; and (8) $RGR = r_m/0.86$, the relative growth rate, (No offspring/day/No. Female).

2. Materials and Methods

2.1. Plants, Insects, and Screenhouse Conditions

Seeds of *Brassica napus* cv. Faisal Canola were sown in sterilized plastic pots (25 cm diameter and 23 cm height) filled with 8 kg of sieved soil. Soil characteristics were sandy clay loam, containing nitrogen 0.05%, potassium 150 ppm, and phosphorous 7.47 ppm. Nitrogen (90 kg/ha), phosphorus (60 kg/ha), and potash (50 kg/ha) were applied using urea, $(NH_4)_2HPO_4$, and sulfate of potash, respectively. Screenhouse environmental conditions for temperature, relative humidity, and photoperiod were 21 ± 5 °C, 65 ± 5% relative humidity (RH), and 10:14 (L:D) hours, respectively. Temperature and RH were recorded using an LCD thermo-hygrometer (Digital HTC-2, HTC, China).

B. brassicae was collected initially from local *B. oleracea*, *B. juncea*, and *B. napus*. The insects thereafter were maintained for >15 generations on *B. napus* before treatments. To collect adults of the same age, individual adult female aphids were confined under clip cages (3 cm diameter, depth 1.5 cm) for 24 h. The adults were then removed, and new nymphs were kept on individual plants until the adult stage when they were used in bioassays. The experiments proceeded using 5-week-old plants, as they have enough strength to hold three clip cages per plant effectively. The positions of the plants were randomized daily.

2.2. Treatment Preparation and Application

Under all resistance induction approaches, treatments were applied either via foliar or soil application to potted *B. napus* plants. Carbosulfan (Advantage 20% EC-FMC PA, USA) was prepared at the rate of 8.33 mL/L and was adjusted with respect to the manufacturer's recommended dose rate of 1 L per 120 L water for coverage of a 0.405 ha surface area. The induced resistance (IR), nutrient deterrence (ND), and soil amendment (SAM) treatments were tested in separate groups as described below, each grouping of which included an insecticide and a control (distilled water only).

The IR grouping included SA and CA, each tested at 0 (control), 0.5, and 1 mM, a total of 8 different treatments. The SA was dissolved in 0.1% ethanol, and the CA in distilled water, respectively. Solutions of 0, 0.5, and 1 mM each for SA and CA were prepared per the recommendations of Afshan et al. [52] and Syeed et al. [53]. For the 1 mM SA treatment, 138.121 mg of SA, and for the 0.5 mM SA treatment, 69.06 mg SA were slowly dissolved in (1 L of 0.1% ethanol). Each mix was added to 1 L of distilled water. For 0 mM SA, 0.1% ethanol was used. In the same way, for 1 and 0.5 mm CA, 192.12 mg and 96.06 mg of CA were dissolved in one liter of distilled water. Distilled water was used for the 0 mM CA (control) treatments. The foliar spray was applied manually with a hand sprayer (Type: Top gun—manual; Volume: 1000 cm^3). The 1 mM concentrations of SA were sprayed at the rate of 41.45 g/ha, adjusted with respect to 300 L volume. Each plant was dosed with 30 mL of the 138.121 mg/L solution. Similarly, for 0.5 mM SA, the dosage was 20.72-g/ha and 69.06 mg/L. For 1 and 0.5 mm CA, 192.12 mg and 96.06 mg of CA were applied at the rate of 57.63 g/ha (1 mM CA) and 28.81 g/ha (0.5 mM CA), respectively, adjusted according to 300 L/ha spray volume.

The ND grouping included Si and AS, each tested at 0.5 and 1 kg/ha, with a total of 6 different treatments, including the insecticide and its control. Si and AS were applied to soil at the rate of 25 and 50 kg/ha. For 25 kg/ha, 100 mg of either Si or AS was added to 8 kg of soil, based on 12.5 mg per kg of soil. Similarly, for 50 kg/ha, 200 mg of nutrients was added to the pots at the rate of 25 mg nutrient per kg of soil.

The SAM grouping included elemental sulfur (ES), bio-sulfur (BS), and compost (Cp) treatments, applied alone or in the combinations, ES + Cp or BS + Cp, with a total of 7 different treatments, including the insecticide and its control. Cp was prepared by incubating animal manure with 0.1% molasses. The material was composted in a 500 kg, locally manufactured composter. The composter was run continuously for seven days until the achievement of desired composition. Soil application was carried out by manual mixing of treatments in the potting soil. The ES and BS sulfur were applied at the rate of

8000 kg/ha, and Cp at the rate of 16,000 kg/ha. ES and BS were mixed at the rate of 4 g/kg (32 g/8 kg pot) and Cp was mixed at the rate of 8 g/kg (64 g/8-kg/pot). In integrated treatments of ES + Cp and BS + Cp, sulfur formulation and compost were applied at the rate of (4g + 8g)/kg/pot.

2.3. Fitness Bioassays

Individual apterae *B. brassicae* females (9–10 days old) were placed on middle, fully expanded healthy leaves on the adaxial surfaces of 5-week-old *B. napus* plants. The females were confined for 24 h under clip cages to produce nymphs, and the time of confinement was marked as the start of each bioassay. One newly produced nymph was retained per clip cage and all others were removed. The adult females were removed from the clip cage after laying nymphs so that only one nymph remained per clip cage. Eggs were not included in the study due to availability of only parthenogenically producing *B. brassicae* females during that time. Eggs were only available near the end of the cropping season.

The biological and life history attributes of the nymphs produced by treated females were monitored and timed according to the day of occurrence. The day when each of the treatment aphids produced its first nymph was marked as the pre-reproductive period. From that day forward, the additional nymphs were counted and removed regularly until the day each treatment aphid stopped reproducing, which was marked as its reproduction time. The effective fecundity, M_d, was calculated as the number of nymphs counted during a time equal to the pre-reproductive period [49,50]. However, the instar durations for nymphs were not calculated, as the different nymphal stages could not be identified easily.

The timed attributes above enabled calculations of r_{dp}, the ratio of aphid development period to the pre-reproductive period, M_d, and the mean number of young produced during the reproductive period, d [48]. The *B. brassicae* intrinsic rate of natural increase (r_m) was estimated using $r_m = 0.738\ (\ln M_d)/r_{dp}$. The *B. brassicae* relative growth rate (RGR) was estimated as $RGR = r_m/0.86$, and the aphid mean generation time as $T_d = r_{dp}/0.738$. Total adult progeny production and the numbers and percentages of progeny nymphs surviving to adulthood were also recorded as in Ahmed et al. [54], where percent progeny survival was determined as (progeny nymph survived per treatment/total progeny in that treatment) × 100. Progeny nymph mortality was calculated by subtracting the percent progeny survival from 100 percent. Observations were recorded daily until the completion of the experiment, as in [55].

For the insecticide treatment, only aphid survival was computed, as all aphids died before reproducing. If an insect either escaped or was accidentally squashed, it was replaced either with a new individual or the data were censored. The data were censored for Kaplan–Meier survival analyses in different treatments, and censored points were displayed with barbed inscriptions on the survival curves.

2.4. Data Analyses

Data were analyzed using GraphPad Prism Version 8.0.2 (263) (GraphPad Software Inc., San Diego, CA, USA). First, the measurements were subjected to tests of Gaussian distribution/normality (Shapiro–Wilk) and homoscedasticity (Bartlett) to determine if they fulfilled the normal distribution assumptions of ANOVA [56]. Data that satisfied these assumptions were analyzed using one-way ANOVA followed by Tukey's multiple comparison tests ($p < 0.05$). Otherwise, data were transformed or were subjected to the non-parametric Kruskal–Wallis test followed by Dunn's multiple comparison test [56]. The *B. brassicae* survival was estimated using Kaplan–Meier survival functions. Means of applied treatments were differentiated using the Mantel–Cox test ($p < 0.05$). Here, the censored data points were those where aphids remained either alive or escaped/crushed during the experiment [57].

Transformation with the log (Y) function was performed to decrease homoscedasticity of data for development period (AS-50, BS and ES), reproduction time (0 mM CA, AS-25, AS-50 and ES), and total progeny production for treatments with 0.5 mM SA, Cp, ES + Cp, BS + Cp and BS + Cp, and for progeny nymph survival (0 mM SA Cp, ES + Cp), as well as for M_d (ES, ES + Cp, BS + Cp), r_m (0.5 mM SA, 1 mM CA, AS-50, Cp, BS + Cp), generation time (1 mM CA, AS-50, BS, ES + Cp), and *RGR* (0.5 mM SA, 1 mM CA, AS-25, AS-50, ES, Cp, BS + Cp).

The measurements that followed a Gaussian (normal) distribution after transformation were analyzed using one-way ANOVA ($p < 0.05$). Post hoc comparisons of percent progeny survival were performed using non-parametric, Kruskal–Wallis tests [58].

3. Results

The *B. brassicae* survival and reproduction fitness attributes measured under the different treatments were determined and differences among the attributes were statistically analyzed in the IR, ND, and SAM sections below.

3.1. Induced Resistance (IR) Responses against Brevicoryne Brassicae

The Kaplan–Meier survival functions revealed significant differences ($\chi2 = 137.9$, df = 7, $p < 0.0001$) from the control for both SA and CA treatments (Figure 1). In addition, the pre-reproductive period was delayed significantly compared to the control treatment for both the SA and CA treatments ($F_{6, 63} = 81.85$, $p < 0.0001$). However, SA was more effective in delaying aphid development compared to CA treatments. The 1 mM SA concentrations delayed the development period by 30.08%, followed by 1 mM CA (10%), 0.5 mM SA (3%), and 0.5 mM CA (2%) (Figure 2a). The *B. brassicae* reproduction time was also reduced significantly compared to the control ($F_{6, 63} = 680.9$, $p < 0.0001$), and was reduced by the greatest amount under the 1 mM SA (30.63%) treatment, followed by 1 mM CA (22.07%). Intermediate concentrations (0.5 mM) reduced the aphid reproduction time by 8.26% (Figure 2b). Effective fecundity, M_d, was also significantly decreased compared to the control ($F_{6, 63} = 62.08$; $p < 0.0001$). Here, 1 mM SA was more efficient in reducing M_d up to 43%. Other treatments, 1 mM CA (32%), followed by 23% in 0.5 mM SA and 22% in 0.5 mM CA were significantly different from the control (Figure 2c).

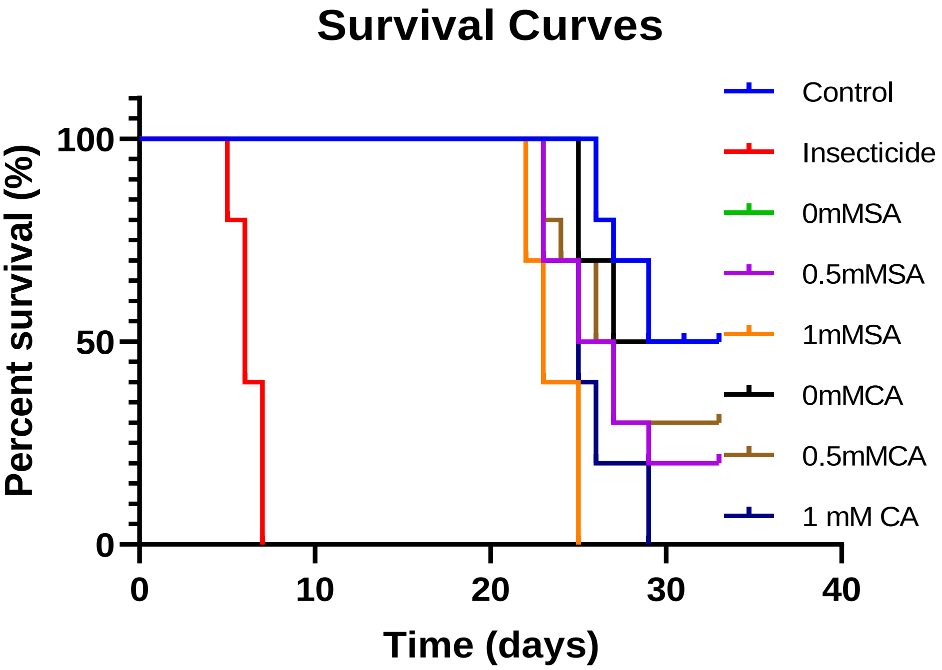

Figure 1. Kaplan–Meier survival functions of *Brevicoryne brassicae* under induced resistance treatments of SA (Salicylic Acid) and CA (Citric Acid) (Mantel–Cox test, $p < 0.0001$).

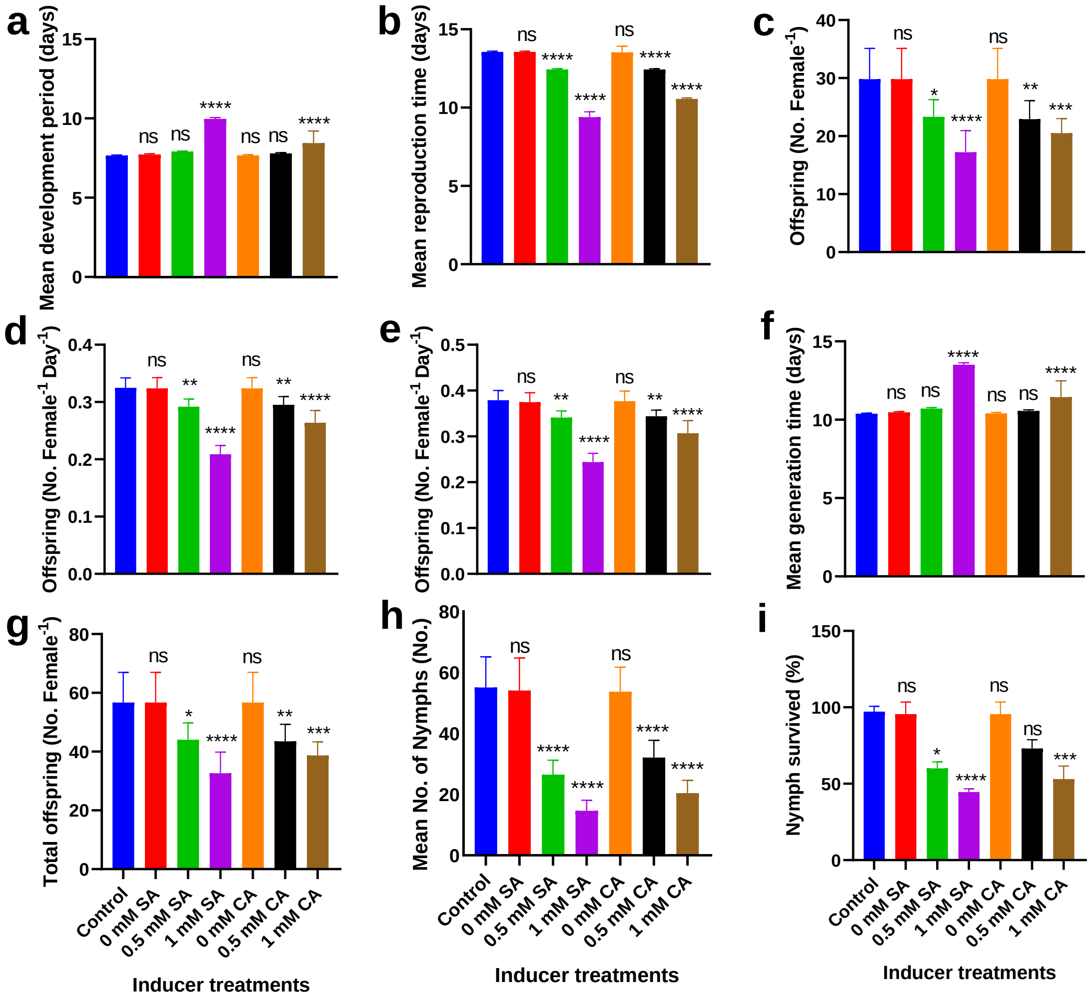

Figure 2. Effects of SA (Salicylic Acid) and CA (Citric Acid) on different fitness attributes of *Brevicoryne brassicae* (Mean ± SD, n = 10). (**a**) Development period (Days). (**b**) Reproduction time (Days). (**c**) Effective fecundity (No. Female Day^{-1}). (**d**) Intrinsic rate of natural increase (No. Female^{-1} Day^{-1}). (**e**) Relative growth rate (No. Female^{-1} Day^{-1}). (**f**) Generation time (Days). (**g**) Total progeny production (No. Female^{-1}). (**h**) Immature becoming adults (No Female.). (**i**) Progeny nymph survival (%). Statistical significance of P in post hoc tests: ns ≥ 0.05, * <0.05, ** <0.01, *** <0.001, **** <0.0001.

The intrinsic rate of natural increase, r_m, also displayed statistically significant differences from the control treatment ($F_{6,\,63} = 62.08$, $p < 0.0001$). The rates were lowest for 1 mM SA (37%) and 1 mM CA (20%). The 0.5 mM concentrations of SA and CA showed 11% and 10% reductions compared to the control (Figure 2d). Treatments tested under the induced resistance approach also influenced *RGR* significantly ($F_{6,\,63} = 58.82$; $p < 0.0001$), with decreases of 36% for 1 mM SA treatments, followed by lower decreases of 19% for 1 mM CA (Figure 2e). Mean generation times were also found to vary significantly from the control ($F_{6,\,63} = 81.86$; $p < 0.0001$) (Figure 2f).

Total *B. brassicae* progeny, progeny per adult, and progeny nymph survival were also affected by SA and CA treatments, with statistically significant differences from the control observed for total progeny ($F_{6,\,63} = 14.79$, $p < 0.0001$), progeny per adult ($F_{6,\,63} = 57.9$, $p < 0.0001$), and progeny nymph survival ($p < 0.0001$ and Kruskal–Wallis statistic = 60.891). Total progeny production was reduced significantly in the 1 mM SA treatment (43%), followed by 1 mM CA (32%), 0.5 mM CA (23%), and 0.5 mM SA (22%), compared to the control treatments (Figure 2g). Additionally, progeny nymph survival was significantly decreased under the 1 mM SA treatment (74%), followed by 1 mM CA (63%), 0.5 mM SA (52%), and 0.5 mM CA (42%), compared against the control treatments (Figure 2h). Percent progeny nymph survival decreased significantly by 55% in the 1 mM SA treatment, 47% in 1 mM CA, 39% in 0.5 mM SA, and 26.23% in 0.5 mM CA, compared with the control (Figure 2i). Similarly, percent nymph mortality increased to 55% in the 1 mM SA treatment, followed by 47% in 1 mM CA, 40% in 0.5 mM SA, and 27% in 0.05 mM CA, compared to 3% in the control (as the percentage mortality mathematically is 100%—percentage survival) treatments (Mantel-Cox test: $\chi^2 = 137.9$, df = 7, $p < 0.0001$). Insecticide treatment killed all *B. brassicae* aphids within 5–7 days of application (Figure 1). Decreases in survival were greatest at the highest rates of SA and CA treatments.

3.2. Nutrient Deterrence (ND) Responses against Brevicoryne Brassicae

The ANOVA followed by post hoc tests revealed significant differences among Si and AS treatments hindering *B. brassicae* fitness attributes compared to the control, including the development period ($F_{4, 45} = 9475$; $p < 0.0001$), reproduction time ($F_{4, 45} = 26.73$; $p < 0.0001$), and effective fecundity ($F_{4, 45} = 14.21$; $p < 0.0001$), r_m ($F_{4, 45} = 139.8$; $p < 0.0001$), *RGR* ($F_{4, 45} = 153.8$; $p < 0.0001$), and generation time ($F_{4, 45} = 9133$; $p < 0.0001$). Additionally, total progeny production ($F_{4, 45} = 14.17$; $p < 0.0001$), progeny nymph survival to adulthood ($F_{4, 45} = 53.61$; $p < 0.0001$), and percent progeny survival ($p < 0.0001$, Kruskal–Wallis statistic = 51.63) revealed statistically significant decreases from the control treatments.

The Si and AS treatments had significantly different Kaplan–Meier survival functions from the control (Mantel–Cox test: $\chi^2 = 102.9$, df = 5, $p < 0.0001$). Insecticide application killed all *B. brassicae* within 7 days of application; conversely, the 50 kg/ha dose of SI did not produce *B. brassicae* mortality until day 26. At day 33, 30% of aphids remained alive at the 50 kg/ha dose of AS. The moderate doses of both nutrients (25 kg/ha) were least effective and 30% of aphids still survived until the end of testing in these treatments (Figure 3).

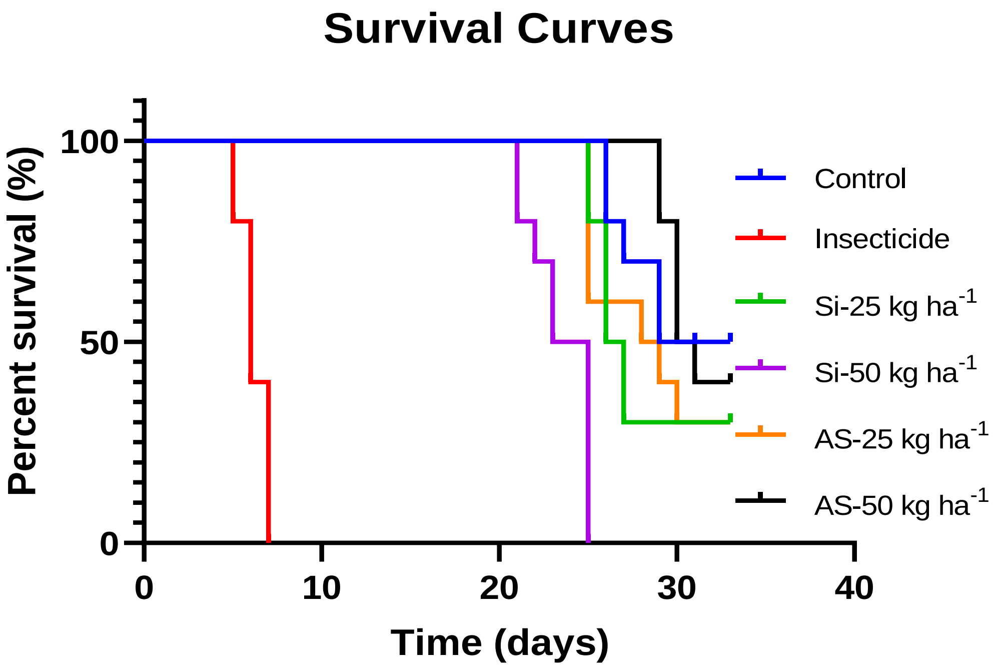

Figure 3. Kaplan–Meier survival functions of *Brevicoryne brassicae* under nutrient deterrence treatments of Si (Silicon) and AS (Ammonium Sulfate) (Mantel-Cox test, $p < 0.0001$).

The AS treatments were not significantly different from the control for the development or pre-reproductive period of *B. brassicae*, which reduced to 0.33% at 25 kg/ha and increased to 1.26% at the 50 kg/ha dose. In contrast, the developmental period was lengthened significantly in the Si treatment in proportion to the applied dose. Compared with the control, the 25 kg/ha dose of Si had lower effect (18%) on developmental time than the 50 kg/ha dose (22%) (Figure 4a). Reproduction time was reduced significantly by 16% and 5% compared with the control at the 25 kg/ha dose of Si and AS, respectively. Likewise, stronger reductions in response were observed for the 50 kg/ha dose of AS (24%) than from the same dose of Si, which reduced it up to 22% (Figure 4b). Statistically significant reductions in effective fecundity were observed relative to the control in Si treatments but not in AS treatments. The Si 50 kg/ha treatment reduced effective fecundity up to 40% compared to the control. The effective fecundities under other treatments were reduced by 22% at the Si 25 kg/ha dose compared to the control and by even lower percentages as the Si doses decreased (Figure 4c). The intrinsic rate of natural increase in Si treatments was also found to differ significantly from the control. The r_m decreased by 41% in the Si 50 kg/ha treatment and by 22% in the Si 25 kg/ha (Figure 4d).

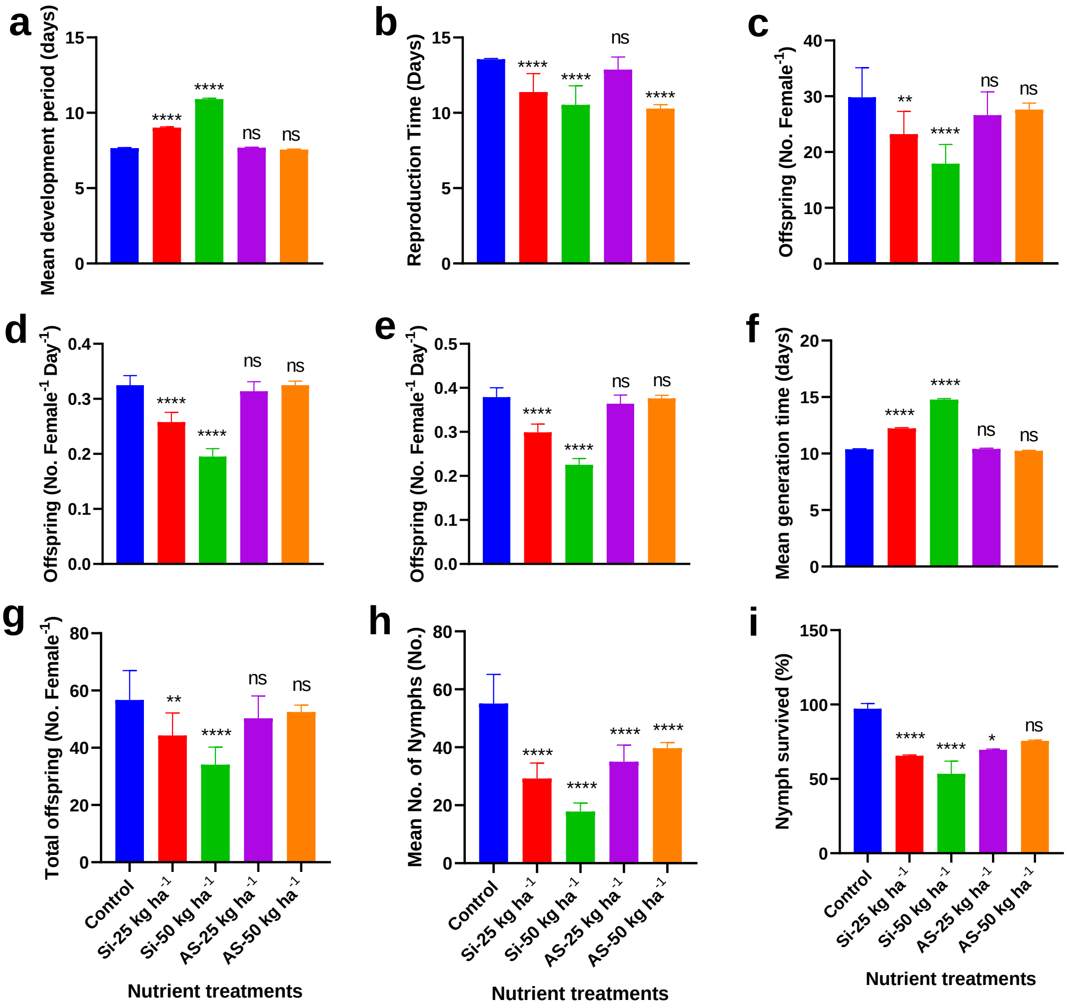

Figure 4. Effects of Si (Silicon) and AS (Ammonium Sulfate) on different fitness attributes of *Brevicoryne brassicae* compared to the control (Mean ± SD, $n = 10$). (**a**) Development period (Days). (**b**) Reproduction time (Days). (**c**) Effective fecundity (No. Female^{-1}). (**d**) Intrinsic rate of natural increase (No. Female^{-1} Day^{-1}). (**e**) Relative growth rate (No. Female^{-1} Day^{-1}). (**f**) Generation time (Days). (**g**) Total progeny production (No. Female^{-1}). (**h**) Progeny nymphs surviving to adulthoods (No. Female). (**i**) Percent progeny nymph survival (%). Statistical significance of P in post hoc tests: ns ≥ 0.05, * <0.05, ** <0.01 **** <0.0001.

The Si treatments significantly affected *B. brassicae RGR* and generation time relative to the control (Figure 4e,f). The treatment of Si 50 kg/ha reduced generation time by 42%, followed by 18% at the Si 25 kg/ha dose.

Total progeny production was also reduced in Si treatments compared with AS and the control. A progeny reduction of 40% compared to the control was observed for the 50 kg/ha dose of Si and 22% reduction at the 25 kg/ha dose. AS was slightly effective and reduced total progeny production 11% in the 25 kg/ha treatment compared to the control. However, doubling the dose to 50 kg/ha improved *B. brassicae* performance, and reduced total progeny only by 7% (Figure 4g). Nevertheless, the number of progeny that became adults was reduced by 36% and 28% at 25 and 50 kg/ha AS treatments. This fitness attribute was also reduced significantly in Si treatments compared to the control. At 25 kg/ha, Si showed a greater percentage reduction of progeny production than that of the same dose of AS (48%). Yet, Si produced a much greater effect at 50 kg/ha, to a 67% reduction (Figure 4h). In addition, progeny survival was reduced significantly under both doses of Si, up to 46% and 33%, and under the AS treatments, up to 30% and 24%, compared to the control (Figure 4i).

3.3. Soil Amendment (SAM) Treatments against Brevicoryne Brassicae

The Kaplan–Meier survival functions showed statistically significant differences among SAM treatments (Mantel-Cox test: $\chi 2 = 99.29$, df = 6, $p < 0.0001$). In the insecticide treatment, no *B. brassicae aphids* survived and all died within 7 days of application. Conversely, 20% and 50% aphids survived in ES and BS treatments, respectively, until day 33, the last day of observation. The Cp treatments resulted in 30% survival until day 33. About 40% survived until day 33 in the ES + Cp treatment. In the BS + Cp treatment only 10% remained alive until the last day, and aphid survival was 30% in the control treatment (Figure 5).

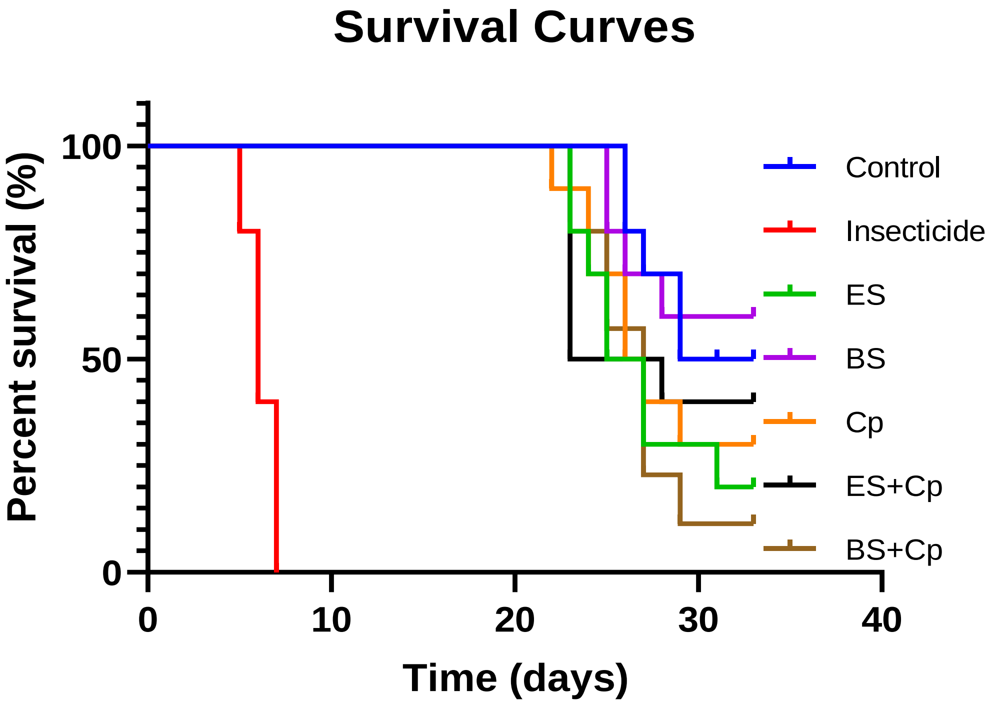

Figure 5. Kaplan–Meier survival functions of *Brevicoryne brassicae* under soil amendment (SAM) treatments of ES (Elemental Sulfur), BS (Bio Sulfur), and Cp (Compost) (Mantel–Cox test, $p < 0.0001$).

The developmental period changed significantly in the different SAM treatments compared to the control ($F_{5, 54} = 128.3$; $p < 0.0001$). BS shortened the development period by 13%, while ES and Cp lengthened it up to 15% and 7%, respectively. Adding Cp gave additional effect for BS but a negative effect for ES, when compared with the control. BS + Cp lengthened the developmental time by 19% (Figure 6a). Reproduction time was reduced by similar amounts with ES (16%) and Cp (19%) and the difference was statistically significant compared with the control ($F_{5, 54} = 33.81$; $p < 0.0001$). However, no additional benefits were observed from the BS treatment, which accelerated the aphid reproduction time by 4%. Integrated treatments of either ES or BS with the compost reduced the reproduction time by 17% and 27%, respectively, both of which were significant decreases from the control (Figure 6b).

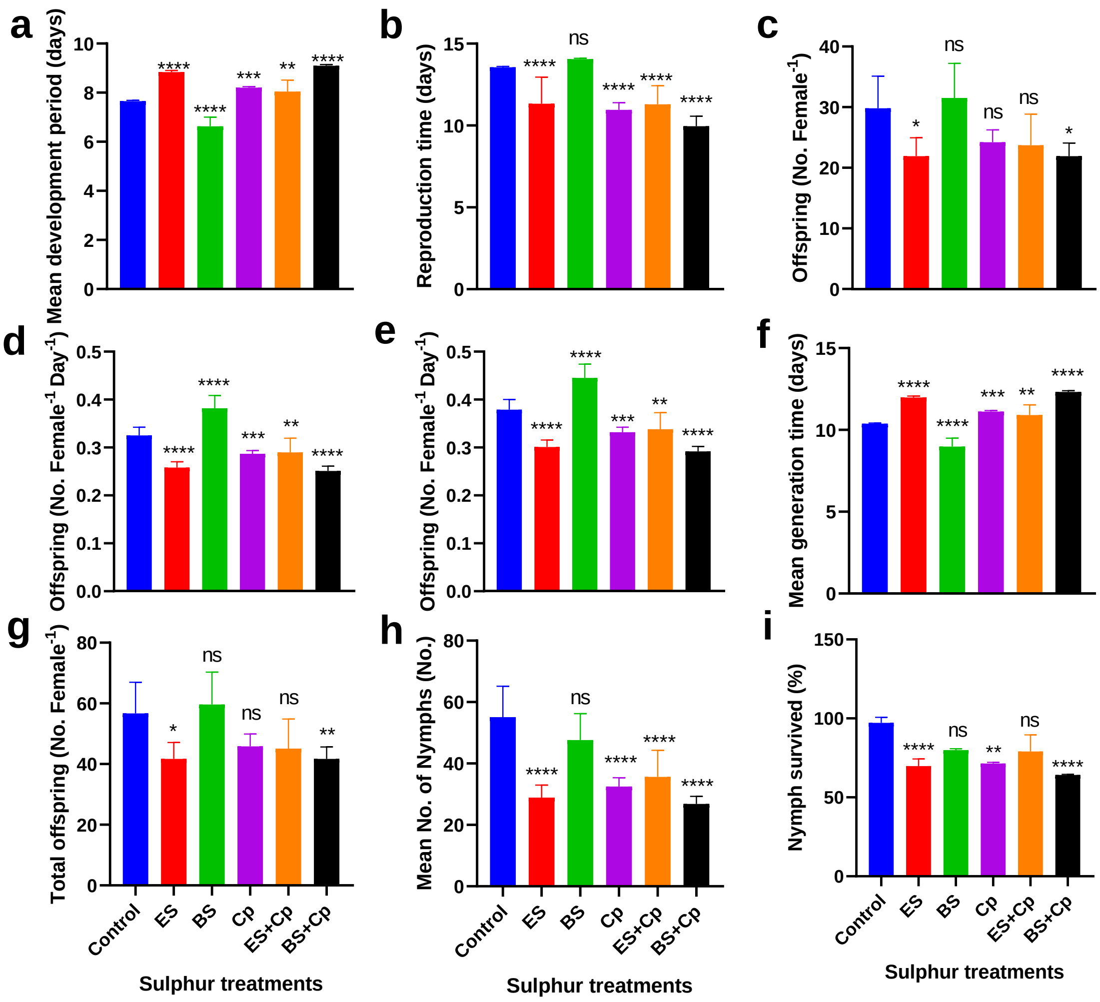

Figure 6. Effects of soil amendment treatments, ES (Elemental Sulfur), BS (Bio-Sulfur), and Cp (Compost) on different fitness attributes of *Brevicoryne brassicae* (Mean ± SD, $n = 10$). (**a**) Development period (Days). (**b**) Reproduction time (Days). (**c**) Effective fecundity (No. Female^{-1}). (**d**) Intrinsic rate of natural increase (No. Female^{-1} Day^{-1}). (**e**) Relative growth rate (No. Female^{-1} Day^{-1}). (**f**) Generation time (Days). (**g**) Total progeny production (No. Female^{-1}). (**h**) Progeny nymphs reaching adulthood (No. Female). (**i**) Progeny nymph survival (%). Statistical significance of p in post hoc tests: ns ≥ 0.05, * <0.05, ** <0.01, *** <0.001, **** <0.0001.

Effective fecundity in both ES and BS + Cp treatments was found to have a statistically significant difference from the control ($p < 0.0001$; Kruskal–Wallis statistic = 31.69) (Figure 6c). Several measurements of r_m in Figure 6d were also found to have statistically significant differences compared to the control ($F_{5,54} = 65.31$; $p < 0.0001$). Here, BS + Cp treatments were found to reduce the r_m up to 24% compared to the control. Interestingly, BS alone improved r_m by 16% (Figure 6d). Relative growth rates were also reduced significantly ($F_{5,54} = 65.47$; $p < 0.0001$) in the order: BS + Cp (23%) > ES (21%) > Cp (12%) (Figure 6e; however, in contrast to other treatments, BS increased aphid *RGR* by 17%. Generation time exhibited statistically significant differences between all treatments and the control ($F_{5,54} = 128.1$; $p < 0.0001$), increasing by 19% in the BS + Cp treatment and then by 15% under ES treatment. The use of Cp alone revealed a 7% increase in generation time, and with ES + Cp, there was a 5% reduction, compared with the control. BS decreased the generation time up to 13% against the control (Figure 6f). Total progeny production was significantly reduced compared to the control in the ES and BS + Cp treatments ($p < 0.0001$; Kruskal–Wallis statistic = 31.46). The effect of Cp alone (19% reduction) was not different from the effect in combined treatments with ES + Cp (20% reduction) but the effect was greater for BS + Cp (26% reduction), when compared to the control (Figure 6g).

The number of nymphs from progeny that survived to adulthood was also significantly reduced compared to the control under the SAM treatments of ES (47%) and Cp (41%) ($F_{5, 54} = 26.57$; $p < 0.0001$). However, the benefit obtained from BS (14% reduction) was not significant compared with the control treatment. When Cp was integrated with BS (BS + Cp), it reduced the percentage of progeny reaching adulthood by 51% compared to the control, both of which were significantly greater than the reduction in the ES + Cp treatment (35%), which was also a statistically significant reduction compared to the control (Figure 6h). Progeny percent survival of *B. brassicae* nymphs was reduced significantly in some but not all BS treatments compared to the control ($p < 0.0001$; Kruskal–Wallis statistic = 51.63). Survival was lowered by 27% in ES, followed by 28% in Cp and 20% in BS, compared to the control. No additional benefit was found from the integration of Cp into ES sulfur formulations (ES + Cp). However, BS + Cp reduced progeny survival significantly (35%) and by more than ES + Cp (20%), against the control (Figure 6i).

4. Discussion

Aphids are managed primarily through the application of synthetic insecticides. However, these have caused negative impacts such as insecticide resistance, toxic residues in food commodities, and harm to the environment, humans [10,11], and pollinators [13]. Therefore, alternative methods were addressed in this report, i.e., induced resistance, nutrient deterrence, and soil amendments [18,19,59]. The effectiveness of IR, ND, and SAM approaches have been assessed by examining their impacts on different bio-fitness parameters of *B. brassicae*. The parameters included survival curves, development or pre-reproductive periods, reproduction time, effective fecundity (M_d), intrinsic rate of natural increase (r_m), relative growth rate (RGR), generation time, total progeny production, number of progeny per adult, and percentage of progeny survival of nymphs.

4.1. Induced Resistance Treatments

The SA and CA affected *B. brassicae* survival and reproduction attributes compared with the control as observed for SA applications to canola in Khoshfarman-Borji et al. [24], see also Yali and Sattari-Nassab [46]. The SA was found to be more effective than CA in this study and higher dosages of both SA and CA were more effective resistance inducers than the control at different inducer doses, as in Afshan et al. [52] and Yali and Sattari-Nassab [46]. As expected in insecticide treatments, no aphids survived longer than 7 days unlike in the IR treatments, also observed by Ahmad et al. [60]. However, in a series of IR treatments at increasing dosages, *B. brassicae* first displayed decreased levels of fitness at moderate treatment levels compared to the control, and then increased levels of fitness at higher treatment doses in Nasab et al. [59].

In previous studies by Nasab et al. [59] and Khoshfarman-Borji et al. [24], SA delays *B. brassicae* development time and intrinsic rates of natural increase, r_m, as well as RGR. Our results from IR treatments were in agreement with such results. Similarly, Moreno-Delafuente et al. [18] found that, after SA treatment, total progeny production, progeny to adults, and progeny survival showed significant declines against the control treatment.

The occurrence of a significant decrease in *B. brassicae* survival and reproduction attributes after IR treatments has been ascribed to the activation of plant biochemical defense, e.g., phenolics, glucosinolates, and nutrients [46,61,62]. Several recent studies showed the direct impact of phenolics and glucosinolates on *Brassica* spp. in decreasing economic harm caused by *B. brassicae* and other aphids [24,46,59,62–64]. Improvements in plant growth, nutrient uptake, and chloroplast ultrastructure attributes under SA and CA treatments have also been linked to activation of plant biochemical defenses [55,61,65]. Afshan et al. [52] reported that *B. napus* antioxidant and other biochemical defenses were activated under the application of CA to increase defense against abiotic stress. More recent studies also confirmed a role of CA in promoting plant antioxidant activity under stress [66,67].

It has been suggested that reductions in insect survival, longevity, reproduction time, and total progeny production to counter *B. brassicae* infestation may be linked to the activation of phenolics [24]. Cooper et al. [68] revealed that application of SA analogue could induce R-gene-mediated resistance in susceptible tomato cultivars to decrease *Macrosiphum euphorbiae* (Hemiptera: Aphididae) performance.

Moreno-Delafuente et al. [18] suggested that induced resistance may contribute to the disruption of insect hervibory on *B. brassicae*. At 1 mM concentration, the SA analogue, BTH, efficiently delayed *Aphis gossypii* (Hemiptera: Aphididae) feeding time, with a significant increase in the time needed for stylets to reach phloem. Additionally, survival curves, pre-reproductive period/development time, effective fecundity, intrinsic rate of natural increase, population relative growth rate, and generation time were disturbed [18].

This report found reductions in IR, treatments on *B. brassicae* survival and reproduction fitness attributes, and contributes additional information that CA treatments can assist plants in defending against biotic stress. It should also be noted that many investigations which had described the bio-toxic impact of the IR approach only showed the effects of inducers on the exposed generation of a pest, and trans-generational impacts had not been considered. It was found in this study that trans-generational impacts also occur, including reductions in the number of progeny nymphs that became adults, their survival, and increases in progeny percent mortality.

4.2. Nutrient Deterrence Treatments

Both Si and AS were applied to canola at doses of 25 and 50 kg/ha to determine their responses against *B. brassicae*. Other researchers [19,30,32] have also considered applications of Si against aphids and other insect pests [19,30,32]. Because AS is a widely used fertilizer in different brassica crops [38,69], we considered its combined and separate effects for the first time in this report. Previously, Si and AS have been noted as providing important nutrients that could increase plant resistance, enabling the plants to withstand harm from insects feeding on the plants [30,33,70]. The Si treatment reduced aphid fitness most effectively at 50 kg/ha, whereas AS was more effective at 25 kg/ha. There was a significant delay in aphid development time and there were significant reductions in reproduction time and effective fecundity compared to the control. Additionally, the intrinsic rate of natural increase and the *RGR* also decreased significantly relative to the control.

A significant decline in *B. brassicae* biological and life history attributes under the ND treatments has been linked to the variations of nutrient contents in plants and insect food digestion [33,71]. The role of Si in inducing deterrence against aphids by activating plant resistance has been highlighted [30,33,70]. The deterrence partly involved a reduction in the structural integrity of aphid feeding organs and impairment of phloem intake ability but reductions in food digestion and assimilation may also play a role in deterrence [72,73].

Reductions in survival and other measures of insect fitness at increased levels of Si have also been reported previously [43,74]. The roles of plant defense biochemicals, e.g., glucosinolates and phenolics, causing either reduced food intake or less-efficient digestion have been documented as contributing to nutrient deterrence effects in different plants [75,76]. The ND treatments may activate either salicylic or jasmonic acid defense pathways as well [77,78].

The AS treatments contained sulfur nutrients which have been reported to play roles in plant defense [38,69]. Sulfur has a role in the synthesis of glucosinolates, which are major deterrent compounds produced by *Brassica* against aphids and other insects [35,36]. The AS may facilitate reductions in aphid fitness by enabling increases in such defense chemicals. However, at 50 kg/ha, the increasing effects of ammonia toxicity may be decreasing the effectiveness of increased AS against aphids [31,79]. Ammonia toxicity may lead to declines in synthesis of plant defensive compounds due to a higher level of NH_4+ ions. Another potential concern is that increasing doses of sulfur along with nitrogen have been reported to increase plant nitrogen uptake [31] and hence can increase the availability of amino acids for feeding insects [80]. Further studies are needed to resolve such concerns.

This study contributes additional support for applications of Si in pest management and the balanced usage of fertilizer to manage insect pests. Furthermore, it identifies a dose-dependent protective role of AS against *B. brassicae* that has not been studied previously. In association with IR, the ND approach also revealed trans-generation reductions in aphid fitness relative to the control.

4.3. Soil Amendment Treatments

Elemental sulfur (ES) and bio-sulfur (BS) formulations were studied alone and with compost (Cp) to assess their effectiveness against *B. brassicae* fitness attributes. Sulfur has been reported previously to increase oil content in *B. napus* [37,39], but studies have not been conducted to determine such impacts on aphid fitness attributes. In this study, the BS was not effective as a single treatment, as it increased rather than decreased the fitness of *B. brassicae* relative to the control. However, the use of ES + BS did reduce fitness relative to the control. The ES was expected to be effective alone, as it had a documented role of increasing plant glucosinolates and related defense chemicals [35,36]. Other researchers have reported that phosphorous and potassium negatively influence herbivory by aphids and other insects [81–83]; consequently, the Cp treatment was hypothesized to have the potential to reduce aphid fitness attributes through the inclusion of these elements in its soil amendment. Moreover, the supply of carbon and beneficial organisms from Cp also may improve plant health and photosynthesis [17,45,82]. The result that integrated treatments of both ES and BS with Cp, enhanced their effectiveness against *B. brassicae* may be due also to the presence of microbial communities in compost that help the plants indirectly to improve sulfur availability [84]. Additionally, the presence of N, P, K, and carbon in compost may also potentiate ES and BS oxidation [81,85,86]. Chaudhary et al. [7] reviewed various reports where the low availability of sulfur to plants resulted in reduced performance. The use of SAM may ameliorate such problems; therefore, combined use of sulfur with other amendments may augment the role of plant sulfur in insect control.

In this study, *B. brassicae* was restricted to feeding on plant leaf areas, as it was difficult to find a way to restrict it solely to plant stems. However, a more comprehensive understanding of the effects of these treatments on *B. brassicae* fitness may be obtained by conducting a similar study with aphids restricted to *B. napus* stems, as this insect is a more efficient feeder on stems and inflorescences than on leaves. The results of the study also suggest further investigation of plant defense pathways at molecular levels. This may involve exploration based on pathogenesis-related protein genes for salicylic acid defense, and proteinase inhibitor genes of the jasmonic acid plant defense pathway. Moreover, variation in biochemical defenses linked to reactive oxygen species generation and antioxidants production would also benefit from further evaluation. An associative study of electrical penetration graph (EPG) measurements should also be carried out to consider the impacts of different treatments on the mechanics of aphid feeding.

This study showed the potential of multiple alternative approaches to aphid management on canola that could be integrated into eco-friendly control treatments. However, in-depth studies on applied inducer/nutrient rates and their relevant compatibility for integration during different stages of crop growth remain to be assessed.

5. Conclusions

Plant resistance-activating treatments based on induced resistance, nutrient deterrence, and soil amendment treatments induced dosage-dependent decreases or increases in survival and reproductive fitness attributes of *B. brassicae* relative to the controls. The results of the study have potential value for eco-friendly integrated pest management of aphids and other insect pests on *B. napus* and other crops. However, further molecular investigations are needed to fully understand the physiological interactions underlying the results and develop *B. napus* crops more resistant to *B. brassicae* herbivory.

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