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Aphid-host plant interaction

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ABSTRACT

Black bean aphid, *Aphis fabae* (Homoptera; Aphididae) is a serious pest causing crop loss. Plant-aphid interaction is a dynamic system subjected to continual variation and changes. Host plants induce various biochemical and physical defense mechanisms due to aphid feeding. Aphids can overcome plant defenses by enzymatic adaptations and sequestering secondary metabolites produced by the plant within their bodies as a defense against their enemies. Many strategies were developed and evolved by aphids in order to overcome plant defense barriers which allowed them to feed, grow and reproduce on their host plants. This study aimed to aid in better understanding of the effect of altering host plant on specialist and generalist aphid fitness.The influence of plant defense on population development of *Aphis fabae* was also investigated. Analyses for insect enzymes were also demonstrated in addition to further biochemical studies on host plant defences. Generalists showed different ecological and enzymatic adaptations towards host plants than specialist *Aphis fabae*. The results were fully discussed in details.

Keywords: Insect-Plant Interaction; *Aphis fabae*; Specialists; Generalists; Enzymes; Secondary Metabolites

1. INTRODUCTION

Aphis fabae has a host-alternating life cycle [1], overwintering on its winter host, spindle (*Euonymus europaeus*) [2], and migrating in the spring to a wide variety of summer hosts that include bean, sugar and beet [3,4]. After settling on the summer host plant, the spring migrant aphid gives birth to wingless females (generalist wingless virginoparae) that undergo several generations of parthenogenetic viviparous reproduction. High population densities on the secondary host plant cause the production of specialist winged virginoparae [4], which migrate to other summer host plants to start new colonies.

The first activity of aphids is to determine whether a plant is suitable for them or not [5]. After landing on a plant, aphids ingest phloem sap from their hosts through narrow piercing-sucking mouthparts called stylets [5]. During probing, aphids' stylets form mechanical damage that may influence plant responses to infestation [6].

As a result of aphid stress on plants, plants possess a variety of biochemical and physical defense mechanisms that can deter or poison feeding insects on them [7,8]. Many plant secondary metabolites have direct toxic effects on a variety of herbivores and pathogens, known from studies *in vitro*, while other plant defenses appear to have indirect effects upon pests and pathogens, such as in attracting predators and inhibiting insect oviposition [9]. Secondary metabolites have antixenotic or antibiotic properties [10] and plant volatiles that repel PFIs (phloem feeding insects) or attract their natural enemies [11].

Special herbivores including aphids can overcome certain plant defenses by releasing certain enzymes such as mixed function oxidases (MFOs) group in order to detoxify harmful plant compounds by catalyzing oxidative reactions [12,13]. In addition, sequestering chemical toxins as secondary metabolites produced by the plant within their bodies to overcome plant defense barriers which allow them to feed, grow and reproduce on their host plants [14,15] and use as a defense against their own enemies [16].

Aphids that feed on plant sap have several enzymatic proteins as phenol oxidases, hydrolases, peroxidases, acetyl choline esterases, glucosidases and esterases [17]. Enzymatic studies reveal that generalist and specialist *Aphis fabae* have different enzymes according to their host plant. This study aims to aid in better understanding of the effect of altering host plant on specialist and generalist aphid fitness.

2. MATERIALS AND METHODS

2.1. Plants and Insect Rearing

Fresh plants or planted seeds for *Vicia fabae*, *Zea mays* and *Cynanchum acutum* were obtained approximately every two weeks from field and some aphids were transferred to the new plants in order to keep stock colony of aphids healthy. 15 plant pots were set up under the laboratory conditions for each experiment. Aphids from El-sharkya governorate were brought to supply this study. A stock culture of *Aphis fabae* was reared on tick bean seedlings (*Vicia faba* L.) in environmental cabinets at 15˚C - 20˚C and LD 16:8 h photoperiod [18]. After colony establishment for both specialist and generalist on the new plant, *Aphis fabae* population growth and different signs of infestation stress on host plants were monitored.

2.2. Aphid Growth Rate Experiments

The whole related experiments were conducted in a growth chamber under the 20˚C, 60% ± 10% R.H. and a photoperiod of 16:8 (L:D). In order to assess the development duration and survivorship of immature stages, birth weight , adult weight, number of molts and adult longevity, adult apterous aphids were randomly selected from the *Aphis fabae* stock and placed on the leaf surface inside the leaf Petri cages (9 cm in diameter 1.5 cm in height) using a fine-hair brush [19]. They were then allowed to produce nymphs for 24 hours period. After this time, the adults were omitted and only a cohort of three or four newly born nymphs retained together into each Petri cage [20]. These remaining nymphs were monitored daily in Cellulase cage [20] until reaching adult to assess developmental time and survivorship on all cultivars. The immature become adults, they were observed for reproduction and survival. The same experiment was carried out for winged alatae under specialist rearing conditions.

Five pots (replica) per entry, each containing one plant, were used in a randomized block design in the greenhouse conditions (no overcrowding—16L:8D intervals-20˚C temperature for generalist apterous *Aphis fabae*) and (overcrowding—>25˚C temperature—12L:12D for specilalist winged alatae). Each plant was covered with cellulase cage with large ventilation windows, covered with gauze.

Hundred fifty apterous *A. fabae* were distributed evenly over each of the plant trays giving a mean infestation of five *A. fabae* per plant. The Chi-squared test using SPSS procedure was used to compare the observed number of *A. fabae* per test entry, per pre-conditioning treatment, using a 4 × 4 contingency table.

2.3. Aphid Reproductivity Measurements

Daily maintenance was performed to keep the Petri dish habitat healthy. Adequate light is necessary, but we avoided direct sunlight as this may result in overheating.

Aphids were placed in sterile plastic Petri dishes, 25 individuals per each treatment. One feeding plant was added daily in Petri dishes during Experimental Petri dishes were provided with 20˚C temperature and 16 hours lightning [21]. Aphids were observed for 72 hours, the number of living and dead insects was noted in the protocol daily. In this regard, we selected and transferred only one newly emerged adult to another new leaf Petri cage. Mortality and the number of nymphs produced by the apterous aphid were recorded and the off springs discarded daily until the death of the adult. In this way we evaluated the fecundity of 21 - 25 adult aphids per each cultivar and the same experiment was carried out for winged alatae.

2.4. Biochemical Analysis of Aphid Enzymes

Insects were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST-2 mechanic-Preczyina, Poland). After homogenation, supernatants were kept in a deep freezer at –20˚C till use for biochemical assays. Double beam ultraviolet/visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

Insects were prepared for analysis by being homogenized in distilled water (50 mg/1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 5˚C in a refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer till use.

All expermints contained 3 - 4 replicates (insects homogenates), and the results of biochemical determinations were pooled from triplicate determinations. The results were analyzed by one-way analysis of variance (ANOVA) using costat statistical software (cohort software, Berkeley). When the ANOVA statistics were significant ($p < 0.01$), means were compared by the Duncan's multiple range test. .

2.4.1. Quantitative Determinations of Peroxidase

Peroxidase activity was determined according to Vetter *et al*. [22]. To the sample (220 µl), in which the color is to be formed, the following reagents are added: 1ml. of 1% o-phenylenediamine (in 95% ethyl alcohol, fresh every 4 hours) and 1 ml of 0.3% hydrogen peroxide (in distilled water).the reaction is allowed to proceed for 5 minutes at which time is stopped by adding 2 ml of saturated sodium bisulfite. The reagent blank for each sample is prepared by adding the dye, followed by the sulfite, and then the hydrogen peroxide. The enzyme is inhibited by the sulfite so that it is inactive when the hydrogen

peroxide is added.

The starch in the sample and the blank is flocculated by adding 25 ml of 95% ethyl alcohol. The starch suspension must be swirled continuously during addition of alcohol, so that good flocculation occurs. The samples were then centrifuged at approximately 3000 r.p.m. for 5 minutes [22]. The clear supernatant is decanted into a colorimeter tube and its absorbance recorded at 430 mμ. The colorimeter is set at 100% transmittance with the corresponding blank for each sample. The enzyme activity was expressed as the change in absorbance at 430 m (ΔOD_{430})/minute/gm fresh weight.

2.4.2. Phenoloxidase Activity

Phenoloxidase activity was determined according to a modification of Ishaaya (1971), in a reaction mixture consisting of 0.5 ml phosphate buffer (0.1 M, PH 7), 200 µl enzyme solution and 200 µl catechol solution (2%). Prior to the initiation of the reaction, the substrate and other ingredients of the reaction mixture were separately incubated at the optimum temperature of the reaction (25˚C). Enzyme reaction was initiated by adding catechol solution. Then after exactly 1 min, the optical density was determined. Zero adjustment was against sample blank. The phenol oxidase activity was determined as O.D. units × 10^3 at an absorbance of 405 nm [23].

2.4.3. B-Glucosidase Activity

β-glucosidase activity was measured by assaying glucose liberated by enzymatic hydrolysis of salicin as described by Lindorth (1988). One ml of the reaction mixture consisted of 200 µl enzyme solution, 0.1 M phosphate buffer (pH 6) and 50 µ mole salicin. Mixtures were incubated at 35˚C for 30 min, then boil for 2 min to stop the reaction [24]. Glucose that liberated by salicin hydrolysis was measured enzymatically by a glucose kit (sigma kit, sigma co.). Optical densities were measured against blank containing boiling enzyme. Enzyme activity was expressed as ug glucose liberated/min/mg protein.

2.4.4. Esterases Activity

Alpha esterases (*α*-esterases) and beta esterases (*β*-esterases) were determined according to Van Asperen (1962) using *α*-naphthyl acetate or *β*-naphthyl acetate as substrates, respectively. The reaction mixture consisted of 5 ml substrate solution (3×10^{-4} M *α*- or *β*-naphthylace-tate, 1% acetone and 0.1 M phosphate buffer, pH 7) and 20 µl of larval homogenate.

The mixture was incubated for exactly 15 min at 27˚C, then 1 ml of diazoblue color reagent (prepared by mixing 2 parts of 1% diazoblue B and 5 parts of 5% sodium lauryl sulphate) was added. The developed color was read at 600 or 555 nm for *α*- and *β*-naphthol produced from hydrolysis of the substrate, respectively [25].

α-and *β*-naphthol standard curves were prepared by dissolving 20 mg *α*-or *β*-naphthol in 100 ml phosphate buffer, pH7 (stock solution). Ten milliliters of stock solution were diluted up to 100 ml by the buffer. Aliquots of 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml and 1.6 ml of diluted solution (equal to 2 µg, 4 µg, 8 µg, 16 µg and 32 µg naphthol) were pipetted into test tubes and completed to 5 ml by phosphate buffer. One milliliter of diazoblue reagent was added and developed color was measured as mentioned before.

2.4.5. Acetyl Cholinesterase Activity

Ach-E (acetyl cholinesterase) activity was measured according to the method described by Simpson *et al*. [26], using acetylcholine bromide (AchBr) as substrate. The reaction mixture contained 200 µl enzyme solution, 0.5 ml 0.067 M phosphate buffer (pH7) and 0.5 ml AchBr (3mM). The test tubes were incubated at 37˚C for exactly 30 min. 1 ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes. Then 0.5 ml of Hcl (1 part of conc. Hcl and 2 parts of ΔH_2O) was added.

The mixture shaken vigorously and allowed to stand for 2 min. 0.5 ml of ferric chloride solution (0.9 M $Fecl_3$ in 0.1 M Hcl) was added and mixed well [26]. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

3. RESULTS

3.1. Ecological Studies

3.1.1. Aphid Fitness

♦ Mortality

Significant variation in net mortality rate of generalist *A. fabae* was identified among various host plants (x^2 = 15.94; d.f. =2; P < 0.0003). The generalist aphids reared on *Vicia fabae* had the lowest mortality than those on *Maize* that had the highest mortality while on *Toxic spindle* mortality was mild (**Figure 1(a)** and **Table 1**). The mortality of specialist of *A. fabae* indicated to be significantly different (x^2 = 15.18; d.f. =2; P < 0.0005) with highest mortality on *Zea mays* and *vicia fabae*, respectively but lowest mortality on *Cynanchum acutum*.

♦ Fecundity

There was significant differences in generalist *A. fabae* number of offspring observed on three tested host plants (x^2 = 1067.22; d.f. = 2; P < 0.0001). The mean numbers of offspring per aphid were recorded in **Figure 2(a)** and **Table 1** that illustrates the highest reproductivity of generalist on *V. fabae*. Similarly, the number of specialist *A. fabae* offspring was significantly different (x^2= 68.13;

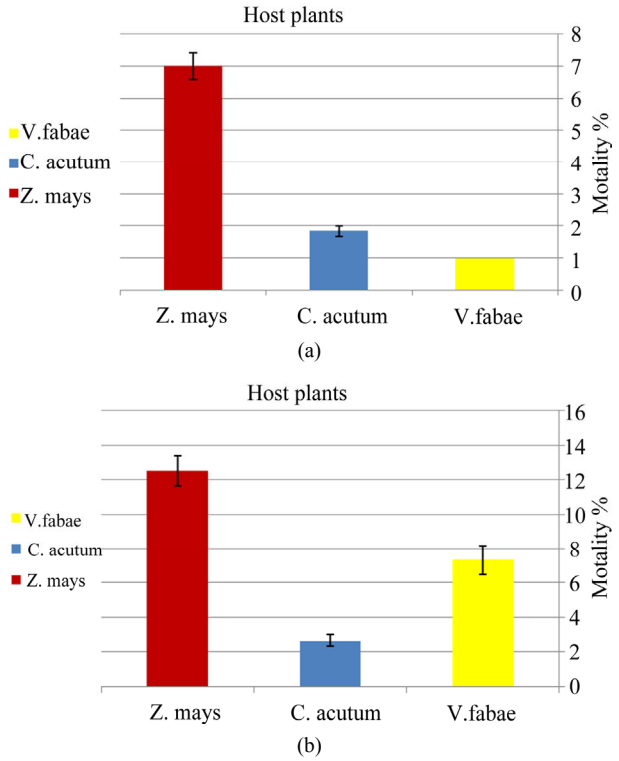

Figure 1. Difference in mortality % of generalist *Aphis fabae* (a) and specialist *Aphis fabae* (b) on different host plants (mean value ± S.E.; (P ≤ 0.05)).

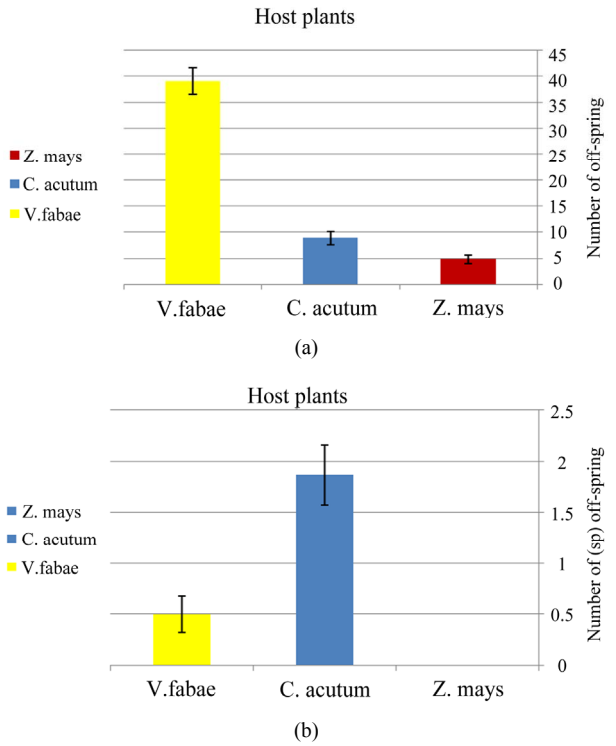

Figure 2. Reproductivity (number of off spring) of generalist *Aphis fabae* (a) and specialist *Aphis fabae* (b) on different host plants under laboratory conditions (mean value ± S.E.; P ≤ 0.05).

Table 1. Effect of altering host plants on generalist (G) *A. fabae* fecundity (number of off-spring along life span), mortality, growth rate (G.R), Birth weight (B.W) (nymph length and width after birth), Adult weigtht (A.W) (adult length and width after maturity), number of mature aphid (M) and developmental time (DT).

parameters	Host plants		
	V. fabae	*C. acutum*	*Z. mays*
	(G)	(G)	(G)
Fecundity	39.12 ± 2.57	8.80 ± 1.30	4.84 ± 0.82
Mortality	1.10 ± 0.00	1.83 ± 0.16	7.00 ± 0.80
G.R	24.57 ± 1.02	20.28 ± 1.54	0.85 ± 0.28
B.W (L)μ	76.80 ± 4.74	76.6 ± 5.41	42.15 ± 2.07
B.W(W)μ	43.85 ± 2.84	42.45 ± 2.38	23.05 ± 1.25
A.W (L) μ	113.68 ± 8.10	67.31 ± 4.79	33.40 ± 4.44
A.W(W) μ	67.26 ± 5.04	40.21 ± 2.16	20.3 ± 2.65
M	5.00 ± 0.33	3.25 ± 0.39	1.5 ± 0.23
DT	11.90 ± 0.37	4.15 ± 0.36	2.83 ± 0.16

Data represents the mean value ± S.E. from 25 aphid/group with significance difference between the three different host plants, using Chisquare test (P ≤ 0.05).

d.f. = 2; P < 0.0001) among cultivars with highest number on *Cynanchum acutum* than on *vicia fabae* but no offspring on *Zea mays* (**Figure 2(b)** and **Table 2**).

3.1.2. Growth Rate

The laboratory experiments were conducted in a growth chambers for the three host plants indicating that there was significant difference between the measured parameters on generalist and specialist *Aphis fabae*.

♦ **Developmental time**

The development time of generalist immature stages (time from birth until beginning first reproduction) of *A. fabae* varied significantly among the three examined host plants (x^2 = 9.10; d.f. = 2; P < 0.01). The mean number of developmental time ranged from 11.9 on *Vicia fabae*, 4.15 on *Cynanchum acutum* and 2.83 days on *Zea mays* (**Figure 3(a)** and **Table 1**). The table also demonstrates that the highest number of mature aphid generalist was on bean (x^2= 7.68; d.f. = 2; P < 0.02) (**Figure 4(a)** and **Table 1**).

Specialist *A. fabae* immature stages reach maturity on *Cynanchum acutum* in 10 days slower than other host plants with significance difference (x^2 = 7.6; d.f. = 2; P < 0.02) (**Figure 3(b)** and **Table 2**). The number of specialist *A.fabae* that reached maturity was on *Cynanchum acutum* higher than number on *Zea mays* and *vicia fabae*

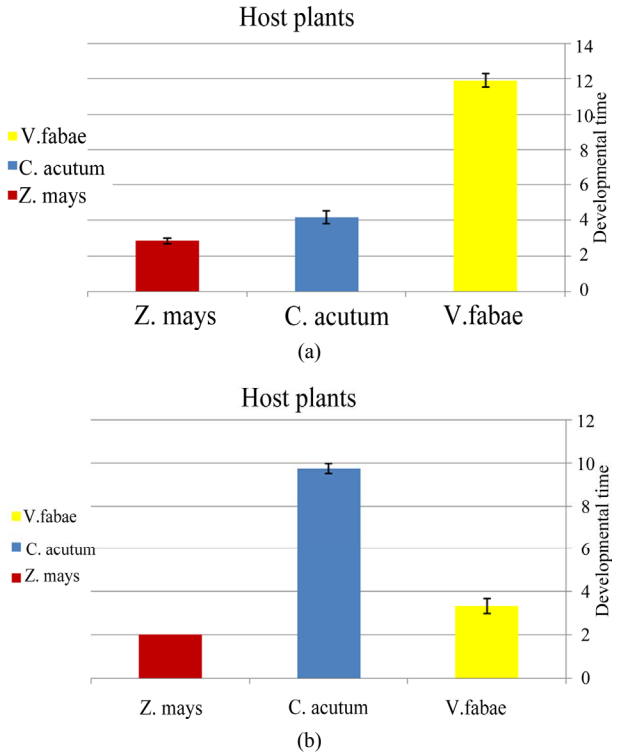

Figure 3. (a) Difference between generalist developmental time *of Aphis fabae* (time from birth until beginning first reproduction) and specialist *Aphis fabae*; (b) on different host plants (mean value ± S.E.; P ≤ 0.05).

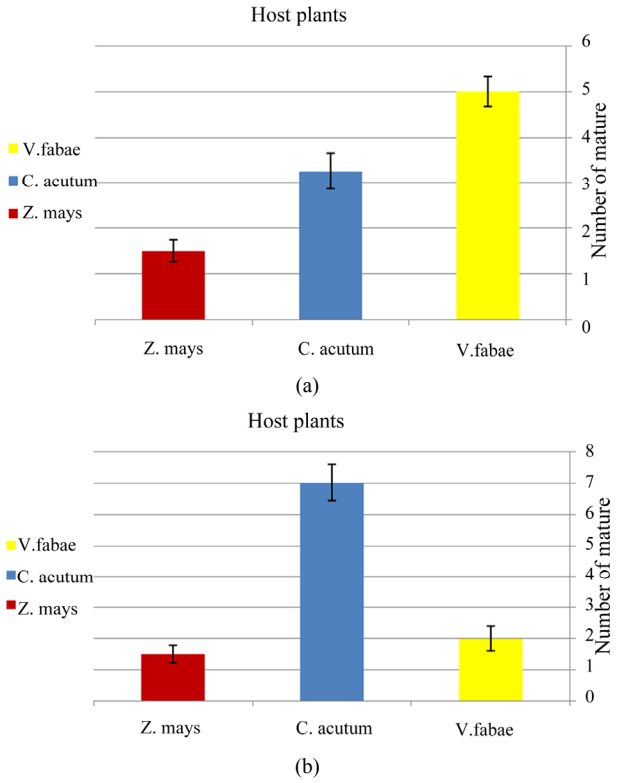

Figure 4. Difference between number of mature individuals of generalist *Aphis fabae*/days (a) and specialist *Aphis fabae* (b) on different host plants (mean value ± S.E.; P ≤ 0.05).

Table 2. Effect of altering host plants on specialist (Sp) *A.fabae* fecundity (number of off-spring along life span), mortality, growth rate (G.R), Birth weight(B.W) (nymph length and width after birth), Adult weigtht (A.W) (adult length and width after maturity), number of mature aphid (M) and developmental time (DT).

parameters	Host plants		
	V. fabae	*C. acutum*	*Z. mays*
	(Sp)	(Sp)	(Sp)
Fecundity	0.5 ± 0.17	1.86 ± 0.28	0.00 ± 0.00
Mortality	7.33 ± 0.84	2.66 ± 0.33	12.50 ± 0.86
G.R	2.66 ± 0.23	4.60 ± 0.36	1.5 ± 0.28
B.W(L)µ	74.25 ± 3.16	82.60 ± 3.99	46.25 ± 0.69
B.W (W)µ	41.25 ± 2.03	45.60 ± 1.79	24.00 ± 0.99
A.W(L)µ	61.75 ± 3.61	120.00 ± 2.10	28.75 ± 2.61
A.W(W)µ	37.75 ± 1.83	59.40 ± 4.02	18.00 ± 1.37
M	2.00 ± 0.40	7.00 ± 0.57	1.5 ± 0.28
DT	3.00 ± 0.33	10.00 ± 0.24	2.00 ± 0.00

Data represents the mean value ± S.E. from 25 aphid/group with significance difference between the three different host plants, using Chi-square test (P ≤ 0.05).

respectively "**Figure 4(b)**" and "**Table 2**".

♦ **Survival of nymphal stages**

Percentage of specialist survivorship varied from lowest on *Zea mays* to highest on *Cynanchum acutum* (**Figure 5(b)** and **Table 2**) (x^2 = 19.12; d.f. = 2; P < 0.0001). The population growth of generalist (number of offspring from nymph to adult until death) was dissimilar on three different cultivars significantly (x^2 = 146.73; d.f. = 2; P < 0.0001) (**Figure 5(a)** and **Table 1**).

♦ **Birth weight and adult weight**

The comparison between aphid generalist growth rate on different host plants shows that the highest growth rate was on *Vicia fabae* and lowest on *maize*. This result was confirmed by the experiment on the aphid size on different host plants which indicate that generalist aphid reared on bean has the largest size (**Figure 6(a)** and **Table 1**) (x^2 = 176.79; d.f. = 2; P < 0.0001 for length and x^2 = 68.1; d.f. = 2; P < 0.0001 for width) in contrast with specialist aphid reared on *C. acutum* that has the largest size with "(x^2 = 53.10; d.f. = 2; P < 0.0001 for length and x^2 = 12.18; d.f. = 2; P < 0.002 for width) (**Figure 6(b)** and **Table 2**) .

3.2. Biochemical Studies

3.2.1. Peroxidase Level

Enzymes analysis revealed that there were high sig-

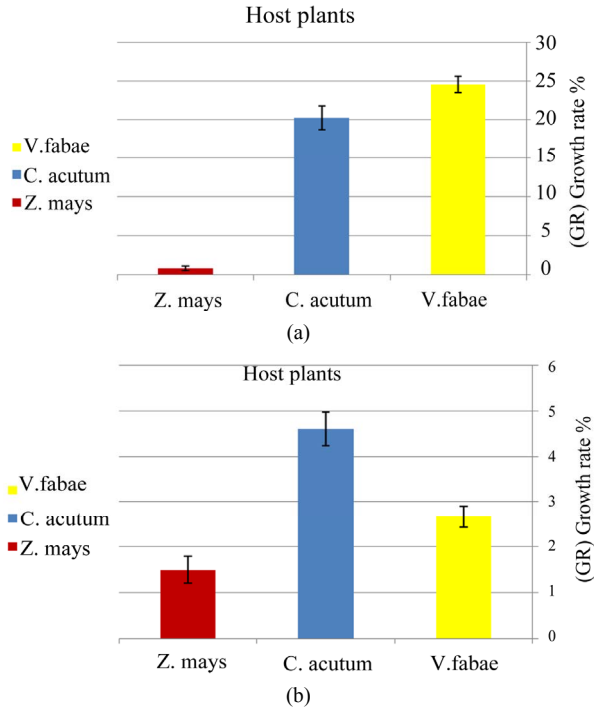

Figure 5. (a) Difference between population growth rate (G.R) (number of offspring from nymph to adult until death) of generalist and specialist *Aphis fabae* (b) on different host plants (mean value ± S.E.; ($P \leq 0.05$)).

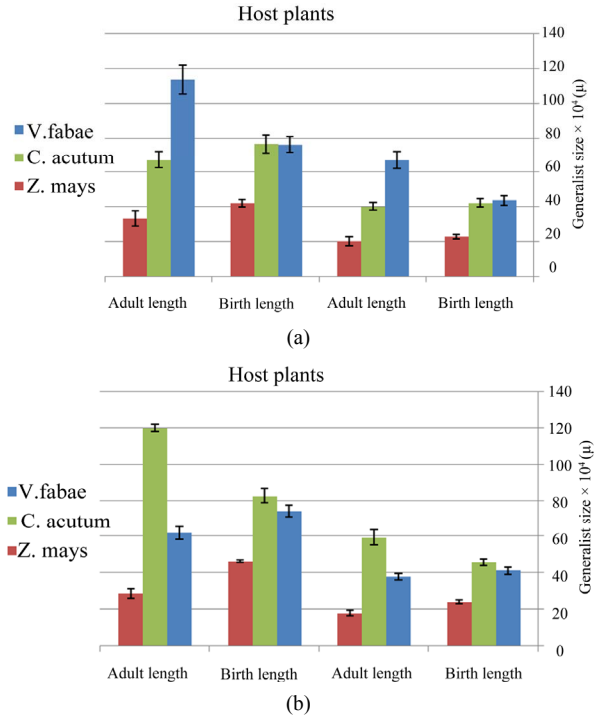

Figure 6. (a) Difference between size of generalist *Aphis fabae* and specialist (b) on different host plants (Birth weight (B.W) (nymph length and width after birth), Adult weight (A.W) (adult length and width after maturity)) (mean value ± S.E.; ($P \leq 0.05$)).

nificant differences ($P \leq 0.001$) in Peroxidase activity of generalist *A. fabae* on the three different host plants. The highest elevation in Peroxidase activity was recorded on *V. fabae* with ($F_{(3,8)} = 377.12$, ($P \leq 0.001$) (**Figure 7** and **Table 3**). Specialist Peroxidase level shows increase on *C. acutum* over that on *vicia fabae* and *Zea mays* the difference was highly significant ($F_{(3,8)} = 187.80$, ($P \leq 0.0001$) (**Figure 8** and **Table 4**).

3.2.2. Phenol Oxidase (PO) Level

Data represented in **Figure 7** and **Table 3** illustrates that there were alterations in PO level with altering host plants for both generalist and specialist *A. fabae*. There was decline in generalist PO level on *Zea mays* but increased on *V. fabae* than *on C. acutum* with high significant difference among the three host plants ($F_{(3,8)} = 47.05$, ($P \leq 0.0002$). In the specialist group on both *C. acutum* and *Zea mays* plants results showed high PO enzyme activities than on *V. fabae* (**Figure 8** and **Table**

Table 3. Effect of altering host plants on generalist (G) A. fabae enzymes level (Peroxidase, Phenol-oxidase, Ach-E, Esterase, *β*-glucosidase).

parameters	Host plants		
	V. fabae	*C. acutum*	*Z. mays*
	(G)	(G)	(G)
Peroxidase	201.33 ± 5.16	55.93 ± 2.05	29.16 ± 1.78
Phenol oxidase	37.56 ± 1.79	24.83 ± 1.00	13.35 ± 0.64
Ach-E	9.47 ± 0.23	9.74 ± 0.26	10.1 ± 0.17
Esterase	93.76 ± 2.57	62.85 ± 2.91	57.6 ± 2.69
β-glucosidase	23.11 ± 0.43	7.59 ± 0.36	3.95 ± 0.21

Data represents the mean value ± S.E. of aphid enzymes/group with significance difference between generalist enzymes level on three different host plants, using Chi-square test ($P \leq 0.05$).

Table 4. Table Effect of altering host plants on generalist (G) *A. fabae* enzymes level (Peroxidase , Phenol-oxidase, Ach-E, Esterase, *β*-glucosidase).

parameters	Host plants		
	V. fabae	*C. acutum*	*Z. mays*
	(Sp)	(Sp)	(Sp)
Peroxidase	136.83 ± 3.12	208.66 ± 6.48	41.42 ± 2.09
Phenol oxidase	5.94 ± 0.29	28.53 ± 1.06	25.34 ± 0.39
Ach-E	8.46 ± 0.23	5.32 ± 0.11	11.8 ± 0.10
Esterase	56.66 ± 1.30	64.98 ± 2.50	45.27 ± 2.08
β-glucosidase	8.81 ± 0.39	7.95 ± 0.18	11.67 ± 0.56

Data represents the mean value ± S.E. of aphid enzymes/group with significance difference between specialist enzymes level on three different host plants, using Chi-square test ($P \leq 0.05$).

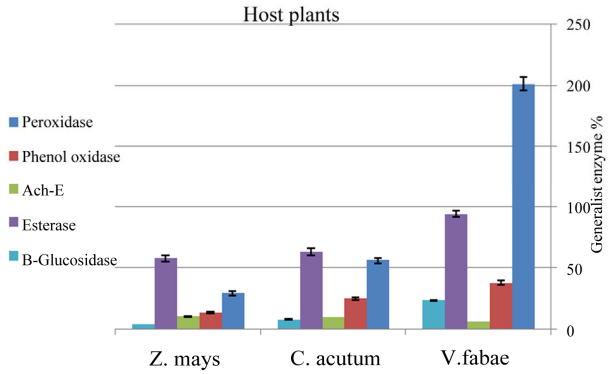

Figure 7. Difference between generalist *Aphis fabae* enzymes level on three different host plants (mean value ± S.E.; ($P \leq 0.05$)).

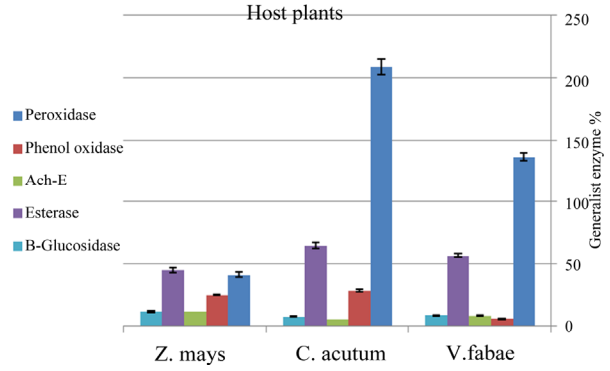

Figure 8. Difference between specialist *A. fabae* enzymes level on three different host plants (mean value ± S.E.; ($P \leq 0.05$)).

4) indicating high significance difference ($F_{(3,8)} = 161.89$, $P \leq 0.0001$).

3.2.3. Acetyl-Choline Esterase (Ach-E) Level

Generalist aphids Infested resistant *Zea mays* plants induced significant increase in Ach-E activity than in susceptible *C. acutum* and *V. fabae* plants ($F_{(3,8)} = 70.79$, ($P \leq 0.0001$) (**Figure 7** and **Table 3**). All specialist aphids shows Ach-E enzyme activities that were detected in low levels on *C. acutum* and *V. fabae* plants with mean value 5.32 and 8.46 respectively, except that on *Zea mays* that is the highest with mean value 11.80 (**Figure 8** and **Table 4**) ($F_{(3,8)} = 188.86$, $P \leq 0.0001$).

3.2.4. Esterase (E) Level

Aphid esterases content showed significant change with altering host plants. Highly significant increase were recorded in generalist esterases on *V. fabae* while *on C. acutum* induced mild esterase content than on *Zea* (**Table 3**). There were significant differences in total specialist esterses contents among different host plants (**Figure 8** and **Table 4**) ($F_{(3,8)} = 11.92$, $P \leq 0.008$). Aphids reared on *C. acutum* induced more E than on *V. fabae* and *Zea mays* respectively.

3.2.5. *β*-Glucosidase (*β*-G) Level

The results of *β*-Glucosidase enzyme assays supported the results of whole-aphid assays representing that (*β*-G) activity (**Figure 7** and **Table 3**) was highly detected in *generalist A. fabae* reared on *V. fabae* than on *C. acutum,* but in low content for those on *Zea mays* showing high significance difference ($F_{(3\ 8)} = 255.31$, $P \leq 0.0001$). There were slightly reductions in specialists (*β*-G) content reared on *C. acutum* than those reared on *V. fabae* and *Zea mays* with highest value (**Figure 8** and **Table 4**) that vary significantly ($F_{(3,8)} = 14.10$, $P \leq 0.005$).

4. DISCUSSION

The black bean aphid; *Aphis fabae* (Homoptera; Aphididae) attacks a large number of host species from many plant families such as Leguminoseae and Chnopodiaceae as well as a quantity of weeds as secondary hosts [27,28]. The goal of our study was to investigate *A. fabae* population growth and fitness on three different host plants. The study clearly showed that *V. fabae* was the most suitable host for apterous aphid *A. fabae* fecundity and longevity due to including the best host quality [29], this is in agreement with [30,31].

The present study revealed that significant induction in the generalist *A. fabae* reproductivity was noticed on *V. fabae*, in addition to significant reductions in the number of specialist *A. fabae* offspring likewise [32] study on *A. fabae* populations indicating that all measured fitness indices are higher on broad bean.

Generalist aphids can cope with induced plant resistance [33] which results in facilitated settling and thus in increased population growth in agreement with [34] on *Myzus persicae* and [35] demonstrated that *Megoura viciae* [Buckton) aphids are able to feed over longer periods on *Vicia faba* that was confirmed within our data. A similar effect was not observed for *M. persicae* on *Arabidopsis thaliana* resistance 12 h after infestation [36].

The represented data show that aphid fecundity on *C. acutum* represents mild number of generalist offspring than number on *V. fabae* that may be due to the plant *C. acutum* containing toxic latex and chemical compounds as terpenes, alkaloids and glucosinolates present in dicotyledones families [37] that affect both specialist/generalist aphid species causing changes of reproductive rates, development and performance that are in agreement with [38-40] studies.

Zea mays were the least suitable plant for the development of black bean aphid, it may be due to the fact that the plant containing toxins can reduce the infestation of aphids [28] which are in agreement with [31].

Specialist immature survivorship and growth rate varied from lowest on *Zea mays* as documented in our

data and supported by Ogenga [89] showing that maize decrease *A. fabae* infestations to the highest on *Cynanchum acutum* that agrees with [41] study on specialist *Acyrthosiphon kondoi* on *M. truncatula* Jester and Jemalong indicating that specialist and generalist *A. fabae* infestations increase according to host quality as some chemicals deter alatae from settling and antibiosis (reduced longevity, growth, and fecundity) as in [41-43]. [17] also investigated the ability of specialist *Brevicoryne brassicae* to sequester toxic glucosinolates from their host plants specificity in plant molecular, biochemical, and physiological responses to insects which are observed frequently in volatile production [17] act directly to decrease fecundity, enhance or deter feeding, that clearly explains the study results .

Generalist *A. fabae* reared on *Vicia faba* and *C. acutum* inducing 8% and 44% mortality respectively with highest immature survival while on resistant *Z. mays* showing 84% mortality with lowest immature survival since generalists tended to respond to a large array of different plant chemicals and proteins [44-46] that agree with study of Hunter *et al*. [47] and it was shown by [48] only due to generalist feeding strategy that were able to grow and develop on a variety of host plant species [49]. Specialist *A. fabae* showed highest mortality on both *Vicia faba* and *Z. mays* with 88% and 100%, respectively and lower mortality 16% on *C. acutum* with highest immature survival. Specialist insect hosting only on a few related plant species might be expected to have a more efficient form of adaptation, either involving the production of large quantities of an enzyme to detoxify their food, or evolve storage mechanisms [50-52]. Shen *et al*. [53] study revealed that Maize contains both indol and terpene volatiles in addition to having less favorable PH and nutrient content for aphid than *V. fabae* as [54] .

The present study indicated that the development time of generalist on *V. fabae* was longer with higher birth weight and adult weight than the specialist aphids developed on *C. acutum* with reduced birth weight and adult weight as delay in adult emergence may be correlated to ability for prolonged feeding on phloem sieve elements keeping these cells alive and their sieve plate pores open by preventing coagulation of phloem or even days from a single sieve element [55] and proteins [p-proteins] [56] that allow aphids ingest phloem sap [57] and their ability to adapt feeding strategies and avoid or deter many plant defenses.

Aphids avoid plant chemicals and therefore, do generate detoxification enzymes and reduce total glucosinolate levels [44-46]. A reduction in glucosinolate levels creates a more insect-friendly environment for generalists, which are repelled by glucosinolates. However, reduced glucosinolates may be advantageous for specialist aphids that are attracted to and utilize these compounds for their own defense [58]. [17] investigated the ability of specialist *Brevicoryne brassicae* to sequester toxic glucosinolates from their host plants.

Specialist herbivores adapted to plant chemical defense developing mechanisms. These insects frequently detoxify or sequester plant defense compounds [59] and, sometimes, they result in protection against parasitoids and predators being used as toxic or unpalatable at defense [15,60]. Findings in our results indicated insects release antioxidant enzymes after aphid feeding to detoxify these potentially dangerous reactive secondary metabolites. Generalist *A. fabae* enzymes level infesting *V. fabae* increased in Peroxidase and Phenoloxidases activity than others on other non host plants that confirmed by [61]. The reviewed current aphid saliva protein components show some contradictions, not only between species but also within aphid species [62-66] that investigate the variation in enzymes level of both specialist and generalist *A. fabae*.

The signals responsible for the activation of plant defenses to aphid feeding are not only mechanical, but also chemical, through the action of particular enzymes commonly called elicitors which are present in saliva [67]. Goggin (2007) [68] that explain the high level of peroxidase enzyme in generalist aphids feeding on *V. fabae* as a susceptible host plant than *C. Acutum* and *Z.mays* respectively and specialist *A. fabae* on *C. acutum*. [61,69-71] revealed that The salivary enzymes of aphids are similar to enzymes with identical functions in plants, *i.e.* oxidases and enzymes that depolymerize polysaccharides are injected in very small amounts relative to their counterparts in the plant that agree with peroxidase enzyme level increase on secseptible *V. fabae* for generalist and *C. acutum* for specialist than other resistant host plants. [72,73] study on Wheat aphid confirmed PO results in order to oxidize plant phenolics and other allelochemicals in its food plants [74] and remove hydrogen peroxide from plants as oxido-reductases, thus creating a more favorable media for reproduction and development that agree with [75].

The same for Phenoloxidase, β-Glucosidase and Esterases of generalist *A. fabae* enzyme content was indicated in our study coping with [71,76] studies on β-Glucosidase induction due to *Pieris brassicae* infestation that induced the release of volatile organic compounds in host plants in present seeds and vegetative organs of leguminous plants [77-79]. The increase in β-G to overcome the plant induced defensive compounds activity for generalist *A. fabae* but β-G level decreases for specialists as these compounds increase were more preferable for specialist attraction by suitable host plant as indicated by our study.

Our data showed that Esterases increase in general-

ists with highest level on *V. fabae* and the same increase for specialist *A. fabae* but with highest level on *C. acutum* due to their detoxification ability to plant defence compounds in order to adapt to these host plants in agreement with [29] and was investigated in our study results.

Phenol oxidase presented within generalist *A. fabae* on *V. fabae* which is higher than *C. acutum* and *Z. mays* is likely to occur since phloem sap may contain phytochemicals that are produced by the plant for defensive purposes [39] as shown in [80] study in order to deter phenolic compounds that are toxic to aphids and impair their growth, development and fecundity [81-83]. In contrast, specialist Phenol oxidases are higher on *C. acutum* due to their high toxicity and alkaloid content, this is due to ability to sequestering and deploying the poisons as indicated by [84] as well as on Maize due to their high phenolic content that may detoxify them and convert their anti-probing activity in agreement with Studying *Papilio polyxenes* behavior [34,79,85,86].

ACh-E result coincided with the finding of [87,88] reported decrease in ACh-E in *R. padi* that was correlated to their role in transmission of nerve impulses in order to decrease effect of plant toxins on *A. fabae* performance and fitness on host plants that were observed to be the same for both generalists and specialists. Observation of enzymatic secretions of alatae (the winged, migratory morph) and gynoprae can reveal clues to ecological studies on aphid resistance, such as whether antixenotic (deterrent) factors present and the influence on aphid foraging behavior.

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