Journal of Plant Sciences
2019; 7(1): 27-35
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20190701.15
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Category of Resistance, Antixenosis, Antibiosis, and Tolerance to *Acyrthosiphon pisum* (H.) (Hemiptera: Aphididae) in Selected Lentil Genotypes (*Lens Culinaris* M.)

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To cite this article:

Geteneh Mitku, Tebkew Damte, Mulatu Wakgari. Category of Resistance, Antixenosis, Antibiosis, and Tolerance to *Acyrthosiphon pisum* (H.) (Hemiptera: Aphididae) in Selected Lentil Genotypes (*Lens Culinaris* M.). *Journal of Plant Sciences*. Vol. 7, No. 1, 2019, pp. 27-35. doi: 10.11648/j.jps.20190701.15

Received: March 19, 2019; **Accepted**: April 26, 2019; **Published**: May 17, 2019

Abstract: The pea aphid (*Acyrthosiphon pisum* Harris) is an economically important insect pest of lentil in Ethiopia. The development of pea aphid resistant lentil genotypes with known resistance mechanism is an economical and effective way to manage this pest. Hence, the current study was conducted to determine the mechanisms (antibiosis, antixenosis, and tolerance) of resistance in six lentil (*Lens culinaris* Medikus.) genotypes to pea aphid. Three released varieties (Alemaya, Chalew, and R-186), three accessions (ILL-2595 and ILL-4422, and ILL-7664) and one susceptible accession were included in this study. A no-choice study was conducted to determine the categories of antibiosis and tolerance while free choice studies were conducted to determine antixenosis resistance mechanism. In the antibiosis test, there were significant differences in life table characteristics and demographic statistics among the resistant and susceptible lentil genotypes. ILL-7664 had high levels of an antibiotic effect than the others. Alemaya had high levels of tolerance to pea aphid. Choice studies indicated the presence of antixenosis resistance in the lentil genotype, Chalew, ILL-2595 and ILL-4422. The evaluated lentil genotypes exhibited different types of resistance mechanism and level of expression. Chalew showed a three mode of resistance, i.e., antibiosis, antixenosis, and tolerance. Further research should concentrate on levels of antibiosis, i.e., toxins and growth inhibitors to A. pisum.

Keywords: Antibiosis, Antixenosis, Lentil, Tolerance

1. Introduction

Among the insect pests of lentil (*Lens culinaris* Medikus) the pea aphid (*Acyrthosiphon pisum*) is one of the economically important pests in different parts of the world [1]. In Ethiopia, although the grain yield losses are variable between the time of sowing, seasons, production system and locations, the pea aphid causes an average loss of 4 to 72% annually [2].

The *A. pisum* is a small, oligophagous herbivore that feeds by removing sap from the vascular bundles of many species of legumes in the family Fabaceae [3]. Like most aphids in the tropics, *A. pisum* displays cyclical parthenogenesis [4]. Pea aphid passes through four nymphal instars before reaching adulthood [5]. *Acyrthosiphon pisum* generally takes 9 to 11 days to reach the adult stage and then begins producing live young.

Various methods have been used to combat the damage caused by aphids. These include the unilateral investigations on host plant resistance, biological, cultural, and chemical control methods and more interestingly the amalgamation of one or more control methods presently known as integrated insect pest management [6]. Although insecticides effectively control pea aphid, resistant plant offers an attractive alternative pest management option, it provides an economical and environmentally friendly approach for effectively managing this pest.

Deployment of resistant genotypes has been not only the

most effective pest management practice for reducing pest damage but also it can reduce the need for chemical insecticides as a component of an integrated pest management program [7]. An improved understanding of host plant resistance, however, requires knowledge of the modes of resistance of host plants [8]. Resistance (i.e., tolerance, antibiosis, and antixenosis) is the relative amount of heritable qualities of a plant that reduces the degree of damage done by pests [9]. Antixenosis is the resistance mechanism employed by the plant to deter colonization by an insect [10]. Tolerance is a genetic trait of a plant that enables the plant to tolerate higher pest populations before damage occurs compared with a susceptible cultivar while antibiosis is a heritable quality possessed by a plant that adversely affects the life history or biology of the insect [11] reported that insects fed on resistant plants may manifest antibiotic symptoms, which range from lethal or acute to very mild. He further stated the physiological explanations for these symptoms as the presence of toxic metabolites (alkaloids, glucosides, and quinones) and the absence or suboptimal amounts of some essential nutrient. This component of resistance can be caused by physical or chemical plant factors that repel insect herbivores from feeding or oviposition [7]. According to [11] report the appearance of new pest biotypes when antibiosis is the major component of resistance. Host plants that possess different categories of resistance are considered more beneficial than the effect of individual categories of resistance that may increase selection pressure [7].

In Ethiopia, some lentil genotypes with resistance to *A. pisum* have been identified [13]. Although numerous studies have identified different levels of resistance to insects among the major pulse crops to pea aphid grown in the world [14], only limited information is available on the mechanisms (antibiosis, antixenosis, and tolerance) of resistance present in this crop in Ethiopia. Knowledge of the mechanism of resistance in lentil is crucial for preserving these resistance traits and facilitating the development of appropriate resistance management plans for the pea aphid. This study presents the results on category underlying resistance (antibiosis, tolerance, and antixenosis effects) of these genotypes to *A. pisum*.

2. Material and Method

2.1. Description of Study Area

The resistance mechanism experiments were conducted in a lath house (22.5 to 23.6 ^{O}C mean temperature and 60% to 70% relative humidity) at Debre Zeit Agricultural Research Center (DZARC).

2.2. Plant Material

Lentil genotypes Alemaya, Chalew, R-186, ILL-2595, ILL-4422, and ILL-7664, which were identified as resistant to pea aphid in a field study and one susceptible genotype i.e.; ACC-21688 were included in the lath house. The evaluations under field conditions were conducted between 1999 and 2011 during which time some pea aphid resistant genotypes were identified [13]. Alemaya, R-186, and Chalew have released varieties. Black soil, the common soil type in the area, on which lentil crops are traditionally grown, was used for pot experiment.

2.3. Insect Culture

Pots (20 cm diameter) were filled with black soil and seeds of EL-142 (susceptible variety) were planted at a rate of 2g per pot for aphid colony establishment. Pots were watered as required. Pea aphids were collected in August 2017, from the lentil field near to Debre Zeit Agricultural Research Center and potted seedlings were infested with single Aptera adult pea aphid, which was allowed for 24 hours to deposit nymphs. After 24 hours, all the nymphs and the adult mother were removed from the pot, leaving only one newborn nymph. The colony established from this single nymph was raised on El-142 in the lath house by replacing old infested pots with new pots at seven- and ten-days interval. The colonies were maintained under natural light and photoperiod condition at an average temperature of 22.5 to 23.6°C, 12:12 hours light/dark cycle and relative humidity of 60% to 75%. Adult apterous *A. pisum* found in these colony were used for all lath house (antibiosis, tolerance, and antixenoss) experiments.

2.4. Experimental Design and Procedure

Separate experiments were conducted to detect tolerance and antibiosis by no-choice tests and antixenosis via choice tests [15].

2.4.1. Antixenosis Experiment

The free-choice experiment was conducted on seedling trays, which are rectangular wooden seed box of (60cm length, 40cm width, and 10cm depth). Each wooden seedling box was filled with black soil, where the pulse crops are traditionally grown. On each wooden seedling box, there were 2 circles of each 15 cm in diameter. In each circle, three seeds of each genotype, which was spaced at 2.14 cm interval, were sown on the edge of each circle and labeled with the name of the genotype. A week after germination seedlings were thinned to one vigorous seedling per genotype. Genotypes were arranged in a completely randomized design with 10 replications. The layout and randomization were done as per the standard procedure. Plants were grown in the lath house until infestation time. [16] stated that antixenosis tests with apterous *Schizaphis graminum* (Rondani) closely approximated the results obtained with alates. Two weeks after planting, 700 adults apterous of roughly the same ages were placed in ten petri dish and each petri dish with 70 aphids was put at the center of each circle with the teste genotypes, providing an equal chance for each genotype to be selected. Each circle (with the test genotypes) was covered by plastic cages with ventilation side windows and top. To avoid phototaxis effect, the insects released in the evening to [15]. The aphids were given 48h to

select their host plant. After 48 hours aphids on each plant were counted and recorded. The pea aphids that settled on each genotype 48hours after the release were collected for each genotype per replication, new nymphs were not counted, because newly born nymphs cannot easily move from plant to plant. Data were analyzed using one-way analysis of variance and means were compared using Tukey SHD test at a probability of 0.05 [19] (MSTAT-C 1990).

A)

B)

Figure 1. *Antixenosis experiment (A - before infestation and B – after infestation).*

2.4.2. Antibiosis Experiment

The experiment was carried out in a randomized complete block design (RCBD) using seven lentil genotypes each replicated 13 times. Three seeds of each genotype were grown per pot (20cm diameter and 40cm depth) and seedlings were thinned to one vigorous plant for infestation. About two weeks after sowing, the seedling of each genotype was infested each with one adult *A. pisum* and the infested seedling was covered by a cylindrical transparent plastic cage (15 cm diameter and 80 cm height) with top and side windows covered with nylon cloth for ventilation. The cage was tightly slated into the soil to protect insects escaping and parasitism. The aphids were then allowed to reproduce for 24 hours, after which the adults and newborn nymphs were removed leaving only one newborn nymph per plant per pot. When the aphid failed to deposit nymph within 24 h, an extra single nymph from the other replication was transferred to that test plant. Then the nymphs could develop on each genotype, data on life history parameters such as developmental period, (birth to onset of reproduction), reproductive period (days in reproduction), fecundity (total number of nymphs produced), fecundity per day, and longevity were recorded. Body size and exuviae from molting were the criteria used to discriminate the instars of the pea aphid. The survivorship of each nymph was recorded at 24 hours intervals, and the nymphs produced by the adults were counted and removed daily until the female died. Pea aphid life history characteristics data were collected on each single mother aphid per genotypes. First infestation date, developmental period, Pre- reproductive period, Reproductive period, Aphid survival, Daily nymph production by each single mother pea aphid and Post reproductive period recorded. Aphid demographic statistics such as the intrinsic rate of natural increase (rm) and was obtained by recording number of progeny produced in a time equivalent to pre-nymph positional time (time from the first newborn and first reproduction), then rm was calculated as rm = 0.738 (loge Md)/d where 0.74 = correction constant, d = developmental period (pre-nymph positional time) and Md =number of progeny produced in a time equal to the pre-nymph positional time [17]. Finite rates of increase (λ) (number of individuals added to the population/female/day, or the population capacity to multiply the number of times per female per day), lambda (λ) is a function of rm and was estimated using the formula λ=antilog of rm [18]. The mean time required for a given population to complete one generation is Tc and was calculated using the formula Tc = d/0.738 [17], where d is developmental period; whereas, Td is the time required by a population to double its numbers and is also a function of rm. It was calculated using the formula Td = [loge (2)]/rm [18]. Data were analyzed using one-way analysis of variance and means were compared using Tukey SHD test at a probability of 0.05 [19].

A)

B)

Figure 2. *Antibiosis experiment (A - before infestation and B – after infestation).*

2.4.3. Tolerance Test

The tolerance trial was conducted under a completely randomized design with five replications. Two seeds of each genotype were grown per pot (20-cm diameter and 30cm height) and thinned to one vigorous plant for infestation. Two identical but separate infested and un-infested experiments were conducted. The infested groups were infested with 10 adult *A. pisum* per plant when seedlings were 7.3 cm average height (about two weeks after planting), whereas control groups were left un-infested. Each pot was covered with a cylindrical transparent plastic cage of 15 cm diameter by 80 cm height as described in antibiosis test. Infested and un-infested pots were placed side by side to expose them to the same environmental conditions. Infested plants were examined every 24 hours and newborn excess aphids were removed to maintain a constant number of 10 adult aphids per plants. Tolerance can be quantified by comparing the height of regrowth, dry weight, and yield of plants [20]. Hence height of regrowth, dry weight, and other related parameters was used to quantify tolerance as a mechanism of resistance. All tolerance test data were collected on two identical experiments, infested with pea aphid and un-infested plants after 12 days of infestation. The height of each plant, whether infested or not, was measured before infestation at the onset of the tolerance test and at the end of the test period, which was 12 days. To provide a relative degree of height and weight reduction, plant height and weights of each entry per replication were standardized using the formula percent reduction = [(1-(infested/un-infested) *100)], [21]. Data were analyzed using one-way analysis of variance and means were compared using Tukey SHD test at a probability of 0.05 [19].

3. Result and Discussion

3.1. Antibiosis Test

3.1.1. Life History Parameters

i Developmental time of pea aphid

The developmental time i.e., time from birth until the last molt was highly significant ($p < 0.01$) among tested genotypes (Table 1). However, the developmental time among Alemaya, Chalew, ILL-2595, R-186, and ILL-4422 was statistically non-significant. Pea aphids developed faster on ACC-21688, which required about seven days to reach the adult stage, whereas the developmental time of pea aphids reared on the genotype ILL-7664 required eight and half days to reach the same stage. The nymphal development period was prolonged in resistant varieties than in susceptible ones; thus, pea aphid required a relatively longer developmental period to develop on ILL-7664, which suggests that this genotype had high antibiosis resistance to pea aphid. The range of a developmental period of the pea aphids found in this study (7.08 to 8.69) was closer to the developmental period reported by [22], which is 8.13- 8.81 days. A similarity of these results may be due to a similarity in pea aphid bio type (Debre Zeit) and test genotypes (Alemay, Chalew, and R-186).

ii Reproductive period (NP) of the pea aphid

Reproductive period, i.e., the number of reproductive days of pea aphids on tried genotypes was non-significant ($p > 0.05$) different (Table 1). Even though there was a statistically non-significant difference among tested genotypes, a small difference in NP can influence population growth. The differences in the reproductive period (even as small as 24 hours) have a considerable effect on the population growth of aphids [23]. For instance, the NP of pea aphids raised on ILL-2595 was three and a half days shorter than pea aphids raised on ACC-21688; thus, a single adult pea aphid could have additional 9.38 nymphs (3.5 days x 2.68 number of nymphs per female per day) on ACC-21688 compared to ILL-2595. The number of nymphs per female per day (2.268) was taken from section 4.1.1.4 (Table 1). Similarly, the NP of pea aphids on ILL-7664 was three and quarter day smaller than pea aphids raised on ACC-21688, the additional number of pea aphid progenies on ACC-21688 would be around nine that is more than on ILL-7664. Among the resistance genotypes, the NP of pea aphids on ILL-7664 was two and half days shorter than on ILL-4422 and similarly, it was shorter by two and quarter days when compared to pea aphids reared on R-186. The mean reproductive period of pea aphids in this study (18.15-21.69) was greater than the mean fruitful period reported by [22] who found mean value (14.35-17.18 days). Difference between our current and previous result might be due to genotypes difference, an environmental condition of the experiment and aphid bio type difference. Antibiosis mechanism in resistant varieties reduces the length of pest population reproduction [24] thus, ILL-2595 and ILL-7664 had comparable antibiotic resistance, based on pea aphid reproductive period.

iii Mean number of nymphs produced per female per day (DNP)

The mean number of nymphs per female per day was statistically highly significant ($p < 0.01$) among tested genotypes (Table 1). The lower number of nymphs per day was recorded on ILL-7664 (2.24), whereas the higher DNP was on ACC-21688 (3.28). The difference among genotypes

in the mean number of nymphs/female/days might be due to genotype difference in type and level of resistance, which inhibits aphid population build-up. [25] found that the growth, survival, and fecundity of three aphid species on Fabaceae plants were suppressed on resistant varieties as compared to susceptible varieties. These authors concluded that the mechanisms of resistant species affected the growth, survival, and possibly reproduction of aphids. Antibiosis was expressed in terms of reduced feeding and oviposition when insects feed and develop on resistant varieties thus, pea aphids raised on genotype ILL-7664 had the lowest number of nymph/days, which is an indicator of antibiosis resistance mechanism to pea aphid in this lentil genotype.

iv Adult longevity of pea aphids

The longevity of the female adult pea aphids raised on different lentil genotypes was statistically non-significant ($p > 0.05$) (Table 1). Even though pea aphid full-grown longevity was statistically non-significant, a small difference in adult longevity can have a considerable effect on population growth. Pea aphid raised on ILL-7664 had two and half-day shorter adult female longevity than pea aphid raised on ACC-21688 (Table 1). Similarly, mature female longevity on ILL-4422, R-186 and Chalew lived 3, 2 and 1.5 additional days, respectively, when compared to pea aphid raised on IL L-7664 (Table 1). Report on pea aphid biology and bio type on lentil showed that 34.99 days mature female longevity on Alemaya and 32.26 days on Chalew and the comprehensive mean longevity was 32.67 [22]. This report was closer with the current study (32.69 and 33.38 days female longevity for Alemaya and Chalew, respectively) and comprehensive mean longevity was 33.41 days. Genotypes like ILL-7664 that reduced longevity had antibiosis resistant to pea aphid.

v Fecundity of pea aphid

The fecundity of pea aphids was highly significant different ($p < 0.01$) among tested genotypes (Table 1). Fecundity of the pea aphid was tiny on the resistant genotypes when compared with the susceptible control. The mean fecundity of pea aphid over the reproductive period ranged from 56 nymphs/female on ILL-7664 to 82 nymphs/female on ACC-21688. These differences in fecundity among tested genotypes suggested that resistant genotypes are not suitable for the reproduction of pea aphid. Minimum fecundity of 33 was recorded on resistance genotypes and the maximum mean value of fecundity (80 nymphs /female on susceptible control [6]. In another study, [5] found mean fecundity 58 on pea aphid reared on resistant field pea genotype. When different plant species and cultivars are compared, the pea aphid can exhibit differences in fecundity and rm. Hence, the difference in fecundity in the present study and previous report on fecundity might be due to the difference in insect biotype, host plant, genotype influences or from a combination of all these factors. Low fecundity on resistant varieties is due to a potential antibiotic factor in rice leading to considerable reduction in the population buildup of brown planthopper compared with a susceptible variety. In the present study fecundity of pea aphids was significantly reduced on three resistant genotypes, ILL-7664, ILL-2595 and Chalew compared with susceptible control and other resistance genotypes. Hence genotypes ILL-7664, ILL-2595 and Chalew had antibiosis resistance to pea aphid in terms of fecundity.

Table 1. *Mean life history parameters of Acyrthosiphon pisum raised on different genotypes of lentil- antibiosis test.*

Genotype	Developmental time	Pre-NP^{NS}	NP^{NS} Adult	Longevity^{NS}	Fecundity	Post-NP*
Alemaya	8.15ab	9.20	19.85	32.69	68.62ab	3.31
Chalew	8.39ab	9.15	19.69	33.38	62.31a	3.02
R-186	7.46ab	9.20	20.54	34.38	70.69ab	3.62
ILL-2595	7.85ab	8.69	18.15	32.15	60.77a	3.10
ILL-4422	7.36ab	9.23	20.85	34.92	68.54ab	3.85
ILL-7664	8.69b	9.15	18.31	31.92	56.23a	3.23
ACC-21688	7.08a	8.46	21.69	34.38	82.15b	3.92
Mean	7.86	9.00	19.87	33.41	67.00	3.44
CV (%)	14.8	11.1	20.0	14.4	18.1	27.5

Means within columns followed by the same letter are not statistically significant ($P < 0.05$, Tukey's HSD test). NS= statistically non-significant at $p>0.05$
Fecundity =the number of total offspring produced during the nymph positional period, Post-NP= number of days after the aphid ceased reproduction, Longevity= total lifespan of the aphid.
NS= statistically non-significant ($p>0.05$). Pre-NP (pre-nymph positional period) is the number of days prior to reproduction and NP= nymph positional period or number of reproductive days.

3.1.2. Demographic Statistics of Pea Aphids

i Intrinsic rates of the natural increase

The intrinsic rate of the natural increase (rm) of pea aphid was highly significant ($p < 0.01$) among tested genotypes (Table 2). The rm values of pea aphid raised on ILL-7664 and Chalew were significantly less than the rm values on the remaining tested genotype, whereas the highest rm value was recorded on ACC-21688. The differences in rm values might result from difference in resistance to pea aphid among the tested genotypes. The rm has been used to measure aphid performance on different host plant crop cultivar [26]. The higher rm values indicated that aphid had relatively greater potential to reproduce on some genotypes. The low rm value on the other cultivars, especially suggests that these cultivars have comparatively higher antibiosis causing reduced survival [27]; thus, the lowest rm value on ILL-7664 is an indicator of antibiosis resistance. The rm values for the pea aphid range from 0.324-0.402 and 0.288-0.318 when different pea cultivars are compared [28]. Intrinsic rates of increase indicate increasing populations on genotypes. Lower

intrinsic rates of increase were consistent with host genotypes in which aphids exhibited the lowest fecundity and longevity.

ii Finite rate of the increase

The finite rate of increase (λ) value of pea aphids was significantly ($p < 0.05$) different among tested genotypes (Table 2). But there was no significant difference among Alemaya, ILL-2595, R-186 and Chalew. Pea aphid on ILL-4422 and ILL-7664 showed tiny λ value compared with all the remaining tested lentil genotypes. *A. pisum* λ value on ACC-21688 was greater than the λ on all the remained tested genotypes. Differences between tested genotype in finite rate of an increase of pea aphid indicate that tested lentil genotypes had different level and types of resistance to A. pisum. The lowest λ values on ILL-7664 (1.90) and ILL-4422 (1.91), are indicator of antibiosis resistance mechanism. The range of finite rate of increase recorded in our study was (2.12 nymphs /female/day on susceptible control to 1.91 on ILL-7664 and ILL-4422). Report of [6], found mean finite rate of increase value of 1.31 to 1.42 nymphs /female/day of a pea aphid reared on resistant genotype. Our study value of finite rate of an increase was greater than previous one. This may due to that the resistance level of previous genotypes was more resistant than our current one based on this parameter.

iii Mean generation time

Mean generation time of pea aphid was statistically non-significant ($p>0.05$) on tested genotypes (Table 2). Even though there were statistically non-significance variability on tested genotypes mean generation time of the susceptible control was shorter than mean generation time on all tested resistant genotypes. The me mean generation time of pea aphid in our current study ranged from 11.47 to 12.48 days. According to [5], the mean generation time of pea aphid ranged from 14.3 to 18.1 days, which is slightly greater than our current study. In another study [14] mean generation time of pea aphid ranged between 11.5-13.1 days which is closer to our study. The similarity between our current and previous study implies that genotypes may have similar level of antibiosis resistance based on this parameter.

iv Doubling time

Doubling time of pea aphid was significantly ($p < 0.05$) different among tested genotypes (Table 2). Although time of pea aphids to double itself on Alemaya, Chalew, R-186, ILL-4422, ILL-2595 and ILL-2595 showed non-significant difference. Required time of pea aphid to double itself on ILL-7664 showed significantly longer compared with all tested genotypes. Differences in average of Td values in current study, might be caused by host plant resistance and resistance mechanism difference on pea aphid. The range of mean doubling time of pea aphid in this study was 2.15-2.43 days on ACC-21688 and Chalew, respectively which was similar with previous study [14] who found mean value (2.1-2.8 days) susceptible and resistant field pea genotypes. Similarly, our current study was comparable to the report [6], who found mean value of 1.99- 2.64 days on susceptible and resistant genotypes. Resistance genotypes i.e. unsuitable to pea aphid reproduction take longer time to pea aphid doubling, from this ILL-7664 which showed longest doubling time had antibiosis resistance.

Table 2. *Reproductive performance and longevity of Acyrthosiphon pisum reared on different genotypes of lentil- antibiosis test.*

Genotype	rm	λ	Tc^{NS}	Td
Alemaya	0.29ab	1.97ab	12.40	2.39ab
Chalew	0.29a	1.95ab	12.40	2.43ab
R-186	0.30ab	2.00ab	12.40	2.34ab
ILL-2595	0.29ab	1.98ab	11.78	2.35ab
ILL-4422	0.29ab	1.91a	12.48	2.41ab
ILL-7664	0.28a	1.91a	12.40	2.52b
ACC-21688	0.33b	2.12b	11.47	2.15a
Mean	0.297	1.98	12.19	2.37
CV (%)	9.8	8.4	11.14	10.3

Means within columns with different superscript to numbers were statistically significant (P =0.05, Tukey's HSD test). NS= statistically non-significant ($p>0.05$). rm= intrinsic rate of natural increase, λ = finite rates of increase, Tc= the mean time required for a given population to complete one generation, whereas, Td is the time required by a population to double its numbers.

3.2. Antixenosis Test

From 700 *A. pisum* introduced into caged seedlings, 568 (81.14%) were recovered at the end of the test. Pea aphid number after 48hours of release was highly significant ($p<0.01$) among tested genotypes (Table 3). But, pea aphid number 48 hours after release on ILL-4422 and ILL-2595 was not significantly different, similarly, there was the non-significant difference among Alemaya, R-186, and ILL-7664. Resistance genotypes had significantly fewer adult pea aphid when compared with susceptible control after 48hours of pea aphid release. The least preferred genotypes by pea aphid were ILL-2595, ILL-4422 and Chalew, with significant difference among them, these genotypes showed antixenosis resistance mechanism to pea aphid. Antixenosis may be an important resistance modality in crops against aphid, as this modality can deter or delay aphid colonization and reduce the potential of infestations reaching economically injurious levels [29].

Table 3. *Preference of Acyrthosiphon pisum to lentil genotypes measured as mean number of aphids on each genotype.*

Genotype	Mean number of pea aphid /plant 48hours after insect release
Alemaya	8.60ab
Chalew	7.10a
R-186	8.50ab
ILL-2595	7.00a
ILL-4422	7.10a
ILL-7664	8.30ab
ACC-21688	10.20b
Mean	8.11
CV (%)	19.1

Means within columns followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD test.

3.3. Tolerance Test

3.3.1. Plant Height

Plant height after infestation (infested group) showed highly significant ($p<0.01$) among tested genotypes (Table 4). Under

infested condition the genotype ACC-21688 had the shortest height; genotypes ILL-4422, ILL-2595 and R-186 had intermediate, while Alemaya and ILL-7664 had the tallest height. The difference between an infested and un-infested plant of a genotype was significantly different in all the genotypes. Plant height after infestation in an infested group of among tested genotypes ranged from 7.65 cm in genotype ACC-21688 to and 11.77cm in ILL-7664. The overall mean plant height was 9.53cm. Tolerance refers to a situation where a host plant shows an ability to grow, reproduce itself, or to repair an injury to a marked degree despite supporting a population equal to that damaging a susceptible host [9]; thus ILL-7664 and Alemaya significantly grew faster than the remaining genotypes, implied that they have a high level of tolerance to pea aphid feeding based on plant height.

i Plant height of un infested groups.

Plant height in un-infested group showed highly significant ($p<0.01$) variation among tested genotypes (Table 4). ILL-4422 had significantly shortest plant height when compared with all the remaining genotypes. On the other hand, ILL-7664 had the tallest plant height (Table 4). Plant height difference in un-infested group of among tested genotypes in current study indicated that tested genotypes were not genetically similar in plant height.

ii Plant height percent reduction

Plant height reduction (%) was significantly ($p<0.05$) different among tested genotypes (Table 4). All resistant genotype had significantly less percent of plant height reduction than the height reduction in susceptible control. Smallest plant height reduction caused by pea aphid feeding was on Alemaya, followed by ILL-7664 and Chalew; whereas the highest plant height reduction was recorded on ACC-21688 (Table 4). Tolerance is an ability of the plant to grow and reproduce and even repair an injury to a marked degree despite supporting a population approximately equal to that damaging a susceptible host. The small plant height reduction is an indicator of tolerance, plants which withstand insect feeding; thus, Alemaya, ILL-7664 and Chalew which had the smallest percent of plant height reduction, are tolerant of pea aphid based on this parameter. After 12 days of infestation, all infested groups had shorter plant height than un-infested genotypes implying that aphid infestation reduces plant height.

Table 4. *Effect of Acyrthosiphon pisum infestation on plant height of different lentil genotypes.*

Plant height after infestation			
Genotype	**Un-infested**	**Infested**	**% Reduction**
Alemaya	13.92b	11.75c	15.63a
Chalew	11.99ab	9.49b	20.61b
R-186	12.25a	8.63ab	28.42c
ILL-2595	12.25a	8.95a	26.85bc
ILL-4422	11.17a	8.48ab	23.14bc
ILL-7664	14.36b	11.77c	18.03ab
ACC-21688	13.98b	7.65a	45.14d
Mean	12.83	9.53	25.40
CV (%)	10.3	9.5	21.3

Means followed by the same letter in a column for a given are not significant (Tukey's HSD test P >0.05)

3.3.2. Plant Biomass

Dry biomass after infestation (infested group) showed highly significant ($p<0.01$) among tested genotypes (Table 4). The largest dry weight was recorded on Alemaya and ILL-7664, these genotypes withstand the insect damage and gave largest dry weight when compared to others tested genotypes; thus, genotypes which had the largest dry weight, ILL-7664 and Alemaya in our study showed that tolerance resistance mechanism regarding dry biomass. Dry biomass in un-infested group experiment showed significant ($p<0.05$) variability among tested genotypes (Table 4). Dry biomass on ILL-7664 in the un-infested group was significantly highest ($p<0.05$) when it compared with all the remaining genotypes, whereas lower dry biomass was on ILL-4422 compared with all the remaining tested genotypes. There was no dry biomass difference in dry biomass among Chalew, ILL-4422, R-186, Alemaya, and ACC-21688. Dry biomass difference in the un-infested group of among tested genotypes in the current study indicated that the genotypes were not similar.

Dry biomass in un-infested group experiment showed significant ($p<0.05$) variability among tested genotypes (Table 5). Dry biomass on ILL-7664 in un-infested group was significantly highest ($p<0.05$) when it compared with all the remaining genotypes, whereas lower dry biomass was on ILL-4422 compared with all the remaining tested genotypes. There was no dry biomass difference in dry biomass among Chalew, ILL-4422, R-186, Alemaya and ACC-21688. Dry biomass significance difference in un-infested group of tested genotypes in current study suggest that presence of inherent differences in plant dry biomass among tested genotypes within the same growth stage.

i Dry biomass percent reduction

Dry biomass percent reduction showed that highly significant ($p<0.01$) among tested genotypes (Table 5), even though percent reduction on ILL-4422 and ILL-2595 showed non-significant ($p<0.05$). Significantly the smallest dry biomass reduction caused by pea aphid feeding on Alemaya followed by Chalew and ILL-7664 whereas the highest was on the ACC-21688. Tolerant plants support large insect population with little damage or yield loss and have value in maintaining predator and parasite population [30]. Hence least dry biomass percent reduction on Alemaya and intermediate reduction on Chalew and ILL-7664 indicated that they have comparable levels of tolerance when compared to remaining resistant tested genotype based on this parameter.

ii Fresh biomass percent reduction

Fresh biomass percent reduction was significantly ($p<0.05$) variable among tested genotypes (Table 7), except R-186, ILL-4422, Chalew and ILL-7664. The largest fresh plant biomass percent reduction was on ACC-21688, whereas the smallest reduction was on ILL-2595 and Alemaya without significant difference. The fresh biomass percent reduction difference among tested genotypes may due to resistance level and type difference. Alemaya and ILL- 2595 which had smallest fresh biomass percent reduction had tolerant resistance mechanism based up on fresh biomass

percent reduction. Fresh biomass percent reduction may not exactly quantify tolerance because plants may have different potential to maintain moisture content, this moisture content may have confounding effect, so observing and considering dry biomass and plant height is wise decision to quantify tolerance. Correlations between plant height reduction, fresh biomass reduction and dry biomass reduction showed strong positive relationship.

Table 5. *Fresh and dry plant biomass of lentil genotype after infestation with A. pisum.*

Fresh biomass				**Dry biomass**		
Genotype	**Un- infested**	**Infested**	**% Reduction**	**Un-infested**	**Infested**	**% Reduction**
Alemaya	4.48abc	3.36c	24.13a	0.13ab	0.11c	20.14a
Chalew	4.56cd	3.09bc	31.06ab	0.13ab	0.09bc	29.44ab
R-186	3.55ab	2.27a	35.63ab	0.11ab	0.06a	45.00bc
ILL-2595	3.25 a	3.50a	23.50a	0.10a	0.06ab	34.99abc
ILL-4422	3.88b	2.59ab	32.99ab	0.11ab	0.07ab	35.02ab
ILL-7664	4.95d	3.00abc	38.54ab	0.16b	0.11c	31.45ab
ACC-21688	4.4abc	2.43ab	43.00a	0.11ab	0.05a	49.95c
Mean	4.33	2.75	32.7	0.12	0.08	35.1
CV (%)	12.8	14.3	31.1	25.2	19.6	24.6

Means within columns with different lower-case letter were statistically non-significant ($P < 0.05$, Tukey's HSD test).

4. Conclusion and Recommendation

Lath house studies were conducted for a better understanding of the category of resistance in lentil genotypes to *A. pisum*. Modalities of resistance were identified to ascertain the degree of resistance in lentil genotypes and it was essential for the development of durable resistant genotype. The current research classified the resistance mode of six resistant lentil genotypes (Alemaya, Chalew, ILL-7664, ILL-4422, ILL-2595, and R-186) to pea aphid by assessing levels of antibiosis, antixenosis, and tolerance. It was found that all tested genotypes had at least one category of the resistance mechanism. In this study, it was found that an individual resistance genotype might have singular, double or triple modalities of resistance.

The antibiosis resistance in ILL-7664 on *A. pisum* was manifested by reduced fecundity, nymph production per female per day, intrinsic rate of the natural increase (rm). The case was similar in Chalew. Reduced pea aphid population in genotypes with antibiosis resistance in the current study may due to depends on the presence of host-specific chemical compounds, the allelochemicals of the plant. Smaller fecundity and the smallest rm value on ILL-7664 suggest these both parameters are the best to estimate antibiosis property of resistance to pea aphid. Pea aphid fecundity on Chalew was also low, which leads to lower rm value. The smaller rm values on tested genotypes suggest that pea aphid had a little potential to reproduce on genotypes that had antibiosis resistance.

Tolerance in released resistant variety, Alemaya decrease pea aphid damage cumulatively by the reduced percent dry biomass, percent plant height and increased dry biomass, plant regrowth after 12 days of infestation compared with other tested genotypes; thus, Alemaya had tolerance resistance mechanism to *A. pisum*. Similarly, comparable tolerance was found on ILL-7664, which exhibited longer plant regrowth, increased dry biomass after 12 days of infestation compared with other tested genotypes, except Alemaya. Chalow also showed reduced percent plant growth and dried biomass reduction and increased leaf number after 12 days of infestation. ILL-2595 and ILL-4422 had some level of tolerance; the remaining genotype R-186 seems to have a lower level of tolerance based on most tolerance parameter and tolerance index value. The genotypes with the highest whole number of aphids after 48-hours of release were the best aphid-preferred whereas those with a low number of pea aphid after 48-hours of release was the least. The entire number of aphids after 48-hours of release was, therefore, considered as an indicator of antixenosis resistance. Free choice antixenosis test showed that Chalew, ILL-4422, and ILL-2595 as the least preferred genotypes.

It is recommended to cross ILL-4422 or ILL-2595 (antixenosis resistant genotypes) with Alemaya (tolerant genotype) to limit aphid populations to acceptable levels on the crop and to reduce selection pressure for the development of resistance in aphids.

Tolerance should be more important in a pest management strategy compared with antibiosis or antixenosis because of compatibility with other control options and has no selection pressure to insect biotypes. It is recommended that using genotypes that had tolerance for pea aphid management been a wise decision as it reduces selection pressure on *A. pisum*.

Alemaya (genotype which had a tolerant resistance mechanism) is more important for an integrated pest management program (IPM) than all the remaining teste genotypes. However, Chalew with three components of resistance may reduce the development of new insect biotypes, which can be more aggressive in the production area.

An additional advanced study should be conducted for a better understanding of morphological and biochemical basis of resistance. Genotypes which had antixenosis resistance mechanism like Chalew, ILL-4422 and ILL-2595 needs additional exploration to identify plant characters that may be involved in hindering feeding preference by adult pea aphids. Besides, the study of the chemical ecology of this pest namely isolating the secondary metabolites which favor/disfavor pea aphid requires further investigation.

Acknowledgements

I would like to express my gratitude and appreciation to the Ethiopian Institute of Agricultural Research for financial assistance. The support and cooperation provided by Mr. Niguse Girma, Coordinator of National Chickpea and Lentil Research Program at Debre Zeit Agricultural Research Center are also highly appreciated.

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