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As a Stress Factor of Acanthoscelides Obtectus Say. (Coleoptera: Chrysomelidae: Bruchidae) Pest in Common Bean (Phaseolus vulgaris)

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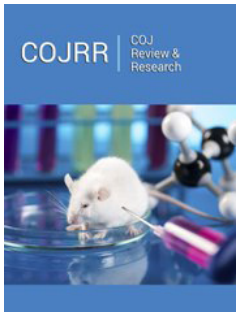

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Abstract

This research was conducted in 2019 year at Bayburt University, Aydintepe Vocational School experimental area (40°24'05.7"N 40°08'31.3"E) that it is in Turkey/Middle East and in the study Aydintepe local bean genotype was used. As a botanical and yield component features stem diameter, branch number, first pod height, pod length, pod width, pod number per plant, seed number per pod, 1000 seed weight, seed yield and as a stress features Proline concentration and Malondialdehyde (MDA) concentration parameters were examined in 0 (control), 1, 2, 3, 4, 5 and 6 Acanthoscelides obtectus Say. pest infected bean seeds. In addition to basic statistics, correlation coefficients between properties were calculated and variance analysis was also performed. According to the results obtained from the study, Acanthoscelides obtectus Say. beetle negatively affected the botanical and yield characteristics of the bean plants and it caused an increase in the concentration of stress substances such as Proline and MDA in the bean plants.

Keywords: Acanthoscelides obtectus Say; Pearson's correlation; Phaseolus vulgaris; Common bean; Yield components; Botanical properties

Introduction

In the world, the most produced and consumed edible legume plant is beans. Food and Agricultural Organizations of United Nations statistical data pointed out that pulses harvest area was 95.720.198ha, production quantity was 9.640kg ha-1 and yield mean was 92.277.859tons. The proportion of dry beans in this amount is as follows; harvest area was 34.495.662ha, production amount was 30.434.280 tonnes and yield value were 8823kg ha-1 [1]. Legume seed beetles create cavities and decrease the nutritional value of the seed. As a result of feeding legume seed beetles, decreased quality, loss of germination and weight loss in seed. Damaged in this way the market value of legumes declines [2,3]. There are 58 different genus and 1500 species of Bruchid beetles (Bruchidae, Coleoptera) which is seed eating [4]. Since the evolution of beans, weevil has been the most important pest in granaries [5]. The Latin name of the insect that harms the bean seed is Acanthoscelides obtectus Say. This species was described by Thomas Say in 1831. English names of the beetle, which belongs to Bruchidae family, is also known as American seed beetle, bean beetle, bean bruchid, bean weevil, common bean weevil, dried bean beetle, dried bean weevil [6]. It is reported that warehouse pests have a negative effect [7]. The bean seed beetle continues to fertilize in the warehouse. Due to Acanthoscelides obtectus Say. species give continuous generation, bean seeds lose a lot of weight and germination rate [8,9]. The weevil can give 2-3 or even 3-4 generations per year and it damages bean seeds a lot [10]. The negative effect of legume seed beetle on seed has been reported in many studies and it was reported to cause loss

of yield [11-13]. This insect may even cause the entire product to be damaged [14-16]. In this study, the effects of Acanthoscelides obtectus Say. species, which damage bean seeds, were investigated in terms of yield characteristics and some substances (Proline and MDA) synthesized in the stress.

Material and Methods

Seed materials

Acanthoscelides obtectus Say. insects to be used in the study were collected from nature and the common bean seeds were infected with 1, 2, 3, 4, 5 and 6 insects based on the controlled conditions (27±1 °C, 14:10 h L:D, 60±5% RH) applied by other researchers [17,18] for the development of larvae at the Laboratory of Bayburt University, Aydintepe Vocational School. Seeds infected with insect larvae were then taken to the field treatment.

Field treatment

Turkey's province of Bayburt, Aydintepe district (40°24'05.7"N 40°08'31.3"E) where the research is conducted is in the Black Sea Geographic Region. However, it has a transition climate between the Eastern Black Sea climate and the Eastern Anatolian climate. According to the average of long years (1960-2017) in Bayburt province, annual rainfall amount is 440.6mm, annual average temperature is 7.1 °C (MGM, 2018).

In the research, 1, 2, 3, 4, 5 and 6 Acanthoscelides obtectus Say. pest infected bean seeds and insect-free seeds were prepared and planted according to Randomized Complete Block Design (RCBD). The plots consisted of 50cm between the rows, 10cm above the row, 4 rows, the length of the parcel was 5m, the area of each parcel was determined as 10 m2 (4 × 0.5 × 5m) and 3 replicates were planted. Struggling with weeds occurred. According to IBPGRI (1982), as a botanical and yield component features stem diameter, branch number, first pod height, pod length, pod width, pod number per plant, seed number per pod, 1000 seed weight, seed yield.

Determination of proline amount in leaf tissues

Proline content was made according to the method of [19]. Collected samples of 0.1-0.3g were extracted with 1ml of 3% sulfosalicylic acid by homogenization with liquid nitrogen. The extracts were centrifuged at 14,000rpm for 5 minutes at 4 °C. 0.1ml of supernatant was taken to a new ependorpha and 0.2ml of acid ninhdrin, 0.2ml of 96% acetic acid, 0.1ml of 3% sulfosalicylic acid was added to each sample and incubated at 96 °C for 1 hour and all protein hydrolysis was performed. After incubation, 1ml of toluene was added to each eppendorf tube and samples were vortexed and centrifuged at 14,000rpm for 5 minutes at 4 °C. The overlying red part was separated and 520nm absorbances were measured using toluene blank. A standard curve was drawn to determine the proline concentration in the range of 5-500µm. Results are expressed as µmol/g TA.

Determination of Malondialdehyde (MDA) amount in leaf tissues

0.5g leaf sample was homogenized with 10ml 0.1% Trichloroacetic Acid (TCA). The homogenate was centrifuged at 15.000 g for 5 minutes, 1ml was taken from the supernatant phase of the centrifuged extract and mixed with 0.5% Thiobarbituric Acid (TBA) dissolved in 4ml of 20% TCA. After the mixture was kept at 95 °C for 30 minutes and cooled rapidly in an ice bath, it was centrifuged for 10 minutes at 10.000g. The Malondialdehyde (MDA) content was calculated by determining the absorbance at 532nm wavelength of the supernatant part obtained after centrifugation [20]. Results are expressed in nmol/g TA.

Statistical analysis

Analysis of the variance of the data obtained at the end of the research was made using the SAS package program v.9.1. according to the RCBD experiment pattern, simple statistics, correlation coefficients and Duncan Multiple Range Test was (DMRT) used to compare the averages [21].

Result and Discussion

Table 1: Basic statistical properties for all measurement characteristics.

Variable	Minimum	Maximum	Mean	Std. Deviation
Stem Diameter (mm)	4.161	6.453	5.023	0.781
Branch Number (number)	1	2.933	1.276	0.731
First Pod Height (cm)	8.5	14.473	10.434	2.014
Pod Length (cm)	6.501	9.867	8.178	1.3
Pod Width (cm)	1.203	1.477	1.323	0.085
Pod Number Per Plant (number)	2.5	15.133	8.112	4.924
Seed Number Per Pod (number)	2.5	3.867	3.319	0.518
1000 Seed Weight (g)	0	389.333	266.39	128.39
Seed Yield (kg/da)	0	121.933	32.176	42.14
Proline (µg/g)	1.285	3.448	2.354	0.754
MDA (µmol/g)	2.215	5.462	4.022	1.22

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Table 2: Averages and Statistical Groups of Stem Diameter, Branch Number, First Pod Height, Pod Length and Pod Width According to Duncan Multiple Range Test (DMRT).

Number of Acanthoscelides Obtectus	Stem Diameter**	Branch Number**	First Pod Height*	Pod Length**	Pod Widths
0	4.403 C	2.93 A	14.47 A	9.867 A	1.477
1	4.160 C	1.00 B	10.00 B	6.767 CD	1.203
2	4.587 CB	1.00 B	9.67 B	8.393 ABC	1.373
3	5.377 B	1.00 B	8.93 B	8.550 AB	1.267
4	5.377 B	1.00 B	11.47 AB	9.577 A	1.31
5	6.453 A	1.00 B	10.00 B	7.593 BCD	1.323
6	4.807 CB	1.00 B	8.50 B	6.500 D	1.31
Coeff. Var.	10.2278	12.3303	17.4601	11.4065	6.9643
R-Square	0.8062	0.9701	0.6647	0.7627	0.6126
Min.Sign. Difference	0.9235	0.2562	3.5756	1.7115	0.1919

Means followed by the same letter within columns are not statistically significantly different at the *0,05 and **0,01 level

According to the results obtained from the research, stem diameter, branch number, first pod height, pod length, pod width, pod number per plant, seed number per pod, 1000 seed weight, seed yield, Proline and MDA varied between 4.161-6.453mm, 1.000-2.933 number, 8.500-14.473cm, 6.501-9.867cm, 1.203-1.477 cm, 2.500-15.133 number, 2.500-3.867 number, 0.000-389.333g, 0.000-121.933kg/da, 1.285-3.448µg/g, 2.215-5.462µmol/g respectively (Table 1). Similar results gained by Kazai et al. [20] in stress bean plant.

Table 3: Averages and statistical groups of pod number per plant, seed number per pod, 1000 seed weight and seed yield according to Duncan Multiple Range Test (DMRT).

Number of Acanthoscelides obtectus	Pod Number Per Plant**	Seed Number Per Podns	1000 Seed Weight**	Seed Yield**
0	16.53 A	3.87	389.33 A	121.93 A
1	3.47 E	2.93	339.10 BA	10.20 CD
2	12.87 BC	3.87	322.00 BC	40.10 B
3	15.13 BA	3.53	280.00 C	30.30 BC
4	9.07 D	3.53	313.30 BC	19.90 BCD
5	9.75 DC	3	221.00 D	2.80 D
6	2.50 E	2.5	not available	0.00 D
Coeff. Var.	21.5045	19.2883	9.1525	38.4996
R-Square	0.9094	0.5322	0.8585	0.9459
Min. Sign. Difference	3.561	1.3974	50.44	20.369

In terms of stem diameter, there has been a considerable increase in the number of insects in bean plants (Table 2). The maximum stem diameter value was obtained from seeds with 5 bugs (6.45mm). This shows that plants that do not die and survive due to Acanthoscelides obtectus insects form a thicker stem. Branch number values were found low in all insect-infected beans (1.0 number) and high in non-insect bean plants (2.93 number per plant). This is normal, if the plants are insect-free, they have lived a comfortable life. The first pod height values were all included in a statistical group, except for the seeds without Acanthoscelides obtectus insects. Plants from seeds that were not infected with insects were formed the pods higher up. The highest first pod height was 14.47cm and it was gained from insect-free beans (Table 2). This showed that when the bean plant was not in stress, it started to form its pods from above. In terms of pod length, insect-free seeds (9.867cm) and 4-insect seed (9.577cm) applications were included in the same statistical group. These had the highest values. However, the application of insect-free was higher. The highest pod number per plants value was gained from 0 number of Acanthoscelides obtectus insect (16.53 number plant-1). The values obtained from insect infected seeds were lower (Table 3). When the bean plant was not in stress due to Acanthoscelides obtectus insect, it formed more pods. Thousand seed weight values generally gave similar results. However, 6 insect-infected applications could not obtain seeds. Therefore, one thousand weight value could not be calculated. Nevertheless, the highest value was obtained from the application without insect contamination (389.33g). Because of the bean seeds were not contaminated with insects and the plant grew

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comfortably, the weight of one thousand seed was higher in zero insects application.

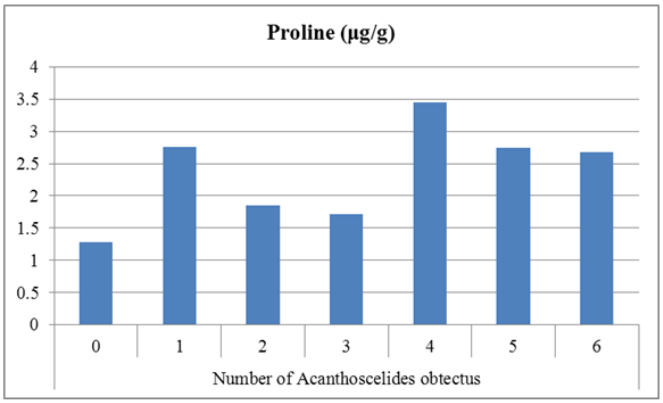

Figure 1: According to number of Acanthoscelides obtectus change of proline concentration.

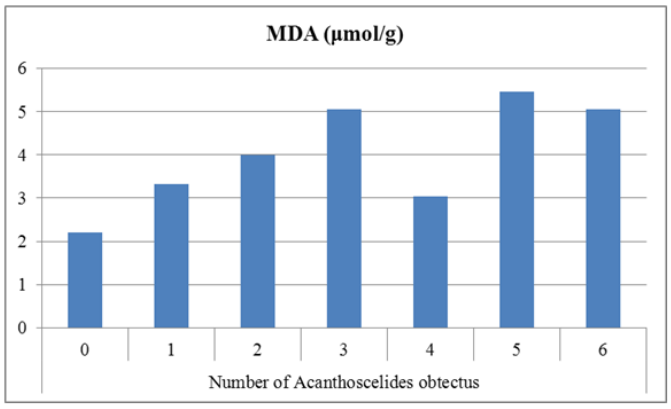

Figure 2: According to number of Acanthoscelides obtectus change of MDA concentration.

The seed yield value obtained from the insect-free application gave the highest yield (121.93kg da-1). It is reported that legume seed beetle was cause losses on yield [13]. Sozen and Karadavut et al. [21] reported that environmental factors had an impact on the development of bean plants. It is reported that warehouse pests have a negative effect [22]. On the other hand Kazai et al. [20] reported that seed yield values were lower in stress plants. Similar opinions have been reported by Asfaw and Blair [23]. On the other hand it is reported that the entire product in the warehouse could be damaged due to Acanthoscelides obtectus Say. insect [24,25]. In terms of the stress chemicals Proline and MDA values were found low when the plants were not in stress. Proline and MDA values were the lowest in zero number of Acanthoscelides obtectus insect 1.2853µg/g and 2.2153µmol/g respectively. In general, as insectication increased, the level of stress chemicals increased (Table 4). Especially when there are many Acanthoscelides obtectus beetles, the synthesis of proline has increased considerably. And if the plants are comfortable, only a small amount of proline has been synthesized except one number plant-1 Acanthoscelides obtectus application (Figure 1). Proline accumulation is a plant resistance mechanism against various stress factors [26]. Proline shows more accumulation in stressful plants than other amino acids [27]. MDA values tended to increase as insect numbers increased except 4 number plant-1 Acanthoscelides obtectus application. (Figure 2). Similar results gained by Kabay [28]. On the other hand, it was reported that MDA level increased in progressive stress conditions [18]. According to correlation coefficient analysis [29], there were positive and significant correlation between branch number and first pod height (0.884), branch number and pod width (0.795), pod length with and number per pod (0.872), branch number and seed yield (0.939), first pod height and seed yield (0.847), pod width and seed yield (0.811), seed number per pod and 1000 seed weight (0.790). on the other hand, there was a negative and significant correlation between first pod height and MDA concentration (-0.826). Other correlation coefficients were insignificant (Table 5). These results like the findings of Kulaz and Ciftci [30].

Table 4: Averages and statistical groups of proline concentration and MDA (Malondialdehyde) concentration according to Duncan Multiple Range Test (DMRT).

Number of Acanthoscelides obtectus	Proline**	MDA**
0	1.2853 D	2.2153 E
1	2.7580 B	3.3333 D
2	1.8507 C	4.0000 C
3	1.7220 C	5.0537 B
4	3.4480 A	3.0320 D
5	2.7400 B	5.4620 A
6	2.6740 B	5.0537 B
Coeff. Var.	10.0726	5.1825
R-Square	0.9396	0.9811
Minimum Significant Difference	0.3911	0.3379

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Table 5: Pearson's correlation coefficients matrix for all measurement parameters.

1.Stem Diameter, 2. Branch Number, 3. First Pod Height, 4. Pod Length, 5. Pod Width, 6. Pod Number Per Plant, 7. Seed Number Per Pod, 8. 1000 Seed Weight, 9. Seed Yield, 10. Proline conc., 11. MDA conc., Values in bold are different from 0 with a significance level alpha=0,05.

Variables	1	2	3	4	5	6	7	8	9	10	11
1	1										
2	-0.35	1									
3	-0.228	0.884	1								
4	0.039	0.573	0.742	1							
5	-0.092	0.795	0.706	0.63	1						
6	0.471	-0.368	-0.297	0.339	-0.075	1					
7	-0.153	0.466	0.572	0.872	0.57	0.517	1				
8	-0.247	0.422	0.635	0.66	0.22	0.259	0.79	1			
9	-0.406	0.939	0.847	0.723	0.811	-0.098	0.722	0.591	1		
10	0.335	-0.626	-0.309	-0.332	-0.572	-0.199	-0.556	-0.327	-0.749	1	
11	0.625	-0.653	-0.826	-0.595	-0.407	0.372	-0.535	-0.688	-0.689	0.148	1

Conclusion

According to the results obtained from the research, Acanthoscelides obtectus Say. (Coleoptera: Chrysomelidae) beetle negatively affected the botanical parameters and yield characteristics of the bean plants and it caused an increase in the concentration of stress substances such as Proline and MDA in the plants. On the other hand, branch number, first pod height and pod width reliable selection criterion for high yielding in stressed bean plants by Acanthoscelides obtectus Say.

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