

Researches on feeding behaviors of *Nezara viridula* (Linnaeus, 1758)(Hemiptera: Pentatomidae) adults in intermittent CO₂ applications

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Abstract

In this study, the effects of 3 different doses (430, 600 and 670 ppm) of intermittent CO₂ applications on the feeding behaviors of *Nezara viridula* L. (Hemiptera: Pentatomidae) Adults were determined. As nutritional behavior, the total nutrition duration of adult male and female individuals other than nutrients was examined. As a result of the study, no statistically significant difference was found between the total feeding times of female and male individuals of *N. viridula*. A statistically significant difference was found between the duration of stay on the nutrients of female and male individuals of *N. viridula* who received 600 ppm and 670 ppm doses of CO₂. The duration of stay on the nutrients of male individuals who received 600 ppm and 670 ppm doses of CO₂ was found to be significantly lower than the duration of stay on the nutrients of female individuals. The duration of stay on the nutrients of male individuals who received 670 ppm doses of CO₂ was found to be significantly lower than the duration of stay on the nutrients of female individuals. No statistically significant difference was found between the CO₂ dose groups of *N. viridula* and the total feeding times of the control group. A statistically significant difference was found between the CO₂ dose groups of *N. viridula* and the duration of stay apart from the nutrients and the duration of stay on the nutrients of the control group. The duration of stay on the nutrients of individuals exposed to 600 and 670 ppm CO₂ doses was found to be significantly lower than the duration of stay on the nutrients of individuals in the control group. In addition, the duration of stay apart from the nutrients of the individuals to whom 600 and 670 ppm CO₂ doses were applied was found to be significantly higher than the duration of stay apart from the nutrients of the individuals in the control group. As a general result of this study, it was determined that as the application dose increased, the feeding behaviors of male and female individuals changed, male individuals reacted more to dose administration and remained on nutrients less than female individuals.

Keywords: *Nezara viridula*, Feeding Behaviors, Intermittent CO₂

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INTRODUCTION

Southern green stink bug, *Nezara viridula* (Linnaeus, 1758) (Hemiptera) (Southern green stink bug), one of the important pests belonging to the Pentatomidae family, is a polyphagous and cosmopolitan pest (Figure 1). *N. viridula*, which has spread to many parts of the world, is spreading to new areas because it is well adapted to changing climatic conditions and has the ability to fly well (Lye and Story, 1988; Panizzi, 1997; Çetin and Karsavuran 2000; Birgücü, 2012). This pest, which is widely found in our country (Lodos, 1986; Lodos et al.,

1998), can be suppressed by its natural enemies under normal conditions. In addition to legumes, the pest is also fed on tomatoes, peppers, beans, hemp, cotton, soy, sesame, alfalfa, tobacco, nuts, citrus fruits, other fruit trees and many wild and cultivated plants (Lodos, 1986; Lodos et al., 1998). With unconscious agricultural pest control practices, it causes serious economic losses in areas where there are no natural enemies. In an environment where there is no natural enemy pressure, the pest population causes significant economic damage (Todd, 1989). With global warming, it is thought that the populations of pests will increase in the next 50 years. Because; atmospheric gases have significant effects on harmful-hostile and natural enemy species. CO₂ has an important place in these gases. How and to what extent the change effects of the amounts of carbon dioxide with global warming will be determined should be determined by sample simulation studies. In the coming years, changes in atmospheric gas levels, especially with temperature change, will cause pests to form different biotopes. These biotopes are expected to increase their damage status by being affected by physiological changes in the plant (Vailla et al., 2019). This study was conducted to determine the effects of CO₂ applied in three different doses to *N. viridula* adults reared under laboratory conditions on the adult feeding behaviors.



Figure 1. *Nezara viridula* adults being reared

MATERIALS AND METHODS

Mass Rearing of *Nezara viridula* and Determination of Feeding Behaviors

Insect rearing and trials were carried out in a climate room prepared according to conditions with a temperature of $25\pm 1^{\circ}\text{C}$, a humidity of $45\pm 5\%$, and a lighting time of 16:8 hours (Figure 2). The cultures were fed with tomato, tobacco and *Datura* seeds, sunflower seeds, green beans, peanuts and soya beans.



Figure 2. Mass rearing conditions of *Nezara viridula*

In the study, a total of 30 adult individuals, 5 adult males and 5 preoviposition females and 5 adult (female/male) controls, were used in *Nezara viridula* adults for each dose. The experiments were repeated with 10 replicates for each of the three CO₂ doses including the control treatment and 30 individuals for each of the three periodic feeding observations, totalling 90 replicates. Individuals were observed by administering carbon

dioxide in two different doses (600 and 670 ppm) in the CO₂ unit (Figure 3) where controlled conditions were provided. The applications were compared with the control (430 ppm), and the nutritional behaviors of the adults were observed and recorded in the cage within 1 hour in the observations made through the camera. Observations were recorded in seconds (3600 seconds). During the observation period, the duration of stay apart from the nutrients, the duration of stay on the nutrients and the total feeding time of the individuals were determined separately by the camera and eye control placed in the cabin in seconds (sec.).



Figure 3. Carbondioxide unit of applications

The Periods Determined for Nutritional Behavior;

Duration of Stay Apart From The Foods: The time taken for activities of *Nezara viridula* individuals such as waiting, resting, and foraging,

Duration of Stay on The Foods: The time elapsed until it extends its hose to the nutrients and/or leaves the nutrients without being fed on the nutrients,

Total feeding time: The time it takes from the moment it starts to extend vertically to insert its hose into the nutrients until it pulls its hose out of the nutrients

Statistical Analysis

"Shapiro-Wilk Goodness-of-Fit Test and Kolmogorow-Smirnov test" were used to test whether the obtained data fit the normal distribution. Descriptive statistics such as mean, median and standard deviation are given in the data in the study. Since it does not meet the normality assumptions, Mann Whitney U test was used to compare binary groups, and Kruskal Wallis Analysis was used to compare more than two groups. In all analyses, the statistical significance level was accepted as $p < 0.05$. Statistical analyzes were performed using SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) 22 package program.

RESULTS AND DISCUSSION

Statistical analyzes of the distribution of the duration of *Nezara viridula* adult individuals on and apart from the nutrients at two different carbon dioxide concentrations in a 1-hour period according to male and female individuals are shown in Table 1.

Table 1. Statistical comparisons of different feeding behaviors of *Nezara viridula* adult individuals

	Dosage (ppm)	Gender	N	Mean (sec.)	Std. Deviation	Median	U	P value
Total Feeding Time	600	Male	5	1430,00	74,161	1410,26	20,00	0,117
		Female	5	1552,43	126,222	1580,50		
	670	Male	5	1316,28	90,229	1350,30	19,00	0,173
		Female	5	1390,36	102,901	1420,30		
Duration of Stay On The Foods	600	Male	5	150,63	9,587	151,30	25,00	0,009
		Female	5	177,55	8,742	179,44		
	670	Male	5	142,90	9,378	142,20	25,00	0,009
		Female	5	182,39	6,983	181,40		
Duration of Stay Apart From the Foods	600	Male	5	823,67	6,934	820,66	16,00	0,548
		Female	5	828,26	12,548	832,36		
	670	Male	5	824,06	13,219	822,50	22,00	0,047
		Female	5	843,25	9,915	842,50		

When Table 1 was examined, no statistically significant difference was found between the female and male groups of *N. viridula* in terms of total feeding times ($p>0.05$). A statistically significant difference was found between the female and male individuals of *N. viridula* in terms of the duration of stay on the nutrients and the duration of 670 ppm apart from nutrients ($p<0.05$). The duration of stay on the nutrients of male individuals who received 600 ppm and 670 ppm carbon dioxide doses was significantly lower than the duration of stay on the nutrients of female individuals ($p<0.05$). The duration of stay apart from the nutrients of male individuals who received 670 ppm carbon dioxide doses was significantly lower than the duration of stay apart from the nutrients of female individuals ($p<0.05$).

The statistical results showing the differences in the duration of *Nezara viridula* adult individuals on and apart from nutrients at two different carbon dioxide concentrations in a 1-hour period compared to the control are shown in Table 2.

Table 2. Statistical Comparison of Different Nutritional Behaviors of *Nezara viridula* Adult Individuals with Control Applications

	Groups	n	Mean (sec.)	Std. Deviation	Median	H	Pvalue
Total Feeding Time	Control	10	1772,50	58,27	1780,50	21,399	0,117
	D600	10	1491,22	116,99	1495,40		
	D670	10	1353,32	99,24	1365,40		
Duration of Stay On The Nutrients	Control	10	203,06	30,53	196,25	8,715	0,013
	D600	10	164,09	16,62	164,23		
	D670	10	162,64	22,22	164,35		
Duration of Stay Apart From The Nutrients	Control	10	738,56	14,62	734,45	20,042	0,000
	D600	10	825,96	9,86	825,50		
	D670	10	833,65	14,95	833,55		

When Table 2 was examined, the differences between the total feeding times of *N. viridula* were not statistically significant according to the application groups ($p>0.05$). The differences between the duration of stay on the nutrients of pests were found to be statistically significant according to the application groups ($p<0.05$). It was observed that the duration of stay apart from the nutrients of pests was statistically different according to the application groups ($p<0.05$).

Boxplot graphs demonstrating the differences between control and dose applications of the pest are shown in Figure 4-6.

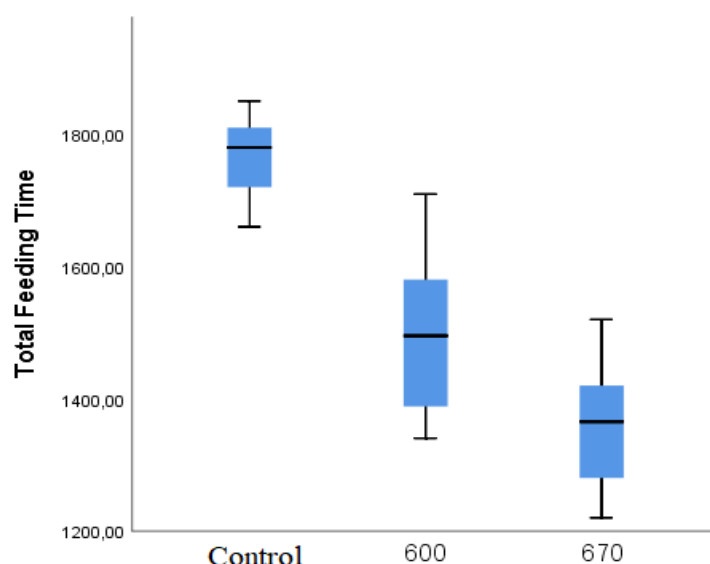


Figure 4. Distribution of total feeding times of *Nezara viridula* individuals exposed to doses of 600 and 670 ppm carbon dioxide and not treated with carbon dioxide (control).

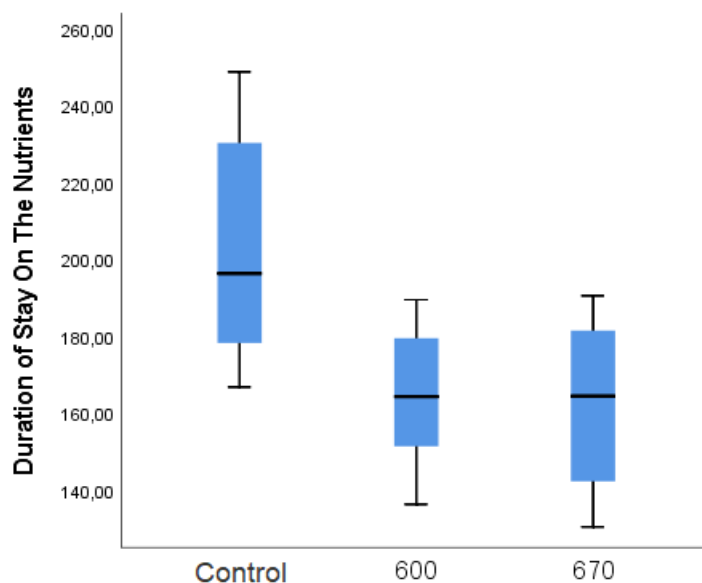


Figure 5. Distribution of duration of stay on the nutrients of *Nezara viridula* individuals exposed to doses of 600 and 670 ppm Carbon Dioxide and not treated with carbon dioxide (control).

When the pairwise comparisons are examined, it is seen that the duration of stay on the nutrients of the individuals exposed to 600 and 670 ppm application doses is significantly lower than the duration of stay on nutrients of the individuals in the control group ($p < 0.05$) (Figure 5).

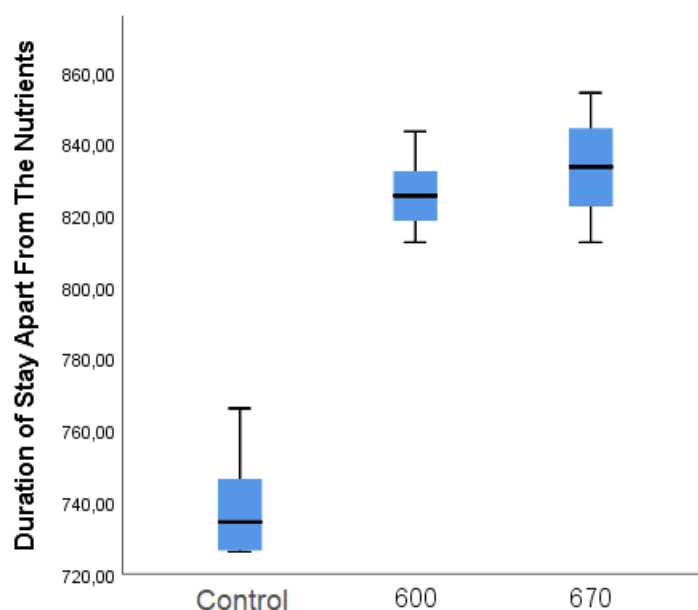


Figure 6. Distribution of the duration of stay apart from nutrients of *Nezara viridula* individuals exposed to 600 and 670 ppm doses of carbon dioxide and not treated with carbon dioxide (control).

Considering the pairwise comparisons, the duration of stay on the nutrients of the individuals to whom 600 and 670 ppm application doses were applied was found to be significantly higher than the duration of stay apart from the nutrients of the individuals in the control group ($p < 0.05$) (Figure 6).

CONCLUSION

In a study conducted by Birgüçü and Karsavuran (2013) on the nutritional behavior of *Nezara viridula* in bean pods, it was reported that the duration of stay on the nutrients of male individuals from the plant in their adult periods was lower than that of female individuals in the oviposition period, and the duration of stay on the plant was higher. However, they stated that the total feeding time of the pest was longer than the duration of stay apart from the nutrients and the duration of stay on the plant in all biological periods. In a study conducted by

Yiğit (1988) on the main nutritional characteristics of *Liorhyssus hyalinus* (F.) adults, it was reported that the active feeding time of male individuals in the hibiscus plant of the pest constituted 19.39% of the observation time and female individuals constituted 18.97%. In addition, in the same study, it was stated that the observation period of the active nutrition of male and female individuals constituted 14.98% and 22.13%, respectively, on the wild lettuce plant.

In a study conducted on adult individuals of *Halyomorpha halys* Stål (Pentatomidae), *Riptortus clavatus* Thunberg (Alydidae), *Nezara antennata* Scott (Pentatomidae) and *Piezodorus hybneri* Gmelin (Pentatomidae) belonging to the Hemiptera team, the nutritional behaviors of the pests on the soybeans of male and female units were observed. According to the results of this study, in which the duration and frequency of daily feeding were examined separately for each species, adult female individuals of *H. halys* performed feeding activities 3.65 times a day. The pest was fed for 1.70 h in each feeding activity. The total daily feeding time of female individuals is 6.20 hours. Male individuals, on the other hand, performed feeding activities 2.71 times a day and showed nutritional behavior for 1.99 hours in each nutritional activity. The total daily feeding time of male individuals is 5.39 hours. Adult female individuals of *R. clavatus* performed feeding activities 2.65 times a day and the pest was fed for 2.02 h in each feeding activity. The total daily feeding time was determined as 5.36 hours. Male individuals, on the other hand, performed nutritional activities 4.00 times a day and performed nutritional behavior for 2.13 hours in each nutritional activity. The total daily feeding time of male individuals is 8.50 hours. Adult female individuals of *N. antennata* performed feeding activities 3.80 times a day and it was observed that the pest was fed for 2.97 hours in each feeding activity. The total daily feeding time was 11.29 hours. Male individuals, on the other hand, performed nutritional activities 4.00 times a day and the pest was fed for 4.04 hours in each nutritional activity. The total daily feeding time of male individuals is 16.17 hours. Adult female individuals of *P. hybneri* were fed 2.67 times a day for 3.94 hours in each feeding activity. The total daily feeding time is 10.52 hours. Male individuals, on the other hand, performed nutritional activities 4.50 times a day and the pest has engaged in nutritional behavior for 2.89 hours in each nutritional activity. The total daily feeding time of male individuals is 13.00 hours (Kawamoto et al., 1987).

According to the results of a study conducted by Birgücü and Karsavuran (2013) on the nutritional behavior of *N. viridula*, it was observed that the nutritional duration of the pest was 16.97 h when calculated from the total nutritional duration of adult male individuals within one hour of observation. Daily feeding times of female individuals in the preoviposition and oviposition period were determined as 16.46 h and 15.02 h, respectively. In this case, it is possible to say that adult male and female individuals of *N. viridula* feed more in a day than the pests in the study conducted by Kawamoto et al. (1987) and that adult individuals of *N. viridula* show more frequent feeding activities in a day. In addition, adult individuals of *N. viridula* showed a total feeding time of 1,924.92 seconds (0.53 h), with an initial period of 25.82 seconds (0.007 h), probing time of 32.54 seconds (0.009 h), and active feeding time of 1,866.59 seconds (0.51 h) in a single repetition. Accordingly, it can be said that adult individuals of *N. viridula* fed for less time at a single time than the pests in the study conducted by Kawamoto et al. (1987). All these results support the opinion that *N. viridula* is one of the most harmful species among pentatomids (Lodos et al., 1978).

As a general result of this study, it was determined that as the application dose increased, the nutritional behaviors of male and female individuals changed, male individuals reacted more to dose administration and remained on nutrients less than female individuals.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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