J.Het.Turk.
Journal of the Heteroptera of Turkey
ISSN 2687 - 3249 (online edition)
May, 2025
Volume 7, Issue 1

Research Article

Evaluation of Feeding Behavior of Carbon Dioxide-Applied *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Heteroptera: Pentatomidae) Using Response Surface Methodology

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ABSTRACT: In this study, a new air conditioning chamber was developed and different carbon dioxide concentrations were applied to examine the feeding behavior of *Nezara viridula*. The feeding behavior of individuals was observed at the concentrations different from normal atmospheric conditions with the response surface methodology. With this method, the duration of individuals staying away from food, the duration of staying on food, and the total feeding time were evaluated. According to the results obtained, it was observed that the total feeding time of individuals decreased with the increase in carbon dioxide concentration. It was determined that the duration of individuals staying on food decreased as the concentration raised in female and male individuals. The duration of individuals staying away from food increased in both sexes as the carbon dioxide concentration increased. When the response surface methodology results were evaluated statistically, it was understood that the total feeding time of male individuals with carbon dioxide application was compatible with the model values and real data. According to the applied the model, the correlation (determination) coefficients for adult male individuals were found to be 0.9935, 0.9854, and 0.9719, respectively. The model's quadratic prediction suggests that at approximately 430 ppm, *Nezara viridula* males achieve optimal feeding behavior with 1809 seconds of total feeding, 178 seconds on food, and 730 seconds off food, identifying 430 ppm as the most suitable concentration

To cite this article: Özgen, İ., Aydoğmuş, E., 2025, Evaluation of Feeding Behavior of Carbon Dioxide-Applied *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Heteroptera: Pentatomidae) Using Response Surface Methodology, *J.Het.Turk.*, 7(1):161-178
DOI: 10.5281/zenodo.15313361
To link to this article: https://www.j-ht.org/wp-content/uploads/2025/05/V71-A6.pdf
Received: Nov 29, 2024; **Revised:** Apr 12, 2025; **Accepted:** Apr 13, 2025; **Published online:** May 7, 2025

under optimal conditions. According to this model, the determination coefficients for adult females were found to be 0.9426, 0.9262, and 0.8666. According to the model, the optimum carbon dioxide concentration for females was determined to be 430 ppm, with optimal results including a total feeding time of 1860 seconds, 214 seconds on food, and 729 seconds off food.

KEYWORDS: Air conditioning chamber, Carbon dioxide concentration, Feeding behavior, *Nezara viridula*, Response surface methodology

INTRODUCTION

Over the past 50 years, rising atmospheric carbon dioxide (CO_2) levels have significantly influenced plant physiology and interactions with herbivorous insects. Elevated CO_2 enhances photosynthesis and carbohydrate accumulation while reducing nitrogen content, potentially altering plant nutritional quality and defense mechanisms. Studies indicate that high CO_2 levels suppress jasmonic acid (JA) accumulation but increase salicylic acid (SA), weakening plant resistance to certain herbivores like *Helicoverpa armigera*. Additionally, increased CO_2 can affect insect feeding behavior, growth, reproduction, and survival rates. While some insects, such as Popillia japonica and *Diabrotica virgifera virgifera*, benefit from these conditions, others experience reduced growth rates due to lower nitrogen availability in plants. The effects of elevated CO_2 on aphids vary, with increased metabolism and feeding rates but inconsistent impacts on development time. These findings suggest that rising CO_2 levels could necessitate adjustments in crop management strategies, such as planting and harvesting schedules, to mitigate pest-related risks (Küçük, Tunca, 2024).

Nezara viridula is one of the important pests belonging to the Pentatomidae family and is a polyphagous and cosmopolitan pest. *N. viridula*, which has spread to many parts of the world, is spreading to new areas due to its good adaptation to changing climatic conditions and good flying ability (Panizzi, 1997; Çetin, Karsavuran, 2000). *N. viridula*, commonly known as the green stink bug, is a species belonging to the Hemiptera order. This insect, which has a cosmopolitan distribution, is found in-tensively in tropical and subtropical regions, and can also be found in temperate climate zones. Known as a pest of high agricultural importance, *N. viridula* is a polyphagous with the capacity to feed on a large number of plant species, and this feature draws attention. It is an important subject of study both biologically and economically due to the damage it causes to agricultural products (Birgücü, Karsavuran, 2013 ; Ren et al., 2022).

The biological characteristics of this species, the expansion of its distribution areas and its ability to adapt to environmental factors create serious problems in agricultural ecosystems. *N. viridula* is a direct pest of economically important agricultural products such as beans, corn, cotton, tomatoes and soybeans. It sucks sap by piercing plant tissues during feeding, which leads to product loss, loss of quality and the transmission of diseases. This species, which is especially sensitive to environmental parameters such as temperature and humidity, has the potential to spread to new areas with global climate change (Subhasree, 2013).

N. viridula is a bright green insect, approximately 12-15 mm long. Its body structure is pentagonal and its antennae usually have black and white bands. Its eggs are usually round and cream colored, and are laid in clusters under the leaves by the females. This species, which shows different coloration and patterns in the nymphal stages, undergoes various biological changes throughout its life cycle. This situation provides important clues in the identification of the species and the development of biological control methods (Barrion, Litsinger, 1994).

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N. viridula, which is widespread throughout the world, is an important focus in integrated pest management strategies. Efforts are being made to reduce the impact of this species with methods such as biological control, cultural practices and chemical control. In particular, parasitoid wasps can control the populations of this insect as natural enemies and provide balance in the ecosystem. However, more sustainable and environmentally friendly approaches are needed due to problems such as pesticide resistance (Knight, Gurr, 2007).

N. viridula poses a serious threat to agricultural ecosystems with its wide host range, high reproductive capacity and rapid adaptation ability. Studies on the biology, behavior and control methods of the species are of great importance, especially for the sustainability of agriculture. In this context, as the life cycle of the species and its responses to environmental factors are better understood, it will be possible to develop effective control strategies (Karpun et al., 2022).

This pest, which is widely found in our country, can be suppressed by its natural enemies under normal conditions. In addition to harmful legumes, it also feeds on tomatoes, peppers, beans, hemp, cotton, soy, sesame, clover, tobacco, hazelnut, citrus fruits, other fruit trees, and many wild and cultivated plants. Unconscious agricultural pest control practices cause 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 losses (Todd, 1989).

It is thought that the populations of pests will increase in the next 50 years with global warming. Atmospheric gases have significant effects on pest-enemy and natural enemy species. Carbon dioxide gas has an important place among these gases. In their study on the effects of different CO_2 doses on *N. viridula* feeding behaviors, Ozgen et al.,(2024)_ stated that the feeding behaviors of male and female individuals changed as the application dose increased. Adult male individuals reported that they responded more to CO_2 application and stayed on the food less than female individuals (Ozgen et al., 2024). It is necessary to determine how and to what extent the effects of carbon dioxide amounts changing with global warming will be in different species with sample simulation studies. Because in the coming years, especially with temperature changes, there is a possibility that pests will form different biotopes due to changes in atmospheric gas levels. It is expected that these biotopes will increase their damage status by being affected by physiological changes in the plant (Vailla et al., 2019).

The extent to which different feeding behaviors are affected by atmospheric gases and at which CO_2 concentrations optimum feeding behaviors specific to the species occur will be examples for different simulation studies. In this research, the effects of three different CO_2 concentrations on adult feeding behaviors of *N. viridula* adults reared under laboratory conditions were evaluated with RSM. This method evaluates the effect of one or more independent variables (factors) on the dependent variable (response) and the optimum conditions. The experimental design type commonly used in RSM was simulated for this study according to the central composite design (CCD) and Quadratic model.

MATERIALS AND METHODS

In this study, *N viridula* individuals were fed and reared using green beans (50 wt.%), sunflower seeds (10 wt.%), peanuts (20 wt.%), tobacco (15 wt.%), and tomato seeds (5 wt.%) as food . A total of 15 adult individuals were used in the climate chamber for different CO_2 application concentrations, including 5 adult males, 5 pre-

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oviposition females, and 5 adult (female/male) controls (Figure 1). The experiments were carried out at three CO_2 concentration applications, 430 ppm (control), 600 ppm, and 670 ppm. Considering the general CO_2 averages of the provinces in Türkiye's conditions, 430 ppm was selected as control. Considering the increasing CO_2 ratios in the next century, maximum application concentrations of 600 and 670 ppm were preferred. A total of 900 repetitions were made for 15 individuals, with 10 repetitions and for each experiment. Individuals were observed at different CO_2 concentrations (430, 600, and 670 ppm). The treatments were compared with the results in the control (430 ppm) CO_2 gas atmosphere. The feeding behaviors of the adults were recorded in 1-h periods by using a sensitive camera in the carbon dioxide chamber. Observations were recorded in seconds (3600 s). During the observation, the time individuals were away from food, the time they remained on food, and the total feeding time were determined separately in seconds (s) by a camera placed in the cabin and eye control.

Figure 1. Feeding conditions of *N. viridula* and CO_2 application chamber system

N. viridula Feeding Behavior Times

The time spent by *N. viridula* individuals for activities such as waiting, resting, and searching for food is defined as the time spent away from food. The time spent on food is defined as the time spent by individuals extending their proboscis to the food and/or leaving the food without taking the food. The total feeding time is defined as the time spent by individuals from the moment they start extending their proboscis vertically to insert their proboscis into the food until they withdraw their proboscis from the food.

RESULTS AND DISCUSSIONS

Response Surface Methodology (RSM) used in this study is based on the Central Composite Design (CCD), a widely applied statistical technique for optimizing processes and modeling complex relationships between independent variables and response variables. CCD is particularly effective for exploring quadratic effects and interactions between factors, making it suitable for experiments where a nonlinear response is expected. This design consists of factorial points, axial points, and a

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central point, ensuring a well-balanced distribution of experimental runs. One of the main advantages of CCD is its ability to provide accurate predictive models while minimizing the number of experimental trials, thus reducing cost and time. The model generated through this design helps identify optimal conditions by analyzing response variations and evaluating significant parameters. Furthermore, the adequacy and reliability of the model are assessed using statistical measures such as R^2, adjusted R^2, and adequate precision, ensuring that the predictions align closely with experimental results (Özgen et al., 2024)

According to the RSM, the change in total feeding time with carbon dioxide application for adult males is shown in Figure 2. It is understood that the total feeding time of individuals decreases with the increase in carbon dioxide concentration. Figure 3 shows the change in total feeding time with carbon dioxide application for adult females. Similarly, it was determined that the total feeding time decreases as the concentration increases in carbon dioxide applications.

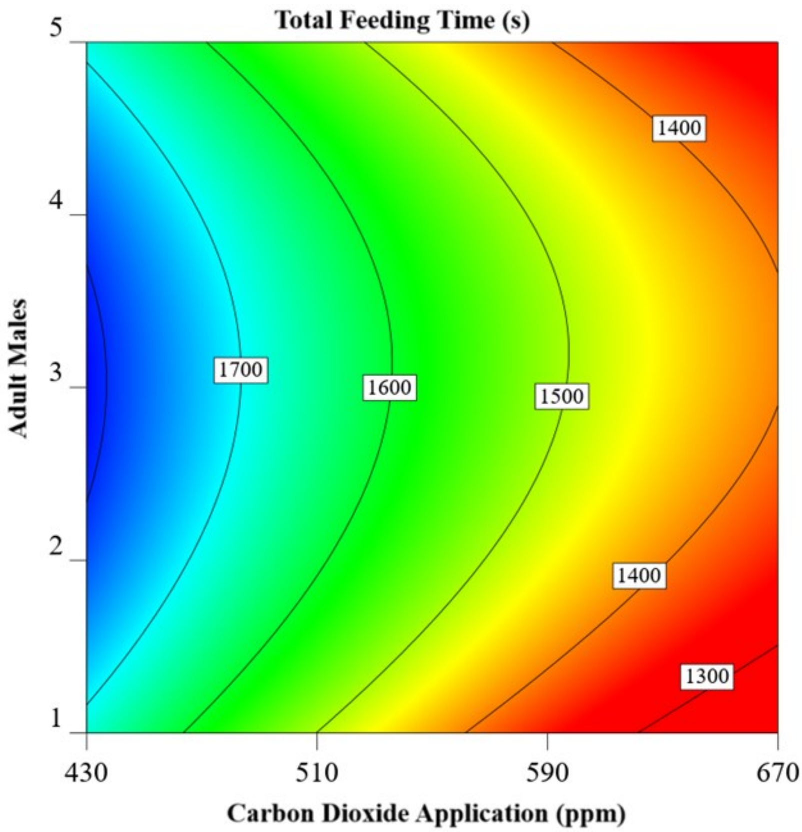

Figure 2. Change in total feeding time with carbon dioxide application for adult males

Figure 4 shows the change in the duration of adult males on food with carbon dioxide application. It is seen that increasing the CO_2 concentration from 430 ppm to 670 ppm reduces the duration of adult males on food.

Figure 5 shows the change in the duration of adult females on food with carbon dioxide application. Similarly, as the CO_2 concentration increases in females, the duration of individuals on food decreases.

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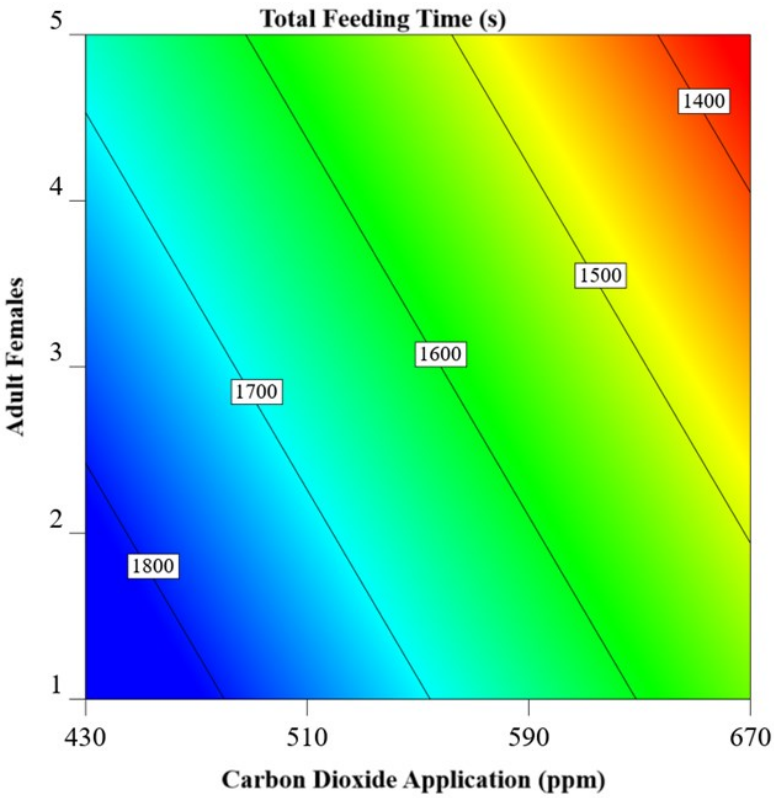

Figure 3. Change in total feeding time with carbon dioxide application for adult females

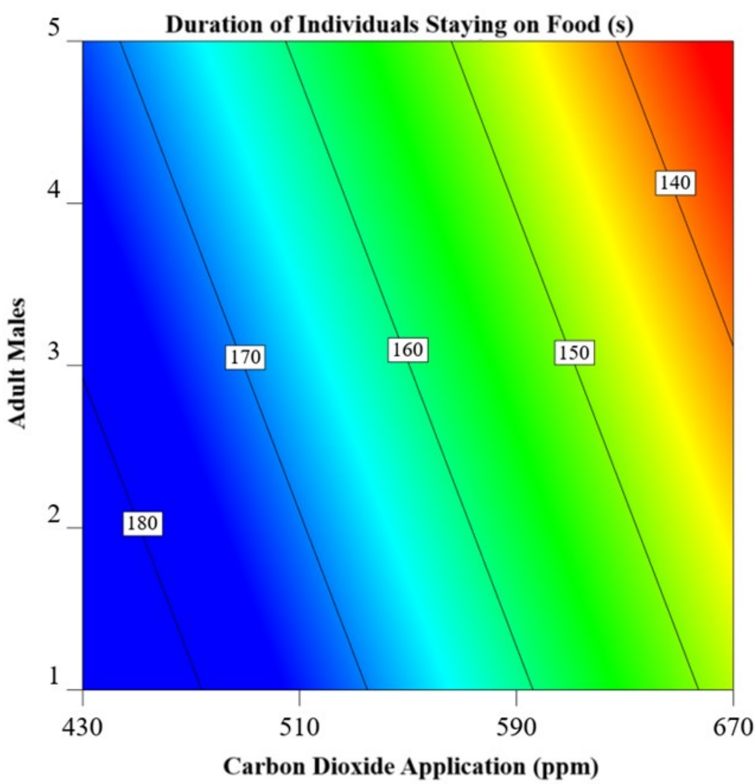

Figure 4. Change in the duration of food retention by CO_2 application for adult males

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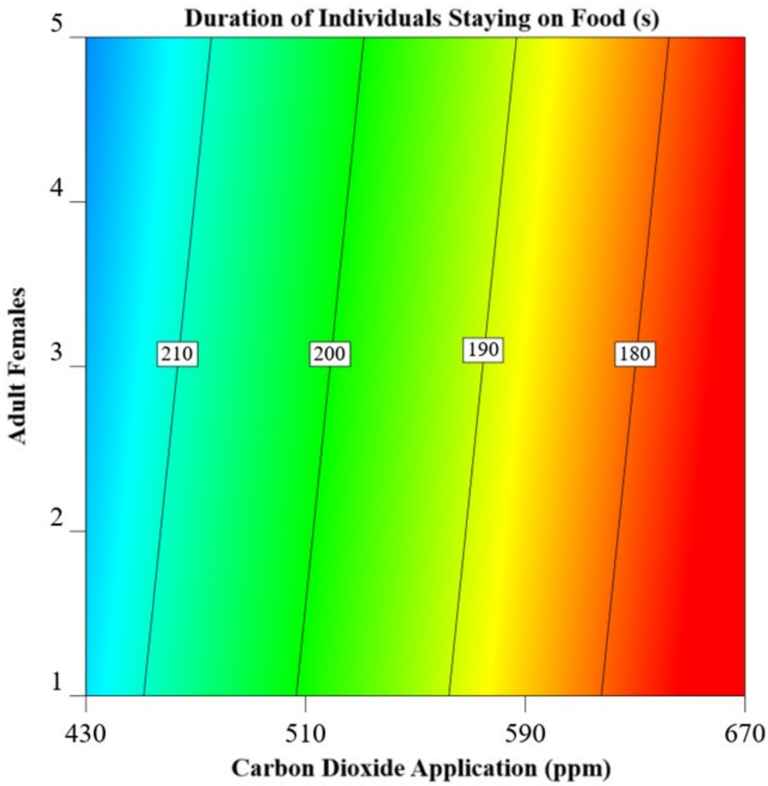

Figure 5. Change in the duration of food retention with CO_2 application for adult females

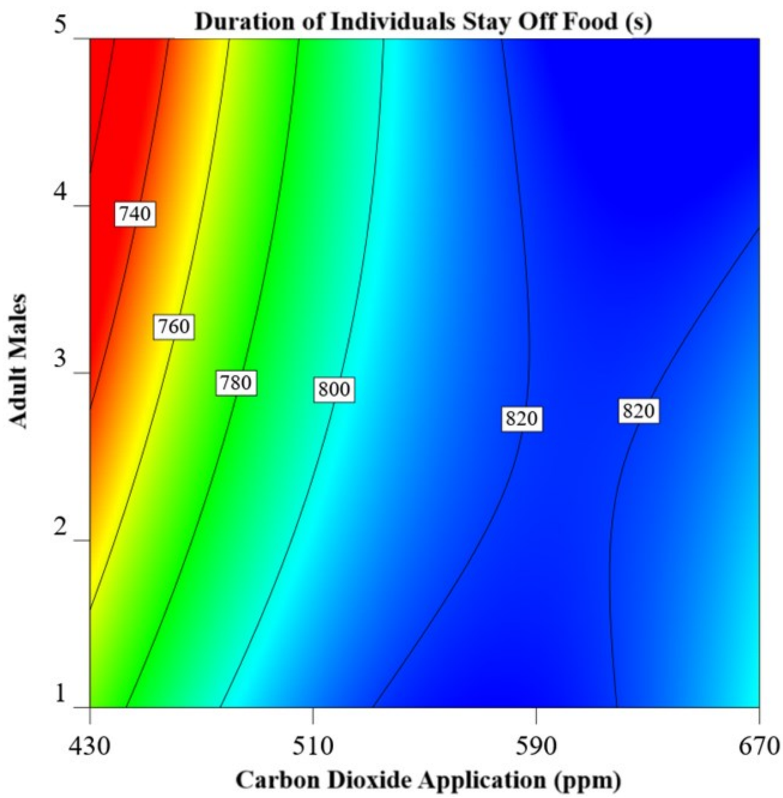

Figure 6. Change in the duration of food exclusion by CO_2 application for adult males

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Figure 6 shows the change in the duration of food exclusion with carbon dioxide application for adult males. As the carbon dioxide concentration increases, the duration of food exclusion increases. Figure 7 shows the change in the duration of food exclusion with carbon dioxide application for adult females. Similarly, as the CO_2 concentration increases from 430 ppm to 670 ppm in adult females, the duration of food exclusion raises.

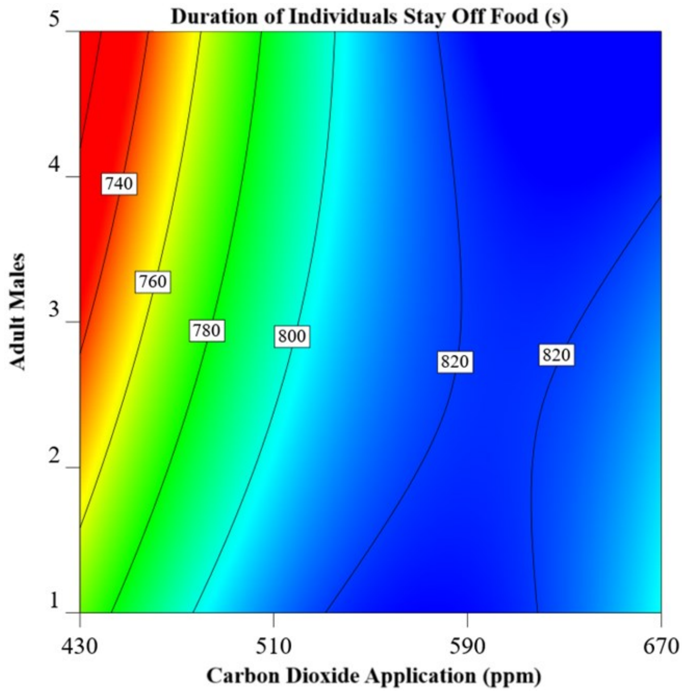

Figure 6. Change in the duration of food exclusion by CO_2 application for adult males

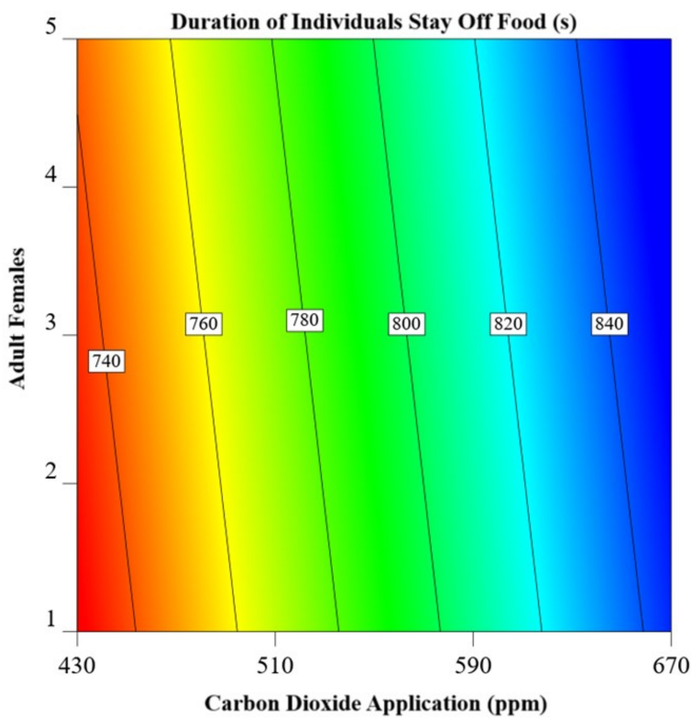

Figure 7. Change in the duration of food exclusion with CO_2 application for adult females

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Statistical Analysis of RSM Results

Within the scope of response surface methodology (RSM), variance analysis (*ANOVA*) is mostly used to evaluate the results statistically. *ANOVA* tests whether the model has a significant effect on the dependent variable and helps to determine which factors and interactions are important. In RSM, the F-value, which tests the general significance of the model, is examined with *ANOVA* analysis. A high F-value and a low p-value (generally $p < 0.05$) indicate that the model is significant. In addition, the coefficient of determination (R^2) and adjusted R^2 value of the model express the explanatory power of the model. These values show how much data fits the model. The *ANOVA* output also evaluates the significance status of the factors and factor interactions with p-values, revealing which variables have a strong effect on the response variable.

Statistical Analysis for Adult Male Individuals According to RSM

Figure 8 shows that the total feeding time of male individuals with carbon dioxide application is consistent with the RSM model and real data.

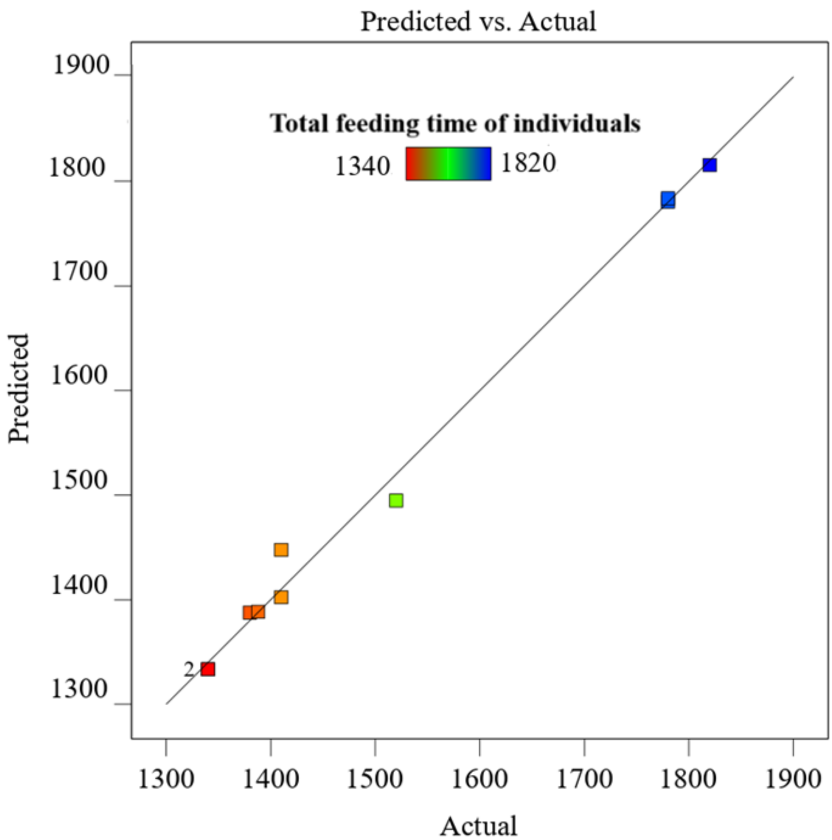

Figure 8. Compatibility of total feeding time of male individuals with CO_2 application between RSM model and real data

Figure 9 shows the distribution of the total feeding time of male individuals with CO_2 application. Figure 10 shows the distribution of the time male individuals stayed on food with carbon dioxide application. Figure 11 shows the distribution of the time male individuals stayed off food with CO_2 application. It is understood that individuals showed a distribution especially at 430 ppm, 600 ppm, and 670 ppm carbon dioxide concentrations.

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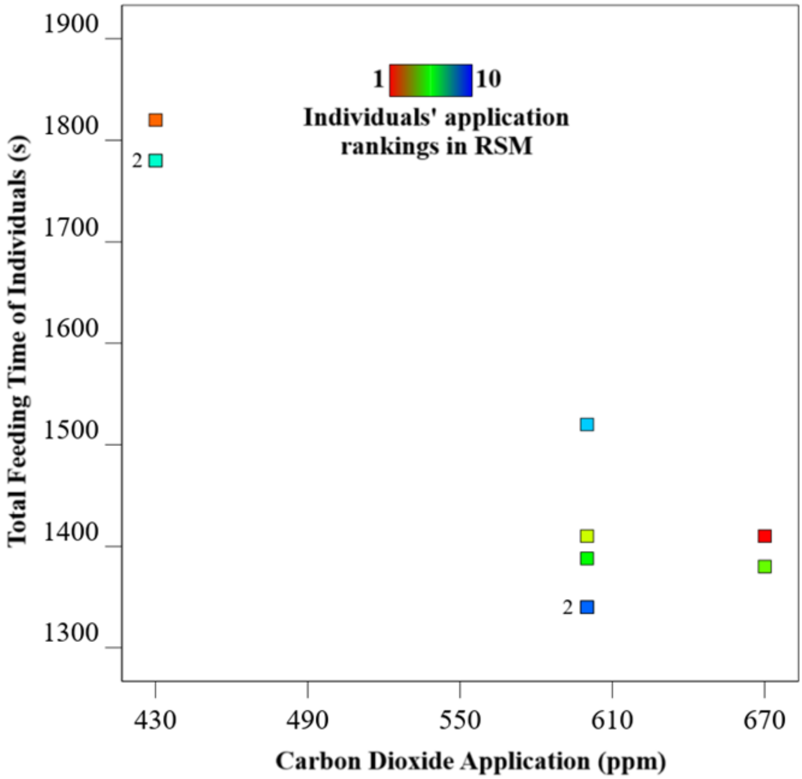

Figure 9. Distribution of total feeding time of male individuals with CO_2 application

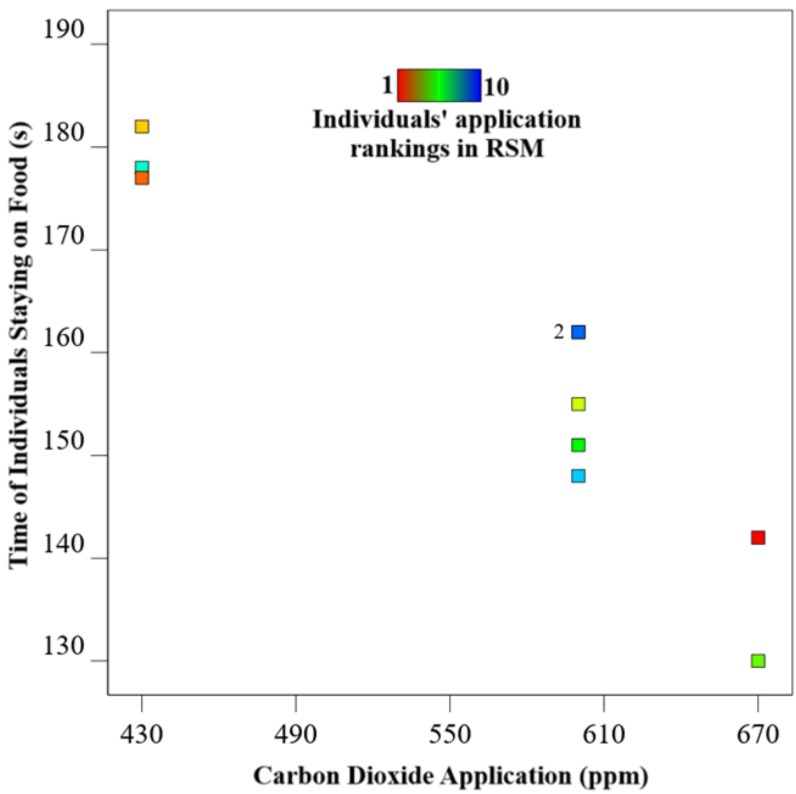

Figure 10. Distribution of the time of male individuals staying on the food by CO_2 application

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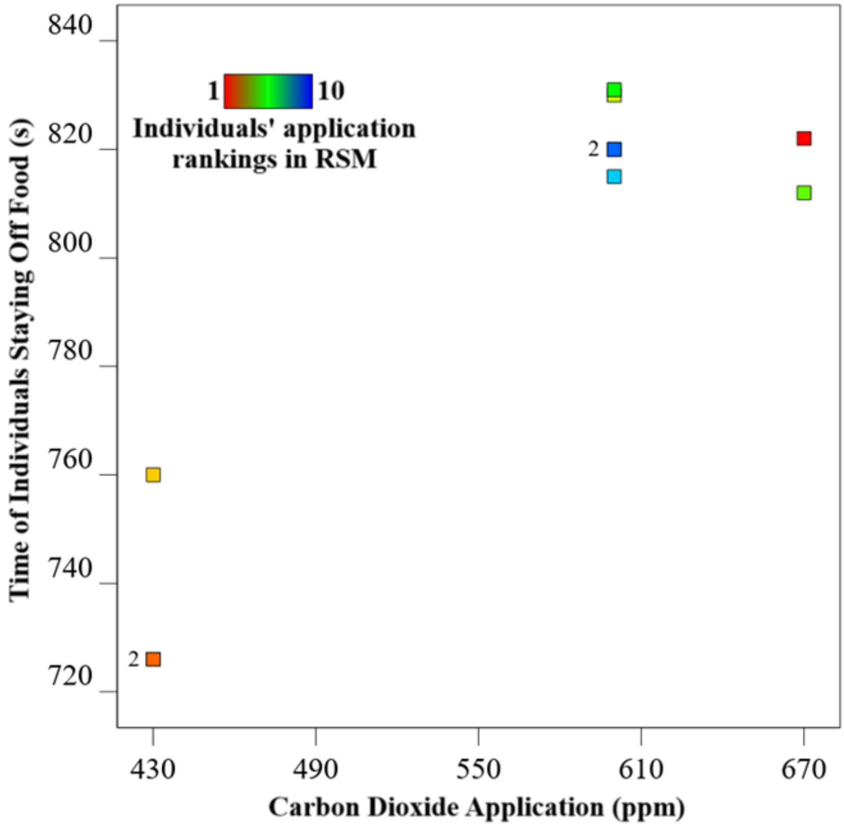

Figure 11. Distribution of time of male individuals remaining without food by the application

The correlation coefficients, standard deviations and other statistical results of *N. viridula* males according to the RSM model. ANOVA results and the significance of the RSM model are also expressed. Carbon dioxide application and adult male parameters were found to be significant according to the RSM model (p-value <0.05). The presents the correlation coefficients, standard deviations, and other statistical outcomes for *N. viridula* males based on the RSM model. The results indicate a strong agreement between the actual experimental data and the predicted values generated by the model. The standard deviation of the data was found to be 23.90, and the coefficient of determination (R^2) was calculated as 0.9935, suggesting that 99.35% of the variation in the response is explained by the model. The adjusted R^2 value of 0.9854 further confirms the model's high accuracy by accounting for the number of independent variables used in the analysis. The coefficient of variation (C.V. %) was recorded as 1.58%, indicating a low level of variability relative to the mean value of 1516.80. Additionally, the predicted R^2 value of 0.9719 demonstrates strong predictive capability, while the adequate precision value of 26.0432 confirms that the model provides a robust signal-to-noise ratio, making it reliable for further analysis.

ANOVA (Analysis of Variance) results, which assess the statistical significance of the RSM model. The model itself was found to be highly significant, with an F-value of 122.17 and a p-value of 0.005, indicating a strong correlation between the independent variables and the response. The effect of carbon dioxide application (Factor A) was particularly significant, with a p-value of less than 0.002 and an F-value of 334.95, demonstrating its substantial influence on the response variable. However, the adult

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male individual parameter (Factor B) was not found to be statistically significant ($p = 0.2242$), suggesting that its contribution to the model was relatively weak.

The interaction term (AB) and the quadratic term for Factor A (A^2) also showed no statistical significance, while the quadratic term for Factor B (B^2) was found to be significant ($p = 0.0048$), indicating a non-linear effect of this factor on the response. The residual sum of squares was 2284.36, with a mean square value of 571.09, further confirming the model's accuracy. Additionally, the lack of fit was not considered problematic, as the pure error was recorded as 0.0001. These results highlight that the RSM model effectively captures the relationship between carbon dioxide application and *N. viridula* males, with Factor A playing a dominant role in influencing the response.

Table 1. The model suggested by RSM for *N. viridula* males

Source	Sum of Squares	Mean Square	F-value	p-value	
Mean vs Total	2.301E+07	2.301E+07			
Linear vs Mean	3.127E+05	1.563E+05	28.45	0.0019	
2FI vs Linear	2295.61	2295.61	0.3809	0.5598	
Quadratic vs 2FI	**33880.58**	**16940.29**	**29.66**	**0.005**	**Suggested**
Cubic vs Quadratic	2284.36	761.45			Aliased
Residual	0.0000	0.0000			
Total	2.336E+07	2.336E+06			

Table 2. Optimum CO_2 application results for *N. viridula* males according to the RSM model

No	CO_2 Application (ppm)	Total Feeding Time (s)	Time on Food (s)	Time off Food (s)	Desirability
1	**430**	**1809.220**	**177.984**	**729.912**	**0.954**
2	432	1809.337	177.999	729.969	0.953
3	431	1808.899	177.944	729.759	0.951
4	430	1809.724	178.050	730.162	0.952
5	433	1808.464	177.890	729.557	0.950
6	432	1810.130	178.104	730.370	0.953
7	430	1810.381	178.139	730.504	0.952
8	431	1807.730	177.804	729.232	0.951
9	430	1810.908	178.215	730.795	0.952
10	433	1811.268	178.270	731.005	0.954

Statistical Analysis Results of Adult Female Individuals According to RSM

The response surface methodology (RSM) was employed to analyze the impact of carbon dioxide applications on *N. viridula* females.

A Central Composite Design (CCD) was selected as the experimental framework, which is a commonly used design for optimizing and modeling quadratic relationships

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between variables. The study followed a randomized approach with a total of 10 experimental runs to ensure accuracy and minimize bias.

The quadratic model was chosen for the analysis, as it allows for a more precise estimation of the interactions and curvatures in the response.

Additionally, the study was conducted without blocks, and the computational build time for the model was recorded as 1 millisecond, indicating high efficiency in data processing.

Figure 12 shows the compatibility between the actual data and the RSM model values for the duration of female individuals staying off food. Figure 13 shows the percentage distribution probability of the duration of female individuals staying off food according to RSM.

According to these results, it is understood that there is an agreement between the model data and the actual value. Figure 14 shows the distribution of the total feeding time of female individuals by CO_2 application. Figure 15 shows the distribution of the duration of female individuals staying on food with carbon dioxide application. Besides, Figure 16 shows the distribution of the duration of female individuals staying off food with CO_2 application. It is understood that individuals are distributed in the concentrations of 430 ppm, 600 ppm, and 670 ppm in these figures.

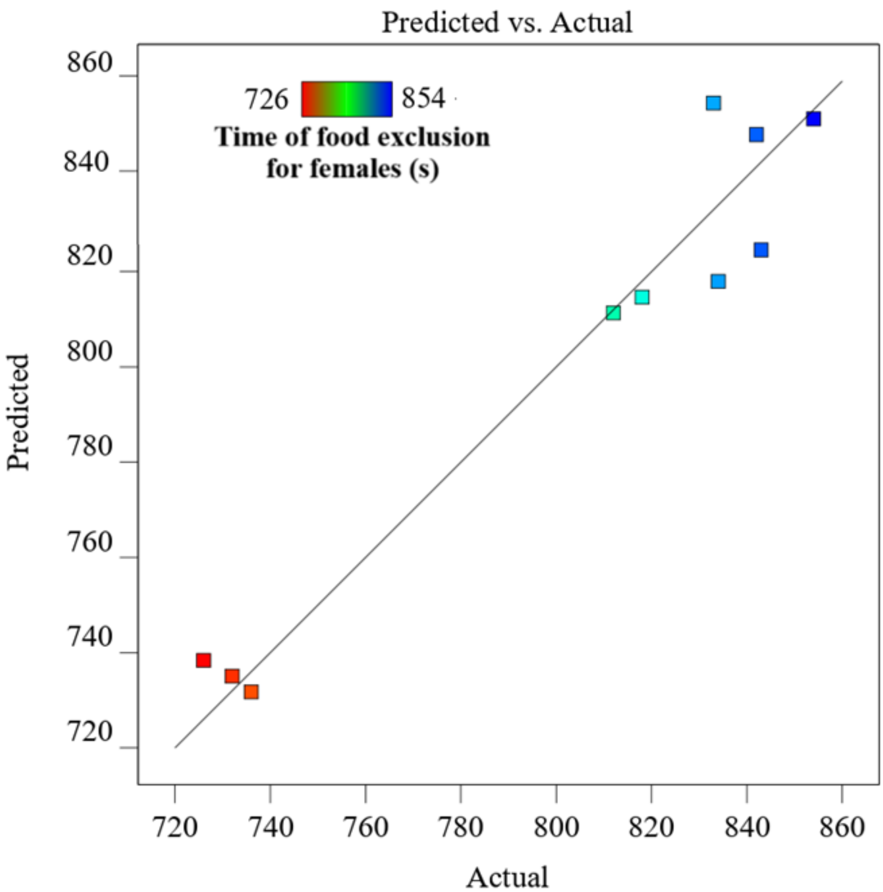

Figure 12. Compatibility of the time of food exclusion for females between real data and the model

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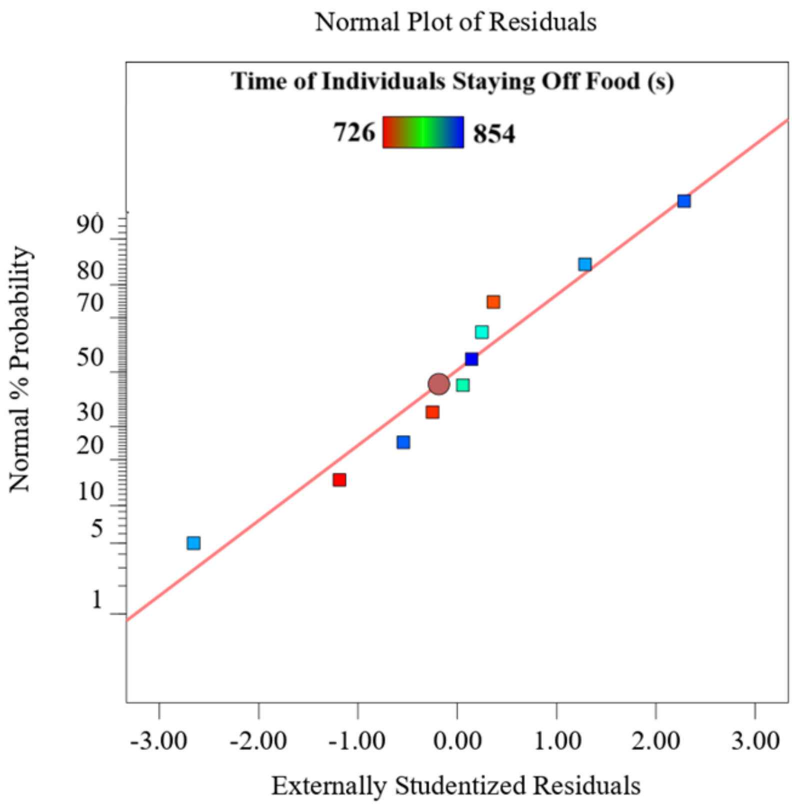

Figure 13. Distribution probability of food abstinence time in females according to RSM

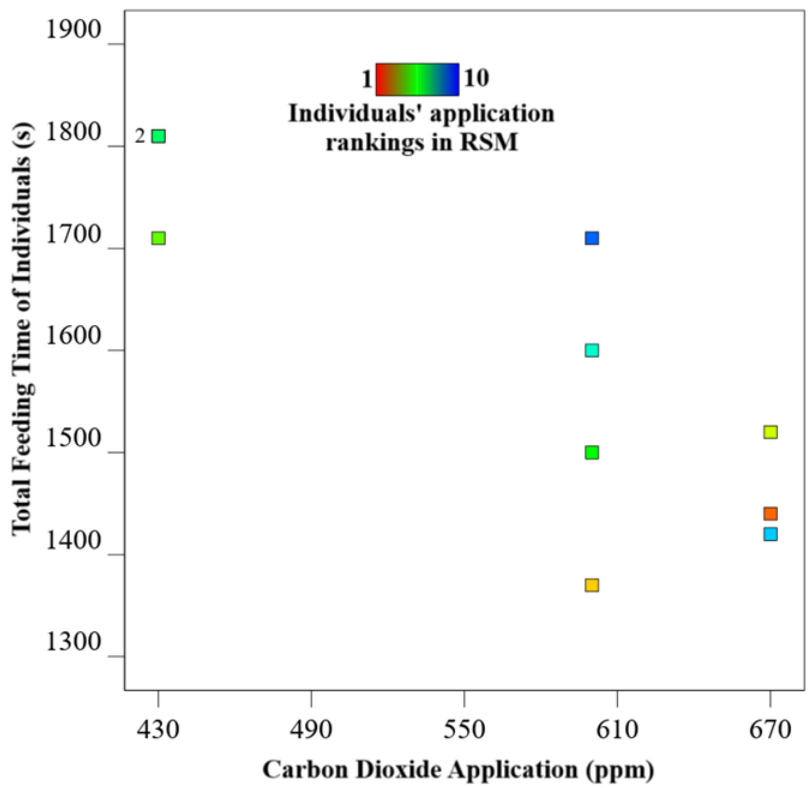

Figure 14. Distribution of total feeding time of female individuals with CO_2 application

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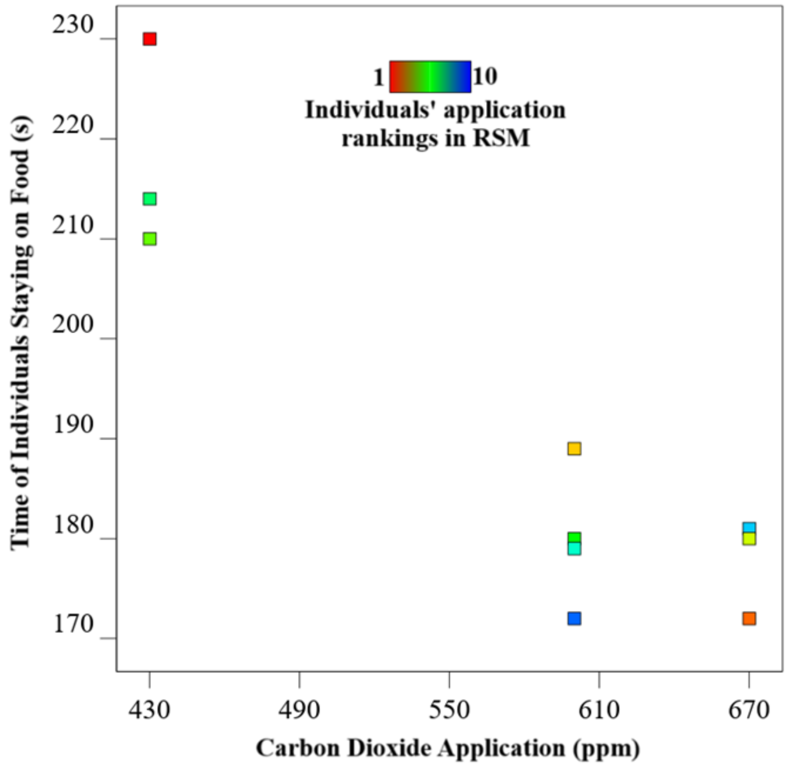

Figure 15. Distribution of the duration of female individuals staying on the food with CO_2

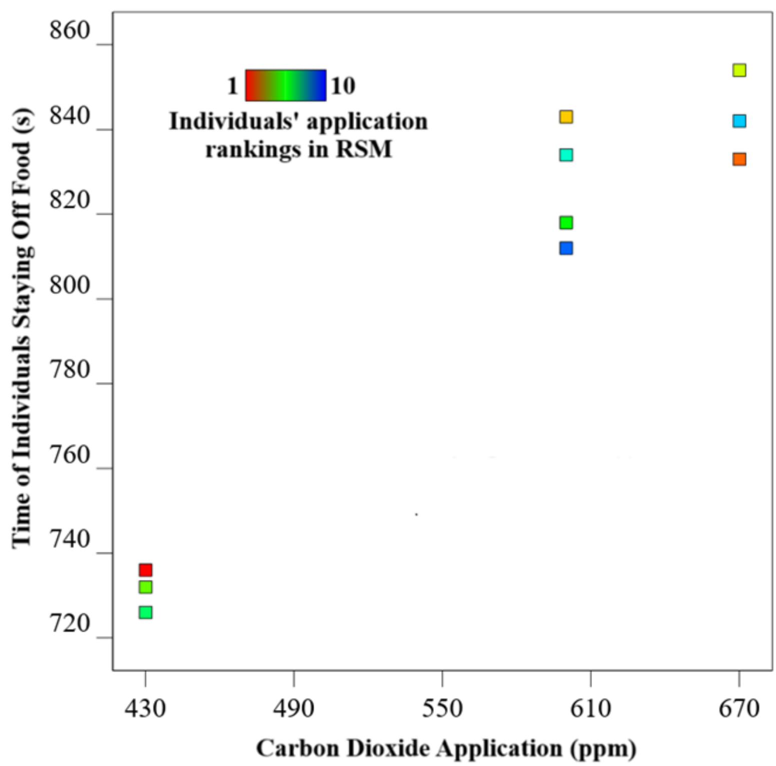

Figure 16. Distribution of the time of the individuals staying away from food by the application

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In carbon dioxide applications for *N. viridula* females, the correlation coefficients, standard deviation, and other statistical data found in RSM for *N. viridula* females are expressed. The statistical evaluation of the model revealed a standard deviation of 13.84, suggesting the degree of variation in the data. The coefficient of determination (R^2) was found to be 0.9426, indicating that approximately 94.26% of the variation in the response could be explained by the model.

The adjusted R^2 value of 0.9262 further confirms the model's robustness by considering the number of predictors used. The coefficient of variation (C.V. %) was calculated as 1.72%, highlighting the model's reliability and consistency. The predicted R^2 value of 0.8666 suggests a good agreement between predicted and actual values.

Furthermore, the adequate precision of 16.3105 indicates a strong signal-to-noise ratio, demonstrating the model's suitability for navigating the design space effectively.

Table 3 shows the *ANOVA* results for *N. viridula* females. According to these results, carbon dioxide application and adult female parameters were found to be significant (p-value <0.05). Also, Table 4 shows that the model suggested in RSM for *N. viridula* females is Linear vs Mean.

Table 3. ANOVA results for *N. viridula* females

Source	Sum of Squares	Mean Square	F-value	p-value
Model	22007.66	11003.83	57.47	< 0.05
A-CO_2 Application (ppm)	21946.88	21946.88	114.62	< 0.005
B-Adult Female	140.75	140.75	0.7351	0.4196
Residual	1340.34	191.48		
Cor Total	23348.00			

Table 4. Recommended models for *N. viridula* females according to RSM

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	6.448E+06	1	6.448E+06			
Linear vs Mean	**22007.66**	**2**	**11003.83**	**57.47**	**< 0.005**	**Suggested**
2FI vs Linear	46.45	1	46.45	0.2154	0.6589	
Quadratic vs 2FI	704.30	2	352.15	2.39	0.2076	
Cubic vs Quadratic	479.52	3	159.84	1.45	0.5325	Aliased
Residual	110.08	1	110.08			
Total	6.471E+06	10	6.471E+05			

Table 5 shows the optimum carbon dioxide results for *N. viridula* females according to the RSM model.

According to the results obtained, approximately 430 ppm CO_2 concentration, 1860 s total feeding time, 214 s on food time and 729 s off food time are given as optimum results.

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Table 5. Optimum CO_2 application results for *N. viridula* females according to the model

No	CO_2 Application (ppm)	Total Feeding Time (s)	Time on Food (s)	Time off Food (s)	Desirability
1	**430**	**1860.256**	**213.949**	**728.954**	**0.891**
2	432	1859.858	213.959	728.981	0.890
3	434	1862.369	213.900	728.806	0.887
4	431	1858.823	213.983	729.053	0.889
5	433	1861.860	213.912	728.842	0.890
6	432	1858.605	213.988	729.069	0.888
7	431	1860.986	213.932	728.903	0.890
8	435	1857.313	214.018	729.159	0.889
9	433	1857.907	214.004	729.117	0.887
10	434	1859.558	213.966	729.002	0.886

CONCLUSIONS

This study demonstrated the impact of varying CO_2 concentrations on the feeding behavior of *N. viridula* individuals using a newly developed air conditioning chamber and RSM. The findings revealed that increased CO_2 levels significantly reduced the total feeding time and the duration of staying on food while increasing the time spent away from food in both male and female individuals. The RSM models provided statistically significant and compatible predictions, with high determination coefficients indicating robust model reliability. The optimum CO_2 concentration for feeding behavior in both sexes was determined to be 430 ppm, with male individuals showing 1809 seconds of total feeding time and female individuals demonstrating 1860 seconds under these conditions. These results underline the potential of CO_2 concentration as a variable affecting the behavior of *N. viridula* and highlight the applicability of RSM for evaluating feeding parameters in other members of the Pentatomidae family, offering valuable insights for further ecological and behavioral studies.

According to the applied model, it was found that the model suggested by RSM for male adults was Quadratic model, and the model suggested by RSM for female individuals was Linear vs Mean. for *N. viridula* male individuals at approximately 430 ppm, the optimum carbon dioxide results according to the RSM model were 1809 s total feeding, 178 s on food and 730 s off food, and for female individuals, the optimum carbon dioxide results according to the model were 1860 s total feeding, 214 s on food and 729 s off food for approximately 430 ppm CO_2 concentration. The results of this study showed that all parameters that may affect the feeding behavior of other species of Pentatomidae family can be interpreted in line with explanatory and compatible models with the surface response system.

ACKNOWLEDGEMENT

This study was supported by Scientific and Technological Research Council of Türkiye under the Grant Number 123O061 (TÜBİTAK 1001). Besides, authors would like to thank Dr. Barış ÇERÇİ for his contributions and help with the diagnosis.

J.Het.Turk., 7(1), May, 2025 (eISSN 2687-3249)

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J.Het.Turk., 7(1), May, 2025 (eISSN 2687-3249)