Remittances Review
August 2024,
Volume: 9, No: 4, pp.2350-2368
ISSN: 2059-6588(Print) | ISSN 2059-6596(Online)

Received: 20 April 2024, Accepted: 15 September 2024
DOI: https://doi.org/10.33282/rr.vx9i2.128

THE IMPACT OF LEG LOSS OF COMMON WEB SPIDER *(Neoscona Theisi)* ON ORB WEB: UNRAVELING THE THREADS

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Abstract

Spiders, those crafty arthropods, are famed for their widespread web-building skills. Found everywhere from homes to fields, they use their spinnerets and legs to weave intricate webs. With their ability to survive leg loss and even perform autotomy to escape predators, spiders adapt swiftly despite the costs of their web-building behavior. This study aimed to assess the effect of autotomy on web-building behavior so that we can measure altered web parameters to determine how autotomy affects spiders in the wild. To investigate the impact of leg loss on spider behavior, three groups were formed: control, forelegs removed, and hind legs removed. Spiders from the city Farooka, district Sargodha were housed in custom wooden boxes, with control spiders having intact legs, forelegs removed spiders missing the first two pairs, and hind legs removed spiders missing the last two pairs. Daily observations, feeding, and web parameter measurements were conducted to assess how leg loss affected prey capture behavior. Results showed that the removal of forelegs had no significant impact on the web parameters of the orb-web spider whilst hind legs removal caused a considerable change in the web parameters altering the number of spirals, number of radii, and anchoring thread length. The results of this study have provided data to conduct in-depth future research to find study detailed impacts of loss of legs on the web architecture of spiders.

Keywords: Spider web, Spider leg, Autotomy

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INTRODUCTION

Spiders are among the oldest, most omnipresent, and numerous predators in both agricultural and natural ecosystems. Many spiders are specialized web spiders, whereas others hunt their victims. Their webs serve the purpose of catching the prey by trapping it in the intricately built webs. Insects constitute the major source of prey for spiders, but certain other arthropods are often consumed as well. Thus, spiders are regarded as high in value for pest control (Foelix, 1996).

Spiders are predators that have developed a great variety of different ways to capture prey. All spiders produce silk and several species use this ability to construct webs as catching devices. Among the various kinds of web-building spiders, orb weavers have specialized in building webs that catch airborne prey (Craig, 1986).

Only web spiders use their ability to produce silk for the construction of webs. They mostly use the fourth leg to build the web using silk ejected from silk glands. The well-known orb-shaped web is one of the many kinds of webs built by different spider families (Herberstein, 2000). There are significant variations within species and it affects prey capture ability. Webs of different sizes, inclinations, and mesh heights will capture different types and sizes of prey at different rates. (Long, 2020). The orb web is certainly the best-known of all webs. Essentially it is made up of three components which are radial threads, spiral threads, and frame threads. Web building is an innate behavior of spiders yet experience and learning also play a role in web building (Chacon *et al.*, 1980).

Many spiders lose their legs when they come across predators. (Roth, 1984) Almost 10% of all orb-web spiders lose one or more legs during their life with serious consequences for their fitness (Gregoric, 2020). Loss of an appendage can impair foraging abilities, locomotor performance, competitive abilities, and mating. With the loss of one or more legs, female orb-weaving spiders can be penalized twice: firstly, because the legs are necessary for web construction and secondly, the legs are essential for the control of the prey after its interception by the web. In orb-weaving spiders, the use of the eight legs is of extreme importance during web construction. Indeed, a full set of legs is utilized during the installation of various types of threads (Pasquet *et al.*, 2011; Ahmad *et al.*, 2023).

It has also been found that Autotomy reduces spider fitness by adversely affecting their capacity and success at foraging. Losing a limb can affect a spider's ability to move, as seen by the much slower running speeds of spiders with fewer than eight legs (Amaya *et al.*, 2001; Apontes *et al.*, 2005; Brown *et al.*, 2012). Although studies testing foraging performance in spiders with autonomies have shown mixed findings, limb loss may also influence a spider's capacity to acquire prey and adversely impact the web architecture. While Brueseke et al. (2001) discovered that autotomized *Pardosa milvina* consumed smaller crickets than their intact counterparts, Amaya et al. (2001) found no difference in the number of attacks required to capture prey items between intact and autotomized spiders of two species. When examined on a diverse, natural substrate, scientists discovered that *Schizocosa ocreata* ones with autotomized legs had lower prey catch rates and their web architecture plus the ability to make web gets poor (Long, 2020).

When orb-weaving spiders reach adulthood, autotomy may first change their construction behaviors, which may then modify the web's characteristics and reduce the effectiveness of their prey interception. Second, spiders with disabilities may be less effective in catching prey through locomotor activity. Since males do not produce webs as adults, autotomy in these situations may impair female fitness, particularly by limiting the accumulation of reserves required for egg development (Wrinn *et al.*, 2007).

More than half of the 40,000 spider species make webs that serve as a trap, composed of a silky structure that can catch and hold prey (Foelix, 2011). These structures come in a wide variety, but the geometrical webs have predictable structural patterns. Variations in the establishment of the attachment points, frame, and radial threads, which involve modifications of the behavioral sequences such as thread addition and removal, are known to occur in these webs. The sticky and auxiliary spirals, composed of a series of silk lines between two radii, result from repetitive behaviors and the help of legs (Lim and Kang, 2022).

The spider web is something worth noticing. It has been argued that if a spider loses a pair of legs, it becomes difficult for it to weave webs, just as getting food and movement is difficult for them. Orb webs have stunning architecture, especially with their geometric uniformity. The following is a description of how such webs are made: First, the spider attaches silk threads to the supports that make up the framework for the web. Next, the spider weaves the web. The spider builds the radii first, followed by an auxiliary spiral that is formed from the center out. The spider then places a sticky spiral (capturing thread) by traveling toward the center of the web from the edges while utilizing the auxiliary spiral as a guide (Foelix, 2011).

After autotomy, juvenile individuals may regenerate the lost appendage during a subsequent molt. It is known however that a regenerated leg, which is generally shorter than a normal one, generates structural modifications of the web (Witt et al. 1968). For orb-weaving spiders during their adult life, autotomy could first result in the modification of construction behaviors, inducing possible alterations of the web properties, and thus decreased prey interception efficiency. Secondly, handicapped spiders could be less efficient in locomotor activity to capture the prey (Pasquet *et al.*, 2011; Shah *et al.*, 2022).
The effect of leg autotomy differs with the habitat of the spider species as well. Their foraging ability and walking speed vary after leg loss depending on the type of habitat in which they live but it is generally observed that the walking speed of the spiders becomes less after losing their legs. (Amaya, 2001; Sattar *et al.*, 2024).
Leg autotomy occurs in spiders when a leg is pulled or injured. It occurs consistently at the level of the coxa-trochanter joint near the base of the leg. A special mechanism provides for the minimization of bleeding at the site of leg detachment, and spiders can withstand the loss of one or two pairs of legs (Camazine, 1983; Sajjad *et al.*, 2024).
So, keeping in view, the importance of legs for web building our study aims were to check the effect of leg loss on the architecture of web built by spiders as well as to assess the altered web characteristics due to loss of legs.

MATERIALS AND METHODS

Spider Collection

Spiders *Neoscona theisi* were collected from field areas in Sargodha. The areas were selected randomly based on the presence of fields. Areas that were selected to collect spiders from the city of Farooka, district Sargodha (31.88 ^{O} N, 72.41 ^{O} E). Spiders were collected during normal weather conditions when it is neither rainy nor windy. The sampling was completed in the duration of four months from June to September 2022.

Capturing Spiders

To capture an orb-web spider from its web, a small jar which was (4' x'6") was placed beneath the web. Then the web was slightly tapped to drop it into the jar and the cover was placed from the other side of the web. Before transferring live spiders to the wooden arena designed for their captivity, many twigs, leaves, and wet cotton swab was placed into the

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jars. The small twigs and leaves were added to give them the sense of a natural environment and cotton swabs provided the necessary humidity. For each sample, a separate jar was used so that they do not eat each other as spiders are known for cannibalism. Spider samples were then divided into three groups; Control Group, Group I (Two pairs of forelegs removed), and Group II (Two pairs of hind legs removed). The samples were carefully observed to ensure the presence of all four pairs of legs as the first two and last two pairs were removed to perform the experiment and find out the results.

Enclosure to Keep the Spiders

Wooden frames of 2/2 feet were used to keep the spiders alive and study the web-building of the spiders and the behavior related to it. To keep the web safe and prevent the spiders from escaping, the frame was covered with thin, slightly transparent, and rather flexible PVC sheets from both sides. On the inside, there were hooks to attach a thread to provide anchoring support to the spider to build the web by attaching the anchoring thread of the web. The spiders were kept hungry for three days before introducing them into the frames so that the strongest spider survives and is used in the experiment.

As frames were kept in the same place they were separated from each other by thin PVC sheets. To prevent silk threads from getting attached to the sheets, the sheet was smeared with Vaseline from both sides. The sheet could be removed easily from both sides to introduce the spider and provide feed to the spider. Additionally, wet cotton swabs were placed in the corners of the wooden box to provide the required humidity that ranges from 65-70%. Then the samples were carefully transferred into the wooden frames. All wooden arenas were kept in a place that did not receive direct sunlight and the temperature was 27±3 with the light and dark cycle of 11:13 hours.

Feeding the Spiders

Spiders were given two live houseflies (*Musca domestica*) and the feed was kept constant throughout the experiment. The flies were captured using honey jars by quickly closing the lid as soon as they entered the jar. The live and buzzing prey acted as bait to lure the spider to build the web and trap that prey. Then they were transferred to the wooden boxes by removing the PVC sheet from one side and tapping the jar onto the edge.

Cutting the limbs

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As we have to study the effect of leg loss thus the spiders must be handicapped. They have an inbuilt mechanism of autotomy so the loss of legs will be induced in them. Their legs will be stretched from the coxa-trochanter joint using a bicep so that they self-amputate their legs. But if they do not then the leg will be cut from the coxa-trochanter joint.

Grouping of Spiders

Spiders were subdivided into three groups to study the effect of leg loss for two subsequent pairs. Thus, one will be a control group with intact legs. There were two test groups with legs removed.

In group I, the first two pairs (I & II) of legs were removed. In group II, the last two pairs of legs were removed. The two pairs were removed to study the significant difference as spiders can easily survive with the loss of one pair.

Web Parameters and Imaging

Pictures of webs were taken with Oppo F19 against a dark background and every other day web parameters were noted after observing the web building at each observation. The images were taken at dawn using an Oppo F19 android cellphone. At every observation, the parameters recorded were the number of spirals, number of radii, diameter of the web (vertical and horizontal), radius of the web, mesh height, anchoring thread length, and capture area. As the web of the spider is elliptical that is why it was calculated using the "Ellipse formula". The total surface area of the web of the spider is called as capture area. (Herberstein and Tso, 2000).

$$\text{Capture Area} = (dv/2)\,(dh/2)\pi$$

Where dv stands for vertical diameter and dh for horizontal diameter.

When every observation was done and parameters were recorded, the previous web was destroyed and spiders were kept in the wooden box again to let them build another web. All the conditions were maintained throughout and food supply was given constantly. The prey entanglements and capturing were also observed for every group at each observation.

Statistical Analysis

To compare the effects of leg loss on the web building and web parameters of test groups with controls, a one-way ANOVA was applied followed by Tukey's test. To compare the effect of leg removal of both groups with the control group, two sample t-tests were used. Both of these tests were applied using GraphPad Prism 8.0.1.

RESULTS

Web building and web parameters

First of all, observations of controlled groups were made that had all normal parameters and web-building. It showed no degree of difference as all the legs were intact and the conditions provided were normal, nearly similar to the ones in the wild. The results of the observations varied for the treated groups with spider samples. Their legs were removed and they showed different web-building and web parameters from the controlled group. The results of group I with forelegs removed did not show much variation from the control group. The results varied very little from normal and they showed not much difference in building the web. When the webs of the first group were observed, only the number of spirals slightly varied and that too was negligible. This study did not show the significant importance of forelegs in the building of the web except for one parameter. The parameter that was altered due to the loss of forelegs was the length of the anchoring thread which is considerably greater than the control group. The results through comparison of the control group and group I through T-test showed a significant difference for several spirals (P-Value=0.0055) while many radii showed a slightly significant difference (P-Value=0.0065). The anchoring thread length was also significant (P-Value=0.0038).

When the group II was observed, the results were significant. This group has spiders with two pairs of hind legs removed. The results showed much impact on the web architecture of the spider as a few parameters were significantly altered. These parameters that varied considerably were the number of spirals, the number of radii, and the anchoring thread length. The rest of the parameters did not show much variation. The results through comparison of the control group and group II through T-test showed a highly significant difference for the number of spirals (P-Value=0.0409) while the number of radii also showed a significant difference (P-Value=0.00260). The anchoring thread length was also significant (P-Value=<0.0001)

The prey capture was observed in all three groups. All spiders were able to capture spiders in their webs, but the observations for prey handling were a little different for the group I with forelegs removed. The spiders in group I with missing forelegs were observed to be slow to seize the fly, and in 20% of webs, the fly was found to be just trapped in the web but not eaten. It was confirmed through continuous observation that the group I spiders were slow in seizing their prey.

Figure 1: Web built by a spider in control group

Figure 2: Web built by a spider in group II

Figure 3: Web built by a spider in group I

Table No 1: Comparison of web building between Forelegs Removal group (Group I) and Hind Legs Removal (Group II) using ANOVA

Sr. No	Parameters	Treatments	Mean±SE	Df	P-Value	F-Value
1	No. Of Spirals	Control Group	28.50±1.241	2.27	0.0140	5.022
		Group I	26.90±1.206			
		Group II	23.40±1.035			
2	No. of Radii	Control Group	26.70±1.325	2.27	0.0122	5.214
		Group I	25.30±1.174			
		Group II	21.40±1.097			
3	Mesh Height	Control Group	3.165±0.065	2.27	0.4337	0.8618
		Group I	3.258±068			
		Group II	3.141±0.065			
4	Diameter	Control Group	19.69±0.389	2.27	0.3948	0.9622
		Group I	19.36±0.370			
		Group II	18.96±0.57			

5	Radius	Control Group	9.880±0.183	2.27	0.3093	1.226
		Group I	9.730±0.184			
		Group II	9.480±0.178			
6	Capture Area	Control Group	314.1±12.73	2.27	0.7587	0.2790
		Group I	310.4±12.64			
		Group II	301.5±11.44			
7	Anchoring Thread Length	Control Group	39.05±0.8083	2.27	<0.0001	20.22
		Group I	42.20±0.6864			
		Group II	35.13±0.8583			

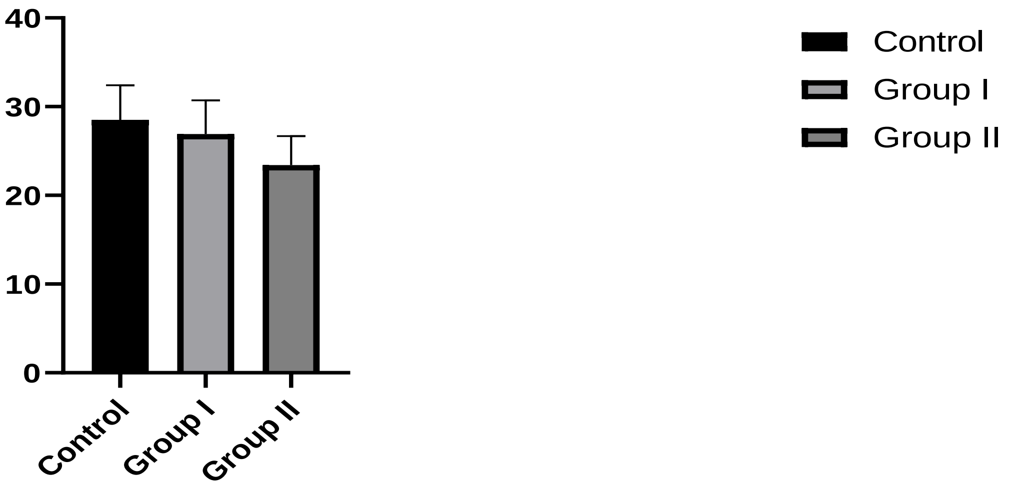

Figure 4: Comparison of Number of Spirals between groups

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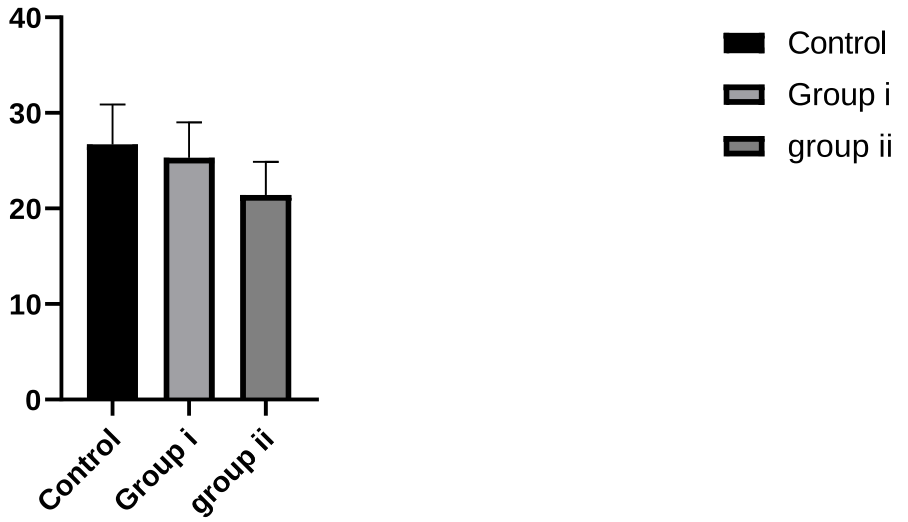

Figure 5: Comparison of the Number of Radii between groups

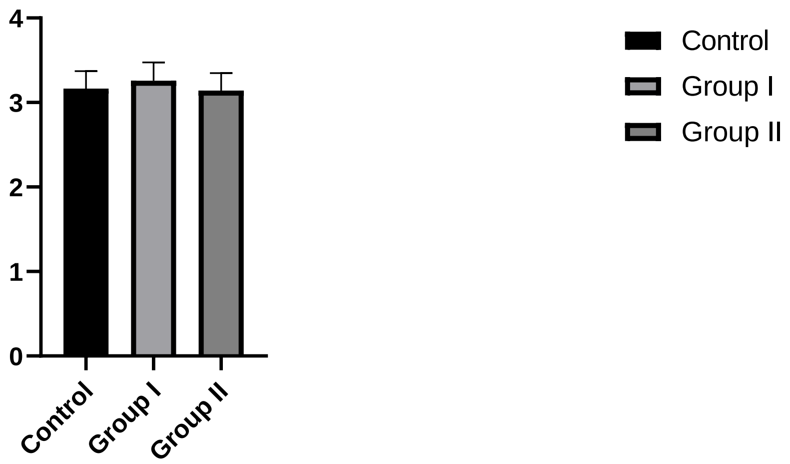

Figure 6: Comparison of Mesh Height between groups

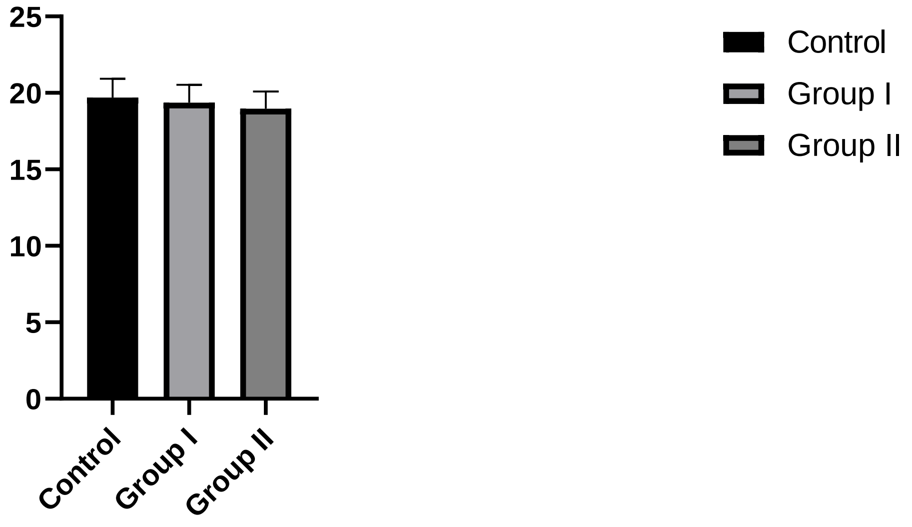

Figure 7: Comparison of Diameters between groups

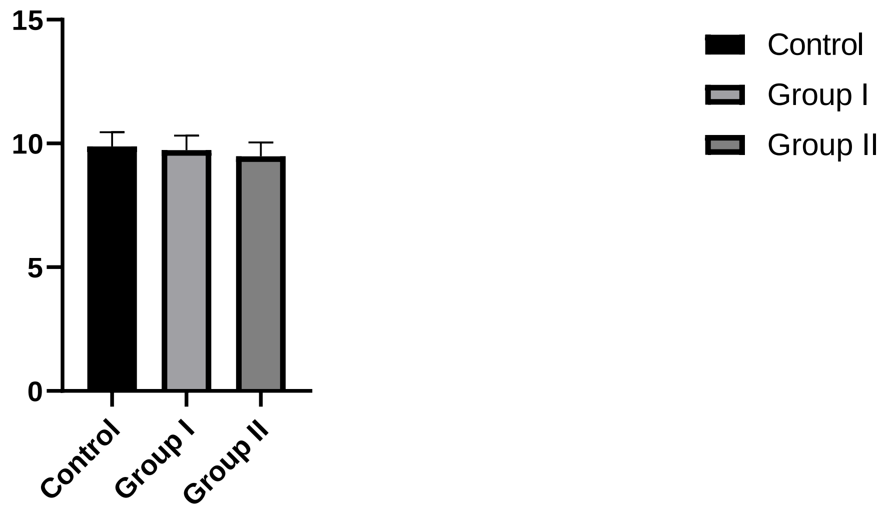

Figure 8: Comparison of Radius between groups

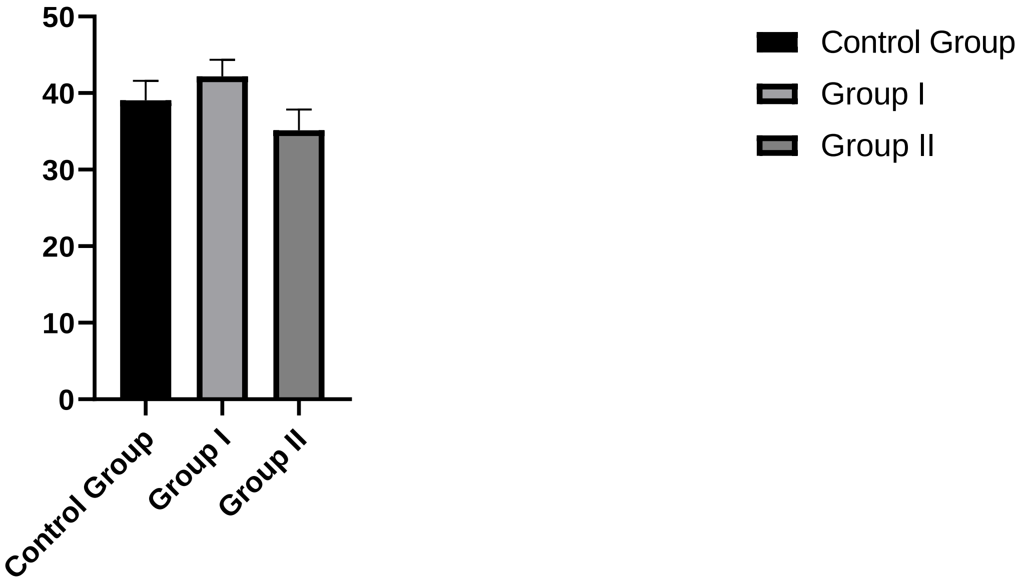

Figure 9: Comparison of Anchoring Thread Length between groups

Table No 2: Comparison of web building between the control group and Forelegs Removal (Group I)

Sr. No	Parameters	Treatments	Mean±SE	Df	P-Value	T-Value
1	No. of Spirals	Control Group	28.50±1.241	18	0.3673	0.924
		Group I	26.90±1.206			
2	No. of Radii	Control Group	26.70±1.325	18	0.4395	0.790
		Group I	25.30±1.174			
3	Mesh Height	Control Group	3.165±0.06522	18	0.3385	0.893
		Group I	3.258±0.06850			
4	Diameter	Control Group	19.69±0.3894	18	0.5467	0.614
		Group I	19.36±0.3700			
5	Radius	Control Group	9.880±0.1837	18	0.5720	0.575
		Group I	9.730±0.1849			
6	Capture Area	Control Group	314.1±12.73	18	0.8379	0.207
		Group I	310.4±12.64			
7		Control Group	39.05±0.8083			

	Anchoring Thread Length	Group I	42.20±0.6864	18	0.0082	2.971

Table No 3: Comparison of web building between the control group and Hind Legs Removal (Group II)

Sr. No	Parameters	Treatments	Mean±SE	Df	P-Value	T-Value
1	No. of Spirals	Control Group	28.50±1.241	18	0.0055	3.157
		Group II	23.40± 1.035			
2	No. of Radii	Control Group	26.70±1.325	18	0.0065	3.080
		Group II	21.40±1.097			
3	Mesh Height	Control Group	3.165±0.0652	18	0.7987	0.258
		Group II	3.141±0.0659			
4	Diameter	Control Group	19.69±0.3894	18	0.1844	1.380
		Group II	18.96±0.3578			
5	Radius	Control Group	9.880±0.183	18	0.1362	1.560
		Group II	9.480±0.178			
6	Capture Area	Control Group	314.1±12.73	18	0.4701	0.737
		Group II	301.5±11.44			
7	Anchoring Thread Length	Control Group	39.05±0.8083	18	0.0038	3.325
		Group II	35.13±0.8583			

Table No 4: Comparison of web building between Forelegs Removal group (Group I) and Hind Legs Removal (Group II)

Sr. No	Parameters	Treatments	Mean±SE	Df	P-Value	T-Value
1	No. of Spirals	Group I	26.90±1.206	18	0.0409	2.202
		Group II	23.40±1.035			
2	No. of Radii	Group I	25.30±1.174	18	0.0260	2.426
		Group II	21.40±1.097			
3		Group I	3.258±0.068			

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	Mesh Height	Group II	3.141±0.065	18	0.2343	1.231
4	Diameter	Group I	19.36±0.370	18	0.4472	0.777
		Group II	18.96±0.3578			
5	Radius	Group I	9.730±0.184	18	0.3441	0.971
		Group II	9.480±0.1789			
6	Capture Area	Group I	310.4± 12.64	18	0.6079	0.522
		Group II	301.5±11.44			
7	Anchoring Thread Length	Group I	42.20±0.686	18	<0.0001	6.433
		Group II	35.13±0.8583			

DISCUSSION AND CONCLUSION

In our study, the effects of the loss of legs by removal of two pairs of forelegs and two pairs of hind legs on the web architecture of an orb-web spider *Neoscona theisi* were studied. It is an orb-weaving spider that uses all of its eight legs to construct the web. While installing various types of silk, they use the full set of legs for that purpose and make a framework of the web (Foelix, 1996). Another aspect of the leg loss that we observed was prey capture, which did not vary significantly with the loss of legs except for the negligible number of webs. It is because left forelegs (I & II) have a considerable role in touching and exploring the prey (Govind, 1988; Ades, 2002; da Silva *et al.*, 2011).

Leg autotomy is a good strategy for escaping from predators in several animals. In spiders, autotomy is common (5–40% of spiders may have missing legs) and it reduces their speeds and can affect their ability to find mates, food, mates, etc (Gerald, 2017; Bilal[a,b], 2021).

Our results showed that the removal of group I has not much effect on the web characteristics of the spider. There was some distance between the turns of the spiral for a few webs but the results were not impacted by that much difference. This result is also supported by the spare-leg hypothesis (Guffey, 1999). Our results were also following the study conducted by Alain *et al.*, 2011. Spiders can easily lose two legs and still survive in the wild with web parameters almost remaining unaffected.

Spiders use all of their legs present on one side of the body to measure the distance between subsequent spirals. The role of forelegs is only that they find the point where the capture spiral is to be placed and that is why the web parameters were not much affected with the removal of forelegs. So, when the first two pairs of legs are removed there is no effect on web parameters like the number of spirals and mesh height (Gregoric, 2020).

The prey capture though was slightly affected by the removal of forelegs as it is proved that legs I and II are important in chasing the prey. When it comes to sensing and capturing the prey leg, I have a significant role in doing that. Though spiders can effectively capture prey despite the removal of legs because the rest of the legs are capable of performing all functions to survive according to the "Spare-leg hypothesis", I & II have been shown to more effectively capture the prey according to the study of Ades (2002).

Another study also confirms that spiders with regenerated short forelegs did not differ greatly in web-building behavior from normal spiders and the webs had functional parameters (Krink *et al.*, 1999). In *Araneus diadematus*, like most other orb spiders that have all their legs, the two pairs of front legs detect the presence of threads and measure distances while the spinneret lays and affixes the silk assisted by the fourth pair of legs with the third pair holding onto the supporting threads (Vollrath and Krink, 2020; Jawad *et al.*, 2023&2024).

The second group which had spiders with hind legs removed showed significant differences in web parameters. According to our results, the web parameters that differed from the control group were the number of spirals, number of radii, and anchoring thread length. The study of Vollarth (1987) supports these results as they worked with spiders who had grown shortened hind legs after autonomy. According to that study, spirals are fixed by legs III & IV. It measures the placement of the next spiral from the capture spiral and so on, thus the difference in the number of spirals (Vollrath, 1987).

This study concludes that forelegs have no significant impact on web building and without them, spiders can build a normal web. The removal of hind legs causes significant changes in web parameters particularly the number of spirals as legs III & IV are important in the placement of spirals. Still, this topic needs further elaborate studies both in the wild and in the laboratory because the building behavior of spiders is subject to a lot of factors.

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