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Biology, Behaviour and predatory efficiency of *Scybanus galbanus* Distant. Hemiptera: Reduviidae: Harpactorinae recorded in Cashew plantations

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Abstract

Reduviids are one of the important predatory groups encountered in cashew plantations, which predate on several insect pests including *Helopeltis* spp. *Scybanus galbanus* Dist. (Hemiptera: Reduviidae) was observed to be frequently preying on *H. antonii*. *S. galbanus* was collected from cashew plantations and reared under laboratory conditions on the larvae of greater wax moth, *Galleria mellonella* L. *S. galbanus* laid 80-110 eggs in clusters. The incubation period was 17.00 ± 0.28 days. The stadial durations of I, II, III, IV and V nymphs were 9.24 ± 0.18 , 7.72 ± 0.22 , 8.32 ± 0.17 , 10.40 ± 0.21 and 16.04 ± 0.19 days, respectively. Adult males and females survived for 74.00 ± 1.29 and 81.10 ± 1.06 days, respectively and the sex ratio was 1: 0.93. Many desirable biological and behavioral traits noticed in *S. galbanus* imply that this species can be mass reared under laboratory conditions and included in integrated pest management in cashew.

Keywords: Reduviids, *Helopeltis antonii*, wax moth, biological control

Introduction

Various insect pests have been recorded on cashew (*Anacardium occidentale* L.) in India^[1], of which one of the major widespread pest is the tea mosquito bug (TMB), *Helopeltis* spp. (Hemiptera: Miridae). Among the several abundant and thriving predators, the reduviids (Hemiptera: Reduviidae) play a vital role in the control of insect pests of cashew^[2]. The nymphal instars and adults of *Helopeltis* damage tender shoots, inflorescences, immature nuts and cashew apples at various stages of development, resulting in a yield loss of 30–50%^[3] and even 100%^[4] and cause economic loss to the farmers. Five species of Reduviidae: *Scybanus collaris* F., *Sphedanolestes signatus* Dist., *Endochus inornatus* Stal, *Irantha armipes* Stal and *Occamus typicus* Dist., reported as predators of *H. antonii* on cashew in India^[5, 6]. Under lab conditions all these reduviid predators are capable of catching and devouring 1-5 adult *Helopeltis* within 24 hours. First attempts were made in Southeast Asia to manage *Helopeltis* spp. Using reduviids^[7]. *S. galbanus* has been recorded as a predator of *H. antonii* during the present study. The biology and behavior of several reduviids like *E. plagiatus*^[8], *I. armipes*^[9], *S. signatus*^[6], *Endochus albomaculatus* Stal, *Epidaus bicolor* Distant, *Panthous bimaculatus*^[10], *S. dichotomus*^[11] were reported on different hosts/diets from different geographical regions. However, there are no reports available for *S. galbanus*.

In the present study, this reduviid species was reared using larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), to document its biology, behavior and predatory efficiency. Functional response was estimated to determine its predatory efficiency. Biology and mating behavior have been documented as baseline information to be used for conservation and augmentation of reduviids for biological control especially in cashew.

2. Materials and Methods**Biology**

The nymphs of *S. galbanus* were collected from cashew plantations at the Directorate of Cashew Research, Puttur (12.5° N; 75.4° E; 90 amsl), in Karnataka State of South India. These were reared under laboratory conditions; individually in glass bottles (500 ml cap.) using larvae of greater wax moth as prey. (Temp. 24–32 °C; 89–94% RH; photoperiod 10:14 h (L: D). The males and females that emerged from these laboratory cultures were allowed for mating in glass rearing bottles (500 ml cap.).

Only those adults that emerged from the nymphs in the laboratory cultures were used for further observations.

Successful copulation was confirmed by ejection of spermatophore capsules by mated females [12]. The bottles were carefully examined twice daily to record the number of eggs laid by the females. Eggs were allowed to hatch in the rearing bottles; wet cotton swabs were placed inside the bottles to maintain optimum relative humidity. The cotton swabs were changed periodically in order to prevent fungal incidence on the eggs. Later, mated females were separated and maintained individually in order to record the number of egg batches and eggs per batch. Soon after eclosion, the reduviids were reared individually on first and second instars of wax moths. When the reduviid nymphs grew larger, fourth and fifth instar wax moth larvae (10–15 mm) were supplied as prey. Subsequently, incubation period, stadia period, nymphal mortality, fecundity, longevity and sex ratio were recorded for two generations.

Behaviour

The predatory and mating behaviour of *S. galbanus* species was studied under laboratory conditions (24–32 °C; 89–94% RH). Predatory behaviour was assessed in prey-deprived (24h) reduviid bugs separately against wax moth larvae and their natural prey, *H. antonii* adults. For documenting the mating behaviour, freshly emerged males and females were released as pairs into the glass containers (500 ml cap.) provided with fresh cashew twigs along with leaves (to simulate natural conditions) and wax moth larvae (15 -20 days old) as prey and the sequences of mating behaviour were observed.

Predatory Efficiency

The predatory efficiency of *S. galbanus* was assessed separately at different prey densities. *i.e.* 1, 2, 3, 4 and 5 prey per predator separately for both wax moth larvae and its natural prey *Helopeltis* spp. for 5 continuous days in a 500 ml glass rearing bottles. For each trial, 5 replicates were maintained. The number of prey killed was recorded at interval of 24 h. Prey number was maintained constant in the experiment by the introduction of new prey.

The following parameters were recorded for obtaining the Holling's 'disc' equation:

x = prey density

y = total number of prey killed in given period of time (Tt)

y/x = attack ratio

Tt = total time in days when prey was exposed to the predator

b = time spent handling each prey by the predator (Tt/k)

a = rate of discovery per unit of searching time [$(y/x)/Ts$]

The handling time 'b' was estimated as, the time spent for pursuing, feeding and subduing each prey. The maximum predation was denoted by 'k' and it was restricted to the higher prey density. 'a' was the rate of discovery and was defined as the proportion of the prey attacked successfully by the predator per unit of searching time. Assuming that the predatory efficiency is proportional to the prey density and to the time spent by the predator in searching the prey (Ts), and is expressed in a relationship as:

$$y = a Ts \times (1)$$

Since time available for searching is not a constant, it is deducted from the total time (Tt) by the time spent for

handling the prey. If one presumes that each prey item requires a constant amount of time 'b' for consumption, then $Ts = Tt - by$ (2)

Substituting (2) in (1), we get Holling's 'disc' equation *i.e.*, $y = a (Tt - by) \times (3)$

The data were later subjected to linear regression analysis [13].

Data analysis

All data were expressed as mean \pm SE using SPSS 22 software (IBM Corp., Armonk, NY) [14].

3. Results

Morphology of eggs, nymphs and adult of *S. galbanus*

Eggs

Eggs were laid on the bottom and sides of the rearing bottles and also underneath the muslin cloth cover (Fig. 5). Eggs were brownish yellow in colour, endo-lateral of chorion yellow, exo-lateral brown and operculum being transparent white. Cylindrical shape, basal portion was wider than the apical portion, smooth and shiny outer surface. The operculum is extended and serrated (Fig. 1). The fertilized egg turned reddish brown in colour as the hatching time approached whereas, the unfertilized eggs became black and shrunken after few days. The eclosion duration lasted for about 5 to 8 minutes. The eggs hatched in 17.00 ± 0.28 days (Fig. 6) and mean percent egg hatchability was 95.5.



Fig 1: *S. galbanus* eggs in clusters

Nymphal Instars

There were totally five nymphal instars (Fig. 2). The stadia durations of I, II, III, IV and V nymphs were 9.24 ± 0.18 , 7.72 ± 0.22 , 8.32 ± 0.17 , 10.40 ± 0.21 and 16.04 ± 0.19 days, respectively (Table.1).

First Instar

Body size varied from 0.4 cm - 0.5 cm in length. Colour varied from yellow to brown with dark brown colouration at the tip of the abdomen. Basal three antennal segments were brown while the last segment was red colored. Tibiae were longer than femora. Head is much longer than the pronotum, post-ocular region is longer when compared to ante-ocular portion. Ocelli were not noticed. Cannibalism is not noticed in this stage of development. Nymphal period is 9.24 ± 0.18 days and 90.24% of nymphs were survived during moulting into next instar.

Second Instar

Body size varies from 0.6 cm - 0.7 cm in length. Colour varies from yellow to brown with dark brown colouration at the tip of the abdomen. Nymphs have 4 black spots on the abdominal region. Basal three antennal segments are brown and the last segment is red colored. Shape of head is similar to that of adult. Post-ocular region is longer when compared to ante-ocular portion. Ocelli were absent. Shape of pronotum is same as the first instar. Cannibalism was not noticed in this stage of development also. Nymphal period was 7.72 ± 0.22 days and 89.19% of nymphs survived during moulting into next instar.

Third Instar

Body size varied from 0.9 - 0.10 cm in length. Colour varied from orange to red with dark brown colouration at the end tip of the abdomen. Abdomen is erect and short. Shape of the head was similar to that of adults. Ocelli were absent. Mid and hind femora was equal in length and mid femora was shorter. Cannibalism was not noticed in this stage of development. Nymphal period was 8.32 ± 0.17 days and 95.80% of nymphs survived during moulting.

Fourth Instar

Body size varied from 1.4 - 1.5 cm in length. Body colour ranges from brown with blackish spots on the abdomen. Abdominal region was bulged. Shape of the head resembles that of adults. Ocelli were absent. Wing pads appeared but

still in the initial stages of development. Cannibalism is not noticed in this stage of development. Nymphal period is 10.40 ± 0.21 days and 96.90 per cent of nymphs survived during moulting.

Fifth Instar

Body size varied from 1.9 - 2.2 cm in length. Colour varied from yellow to brown with black colouration at the tip of the abdomen. Body was long and erect. Shape of the head was similar to that of adults. Ocelli were appeared in this stage of development. Further development of distinct wing pad which was blackish in colour was noticed. Cannibalism was not noticed in this stage of development. Nymphal period was 16.04 ± 0.19 days and 96.33 per cent of nymphs survived during moulting.

Adults

S. galbanus exhibited distinct sexual dimorphism. Generally, males and females were similar in their external appearance with black body with yellow band in the wing, but varied in size and shape. Females were larger (2.8 cm length) with a round bulged abdominal base, whereas males were relatively small (2.4 cm in length) and lean, with a pointed abdominal base (Fig. 2). The adult male longevity and total duration of male life cycle was (74.00 ± 1.29 days and 142.92 ± 2.56 days) and that of female was (81.10 ± 1.06 days and 150.02 ± 2.33 days) which indicates that females survived for a longer period when compared to the males.

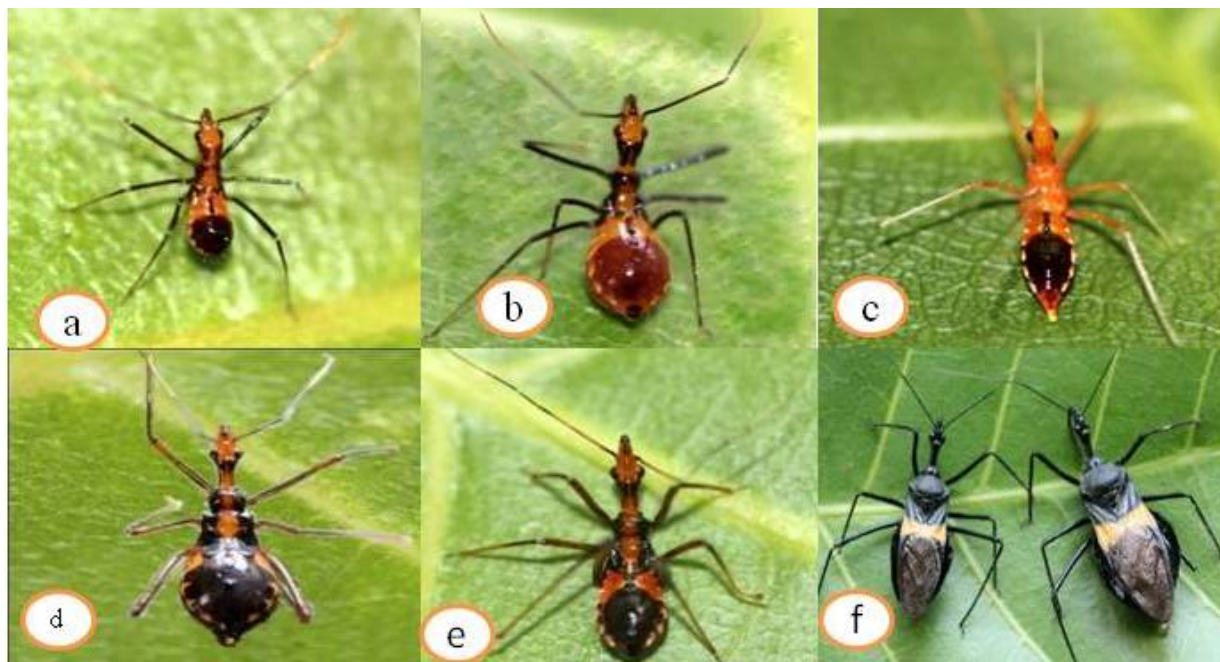


Fig 2: a) First instar (0.4 cm), b) Second instar nymph (0.6 cm), c) Third instar nymph (0.9 cm), d) Fourth instar nymph (1.4 cm), e) Fifth nymphal instar (1.9 cm) and f) Adults of *S. galbanus* (male: 2.4cm; female: 2.8 cm).

Mating Behaviour

The sequential acts of mating behavior observed in *S. galbanus* were arousal, approach, riding over, and copulation.

Arousal and approach

The excitation of mating partners was initiated by the sight of opposite sex. Freshly emerged reduviid males were aroused immediately after sighting females of their own species. Males approached females with extended antennae and rostrum. The approach response was completed once the

males touched the females with their antennae and placed their legs over them.

Riding over

The male clasped the female with his forelegs and pressed her pterothorax region with his labial tip, and they remained in the riding-over dorsoventral position (Fig. 3). This act lasted for nearly 15 to 20 min.



Fig 3: Mating of *S. galbanus*

Copulation

At the culmination of riding over, males extended their genitalia and established copulation with the females, and the mating pair remained motionless during copulation. The duration of copulation lasted for 25 to 30 min. The end of copulation was characterized by the drooping down of antennae by both males and females, followed by separation of mating partners. The successful completion of copulation was evidenced by the ejection of the spermatophore capsule by females after termination of copulation. Post-copulatory cannibalism of the males by the females was not observed in this species.

Predatory Behaviour

The reduviid predators killed more wax moth larvae and TMB at higher densities (Table 2 and 3). Predation exhibited a typical functional response and thus established the applicability of the second model of Holling’s ‘disc’ equation. Typically, most heteropteran predators exhibited type II functional response [15].

Table 1: Biological parameters of *S. galbanus* on wax moth larvae under laboratory conditions.

Incubation period (days)		17.00 ± 0.28
Stadial period (days)	I instar	9.24 ± 0.18
	II instar	7.72 ± 0.22
	III instar	8.32 ± 0.17
	IV instar	10.40 ± 0.21
	V instar	16.04 ± 0.19
I-V instars		68.92 ± 1.27
Fecundity/female (no.)		96.60 ± 5.65
Hatchability (%)		95.5
Survival rate (I-V) (%)		87.63
Sex ratio (male: female)		1:0.93
Pre-oviposition period (days)		14.00 ± 0.70
Oviposition period (days)		9.40 ± 1.20
Post-oviposition period (days)		13.90 ± 0.78
Adult longevity (days)	Male	74.00 ± 1.29
	Female	81.10 ± 1.06
Total longevity (days)	Male	142.92 ± 2.56
	Female	150.02 ± 2.33
(n=25; x ± SE)		

Table 2: Functional response of *S. galbanus* to TMB.

Prey density (x)	Prey attacked (y)	Max 'y' (k)	Days/y b=Tt/k	All y's days (by)	Searching days Ts=Tt-by	Attack ratio y/x	Rate of discovery ((y/x)/Ts)=a	Disc equation y'=a(Tt-by)x
1	0.73	4.6	1.09	1.49	3.51	0.73	0.21	y'=0.81(5-1.09y)x
2	1.40			0.78	4.22	0.70	0.17	
3	1.16			0.94	4.06	0.39	0.10	
4	2.60			0.42	4.58	0.65	0.14	
5	4.60			0.24	4.76	0.92	0.19	

Table 3: Functional response of *S. galbanus* to wax moth larva.

Prey density (x)	Prey attacked (y)	Max 'y' (k)	Days/y b=Tt/k	All y's days (by)	Searching days Ts=Tt-by	Attack ratio y/x	Rate of discovery ((y/x)/Ts)=a	Disc equation y'=a(Tt-by)x
1	0.08	1.68	2.98	0.24	4.76	0.08	0.02	y'=1.99(5-2.98y)x
2	0.34			1.01	3.99	0.17	0.04	
3	0.82			2.44	2.56	0.27	0.11	
4	1.40			4.17	0.83	0.35	0.42	
5	1.62			4.82	0.18	0.32	1.81	

4. Discussion

Biological studies on reduviids, their conservation, augmentation and their utilization in biological control of insect pests especially *Helopeltis* spp. have been gaining momentum in recent years [16, 17]. In the present study, attempts were made to record the biology, behaviour and predatory efficiency of *S. galbanus*. *S. galbanus* laid elongate oval reddish-brown eggs (96.60 ± 5.65/female) on the bottom and sides of the culture bottles and on muslin cloth. Eggs were laid in groups of 80-110 eggs in 1-3 clusters per female as observed in other Harpactorines [18, 19]. The fecundity rate of *S. galbanus* was higher (96.60 ± 5.65 eggs) when compared to other Harpactorines like *Sphedanolestes* spp. (15.33 ± 6.41 eggs) [6], but lower than that of *R. marginatus* Fab. (208.3 ± 3.9 eggs) [20] and *Sphedanolestes signatus* Distant. [10]. The pre-oviposition period of *S. galbanus* was

shorter (14.00 ± 0.70 days) than that of *R. kumari* (26.0 days) and *R. marginatus* (33.3 days) [21].

The incubation period of *S. galbanus* (17.00 ± 0.28 days) was longer than *Clavigrallaria spiniscutis* Berg Roth (4.66 ± 0.77 days) [22], *S. signatus* (9.6 ± 0.86 days) [6] and *Sycanus collaris* Fab. (15.0 days) but shorter than *Panthous bimaculatus* Dist. (21.0 days) [21]. The eclosion duration lasted for about 5 to 8 minutes. The eggs hatched in 17.00 ± 0.28 days and mean percent egg hatchability was 95.5. The higher hatching percentage is a diagnostic key feature of Harpactorines [18, 19]. After 4 to 5 hrs of eclosion, the newly hatched nymphal instars started feeding on small (0.2 - 0.4 cm) wax moth larvae (*G. mellonella*) showing a preference for small and active prey. The subsequent nymphal instars fed on large sized (1.5- 2.00 cm) wax moth larvae (Fig. 4a) and also on adult TMB, *H. antonii* (Fig. 4b).

The total developmental period of *S. galbanus* D. from egg to adult lasted for 68.92 ± 1.27 days. It was shorter than that of *P. bimaculatus* Distant (101.12 ± 2.30 days), *R. kumarii* (88.30 ± 3.60 days) and *S. collaris* (75.67 ± 9.06 days) [21]. Abnormal hatching and moulting induced a total of 12.37% nymphal mortality, from I to V instars and thus the nymphal instars had a mean survival rate of 87.63%.

The nymphal mortality of *S. galbanus* was lower when compared to *Sphedanolestes pubinotum* Reuter (89.30%), *S. minusculus* Bergroth (21.06%) and *S. himalayensis* (13.0%) [18]. The adult male longevity and total male longevity was (74.00 ± 1.29 and 142.92 ± 2.56 days) and that of female was (81.10 ± 1.06 and 150.02 ± 2.33 days) indicates that females survived longer period when compared to males. Females living longer than males are common in harpactorines, a mechanism that promotes multiple matings with males of different age groups, which subsequently facilitates enhanced fecundity [18, 23].

The pre-oviposition and post-oviposition period in *S. galbanus* were 14.00 ± 0.70 and 13.90 ± 0.78 days, respectively. The oviposition period of *S. galbanus* lasted for a period of 9.40 ± 1.20 days. The laboratory-emerged adults exhibited male biased sex ratio (1.00: 0.93). But among most of the harpactorines female biased sex ratio was reported viz., *S. collaris* (0.67:1.00), *R. kumarii* (0.50: 1.00) and *P. bimaculatus* (0.60: 1.00) [21, 10].

Predatory behaviour of reduviids generally consists of stimuli-response mediated sequences of events, always initiated by a moving prey [24]. The same sequential pattern of

the pin and jab mode of predation was observed in this species also. Prey movement was an important stimulus in the primary sensory input for arousal in predation by reduviids [18, 25]. The sensory hairs of the forelegs [26], tibial pads [27] and antennal perception of kairomones and allomones [28] play a major role in prey capturing. *S. galbanus* attacked prey in a sequential pattern; arousal - approach - capturing - probing - piercing and sucking (Fig. 4a, b and c).

The reduviid responded to increasing prey density of wax moth larvae and TMB by killing more prey than at lower prey densities (Table 2 and 3). It exhibited a typical functional response and thus established the applicability of the second model of Holling's 'disc' equation. The type II functional response is typical of most heteropteran predators [15]. As the prey density increases (x), the number of prey killed (y) by the individual also increases. Similar results were obtained [30] i.e., positive correlation between prey density and prey killed. Earlier reports of [30, 31] also confirmed increase in the number of prey killed by an individual predator as a function of increasing prey density 'k' value represents maximum predation ($k = 1.68$ for wax moth larvae and $k = 4.6$ for TMB). The highest attack ratio was observed at 5 prey/predator in case of TMB provided as a prey and in case of wax moth supplied as a prey, then highest attack ratio was observed in 4 prey/predator. Thus, it could be concluded that *S. galbanus* released at a ratio of 1:5 (predator-prey) could help in managing the TMB population in the cashew ecosystem.



Fig 4: Pin and jab predation of *S. galbanus* nymphs (a) on wax moth larva, (b) On TMB and (c) adult *S. galbanus* on TMB.



Fig 5: *S. galbanus* laying egg on cloth



Fig 6: Nymphs hatching from eggs

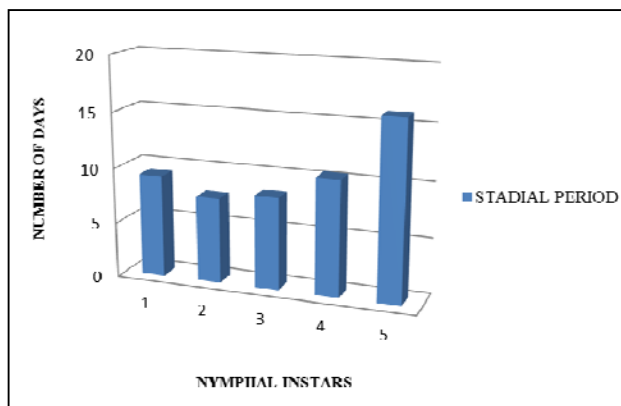


Fig 7: Stadia period of *S. galbanus*

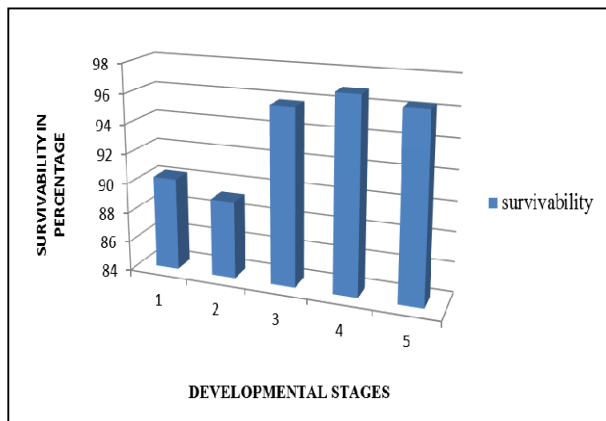


Fig 8: Percentage survival of *S. galbanus* nymphs at developmental stages.

5. Conclusion

S. galbanus was most commonly found preying on *H. antonii*, which is a serious pest of Cashew ecosystems. Upon noticing the prey, aggressiveness for prey capturing and rostral thrusting was witnessed in both nymphs and adults of *S. galbanus*. It can be easily mass reared in the laboratory conditions. Many desirable biological and behavioral traits noticed in *S. galbanus* imply that this species can be successfully included in biocontrol module of integrated pest management of *H. antonii* in cashew.

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