BIOLOGY OF *HELICOVERPA ARMIGERA* (HUBNER) ON SUNFLOWER TREATED WITH DIFFERENT CONCENTRATION OF NEEM EXTRACTION IN LABORATORY

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ABSTRACT:*Sunflower field collected 30 eggs of H. armigera were kept in the cage. Up on hatching, the newly emerged larvae were shifted to fresh sunflower leaves treated with the concentrations of neem leaves extracts viz. 2% (T1), 5% (T2), 10% (T3), 15% (T4) and T5 control (untreated). Observations on time span by the larvae, their survival percent and adult life span i.e. pre-mating, mating, pre-oviposition, oviposition and post-oviposition were determined, female fecundity and eggs fertility were also recorded. The result showed that the larvae of H. armigera passed through 6 instars in all treatments. The number (20.00±091-20.8±1.38) eggs were non-significantly hatched on all treatments. All life stages from 1st larva to adult stage survived non-significantly on T1 and T5. The least number of larvae reached to adult stage on T3 followed bt T2. No survivorship was recorded after 5th instar larva in T4. The result further indicated that H. armigera spent minimum time on T3. The pre-mating, mating, pre-oviposition, oviposition and post- oviposition periods (1.25±0.25days), (21.00±0.91hours), (1.75±0.41days), (10.00±0.41days) and (3.00±0.41 days), respectively were recorded of the adult emerged from the larvae fed on T1 and (4.75±0.25 days) (15.75±1.70) (3.5±0.29 days) (6.00±0.41 days) (2.75±0.25 days), respectively on T3 as compared to (1.00±0.00days) (22.25±0.85±1.70) (1.75±0.25days) (11.00±0.71days) (3.75±0.25days), respectively on T5. The females emerged from the larvae fed on T1 laid 770.0±30.99 eggs, which hatched in 3.75±0.25days with 571.75±37.87 fertility. The fecundity, incubation period and fertility 479.75±22.70, 5.75±0.48 days and 375.5±19.26, respectively of the females emerged from the larvae fed on T3 as compared to the females of T5, which laid 956.5±29.05eggs that hatched in 3.25±0.25 days with 675.25±56.93 fertility. The survival of 1st, 2nd, 3rd, 4th, 5th, 6th, pupa and adult was recorded 66.67, 56.67, 50.00, 47.50, 36.67, 28.33, 26.67 and 18.33 percent, respectively when fed on T1. The Least survival percent 68.33, 35.00, 25.00, 24.17and 15.83 was recorded only from 1st to and 5th instar larvaeon T4. Maximum survival of all life stages was recorded on untreated sunflower leaves.*

Keyword:*Helicoverpaarmigera,* neem extracts, Biology, Survivorship, Sunflower.

INTRODUCTION

Members of the noctuid genus Heliothis comprise some of the most important of all phytophagous insect pests with word wide distribution and significance with more than 300 species currently recognized [1] only *Helicoverpa armigera (Heliothisarmigera)* and *Heliothiszea* have achieved major pest status [2]. *H. armigera* Hb. is a highly mobile and polyphagous pest insect. It has been recorded damaging plant species in the 39 families; it causes most damage in the semi-arid tropics [3,.5] recorded its damage on 41 plant species belonging to 14 plant families in Punjab (Pakistan). Wild plants play an important role in the carry-over of this insect during hot weather [6]. It is very difficult to precisely estimate the amount of damage by *H.armigera* throughout its entire geographical distribution, but rough estimates may run into billions of dollars per years. For example, in India, annual losses to two major pulse crop, chickpea and pegeonpea may exceed 300 million dollars per year [7].In Pakistan, *H. armigera* is one of the most important and serious pests on cotton, tomato and chickpea [8]. It has also been recorded on maize, tobacco, and sunflower and many other vegetables, field and fodder crops throughout the year. There are no systematic survey and studies conducted on the extent of damage in Pakistan, but probably the damage may be in millions of dollars on different crop per annum [5]. With rapid emergence of resistance in insect pests to commercially available insecticides, there is a need for the development of management strategies that are less dependent on chemical insecticides and/or less conducive to the development of resistance to present chemical control measures. Another complicating factor with pesticides treatment is that errant applications of pesticide persist in soils and pollute ground water, streams and river. Therefore, some new methods are needed, which should be effective at disrupting the behavior and physiology of these pests while still preserving the balance and cleanliness of the agro-ecosystems. Biodiversity is of immense importance having wide range of biomolecules, thus offering great opportunity for searching more environment friendly biomolecules for pest control.Plant biodiversity is of immense importance for finding a wide range of biomolecules. Plants have remained source for many important pesticides such as rotenoids, nicotine, pyrethroids, neem etc. The discovery of many synthetic pesticides also finds its origin from plant-based chemicals. The need for newer pesticides remains ever persisting in order to combat the problem of resistance in the insects. The growth inhibitory effects of *Aristolochia* spp on third instar larvae of *H. armigera*, which were reared on pechay leaves treated with 5 ml of 0.1 g/ml of the solution that resulted a number of normal and abnormal pupae and adults. The above information led to conduct an experiment to ascertain the effects of neem leaf extracts on the life cycle of Helicoverpa *amigera*in laboratory conditions. The output

information of the present studies will be utilized in better management of *Helicoverpa amigera* on sunflower crop.

MATERIALS AND METHODS

The experiment on Biology of *H. armigera*on on sunflower leaves treated with neem leaves extract was conducted in laboratory, Department of Entomology, Sindh Agriculture University, Tandojam during spring, 2013.

Extraction process of neem leaves: Fresh leaves of neem were brought in the laboratory. The leaves were ground in the local grinder (manually used). The neem leaf extract was obtained by putting grounded leaves in the muslin cloth and squeezed them. The 100% of neem extract was then put into a well-cleaned bottle.

Concentrations of extract used: (T1) 2% = 2 ml pure neem extract+ 98ml distilled water, (T2) 5% = 5 ml pure neem extract+ 95ml distilled water, (T3) 10% = 10 ml pure neem extract+ 90ml distilled water, (T4) 15% = 15 ml pure neem extract+ 85ml distilled water and (T5) Control (untreated leaves).

Collection of egg and released: The eggs of *H. armigera* were collected from peas crop. Thirty eggs were kept in the cage along with the leaves of sunflower plant. Care was taken that the leaves would not dry up until the hatching of the eggs. If leaves were found less fresh were changed with new ones and the eggs were transferred onto them. Up on hatching the newly emerged larvae were shifted to the sunflower leaves, which were already dipped in the above mentioned concentrations of neem leaf extracts and dried.

Survival, Larval and Pupal Period: The hatched larvae were kept of sunflower leaves treated with different concentrations of neem leaf extracts in glass jars (12 cm × 15 cm) to record the survival of all life stages, and time span of larva and pupa. There were three replications of each treatment. The jars used were cleaned daily bases such as removal of faces and other unwanted material. Treated sunflower leaves were changed daily and replaced with new ones.

Premating, Mating, per-oviposition, oviposition, post-oviposition periods, fecundity, and fertility.

Two pairs of adults emerged from the pupae of each treatment were introduced into the glass jar and the mouth of the jar was covered with muslin cloth for aeration in the jars. Inside each jar a piece of clean muslin cloth (20 cm × 10 cm) was kept to facilitate females for oviposition. The adults were allowed to feed on 15% sucrose diet. Premating, Mating, per-oviposition, oviposition and post- oviposition periods were recorded of the adults emerged from the pupae of each treatment. The number of eggs laid on the muslin was counted for each treatment separately. The Egg Hatching % was calculated by using the following formula:

$$\text{Hatching\%} = \frac{\text{Total eggs hatched}}{\text{Total eggs laid by female}} \times 100$$

The experiment was replicated thrice.

Statistical analysis

The data were statistically analyzed for analysis of variance (ANOVA) using a factorial completely randomized design to determine critical difference (CD) among treatments/concentrations. The difference of two means between treatments/concentrations exceeding CD value was significant at 5% level [10].

RESULTS

The data in Table-1 show that the larvae of *H. armigera* passed through six instars in all treatments. The egg hatched into Ist instar ranged 20.00±091-20.8±1.38 out of 30 eggs in all treatment on sunflower leaves. However, the differences in hatching of eggs on all treatment were non-significant. Maximum (17.00±0.91) of second instars, were survived on sunflower treated with T1 that was followed by T2 (16.5±1.19), T3 (14.75±0.84) and T4 (10.5±0.87) as compared to T5 (18.75±0.25). The 3rd instar larvae displaced similar trend of survival. The highest survival 17.0±0.41was recorded in T_5 and least 7.5±0.66 in T_4. The individuals (7.25±0.63) of 4th instar survived on T_4 and 9.0±0.71 survived on T_3 and it was followed by T_2 (11.8±0.63), T_1 (14.25±0.63) and T5 (16.5±0.29). The least number of 5th instar larvae 4.75±0.25 survived on T_4 then 7.25±1.31 individuals on T3, much better survival was recorded on T_2 (8.5±1.04) and T_1 (11.00±0.91), respectively as compared to T_5 (14.25±0.25). No individuals survived after 5th instar on T4.The same non-significant number of individuals 8.50±0.87and 7.5±0.96 was recorded on T_1 and T_2, respectively.

The highest number of larvae (9.25±0.95) was transformed in to papae of T5 followed by T_1 (9.00±0.91), T_2 (6.00±0.71) and T_3 (3.5±0.87) none of the larvae transformed into pupae in T4. Similarly, the highest survival of adult (9.25±0.85) was recorded in T5, which was followed by T1 (5.50±0.65), T2 (4.75±0.84) and T3 (2.75±0.48). As none of the 6th instar larvae were survived on T4, therefore, survival of subsequent life stages was recorded zero. It was observed from the result that the leaves treated with 15% neem leaf extract was proved lethal for the survival of all life stages. Whereas, the survival rate on 1% and untreated leaves was non-significant with untreated leaves. It was also observed that more that 60% individuals could not survive even on normal food i.e. untreated sunflower leaves.

Time span by instars: Data in Table-2 showed the time span by each larval instar on treated sunflower leaves. It was observed that minimum time was taken by each larval instar to transform in to next stage when fed on untreated sunflower leaves. i.e. 3.25±0.25, 5.00±0.71, 4.25±0.25, 5.00±0.41, 3.75±0.63 and 4.25±0.48 days for 1st, 2nd, 3rd, 4th, 5th, and 6th larval instars, respectively. A little more time was taken to transform into subsequent larval instar when fed on sunflower leaves treated with 2 percent solution of neem leaf extract. The 1st, 2nd, 3rd, 4th, 5th, and 6th instar larvae lived for 3.50±0.29, 6.75±0.48, 4.75±41, 5.25±1.03, 6.25±0.48 and 5.25±0.25 days, respectively. That indicated a little disturbance in the life of each larval instar. It was also observed that as concentration of neem leaf extract in the solution increases that leads more disturbance in life span of each instar . As in case of 5% solution, the time span of 1st, 2nd, 3rd, 4th, 5th, and 6th instar increased a little than the time taken by the larva fed on the leaves treated with 2% neem solution. They lived for 5.00±0.58, 7.00±0.71, 5.50±0.29, 7.00±0.41, 6.75±0.48 and 5.75±1.08 days, respectively.

Further enhance in life span was recorded when the larvae were fed on the sunflower leaves treated with 5 percent neem solution. They lived for (6.00±0.41) days in 1st instar, (8.5±0.87) 2nd instar, (7.5±0.29) 3rd instar (, 8.75±0.63) 4th instar, (8.75±0.63) 5thinstar and (8.5±0.50) days in 6th instar. The most toxic effective of neem leaf extract was observed when these larvae were fed with sunflower leaves treated with 15 percent solution. Clear and obvious impact on larval life span and survival was recorded when the larvae fed on leaves treated with T4. The time span of 1st, 2nd, 3rd, 4th and 5th larval instar was recorded as (8.75±0.63), (10.25±0.63), (9.00±0.71), (9.5±0.87) and (10.25±1.31), respectively; whereas, 6th instar did not live for a day.

Pupal period: The data in Table-2 also revealed that minimum pupal period was recorded on untreated leaves of sunflower (T5). It was 6.25±0.25 days. The next minimum pupal period (9.00±0.91 days) was recorded of the pupae transformed from the larvae fed on 5 percent solution that was followed by 2 percent solution (10.25±0.48 days) and 10 percent solution (11.00±0.71 days) none of the pupae survived on 15 percent solution.

Adult activities: Data in table-3 show that normal activities were observed of those adults, who were fed the untreated leaves of sunflower crop in their larval stage. Their pre-mating, mating, pre-oviposition, oviposition and post-oviposition periods were recorded as (1.00±0.00), 22.25±0.85), 1.75±0.25), (11.00±0.71) and (3.75±0.25) days, respectively. The adult of the larvae fed on the leaves treated with 2 percent solution, spent (1.25±0.25), (21.00±0.91), (1.75±0.14), (10.00±0.41) and (3.00±0.41) days for pre-mating, mating, pre-oviposition, oviposition and post-oviposition activities, respectively. Whereas, time span 2.25±0.25, 18.3±1.31, 2.5±0.29, 8.75±0.85 and 3.75±0.63, respectively, was recorded of the adults emerged from the larvae fed on sunflower leaves treated with 5 percent neem leaf extract. Huge variation in the time span for these activities was recorded when adults emerged from the larvae fed with 10 percent neem solution. They spent 4.75±0.25 day in pre-mating period (15.75±1.701days) mating, (3.5±0.29 days) pre-oviposition (6.00±0.41 days) oviposition and (2.75±0.25 days) post oviposition. None of the adults were emerged from the larvae fed on the leaves treated with 15 percent neem solution.

Egg: Due to these solutions, impacts on fecundity, hatching period and fertility was also observed. The data in table-4 shows that the females emerged from the larvae fed with 2 percent solution laid 770.0±30.99 eggs out of that 571.75±37.87 hatched in 3.75±0.25 days with 70.44 percent fertility. The females emerged from the larvae fed with 5 percent neem solution produced 608.00±36.20 eggs only 415.0±138.41eggs were hatched in 4.25±0.75 days with 56.85 percent fertility. Minimum eggs were produced by the females emerged from the larvae fed with 10 percent neem solution. The female produced 479.75±22.70 eggs among them 375.5±19.26 were hatched in (5.75±0.48) days with 52.28 percent fertility. None of the adults was survived from the larvae fed with 15 percent solution. Therefore, no further activities were recorded. The females, who were emerged from the larvae fed on untreated leaves of sunflower, produced the maximum 956.5± 29.05 numbers of eggs. These eggs hatched in 3.25±0.25 days, and the mean 675.25±56.93 number of egg hatched into larvae with 78.85 percent fertility.

Survival of various life stages: Table-5 shows the survival percent of various life stages of *H. armigera* against different concentrations of neem solution. It was observed that the hatching percent was more or less the same that range from 66.67-69.33 percent on the sunflower leaves treated with different solution of neem extract. First instar larvae fed on the leaves treated with 2, 5, 10 and 15 percent neem extract, their survivorship % in 2nd instar were recorded as (56.67), (55.00), (49.17) and (35.00), respectively as compared to untreated leaves (62.50). The survivorship% (50.00), (46.00), (38.33) and (25.00) of 3rd instar larvae was recorded on 2, 5, 10 and 15 percent neem solution, respectively. The survival of 4th instar was 47.50, 39.33, 30.00 and 24.17 percent on 2, 5, 10 and 15 percent neem solution, respectively. The least survivorship was recorded in 5th instars when fed with 2, 5,10 and 15% neem solution, the survivorship on these solution was recorded as (36.67), (28.33), (24.17) and (15.83) percent, respectively, as compared to untreated leaves (47.50%). The survival of 6th instar was 28.33,25.00 and 15.83 percent on 2, 5, 10 percent solution. None of the larvae survived after 5th instar when fed on 15 percent solution. The pupae 26.67,20.00 and 11.67 percent were transformed from the larvae fed on 2, 5 and 10 percent solution, respectively as compare to 30.83% on untreated leaves. Adult survivorship% was 18.33, 15.83 and 9.17 percent from these solutions as compared to untreated leaves (30.87).

Table 1. Mean number of *H. armigera*survived on various concentrations of neem leaf extract on sunflower leaves.

Life Stage	No. survival				
	T_1	T_2	T_3	T_4	T_5
1st instar	20.00±0091	20.8±1.38	20.25±0.63	20.5±0.87	20.25±0.63
2nd instar	17.00±0.91	16.5±1.19	14.75±0.84	10.5±0.87	18.75±0.25
3rd instar	15.00±0. 41	13.8±0.25	11.5±1.44	7.5±0.66	17.0±0.41
4th instar	14.25±0.63	11.8±0.63	9.0±0.71	7.25±0.63	16.5±0.29
5th instar	11.00±0.91	8.5±1.04	7.25±1.31	4.75±0.25	14.25±0.25
6th instar	8.50±0.87	7.5±0.96	4.75±1.18	0	11.25±1.03
Pupa	9.00±0.91	6.00±0.71	3.5±0.87	0	9.25±0.95

adult	5.50±0.65	4.75±0.84	2.75±0.48	0	9.25±0.85

Table 2. Mean time consumed by different life stages of *H. armigera*after feeding the leaves treated with different concentrations of neem leaf extract on sunflower leaves

Life Stage	Time consumed in days				
	T_1	T_2	T_3	T_4	T_5
1st instar	3.50±0.29	5.00±0.58	6.00±0.41	8.75±0.63	3.25±0.25
2nd instar	6.75±0.48	7.00±0.71	8.5±0.87	10.25±0.63	5.00±0.71
3rd instar	4.75±41	5.5±0.29	7.5±0.29	9.00±0.71	4.25±0.25
4th instar	5.25±1.03	7.00±0.41	8.75±0.63	9.5±0.87	5.00±0.41
5th instar	6.25±0.48	6.75±0.48	8.75±0.63	10.25±1.31	3.75±0.63
6th instar	5.25±0.25	5.75±0.25	8.5±0.50	0	4.25±0.48
Pupae	10.25±0.48	9.00±0.91	11.00±0.71	0	6.25±0.25
Mean±S.E	42.00±0.48	46.00±3.63	59.00±4.04	47.75±3.97	31.75±2.44

Table 3. Mean time spent in hour/day by adult of *H. armigera*during various activities on sunflower leaves

Adult Activities	Time Taken				
	T_1	T_2	T_3	T_4	T_5
Pre-mating period	1.25±0.25	2.25±0.25	4.75±0.25	0	1.00±0.00
Mating period (hours)	21.00±0.91	18.3±1.31	15.75±1.70	0	22.25±0.85
Pre-oviposition	1.75±0.14	2.5±0.29	3.5±0.29	0	1.75±0.25
Oviposition	10.00±0.41	8.75±0.85	6.00±0.41	0	11.00±0.71
Post-oviposition	3.00±0.41	3.75±0.63	2.75±0.25	0	3.75±0.25

Table 4. The mean fecundity, hatching and fertility of the eggs of *H. armigera* recorded due to various neem solutions on sunflower leaves

Adult Activities	Time Taken				
	T_1	T_2	T_3	T_4	T_5
Fecundity	770.0±30.99	608.00±36.20	479.75±22.70	0	956.5±29.05
Hatching period	3.75±0.25	4.25±0.75	5.75±0.48	0	3.25±0.25
Fertility	571.75±37.87	415.0±138.41	375.5±19.26	0	675.25±56.93

Table5. Survival percent of each life stages against the neem solutions on sunflowerleaves

Life Stages	Survival percent				
	T_1	T_2	T_3	T_4	T_5
1st instar	66.67	69.33	67.50	68.33	67.50
2nd instar	56.67	55.00	49.17	35.00	62.50
3rd instar	50.00	46.00	38.33	25.00	56.67
4th instar	47.50	39.33	30.00	24.17	55.00
5th instar	36.67	28.33	24.17	15.83	47.50
6th instar	28.33	25.00	15.83	0.00	37.50
Pupa	26.67	20.00	11.67	0.00	30.83
adult	18.33	15.83	9.17	0.00	30.87

DISCUSSION

Result of the experiment revealed that maximum reduction in number of survivors was recorded in the second instar larvae of *H. armigera* fed on the leaves treated with 15% solution of neem leaf extract and minimum in the larvae fed on 2% solution as compared to the untreated leaves of sunflower crop. Similar pattern in reduction in number of survivors by these solutions was recorded until adult stage. However, no survivors were recorded after 5th instar larvae fed on the sunflower leaves treated with 15% solution. The results are in agreement with those of [11] they reported that recently, plant extracts are frequently used as antifeedent and cepelent for *H. armigera*.[12] determined the efficacy of various plant extracts against the insect pests of cowpea. Aqueous extracts of *Annona senegalensis* root bark, *Azadirachtaindica* seeds, *Clausenaanisata* leaves, or *Zanthoxylumzanthoxyloides* leaves and root bark applied three times at 5% at 10-day intervals significantly reduced the population densities of *Megalurothripssjostedti* (33.7-66.1% in 1997 and 76.2-81.7% in 1998). [13] evaluated neem (*Azadirachtaindica A. Juss*.) formulations against castor semi-looper (*Achaea janata L*.) indicated that reduction in the larval population 46.45 to 58.52% by neem seed kernel extract 1500 ppm. [14] tested three botanicals, Sitaphal (*Annona squamosa*), Sadaphuli (*Catharanthusroseus*) and Kaner (*Nerium oleander*) @ 0.5, 1.0 and 1.5 per cent, against second-instar larvae of *H. armigera*the larval mortality increased with an increase in concentration. Sitaphal seed extract at 1.5 per cent concentration recorded the highest mortality in *H. armigera* (43.33%). [15] mentioned that 5% concentration Neem oil and cake brought 100% mortality in *H. armigera*. The significant mortality counts were also observed at pupal stage. The per cent antifeedancy of mustard cake was lower than the Neem oil and cake.Result further revealed that *H. armigera*spentusual days to complete it life on untrated leaves of sunflower. [16] mentioned the same life span of *H. amigera* on cotton in normal conditions. During present investigation, it was recorded that higher concentrations prolonged the life span of *H. armigera* this could be due to insectisidal or antifeedant properties of higher concentrations of neem leaf extract. [17] tested the antifeedant property of *Azadirachtaindica* (neem) against the maharukha webworm, absolute antifeedancy was exhibited by seed extract of neem at 3%. [18] observed slow growth and development of larvae of *Helicoverpaarmigera* when fed on artificial diet treated with leaf extract of *Eucalyptus camaldulensis* and *Callistemon lanceolatus*. [19] reported that extracts from neem (*Azadirachtaindica A.* Juss.) exhibit significant control of many crop pests. 100, 100, 60, 66 for *H. armigera*. In addition to larval mortality, the extracts also reduced the larval growth and total development, prolonged larval duration to reach pupation, and lowered pupal weights, resulting in the formation of deformed individuals. [20] mentioned that biological activities of the salannin type of limonoids isolated from *Azadirachtaindica* A. Juss using the gram pod borer *Helicoverpaarmigera*(Hubner Inhibition of larval growth was concomitant with reduced feeding by neonate and third instar larvae.Since, it was hard to *H. armigera*to survive until 5th larval instar on the leaves treated with 15% solution, therefore, no further life activities were recorded in that treatment as indicated by the result of present experiment. However, less time was taken by the adults for premating, mating, pre-oviposition and post oviposition activities when they were fed on sunflower leaves treated with 5% neem leaf extract in their larval stage as compared to the 2% solution. Non-significant difference in time taken was recorded in the activities of adults emerged from the larvae fed on 2% solution and untreated sunflower leaves. [18] Observed that the larvae of *H. armigera*could not survive beyond L_3 stage at 2% level of *Eucalyptus* leaf powder while in case of *Callistemon* at 2% level the per cent survival was 20% at pre-pupal stage. [21] mentioned that the effect of dried leaf powders of Piper nigrum, *Annona reticulata, Azadirachtaindica* and Capsicum annuum and dried peel of lemon (*Citrus limon*) on oviposition, adult emergence and adult mortality of *CallosobruchusmaculatusAzadirachtaindica* gave the highest reduction in oviposition of *C. maculatus* (37.5%). Moreover, the result also indicated that higher fecundity, fertility present and lesser hatching period in the eggs were recorded of those adults emerged from the larvae fed on the leaves treated with 2% solution than the adults emerged from the larvae treated with 5% solution. Non-significant difference was also recorded in 2% solution and untreated leaves. [22] determined the efficacy *Azadirachtaindica*, against the eggs of diamondback moth (*Plutellaxylostella*). Azadirachtaindica exhibited the highest egg hatching inhibition (47.91%).

CONCLUSIONS

It is concluded from the result that neem leaf extract had impact on life span and survival of *H. armigera*. The solution with 15% neem leaf extract found lethal for lifespan, survivor, fecundity, hatching period and fertility of *H. armigera*.

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