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Phago-repellent biopotency of *Cichorium intybus, Inula racemosa, Tagetes minuta* and *Chrysanthemum cinerariaefolium* as herbal alternatives to synthetic insecticides against *Earias vittella* Fabricius (Lepidoptera: Noctuidae) on Okra in Kanpur region

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Abstract
To evaluate the effectiveness of selected asteraceous botanical extractives in management of spotted bollworm, *Earias vittella* Fabricius (Lepidoptera: Noctuidae) an experiment was conducted in the plant product laboratory, Department of Zoology, D.B.S. College, Kanpur Nagar during 2021.Phago-repellent effectiveness of ten indigenous naturally occurring asteraceous plant extracts *viz*., aerial parts of *Cichorium intybus* (L.), *Chromolaena odorata* Linn., *Chrysanthemum cinerariaefolium* (trev.) Vis., *Inula racem*osa Hook. F., *Mantisalca duriaeri* Birq. Et Cavill., *Reichardia tingitana* (L.) Roth, *Rhaponticum acaule* (L.) DC, *Scorzonera undulate* Vahl, *Spilanthes paniculata* Well ex DC and *Tagetes minuta* Linn. were prepared under the laboratory conditions. For testing the phago-repellent effect, the okra leaves were used as food against larvae of *Earias vittella*. In each set of extract and one control was introduced, where the leaves pieces were dipped in Benzene + emulsified water only. The okra leaf pieces fastened under clip and left under electric fan for about thirty minutes, so as to complete dry up the extract. The treated foods were kept in jar (23cm x 10cm) on moist filter paper. Then third instars, 24 hours starved larvae of *E. vittella* were released in each jar. After four hours of the release of larvae the data was collected on the number of larvae reached and repelled at each treated food. Three replication of treatment were made. The data depicted from the results in the present experiment of repellent test against larvae of *Earias vittella*. All the plant extracts showed a good repellent property, when compared with the control. The repellent property was observed based on their EC_{50} values as: *C. intybus* (0.0823) > *I. racemosa* (0.1043) > *T. minuta* (0.1620) > *C. cinerariaefolium* (0.1677) > *M. duriaeri* (0.1788) > *S. undulate* (0.3127) > *A. paniculata*, (0.3805) > *R. acaule* (0.4405) > *R. tingitana* (0.6368) > *C. odorata* (0.8870), respectively. The relative EC_{50} values of the above extracts are in descending order, 10.7700 > 8.5043 > 5.4753 > 5.0227 > 4.9608 > 2.8365 > 2.3311 > 1.3929 > 1.0000 times as repellents, where as *C. odorata* is taken as a unit.

Keywords: *Earias vittella, Cichorium intybus, Inula racemosa, T. minuta,* Bhindi (Okra)

1. Introduction
Bhindi, *Abelmoschusm esculentus* (Linn.) Moench commonly known as okra is an important summer vegetable crop grown allover Indian sub Zoogeographical region. Okra is nutritious vegetable which plays an important role to meet the demand of vegetable of the country when vegetable are scanty in the market. Okra contains large quantities of carbohydrate, potassium, vitamin B, vitamin C, protein, folic acid and calcium. It's low in calories and has high dietary fiber content. Okra is a nutritious vegetable which plays an important role to meet the demand of vegetables of the India when vegetable are scanty in the market. Okra mucilage is suitable for medicinal and industrial application (Radake and Undirwadem 1981, Kumar *et al.* 2013) [1, 2].
The spotted bollworm, *Earias vittella* Fabricius (Lepidoptera: Noctuidae) is a serious pest of vegetables ie. Okra, eggplant, pepper melon, cucumber, pumpkin and cotton. The crop is vulnerable to attack of many insect pests, among them *Earias vittella* Fabricius are the most important pest causing direct damage to the marketable fruits (Konar and Rai, 1990) [3]. *Aphis gossypii* and *Earias vittella* is alone reported to cause 57.1% fruit infestation and 54.04% yield loss in okra (Chaudhary and Dadheech, 1989) [4] Leaves can be damaged to such an extent that they wilt and fall off.

This impairs photosynthesis, thereby weakening the plant even further, and may render fruits unmarketable.

The synthetic chemical insecticides are very effective and used in okra insect pest management for a long time and to ensure higher crop and vegetable yield. The excessive and injudicious use of these chemical pesticides led to many problems like development of resistance, induction of resurgence, environment pollutions and human health (Park *et al.* 2011, Chandel *et al.* 2018) [5, 6]. The side effects have forced to look for naturally occurring ecofriendly indigenous asteraceous herbal alternatives to chemical insecticides especially for vegetables like okra where fruits are plucked at an interval of every 2-3 days.

Various plant extractives have been studied for insecticidal activity globally (Dubey *et al.* 2004, Chandel and Sing) [7, 8] and majority of them are insect feeding deterrent (Dang *et al.* 2010, Antonius and Hagazy 1987) [9, 10]. More than 140 compounds, which are chemically diverse and structurally complex, have been isolated from the leaves, seed oil and bark of neem (Ahmed *et al.* 1999 and Al-Lavati *et al.* 2002) [11, 12]. Phytochemicals are often distasteful and toxic to many agricultural insect-pests. They can modify feeding deterrence behaviour of an insect (Schmidt and Streloke, 1994) [13]. Moreover, as most spotted bollworm species have become resistant to many insecticidal agents (Pavela, 2006, Rashid *et al.* 2013) [14, 15], managing these pests in greenhouses and in the field is becoming problematic (Srivastava and Awasthi 1958, Mendes, *et al.* 1997, Kim *et al.* 2011) [16, 17, 18]. Therefore, in the present investigation the selected asteraceous naturally occurring plant extractives are efficient and environmentally friendly pest control alternatives must be developed to replace synthetic insecticides.

2. Materials and Methods

Experiments were conducted in the Entomology Research plant product Laboratory and experimental field of Department of Zoology, D.B.S. College, Kanpur, U.P., India. Geographically, the districts Kanpur is located in between latitudes 25.26º and 26.58º North and longitudes 19.31º and 84.34º East, Kanpur is situated at an elevation of about 127.117º metres above the mean sea level and has a semi-arid subtropical climatic conditions.

2.1 Procurement of raw herbal materials

The plants parts used for extracts were surveyed, identified and collected mainly from wasteland and wild areas and some plants were collected from cultivated fields of the farmers. The investigations on the screening of various available indigenous naturally occurring ten aesteraceous plant extracts *viz*., aerial parts of *Cichorium intybus* (L.), *Chromolaena odorata* Linn., *Chrysanthemum cinerariaefolium* (trev.) Vis. *Inula racem*osa Hook. F., *Mantisalca duriaeri* Birq. Et Cavill., *Rechardia tingitana* (L.) Roth, *Rhaponticum acaule* (L.) DC, *Scorzonera undulate* Vahl, *Spilanthes paniculata* Well ex DC and *Tagetes minuta* Linn. Were screened for their repellent biopotency against spotted bollworm, *Earias vittella* under laboratory trials.

2.2 Process of Herbal Powder Extraction

Fresh collected ten plant parts (leaves, Flowers and seeds, rhizomes etc) were washed with distilled water and kept in the laboratory for 7 days for air drying followed by one day sun drying before making powder. Electric grinder was used to have coarse powder then these were passed through a 60-mesh sieve to get fine powder. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss. For the extraction, Soxhlet Apparatus was used; about 20g powder of each category of powder were extracted with 300 ml of different solvents (n-hexane, acetone, methanol, petroleum ether and distilled water). Extraction of each category of powder was done in about 12 hrs. After soxhlet extraction, the material was run on rotary evaporator (Jilani, *et al.* 1985, Abubakar *et al.* 2000, Ramamurthy, *et al.* 2002) [19, 20, 21]. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept on water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.

2.3 Stock Solution Preparation and Formulations

For stock solution, 50ml. extract in each case was taken into reagent bottles and 50ml. benzene was added in it to dissolve the constituents of the materials. The mouth of the bottles were stopper with airtight corks after which, these bottles containing the solutions were kept in refrigerator. Three concentrations (0.25, 0.5, 1.0, 1.5, 2.0 percent) were used for experiments on repellent tests in the laboratory conditions. However, only three concentrations (0.5, 1.0 and 2.0 percent) were used for insecticidal test in the laboratory. The different concentrations of the herbal extracts were prepared from the stock solution using benzene as solvent and Triton X-100 as emulsifier. The level of solvent and emulsifier were kept constant (Kamaraj *et al.* 2011, Chandel and Sengar, 2018) [22, 23].

2.4 Experimental Equipments

Small plastic jars (capacity 50 ml) were used for the experiment, there was one set of two jars joined by clear plastic pipe of 1.0 cm diameter at an angle of 180 degree for each replication. One jar of each set was provided with 10 g of grains given the name ‘A’ while the other jar was kept empty and given the name **‘**B**’**. In jar ‘A**’**, the grains treated with extracts were placed, while the jar B remained empty. The jars used for experiment were disinfected with alcohol.

3. Experimental Process

The Phago-repellent test was carried out under laboratory condition against nymph and adults of *A. gossypii***.** The okra leaves were used as food for the spotted bollworm, *Earias vittella* larvae. Leaves were treated with different concentrations (0.25, 0.5, 1.0, 1.5 and 2.0 per cent) for two minutes. The treated leaves were left under electric fan for about half an hour, to make a dry film of the extracts on the leaves for each set of extract and one control. The treated foods were kept in jar (23cm x 10cm) on moist filter paper. The untreated leaves were dipped in Benzene + emulsified water only. Ten starved *E. vittella* larvae were released in each jar along with control. Three replicates per treatments were maintained. The treated jars either repelled the insects or forced them to move from treated jars (T) to an empty jar (C) through the plastic pipe. The ones found in plastic pipe were considered repelled individuals. Number of *E. vittella* larvae reached to the food or repelled to untreated empty jar, were recorded.

Table 1: List of selected asteraceous plants materials used for extraction

S. No.	Scientific Name	Common names	Part Used	Family
1.	*Acmella paniculata* Well ex DC	Toothache Plant	Leaves	Asteraceae
2.	*Chromolaena odorata* Linn.	Siam weed	Leaves	Asteraceae
3.	*Chrysanthemum cinerariaefolium* (trev.) Vis.	Insecticide Daisy	Roots	Asteraceae
4.	*Cichorium intybus* (L.)	Chicory	Aerial parts	Asteraceae
5.	*Inula racemosa* Hook. f	Puskarmul	Roots	Asteraceae
6.	*Mantisalca duriaeri* Birq. Et Cavill.	Spach	Flowers	Asteraceae
7.	*Rechardia tingitana* (L.) Roth	False Sowthistle	Flowers	Asteraceae
8.	*Rhaponticum acaule* (L.) DC.	Coffee plum	Flowers	Asteraceae
9.	*Scorzonera undulate* Vahl	Black Salsify	Aerial parts	Asteraceae
10.	*Tagetes minuta* Linn.	Wild Marigold	Flowers	Asteraceae

Table 2: Summary of Extractives Formulations

Concentration (%)	Amount of Stock Solution (ml)	Amount of Benzene (ml)	Amount of Emulsifiable Water (ml)	Total Amount (ml)
0.25	2.50	22.50	475.00	500.00
0.50	5.00	20.00	475.00	500.00
1.00	10.00	15.00	475.00	500.00
1.50	15.00	10.00	475.00	500.00
2.00	20.00	5.00	475.00	500.00

4. Phago-Repellent Bioassay

For testing the repellent effect of asteraceous extracts okra leaves were used as food for *E. vittella* larvae treated with different concentrations. The treated foods were kept in jar (23cm x 10 cm) on moist filter paper. Thirty (15 male and 15 female), 24 hours starved *E. vittella* larvae were released in each jar along with control. The treated seeds were dipped in Benzene + emulsified water only. After four hours of the release *E. vittella* larvae. Treated *E. vittella* larvae either repelled and forced them to move from treated jars A to an empty jar B through the plastic pipe The ones found in plastic pipe were considered repelled individuals. The repellency data (in treated and untreated jars) and alive (in empty or untreated jars) were recorded for 6hr at an interval of 6 hours for each observation (Sighamony *et al.* 1984, Tripathi *et al.* 2000, 2001, Trivedi and Chandel, 2009) [24, 25, 26, 27]. The data was collected on the number of *E. vittella* larvae which reached the treated food and repellency over control was recorded and calculated using Abott formula (Abbott, 1925) [28].

5. Result and Discussion

The data depicted from the (table 1 and figure 1, 2, and 3) results in the present experiment of repellent test against larvae of *Earias vittella*. All the plant extracts showed a good Phago-repellent property, when compared with the control. The repellent property was observed based on their EC_{50} values as: *C. intybus* (0.0823) > *I. racemosa* (0.1043) > *T. minuta* (0.1620) > *C. cinerariaefolium* (0.1677) > *M. duriaeri* (0.1788) > *S. undulate* (0.3127) > *A. paniculata*, (0.3805) > *R. acaule* (0.4405) > *R. tingitana* (0.6368) > *C. odorata* (0.8870), respectively. The relative EC_{50} values of the above extracts are in descending order, 10.7700 > 8.5043 > 5.4753 > 5.0227 > 4.9608 > 2.8365 > 2.3311 > 1.3929 > 1.0000 times as repellents, where as *C. odorata* is taken as a unit.

In the support of present finding the various asteraceous extractives has been reported by several.

Entomologist among them effect of *Cichorium intybus*, *Inula racemosa*, *Tagetes minuta* and *Mantisalca duriaeri* extracts against pulse beetle, *Callosobruchus chinensis* Linn. (Bhartii and Chandel 2017) [29]. in the same way a number of Entomologist worked on several indigenous plant extractives are tested for their repellent biopotency and exhibited strong biopotency against pulse beetle, *Callosobruchus maculatus* (Maredia *et al.* 1992, Rahman and Talukder 2006 and Tripathi and Upadhyay, 2009) [30, 31, 32].

Similarly, different formulation *Cleome gynandra* extractives gave significant relent efficacy to spider mite (*Tetranychus urticae* Koch). *Ocimum suave* was found to repel the tick *Rhipicephalus appendiculatus* (Esther *et al.* 1995) [33]. Aqueous and methanolic plant extracts of *T. minuta*, which exhibited high repellency (IR = 0.04) against repellent effectiveness against the red flour beetle, Tribolium castaneum (Herbst) (Coleoptera Tenebrionidae). The application of these botanicals may be promising in protecting of stored grains against coleopteran pests. (Padin *et al.* 2013) [34]. *Alpinia galanga* (L.) species of termites, *Coptotermes gestroi* (Wasmann) and *Coptotermes curvignathus* (Holmgren) (Isoptera: Rhinotermitidae). Repellent activity shows that 250 ppm of 1,8-cineol caused 50.00 ± 4.47% repellency for *C. gestroi* (Sagheer *et al.* 2011, Salari *et al.* 2011, Khan *et al.* 2013, *et al.* 2011, Chandel and Singh 2016, Fauziah *et al.* 2015, Chandel and Singh, 2017) [35, 36, 37, 38, 39, 40]. The maximum repellent activity was observed at 500 ppm in methanol extracts of *N. nucifera*, ethyl acetate and methanol extract of *P. nigrum* and methanol extract of *T. ammi* and the mean complete protection time ranged from 30 to 150 min with the different extracts tested. (Liu *et al.* 2013) [41].

Table 3: Calculation of Log Conc./Probit Repellency Regression column of asteraceous extractives against *Earias vittella* larvae

Plant Extracts	Het.*	X^2	Regression Equation	Ec_{50}	Fiducial Limit	Relative Ec_{50}
Acmella paniculata	3	0.96	Y=0.72X +3.93	0.3805	M1=2.9870 M2=1.1284	2.836
Chromolaena odorata	3	1.88	Y=0.90X+3.21	0.8870	M1=2.3866 M2=0.1286	1.000
C. cinerariaefolium	3	1.59	Y=1.08X+2.66	0.1677	M1=2.3362 M2=1.9781	5.022
Cichorium intybus	3	0.28	Y=0.75X +4.1	0.0823	M1=2.1368 M2=1.278	10.770
Inula racemosa	3	4.02	Y=1.07X +2.70	0.1043	M1=2.3256 M2=1.9392	8.504
Manisalca duriaeri	3	0.52	Y=0.88X +4.42	0.1788	M1=1.8678 M2=0.1251	5.022
Reichardia tingitana	3	0.52	Y=0.88X +4.42	0.6368	M1=1.8678 M2=0.1251	1.392
Rhaponticum acaule	3	1.95	Y=0.88X +4.37	0.4405	M1=1.5188 M2=0.0148	2.331
Scorzonera undulate	3	0.20	Y=0.84X +4.50	0.3127	M1=1.9418 M2=0.0432	4.960
Tagetes minuta	3	0.76	Y=1.07X +3.03	0.1620	M1=2.0527 M2=1.6572	5.475

In case of X^2 was found non significant heterogeneous at P=0.05, Y=Probit repellency, X=Log Concentration X 10, D.F. = Degree of Freedom, E.C.$_{50}$ = Concentration Calculated at given 50% repellency.

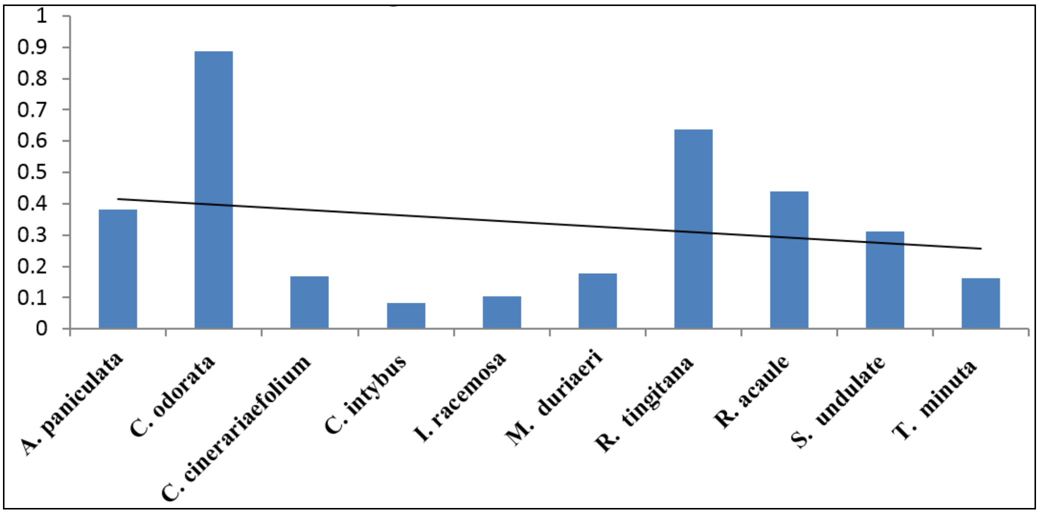

Fig 1: Calculation of log conc./Probit Repellency Regression column of extractives against *E. vittella* larvae based on EC_{50}

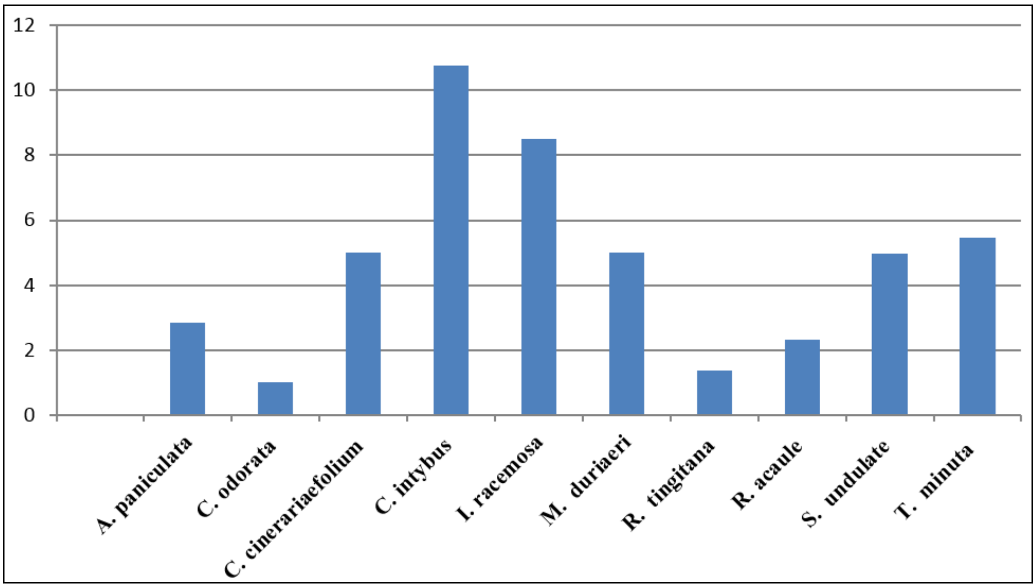

Fig 2: Calculation of log conc./Probit Repellency Regression column of extractives against *E. vittella* larvae based on relative EC_{50}

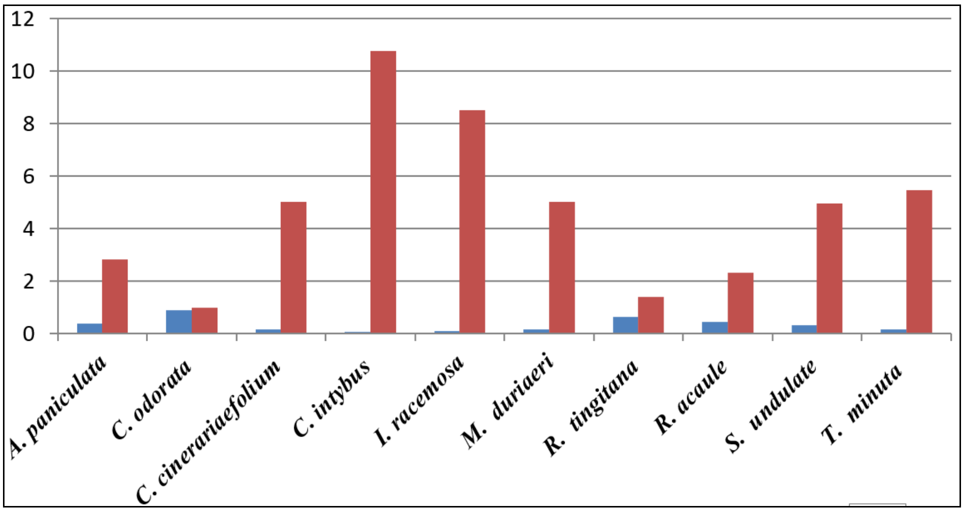

Fig 3: Log concentration probit repellency regression column of comparative Ec_{50} and Relative Ec_{50} values of extractives against *E. vittella* larvae

6. Conclusion

Conclusively, the present investigation revealed that there appears prospects in selected botanicals *Cichorium intybus*, *Inula racemosa* and *Tagetes minuta* was registered promising repellency with minimum EC_{50} values to the *E.vittella* larvae, when compared to other extracts. Among them *Cichorium intybus* elicited a strong defense response to counteract The growth and development of *E.vittella* larvae was significantly retarded when they fed on *Cichorium intybus* followed by *Inula racemosa* and *Tagetes minuta* ectractives as compared to *C. odorata* treated okra leaves. The data depicted in table 1 and figure 1 indicated that result based on their relative EC_{50} values the extracts of *Cichorium intybus* showed highest relative repellency (7.41) to the early emerged treated beetle followed by *Inula racemosa* (6.39), *Tagetes minuta* (5.88) and *Chrysanthemum cinerariaefolium* (5.42) times more repellent than *C. odorata* (1.00), which is taken as unit. The above selected plant materials can be used in protection of okra plant from *E.vittella* larval infestations under laboratory conditions. Thus, *C. intybus* and *Inula racem*osa aerial parts extract significantly reduced the population and damage caused by the major insect pests of okra recorded at Kanpur, India, compared to the untreated control. The *C. intybus*, *Inula racem*osa and *T.minuta* aerial parts extracts can be used effectively by farmers as a component of integrated management of pests of okra in India. This increase might be attributed to the plant's strong repellent properties. Importantly, selected extract from *C.oderata* leaves were also able to retard larval infestation of *E.vittella*. We conclude that defense metabolites from *C. intybus* and *Inula racem*osa possess strong repellent activity against *Plutella xylostella* (Linn.) even at lower concentrations revealed by present study and corroborated by earlier reports (Manish Kumar, 2017) [42].

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