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Original Paper

Insecticidal potentials of dry powder and solvent extracts of *Tithonia diversifolia* (Hemsl.) A. Gray flower against rice meal moth, *Corcyra cephalonica* (Stainton)

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Post-harvest losses of agricultural produce due to storage food grain pests lead to detrimental effects on the economic growth of any nation. However, a solution to this is to rely on adapting necessary cultural operations and effective traditional remedies that are locally available, which are inexpensive for the control of pests. *Tithonia diversifolia* (Hemsl.) A. Gray is an invasive weed that grows along the roadside and wasteland in rural and urban areas. The objective of this study was to evaluate the pesticidal efficacy of powder and extracts of *T. diversifolia* flower by using different solvents such as aqueous, methanol, chloroform, hexane, petroleum ether and ethyl acetate against rice moth, *Corcyra cephalonica*. The dry powder and extracts of *T. diversifolia* flower were tested to check the mortality of *C. cephalonica* eggs, larvae, and adults under the laboratory conditions (28 ±2 °C and relative humidity 65 ±5%). The mortality of the insect increased with an increase in the concentration of extracts. The results showed that the different solvent extracts of *T. diversifolia* flower were significantly more effective than the dry powder and control against different developmental stages of *C. cephalonica*. Methanol and ethyl acetate extracts showed an almost equitoxic lethal effect on all three stages of the life cycle of *C. cephalonica*. The present work suggests that the *T. diversifolia* flower possesses insecticidal properties against *C. cephalonica* eggs, larvae and adults. Thus, it can be recommended for use by the farmers as a potential pesticidal plant.

Keywords: *Tithonia diversifolia*, *Corcyra cephalonica*, larvae, pesticidal plant, weed

1 Introduction

Plant-based products are incorporated into Integrated Pest Management (IPM) strategies and sustainable agricultural practices (Lengai et al., 2020). *Tithonia diversifolia* (Hemsl.) A. Gray, commonly called Mexican sunflower or Japanese sunflower is a member of the Asteraceae family. This plant is native to Mexico, Central America, Cuba and has been spread across the globe in more than 70 countries, and is considered as an invasive weed (Obiakara & Fourcade, 2018). According to the Global Invasive Species Database, it can tolerate heat, drought and swiftly grow to form a large herbaceous shrub (GISD, 2015). It is regarded as one of the less explored plant (Akobundu & Agyakwa, 1987).

The flower of *T. diversifolia* contains sesquiterpene, diterpenes, monoterpenes (Tagitinins A) and acyclic compounds along with α-Pinene, cis-Ocimene, and limonene as reported earlier (Agboola et al., 2016; Menut et al., 1992; Zhao et al., 2012). These phytochemicals are ascribed to their biological activity against insects (Green et al., 2017; Ambrósio et al., 2008). Moreso, this plant has been detailed to have insect feeding deterrent characteristics due to the presence of 6-methoxyapigenin, tagitinins A, B, C, and F, along with diversiform, triotundin, tithonine and sulphurein (Mwanauta et al., 2014). Some of the documented pesticidal properties of *T. diversifolia* are larvicidal and repellent effect on mosquitoes (Langat et al., 2012), insecticidal activity on termite (Séraphin et al., 2018), acaricide (Deka et al., 2017), fungicide (Mapa et al., 2017) and insecticide against storage pest – *Callosobruchus maculatus* (Adedire & Akineye, 2005) and *Sitophilus zeamais* (Adedire et al., 2006; Babarinde et al., 2008).

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Corcyra cephalonica is a global storage pest commonly called as rice meal moth of the family Pyralidae. The pest is pantropical in distribution, mainly seen in tropics and subtropics. The larvae of the rice moth feed on almost all sorts of stored food like cereals, oilseeds, pulses, spices, fruits etc. (Pillai et al., 2017). The enormous losses caused in stored food are mainly by the fully-grown larvae, which feed on grains producing silken webs leaving faecal material. This kind of contamination makes food unhealthy for human consumption (Frenmore & Prakash, 1992; Verma & Pathak, 2018). Prolonged application of chemical pesticides has ultimately resulted in pest resurgence, increase in environmental contamination, harmful effect on non-target organisms and destruction of beneficial insects like pollinators in agroecosystem (Ndakidemi et al., 2016; Aktar et al., 2009). To avoid the toxicity of synthetic pesticides, an attempt was made for the first time to investigate the pesticidal efficacy of powder and aqueous, methanol, chloroform, hexane, petroleum ether and ethyl acetate extracts of *T. diversifolia* flower against rice moth, *C. cephalonica*. The extracts were tested on the egg, larvae, and adult of *C. cephalonica* in laboratory conditions.

2 Material and methods

2.1 Insect rearing

The eggs of *C. cephalonica* were obtained from Indian Council for Agricultural Research (ICAR) – National Bureau of Agricultural Insect Research (NBAIR), Bangalore and reared under the laboratory condition (28 ±2 °C and relative humidity 65 ±5%). The culture was maintained in autoclaved dietary medium kept in a plastic container of 10 kg capacity covered using a muslin cloth and 0.5 cc of eggs were reared on a dietary medium consisting of 2.5 kg broken sorghum, yeast powder (1 g), crushed groundnut powder (100 g) and streptomycin (0.5 g). The subculture and tests were conducted in the same condition (Allotey & Azalekor, 2000).

2.2 Plant collection and Preparation of extracts

Flowers of *T. diversifolia* was collected from the premises of Hindustan Machine Tool Industry Staff Quarters, Bangalore, Karnataka, India and authenticated by Dr. V. Rama Rao, Taxonomist, Regional Ayurveda Research Institute for metabolic disorders (Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India), Bengaluru, Karnataka with the authentication number RRCBI-mus 203. The collected flowers were rinsed in clean water to remove the dirt and impurities. The petals were separated and shade dried in a well-ventilated place for a week. The dried petals were powdered using an electrical mixer. Further, it was sieved, packed in an airtight plastic container and stored in a laboratory cupboard before use.

2.3 Procurement of Rice

Rice used for this study was pesticide-free and obtained from the local farmers of Chamarajanagar, Karnataka, India. Damaged grains were separated by handpicking method and healthy grains were used for the experiment. The clean and healthy seeds were oven-dried at 105 °C for 90 minutes and cooled at room temperature overnight to eliminate any pest infestation coming from the field. Using digital electrical balance, 20 g of rice grains were weighed in 240 ml glass bottles in quadruple of each concentration and stored in a cool, dry place.

2.4 Effect of *Tithonia diversifolia* flower powder on eggs of *Corcyra cephalonica*

Fine flower powder of *T. diversifolia* at concentration 0.5, 1.0, 1.5, 2.0 g mixed with 20 g of rice grain in 240 ml glass bottle and each one was joggled several times to ensure homogenous mixing of powder with grains. Further, 20 un-collapsed eggs (0–24 hr old) of *C. cephalonica* were introduced into each bottle using a fine brush and was covered using muslin cloth. Four replicates of each treatment were set up and a bottle devoid of flower powder was included as control. After a week of incubation, larvae became visible to naked eyes and were counted. The eggs that failed to hatch was noted and percentage of egg hatching inhibition was calculated (Khani et al., 2012; Zambare et al., 2012).

2.5 Effect of flower powder of *T. diversifolia* on larvae and adult emergence

Rice grains (20 g) were admixed with different concentrations (0.5, 1.0, 1.5, and 2.0 g) of *T. diversifolia* flower powder in 240 ml glass bottle. The rice grains without flower powder were set up as control. In each treatment bottle, 20 larvae (16 ±1 day old; 3rd instar) of *C. cephalonica* were introduced. Infested culture bottles were closed using muslin cloth to avoid entry and exit of larvae and were incubated (28 ±2 °C and relative humidity 65 ±5%). The experiment was designed in two sets (A & B) of four replicates of each concentration. In set A bottles, larval mortality was checked after 8 days and the number of dead larvae was noted to figure out the percentage of larval mortality (Meena et al., 2016). In set B bottles, larvae were allowed to feed on to produce adults. The number of adults that emerged was recorded at the end of the experiment. The percentage of inhibition of adult emergence was calculated (Meena et al., 2016).

2.6 Preparation of plant extracts

Twenty grams of the powdered flower were separately extracted with 200 ml of solvents such as methanol, aqueous, ethyl acetate, chloroform, hexane, and petroleum ether using soxhlet apparatus to get the different solvent extracts. The instrument was run for 16 hours at a temperature not exceeding the boiling point of the respective solvent. The extracts were separately collected, filtered and the filtrates were concentrated using a rotary flash evaporator. Different concentration of stock (0.4, 0.6, 0.8, 1.0, 2.0% of extract) was prepared by dissolving the extracts in respective solvent. Extract solutions were stored in the refrigerator at 4 °C (Merculieff et al., 2014).

2.7 Effect of plant extracts on egg hatchability

Two millilitres of different extract solution (methanol, aqueous, chloroform, hexane, petroleum ether, and ethyl acetate) of *T. diversifolia* flowers at various concentration (0.4, 0.6, 0.8, 1.0 and 2.0%) admixed separately with 20 g of rice in 240 ml glass bottle. Rice grains with 2 ml of pure solvent (chloroform, hexane, petroleum ether, and ethyl acetate) alone without extract was separately included as control. Solvents were evaporated to dryness and in each treatment bottle, 20 un-collapsed eggs (0–24 hr old) of *C. cephalonica* was introduced using a fine brush and the bottles were covered using muslin cloth. Four replicates of each treatment were set up and after a week of incubation, larvae became visible to naked eyes and were counted. The eggs that failed to hatch were noted. The percentage of inhibition in egg hatch was calculated (Zambare et al., 2012).

2.8 Effect of plant extracts on larval mortality and adult emergence

Two millilitres of different concentration (0.4, 0.6, 0.8, 1.0 and 2.0%) of different extract solution (methanol, aqueous, chloroform, hexane, petroleum ether, and ethyl acetate) was admixed with 20 g of rice grains in 240 ml glass bottle. In each bottle, 20 larvae (16 ±1 days old; 3rd instar) of *C. cephalonica* were introduced and bottles were covered using muslin cloth and stored at 28 ±2 °C and relative humidity 65 ±5%. The rice grains with solvent alone were set up as control. The experiment was designed in two sets (A & B) of four replicates of each concentration. In set A bottles, larval mortality was checked after 8 days and the number of dead larvae was noted to find the percentage of larval mortality (Pathak & Tiwari, 2012). In set B bottles, larvae were allowed to feed on to produce adults. The number of adults that emerged was recorded at the end of the experiment. The percentage of inhibition in adult emergence was calculated (Khani et al., 2012).

2.9 Statistical analysis

Data were analysed using one- way ANOVA ($p < 0.05$) followed by Tukey's multiple range test using GraphPad Prism 5.01 software. To estimate the LC_{50} and associated 95% confidence interval for each treatment, mortality was corrected by Abbott's formula (Abbott, 1925) and Probit analysis (Finney method) of dose-mortality was conducted (Finney, 1971).

3 Results and discussion

Percentage of egg mortality, larval mortality, and inhibition of adult emergence of *C. cephalonica* in different concentration of dry flower powder of *T. diversifolia* is presented in Table 1. At 0.5 g dosage level, the egg hatchability was inhibited only by 7.5 ±1.44% while at 2 g dosage level highest suppression of 50 ±2.04% was observed. Larval mortality was 5 ±0% at 0.5 g of floral powder which increased to only 33.75 ±6.88% at 2.0 g dosage. Similarly, inhibition of adult emergence was 11.25 ±3.15% recorded at the lowest dosage level 0.5 g

Table 1 Percentage inhibition of egg hatchability, larval mortality and inhibition of adult emergence of *Corcyra cephalonica* exposed to flower powder of *Tithonia diversifolia*

Treatment (g)	% inhibition of egg hatchability	% larval mortality	% inhibition of adult emergence
Control	0.0 ±0.0^{a}	0.0 ±0^{a}	1.25 ±1.25^{ab}
0.5	7.5 ±1.44^{a}	5.0 ±0^{a}	11.25 ±3.15^{b}
1.0	22.5 ±4.33^{b}	10 ±0^{a}	26.25 ±3.15^{c}
1.5	36.25 ±2.39^{c}	11.25 ±3.15^{a}	42.5 ±3.23^{d}
2.0	50 ±2.04^{d}	33.75 ±6.88^{a}	43.75 ±2.39^{d}
F value	68.08	10.43	47.08
LC_{50} (LCL – UCL)	2.09 (1.89–2.29)	6.74 (2.38–11.11)	2.39 (1.83–2.94)

mean ± standard error followed by the same letter with in the column do not differ significantly ($P < 0.0001$). LCL, lower confidence limit, UCL – upper confidence limit

which increased to 43.75 ±2.39% at the 2 g dosage level. As the dosage level of flower powder increased, a significant increase in mortality was noted. The lethal concentration 50% of flower powder on *C. cephalonica* is displayed in Table 1, which depicts that 2.09, 6.74, and 2.39 g of flower powder that could kill 50% of the population of *C. cephalonica* egg, larvae and evoke inhibition of adult emergence, respectively.

The effect of the floral extracts of *T. diversifolia* with different solvents (chloroform, ethyl acetate, hexane, petroleum ether, aqueous, and methanol) on the hatchability of *C. cephalonica* eggs is as shown in Table 2. As the concentration increased, a significant increase in egg mortality was noticed in all the extracts. There was a substantial difference between the egg hatchability in control and floral extracts in different solvents. Among six solvents tested, methanol extract elicited 97.5 ±1.44% mortality of egg at 2% concentration which was significantly highest in inhibiting the hatchability of rice moth eggs. This was followed by chloroform, hexane, and ethyl acetate extract at the concentration 2% which showed 91.25 ±2.39%, 83.75 ±4.27%, and 81.25 ±1.25%, respectively. Ethyl acetate and hexane extract were toxic to the eggs at the lowest concentration of 0.4% where mortality of egg was 46.25 ±2.39% and 45.0 ±2.04% respectively. Significant reduction in egg hatchability showed the detrimental effect of both ethyl acetate and methanol extract having lowest LC_{50} value of 0.507% and 0.539% respectively (Table 2).

The larvicidal effect of different solvent extracts of *T. diversifolia* flower against *C. cephalonica* larvae is presented in Table 3. Noticeably higher mortality was observed in all extracts as compared to the control. Among six extracts, methanol extract at maximum concentration (2%) recorded 100% mortality (LC_{50} = 0.594%), followed by ethyl acetate (91.25%) and aqueous (92.5%) extract. Ethyl acetate extract was effective in causing the death of over half of the total larval population showing LC_{50} of 0.285% (Table 3).

All the treatments strongly suppressed the adult emergence of *C. cephalonica* (Table 4). As the extract

Table 2 Percentage inhibition of *Corcyra cephalonica* egg hatchability in various extracts of *Tithonia diversifolia* flower

Treatment (%)	Chloroform	Ethyl acetate	Hexane	Pet ether	Aqueous	Methanol
Control	10.0 ±3.53^{a}	11.25 ±2.39^{a}	18.75 ±3.15^{a}	6.25 ±2.39^{a}	2.5 ±2.5^{a}	10.0 ±2.04^{a}
0.4	18.75 ±3.75^{ab}	46.25 ±2.39^{b}	45.0 ±2.04^{b}	17.5 ±3.23^{ab}	11.25 ±3.39^{a}	26.25 ±4.27^{b}
0.6	30.0 ±5.40^{bc}	60.0 ±4.56^{c}	58.75 ±3.15^{bc}	30.0 ±4.56^{bc}	12.5 ±3.23^{ab}	51.25 ±4.27^{c}
0.8	38.75 ±4.27^{cd}	68.75 ±2.39^{cd}	61.25 ±4.27^{c}	40.0 ±5.40^{c}	23.75 ±1.25^{bc}	68.75 ±2.39^{d}
1	52.5 ±3.23^{d}	75.0 ±2.04^{de}	77.5 ±1.44^{d}	45.0 ±2.04^{c}	32.5 ±3.23^{cd}	78.75 ±5.54^{d}
2	91.25 ±2.39^{e}	81.25 ±1.25^{e}	83.75 ±4.27^{d}	65.0 ±4.56^{d}	41.25 ±2.39^{d}	97.5 ±1.44^{e}
F value	56.41	89.63	48.84	28.65	31.68	97.63
LC_{50} (LCL – UCL)	0.992 (0.869–1.114)	0.507 (0.394–0.619)	0.747 (0.654–0.841)	1.426 (1.034–1.818)	2.439 (2.244–2.635)	0.539 (0.419–0.659)

mean ± standard error followed by the same letter within the column do not differ significantly (P <0.0001). LCL, lower confidence limit, UCL – upper confidence limit

Table 3 Percentage larval mortality of *Corcyra cephalonica* in various extracts of *Tithonia diversifolia* flower

Treatment (%)	Chloroform	Ethyl acetate	Hexane	Pet ether	Aqueous	Methanol
Control	5.0 ±2.04^{a}	8.75 ±3.75^{a}	6.25 ±2.39^{a}	8.75 ±1.25^{a}	2.5 ±1.44^{a}	5.0 ±2.04^{a}
0.4	17.5 ±3.23^{b}	61.25 ±5.54^{b}	33.75 ±3.15^{b}	20.0 ±2.04^{ab}	12.5 ±1.44^{ab}	30.0 ±4.56^{b}
0.6	38.75 ±3.75^{c}	65.0 ±3.5^{b}	40.0 ±4.56^{bc}	33.75 ±4.27^{bc}	22.5 ±3.23^{b}	51.25 ±4.27^{c}
0.8	56.25 ±2.39^{d}	67.5 ±5.95^{b}	53.75 ±1.25^{cd}	41.25 ±4.27^{cd}	56.25 ±5.15^{c}	67.5 ±3.23^{d}
1	61.25 ±2.39^{de}	77.5 ±3.23^{bc}	62.5 ±5.20^{d}	55.0 ±2.89^{d}	67.5 ±3.23^{c}	85.0 ±2.04^{e}
2	72.5 ±1.44^{e}	91.25 ±5.15^{c}	88.75 ±2.39^{e}	78.75 ±3.75^{e}	92.5 ±1.44^{d}	100 ±0.0^{f}
F value	99.09	36.74	66.28	58.39	139.0	128.8
LC_{50} (LCL – UCL)	0.927 (0.853–1.002)	0.285 (0.087–0.484)	0.748 (0.664–0.831)	1.052 (0.934–1.169)	0.836 (0.797–0.875)	0.594 (0.557–0.632)

mean ± standard error followed by the same letter with in the column do not differ significantly (P <0.0001). LCL, lower confidence limit, UCL – upper confidence limit

Table 4 Percentage inhibition of *Corcyra cephalonica* adult emergence in various extracts of *Tithonia diversifolia* flower

Treatment (%)	Chloroform	Ethyl acetate	Hexane	Petroleum ether	Aqueous	Methanol
Control	11.25 ±4.27^{a}	15.0 ±4.56^{a}	17.5 ±3.23^{a}	12.5 ±3.23^{a}	17.5 ±3.23^{a}	7.5 ±3.23^{a}
0.4	21.25 ±5.54^{ab}	25.0 ±2.04^{ab}	48.75 ±4.27^{b}	27.5 ±1.44^{b}	28.75 ±2.39^{ab}	67.5 ±5.95^{b}
0.6	32.5 ±3.23^{ab}	41.25 ±5.90^{ab}	57.5 ±3.23^{bc}	46.25 ±2.39^{c}	42.5 ±3.23^{b}	80.0 ±2.04^{bc}
0.8	67.5 ±3.23^{c}	83.75 ±4.27^{c}	65.0 ±5.4^{bc}	70.0 ±3.54^{d}	63.75 ±4.27^{c}	87.5 ±1.44^{cd}
1	92.5 ±3.23^{d}	90.0 ±3.54^{c}	72.5 ±3.23^{cd}	96.25 ±2.39^{e}	71.25 ±2.39^{c}	95.0 ±2.04^{d}
2	96.25 ±1.25^{d}	97.5 ±1.44^{c}	87.5 ±4.79^{d}	98.75 ±1.25^{e}	93.75 ±3.75^{d}	100.0 ±0.0^{d}
F value	99.95	83.65	33.74	202.1	75.58	123.6
LC_{50} (LCL – UCL)	0.740 (0.598–0.882)	0.651 (0.590–0.711)	0.602 (0.544–0.659)	0.660 (0.594–0.727)	0.819 (0.716–0.922)	0.289 (0.209–0.369)

mean ± standard error followed by the same letter with in the column do not differ significantly (P <0.0001). LCL, lower confidence limit, UCL – upper confidence limit

concentration increased, a remarkable increase in inhibition of adult emergence of *C. cephalonica* was observed. The methanol extract showed 100% inhibition of adult emergence and the concentration of 0.4% brought 67.5% inhibition of adult emergence with an LC_{50} value of 0.289% (Table 4). On a general note, the toxicity of both methanol and ethyl acetate extracts against all three stages of the insect was almost equitoxic and superior to the toxicity of other extracts.

Powdered leaves of *Lantana camara*, *Clerodendrum inerme*, *Citrus limon* and *Azadirachta indica* exhibited significant mortality of *C. cephalonica* (Morya et al., 2010; Pathak & Tiwari 2010). The present result shows that flower powder of *T. diversifolia* was less effective to the test organism – *C. cephalonica* compared to all the solvent extracts. This performance corresponds to Adedire and Akinneye (2004) who reported the moderate potential of *T. diversifolia* leaf powder in control of *C. maculatus* and *S. zeamais* (Babarinde et al., 2008).

In the current study, the insecticidal activity of different solvent extracts of *T. diversifolia* flower was tested on *C. cephalonica*. A similar study was carried out by Pantoja-Pulido et al. (2020) who evaluated insecticidal activity of various solvent extracts of *T. diversifolia* leaves, in which dichloromethane extract showed best activity against *Atta cephalotes* and Ambrósio et al. (2008) reported antifeedant activity against *Chlosyne lacinia*. Hexane and methanol extract of *T. diversifolia* was effective against the insect pests of honeybees: *Crematogaster lineolata*, *Aethina tumida*, *Achroia grisella* and *Galleria mellonella* (Pitan et al., 2015). Thus, solvents used for extraction has an influence on the bioactivity of the extracts (Lale & Maina, 2003; Akinyemi et al., 2016).

Among all the samples tested, methanol extract from the flower of *T. diversifolia* showed a remarkable detrimental effect on eggs, larvae, and adults of *C. cephalonica*. The present study supports the earlier work of Kangade and Zambare (2013) where 100% mortality of rice moth was observed in methanol extract of *Argemone mexicana*. Similar results were also obtained by Tavares et al. (2014) that ethanol extract of *T. diversifolia* flower caused higher mortality and repellency of *Sitophillus zeamais* and *Sitotroga cerealella* (Fouad et al., 2014). The methanol extract of *T. diversifolia* leaves were toxic to *C. maculatus* (Green et al., 2017) and *Anopheles gambiae* (Ileke et al., 2019).

Thus, the pesticidal property of *T. diversifolia* extracts can be attributed to its rich source of phytochemicals, especially the sesquiterpene lactone (Ayokunnun & Moteetee, 2017). Sesquiterpene lactones are characteristic constituents of the family Asteraceae possessing biological activities such as insect antifeedant, antibacterial, molluscicidal, antiprotozoal, antispasmodic, protection of plants against pathogens, herbivorous insects and allelopathic agents (Choudary & Mishra, 2019). Tagitinin C, a sesquiterpene mainly present in the genus *Tithonia*, revealed great antifeedant activity on the caterpillar (Pavela, et al., 2016) and several arthropod pests (Susurluk et al., 2007). This indicates that *T. diversifolia* having an inhibitory effect on eggs, larvae, and adults can be exploited to control *C. cephalonica* infestation of stored rice and other food grain. This plant is a global invasive weed, where its collection and utilization as pesticide could reduce its environmental impact. The results obtained from this study foretell greater utility of *T. diversifolia* as a source of botanical formulation capable of protecting stored rice from infestation by *C. cephalonica*.

4 Conclusions

The finding of the present study indicates that the methanol extract from the flower of *T. diversifolia* was toxic to *C. cephalonica* showing a promising effect in the control of the rice moth. The outcome of this research suggests that *T. diversifolia*, a locally available weed,

which can be utilized as a potential bioresource for the development of biopesticide. It can be an economically effective and environmentally friendly pest control resource against stored grain pests. However, further studies are necessary for the isolation, identification, and bioassay of the phytocompounds present in the plant with special reference to tagitinin C and tagitinin A for confirming their potential pesticidal property.

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