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# Efficacy of Thai indigenous entomopathogenic nematodes for controlling fall armyworm (*Spodoptera frugiperda*) (J. E. Smith)(Lepidoptera; Noctuidae)

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## Abstract

**Background:** Under laboratory and greenhouse conditions, the virulence of 2 isolates of Thai indigenous entomopathogenic nematodes (EPNs) in controlling the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera; Noctuidae), was demonstrated. Six EPNs dosages were tested against 2 larval instars of FAW under the laboratory conditions, while 2 different concentrations were tested under the greenhouse conditions.

**Results:** The results of a laboratory experiment revealed that 2 Thai indigenous EPNs isolates (*Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL 12.3) were efficient against the FAW, 2nd and 5th larval instars. Six different nematode concentrations (50,100, 150, 200, 250 and 300 infectious juveniles (IJs) ml<sup>-1</sup>) were evaluated, and all were proven to be effective, with the mortality rate associated with concentration. Inoculated larvae in the 2nd instar was more vulnerable than that in the 5th instar. *H. indica* isolate AUT 13.2 was more destructive than *S. siamkayai* isolate APL 12.3. The greatest mortality rate of 2nd instar larvae was 83% when *H. indica* AUT 13.2 was applied at the concentration of 250 IJs ml<sup>-1</sup>, and 68% when the nematode *S. siamkayai* APL 12.3 was used at the concentration of 300 IJs ml<sup>-1</sup>. At 250 IJs ml<sup>-1</sup>, the highest mortality rate of the 5th instar larvae was 45% for *H. indica* AUT 13.2 and 33% for *S. siamkayai* APL 12.3, respectively. To customize the concentration and volume of nematodes suspension evaluated in the greenhouse settings, the most sensitive stage of FAW and the optimum concentration that caused the highest mortality were used. The concentrations of both indigenous nematodes' isolates were 20,000 and 50,000 IJs ml<sup>-1</sup> per pot, respectively, and the results showed that the mortality rates were lower than that in the laboratory. FAW mortality rate was the highest (58%) in case of the nematode *H. indica* isolate AUT 13.2, against (45%) in case of *S. siamkayai* isolate APL 12.3, at the 50,000 IJs ml<sup>-1</sup> concentrations.

**Conclusions:** The study revealed the 2 Thai indigenous EPNs isolates (*H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL 12.3) were capable of controlling the FAW in both laboratory and greenhouse environments. The 2 Thai EPNs showed the potential to be considered as a biological control agent.

**Keywords:** *Spodoptera frugiperda*, Indigenous entomopathogenic nematodes, *Heterorhabditis indica*, *Steinernema siamkayai*, Virulence

## Background

In Thailand, maize forms an essential part of food and feed system and contributes significantly to income generation for rural households (Ekasingh et al. 2004). Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith)

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(Lepidoptera: Noctuidae), is indigenous to tropical and subtropical regions of the Americas (Day et al. 2017). This pest has not been reported outside America until 2016, when it was reported for the first time in Africa (Goergen et al. 2016). Since its invasion, the pest is causing considerable economic losses in Africa (De Groote et al. 2020). This pest was first detected in Thailand in December 2018, in a few sub-districts of Kanchanaburi and Tak provinces in the west of the country, near the Myanmar border (IPPC 2018). This insect is a polyphagous pest that has been documented to impact over 353 plant species from 76 plant families (Montezano et al. 2018). They are said to be a significant economic pest in maize and other Poaceae crops (Silva et al. 2017). Younger larvae feed on the tissue of the leaves, while older ones cause severe defoliation. It also burrows into the growing point (bud, whorl, etc.), leading "dead heart," wilting and death of the unfurled leaves (Day et al. 2017).

Pesticides are currently being utilized to control and minimize the spread of FAW in maize crops. Chemical pesticides may reduce insect pest attacks in the short term, but they may not be sustainable in the long run. Many studies have showed insecticide-resistant populations in FAW (Yu et al. 2003). Furthermore, synthetic chemical pesticides have the potential to harm both humans and the environment (Carvalho 2017). As a result, a new method of controlling FAW is required to decrease the damage caused by this destructive insect pest. Entomopathogens, such as bacteria, nematodes, fungi and virus, are an essential option for the management of diverse arthropod species and are appropriate approaches for a long-term sustainability of the ecosystem (Charnley and Collins 2007). Entomopathogenic nematodes (EPNs) are roundworms that live as parasites in insects (Lacey and Georgis 2012). Out of 23 nematode families, Steinernematidae and Heterorhabditidae are the two most prevalent nematode families studied as biological agents (Lacey and Georgis 2012). The two most important genera within the family are *Steinernema* and *Heterorhabditis*. All *Steinernema* species are symbiotic with *Xenorhabdus* bacteria, whereas all *Heterorhabditis* nematode species are symbiotic with *Photorhabdus* bacteria (Boemare et al. 1993). These symbiotic bacteria are vital in the death of insect hosts (Hominick et al. 1996). These EPNs have a wide host range, making them a viable option for biological insect pest control (Arthurs et al. 2004). The EPNs can be used solely as a biological control agent or combined with other biocontrol agents, such as entomopathogenic bacteria and fungi in order to improve their efficacy in controlling insect pests (Laznik et al. 2012).

In the Americas and freshly invaded areas like Africa, FAW larvae have been found to be vulnerable to EPNs

species (Lacey and Georgis 2012). Noosidum et al. (2010) documented the existence of EPNs in Thailand. Other researchers have recently discovered several species of those indigenous entomopathogenic nematodes in Thailand (Thanwisai et al. 2021).

The goal of this study was to test the efficacy of several Thai indigenous EPNs isolates against the FAW in both laboratory and greenhouse environments.

## Methods

### Fall armyworm collection and rearing

Fall armyworm larvae were collected from maize fields in Thailand's Phitsanulok (16°49'29.32" N, 100°15'30.89" E, elevation: 50 msl.), Sukhothai (17° 19'1.6608" N, 9°33'42.12" E, elevation: 93.54 msl.) and Uttaradit provinces (17°54'1.4537" N, 100°30'48.89" E, elevation: 108 msl.). The larvae were identified and confirmed according to the identification procedures provided by Visser (2017). Larvae were placed in a 20-ml plastic container and fed on fresh maize leaves grown without the use of chemical pesticides. The pupae were collected and placed in a plastic container inside a rearing cage (30 × 30 × 30 cm) once they had developed. Adults were fed on a 10% sugar solution in a rearing cage when they emerged, and then transferred for the experiments. For egg-laying, a young plant was dipped in a glass of water and placed inside the chamber. The larvae were transferred for the experiments when they had reached the relevant stages.

### Entomopathogenic nematodes collection and multiplication

The study used 2 EPN isolates, *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL 12.3. These EPNs were collected from agricultural areas which *H. indica* isolate AUT13.2 was collected from a mango orchard (17°26'13.4" N, 100°05'40.4" E, elevation 57 msl.), while *S. siamkayai* isolate APL12.3 was collected from a vegetable garden (17°02'12.8" N, 100°10'01.0" E, elevation 48 msl.). Final instar larvae of the Greater wax moth, *Galleria mellonella* L., were used to multiply the EPNs. The White trap technique (White 1927; Kaya and Stock 1997) was utilized to obtain infective juveniles (or IJs) of the EPNs from dead larvae to be used in the experiments.

### Testing of the efficacy of EPNs in the laboratory

#### Experimental design and application of the EPNs

The experiment was designed in a completely randomized design (CRD), with 6 treatments consisting of 6 different numbers of IJ nematode suspension, namely: 50, 100, 150, 200, 250 and 300 IJs ml<sup>-1</sup>, and a control consisting of the same volume of sterilized distilled water. The 2nd and 5th larval instars were tested separately on each EPN

isolate and nematode suspension. The tests were repeated 4 times (4 replications), with 10 larvae/replication. Individually, the larvae were placed in a 5.5-cm-diameter Petri dish with a detached maize leaf as food. One milliliter of nematode suspension containing a different density of IJs was applied topically to the larvae and maize leaf in each treatment, with a similar application in the control treatment. The food was changed daily and the larvae were kept at 25 °C under a 14:10 (light: dark) photoperiod with  $60 \pm 10\%$  relative humidity in the insect rearing room.

#### Assessment of mortality

Mortality of the larvae was assessed 48 h after inoculation, and the observations were recorded for 10 days. When larvae failed to respond to the forceps' touch, they were marked as dead. The dead larvae were kept separately to observe emergence of nematodes from the cadaver using the White trap technique. Only those larvae that showed evidence of nematode emergence were recorded as nematode killed. The following formula was used to compute the 10-day accumulated mortality percentage of the tested samples.

$$\text{Observed mortality} = \frac{\text{Total number of dead larvae}}{\text{Total exposed larvae}} \times 100$$

The tests were rejected if the control treatment mortality was more than 20%. When control mortality was less than 20%, Abbott's (1925) formula was used to correct observed mortality, as shown below.

$$\text{Corrected Mortality} = \frac{\% \text{test mortality} - \% \text{control mortality}}{100 - \% \text{control mortality}} \times 100$$

#### Testing of the efficacy of EPNs in the greenhouse Planting maize in greenhouse and release of the FAW

Super-sweetcorn maize variety was grown in the earthen pot (50 cm in diameter), and planting soil was added in a pot at 2/3 in each pot's capacity. Initially, 10 maize seeds were grown in each pot and watered daily and the seedlings were reduced to 5 per pot after germination. The seedlings were ready to utilize in testing when they reached the 4 leaf fully emerged stage, which took around 2 weeks following emergence. Prior the larvae reach their 2nd instar, 10 fully grown 1st instar larvae were manually placed into the maize plant in each container with some detached maize leaves. The pots were caged and covered vertically and from the top with insect mesh when they were infested.

#### Experimental design and application of the EPNs

The greenhouse experiments were carried out using a randomized complete block design (RCBD). There were

3 treatments, each with 2 different densities of EPNs, 20,000 IJs ml<sup>-1</sup>, 50,000 IJs ml<sup>-1</sup>, and a sterile water as a control. Each treatment was carried out 8 times. Because the FAW was in its dispersal stage, only 2nd instar larvae were chosen to be studied. The nematode suspension was applied 24 h after the FAW larvae were released. Using a hand sprayer, each pot was sprayed by 100 ml of the described densities of EPNs suspension directly on the entire leaves. EPNs were applied 3 times, with two-day interval between applications. In the greenhouse experiment, the assessment of larval mortality followed a similar procedure to that used in the laboratory experiment.

#### Data analysis

Mortality percentages the FAW caused by the EPNs both from the laboratory and the greenhouse conditions were normalized using square root transformation. The number of dead larvae in different treatments (density of EPNs) was subjected to statistical analysis of variance (ANOVA). The mean comparison of the laboratory condition was made using Duncan multiple range tests (DMRT) to find a significant difference between treatments ( $p \leq 0.05$ ). The Tukey test was carried out to determine a significant difference of the means between treatments ( $p \leq 0.05$ ) of the greenhouse experiment.

## Results

#### Efficacy of EPNs in the laboratory

In laboratory experiments, the efficacy of EPNs, *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL12.3 on the mortality of 2nd and 5th larval instars of FAW was determined. Both isolates were found to be capable of infecting and killing larvae at varied densities of EPNs utilized in the investigation. After 48 h, death rate of the larvae was observed. It was influenced by the EPN isolates applied and the densities of IJs used. One-way ANOVA revealed a statistically significant difference between treatments for *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL 12.3, with  $F(6,21) = 118$ ,  $p = 0.05$  for *H. indica* isolate AUT 13.2 and  $F(6,21) = 102.7$ ,  $p = 0.05$  for *S. siamkayai* isolate APL 12.3. Over 10 days of the exposure time, *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL12.3 caused 27.5 and 17.5% mortality at 50 IJs ml<sup>-1</sup>, respectively (Table 1). As the concentration was raised, the death rate risen. When infected at the concentration of 250 IJs ml<sup>-1</sup>, the EPNs *H. indica* isolate AUT 13.2 killed (82.5%) of the FAW. It was non-significantly different from those infected at 300 IJs ml<sup>-1</sup>. However, when using the highest concentration of 300 IJs ml<sup>-1</sup>, *S. siamkayai* isolate APL12.3 delivered the highest mortality percentage of 67.5%, which was non-significantly different from the density at 250 IJs ml<sup>-1</sup>.

**Table 1** The mean of accumulated mortality percentage of the 2nd instar larvae of FAW at 10 days after application of the EPNs *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL12.3 in the laboratory condition

Larval stage	Concentrations of EPNs (IJs/ml)	EPNs	
		<i>H. indica</i> AUT 13.2	<i>S. siamkayai</i> APL 12.3
		Mean (%)	Mean (%)
2nd instar	Control (distilled water)	0.0a	0.0a
	50 IJs/ml	27.5b	17.5b
	100 IJs/ml	42.5c	27.5c
	150 IJs/ml	55.0d	35.0d
	200 IJs/ml	65.0e	47.5e
	250 IJs/ml	82.5f	65.0f
	300 IJs/ml	80.01f	67.5f

The means in each column followed by a common letter are not significantly different by the Duncan multiple range tests at 5% level

**Table 2** Mean of accumulated mortality percentage of the fifth instar larvae of FAW at 10 days after application of the EPNs *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL12.3 in the laboratory condition

Larval stage	Concentrations of EPNs (IJs/ml)	EPNs	
		<i>H. indica</i> AUT 13.2	<i>S. siamkayai</i> APL 12.3
		Mean (%)	Mean (%)
5th instar	Control	0.0a	0.0a
	50 IJs/ml	17.5b	15.0Bb
	100 IJs/ml	25.0c	20.0b
	150 IJs/ml	32.5d	22.5b
	200 IJs/ml	35.0e	27.5c
	250 IJs/ml	45.0f	32.5d
	300 IJs/ml	42.5f	30.0d

In each column, the mean followed by a common letter is not significant. Mean separated by Duncan multiple range tests at 5% level

The efficacy of *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL12.3 against the 5th instar larvae of FAW gave a high mortality percentage when they were treated with a high density of the EPNs (Table 2). The mortality percentage was statistically significant between treatments as determined by one-way ANOVA ( $F(6, 21) = 13.75$ ,  $p = 0.05$  for *H. indica* isolate AUT 13.2 and  $F = 39.25$ ;  $df = 6, 21$ ;  $p = 0.05$  for *S. siamkayai* APL 12.3, respectively). The highest mortality occurred in the FAW larvae (45.0%) when they were infected by the EPN *H. indica* isolate AUT 13.2 at the density of 250 IJs ml<sup>-1</sup>, which was non-significantly different from the application at 300 IJs ml<sup>-1</sup>. The EPN, *S. siamkayai* isolate APL12.3 caused the highest mortality percentage when they were infected with 250 IJs ml<sup>-1</sup> (at 32.5%), but was not different from those at the density of 300 IJs ml<sup>-1</sup> (42.5%). However, when compared to the mortality of

**Table 3** The mean of accumulated mortality percentage of the 2nd instar larvae of FAW at 10 days after application of the EPNs *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL12.3 in the greenhouse condition

Treatments	IJs/ml	% mean mortality
<i>H. indica</i> AUT 13.2	20,000 IJs/ml	37.92a
<i>S. siamkayai</i> APL 12.3	20,000 IJs/ml	28.75a
Control		6.25b
<i>H. indica</i> AUT 13.2	50,000 IJs/ml	57.78b
<i>S. siamkayai</i> APL 12.3	50,000 IJs/ml	44.72a
Control		7.50c

The mean followed by a common letter in each density is not significant. Mean separated by Tukey tests at 5% level

2nd instar larvae, this larval stage had a low mortality rate.

### Efficacy of EPNs in the greenhouse

By comparing mean percentage mortality of FAW larvae among the treatments at the end of 10 days, the efficacy of *H. indica* isolate AUT13.2 and *S. siamkayai* isolate APL12.3 on mortality rate of FAW larvae at the 2nd instar larvae under greenhouse conditions was determined. After the application for 10 days, the results showed non-significant difference between those two EPN isolates (Table 3) at the EPN density at 20,000 IJs ml<sup>-1</sup>. The EPN *H. indica* isolate AUT 13.2 caused a high mortality rate (37.92%), while *S. siamkayai* isolate APL12.3 killed only (28.75%) ( $F = 28.63$ ;  $df = 2, 21$ ;  $p = 0.000$ ). Mortality percentages of the FAW raised when the EPN density was increased to 50,000 IJs ml<sup>-1</sup>, and significant difference between the two isolates was observed. *H. indica* isolate AUT 13.2 had a high mortality rate (57.78%), compared to (44.72%) *S. siamkayai* isolate APL 12.3 ( $F = 64.09$ ;  $df = 2, 21$ ;  $p = 0.05$ ).



## Discussion

Entomopathogenic nematodes (EPNs) have already been found to be effective against a wide range of insect pests. They have been widely utilized to manage pests both below and above ground (Bhairavi et al. 2021). Many researchers previously reported the FAW's susceptibility to EPNs (Caccia et al. 2014). However, the efficacy of EPNs is governed by their virulence and their capability to find out their hosts (Cutler and Webster 2003). In Thailand, several isolates of indigenous EPNs have been documented (Thanwisai et al. 2021).

This is the first study evaluating the efficacy of Thai indigenous EPNs; *H. indica* isolate AUT13.2 and *S. siamkayai* isolate APL12.3 against the FAW. The result of the study demonstrated that *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL12.3 were capable of infecting and killing both the 2nd and 5th larval instars of FAW under laboratory conditions. Both isolates showed varied efficacy depending on the different concentrations used. The isolate *H. indica* AUT 13.2 caused more mortality larvae than *S. siamkayai* APL 12.3. With an increase in the density of infective juveniles per milliliter of pre-sterilized distilled water, there was a proportional increase in FAW larval mortality; however, increasing concentration above 250 IJs ml<sup>-1</sup> had non-significant difference. The 2nd instar larvae were particularly more sensitive to both EPN isolates examined.

Environment, stages of the host and innate characteristics of the nematodes, like the capability to find the host and the presence of symbiotic bacteria, all influence the potentials of EPNs (Batalla-Carrera et al. 2010). The discrepancies in efficiency reported in the laboratory where most settings are controlled and uniform may be attributable to the potential of the nematode isolates to infect the host and the efficacy of the symbiotic bacteria that is responsible for killing the host. Presence of symbiotic bacteria *Photorabdus* and *Xenorhabdus* in the nematode *H. indica* isolates AUT 13.2 over *S. siamkayai* isolate APL 12.3 in this study may explain the increased mortality reported by *H. indica* isolates AUT 13.2 over *S. siamkayai* isolate APL 12.3. As a result, it appears that the ability of *H. indica* isolates AUT 13.2 to kill FAW larvae over *S. siamkayai* isolate APL 12.3 was the most likely owing to the symbiotic bacterium *Photorabdus* contained within these EPNs. The mortality of FAW larvae has been demonstrated to vary in previous studies using different strains and isolates of *Heterorhabditis* spp. and *Steinernema* spp. Acharya et al. (2020) investigated the effectiveness of 7 EPN species and found that *H. indica*, *S. carpocapsae*, *S. arenarium* and *S. longicaudum* were highly virulent against various stages of the FAW larvae.

In the present study, the 2nd instar FAW larvae were more vulnerable to both isolates of EPNs than the 5th instar larvae. Some authors have reported differences in susceptibility of different stages of the FAW. For example, Acharya et al. (2020) reported that younger larvae (e.g., first-, second- and third-instar larvae) of the FAW were more susceptible to *H. indica* and *S. carpocapsae*, while elder larvae (e.g., 4th, 5th and 6th larval instars) were susceptible to *S. arenarium* and *S. longicaudum*.

The greenhouse experiment was applied to test how effective EPNs are on 2nd instar larvae that feed on maize plants. The results demonstrated that the EPN isolates were successful in killing the FAW larvae; however, the mortality rate was lower than in the laboratory. It is possible that the lowest mortality of FAW larvae in the greenhouse experiment was attributable to the difference in environmental conditions. UV light, warmer temperatures, desiccation and the features of exposed foliage can promote lesser EPN activation in greenhouse circumstances (Vashisth et al. 2013). These features make it more difficult for infective juvenile nematodes to locate the host larvae, because EPNs' adequate host interaction is crucial in infecting and killing larvae (Kaya and Gaugler 1993). Furthermore, because FAW larvae were placed on maize plants and had the freedom to move from plant to plant, EPNs may have had a difficult time making good host contact and attacking them. The mortality of FAW larvae was a high when the EPNs' concentrations increased to 50,000 IJs ml<sup>-1</sup>, and the EPNs application targeted the feeding larvae site. However, in field conditions, some environmental factors, especially soil moisture and temperature, may affect the efficiency of those EPNs as they need some moisture for survival. According to obtained results, under the greenhouse conditions, the rate and application frequency of EPNs increased, according to providing adequate moisture, to achieve better outcomes. There are many ways to optimize the efficacy of EPNs for greenhouse and field application like preparing EPNs formulation. EPNs can be combined with other control agents which can be some appropriate chemical insecticides and other biological control agents like fungus and bacteria (Koppenhöfer et al. 2020). The effectiveness of EPNs can also be improved by genetic improvement through selection and transgenic methods (Abd-Elgawad 2019). The efficacy of the 2 isolates used in the present study can be improved by developing the formulations or combining EPNs with other control agents.

## Conclusions

Both isolates of Thai indigenous EPNs, *H. indica* isolate AUT13.2 and *S. siamkayai* isolate APL12.3, evaluated against FAW larvae under both laboratory and greenhouse situations. The EPNs performed much effective in the laboratory than in greenhouse. Virulence

of EPNs, the host's susceptible stage and environmental variables all appear to play a role in ensuring effective infections. To use these Thai indigenous EPNs as a biological control agent, appropriate insect stages and environmental conditions must be considered.

#### Abbreviations

EPNs: Entomopathogenic nematodes; FAW: Fall armyworm; IJs: Infective Juveniles; msl: Meter above sea level.

#### Acknowledgements

The authors are highly grateful to the authority of the Department of Biology and Centre of Excellence for Biodiversity, Faculty of Science, Department of Microbiology and Parasitology, Faculty of Medical Science and Department of Agricultural Sciences, Faculty of Agriculture, Natural Resources and Environment, Naresuan University for providing the facilities and support in conducting this research.

#### Authors' contributions

WW designed and planned the experiments, assisted in experiments, major contributor in writing the manuscript. OL carried out all the experiments, analyzed the data and wrote the draft manuscript. AV collected the EPNs samples for experiment purposes and planned the experiments. DW planned the experiments, analyzed the data and critically revised the manuscript. All authors read and approved the final manuscript.

#### Funding

This research was financially supported by the Centre of Excellence on Biodiversity (Project No. BDC-PG4-161011) to W.W. and A.V. The authors would like to acknowledge the Faculty of Agriculture, Natural Resources and Environment, Faculty of Medical Sciences, and Faculty of Science, Naresuan University for providing resources necessary for the study. O. L. was supported by the scholarships from His Majesty the King of Bhutan and Naresuan University.

#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Declarations

#### Ethics approval and consent to participate

This research was approved for the Ethics of Use Animals for Scientific Work from Naresuan University (Approval No. 63–01–003).

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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Received: 8 October 2021 Accepted: 23 November 2021

Published online: 27 November 2021

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