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Int. J. Biol. Chem. Sci. 14(4): 1167-1176, May 2020

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Bio-insecticide potential of three aromatic plants of Burkina Faso (*Ocimum americanum* L., *Cymbopogon schoenanthus* L. and *Hyptis suaveolens* Poit.) and their importance in cowpea seed conservation

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ABSTRACT

Cowpea is an important source of food and income for the populations of sub-Saharan Africa in general and particularly in West Africa. However, farmers face storage and conservation problems. During storage in granaries, cowpea seeds are attacked by insects causing decreased grain weight and quality and sometimes a loss of germination capacity. Traditionally, farmers have used aromatic plants to limit insect damage. The aim of the present study is to test the insecticidal potential of three aromatic plants (*Ocimum americanum* L., *Cymbopogon schoenanthus* L. and *Hyptis suaveolens* Poit.). Cowpea seeds were mixed with increasing amounts of powder from each of the three plants and stored in plastic boxes for 10 month. The results showed that the number of defective seeds varied according to the treatment. *O. americanum* was the most efficient; the smallest effective dose of powder was 12%. For *C. schoenanthus* and *H. suaveolens,* the smallest effective doses were 16% and 18% respectively. With such treatments, no defective seeds and no live or dead weevils were found. Considering all treatments, the cowpea average loss recorded was 6.92 g for *O. americanum*, 29.46 g for *C. schoenanthus* and 36.40 g for *H. suaveolens*. The average loss recorded in the control was 82.23 g. The powders of the three plants had virtually no effect on the seed germination rate after storage. Storage of cowpeas can be considerably improved using all three aromatic species, but *O. americanum* was the most efficient.

Keywords: Africa, insect damage, cowpea, storage, aromatic plants, powder.

INTRODUCTION

West African farmers have to cope with infestation problems of stored seeds and 71% state that the majority of post-harvest losses are caused by insects (Waongo et al., 2013; Kouadio et al., 2017). Insect damage includes decreased weight, quality of grains (Rajendran, 2002; Ashamo, 2006; Kouadio et al., 2017) and sometimes a loss of germination capacity (Dabiré et al., 2008).

Cowpea plays an important role in food production in West Africa as it is protein-rich

DOI : https://doi.org/10.4314/ijbcs.v14i4.1

and contains most essential amino acids (Bressani, 1985). Studies carried out in several countries in West Africa show that most of the cowpea crops are destroyed by the weevils after a few months of storage (Kpatinvoh et al., 2016). The consequences on the quantity and quality of cowpeas are undoubtedly one of the major constraints to the development of cowpea as an income-generating crop.

Recently, the severity of insect attacks has induced a use of synthetic insecticides for the protection of stocks (Dabiré, 2001). However, these insecticides have many disadvantages, including pest resistance (Benhalima et al., 2004), poisoning, environmental pollution and ecological disorders (Regnault-Roger, 2002). In the search for alternative methods of control, the plant kingdom offers many possibilities. Work is being carried out in this context and has shown the effectiveness of plants. According to Bouzouita et al. (2008), plants are a source of natural substances with great potential for application against insects and other parasites of plants and the animal world. Aromatic plants have long been used by farmers to protect crops (Keita et al., 2000; Keita et al., 2001; Savadogo et al., 2016). Farmers introduce aromatic plants into grainaries, where the cowpea is stored, in order to release volatile compounds with insecticidal or repellent properties, which limit the development of insect populations and consequently preserve the crops (Aïboud, 2012). At present, aromatic plants such as *Ocimum americanum* L., *Cymbopogon schoenanthus* L. and *Hyptis spicigera* Poit., which are known for their richness in essential oils, are widely used as bio-pesticides in the agro-food sector (Bouchikhi et al., 2009; Ilboudo, 2009; Kayombo et al., 2014; Ossey et al., 2017). All three species have been tested by the African research network for their insecticidal activities (Ngamo and Hance, 2007). Insecticidal activity of four essential oils extracted from *Lippia multiflora* Moldenke, *C. shoenanthus*, *O. americanum* and *H. suaveolens* on the development of the eggs and adults of *Caryedon serratus* Olivier, a pest of groundnut during storage were determined in Burkina Faso by Ouédraogo et al. (2016). Currently, several plant species with insecticide potentialities are listed in Burkina Faso (Savadogo et al., 2016), but the efficacy of these plants in terms of conservation has not yet been proven.

It is imperative to innovate the traditional methods used by local farmers by testing the insecticidal potential of local plants in order to preserve crops in an inexpensive and sustainable manner. This study aims to test the effect of traditional aromatic plants against insect attack on post-harvest losses.

MATERIALS AND METHODS

Plants used

Three plants species (*O. americanum*, *C. schoenanthus* and *H. suaveolens*) with bioinsectide potential for cowpea storage were selected based on existing literature and preliminary investigations. Moreover, these species often grow near dwellings so cowpea farmers can easily collect the plant material.

Leaves of the three species were harvested around Ouagadougou in October. The leaves were dried in the shade and then crushed. The powders obtained were stored in a refrigerator prior to the experimental runs.

Cowpea and experimental procedure

Before the experiment, the cowpea seeds were carefully sorted by removing defective seeds in order to avoid a bias. Only the best seeds remained. Cowpea seeds were weighed, packed in plastic boxes (250 g per box) and mixed with increasing amounts of powder from the three species in 5 g intervals from 5 g to 50 g giving mixtures of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 percent powder plus 10 controls (all controls are without powder). The boxes were kept for 10 months in a room where the temperature (**°C)** is close to that of cowpea producers' storage attics.

Efficacy of the leaf powder of three species for conservation of cowpea seeds: Variation of the number of defective seeds and seed weight during storage

Following, the number of defective seeds (seeds infested by or with traces of the cowpea weevil, *Collosobruchus maculatus*) were counted and removed. Dead or live weevils were sometimes found in the boxes at the end of the experimental tests, because the seeds without signs of infestations at the beginning of the experiment already sheltered larvae. Pod infestations by insects occur in crops but larval development in seed cotyledons continues in the stocks (Glitho, 1990; Ouedraogo et al., 1996; Sanon et al., 2006; Ilboudo, 2009). After mating, the female weevils lay eggs on the pods or directly on the seeds. The development of *C. maculatus* passes through the following stages: egg, larva (stage 1, 2, 3), pupa and adult individual. The weevils came from eggs that were already in the seeds, as there was no access to the seeds during storage.

The remaining seeds in each box were weighted in order to estimate the biopesticide potential. In addition, live and dead beetles were counted. The yield and percentage of uninfected seeds were calculated as the difference between the initial weight of the seeds and the weight of uninfected seeds at the end of the tests. The experimental trials were carried out under temperature (°C) conditions close to those of the cowpea granaries to make results directly applicable.

Evaluation of the germination rate

In order to test the germination capacity, 100 seeds were taken from each treatment and placed in Petri dishes containing sand. After 72 hours, the germinated seeds were counted and the germination rate was calculated.

The germination formula = (Number of germinated seeds/Total number of seeds) x100.

RESULTS

Variation of the number of defective seeds during the experimentation

The number of defective seeds varied according to treatment for three species. *O. americanum* had the best results in terms of efficacy (Figure 1). From the 12% treatment no defective seeds were found in the boxes. The treatment by *C. schoenanthus* was effective from 16% and that by *H. suaveolens* from 18%. Above these proportions, no defective seeds were found. However, in controls, 160-173 defective seeds were found.

The number of live or dead weevils present in each treatment followed the same trend.

From the 2% *O. americanum* treatment, no live weevils were observed. For *H. suaveolens* and *C. schoenanthus*, the same observation was made from 10% and 18% treatments respectively (Figure 2). This reveals that a quantity of *O. americanum* powder of less than 2% is ineffective to kill eggs, larvae or pupae of the weevils in cowpea seeds. A quantity of *C. shoenanthus* powder of 10% or less is not sufficient to remove eggs, larvae, pupae or adults from cowpea weevils. For *H. suaveolens*, 18% or more powder is required to kill eggs, larvae, pupae or adults of cowpea weevils. These proportions of powder contain sufficient amounts of bioinsecticides to kill the eggs, larvae, pupae or adult individuals of the weevils in the cowpea seeds. In the control, the number of live weevils varied between 76 and 88.

The number of dead weevils differed among treatments (Figure 3). The weight loss was lowest for *O. americanum* L. treatments and reached 0 g with 12% powder (Figure 4).

Variation of seed weight during storage

Considering all treatments, the average losses recorded on each sample about 250 g were 6.92 g for *O. americanum*, 29.46 g for *C. schoenanthus* and 36.40 g for *H. suaveolens*. The average losses recorded in the control were 82.23 g. Table 1 gives the final weights of the seeds as a function of the proportion of the mixture.

Evaluation of the germination rate of the stored seeds after the treatment

The treatment had no effect on seed germination capacity. Overall, the seeds had a good germination rate. The average seed germination rates were 99.6% when seeds were treated with *O. americanum*, 98.9% with *H. suaveolens* and 98.8% with *C. schoenanthus* (Table 2).

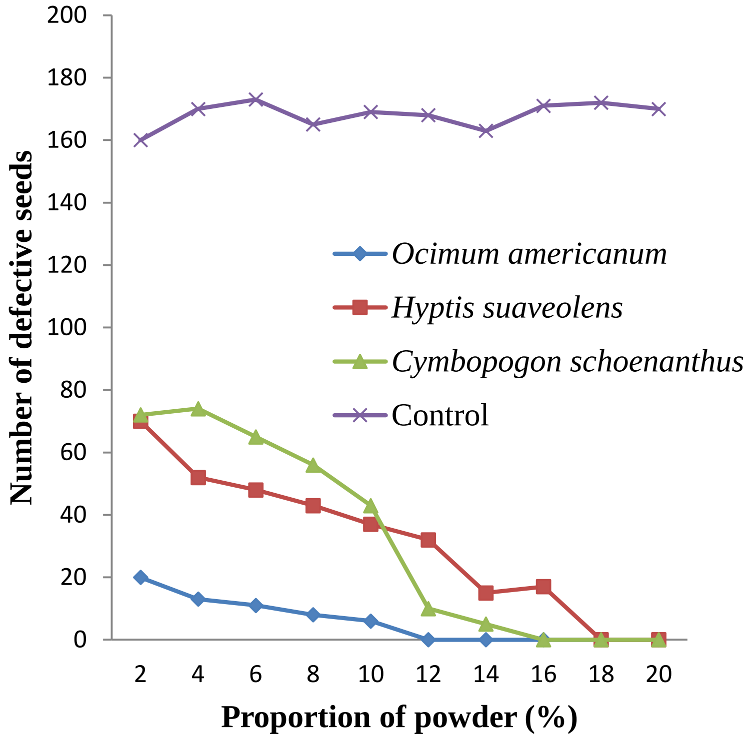

Figure 1: Number of defective seeds according to treatment (control is without powder).

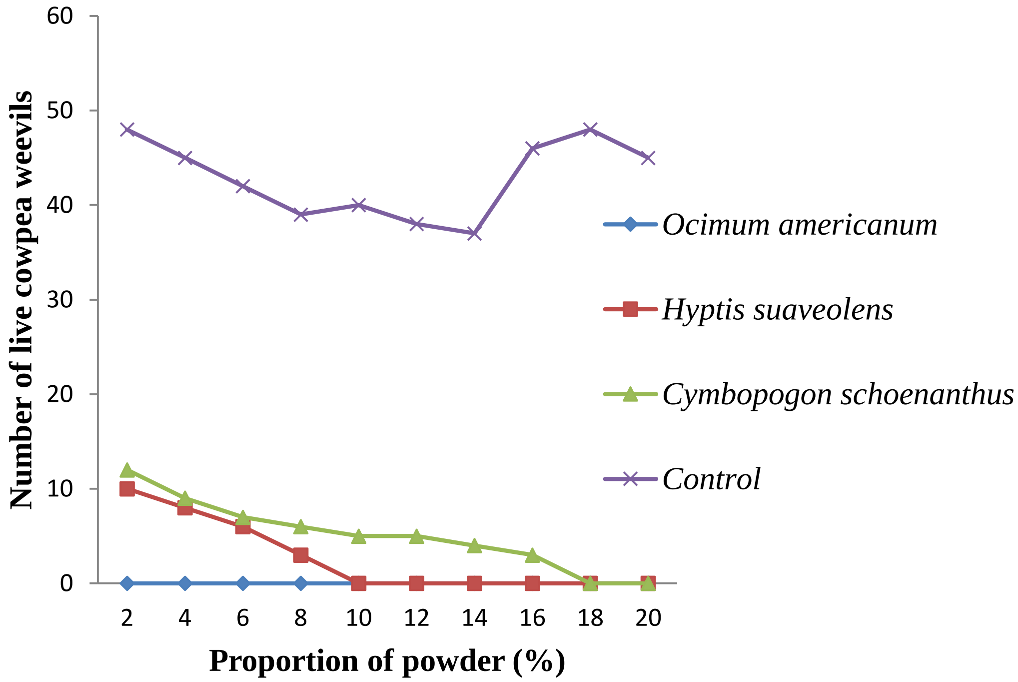

Figure 2: Number of live weevils according to treatment.

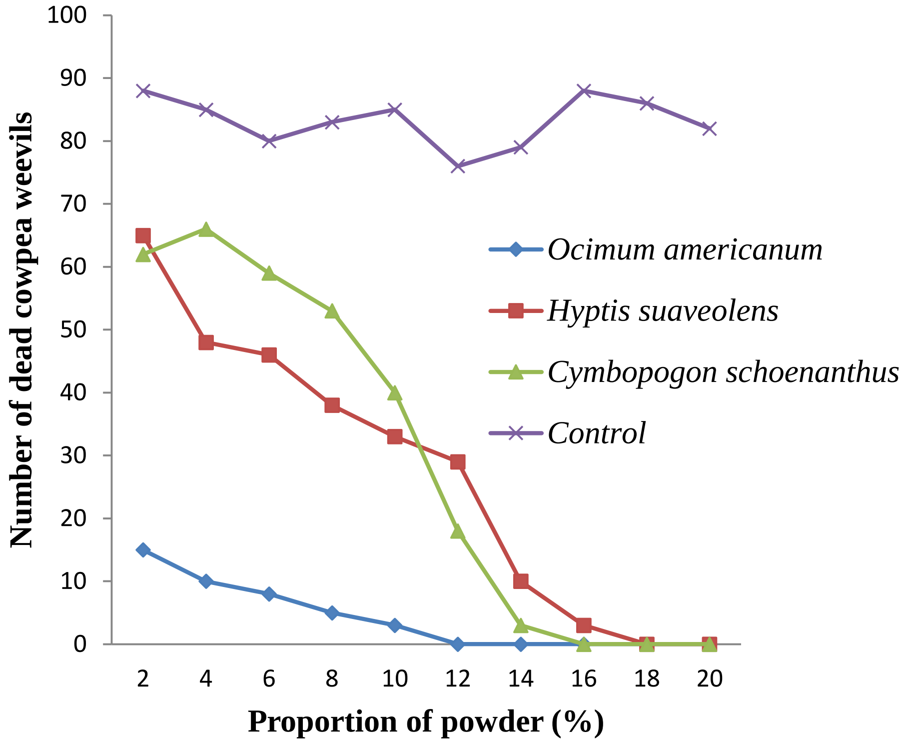

Figure 3: Number of dead weevils according to treatment.

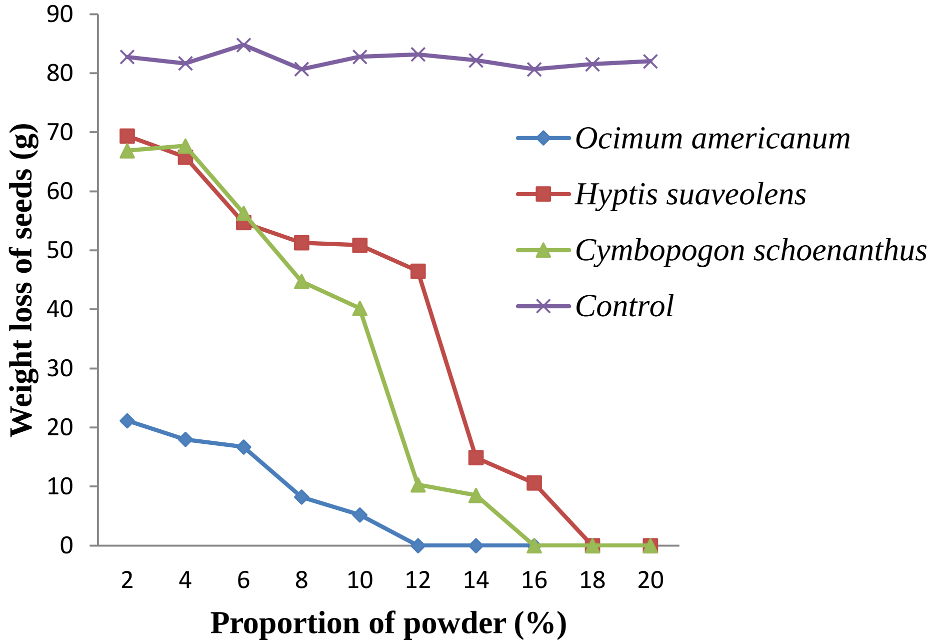

Figure 4: Weight loss of seeds according to treatment.

Table 1: Weight of seeds after 10 month of storage according to treatment.

Proportion of mixture (%)	*Ocimum americanum L. (g)*	*Hyptis suaveolens* **Poit.** *(g)*	*Cymbopogon schoenanthus* **L.** *(g)*	Control (g)
2	228.85 ± 2.3	180.60 ± 3.2	183.12 ± 1.8	167.25 ± 3.5
4	232.02 ± 1.5	184.20 ± 2.4	182.30 ± 1.5	168.32 ± 3.8
6	233.30 ± 1.5	195.30 ± 1.7	193.70 ± 1.8	165.20 ± 2.6
8	241.80 ± 1.5	198.70 ± 2.3	205.26 ± 2.1	169.30 ± 3.7
10	244.80 ± 2.1	199.11 ± 2.7	209.80 ± 2.9	167.22 ± 1.3
12	250 ± 2.3	203.50 ± 2.1	239.70 ± 2.5	166.79 ± 2.4
14	250 ± 2.2	235.12 ± 2.1	241.50 ± 3.0	167.80 ± 2.4
16	250 ± 1.5	239.40 ± 2.1	250 ± 2.4	169.33 ± 1.9
18	250 ± 1.2	250 ± 1.5	250 ± 2.5	168.45 ± 2.9
20	250 ± 1.2	250 ± 1.6	250 ± 1.9	167.97 ± 2.7

Legend: g = weight of seeds in gram

Table 2: Variation of the germination rate after 10 month of storage according to the proportion of the mixture and the species used.

Proportion of mixture (%)	Percentage of germinated seeds (%)		
	Ocimum americanum L.	***Hyptis suaveolens*** Poit.	***Cymbopogon schoenanthus*** L.
2	100	100	97
4	100	98	100
6	100	97	96
8	100	100	98
10	99	100	100
12	100	95	98
14	100	100	100
16	98	100	100
18	100	99	99
20	99	100	100
Average	**99.60**	**98.90**	**98.80**

DISCUSSION

O. americanum, *C. schoenanthus* and *H. suaveolens* have insecticidal effects that can be used in the conservation of cowpea seeds after harvesting. The most potent species in term of biopesticide is *O. americanum*. Previous studies have shown the biopesticidal potential of this species. According to Savadogo et al. (2016), *O. americanum* and *C. schoenanthus* are widely used by farmers in the control of insect vectors of disease and for the conservation of cowpea and cereal seeds. Several plants tested for their effect against weevils attack on maize, cassava, cowpea and bean pests have proved their insecticidal potential (Glitho et al., 2008; Cissokho et al., 2015). *H. suaveolens* is less effective than the other two species. Bouchicki et al. (2009) have shown that *C. schoenanthus* has insecticidal properties against the *Tineola bisselliella*, clothing moth. The insecticidal effects of the three plants studied were due to their aromatic odors. Indeed, ethnobotanical observations have shown that there is a traditional practice in the south-west of France for the protection of leguminous seeds by odorous plants (Regnault-Roger and Hamraoui, 2013). The effectiveness of this method was evaluated in the laboratory. The insecticidal activity of aromatic plants is exerted on two levels: a lethal effect on adult populations and an inhibition of reproduction (Regnault-Roger and Hamraoui, 2013). According to the same authors, aromatic plants harbor within them a true molecular arsenal of insecticidal or insect repellent substances capable of inducing plant protection. Plants are naturally endowed with chemical mediators allowing communication between species and presenting various effects. Many molecules in these compounds are involved in the defense of the plant against pests (Ngamo and Hance, 2007).

H. suaveolens is less fragrant than *O. americanum* and *C. schoenanthus*, so powder-based treatment of its leaves has been less effective. This is noticeable both in the number of defective seeds and in the number of dead beetles. The low efficacy of the *H. suaveolens* leaf powder treatment also results in low storage yield. This species may be more insect repellent than insecticide. In fact, fresh leaves of this species are deposited in houses or applied to the walls of habitats to repel mosquitoes by many peasants during the winter period (Savadogo et al., 2016). The low efficacy of the *H. suaveolens* powder treatment would probably be due to a loss of its insecticidal activity which could occur as a result of temperature variations. Although the tests were carried out under conditions of temperature close to those of the chambers or granaries of the farmers' storage, the annual temperature variations could also induce temperature changes in the chambers or in the cowpea storage attics. Indeed, according to Ilboudo (2009), the rise in temperature during tests can lead to the loss of insecticidal activity of a substance which has an effect on the spawning activity of females. Despite this observation, *O. americanum* and *C. schoenanthus* species whose treatments have proved to be very effective have not lost their insecticidal activities. The decrease in seed weight was considerable in untreated control tubes (328.92g/kg). This is fundamentally very different from the average loss for all treatments (27.68 g/kg, 117.84 g/kg and 145.6 g/kg, respectively for *O. americanum*, *C. schoenanthus* and *H. suaveolens*). These losses are very different from those recorded by Ngamo and Hance (2007), who state that losses of cowpea during storage due to *C. maculatus* are estimated to be more than 800 g/kg after seven months of storage. The treatment has no effect on the seed germination rate after storage. The seeds have substantially the same germination rates for all treatments. These results are close to those of Ilboudo (2009), who demonstrated that the treatment of cowpea seeds based on the essential oils of *O. americanum*, *H. suaveolens*, *Hyptis spicigera* Lam. and *Lippia multiflora* Moldenke has no effect on the germination rate of these seeds after storage. Such results may be of interest to cowpea farmers who struggle to keep their cowpea seeds after harvest, especially for seeds that are destined to be sown. But for seeds that are destined for consumption, it is imperative to make toxicological analyzes of all batches before consuming them.

Conclusion

All three plants have bioinsecticidal potentials. *O. americanum* showed the best results followed by *C. schoenanthus* and *H. suaveolens*. The use of *O. americanum* powder to store cowpea seeds for ten months resulted in fewer defective seeds and therefore less loss than when using powder of the other two plants. The optimal dose of *O. americanum* powder is around 12%. However, for *C. schoenanthus* and *H. suaveolens*, optimal doses are respectively around 16% and 18%. Very little loss is recorded when the seeds are stored with *O. americanum* powder. Treatment does not affect seed germination rate. Cowpea farmers would gain better by using *O. americanum* powder which shows an effective treatment starting at 12% for storage of their seeds after harvest. This three plants species are accessible to farmers because they are wild species which grow naturally in savannas, fields, neighborhoods of human habitats and the edges of roads. The amount of powder used (12%) in this experiment is also reasonable for farmers because of the accessibility of the species. The use of these less expensive methods will allow the storage of large quantities of cowpea seeds for consumption and marketing.

AUTHORS' CONTRIBUTIONS

Myself, Savadogo Salfo, I am the designer of this article. We are the ones who carried out the experimental tests in the laboratory and then collected the data. Anne Mette Lykke and I, we analyzed the data together and we wrote the draft of the article. Traoré Lassina, Sérémé Abdoulaye and Adjima Thiombiano have read and greatly contributed to the improvement of the scientific quality of the article.

COMPETING INTERESTS

For this article, there is no conflict of interest

ACKNOWLEDGMENTS

We thank the authorities of Aarhus University in Denmark who welcomed us into their laboratory for my data analysis. We also thank the technician Nana Théodore who helped us a lot in collecting and grinding the plants.

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