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Original article

Assessing *Spirulina platensis* as a dietary supplement and for toxicity to *Rhynchophorus ferrugineus* (Coleoptera: Dryopthoridae)

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ABSTRACT

Insects are important for humanity; play role in crop pollination, and biocontrol of harmful pests. The red palm weevil, *Rhynchophorus ferrugineus*, is a major pest of date palms and has become a serious threat. Scientists needs ample numbers of insects for bioassays to explore control options. The alga *Spirulina platensis*, is enriched by protein, natural vitamins, minerals, and amino acids, stimulate the development of organisms that feed on it. I assessed the value of *Spirulina* as a nutritional supplement for red palm weevil larvae by adding its various percentages to the artificial diet. Once a week, the larvae were removed from the containers, washed with distilled water, dried, weighed using an electronic scale, returned to a new container, and supplied with *Spirulina* mixed fresh diet. Larvae fed with lower concentrations showed vigorous growth and significant weight gain. Particularly, larvae fed 0.5%, 1%, and 2% *Spirulina* powder supplementation to their diet were healthier and gained more weight than larvae reared with 5% concentration. Overall 40% mortality was recorded in larvae fed with 10% concentration. Higher concentrations from 0.5% to below 5% had a beneficial effect on red palm weevil larval growth but a detrimental effect and even mortality was recorded when used $\geq 5\%$.

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1. Introduction

Insects are important for the survival of humanity as they play a role in crop pollination, biocontrol of harmful pests, and serve as food for thousands of people worldwide (Van Huise et al., 2013; Liu et al., 2019; Schwarz and Frank, 2019). In contrast, thousands of insect species are harmful to field crops, vegetables, and fruit trees. The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryopthoridae), is a serious pest of the date palm (*Phoenix dactylifera* L.) (European and Mediterranean Plant Protection Organization, 2008). The date palm is a prehistoric tree grown globally in warm climates (Chao and Krueger, 2007). The date fruit is nutritious, carbohydrate-rich, and contains minerals

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and vitamins (Ahmed et al., 2014; Assirey, 2015). Unlike most other fruit crops, date palm cultivation is laborious, costly, and requires significant attention. The *R. ferrugineus* has become a significant concern for date palm growers worldwide. Plant protection scientists are attempting to overcome this pest problem using several management techniques. Laboratory studies require the red palm weevil individuals for bioassay; however, rearing is difficult and requires significant attention, and several researchers have proposed specific diets for rearing it in the laboratory for experimental purposes (Al-Ayedh, 2011).

There are several beneficial uses of insects other than crop pollination. Such as, entomophagy is well-known in several parts of the world, particularly in African countries, and could help to alleviate malnutrition in developing countries and food security issues (Van Huis, 2015). The increasing world population might lead to food security risks and require alternative sources of protein. There are approximately 1900 species of insects worldwide reported as edible for humans (Van Huise et al., 2013; Chakravorty, 2014; Bernard and Womeni, 2017; Orkusz et al., 2020). The Coleopteran and Lepidopteran insects are mainly entomophagic, however, termites and grasshoppers are also a favorite food of humans in several parts of Africa, Mexico, and Arabian countries (Chakravorty,

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2014). Previous studies have confirmed that crickets contain 1562 mg of iron per 100 g of dry matter and it had been suggested that to overcome iron deficiency issue adult human males and females can consume 9.5 and 19.5 mg of dry matter per day, respectively (Christensen et al., 2006).

It has been reported that as an estimate one billion people worldwide consume insects as food, including in Africa, Asia, and Latin America. However, if insects are to become an important resource, they need to be farmed (Van Huis, 2015). Insects are not only eaten for their nutritional value but also their taste (Nonaka, 2009). For example, consumers tried and reported liking to eat insect-based products such as insect burgers (Schouteten et al., 2016). Similarly, a survey-based study reported that 72% and 74% of adults living in America and India, respectively, were willing to eat insects as food (Ruby et al., 2015). Insects have a high nutritional value, and the comparison between the nutritional value of insects and other livestock is relevant in that insects have been shown to have high energy levels (Kcal) per 100 g of edible portion. The palm weevil larvae showed 479 Kcal/100 g, while beef, chicken, and pork have been reported having 169, 152, and 186 Kcal/100 g, respectively (Gere et al., 2017). It has been reported that edible insects are a potential food source because of their high energy and protein content (Rumpold and Schlüter, 2013). Palm weevil larvae contain protein up to 36% and are consumed as food in several parts of the world (Van Huis et al., 2013).

Adequate nutrition is important and vital at all stages of an organism's growth and development. Like humans and all other organisms, insects require basic nutritional inputs for proper growth and development, and the proportion of nutrients in insect food is important (House, 1969). Unfortunately, few studies have been reported for nutritional requirements of phytophagous insects (Offor, 2010). The Spirulina platensis is an alga containing high levels of protein, vitamins, minerals, and amino acids and could enhance the growth of a wide range of organisms by supplying appropriate quantities of nutrients (Salmeán et al., 2015). Spirulina powder is a promising resource as a supplement for livestock and animal feed (Holman and Malau-Aduli, 2013), and it has been used in bird, poultry, and fish feed to enhance production (Habib 2008; Swiatkiewicz et al., 2015; Yusuf et al., 2016; Khalila et al., 2018; El-Bahr et al., 2020). In addition, it has been reported that Spirulina has the same nutritional value as pollen and can be used as a dietary supplement for honey bees (Ricigliano and Simone-Finstrom, 2020). It can also reduce the toxic effects of chemicals as it contains antioxidant compounds, and it has been reported that Spirulina supplementation can alleviate the toxic stress of deltamethrin in male mice (Abdel-Daim et al., 2016). Another study assessing the toxicity of Spirulina at high doses (10 and 30 g/kg body weight) in mice reported no abnormality in appearance (Hutadilok-Towatana et al., 2008).

Similarly, rats fed 800 mg/kg showed no mortality and there was no allergic skin reaction when fed 2000 mg/kg body weight (Salmeán et al., 2015); however, toxic effects have been reported in insects. Several studies have reported the effects of fungal isolates as pathogenic to different insects like *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), and *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) (Qasim et al., 2018; Islam et al., 2020; Qasim et al., 2020A, B). Unfortunately, very limited information is available regarding toxic effects of *Spirulina* against field crop pests. Aly and Abdou, 2010; Rashwan and Hammad, 2020, have reported that *Spirulina* can cause 100% mortality in the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), at 5% concentration. In present study, I evaluated the effects of *Spirulina* as a nutritional supplement and its toxicity to red palm weevil larvae.

2. Materials and methods

2.1. Rearing of red palm weevil

In this study, a red palm weevil colony was reared at the Economic Entomology Research Unit, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia, at 25 °C \pm 2 °C, 65% \pm 5% relative humidity, and photoperiod 6:18 h light: dark. The colony was reared on an artificial diet that included chopped date palm fronds, corn, wheat flour, and ascorbic acid as a preservative. Approximately 2-d-old, freshly hatched red palm weevil larvae were collected and used for study.

2.2. Production of red palm weevil larvae

Newly emerged red palm weevil adults were placed in plastic boxes with 10% sugar solution cotton soaked at base of the box to provide the adults with sugar that could boost egg-laying. Female adults laid eggs in the cotton which were collected daily, placed on wet filter paper in a plastic petri dish, and kept in an incubator at 25 °C \pm 2 °C, 65% \pm 5% relative humidity, and photoperiod 6:18 h light: dark. The eggs hatched between 3 and 4 d; the neonates were gathered, placed in 50 g plastic cups, and supplied with 1 g artificial diet. The larvae were taken off the standard artificial diet after 1-d, weighed, and fed a diet mixed with *Spirulina*.

2.3. Diet preparation and feeding protocol

I used different percentages of *Spirulina* powder (Pharma Care Europe Ltd., UK) as a nutritional supplement for the larvae. The six treatments consist of 0.5%, 1%, 2%, 5%, 10%, and 20% *Spirulina* powder mixed with the artificial feed, whereas the control treatment feed contained no *Spirulina* powder (Fig. 1). The artificial diet and *Spirulina* powder for each treatment were measured and mixed with a disposable plastic spoon and the mixed diet was fed to the larvae in the plastic cups. Once a week, the fresh diet was prepared and fed to the larvae.

2.4. Weighing and feeding of larvae

The larvae were weighed using an electronic balance (PGL 3002 Adam Equipment UK). During the first week, larvae were fed 1 g of *Spirulina*-mixed diet; the quantity was increased every week with larval growth (Fig. 2). In the control treatment, the larvae were fed the standard artificial diet. Once a week, they were removed from the cups, washed with sterilized distilled water for 1–2 s, dried on filter paper and weighed. Dead larvae were removed, and the mortality rate was recorded. After weighing the larvae were transferred to a new 50 g plastic cup, and fresh *Spirulina*-mixed diet was provided. The cups were transferred to an incubator at 25 °C ± 2 °C, 65% ± 5% relative humidity, and photoperiod 6:18 h light: dark.

2.5. Data collection and statistical analysis

Observations were made weekly until the larvae reach the prepupal stage. The weight of each larva was recorded and the mean body weight gain was calculated. Initially, there were ten larvae in each treatment, considering each larva as one replicate. The dead larvae from each treatment were removed, the numbers were recorded, and the mean mortality was calculated. For each of the six treatments and the control, the parameters weight gain and mortality were recorded weekly. The data were analyzed using analysis of variance (ANOVA) and the means were separated using the least significant difference (LSD) test at P < 0.05 (SAS 2009).



Fig. 1. Spirulina powder mixed with the diet displaying all the concentrations.



Fig. 2. Amounts of diet given to larvae each week.

3. Results

The present study assessed the nutritional benefits and toxicity of *Spirulina* to the development of red palm weevil larvae. The findings showed that *Spirulina* could be used as a dietary supplement for red palm weevil larval growth. In general, the weeklyrecorded weight gains of larvae fed the *Spirulina*-mixed diet were significantly higher than the control.

The larval weights at the end of week-1 for larvae fed the *Spirulina*-supplemented treatment diets were significantly higher than the artificial-feed-only control diet (Table 1). There was a maximum weight gain of 0.04 g/larvae in larvae fed the 2% concentration diet, followed by the 0.5% and 10% diets, in both of which the weight gain was 0.031 g/larvae. In contrast, the least weight gain of 0.010 g/larvae was in the control treatment larvae.

At the end of week-2, larvae fed with 2% concentration diet gained the most weight of 0.092 g/larva, followed by larvae fed with 0.5% *Spirulina* diet with 0.078 g/larva weight gain. In the control treatment, larval weight gain was 0.040 g/larva. During week-2 with 20% concentration, larval weight was reduced and there was 100% mortality by the end of week-3 (Table 2).

At the end of week-3, the maximum larval weight gains of 0.383 and 0.389 g/larva were recorded in the 0.5% and 2% concentrations, respectively. Larvae fed the 5% and 10% diet did not gain more weight during week-3 and their weight gain was not significant than the larval weight gain in control (Table 3).

At the end of week-4, the maximum weight gain was 0.806 and 0.781 g/larvae for larvae fed the 1% and 2% concentrations, respectively. The least weight gain of 0.068 g/larvae was in the 10% diet. Similar to 3rd week; during the 4th week, larvae fed with 10% diet could not gain more weight. However, during this week, larvae fed in control gain more weight as compared to the 3rd week (Table 4).

Generally, weight gain increased with time and larval growth. At the end of week-5, larvae fed the 5% concentration gained 0.618 g/larvae. This was the first record of a greater increase in weight with the 5% concentration than the 2% treatment. However, the greatest weight increase was in the 1% concentration treatment in which the larval weight was 0.846 g/larvae, followed by 0.5% concentration where the weight gain was 0.721 g/larva (Table 5).

At the end of week-6, the larval weight gain was maximum among the treatments as compared to the previous five-week data. During week-6, larvae in almost all treatments gained more weight than in the same treatments within each of the previous 5 weeks. Following the pattern of larval weight gain, during week-6, there were weight gains of 0.953 and 1.12 g/larvae for the 1% and 2% concentrations, respectively. Although the weight gain was more

Table 1

Mean weight gain (g ± SE) at the end of week-1 in red palm weevil larvae reared on Spirulina-supplemented artificial diet.

Treatment	Weight gain (g)	ANOVA Parameters				
		N	F	df	Р	
0% Spirulina (control)	0.010 ± 0.001c	9	6.68	6, 62	< 0.0001	
0.5% Spirulina	0.031 ± 0.005ab	9				
1% Spirulina	0.028 ± 0.003b	9				
2% Spirulina	0.040 ± 0.002a	9				
5% Spirulina	0.021 ± 0.002b	9				
10% Spirulina	0.031 ± 0.005ab	9				
20% Spirulina	$0.022 \pm 0.003b$	9				

Means followed by the same letters do not differ significantly (at P < 0.05).

Table 2

Mean weight gain $(g \pm SE)$ at the end of week-2 in red palm weevil larvae reared on Spirulina-supplemented artificial diet.

Treatment	Weight gain (g)	ANOVA Parameters				
		N	F	df	Р	
0% Spirulina (control)	0.040 ± 0.007c	8	8.92	6, 55	< 0.0001	
0.5% Spirulina	0.078 ± 0.014a	8				
1% Spirulina	0.077 ± 0.011a	8				
2% Spirulina	0.092 ± 0.006a	8				
5% Spirulina	0.044 ± 0.008bc	8				
10% Spirulina	0.072 ± 0.017ab	8				
20% Spirulina	$-0.00 \pm 0.002d$	8				

Means followed by the same letters do not differ significantly (at P < 0.05).

Table 3

Mean weight gain (g ± SE) at the end of week-3 in red palm weevil larvae reared on Spirulina-supplemented artificial diet.

Treatment	Weight gain (g)	ANOVA Parameters			
		N	F	df	Р
0% Spirulina (control)	0.097 ± 0.021c	8	17.78	5, 47	< 0.0001
0.5% Spirulina	0.383 ± 0.038a	8			
1% Spirulina	0.281 ± 0.025b	8			
2% Spirulina	0.389 ± 0.047a	8			
5% Spirulina	0.111 ± 0.024c	8			
10% Spirulina	0.103 ± 0.036c	8			

Means followed by the same letters do not differ significantly (at P < 0.05).

Table 4

Mean weight gain (g ± SE) at the end of week-4 in red palm weevil larvae reared on Spirulina-supplemented artificial diet.

Treatment	Weight gain (g)	ANOVA Parameters			
		N	F	df	Р
0% Spirulina (control)	0.247 ± 0.027bc	6	20.99	5, 35	<0.0001
0.5% Spirulina	0.389 ± 0.115b	6			
1% Spirulina	0.806 ± 0.057a	6			
2% Spirulina	0.781 ± 0.090a	6			
5% Spirulina	0.216 ± 0.046bc	6			
10% Spirulina	0.068 ± 0.030c	6			

Means followed by the same letters do not differ significantly (at P < 0.05).

Table 5

Mean weight gain $(g \pm SE)$ at the end of week-5 in red palm weevil larvae reared on Spirulina-supplemented artificial diet.

Treatment	Weight gain (g)	ANOVA Para			
		N	F	df	Р
0% Spirulina (control)	0.418 ± 0.038c	6	9.85	5, 35	<0.0001
0.5% Spirulina	0.721 ± 0.64ab	6			
1% Spirulina	0.846 ± 0.056a	6			
2% Spirulina	0.493 ± 0.140c	6			
5% Spirulina	0.618 ± 0.046bc	6			
10% Spirulina	$0.190 \pm 0.049d$	6			

Means followed by the same letters do not differ significantly (at P < 0.05).

Table 6

Mean weight gain (g ± SE) at the end of week-6 in red palm weevil larvae reared on Spirulina-supplemented artificial diet.

Treatment	Weight gain (g)	ANOVA Para	meters				
		N	F	df	Р		
0% Spirulina (control)	0.892 ± 0.101a	6	2.47	5, 35	0.0548		
0.5% Spirulina	0.878 ± 0.143a	6					
1% Spirulina	0.953 ± 0.143a	6					
2% Spirulina	1.120 ± 0.199a	6					
5% Spirulina	0.873 ± 0.106a	6					
10% Spirulina	$0.475 \pm 0.082b$	6					

Means followed by the same letters do not differ significantly (at P < 0.05).

during this week yet there was no significant difference among the treatments except 10% concentration where weight gain was almost 40–50% less than other treatments (Table 6).

After week-6, larval weight gain started to decrease as the peak larval growth stage had been reached, after which larvae stopped feeding and prepared for pupation. In week-7, there was a decrease in weight in the 1% and 2% *Spirulina* diets. Surprisingly, the weight gain in the 5% and 10% concentrations was greater during week-7. The weight gain data at the end of week-7 are presented in Table 7.

The overall weight gain data of the red palm weevil larvae among all treatments were significant (Table 8). These results indicate that the 1% and 2% concentrations supported the growth of the red palm weevil larvae. Although the 5% concentration resulted in greater overall weight gain as compared to the 0% (control) however, to avoid the risk of mortality, it should not be used.

The mean mortality among larvae shows that at the dietary concentration of 20% *Spirulina* was toxic and resulted in 100% mortality by the end of week-3, while 20% and 40% larval mortality rates were recorded in the 5% and 10% *Spirulina* treatments, respectively (Fig. 3). However, apart from the control, there was no larval mortality in the rest of *Spirulina*-mixed diet treatments. In the control treatment, 10% mortality was recorded by the end of week-1.

4. Discussion

The Spirulina powder is a product with opposing effects on insects. While it exhibits high nutritional value to insects at low concentrations, it has been reported to have significant toxic effects on several insect pests at higher concentrations and could be a potential source of natural pesticides against them (Rashwan and Hammad, 2020). I found a reduction in larval weight and mortality rates at concentrations of > 5% of the diet. I observed an increase in larval weight with Spirulina mixed diet during the first week particularly more weight gain with lower concentrations. It has been previously reported that lower concentrations enhanced larval growth of Spodoptera littoralis, the maximum larval weight was recorded with 0.5% and 1% Spirulina concentrations (Aly and Abdou, 2010). These findings align with our results that indicate maximum larval weight gains of 3.64 and 3.17 g/larva in red palm weevil at the 1% and 2% concentrations, respectively (Table 8).



Fig. 3. Red palm weevil larval mortality (%) reared on Spirulina-supplemented artificial diet.

In aquaculture, several research findings have reported Spirulina as the best protein dietary supplement to improve the immune system and boost reproduction (James et al., 2006; Watanuki et al., 2006; Guroy et al., 2012; Jana et al., 2014; Mosha, 2019). Previous studies have reported Spirulina to have a beneficial effect on guppy growth. Guppies fed fishmeal with 40% Spirulina diets displayed improved growth and weight (Dernekbasi et al., 2010). In the present study, I assessed the weight gain in red palm weevil larvae every week and found a continuous increase in larval weight gain in larvae fed on the Spirulina-mixed diet. In contrast to previous results, our results showed the toxic effects of Spirulina on red palm larvae resulted in 100% and 40% mortality at 20% and 10% concentrations, after week-2 and 6, respectively. Similarly, Spirulina fed as a dietary supplement to green tiger shrimps resulted in a higher survival rate of shrimp larvae than the regular diet (Ghaeni et al., 2011). Similar advantages of Spiruling dietary supplementation, such as increased specific growth and increased live weight gain, have been documented in Nile tilapia (Abu-Elala et al., 2016).

In red palm weevil larvae, the maximum weight gain of 0.953 and 1.120 g/larva was recorded during 6-week of larval span for 1% and 2% concentration, respectively. The larval stage is usually completed in 8–9 weeks on the standard artificial diet. During week-6, the larvae fed actively and were provided with the highest

Table 7

Mean weight gain (g ± SE) at the end of week-7 in red palm weevil larvae reared on Spirulina-supplemented artificial diet.

Treatment	Weight gain (g)	ANOVA Para			
		N	F	df	Р
0% Spirulina (control)	0.610 ± 0.060b	6	6.72	5, 35	0.0003
0.5% Spirulina	0.246 ± 0.170c	6			
1% Spirulina	0.640 ± 0.084b	6			
2% Spirulina	0.260 ± 0.162c	6			
5% Spirulina	0.990 ± 0.087a	6			
10% Spirulina	0.885 ± 0.104ab	6			

Means followed by the same letters do not differ significantly (at P < 0.05).

Table 8

Mean of total weight gain (g) throughout larval span in red palm weevil larvae reared on Spirulina-supplemented artificial diet.

Treatment	Total weight gain (g)	ANOVA Parameters				
		N	F	df	Р	
0% Spirulina (control)	2.33 ± 0.120 cd	6	11.05	5, 35	<0.0001	
0.5% Spirulina	3.08 ± 0.253ab	6				
1% Spirulina	3.64 ± 0.165a	6				
2% Spirulina	3.17 ± 0.177ab	6				
5% Spirulina	2.87 ± 0.195bc	6				
10% Spirulina	1.84 ± 0.217d	6				

Means followed by the same letters do not differ significantly (P < 0.05).

quantity of feed (20 g/larva). At week-7, the average larval weight gain started to decrease as compared to the previous week because the larvae had stopped feeding to prepare for the pupation stage. During the observation it was noticed that all the larvae fed with lower concentrations of *Spirulina* supplemented diet were active and healthier as the *Spirulina* has acted like a supertonic for them. In literature, medicinal values of *Spirulina* also have been reported in many animals, including chickens, rats, mice, and even in humans. *Spirulina* possesses significant nutritional value and contains vitamins to support the growth and development of living organisms and protect them from several diseases (Belay 2002; Colla et al., 2008; Ghaeni and Roomiani, 2016). It has been reported that *S. platensis* has the highest source of Vitamin B₁₂ and β -carotene; it is good for human health and could improve the body's defense system (Sindhumole, 2015).

Spirulina has partially replaced soybean and corn as a protein source for chickens. Several studies have documented significant improvements in chicken body weight, growth performance, immunity, fatty acid profile, and biological traits (Kaoud 2012; Bonos et al., 2016; Zeweil et al., 2016; Neumann et al., 2018; Sugiharto et al., 2018; Velten et al., 2018; Sharmin et al., 2020). Additionally, several studies have concluded that microalgae show significant advantages as an economical and eco-friendly food source in the poultry industry.

In the animal production and poultry industries, and aquaculture, Spirulina has become an important dietary supplement with no acute toxic effects. In contrast, toxic effects on insects have been documented in the literature. At low concentrations, Spirulina supplementation resulted in positive effects on insect growth and performance, whereas at higher concentrations, it caused mortality. These findings agree with this study in which I report 100% mortality of red palm weevil larvae at 20% concentration (Fig. 3). In the black cutworm, Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae), 80% mortality was reported in the 2nd stage larval instars when fed with castor bean leaf discs dipped in different microalgae strains solutions (Abdel-Rahim and Hamed, 2013a). Similar findings have been documented in 4th instar larvae of *S. litoralis*, showing 100% mortality when fed with a 5% concentration of Spirulina (Aly and Abdou, 2010). Similarly, when S. litoralis 2nd instar larvae were fed with water and phenolic based extracts of 7% Spirulina, 19% mortality was documented (Rashwan and Hammad, 2020). Similar to our findings, several studies have reported the toxic effects of different algae against insects (Abdel-Rahim and Hamed, 2013b; Saber et al., 2018). There is a need for further studies on the effectiveness of Spirulina as a toxicant at different developmental stages of the red palm weevil to pave the way for its field use as an ecofriendly bio-pesticide against the weevil.

5. Conclusions

The present research findings indicated that *Spirulina*, used in lower concentrations, has a beneficial effect on the growth of red palm weevil larvae. However, when used \geq 5% dietary concentration, it has detrimental effects and can cause mortality. Further studies could explore the effects of *Spirulina* as a dietary supplement on other biological parameters of the red palm weevil and investigate its potential as an eco-friendly bio-pesticide.

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Declaration of Competing Interest

The author declared that there is no conflict of interest.

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