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Research Article

Antioviposition and Reduction of *Callosobruchus chinensis* Pic. 1902 (Coleoptera: Bruchidae) Emergence on *Phaseolus vulgaris* by *Dioscorea sansibarensis* Powder and Its Chemical Composition

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Callosobruchus chinensis causes damage to the *Phaseolus vulgaris* seeds. Traditionally, *Dioscorea sansibarensis* serves as a medicinal plant. Naturally, *D. sansibarensis* has toxins that protect against herbivores and the surrounding invasive plants in its natural habitat. Phytochemical analysis by thin-layer chromatography (TLC) and laboratory experiments was carried out to determine the activity of *D. sansibarensis* leaves, bulbils, and yams powders on antioviposition and inhibition of the F_1 emergence of *C. chinensis*. Bioassay data were subjected to nonparametric (Kolmogorov–Smirnov) statistical analysis and a generalized linear model at $P \leq 0.05$. Statistically, the powders had an antioviposition activity of 34.3% ($R^2 = 0.343$). A recommendable activity on antioviposition was displayed by the yams powder; treatment by 0.8 g of yams powder had a Wald Chi-Square value of 1.291, $P = 0.26$. Inhibition of F_1 emergence was significantly attained by the yams powder; the treatment by 0.6 g of yams powder had a Wald Chi-Square value of 7.72, $P = 0.01$. Statistically, the bulbils powder displayed low antioviposition and inhibition of F_1 emergence. Observations on the TLC exposed compounds with similar R*f* values; saponin with an R*f* value of 0.72 was portrayed in the leaves, bulbils, and yams. A terpenoid and a flavonoid with R*f* values of 0.37 and 0.71, respectively, were observed in bulbils and yams but absent in leaves. A terpenoid with an R*f* value of 0.49 was visualized in leaves and bulbils but not in the yams powder. The study concluded that the *D. sansibarensis* yams and leaves powders are viable for application by the farmers in the protection of stored legumes against attack by *C. chinensis*. However, there may be other diverse interests in other storage insects and other methods of phytochemical analysis that have not been investigated.

1. Introduction

The common bean, *Phaseolus vulgaris* (Fabaceae), is one of the principle staple foods and cash crops in Tanzania and serves as an important crop for food security in many countries in sub-Sahara Africa [1]. It is important for many low-income populations and significantly improves their nutrition, aimed at the prevention and treatment of diseases [2]. Several studies have demonstrated *Callosobruchus chinensis* attacks on *P. vulgaris* in silos, stacks, and barns throughout the world causing qualitative and quantitative damage by both larvae and the adults [3]. *C. chinensis* accounts for 60% loss of weight and 66.3% loss of protein content of *P. vulgaris* [4].

Callosobruchus chinensis Pic. 1902, also termed *Bruchus chinensis* Linnaeus 1758, of the family Bruchidae, has an average lifetime of 22–25 days from egg to the adult stage [5]. The infestation begins in the field during harvest; the bruchid is transported to store with the legumes where it lays eggs on the seed surface at the right atmospheric conditions and its life is completed inside the bean seed as it feeds. Damage by *C. chinensis* occurs after larvae hatch from the eggs as the first instars penetrate the bean seeds. The bruchids develop within the seeds and emerge as adults leaving damage in the

form of the empty feeding chambers [6, 7]. More than one larva can develop in a single grain [8].

Studies are being conducted on strategies to reduce common bean damage and losses caused by *C. chinensis* [4]. Farmers use banned and highly toxic synthetic insecticides in their quest to protect agricultural products, including stored bean seeds. Other setbacks associated with using synthetic pesticides include high costs, health hazards, residue problems, and the development of resistance by treated insects [9].

Farmers are opting for exploiting the readily available natural resources such as plants of medicinal importance and have contributed to the suppression of pests. Pretreatment of *Vigna radiata* seeds with inert clay by farmers led to adult mortality and reduced the oviposition of *Callosobruchus chinensis* [10]. In recent years, researchers have shifted attention to plant powders for control of insect pests in stored bean seeds, due to their biodegradability and being ecofriendly to the environment, thus becoming an alternative to synthetic pesticides in improving food security [11]. Leaves powders of *Ocimum basilica* (basil), *Azadirachta indica* (neem), *Dennittia tripetala* (pepper fruit), *Curcuma longa* (black pepper), *Anacardium occidentale* (nutshell), *Alpinia galangal* (Siamese ginger), *Clusia palmicida* (Clusia), and *Curcuma zedoaria* (zedoary) have been reported to induce antioviposition and reduce the number of emerging F_1 progeny of *C. chinensis* on chickpea, cowpea, and green gram seeds [11–13].

Plants store the active compounds in the form of phenols, alkaloids, flavonoids, tannins, and saponins in different parts of their organs for protection against herbivores and pathogens [8]. The genus *Dioscorea* is the largest genus of the monocotyledonous family Dioscoreaceae. The wild *Dioscorea sansibarensis*, also called (*m*)*chochoni* in Swahili, is a sun-loving ornamental climber that forms a canopy occurring in riverine forests of tropical sub-Sahara Africa, specifically in Madagascar, Zanzibar, and Tanganyika. *D. sansibarensis* documented history in traditional medicine goes to at least 2000 BC [14, 15]. *D. sansibarensis* bulbils, yams, stem, and leaves are found to be toxic to pathogens, herbivores, and monkeys; hunters use its extract on arrows to hunt wild pigs [14, 16]. This study investigated the antioviposition and ability to reduce the F_1 emergence of the *C. chinensis* from stored *P. vulgaris* using *D. sansibarensis* leaves, bulbils, and yams powders, in addition to determining the chemicals present in the plant parts.

2. Materials and Methods

2.1. Materials. Undamaged *P. vulgaris* seeds were brought directly from Kisarawe farm (7° 10′0′ S, 38° 50′0″ E and altitude of 252 m above sea level) after harvest. The seeds were sun-dried and stored with 10 ± 2% moisture content. The commercial synthetic pesticide Actellic 50 EC (100 g/90 kg Syngenta, Switzerland) was purchased from agricultural input suppliers in Dar es Salaam, Tanzania, for use as the check (positive control). Thin-layer chromatography (TLC) plates and analytical grade solvents were purchased from Chem Equip Laboratory supplier in Dar es Salaam, Tanzania.

Fresh leaves, bulbils, and yams of *D. sansibarensis* were identified and harvested from the Zoology-Botany forest thickets at the University of Dar es Salaam (6° 45′0″ S by 39° 15′0″ E, altitude 80 m above sea level). Morphological identification was done at the Herbarium in the Department of Botany, University of Dar es Salaam, where voucher specimens bearing reference numbers FMM 3910a, 3910b, and 3910c for leaf, bulbil, and yam, respectively, are deposited. The plant materials were brought to the Entomology Laboratory of the Department of Zoology and Wildlife Conservation at the University of Dar es Salaam where they were dried under shade in a well-ventilated area for 5 days. After drying, they were pulverized and sieved using a 60 μm mesh sieve to obtain fine powders.

2.2. Qualitative Analysis by Thin-Layer Chromatography

2.2.1. Determination of Alkaloids. Weigh 2.5 g of plant powder into a 100 mL beaker to which 80 mL of 1% acetic acid (1 : 10 w/v) in ethanol at a ratio of 1 : 1 was added and allowed to stand for 5 hours, followed by filtration. The resulting filtrate concentrated on a water bath to one-third of the original volume. Concentrated ammonium hydroxide was added dropwise to precipitation. The precipitate was collected, washed with dilute ammonium hydroxide, and filtered. The dry residue was weighed and calculated as a percentage alkaloid. Thin-layer chromatography (TLC) of isolated alkaloids was performed by wetting the powdered test samples with a solvent mixture of chloroform and methanol at a ratio of 15 : 1 which was also used as the mobile phase for running thin-layer chromatography at room temperature. The TLC plate was visualized under UV light and further detection of alkaloid was aided by spraying with Dragendorff's reagent [17].

2.2.2. Determination of Flavonoids. Plant powder (2.5 g) was soaked repeatedly with 50 mL of 75% aqueous methanol and filtered through Whatman filter paper at room temperature. The filtrate was dried in vacuo and weighed to calculate the percentage flavanoid. The filtrate was mixed with methanol in the ratio of 1 : 8 (g/mL) and heated on a water bath at 60°C for 5 minutes. Chloroform and methanol at a ratio of 18 : 2 were used as the mobile phase [18].

2.2.3. Determination of Saponins. Weigh 2.5 g of plant powder into a conical flask and 50 mL of 20% aqueous ethanol added. The mixture was subjected to heating over a water bath at a temperature of 57°C for 5 minutes. On cooling, the mixture was filtered and the residue reextracted by 50 mL of 20% ethanol. The alcohol extract was reduced to half the original volume by heating on a water bath at 60°C. On cooling, 25 mL of diethyl ether was added and shaken for 5 minutes. The aqueous layer was recovered and washed with 15 mL of 5% aqueous sodium chloride. The aqueous layer was dried, weighed, and calculated for percentage saponin. TLC analysis was performed by eluting with chloroform, glacial acetic acid, methanol, and water solvent system in a

ratio of 48 : 26 : 9 : 6 and spots were visualized under the UV light. Saponins were detected by placing the TLC plate in contact with the iodine vapour [19].

2.2.4. Determination of Phenolics. Weigh 2.5 g of plant powder and transfer it into a conical flask to which 40 mL of 80% acetone (acetone to water at a ratio of 8 : 2) was added. The mixture was heated at 65°C in a water bath for 20 min. The supernatant was decanted and mixed with 80% acetone (acetone to water at a ratio of 8 : 2). The aqueous residue was dried, weighed, and calculated for percentage phenolic compounds. TLC analysis was done by eluting with butan-2-ol/acetic acid/water solvent system in a ratio of 14 : 1 : 5. Then $FeCl_3$-$K_3Fe(CN)_6$ was sprayed on the TLC plate for the detection of the phenolic constituents [20].

2.2.5. Determination of Terpenoids. Soak 2.5 g of plant powder in 50 mL alcohol for 24 hours and then filter it. The filtrate was placed in a separating funnel with extraction solvents (hexane : ethyl acetate) at a ratio of 85 : 15 (v/v). The separating funnel was vigorously shaken for 5 min and then left to settle overnight. The hexane layer was decanted to a beaker for air drying. The hexane extract was treated as total terpenoids. TLC analysis was done using *n*-hexane : ethyl acetate eluting solvent system in a ratio of 6.2 : 2.4. The TLC plate was visualized under the UV and light then heated to 105°C on a hot plate after spraying with freshly prepared *p*-anisaldehyde reagent [21].

2.2.6. Calculation of Retention Factor Values. The retention factor (R*f*) values of different spots were calculated as follows:

$$\text{R}f \text{ value} = \frac{\text{distance traveled by solute}}{\text{distance traveled by the solvent}}. \quad (1)$$

2.3. Preparation of Working Concentrations of the Powders. Fine powders of the leaves, bulbils, and yams at 0.2, 0.4, 0.6, and 0.8 g/50 g of bean seed were measured for bioassays. The standard 0.05 g/50 g of the commercial pesticide, Actellic 50, EC was used in the bioassay as the positive control. Clean, undamaged, and sun-dried *P. vulgaris* seeds were used as the negative control (untreated seeds).

2.4. Stock Culture of Callosobruchus chinensis. In a glass jar (10.3 cm wide and 15 cm deep) containing 350 g of dry and undamaged *P. vulgaris* beans, approximately 200 females of *C. chinensis* were placed and the mouth was covered with a muslin cloth. The jar was incubated for 1 week in darkness at 28 ± 2°C and relative humidity of 65 ± 5% to facilitate egg-laying by the *C. chinensis*. The bruchids were removed and the beans were incubated for mass culturing of the F_1 progeny of *C. chinensis*. The pure culture of the F_1 breed was used in experimentation.

2.5. Antioviposition on P. vulgaris. Twenty females of 3-day-old *C. chinensis* were selected from the culturing jar and added into different jars measuring 6.5 cm wide and 10 cm deep, with 50 g of *P. vulgaris* seeds treated with the different doses of the leaves, bulbils, and yams powders. Actellic 50 EC was used as the positive control and the untreated seeds were used as a negative control. The experiment was replicated in quadruples. The jars were incubated for 5 days in the dark at 28 ± 2°C with a relative humidity of 65 ± 5% for egg-laying. On the 5th day of incubation, all bruchids were carefully isolated and the eggs on the *P. vulgaris* seed coat were counted with the aid of a dissecting microscope.

2.6. Prevention of the Emergence of F_1 Progeny. Twenty females of 3-day-old *C. chinensis* were selected from the culturing jar and added into jars of 6.5 cm width and 10 cm depth with 50 g of undamaged and dry *P. vulgaris* seeds. Under incubation at 28 ± 2°C with a relative humidity of 65 ± 5% in darkness, the weevils were left to lay eggs for 5 days and the weevils were carefully removed from the jars. Different doses of the leaves, bulbils, yams powders, and the positive control were added to their respective jars and then incubated at 28 ± 2°C with a relative humidity of 65 ± 5%. The experiment was monitored daily until the first adults emerged from the negative control. Emerging *C. chinensis* were isolated and counted daily for 5 weeks to avoid overlap with their F_1 generation.

2.7. Statistical Analyses. Antioviposition and reduction of F_1 emergence analyses were performed using the SPSS software (version 20). The analysis involved determination of Poisson distribution by nonparametric (Kolmogorov–Smirnov) test and descriptive statistics of the mean variance. The Poisson distributed data from the antioviposition and F_1 emergence were subjected separately to generalized linear model analysis at $P \leq 0.05$. In the model of analyzing the antioviposition, the concentrations of the doses were the independent variables as data of antioviposition was the dependent variable. In the analysis of F_1 emergence, the concentration was the independent variable while the data obtained from the emerged F_1 progeny was utilized as the dependent variable.

3. Results

3.1. Phytochemical Profiling. The leaves, bulbils, and yams contained different compounds with different R*f* values (Table 1). Alkaloids were absent on the plant parts. Saponins and terpenoids were spotted in all the plant parts. Two spots of phenolic compounds and three spots of saponins were observed in bulbils. Terpenoids had high intensity in the leaves with an observation of six spots. Five spots of flavonoids were visualized in bulbils (Table 1). The results also showed that the powders had compounds with similar R*f* values; saponin with an R*f* value of 0.72 was depicted in the leaves, bulbils, and yams. Powders of bulbils and yams had terpenoids with an R*f* value of 0.37 and flavonoid of R*f* value of 0.71, but were absent in leaves. The bulbils and leaves

Table 1: R*f* values of chemical compounds found in leaves, bulbils, and yams of *Dioscorea sansibarensis*.

	R*f* values				
	Alkaloids	Flavonoids	Saponins	Phenolic compounds	Terpenoids
Leaves	—	0.73	0.72, 0.84	—	0.18, 0.29, 0.49, 0.63, 0.80, 0.84
Bulbils	—	0.51, 0.60, 0.69, 0.71, 0.81	0.61, 0.72, 0.82	0.37, 0.63	0.37, 0.49, 0.69, 0.78
Yams	—	0.56, 0.62, 0.71	0.61, 0.72	0.52	0.37, 0.75, 0.80

powders showed terpenoid with an R*f* value of 0.49 which was absent in yams powder (Table 1).

3.2. Antioviposition. Tabulated results showed counts of 423, 459, and 479 eggs on the seeds treated by 0.2 g of leaves powder, 0.2 g of yams powder, and 0.4 g of bulbils powders, respectively (Table 2). When the eggs laid on treated seeds are counted against the eggs in the negative control (untreated seeds), 0.2 g of leaves powder, 0.2 g of yams powder, and 0.4 g of bulbils powder exhibited an antioviposition of 36.13%, 30.69%, and 27.61%, respectively (Table 2). Data subjected to statistical analysis ($N = 56$) showed a Poisson distribution; the Kolmogorov–Smirnov test recorded 3.18 and the descriptive statistics recorded a mean of 398.11 with a variance of 20197.30. The generalized linear model reported a Wald Chi-Square value of 1.291, $P = 0.26$ on the treatment by 0.8 g of leaves powder. Statistical analysis showed an overall antioviposition of 34.3% ($R^2 = 0.343$) by the leaves, bulbils, and yams powders. Treatment by 0.2 g of leaves powder exhibited better antioviposition activity than 0.4 g of leaves and 0.4 g of bulbils powders; they recorded 422.5, 441, and 478.75, respectively, at $P = 0.51$. At $P = 0.113$, the antioviposition activity by 0.6 g of yams powder (338.75) had equivalent antioviposition activity to 0.8 g of leaves powder (299.75). Antioviposition was highly displayed in the treatment by 0.8 g of the yams powder with a statistical result of 286 as the bulbils powder scored the lowest antioviposition effect amongst the test powders.

3.3. Inhibition of F_1 Emergence. The first *C. chinensis* adult emerged from the negative control (untreated seeds) on the 28th day of incubation from the untreated bean seeds. Graphical results showed that 362, 368, and 387 weevils had emerged from the treatments by 0.2 g of yams and the leaves and 0.4 g of the bulbils powder, respectively (Figure 1). When emerged weevils in treatments are compared to the negative control, 0.2 g of yams powder, 0.2 g of leaves powder, and 0.4 g of bulbils powder exhibited inhibition of weevil emergence from the seeds by 37.02%, 35.98%, and 32.58%, respectively (Figure 1). Kolmogorov–Smirnov test recorded 2.90 and descriptive statistics recorded a mean of 307.71 with a variance of 13727.66. The Poisson distributed data subjected to a generalized linear model reported a Wald Chi-Square value of 2.37, $P = 0.12$ on the treatment by 0.8 g of leaves powder and 7.72, $P = 0.01$ by 0.6 g of yams powder. Statistically, reduction of the F_1 emergence was highly displayed in the treatment by 0 g of yams powder where 188.5 weevils emerged from the seeds. Significant activity was displayed in the treatment by 0.2 g of leaves and 0.4 g of bulbils powders recorded 367.75 and 387.25 at $P = 0.07$. Treatments by 0.6 g of yams and 0.8 of leaves powders showed a significance in reduction of the emerging F_1 progeny at $P = 0.332$. Statistically, the seeds treated by the bulbils powder recorded a higher number of the emerged weevils than the leaves and the yams powders.

4. Discussion

In the family Dioscoreaceae, there is limited literature review concerning studies on wild *D. sansibarensis* especially on chemical analysis and insecticidal potency. Research on *D. sansibarensis* has surveyed its therapeutic application [22]. In this study, the *D. sansibarensis* leaves, yams, and bulbils powders were found to possess terpenoids, saponins, and phenolic compounds; these compounds might have been attributed to their biological activities on mortality, antioviposition, and reduction of the emergence of the *C. chinensis* from the *P. vulgaris* seeds. Dhandapani and Sabna reported that alkaloids, tannins, and phenolic compounds that are present in *Aegle marmelos, Cynodon dactylon, Eclipta prostrata, Moringa pterygosperma, Pongamia pinnata, Sida acuta*, and *Tridax procumbens* protected the plants against pathogens and herbivores [23]. In this study, terpenoid with R*f* value of 0.37 and flavonoid of 0.71 were identified. According to Lazarević et al. [24] and Omotoso et al. [25], terpenoids located at R*f* values of 0.37 ± 0.03 are associated with the prevention of the egg-laying process of bruchids. Chiluwal et al. [26] state that terpenoids with R*f* values of 0.49 ± 0.05 are involved in the larvicidal activity of bruchids, thus preventing the emergence of F_1 progeny. Flavonoids with R*f* values of 0.71 and 0.72 are associated with antioviposition of *C. chinensis* [27].

The results in this study showed that *D. sansibarensis* powders have significant control of *C. chinensis* in the reduction of oviposition activity and adult emergence. In this study, antioviposition recorded after 5 days on *P. vulgaris* beans treated with 0.6 g of *D. sansibarensis* leaves powder was relatively higher as compared to the treatment by 0.8 g of *Jatropha curcas* leaves powder on cowpea seeds which resulted in a 42% reduction in oviposition after 5 days of infestation by *C. maculatus* and 50.67% in reduction of emerging F_1 progeny [22].

The antioviposition and reduction of *C. chinensis* emergence might have been influenced by the presence of flavonoids in the leaves, bulbils, and yams. Flavonoids of plant sources are highly involved in the defence mechanisms by exerting toxic effects on the bruchids [28, 29]. Upasani et al. reported that flavonoids extracted from the aqueous shade-dried leaves of *Ricinus communis* (Euphorbiaceae) showed excellent ovicidal and oviposition deterrent activity and reduced the number of bean seeds damaged and the *C. chinensis* emerging from cowpeas [30].

Table 2: Oviposition by *Callosobruchus chinensis* cultured on *Phaseolus vulgaris* seeds treated with powders of *Dioscorea sansibarensis* leaves, bulbils, and yams.

Powder	Oviposition ± SE (Conc. g/50 g)				Positive control	Negative control
	0.2	0.4	0.6	0.8		
Leaves	422.5 ± 56.8^{c}	441.0 ± 51.6^{b}	360.5 ± 39.8^{c}	$299.8 \pm 31.2^{c,d}$	144.3 ± 15	661.5 ± 67.8
Bulbils	491.8 ± 59.7^{a}	478.8 ± 54.4^{a}	399.8 ± 46.9^{c}	385.5 ± 37.9^{c}		
Yams	458.5 ± 65.6^{b}	405.0 ± 36.3^{c}	$338.8 \pm 31.6^{c,d}$	286.0 ± 40.8^{d}		

Treatment with Poisson distributed data was subjected to a generalized linear model. Means in the table that do not share a letter are significantly different ($N = 56$; $P < 0.05$).

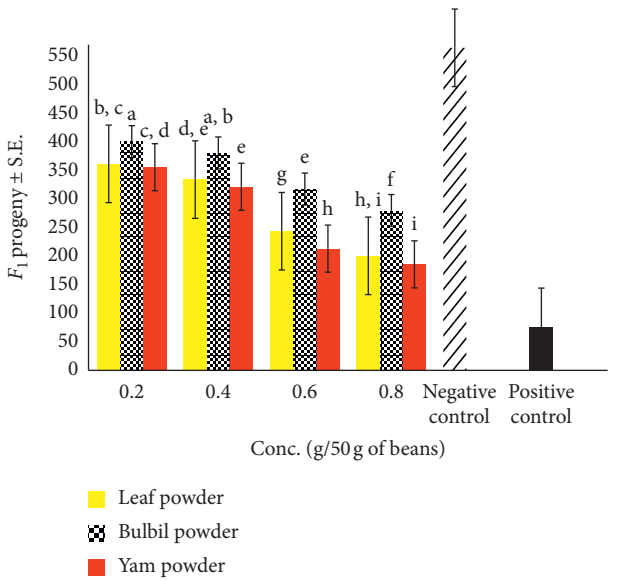

Figure 1: Emerging *C. chinensis* from *P. vulgaris* seeds treated with *D. sansibarensis* leaves, bulbils, and yams powders. Means in the graph that do not share a letter are significantly different ($N = 56$; $P < 0.05$).

A considerable amount of saponins present in the *D. sansibarensis* might have influenced antioviposition and the reduction of the emerging *C. chinensis*. Saponin in form of diosgenin identified in *Trigonella foenum-graecum* prevents *Tribolium castaneum* and *Acanthoscelides obtectus* from laying eggs on the *Zea mays* seed coat and interrupts their larval development that led to the reduction of the emerging F_1 progeny from seeds [24]. Saponic compounds estimated to appear at R*f* values of 0.71 to 0.80 have been highly associated with mortality, antioviposition, larvicidal activity, and emergence of F_1 progeny of the family Bruchidae [31]. Saponins from *Nicotiana tabacum, Tephrosia vogelii*, and *Securidaca longepedunculata* were found to be toxic to the larvae of *C. chinensis* and *C. maculatus* by preventing larval growth and reduction of adult emergence [32]. Saponins isolated from *Vigna unguiculata* seeds reduced the *C. maculatus* F_1 emergence from *P. vulgaris* seeds [33].

5. Conclusion

Based on the findings in this study, *D. sansibarensis* powders possess chemicals and have shown potency on *C. chinensis* in antioviposition and reduction of F_1 emergence. *D. sansibarensis* yams powder is viable for application by the farmers in the protection of *P. vulgaris* against attack by *C. chinensis*.

Data Availability

The datasets generated and analyzed in this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

G. O. Mauti is the sole author of the review article, whereas other authors contributed equally to the literature collection, manuscript documentation, and its revision. All authors read and approved the final manuscript.

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