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Original Article

Antifungal action of Siam weed (*Chromolaena odorata* L.) leaf powder on infected pawpaw fruits (Papaya)

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ABSTRACT

This research is aimed at evaluating the effectiveness of Siam weed (*Chromolaena odorata*) leaf powder on fungi associated with pawpaw fruits. A total number of 15 samples were collected, five infested and ten healthy ones. Infested pawpaw fruits were inoculated into Potato Dextrose Agar and pure cultures were obtained. Identification of isolated fungi were made based on the cultural and characteristics and microscopic examination. The fungi were isolated from the spoilt pawpaw fruits and identified using macroscopic and microscopic features as *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Aspergillus ustus,* and yeast. The pathogenicity test was determined and results showed that fungi isolated were responsible for the spoilage of pawpaw fruits with the yeast causing the most rapid rot and weight loss followed by *A. ustus* and then *A. fumigatus. A. flavus* causes less rot and weight loss while *A. niger* causes the least of rot and weight loss. This implies that the yeast is the most pathogenic while the *A. niger* is the least pathogenic amongst the identified fungi on pawpaw fruits. A sensitivity test was carried out to evaluate the antifungal action of *C. odorata* leaf powder on the identified fungi at different concentration of 0.5 g, 1.0 g, 1.5 g, and 2.0 g. Data collected were subjected to analysis of variance (ANOVA) and means were separated using least significant difference. Results show that the powder is most sensitive on *A. ustus* as 0.5 g can give antifungal activity of 74% and 2.0g can give antifungal activity of 84% while the powder is least sensitive to *A. flavus* and *A. niger* as 0.5 g could only give an antifungal effect of 35% and 2.0 g could give antifungal effect of 57%. This implies that a little quantity of the leaf powder can be used to effectively control *A. ustus* while larger quantity will be required to achieve control on *A. flavus* and *A. niger*. The data analysis of the sensitivity test for all the fungi species shows that there is a significant difference for individual fungus at different gram quantity of leaf powder which implies that at least one of the treatments is responsible for the difference.

Keywords: Antifungal action, pathogenicity, pawpaw, Siam weed

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INTRODUCTION

Fruits and vegetables are very important and have high dietary and nutritional qualities.[1] Studies have evaluated the association of fruit and vegetable consumption with the reduction of risk of specific diseases.[2] Their consumption has dramatically increased by more than 30% during the past few decades.[1] Fresh fruit and vegetable consumption increased by 25.8% and 32.6%, respectively, and far exceeded the increases observed for processed fruit and vegetable products. It is also estimated that about 20% of all fruits and vegetables produced is lost each year due to spoilage.[1] Kuthe and Spoerhase[3] reported that 20 new human fungal pathogens are documented each year. It is estimated that about 20–25% of the harvested fruits are decayed by pathogens during postharvest handling even in developed countries.[4]

Deterioration of foods generally is attributed to two main causes which are natural degradation due to activities of enzymes and growth of microorganisms (bacteria, molds, and yeasts).The adverse effects of microbial activities result in decay, rotting of food, and food poisoning. Bacteria and fungi may also produce waste products which act as poisons or toxins, thus causing the renowned ill-effects.[3]

Papaya (*Carica papaya* L.) is a popular fruit plant grown all over the wetter parts of West Africa, tropical and subtropical regions of the world being one of the most nutritious and

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cheapest fruits grown and consumed in Nigeria.[5] It is usually grown as compound fruit crop or semi-wild fruit crop from discarded seeds.[3] Papaya (*C. papaya* L.) belongs to the family Caricaceae which incorporates 35 latex-containing species in six genera, *Carica*, *Cylicomorpha*, *Jarilla*, *Jacaratia*, *Horovitzia,* and *Vasconcellea*[6] with the genus Carica, consisting of only one species. Although opinions differ on the origin of *C. papaya*, it is likely that *C. papaya* originates from the lowlands of eastern Central America, from Mexico to Panama.[7] The papaya fruit is melon-like, oval to nearly round, elongated club–shaped and is rich in latex when unripe with a green colored skin. The fruit is juicy, sweetish and some types are quite musky.[8] The fruit consist largely of water, sugar, Vitamins A and C, protein, and ash.[5] Papaya has a mild laxative action and the seeds are used medicinally against worms and ulcer.[5] The green fruits, leaves, and flowers may also be used as cooked vegetable.[9]

Papaya also has several industrial applications due to the proteolytic enzyme called papain hence their use in the production of chewing gums, tenderizing meat, and drug preparation for various digestive ailments and for the treatment of gangrenous wounds as well as in the textile and cosmetics industries.[10] The seed is used to expel worms and the flower may be taken in an infusion to induce menstruation.[11]

Nigeria is the third largest producer of papaya in the world with 703,800 metric tons,[12] it's plentiful and sold all year round as well as being globally consumed by people for multiple benefits hence, the need for preservation and to boost its production. This can be achieved among other things by preventing the occurrence of these diseases through studying the fungi responsible for their spoilage and subsequent treatment with botanicals.[12]

Chromolaena odorata popularly known as Awolowo or Independence weed, bitter bush or Christmas bush in Nigeria but generally referred to as Siam weed is an exortic weed specie which poses a fire hazard, even when actively growing and its flammable nature promotes the spread of veld fires in forests.[13,14] The weed is allelopathic and phytotoxins are released through the rain-wash of leaves, exudation from roots and from decomposing plant residues.[15]

Papaya is a useful plant with nutritional, medicinal and health benefits. In spite of all these benefits, the plant is besieged by lots of pathogens both in the field and post-harvest diseases and these diseases result in yield losses thus making its valuable components unavailable.

Papaya fruits are beset with problems of field and storage rot. Gutpa, Pathak[16] identified 22 different fungi in post-harvest decay of papaya fruits which include *Aspergillus niger, Aspergillus flavus, Rhizopus oryzae,* and *Fusarium moniliforme*. Bacterial pathogens involved with rot of papaya include the species of soft-rotting *Erwinia, Pseudomonas, Xanthomonas, Cytophaga,* and *Bacillus*. Besides the losses in income to the papaya fruit marketers, the rotten fruits could also cause a health hazard to consumers. Krogh[17] had earlier reported that most microbes infecting plant tissues often produced secondary metabolites in their hosts, which are known to be hazardous to animals including man. Some of these metabolites include the ergot alkaloids on cereals by *Clavisep spp, fumonisin* on maize by *Fusarium spp*, aflatoxins, and ochratoxins on several plants produced by *Aspergillus spp*.[18] An estimated 4.5 billion people in developing countries may be exposed chronically to aflatoxins though their diet. Exposure to aflatoxins is known to cause both chronic and acute liver diseases and liver cancer.[19] Aflatoxins are extremely carcinogenic, mutagenic and immunosuppressive.[20] Consequently, up to 10% of hospital acquired systemic infections are caused by fungi. Altogether this forces the scientific community, agro-industry and pharmaceutical companies to search for natural compounds that will satisfy consumer requirements.[20]

Furthermore, there is growing concern about chemicals for plant protection because of their undesirable side effects on humans, non-target organisms, and their behavior and fate in the environment.[20]

The aim of this research is to evaluate the antifungal action of *C. odorata* leaf powder on infected pawpaw fruit, to isolate and identify fungi associated with infected pawpaw fruit, to determine the pathogenicity of identified fungi in spoilage of pawpaw fruits and to determine the lowest concentration/dosage of *C. odorata* leaf powder that gives the best action on fungi associated with infected pawpaw fruits.

MATERIALS AND METHODS

Study Site

The study was carried out in Federal College of Forestry, Jos, Plateau State, Nigeria which is located between latitude 8°N, longitude 7°E and 25°F and at an altitude of 1200 m above sea level. The area falls under Natural Region II of Nigerian's agro-ecological zones, the climate of the area is humid with an average annual rainfall and temperature between 140–148 mm and 10–32°, respectively.

Sample Collection

Pawpaw fruits were collected from vegetable markets in Jos North local government of plateau state (Both healthy and infected pawpaw). The samples were placed in sterile polythene bags and labeled appropriately and were taken to the laboratory for further analysis.

C. odorata leaves [Figure 1] were obtained from its natural habitat and identified in the herbarium of Federal College of Forestry Jos, Plateau state.

Figure 1: *Chromoleana odorata* leaves

Preparation of *C. odorata* Leaf Powder

C. odorata leaves collected, washed with water and dried at room temperature. The leaves were pounded using ceramic mortar and pestle and then sieved with a mesh to obtain a fine powder. The powder was weighed into four different concentrations of 0.5 g, 1.0 g, 1.5 g, and 2.0 g. These were labeled and kept safe for use.

Preparation of Medium

Potato Dextrose Agar (PDA) was used to culture the fungi from the spoilt papaw and was also used to obtain pure culture. The commercially produced dehydrated medium was prepared in the laboratory strictly according to manufacturer's instructions. The PDA was sterilized by autoclaving at 121°C for 15 min. It was then allowed to cool at temperature of 40°C. Gentamycin was added when it was cool to inhibit the growth of bacteria, in the medium and it was then poured into appropriate sterile Petri dishes.

Isolation of Fungus

Diseased portion of the pawpaw fruits was cut under aseptic conditions into small bits into a sterile dish with the aid of sterile scissors (flamed over a Bunsen burner flame) and dipped inside a methylated spirit. The bits were placed on Petri dishes containing already prepared solidified PDA. The solidified plates were incubated at room temperature (28 ± 2°C) for 3–7 days in the dark until visible growth was seen on the plates. The fungal colonies grown from the incubated plates were sub-cultured into fresh medium until pure culture was obtained. The fungal colonies that appeared were primarily identified using cultural and morphological features.

Identification of Isolated Fungus

The fungal growths that appeared were primarily identified using cultural and morphological features. The isolates were identified microscopically by staining with Lacto phenol cotton blue. It allows for the identification of various fungal structures such as presence or absence of rhizoids, hyphae, spores as well as other additional structures. And the procedure by Fawole and Oso[21] was used, a drop of lactophenol cotton blue reagent was placed on a clean, grease-free glass slide and a small tuft of the fungus was obtained using sterile inoculating needle and transferred to the glass slide. A cover slip was placed over the preparation and examined under the microscope using magnification of 00D7X10 and X40.

Pathogenicity

This was carried out as described by Baryewu[22] and Chukwuka.[23]

Healthy pawpaw fruits were properly washed with tap water, rinsed with distilled water and surface sterilize with 70% alcohol. With a cool flamed 2 mm cork borer, 2 cm long cylindrical cores were removed from each fruit; discs of pure cultures of each isolate were removed from agar plates and placed in the bored holes on each fruit. Petroleum jelly was smeared to completely seal each hole. The inoculated fruits were then incubated at 28°C for 3–7days. Rot symptoms developed with different fungal isolates were observed. Regular observations were made. During which, the extent of rot caused by the isolate was measured at interval of 24 h with a ruler (in cm) and recorded. Daily weights of the pawpaw fruits were also taken.

Sensitivity Test (Poisoned Food Method)

Poisoned food method reported by Nene and Thapliyal.[24] was used to determine the sensitivity of Siam weed powder on the identified fungi. This method is mostly used to evaluate the antifungal effect against fungi. The *C. odorata* leaf powder at different concentration (0.5 g, 1.0 g, 1.5 g, and 2.0 g) was incorporated into the molten agar per 20 ml of PDA and mixed well. The medium was then poured into Petri dishes. After an overnight pre-incubation, the inoculation was done by taking a bit of the mycelia disc with a sterile needle and deposited in the center of the plate. The plates were allowed to stand on the bench for an hour to perfuse and later incubated at 28° C for 3–7 days. The diameter of the zone of inhibition of each organism by different concentrations of the Siam weed powder was measured using a transparent ruler in millimeters and recorded. A fungal culture without any antifungal agent was used as control.[24] The antifungal effect was estimated by the following formula:

Antifungal activity (%) = [(Dc–Ds)/Dc] ×100[24]
Where Dc = diameter of growth in control plate
Ds = Diameter of growth in the plate containing tested antifungal agent.

Data Collection

The data collected include, initial weight of pawpaw fruit before treatment, initial cut made before inoculation, daily

weight loss at the interval of 24 h for each pawpaw for 3–7 days by subtracting the new weight from the initial weight, daily radial growth at the interval of 24 h for each pawpaw for 3–7 days by subtracting the new radial increase from the initial, using a meter rule.

Experimental Design and Data Analysis

A complete randomized design was used involving four concentrations (0.5 g, 1.0 g, 1.5 g, and 2.0 g) and a control on the fungi subject making a combination of five treatments and replicated 4 times. The data collected were subjected to ANOVA. Means was separated using least significance difference.

RESULTS

The results of the evaluation of antifungal action of Siam weed (*C. odorata*) on infected pawpaw fruits (*C. papaya*) show that four fungi are isolated in Table 1.

Result of the pathogenicity test shows that these fungi are responsible for the spoilage of pawpaw fruits with the yeast causing the most rapid rot and weight loss followed by *A. ustus* and then *Aspergillus fumigatus*. *A. flavus* causes less rot and weight loss while *A. niger* causes the least of rot and weight loss. This implies that the yeast is the most pathogenic while the *A. niger* is the least pathogenic amongst the identified fungi on pawpaw fruits as shown in Table 2.

Result of the sensitivity test shows that the powder is most sensitive on *A. ustus* as 0.5 g can give antifungal activity of 74% and 2.0g can give antifungal activity of 84% while the powder is least sensitive to *A. flavus* and *A. niger* as 0.5 g could only give an antifungal effect of 35% and 2.0 g could give antifungal effect of 57%. The results are represented in Table 3 in which + signifies low effect (below 40%), ++ signifies the effect is moderate (40–60%) while +++ signifies very high effect (70–100%).

Antifungal Activity

Aspergillus fumigatus

- 0.5 g = 47.8%
- 1.0 g = 52.15%
- 1.5 g = 65.05%
- 2.0 g = 65.05%

A. ustus

- 0.5 g = 74.70%
- 1.0 g = 74.70%
- 1.5 g = 83.79%
- 2.0 g = 84.98%

A. flavus and A. niger

- 0.5 g = 35.25%
- 1.0 g = 35.25%
- 1.5 g = 40.28%
- 2.0 g = 57.55%

Yeast

- 0.5 g = 59.11%
- 1.0 g = 61.08%
- 1.5 g = 67.98%
- 2.0 g = 70.44%

The data analysis of the sensitivity test for all the fungi species shows that there is a significant difference for individual fungus at different gram quantity of leaf powder which implies that at least one of the treatments is responsible for the difference

Table 1: Macroscopic and microscopic characteristics of fungi isolates from papaya samples

Sample	Macroscopic characteristics	Microscopic characteristics	Organisms identified
F1	Flat white creamy growth on plate	Gram positive, oval budding and presence of cocci	*Yeast*
F2	Colonies are initially white, quickly becoming black. Reverse is pale yellow	Hyphae are septate and hyaline. Conidial heads are radiate. Conidiophores are long, smooth and hyaline. Conidia are brown to black, very rough and globose. Metulae and phialides cover the entire vesicle	*Aspergillus niger*
F3	Colonies are olive to lime green with a cream reverse. Rapid growth. Texture is wooly to cottony.	Hyphae are septate and hyaline. Conidial heads are radiate to loosely columnar with age. Conidiophores are coarsely roughened, uncolored.	*Aspergillus flavus*
F4	Colonies are smoky gray-green with a slight yellow reverse. Very mature colonies turn slate gray. Texture is wooly to cottony.	Hyphae are septate and hyaline. Conidial heads are strongly columnar. Conidiophores are smooth walled, uncolored, long and terminate in a dome-shaped vesicle.	*Aspergillus fumigatus*
F5	Colonies are white to yellow to gray to brown. Gray is the most common color. Reverse is yellow to brown with a diffusing pigment. Texture is wooly to cottony to somewhat granular.	Hyphae are septate and hyaline. Conidial heads are radiate to loosely columnar and biseriate. Conidiophores are smooth-walled and brown. Metulae and phialides cover the upper portion of the vesicle.	*Aspergillus ustus*

F1=Fungi1, F2=Fungi2, F3=Fungi3, F4=Fungi4, F5=Fungi5

Fungi on Petri plates	Photomicrographs

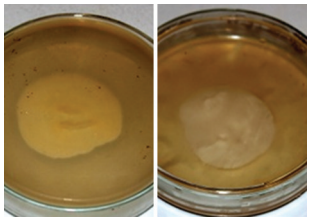
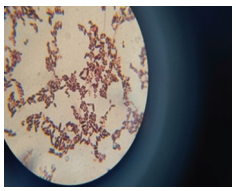
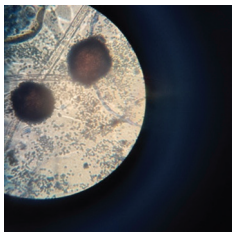

	

Plate 1: Yeast cell	
	
Plate 2: *Aspergillus niger*	

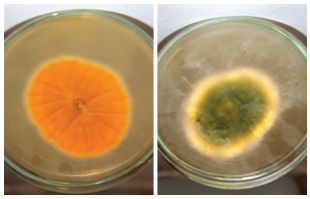
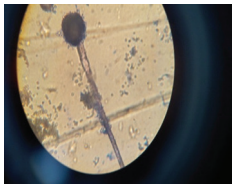

	

Plate 3: *Aspergillus ustus*	

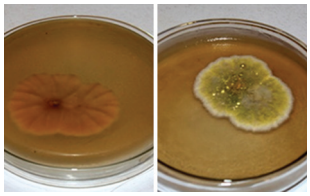
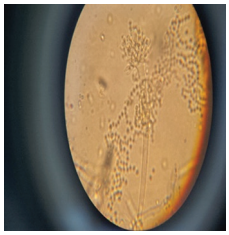

	

Plate4: *Aspergillus flavus*	

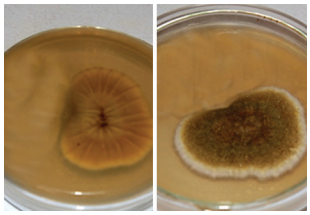
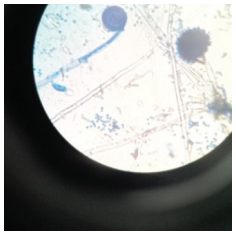

	

Plate 5: *Aspergillus fumigatus*	

DISCUSSION

From the result obtained, it shows that the fungi associated with spoilt pawpaw fruits in Jos North Local Government Area are; *Aspergillus fumigatus, A. ustus, A. flavus, A. niger, and Yeast.* A similar profile of fungal species was obtained from some pawpaw fruits by Gupta and Pathak[16] in South Eastern Nigeria with the inclusion of *Rhizopus oryzae* and *Fusarium monoliforme* and exception of *A. ustus* and yeast. The research of Oniha and Egwari[25] also isolated *A. niger Fusarium solani, and Rhizopus stolonifer* from some pawpaw fruits in the Western Nigeria. A similar work by Yusuf *et al*.[26] in the North Eastern Nigeria also Isolated and identified *A. niger Penicillium expansum, mucor spp and Rhizopus stolonifera.* Comparing these three researches, *Aspergillus spp* is common in this region and the differences may be linked to the differences in soil, climatic and environmental conditions associated with a particular region as well as the prevailing and dominant fungi spores at harvest.

The pathogenicity test result shows that the fungi species grown from the fresh pawpaw fruits had the same characteristics as those isolated from the spoilt fruits indicating that the fungi were responsible for the spoilage of pawpaw fruits. This agrees with Oniha and Egwari[25] who stated that the identified fungi are responsible for the spoilage of pawpaw fruits in a work titled isolation and characterization of microorganisms associated with rot diseases. The daily weight loss caused by each of the fungi species differs accordingly with the yeast giving the most rapid weight loss of 197.3 g in 6 days followed by the *A. ustus* which gave a weight loss of 146.2 g and the *Aspergillus fumigatus* of 112.7 g. This is followed by the *A. flavus* with a weight loss of 101.0 g. *Apergillus niger* gave the least weight loss of 85.4 g. This means that the yeast is the most pathogenic amongst the identified fungi as it gives the most rapid weight loss and also gives the highest extent of rot on the pawpaw fruits, while the *A. niger* is the least pathogenic amongst the identified fungi as it causes less rot and weight loss.

The data analysis of the sensitivity test for all the fungi species shows that there is a significant difference for individual fungus at different gram quantity of leaf powder which implies that at least one of the treatments is responsible for the difference. For *Aspergillus fumigatus*, the 2.0 g of the leaf powder gave

Mean representation of the different treatments for each fungus

Treatments	Yeast	*Aspergillus fumigatus*	*Aspergillus flavus*	*Aspergillus Ustus*	*Aspergillus niger*
Control	5.20^{e}	4.68^{a}	3.48^{a}	6.33^{a}	3.88^{e}
0.5	2.13^{d}	2.43^{cb}	2.25^{eb}	1.60^{e}	2.25^{cb}
1.0	2.03^{bd}	2.23^{b}	2.25^{db}	1.60^{de}	2.48^{ba}
1.5	1.65^{cd}	1.63^{db}	2.08^{cb}	1.03^{ce}	2.28^{cb}
2.0	1.50^{ad}	1.50^{b}	1.48^{bd}	0.95^{be}	1.88^{ac}

Table 2: Daily weight loss of pawpaw fruits (kg) and extent of rot (mm) at Day 6

Samples	Weight loss (kg)		Extent of rot (mm)	
	Total	Control	Total	Control
Yeast	197.3	5.7	40	2.5
Aspergillus ustus	146.2	6.7	35	2.5
Aspergillus fumigates	112.7	2.7	29.7	2.6
Aspergillus niger	85.4	4.1	25.5	2.5
Aspergillus flavus	101.0	2.8	26	3.0

Table 3: Sensitivity test result

	0.5 g	1.0 g	1.5 g	2.0 g
Aspergillus fumigatus	+	++	++	++
Aspergillus ustus	+++	+++	+++	+++
Aspergillus flavus	+	+	+	++
Aspergillus niger	+	+	+	++
Yeast	++	++	++	+++
Control	-	-	-	-

the best inhibitory effect with a total growth of 1.8 cm in 4 days while the 0.5 g gave a 3.2 cm growth, 1.0 g gave 3.0 cm growth, 1.5 g gave 2.0 cm growth, and the control gave 8.5 cm growth. This implies that the powder becomes more effective as the quantity is increased. This seems to be the case with all the fungi as 2.0 g also gave the best inhibitory effect on the yeast and *A. ustus*. For *A. flavus and niger,* they both show the same behavior at the various different leaf powder quantity. The 2.0 g also gave the highest inhibitory activity of 1.9 cm in 4 days. While the 0.5 g, 1.0 g, and 1.5 g gave inhibitory effect of 3.0 cm, 3.0 cm, and 2.7 cm, respectively, and with a control growth of 5.0 cm. This implies that, the Siam weed powder does not show good inhibitory effect on *A. flavus* and *A. niger* compared to the other fungi identified.

From the results obtained and using the Nene and Thapliyal[24] formular for estimating the percentage antifungal activity of a tested substance, it shows that the *C. odorata* leaf powder is most sensitive to *A. ustus*, 1.5 g can give a control as high as 83%, and 0.5 g can give a control of 74%. This implies that a little quantity of the leaf powder is required to give a maximum and effective control on *A. ustus.* On the yeast cell, 2.0g gave an antifungal effect of 70% which means, the leaf powder can be used to effectively control this fungus. For *Aspergillus fumigatus,* 1.5 g and 2.0 g can give a control of 65% which means, more of the leaf powder is required to achieve an effective control on these fungi. The leaf powder at 2.0 g gave a control of 57% on *A. flavus* and *A. niger* while 0.5 g can give a control of as low as 35%. This implies that the leaf powder is least sensitive to *A. flavus* and *A. niger* which means that very much of the leaf powder will be required to achieve an effective control on these two organisms.

CONCLUSION

Finding ways of profitably utilizing this weed is an option. Such profitable option is its use in pest control particularly as a fungicide and this research has revealed that this weed can be used in the control of fungi associated with the spoilage of pawpaw fruits. The occurrence of fungi on pawpaw fruits examined demands that appropriate control measures should be employed during its handling to minimize mechanical damage which leads to loss due to fungi attack that causes spoilage and unacceptability. These organisms are known to be pathogenic and produce disease of man such as Aspergillosis which may be fatal if untreated. Care must be taken during the harvesting, cleaning, sorting, packaging, transport, and storage of the fruits to reduce the incidence of these fungi on the fruits as well as the health risk posed to the consuming public.

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