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ANTIFUNGAL POTENTIAL OF MEDICINAL PLANTS AND ORGANIC PRODUCTS AGAINST *SAROCLADIUM ORYZAE*, THE CAUSAL AGENT OF RICE SHEATH ROT

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ABSTRACT

Sheath rot, caused by *Sarocladium oryzae*, is a destructive disease that poses a significant threat to rice crop worldwide. The extensive use of synthetic fungicides for sheath rot management has raised concerns about their environmental impact and potential health hazards. Therefore, examining alternative control methods, such as organic products and medicinal plant extracts, is essential. This research aimed to evaluate the antifungal properties of organic products and medicinal plant extracts in controlling *S. oryzae* under *in vitro* conditions. Significant differences in the mycelial inhibition (MI) of the *S. oryzae* in all tested organic products and medicinal plant extracts was observed. Among the six medicinal plant extracts tested, Sadabahar (*Catharanthus roseus*) exhibited the highest MI (79.31% at 10% concentration and 74.13% at 5% conc.), followed by Tulsi (*Ocimum sanctum*), which achieved 77.58% and 72.41% MI at 10% and 5 % conc. respectively. Similarly, among three organic products evaluated, Jeevamrut exhibited complete inhibition of mycelial growth (100% at 2% and 5% concentrations) compared to the control, thereby indicating its potential for eco-friendly alternative management to fungicides.
***Keywords*:** Sheath rot, Plant Extract, Sustainable Agriculture, *In vitro*, Bio-efficacy, Organic amendments

Introduction

Rice (*Oryza sativa* L.) is a staple food crop throughout the world providing the major global security for more than 3 billion people (Zhang *et al.* 2022). India ranks as the second-largest rice-producing country after China, contributing nearly one-third of the global rice area and production. Rice cultivation is practiced in almost all states of India, covering more than 30 (%) of the total cultivated area and accounting for over 40 (%) of food grain production. India accounts for the production of 145 million mitric tonns (www.usda.gov). Rice is extensively cultivated in Bihar state with an area of 3.21 million ha. and production of 6.5 million tonns (https://guidely.in/blog/largest-rice-producing-state-in-india).However, several biotic and abiotic factors significantly affect rice cultivation, with diseases being one of the most critical challenges. In addition to reducing yield, diseases also affect the quality and quantity of rice production, thereby affecting agricultural sustainability.

Sheath rot, caused by *Sarocladium oryzae* (*Sawada*) Gams and Hawksworth, has emerged as a major rice disease. Grain yield losses due to sheath rot have been reported to range from 9.6% to 85% across various regions, including India (Panda and Mishra, 2019; Mehta *et al.,* 2023). Weather conditions such as moderate temperatures (20°C–30°C) and high relative humidity (65%–85%) are conducive to the development of the disease (Bigirimana *et al.,* 2015; Mehta *et al*., 2023). The sheath rot pathogen can infect the rice plant at any growth stage, but its impact is most severe when infection takes place during panicle emergence stage and produce distinctive symptoms (Peeters *et al*. 2021; Mehta *et al.* 2023), resulting in the development of grey-brown lesions on the sheath of the flag leaf. These spots expand, merge and eventually cover the entire sheath, with severe infections often associated with moderate temperature and humid weather, This leads to the development of husky, discolored grains, which adversely affect seed viability, quality, and nutritional value resulting in production of empty grains, reduced seed germination, and impaired grain filling, which also diminishes the test weight. Climate change, shifts in cropping patterns, and the widespread adoption of high-yielding, semi-dwarf, and dwarf commercial rice varieties many of which are susceptible to sheath rot are among the key factors believed to be contributing to increased disease pressure (Peeters *et al.,* 2021). Currently, there is a shortage of rice varieties with adequate resistance to sheath rot. Breeding efforts are challenged by the high genetic variability of the pathogen, limited understanding of its infection mechanisms, and the absence of strong resistance sources among existing germplasm (Mvuyekure *et al.,* 2018; Peeters *et al.,* 2021). Consequently, integrated disease management strategies such as the use of fungicides, biological control agents, and optimized agronomic practices may offer more immediate and practical solutions to reduce sheath rot-related yield losses (Sreenivasa *et al.,* 2001).

Researchers have reported the efficacy of various fungicides against sheath rot (*S. oryzae*) (Kumar and Patinda, 2015; Bigirimana *et al.* 2015, Raju, 2020, Mehta, 2022). However, the extensive use of fungicides has led to challenges such as residual accumulation in food and feed, the development of fungicide resistance (Deising *et al*., 2008), and toxicity to non-target organisms, resulting in environmental contamination and pollution (Arcury and Quandt, 2003).

In response to these concerns, the development of organic substances and natural products as alternatives has gained momentum. These products can mitigate the aforementioned issues through direct toxicity to pathogens, induction of systemic resistance, promotion of beneficial organisms, and neutralization of pathogen-produced toxins. Consequently, there is an increasing need to incorporate environmentally friendly formulations into integrated pest management (IPM) approaches to efficiently address these challenges and promote sustainable agriculture. Although, botanicals mediated sheath rot management was carried out for sheath rot management previously (Sharma *et al*., 2013), however holistic approach using combined use organic amendments and botanicals in sheath rot pathogen management is still lacking.

To develop a control strategy for rice sheath rot, comprehensive trials were conducted to evaluate the effectiveness of organic products and medicinal plant extracts as potential alternatives. With this objective, present study was carried out *in vitro* experiments to assess the efficacy of various organic products and medicinal plant extracts in managing sheath rot (*S. oryzae*).

Materials and Methods

Isolation of the Pathogen

The pathogen (*Sarocladium oryzae*) responsible for rice sheath rot was isolated from infected leaf sheaths. Infected portions, along with some healthy tissue, were carefully cut into small bits using a sterilized scalpel and subjected to surface sterilization with 1% sodium hypochlorite. The sterilized tissue bits were aseptically transferred to petri plates containing potato dextrose agar (PDA) medium and incubated at 28°C in a BOD incubator. Pure cultures of the pathogen were obtained and preserved on PDA slants at 4°C. Pathogen identification was carried out based on its pathogenicity, cultural characteristics, and spore morphology.

Preparation of Plant Extracts

Fresh leaves of medicinal plants were used for extract preparation. Leaves were first washed thoroughly with tap water, followed by sterile distilled water. Then, 100 g of leaves were ground with 70% ethanol in a 1:1 ratio using a grinder. The resulting mixture was filtered through muslin cloth and then through Whatman No. 41 filter paper to obtain a crude extract. The filtrate was collected in sterilized conical flasks and considered as 100% concentration.

Procurement of Organic amendments

Three commercially available organic products such as, Jeevamrut, Panchagavya, and Vermivash were collected from the Department of Plant Pathology, RPCAU, Pusa. Each product was tested at 2 and 5% concentrations, with unamended medium serving as the control.

In Vitro Assessment of Organic Products and Plant Extracts Against *Sarocladium oryzae*

Poison Food technique

The antifungal properties of medicinal plant extracts and organic products, was assessed using poison food technique (Schmitz, 1930). PDA medium was autoclaved, cooled, and amended with the required concentrations of plant extracts (5 and 10%) or organic products (2 and 5%) before being poured into Petri plates (90 mm diameter). Once solidified, a 9 mm mycelial disc of actively growing seven days old culture of pathogen was inoculated into each plate. For each treatment, three technical replications were maintained. Plates with unamended PDA served as controls.

The growth of the fungus was recorded by measuring radial mycelial growth (in mm) at three days intervals until 15 days post-inoculation. The percentage mycelial inhibition of fungal growth compared to the control was calculated using the formula (Vincent, 1947).

Growth inhibition (%): $I = \left(\frac{C-T}{C}\right) \times 100$

Where,

C = Colony diameter (mm) in check plate

T = Colony diameter (mm) in the treated plate

Statistical Analysis

The impact of different treatments on mycelial inhibition of pathogen was determined by ANOVA using OP stat software (http://14.139.232.166/opstat). The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for completely randomized design and interpretation of data was carried out. The level of significance used in 'F' and 'T' test was P = 0.05 and P = 0.01. Critical differences were calculated wherever 'F' test was significant. The values percent disease index was subjected to angular transformation according to the table given by Sundarraj *et al*., (1972).

Critical difference (C.D.)

C.D. = S.Ed. × t values at error degrees of freedom t= tabulated t value at '5' per cent probability level.

Results and Discussion

Sheathrot is one of the natorius disease of rice causing significant yield losses and affecting quality parameters. The disease management of sheath rot was solely depended on the fungicides by farmers, that leads to various health hazards to humans and animals and creates environmental pollution. Thus, this study focused on the sustainable management of the disease through use of botanicals and organic amendments. Three organic amendments and six botanicals were tested at different concentrations.

In Vitro* Assessment of Medicinal Plant Extracts Against *Sarocladium oryzae

In this study, six medicinal plant extracts were evaluated for their effectiveness against *S. oryzae*. The findings are summarized in Table 1. All plant extracts demonstrated significant antifungal activity, as indicated by their ability to inhibit colony growth. Among the six plant extracts, *Catharanthus roseus* (Sadabahar) was the most effective, showing the highest inhibition of mycelial growth (79.31%) at 10% concentration. At 5% concentration, it achieved 74.13% inhibition. This was followed by *Ocimum sanctum* (Tulsi), which recorded 77.58% and 72.41% inhibition at 10 % and 5 % concentrations, respectively. Least mycelial inhibiton was recorded in *Centella asiatica* (Mandukaparni) at 5 % concentraion (48.25 %), however it recorded 60.34 % inhibition at 10 % concentration. Other extracts, such as *Bacopa monnieri* (Brahmi), *Andrographis paniculata* (Kalmegh), and *Piper longum* (Pippali)also exhibited considerable antifungal activity.

The experiment showed that all tested plant extracts effectively inhibited the mycelial growth of S. oryzae. Plant extracts such as, Sadabahar and Tulsi, were found promising in inhibition of pathogen in vitro at both 5 and 10 % concentrations, thus we can incorporate these under integrated disease management of sheath rot. Further testing of these medicinal plant extracts can be done under in-planta conditions is necessary by different formulations. Various reports studies reported the bioefficacy of similar botanicals and found to be antimicrobial for controlling fungal and bacterial pathogens such as *Bacopa monneri* (Udgire and pathade, 2012: Alam *et al*., 2011: Indoliya *et al*., 2022; Fazlu *et al*., 2019): *Piper longum* (Pippali) (Srinivasa Reddy *et al*., 2001: Lee *et al*., 2009): Andrographis paniculata (Kalmegh) (Mishra *et al*., 2007: Sule *et al*., 2012: Deepak *et al*., 2014: Nidiry *et al*., 2015). Vineeth *et al*., 2023 have tested medical plant extract, Tulsi in vitro bioefficacy at 5 and 10 % against *Colletotrichum gloeosporioides* and recorded moderate level mycelial inhibition which align our results. Similar results were also found by Dev *et al*. (2016). Apart from this, *C. asiatica* extracts and derivatives was also used in the treatment of neurological conditions of humans. These plant extracts have reported to produce array of bioactive compounds which control phytopathogenic mycelial growth. The utilization of plant-based products as antifungal agents has been widely explored in controlling plant diseases, underscoring their importance in sustainable agriculture. Number of secondary substances like various cell wall degrading enzymes phenols, flavonoids, quinines, essential oils, alkaloids, saponins, steroids, etc were reported to produce these botanicals used in present study. Some of these plant-based metabolites have antimicrobial properties and are toxic to phytopathogens. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, functioning as signal molecules, allelopathic compounds, phytoalexins, detoxifying agents, and antimicrobial defensive compounds. Moreover, plant extracts also have roles against frost hardiness, drought resistance, and may play a functional role in plant heat acclimation, freezing tolerance, and photo protection (Lattanzio *et al*., 2006, Samanta *et al*., 2011: Agati *et al*., 2013: Zeng *et al*., 2018). These results align with previous studies demonstrating the antifungal potential of plant extracts against various pathogens (Mitra *et al*., 1987).

In Vitro Efficacy of Organic Products Against *S. oryzae*

The antifungal properties of three organic product such as, Jeevamrut, Panchagavya, and Vermivash-were evaluated at 2% and 5% concentrations using the poison food technique. Results were summarized in Table 2.

At 2% concentration, Jeevamrut completely inhibited fungal growth of *S. oryzae*, achieving 100% inhibition. Vermivash showed 70.37% inhibition, while Panchagavya achieved 61.11%. At 5% concentration, Jeevamrut and Vermivash both completely inhibited fungal growth (100% inhibition), whereas Panchagavya showed lower efficacy, with 66.66% inhibition.After 15 days of incubation, all organic products significantly suppressed the growth of *S. oryzae* compared to the control. Jeevamrut emerged as the most effective product, displaying complete inhibition at both concentrations. This study underscores the notable environmental benefits of using organic amendments instead of chemical fungicides. By limiting chemical contamination, improving quality, preserving biodiversity, and fostering sustainable farming, organic amendments present a more environmentally responsible method of managing plant diseases. Although their effects may not be immediate, combining them with other sustainable strategies can effectively control diseases while reducing negative impacts on the environment. This finding highlights the potential of organic products as eco-friendly alternatives to synthetic fungicides. Previous studies have also reported the antifungal efficacy of organic products in managing plant diseases (Kumar *et al*., 2010; Jandaik *et al*., 2015: Anandeeswari and Christopher, 2020). Apart from inhibiting plant pathogens, jeevamruth is reported to produce various plant growth promoting activities such as growth, yield, quality nutrient update, nutrient solubilisation and also improves the physical properties of soil in various crops such as, chickpea, Bajra, capsicum, Blackgram, Brinjal, tomato, potato, Clsuter Bean and lablab beans. Also jeevamruth is said to improve the beneficial microbes in soil rhizosphere and phyllosphere (Rathore*et al*., 2023: Deepika*et al*., 2024: Maheswari*et al*., 2015: Chaitanya*et al*., 2021: and Hiremath*et al*., 2023). Vermiwash has inhibited 100 % mycelial inhibition at 5% concentration, hence it can be best alternative for chemical management upon combine with other IDM practices, also it said to improve the plant vigour index (Jandaik*et al*., 2015). Our results were supported by previous reports, where antifungal activities were reported against various phytopathogens (Kumar *et al*., 2010; Jandaik *et al*., 2015).

Conclusion

Managing rice sheath rot caused by *Sarocladium oryzae* in organic agriculture presents significant challenges. To address these challenges, integrated disease management practices are essential. Based on the results obtained in this study, the most effective medicinal plant extract was identified as Sadabahar (*Catharanthus roseus*), which exhibited the highest inhibition of mycelial growth at both tested concentrations. Similarly, Jeevamrut was the most effective organic product, demonstrating complete inhibition of fungal growth at both 2% and 5% concentrations. These findings suggest that the integration of medicinal plant extracts and organic products into sustainable agricultural practices can provide eco-friendly alternatives to synthetic fungicides. The use of such alternatives can reduce environmental contamination and support the long-term sustainability of rice production. Future research should focus on field trials to validate these results and explore the compatibility of these natural products with other components of integrated pest management systems.

Table 1: *In vitro* effect of medicinal plant extracts against *S. oryzae* at different concentrations

Sl No.	Medicinal plant extracts		5%		10%	
			G*	I	G	I
1	Sadabahar	*Catharanthu sroseus* (L.) G.Don	15.00	74.13	12.00	79.31
2	Kalmegh	*Andrographis paniculate* (Burm.f.) Nees	22.50	61.20	18.00	68.96
3	Pippali	*Piper longum* L.	25.00	56.89	23.00	60.34
4	Tulsi	*Ocimum sanctum* L.	16.00	72.41	13.00	77.58
5	Brahmi	*Bacopa monnieri* (L.) Pennell	20.00	65.51	14.00	75.86
6	Mandukaparni	*Centella asiatica* (L.) Urban	30.00	48.25	21.00	63.79
7	Control	-	58.00	-	58.00	-

Factors	SEm (±)	C.D. (P=0.05)
Medicinal plant extracts (A)	1.66	3.42
Concentration (B)	0.88	1.83
Interaction (A X B)	2.35	4.82

Note: *Average of three replications; G- Colony growth (mm); I- Percent growth inhibition.

Table 2: *In vitro* effect of organic products against *S. oryzae* at different concentrations

Sl No.	Organic products	2%		5%	
		G*	I	G	I
1	Jeevamrut	0.00	100.00	0.00	100.00
2	Panchagavya	21.00	61.11	18.00	66.66
3	Vermivash	16.00	70.37	0.00	100.00
4	Control	54.00	-	54.00	-
Factors		**SEm (±)**		**C.D. (P=0.05)**	
Organic products (A)		0.84		2.55	
Concentration (B)		0.59		1.80	
Interaction (A X B)		1.19		3.61	

Note: *Average of three replications; G- Colony growth (mm); I- Percent growth inhibition

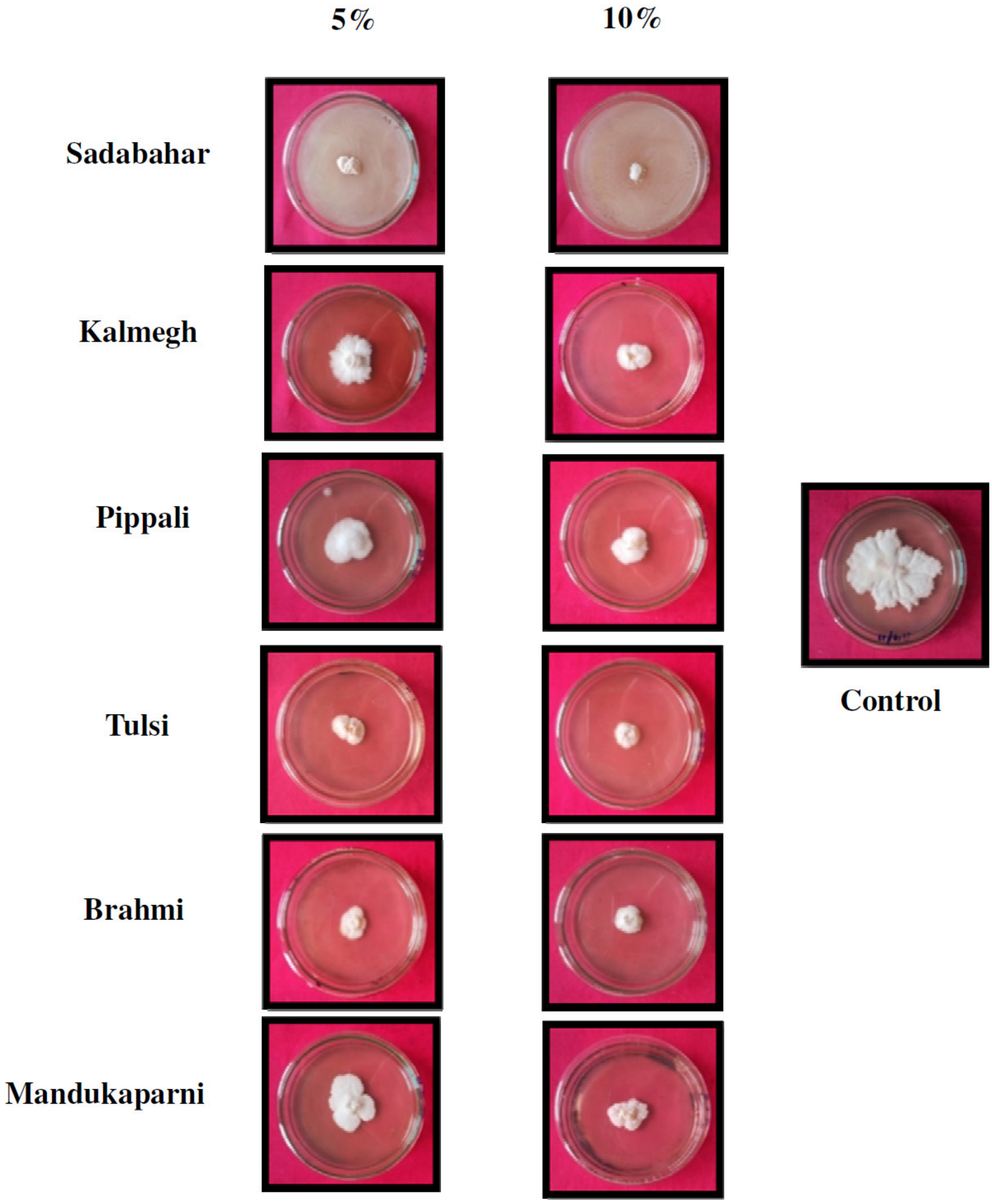

Plate I: *In vitro* effect of medicinal plant extracts against *Sarocladium oryzae* at different concentrations

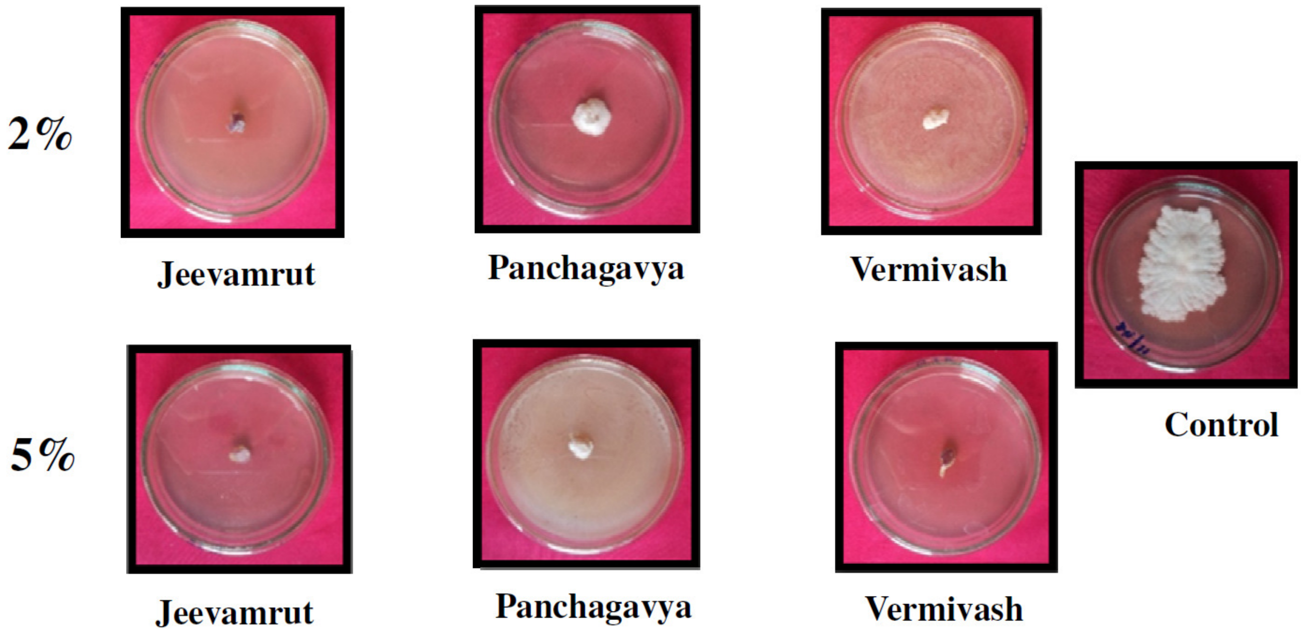

Plate II: *In vitro* effect of organic products against *Sarocladium oryzae* at different concentrations

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Conflict of interest

The authors have no competing interests to declare.

Ethical statement

This article does not contain any studies involving humans/animals/plants that need approval from ethical committee. None of the flora and fauna utilised in current study belongs to endangered species.

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