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Effect of powdered *Moringa oleifera* seeds on the characteristics of surface water

KEYWORDS: *Moringa oleifera* seed, Natural coagulant, Water treatment, Antimicrobial activity

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ABSTRACT *Moringa oleifera tree is indigenous and abundant in India; its seed powder is known to be used as an effective natural coagulant for purification of water. The high cost of treated water makes people live in the rural communities to resort to readily available water sources which are normally in poor quality and expose them to water-borne diseases. Present study deals with the application of M. oleifera seed powder extracted from the matured-dried seeds as a natural absorbent and antimicrobial agent for treatment of surface water for drinking purpose. During this study, water samples from three different rivers were collected and treated with various doses of Moringa seed powder viz, 50, 100, 150 and 200mg/L to understand the efficacy of M. oleifera seed powder on the clarification of raw water. It was observed that in post-treated water samples, most of parameters like turbidity, BOD, TDS, total alkalinity, total hardness, chlorides, sulphate and standard plate count (SPC) were significantly reduced with increased doses of seed powder compared to control or untreated samples. Based on the results, this approach of adopting low cost M. oleifera seed powder as a water purifying agent can be recommended as an eco-friendly, non-toxic simple water treatment system for purification of surface water, where rural and peri-urban people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water.*

Introduction

Highly industrialized and developing countries depend substantially on large-scale industrialization to boost their global economic competitiveness. Concurrently, tremendous economic growth is spurred by robust manufacturing industries, which generate significant quantities of organic, inorganic and metal contaminants. This increases the Influx of anthropogenic-based contaminants into earth's environment, particularly into the surface and ground water resources. The implications of these contaminants in terms of public, ecological and environmental health have been well documented. Unfortunately, the detrimental effects of these contaminants are more apparent and observable in developing countries due to their less stringent environmental regulations and difficulty in constructing, operating and maintaining proper water or wastewater treatment systems because of high fixed costs, especially in the case of rural areas (Montgomery et al., 2007).

Due to the lack of proper water treatment systems in rural or unprivileged areas, the best and immediate option is to use simple and relatively cost-effective point-of-use technologies (POU) such as coagulation (Miller et al., 2008). The process of coagulation is applied to remove dissolved solids and turbidity from water by using conventional chemical-based coagulants like alum ($AlCl_3$), ferric chloride ($FeCl_3$) and polyaluminium chloride (PAC) in treatment of surface, ground and industrial waste water. The effectiveness of these chemicals as coagulants was well recognized (Edzwald et al., 1993; Kang et al., 2003), but their usage was associated with certain disadvantages like ineffectiveness in low-temperature water (Haaroff), relatively high procurement costs, detrimental effects on human health, production of large sludge volumes and they also significantly affect the pH of treated water.

It was evident that aluminum-based coagulants led to the development of Alzheimer's disease in human beings (Flaten et al., 2001). Therefore, it is desirable to substitute these chemical coagulants with plant-based coagulants to counteract the aforementioned drawbacks. The main advantages of opting plant-based natural coagulants are ; they are cost effective, unlikely to alter the pH of treated water and highly biodegradable. In the age of depletion of natural resources, climate change and wide spread environmental degradation; application of natural coagulants is a vital effort in line with global sustainable development initiatives.

M. oleifera (drumstick tree), is a tropical plant found in India, Asia, Sub-Saharan Africa and Latin America (Sanghi et al., 2002; Huang et al.,1996). Its seeds found to contain an edible oil and water soluble substance (Ndabigengesere et al. 1995) which is amiably the most studied natural coagulant by the environmental scientific community. Most frequently, this plant is used as a source of food and medicine within less-developed communities. Anwar and Bhanger in 2003 reported multiple uses of this plant and almost every part of this plant system can be used for different beneficial purposes. Based on the further research, it was also reported that this can be used in low-cost POU water treatment technology (Madsen et al., 1987; Olsen et al., 1987; Muyibi 1995; Ghebremichael et al. 2005; Kwaambwa and Maikokera 2007; Santos et al. 2009; García-Fayos et al. 2010).

The basic coagulation mechanisms of *M. oleifera* applied for turbid water treatment were reported by Ndabigengesere et al. (1995) for the first time and it essentially sparked widespread interests among environmental scientific community. It is suggested that active coagulating agents in *M. oleifera* are dimeric cationic proteins, with molecular mass of 12–14 kDa and isoelectric point (pI) between 10 and 11. The main coagulation mechanism involved is adsorption and charge neutralization. In many cases, impurity particles are nega-

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tively charged and cationic polyelectrolytes are the most efficient coagulants, which bodes well for the usage of *Moringa* as a coagulating agent for water treatment process. It is well-established that strong adsorption in these systems is due to electrostatic interaction which is responsible for neutralization of the particle surface and charge reversal (Bolto and Gregory 2007). All these technical factors ultimately attract the interest of the scientific community to continue research on *Moringa* to treat a wide spectrum of turbid waters or even industrial wastewaters. According to Okuda et al. (2001) coagulating effect of *M. oleifera* extracts can be significantly enhanced with addition of bivalent cations (e.g. Ca2+ and Mg2+) in water treatment process.

The present study deals with the application of *M. oleifera* seed powder as a natural coagulant in treatment of different river water samples. The efficacy of *M. oleifera* seed powder as potent a coagulant was analyzed and its optimum concentration to reduce turbidity, BOD, alkalinity, hardness, TDS, chloride, sulphate and SPC of water samples was determined.

Materials and Methods

Collection of water sample

Water samples used for this study were collected aseptically from three different rivers nearer to Desapatrunipalem village of Visakhapatnam district according to the method mentioned by Cheesbrough, M. (2006). New plastic containers pre-sterilized with alcohol were used for sample collection. Samples were analyzed immediately in laboratory.

Collection of seeds and preparation of *M. oleifera* seed power:

Seeds of *M. oleifera* used in this study were collected from a healthy tree and were de-shelled and the endocarps were air dried at ambient temperatures (23 to 25°C) for a period of five-seven days before milling. Direct sunlight was avoided to prevent degradation of some of the plant photochemicals or antimicrobial constituents. The dried kernels were pulverized using electric blender to obtain powder. The powder was then sieved with a plastic strainer of small pore size to obtain fine powder. The fine powder obtained was stored in a sterile air-tight container in a dark place to prevent oxidation and for further use.

Treatment of water samples using *M. oleifera* seedpowder:

One liter of each water sample was transferred into three clean plastic white containers pre-sterilized with alcohol. Different concentrations of *M. oleifera* seed powder (50 to 200mg/L) was added to the water samples contained in the plastic containers. The mixture (water and *Moringa* seed powder) was stirred rapidly for 60 seconds and then slowly for 2 minutes. The treated water samples were then allowed to stand undisturbed for 24 h after which the top layer was decanted and 100mL was collected for subsequent microbial and physicochemical analysis.

Determination of turbidity:

The turbidity of the water sample was determined using Digital photocolorimeter (312 E, India). Initially, the machine was switched on and calibrated with distilled water to read further water samples. 5 mL of the water sample was poured into a cuvette holder with the vertical line on the cuvette aligning with the horizontal mark on the instrument. The value of the turbidity was read on the crystal liquid display (CLD) with indication of ready signal on the screen.

Measurement of Biochemical oxygen demand:

BOD measurements were done by using a dissolved oxygen (DO) meter. About 1 liter of diluted water sample was prepared by adding 1 mL of phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solution into 996 mL distilled water. About 10 mL of water sample prior to and after treatment was transferred into each BOD bottle. Then, 300 mL of diluted water was added into the BOD bottle. Besides that, the control was prepared from 300 mL diluted water in BOD bottle. The DO was measured for all samples using DO meter. After that, the diluted water was added to the flared mouth of the bottle and covered with aluminum foil to prevent evaporation of the solution. All bottles were put into the BOD incubator for 5 days at 20°C. The DO value was measured after 5 days (Suhartini et al., 2013).

Determination of total dissolved solids (TDS):

This parameter was determined using a mulitmeter analyzer that can inter-change to read different parameters when the 'mode' button is pressed. The instrument was switched on and calibrated with distilled water. Samples (around 5 mL) to be determined were poured into a test tube, then electrode of the instrument was inserted into the test tube and the mode button was pressed for reading each of the parameter. The values were read from the crystal liquid display (CLD) as the instrument indicates ready signal.

Determination of alkalinity:

The alkalinity of water sample can be determined by titrating it with Sulphuric acid of known values of pH, volume and concentrations. Based on stoichiometry of the reaction and number of moles of Sulphuric acid needed to reach the end point, the concentration of alkalinity in water was calculated.

Determination of total hardness:

The total quantity of Ca^{2+} and Mg^{2+} ions dissolved in water sample can be measured by using EDTA reagent. Thus, the total hardness of water sample can be estimated by titration with a standard solution of EDTA.

Results:

The purification potential of *M. oleifera* seed powder was tested by analyzing the physicochemical characteristics and microbiological quality of river water samples. The mean values of various physicochemical parameters and microbial counts corresponding to three different water sources before and after treatment with 50 to 200 mg/L concentrations of *M. oleifera* seed powder were represented in table.1. The mean values of physicochemical parameters before treatment of water samples (S_1, S_2 and S_3) are as follows: Turbidity, 14.4 ± 0.02, 8.6 ± 0.04 and 13.3 ± 0.03 NTU; BOD, 7.8 ± 0.00, 6.9 ± 0.01 and 7.2 ± 0.01 mg/L; TDS, 614 ± 0.57, 108 ± 0.56 and 610 ± 0.54 mg/L; Total alkalinity, 340 ± 0.17, 240 ± 0.15 and 320 ± 0.17 mg/L; Total hardness, 660 ± 0.05, 270 ± 0.03 and 580 ± 0.07 mg/L; Chloride, 100 ± 0.57, 105 ± 0.55 and 118 ± 0.53 mg/L; Sulphate 140 ± 0.73, 80 ± 0.75 and 120 ± 0.71mg/L respectively. Similarly, the mean values of physicochemical parameters after treatment of the water samples with the 50-200 mg/L concentrations of *M. oleifera* seed powder ranged as

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follows: Turbidity (Fig.1), 10.76 ± 0.05 to 2.86 ± 0.04 NTU for the first water sample (S_1) , 6.55 ± 0.04 to 2.38 ± 0.05 NTU for the second sample ($S_{2)}$ and 10.22 ± 0.05 to 2.60 ± 0.02 NTU for the third water sample (S_3); BOD (Fig.2), 5.42 ± 0.32 to 3.0 ± 0.05mg/L for the first sample (S_1), 4.86 ± 0.28 to 2.70 ± 0.04mg/L for the second sample ($S_{2)}$ and 4.98 ± 0.30 to 2.96 ± 0.05 mg/L for the third water sample (S_3); TDS (Fig.3), 521 ± 0.25 to 260 ± 0.05 mg/L for the first sample (S_1), 106 ± 0.20 to 99 ± 0.01 mg/L for the second sample (S_2) and 519 ± 0.22 to 248 ± 0.05 mg/L for the third water sample (S_3); Total alkalinity (Fig.4), 290 ± 0.28 to 186 ± 0.06 mg/L for the first water sample (S_1), 195 ± 0.17 to 73 ± 0.03 mg/L for the second sample (S_2) and 280 ± 0.25 to 160 ± 0.05 mg/L for the third water sample (S_3); Total hardness (Fig. 5), 600 ± 0.57 to 310 ± 0.04 mg/L for the first sample (S_1), 230 ± 0.55 to 111 ± 0.01 mg/L for the second sample (S_2) and 535 ± 0.56 to 275 ± 0.03 mg/L for the third water sample (S_3) ; Chloride (Fig. 6), 90 ± 0.85 to 68 ± 0.57 mg/L for the first water sample (S_1), 96 ± 0.81 to 65 ± 0.55 mg/L for the second sample (S_2) and 106 ± 0.83 to 72 ± 0.57 mg/L for the third water sample (S_3) ; Sulphate (Fig. 7), 130 ± 0.55 to 80 ± 0.05 mg/L for the first sample (S_1), 72 ± 0.05 to 52 ± 0.01 mg/L for the second sample (S_2) and 133 ± 0.57 to 87 ± 0.03 mg/L for the third water sample (S_3) respectively. There was reduction in the values of parameters after treatment with *M. oleifera* seed powder.

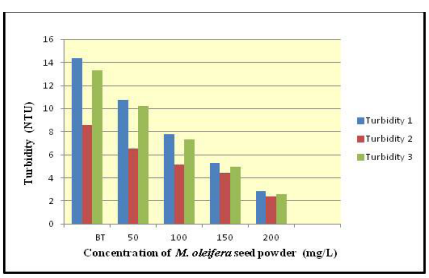

Fig. 1 Effect of *M. olifera* seed powder as a coagulant at varying concentrations on turbidity of river water.

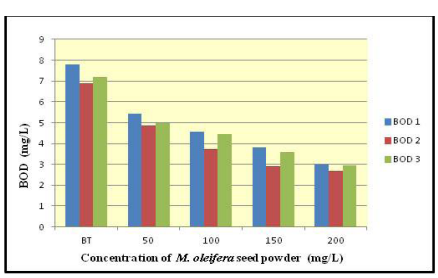

Fig.2 Analysis of BOD levels in river water before and after treatment with varying concentrations of *M. oleifera* seed powder.

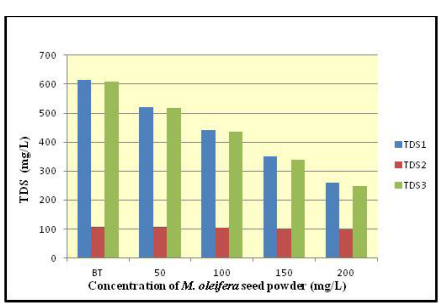

Fig. 3. Total dissolved solids of river water before and after treatment with different concentrations of *M. oleifera* seed powder.

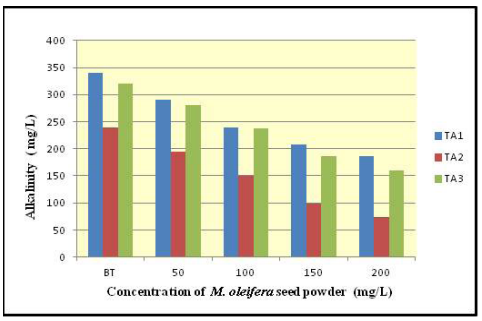

Fig. 4 Analysis of Total Alkanity in river water at different concentrations of *M. oleifera* seed powder

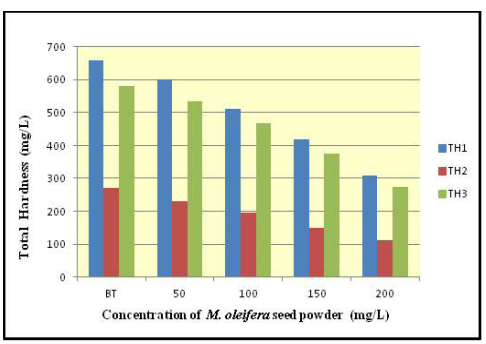

Fig.5 Study of Total hardness of river water before and after treatment with different concentrations of *M. olifera* seed powder.

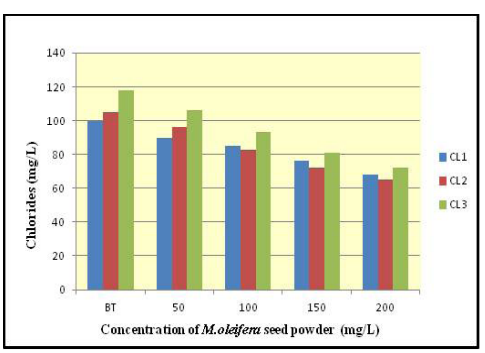

Fig.6 Chloride concentration of river water before and after treatment with *M. olifera* seed powder.

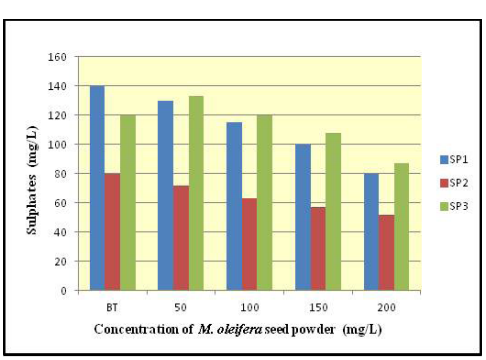

Fig. 7 Concentration of sulphates in river water before and after treatment with *M. olifera seed powder*

Comparatively, the 50 mg/L concentration had the highest values while the 200 mg/L had the least values in all the

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parameters irrespective of control or untreated water samples. The mean microbial count for three different water samples (S_1, S_2 and S_3) before treatment revealed that the Standard plate count was 6.3 × 10^6 ± 0.57, 6 × $10^{5\pm}$ 0.55 and 5.7 × 10^6 ± 0.57 SPC/100 mL respectively. After treatment with 50–200 mg/L concentrations of *M. oleifera* seed powder the mean counts were as follows: 3.0 × 10^6 ± 0.57 to 0.2 × 10^6 ± 0.57 for the first sample (S_1), 2.9 × 10^6 ± 0.53 to 0.1 × 10^6 ± 0.53 for the second sample (S_2) and 2.8 × 10^6 ± 0.57 to 0.1 × 10^6 ± 0.55 for the third sample (S_3) respectively.

Parameter	Before Treatment			After treatment with 50 mg/L of *M. oleifera* seed powder			After treatment with 100 mg/L of *M. oleifera* seed powder			After treatment with 150 mg/L of *M. oleifera* seed powder			After treatment with 200 mg/L of *M. oleifera* seed powder			WHO Values
	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3	
Turbidity (NTU)	14.4 ± 0.02	8.6 ± 0.04	13.3 ± 0.03	10.76 ± 0.05	6.55 ± 0.04	10.22 ± 0.05	7.76 ± 0.03	5.12 ± 0.03	7.32± 0.03	5.26 ± 0.02	4.44± 0.02	4.92 ± 0.01	2.86 ± 0.04	2.38 ± 0.05	2.60 ± 0.02	5
BOD (mg/L)	7.8 ± 0.00	6.9 ± 0.01	7.2 ± 0.01	5.42± 0.32	4.86 ± 0.28	4.98 ± 0.30	4.57 ± 0.57	3.72± 0.50	4.46 ± 0.55	3.81 ± 0.27	2.9 ± 0.1	3.58 ± 0.25	3.0 ± 0.05	2.70 ± 0.04	2.96 ± 0.05	1
TDS (mg/L)	614 ± 0.57	108 ± 0.56	610 ± 0.54	521± 0.25	106 ± 0.20	519 ± 0.22	440 ± 0.03	103 ± 0.02	437 ± 0.03	350± 0.11	100 ± 0.05	338 ± 0.15	260 ± 0.05	99 ± 0.01	248 ± 0.05	500
Total Alkalinity (mg/L)	340 ± 0.17	240 ± 0.15	320 ± 0.17	290 ± 0.28	195 ± 0.17	280 ± 0.25	240 ± 0.57	150± 0.15	237 ± 0.55	208 ± 0.28	98 ± 0.15	186 ± 0.27	186 ± 0.06	73 ± 0.03	160 ± 0.05	200
Total Hardness (mg/L)	660 ± 0.05	270 ± 0.03	580 ± 0.07	600 ± 0.57	230 ± 0.55	535 ± 0.56	510 ± 0.28	195 ± 0.10	468 ± 0.27	420 ± 0.05	148± 0.03	375± 0.05	310± 0.04	111± 0.01	275 ± 0.03	500
Chloride (mg/L)	100 ± 0.57	105 ± 0.55	118 ± 0.53	90 ± 0.85	96 ± 0.81	106 ± 0.83	85 ± 0.75	83 ± 0.71	93 ± 0.75	76 ± 0.28	72 ± 0.25	81 ± 0.23	68 ± 0.57	65 ± 0.55	72 ± 0.57	250
Sulphate (mg/L)	140 ± 0.73	80 ± 075	120 ± 0.71	130± 0.55	72 ± 0.05	133 ± 0.57	115 ± 0.28	63 ± 0.04	120± 0.25	100 ± 0.05	57 ± 0.02	108 ± 0.07	80 ± 0.05	52 ± 0.01	87 ± 0.03	400
SPC/100mL	6.3 × 10^6 ± 0.57	6.0 × 10^6 ± 0.55	5.7 × 10^6 ± 0.57	3.0 × 10^6 ± 0.56	2.9 × 10^6 ± 0.53	2.8 × 10^6 ± 0.57	1.8 × 10^6 ± 0.75	1.7× 10^6 ± 0.71	1.5× 10^6 ± 0.73	1.0 × 10^6 ± 0.28	0.8 × 10^6 ± 0.27	0.6 × 10^6 ± 0.25	0.2 × 10^6 ± 0.57	0.1 × 10^6 ± 0.53	0.1 × 10^6 ± 0.55	1 × 10^6

Table. 1 The mean values of the physicochemical parameters of river water samples before and after treatment with different concentrations of *M. oleifera* seed powder

Discussion:

Water is one of the most essential natural resource and plays an active role in all vital processes of our body (Kawther et al., 2007) and availability of good quality water is an indispensable feature for preventing diseases and improving quality of life. So use of safe, effective and economic water purifying agents has always been a topic of major concern in water treatment process. The use of plant materials as natural coagulants for water clarification process has become a common practice since ancient times (Rao, 2005) and garnered flourishing interests from researchers over the years due to their biodegradability and environmental friendly nature.

The present study reports remarkable coagulant activity of *M. oleifera* seed powder and revealed it as an effective purifying agent in clarification of surface waters. Earlier studies reported the non-toxic nature of *Moringa* (leaves& seeds) (Grabow et al., 1985) and recommended it to use as a coagulant in water purification process (Jahn et al., 1988) due to its low cost, high biodegradability, short shelf, safety to human health and environment compared to synthetic coagulants (Muyibi *et al.* 2002; Katayon *et al.* 2005; Katayon *et al.* 2006). *M. oleifera* seeds act as natural absorbent and antimicrobial agent as they contain 1% of active polyelectrolytes that neutralize the negatively charged colloid in impure waters. All these characteristics prompted us to use *M. oleifera* seed powder as a purifying agent in purification of river water and to evaluate its

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efficiency as a coagulant in treatment of water for drinking purpose.

When water samples from three separate sources were treated with various concentrations of *M. oleifera* seed powder, interestingly, it was observed that there was higher level of reduction in both the values of physico-chemical parameters and mean microbial count with increased concentrations of *M. oleifera* seed powder. There was clear difference between treated and untreated water samples in the exhibition of values for different parameters. Based on analysis, the values of turbidity, total alkalinity, BOD and TDS were reduced upto 80, 70, 61 and 60% in treated water samples. Microbial analysis of water samples revealed that there were drastic reductions in the microbial load after treatment with different concentrations of *M. oleifera* seed powder. On treatment, the rate of reduction increased with increased concentration of seed powder and this is in coordination with earlier reports (Mangale et al., 2012; Alo et al., 2012) where bacterial count was reduced with increased dose of *M. oleifera* powder. In addition, in earlier studies *M. oleifera* was reported as an effective natural coagulant in reducing the microbial count and values of physicochemical parameters in water purification process (Bichi et al., 2012; Mangale., 2012; Eze et al., 2014). In a recent investigation, Bapitista et al. (2015) have shown that saline coagulant of *M. oleifera* is an effective natural coagulant in purification of supply water. Based on the analysis, most of the physicochemical parameters after treatment were within the World Health Organization standards for drinking water quality. It is evident that powdered seed of *M. oleifera* can be used as potent coagulating and antimicrobial agent for effective purification of surface waters.

Conclusion:

This study has revealed that *M. oleifera* seed powder is effective in reducing turbidity, TDS, BOD, Total alkanity, Total hardness and microbial load of water and possess the potential for water purification by possessing coagulation, flocculation, antimicrobial and hardness removal properties. This eco-friendly and economic method of water treatment could be a promising technology to apply, especially in rural areas to provide safe and potable drinking water, where there are no facilities available for treatment of water for drinking purpose. In addition, sludge retained after treatment can be used as bio-fertilizer in agriculture.

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