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RESEARCH PAPER

Plant Based Biocoagulants from *Cucurbita pepo* and *Cicer arietinum* for Improving Water Quality

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ABSTRACT

Field pumpkin (*Cucurbita pepo*) and Chickpea (*Cicer arietinum*) seeds have been explored to improve water quality by reducing turbidity, BOD and attached pathogens. The seed components of these plants have high protein content with biocoagulant-flocculants properties which aids in floc formation and settling turbidity. Very few literature reports are available on the water treatment process of these two popular seeds. In this study, an attempt has been made to evaluate the biocoagulant performance capability of the seeds through the Jar test apparatus. The flocculation process was designed based on different water quality parameters like the seed dose, pH, total dissolved solids, turbidity, sludge volume and coliform count. Simulated water was generated for turbidity reduction studies and coliform count was performed in real water samples. The sludge produced was also much reduced compared to untreated water. Turbidity reduction achieved after 8 h at pH 6.0 was within 91-92% with significant reduction in BOD and coliform count. Our results highlight the feasibility of the biocoagulant property of both *C. pepo* and *C. arietinum* seeds. Further combinatorial treatment of both the seeds could be investigated for their biocoagulant properties as suitable alternatives for chemical coagulants in water purification.

HIGHLIGHTS

- Plant derived Biocoagulants have the potential to improve water quality by turbidity reduction.
- Biocoagulants exhibited antibacterial activity against pathogenic coliforms.
- They are sustainable and cheap alternatives for Chemical coagulants.

Keywords: *Cucurbita pepo, Cicer arietinum*, Turbidity, Coliform, pH

Plant seed proteins are considered as an abundant source of cheap proteins but sometimes discarded as vegetable waste. Apart from the nutritional contribution to humans and animals, these seeds have excellent biocoagulant properties used for obtaining clean water by removing and settling water turbidity particles (Zaman *et al.* 2021; Ray *et al.* 2023). Many literatures have highlighted the use of indigenous plant parts for improving water quality in rural communities of many countries (Konkobo *et al.* 2023). The inherent properties of the plant parts used along with coagulation-flocculation techniques can make the water potable (Khettaf *et al.* 2021). While considering this study two plant seeds were chosen which are either discarded due to their wide consumption in India (*Cucurbita pepo*) or popularly consumed as good protein source (*Cicer arietinum*).

Pumpkin or *Cucurbita* belongs to the Cucurbitaceae family, and widely consumed vegetable globally (Vinayashree and Vasu, 2021). Pumpkin seeds are rich in minerals like Zinc and utilized as culinary ingredient in many recipes. The seed contains 35.18% of proteins with high propensity of Arginine and Glycine (Vinayashree and Vasu, 2021) and also essential fatty acids and dietary fibres (Hussain *et al.*

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2021; Hosen *et al.* 2021). Apart from the nutritional benefit the seeds show efficacy in reducing the turbidity of water due to active bioflocculant components present in the seed (Moniruzzaman *et al.* 2022). Krstić *et al.* (2023) observed antibacterial activity of pumpkin extracts on Gram positive bacteria, *S. aureus* and *S. epidermidis*. Chemically modified *Cucurbita moschata* seed has been used as adsorbent for the abstraction of metal ions like Cu (II) and Ni (II) from aqueous solution (Khan and Rao, 2017). *Cicer arietinum* is commonly referred as Chickpea is a leguminous plant of Fabaceae family. The chemical composition of the seed shows good protein content (18%), rich in lysine and arginine amino acids (Amod *et al.* 2021). Chickpea offers a cheap water treatment process with ability to increase the formation of floc by interacting with the negatively charged dissolved particles like silt or clay causing turbidity in water (Jaramilloa and Epalzab, 2019).

Polysaccharides derived from plant seeds, leaves and fruits peels, acts as coagulant-flocculant agents for remediating various effluents like tannery, oil, paper and leachate from landfills (Desta and Bote, 2021; Zainal *et al.* 2021; Shabanizadeh and Taghavijeloudar, 2023). Even this flocculant agents were blended with chemical flocculants as coagulant aid to improve efficiency in cleaning up the waste water (Kurniawan *et al.* 2020). To prepare plant seeds for extracting proteins, they are usually washed with distilled water and sun dried in open or inside hot air oven. This helps in complete removal of moisture content from the seeds without biocharring them or impairing the biocoagulant property (Kurniawan *et al.* 2020). Grinding and sieving are also important prerequisites to bring uniformity in the biocoagulant particlulate size (Kurniawan *et al.* 2020). According to Yimer and Dame (2021), *Carica papaya* seed powder of 250 mesh size exhibited better performance than 150 mesh. The reason behind the result might be due to more contact of dissolved components in water with the biocoagulant. Both the seeds are obtained easily, economical, biodegradable, and also exhibits antimicrobial activity. Presence of phytochemicals like phenols and flavonoids in the plant products displays antimicrobial properties along with coagulation capabilities (Virk *et al.* 2019; Taiwo *et al.* 2020). Tallying with turbidity removal potential, pathogenic microbes present in water sources were also excluded by the natural treatment process. Another process parameter that contributes to improved water treatment is the removal of oil or lipid components from the seeds before application as it reduces the dissolved organic components and improves coagulation-flocculation (Andressa *et al.* 2017). Use of natural coagulants reduces the need for chemical coagulants in water treatment which are considered as health hazards in long term use (Fouad *et al.* 2021). The sludge generated after chemical treatment cannot be used directly in the ecosystem and needs further re-treatment before use (Kurniawan *et al.* 2020; Ray *et al.* 2023; Shabanizadeh and Taghavijeloudar, 2023). Thus, current need is to focus on the replacement of synthetic chemicals with natural green biocoagulants.

MATERIALS AND METHODS

Extraction of coagulating components from seed and protein determination

The Field pumpkin and Chickpea seeds were procured from local market and they were cleaned, washed and dried prior to use (Fig. 1). The powdered seeds were sieved for use in the biocoagulant assay. To remove the turbidity interfering components (seed oil) from the seed, ethanol treatment was done for 24 h to defat the seed. The seed cake collected after filtration was oven-dried and considered as the final biocoagulant powder used for treatment of water turbidity removal. Bradford method was used to determine the protein content of the seeds pre and post defatting using BSA as standard. In the process, samples were stirred occasionally for 1 h, centrifuged at 15,000 rpm for 10 min and supernatant taken for protein estimation.

Preparation of artificial turbid water for experimentation

In order to make the artificial turbid stock water, five grams of powdered Kaolin Clay powder was mixed with 1 L of distilled water. Prior to start of the experiment the solution was left for 24 h to saturate the clay. High or low turbidity water was derived after diluting the stock solution with desired amount of distilled water for the Jar test experiments (Fig. 2).

Fig. 1: Field pumpkin (*Cucurbita pepo*) and Chickpea (*Cicer arietinum*) seeds

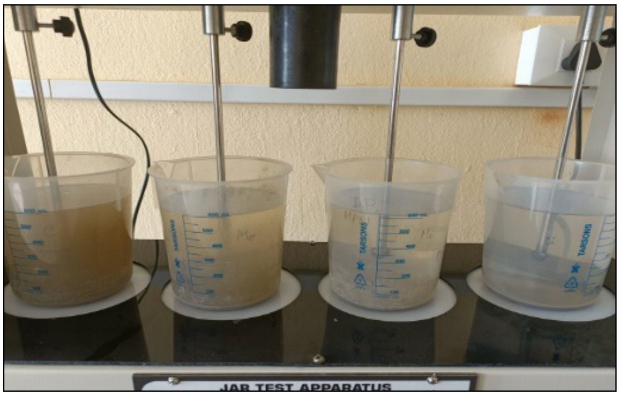

Fig. 2: Jar test apparatus for treatment of turbid water by biocoagulants

Experimental Design

The Jar test apparatus with four paddles were employed to analyse the coagulating nature of the defatted seeds. In 1 L of the simulated water samples, different dosages of the biocoagulant (0.10 g, 0.50 g, 1.0 g, 1.5 g, 2.0 g and 2.5 g) were added to each container and stirred at initially high (20 min) and then low speed (15 min). The rapid mixing disperses the biocoagulant through the turbidity components and interacts with them while slow mixing initiates floc formation and settlement. After settling time of half an hour, the reduction in turbidity in control and experimental sets were observed at UV-Vis spectrophotometer at 500 nm. The optimum dose of the biocoagulants necessary for water turbidity removal was calculated as follows:

$$\%\ Turbidity\ removal = \{(C_i - C_f)/C_i\} \times 100,$$

where C_i and C_f are initial and final concentrations of the simulated water respectively.

Determination of process optimization parameters

Total Solids (TS) was determined by calculating individually the Total Suspended Solids (TSS) and Total Dissolved Solids (TDS). For TS, known volume of sample water was passed through dry filter (W_1). The wet filter paper carrying the suspended unfilterable solids was air dried in oven and weighed again (W_2).

Hence,

$$\text{TSS (mg/L)} = [(W_2 - W_1) \times 1000 \times 1000] / \text{volume of sample (mg/L) (APHA, 1975).}$$

For TDS, the known volume of water sample (filtrate) was poured in the evaporating dish and dried in oven (W_3). Initially, the empty dish was weighed and taken as W_4 (APHA, 1975).

Hence,

$$TDS (mg/L) = [(W_3 - W_4) \times 1000 \times 1000] / \text{volume of sample (mg/L)}.$$

and

$$\text{Total Solids (TS)} = TSS + TDS \text{ (mg/L)}.$$

The turbidity measurement was done in UV-Vis spectrophotometer at 500 nm (OD500 nm) following the protocol of Taiwo *et al.* (2020). Biochemical oxygen Demand (BOD) was done following standard protocol of APHA, 1975.

The sludge generated is calculated in terms of Sludge Volume Index (SVI), measured as follows

$$SVI (mL/g) = \text{Settled sludge volume (mL/L)/ Suspended solids (mg / L)} \times 1000 \text{ (APHA, 1975)}$$

Post water treatment with seed coagulants, the sludge contents of the Jar test were transferred carefully to a graduated measuring cylinder. After one hour of settling, the volume occupied by settling sludge and suspended solids in mixed liquor was observed and noted.

The reduction in bacterial load was observed within 2 hrs post treatment of water with optimum seed dosages of *Cucurbita* and *Cicer*. The supernatant were collected, diluted and used for enumeration by growing them in Eosin-Methylene Blue (EMB) plates, selective media for coliforms. The results were visualized as Colony Forming Units (CFUs) per 100 mL of the water sample tested.

RESULTS AND DISCUSSION

Effect of biocoagulant concentrations on turbidity reduction

In coagulation/flocculation studies, determining the optimum dose of the prepared seed powder is necessary to evaluate its performance in making the water potable or suitable for irrigation purposes. Doses less than the optimum measure will generate less floc while higher doses may increase self-turbidity of the water by re-stabilization of the flocs. The optimum coagulant dosage is considered as that concentration after which the turbidity begins to surge as more coagulant is added. Moreover, defatting process ensures removal of oil contained in the seeds which may inhibit surface contact with the turbidity causing components present in water reducing floc formation and hence decreasing coagulation-flocculation process. Defatted seeds exhibited better coagulation potential than raw seed components (Andressa *et al.* 2017). Fig. 3 shows the optimum dosage of 0.50 g/L for *Cicer* and 0.1 g/L for *Cucurbita*, where 85-86 % reduction in turbidity was observed after 8 hrs. WHO guidelines specify that turbidity should be less than 5 NTU for potable water which corresponds to 0.2 (OD value) measured in spectrophotometer at 500 nm (Yimer and Dame, 2021).

The biocoagulant-flocculant capacity of the seeds was highly comparable with the conventional chemical coagulants available in the market after settling time of 24 h. Rai *et al.* (2023), also observed that *Trigonella* seed exhibited potential attractive properties for turbidity removal with 96.47%

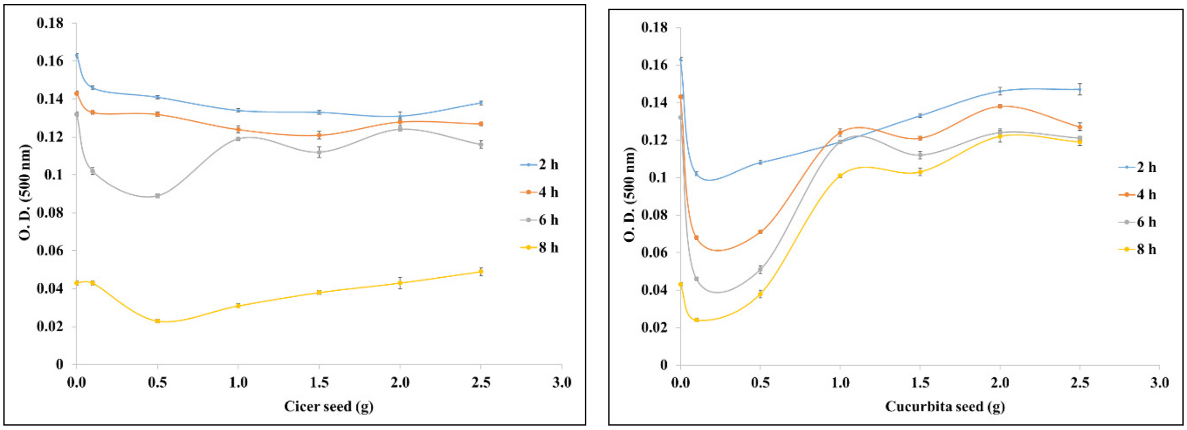

Fig. 3: Changes in turbidity with variation in dose of biocoagulants

turbidity reduction from surface water for a contact time of 24 h. Jaramilloa and Epalzab (2019), obtained the highest percentage of turbidity removal using 90 mg/L of *Cicer* biocoagulant, whereas Desta and Bote (2021), observed 98-99 % turbidity reduction with 0.2 g/L of *Moringa oleifera* seed powder. Asrafuzzaman *et al.* (2011), also achieved highest water clarity in both high (94.1%) and low turbid (60%) conditions with 100 mg/L of *Cicer* seed coagulant. In our study, the extent of turbidity removal progressed with increase in the contact time with the *Cicer* biocoagulant and removal of 76.47% was observed after 8 hrs. Similarly, 83.68% reduction in turbidity was observed after 8 hrs applying *Cucurbita* as biocoagulant. But the evaluated high doses of the coagulant with prolonged retention time of 24 h didn't improve the results but rather an increase in the turbidity trend was observed. Comparing both the coagulants, *Cicer* delivered a little better turbidity reduction compared to *Cucurbita* at 8 h but the biocoagulant concentration needed was less for *Cucurbita* (0.1 g/L) than *Cicer* (0.50 g/L) as evident from Fig. 3.

Effect of biocoagulants on turbidity removal in varied pH conditions

pH of water is a decisive factor of coagulation-flocculation processes. Each coagulant has an optimum coagulation contact time and optimum pH for highest turbidity removal from water. pH variation from 4.0-10.0 was taken into account by adding desired amount of acid or base. There was 78-92% reduction in turbidity when pH was varied from 4.0-6.0, in case of *Cucurbita*. In the same range performance of *Cicer* was also similar with 73-91% decline in turbidity. Whereas low activity was seen by the two biocoagulants when the pH was increased to highly alkaline conditions (pH 10.0). The reason might be that low pH generated more H^+ ions which accelerated the coagulation process. But, as the coagulant dosage increased there was no prominent reduction in turbidity, rather turbidity increased. This was due to more availability of organic components from seeds as the coagulant dosage increased which made the water more turbid. High alkaline conditions also gave no significant results. But again, very low pH of the water will hinder with the consumption, affect solubility of ions and make it unpalatable (Taiwo *et al.* 2020).

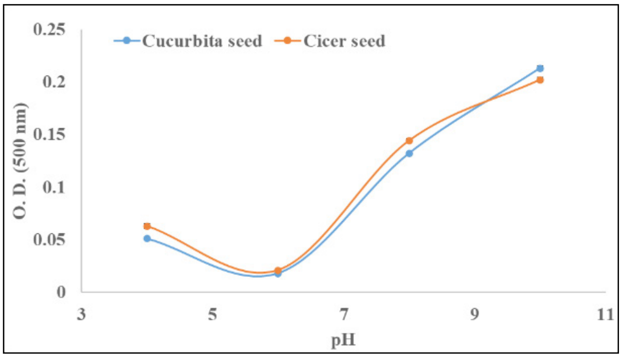

Fig. 4: Changes in turbidity with variation in pH

Effect of biocoagulants on Total Dissolved Solids (TDS)

Turbidity of water body indicates the transparency and is an optical property which is measured by comparing the light intensity scattered by the dissolved components present in water sample with a standard reference. Turbidity is caused by suspended solids and dissolved solids like organic matter, algae, silt, clay, pesticides, dyes, heavy metals, and others. Suspended solids are not filterable and held back by the filter paper whereas the dissolved particles pass through the filter and present in the filtrate. Our observation recorded 62.66%, and 47.34% reduction in TDS after treatment with *Cicer* and *Cucurbita* respectively. In contrast, Taiwo *et al.* (2020) reported slight elevated TDS after treatment with biocoagulants. According to water quality standards, maximum permissible limits for TDS are 200-500 mg/L which satisfies our concern by using these natural biocoagulants. Similarly, there was decline in Total Suspended Solids after treatment with *Cicer* and *Cucurbita* by 79%, and 60.71%, respectively (Table 1). This might be due to floc generation and removal by settling which helped in the removal of suspended solids.

Effect of biocoagulants on BOD

Biochemical oxygen demand (BOD) specifies the requirement of oxygen by the microbial species and indicates highly polluted water body. Bacteria consumes organic substance present to simpler forms utilizing oxygen for their cellular processes. After treatment with *Cicer* and *Cucurbita* biocoagulants there was 30.12% and 17.38 % reduction in BOD as evident from Table 1. Similar results were observed by Taiwo *et al.* (2020), where

Table 1: Changes in Physico-Chemical Parameters and Coliform Load after Treatment

Biocoagulants	TDS reduction (%)	TSS reduction (%)	BOD reduction (%)	Coliform load (cfu/100 mL)		Coliform reduction (%)
				Before treatment	After treatment	
Cicer arietinum	62.66	79.0	30.12	133	2	98.49
Cucurbita pepo	47.34	60.71	17.38	133	13	90.22

74.6% reduction in BOD value was observed using *Moringa* seed protein. BOD values of 10 mg/L indicate severe pollution whereas 3–5 mg/L is considered moderately clean water body. These values obey with drinking water quality standards of 2 mg/L or less (WHO, 2008).

Effect of biocoagulants on Sludge formation

Substantial difference in the sludge volume was noted between untreated water sample and biocoagulant treated ones. The sludge volume was compact and reduced due to the agglomerated and settable flocs produced when biocoagulants were used. Lower SVI indicates better settling performance of the sludge (Chethana *et al.* 2016). *Cicer* treated had SVI in the range of 33-36 mL/g compared to that of *Cucurbita* as 30-32 mL/g, which indicates good sludge stability. Whereas in untreated sample, the floc was huge and took longer time to settle. Moreover, the generated sludge is environment friendly and can be disposed of easily without further treatment procedures (Hadadi *et al.* 2022).

Effect of biocoagulants on Coliform reduction in real water sample

Contaminated water surface shows presence of coliform which are of faecal origin and mostly pathogenic causing diarrhoea and dysentery in humans. Treatment of real water with optimum dose of the biocoagulants did not exhibit any such antibacterial activity but with increasing dose concentration to 10 g/L showed considerable effect. Similar pattern was reported by Azeem *et al.* (2018) while working with papaya seeds. In this study, the total coliform count in real sample water (133 cfu/100 mL) was decreased after treatment with *Cicer* (98.49%) and *Cucurbita* (90.22%), respectively (Table 1). Comparable results were obtained by Virk *et al.* (2019), where 12.5 gm/L *Moringa* seed was used to obtain antibacterial effect against *E. coli*. Taiwa *et al.* (2020), observed purified 40 mg/L of *Moringa* protein was active in eliminating *Pseudomonas aeruginosa* and *Klebsiella* sp. The seed extracts of *Cucurbita maxima* also exhibited significant inhibition zone against *Staphylococcus aureus*, as reported by Moniruzzaman *et al.* (2022). Similar case was reported by Krstić, *et al*. (2023) where 1000 μg/mL of *Cucurbita* extracts were able to supress growth of Gram positive bacteria. The inhibitory effect was ascribed to the high phenol and flavonoid content of pumpkin seed (Mokhtar *et al.* 2021). Reports of Asrafuzzaman *et al*. (2011), also showed 90.47% removal of total coliform by *Cicer arietinum* in their study. Among the two biocoagulants tested, *Cicer* showed better antibacterial activity than *Cucurbita*. The reason could be the inherent properties of the seed composition like presence of tannins and alkaloids showing antibacterial activity or the suspended particles and settling flocs remove the attached microbes. Megersa *et al*., 2016 also suggested the disinfectant role of biocoagulants as combinatorial action of Flocculation process and antibacterial action. For long term storage of water, the biocoagulant performance may reduce the propagation capacity of the pathogens (*Konkobo et al.* 2023).

CONCLUSION

Cicer arietinum and *Cucurbita pepo*, exhibited excellent potentials for water clarity and thus could be harnessed in future as replacement of chemical coagulants or as coagulant aids. Turbidity reduction greater than 90% was achieved within 8 h of treatment with the biocoagulants. Reduction in coliform pathogen could also be seen at higher doses of the biocoagulant. This justifies the effectiveness of these seeds in the treatment of raw water by rural communities without depending on chemical coagulants. Seeds are easily available, eco-friendly and generates nontoxic sludge. Thus, further investigations are needed for their extraction and preservation of the biocoagulant-flocculant protein to increase their shelf-life and retain their efficacy as eco-friendly technology for water treatment.

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