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Evaluation of eco-friendly coagulant from *Trigonella foenum-graecum* seed

Chitteti Ramamurthy[1], Malige Uma Maheswari[1], Natarajan Selvaganabathy[1], Muthuvel Suresh Kumar[2], Venugopal Sujatha[3], Chinnasamy Thirunavukkarasu[1*]

[1]Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Puducherry, India
[2]Centre for Advanced Studies in Bioinformatics, School of Life Sciences, Pondicherry University, Puducherry, India
[3]Department of Chemistry, Periyar University, Salem, India
Email: [*]tchinnasamy@hotmail.com

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ABSTRACT

The ability of seed extracts of *Trigonella foenum-graecum (T. foenum-graecum)* and *Cuminum cyminum* (*C. cyminum*) to act as natural coagulants was tested using natural turbid water. Seed extracts were prepared using distilled water and NaCl (0.5 M and 1.0 M) solution. Only 1.0 M NaCl extract of *T. foenum-graecum* had coagulation capability and did not depend on pH values. Further it showed that natural coagulant obtained from *T. foenum-graecum* is temperature (up to 100˚C) and pH stable (pH 4.0 - 10.0). Extract of *C. cyminum* had very minimal (16 ± 2) coagulation property. The seed extract of *T. foenum-graecum* showed about 80% coagulation properties, where as the best known natural coagulants such as *Strychnos potatorum* and *Moringa oleifera*, and chemical coagulant such as $Al_2(SO_4)_3$ showed around 90%, 65% and 95% respectively, which are used as standards for the present study. When compared with pond water, *T. foenum-graecum* extract treated water shows decrease in alkalinity, turbidity, $KMnO_4$ demand and total coliform. This study reveals that seed extract of *T. foenum-graecum* can be used as natural water coagulant.

Keywords: Natural Coagulants; Water Purifier; *T. foenum-graecum*; Fenugreek

1. INTRODUCTION

Growing population, increased economic activity and industrialization have not only created an increased demand for fresh water but also resulted in severe misuse of natural resource. Water resources all over the world are threatened not only by over exploitation and poor management but also by ecological degradation. According to a survey conducted by United Nation Environment Programme (UNEP), 20% of world's population lacks access to safe drinking water and 50% lacks access to safe sanitation. Polluted water is estimated to affect the health of about 1200 million people and contributes to the death of 15 million children (per year) under the age of five [1].

The use of plant materials as natural coagulants to clarify turbidity of water is common practice since ancient times. Seeds of *Strychnos potatorum* (*S. potatorum*) and *Moringa oleifera* (*M. oleifera*) have shown promising result as the source of natural coagulant in the clarification of turbid water [2-4]. Direct filtration with *S. potatorum* seeds as coagulant appeared effective in clarifying turbid water [5]. This property is attributed due to the presence of polyelectrolytes, proteins, lipids, carbohydrates and alkaloids containing the -COOH and free -OH surface groups in the seed [6]. Among all plant materials investigated, seeds of *M. oleifera* were found to be one of the most effective sources of primary coagulant for water treatment [3].

T. foenum-graecum (Fenugreek) belongs to the family Leguminosae that grows predominantly in Asia, Northern Africa and the Middle East. Fenugreek seed contains 23% - 26% protein, 6% - 7% fat and 58% carbohydrates of which 25% is dietary fiber, saponins [7] and rich in flavonoids. Fenugreek has been widely used as a flavoring agent and in folk medicine. Several beneficial effects, such as appetite stimulation [8], anti-inflammatory, antipyretic [9], antimicrobial [10], antioxidant, antidiabetic [11], anticancer [12] and antiatherogenic properties [13] have been reported. *C. cyminum*, an aromatic plant from the family Umbelliferae is used as a flavoring and seasoning agent in foods [14,15]. To our knowledge no studies have been carried out to find out, whether seeds of *T. foenum-graecum* and *C. cyminum* can serve as water coagulant. Hence, this study was ventured to investigate the applicability of natural coagulants extracted from seeds

[*]Corresponding author.

of above plants that are abundantly available in Asia.

2. MATERIALS AND METHODS

2.1. Preparation of Seed Extracts and Collection of Turbid Water

Seeds of *T. foenum-graecum*, *C. cyminum*, *M. oleifera* and *S. potatorum* were collected locally near the city of Puducherry, India. The whole seeds of *T. foenum-graecum*, and *C. cyminum* were ground to fine powder using a laboratory mill. All ground materials were sieved through 0.4 mm membrane sieve and the fraction with particle size less than 0.4 mm was used in experiments. 10 g of prepared powder was suspended in 1 L of distilled water or NaCl (0.5 M and 1.0 M) solution and the suspension was stirred using a magnetic stirrer for 10 min to extract the coagulation active components. The seeds of *M. oleifera* and *S. potatorum* were made into fine pieces and soaked in water or NaCl for 1 hr, grinded in mortar and pestle, 1% suspension of *S. potatorum* and 5% suspension of *M. oleifera* were made with distilled water [3,4] or 1.0 M NaCl solution [2,16,17]. The suspension was then gravity filtered through a rugged filter paper. The filtered solutions, called extracts, were kept in refrigerator at 4˚C.

Water was collected from pond (stagnant rain water in red soil) near Pondicherry University, Puducherry, India, in the month of February 2010. The turbid pond water was left undisturbed for 48 hr to check for any initial spontaneous particle settlement. But the turbidity of the water did not change with increase in time.

2.2. Coagulation Test

Coagulation efficiency of each seed extract was determined by the jar test. The pond water (300 ml) was filled into the beakers (600 ml) and mixed at 200 rpm at constant room temperature (25˚C). Various doses of seed extracts were added into the beakers and mixed for 1 min. The mixing speed was then reduced to 80 rpm and kept for another 30 min. Then the suspensions were left for sedimentation. After 1 hr, 3 hr, 6 hr, 12 hr and 24 hr of sedimentation, clarified samples were collected from the top of the beakers, and turbidity was measured as TS (Turbidity of Sample). The turbidity in the control was defined as TB (Turbidity of Blank). Turbidity was measured using a turbidimeter (TURB 550 IR) and it was expressed in nephelometric turbidity units (NTU). Coagulation efficiency was calculated as:

$$\text{Coagulation efficiency (\%)} = (TB - TS) \times 100/TB$$

Each experiment was performed as triplicates and the same was repeated three times. The values are expressed as mean ± S.D.

2.3. Analytical Analysis

The phytochemical analysis of the seed extract of *T. foenum-graecum* and *C. cyminum* were done as described by Trease *et al*. [18]. Protein concentration in crude extracts was measured by Lowry *et al.* [19]. The quality parameters of the different water were determined using standard methods [20]. Organic matter concentration in water after coagulation was determined as $KMnO_4$ demand by Kübel-Tiemann [21]. Each experiment was carried out in triplicates and experiment was repeated thrice.

3. RESULTS AND DISCUSSION

3.1. Characteristics of Natural Coagulant

Water extract of *T. foenum-graecum* and *C. cyminum* samples were screened for the presence of phytochemical compounds (**Table 1**). As expected, two plant species (used for the present study) showed the presence of various phytochemicals. Plant sterol and steroid presence was observed in *T. foenum-graecum,* which was absent in *C. cyminum.* The coagulating active agents can be extracted from seeds by water, different salt solutions, buffer solutions or organic solvents [4,22,23]. Numerous researchers reported that proteins are the active coagulating components in plant extracts [22,24]. Ndabigengesere *et al*. [4] reported that the coagulation active agents in the seed extract of *M. oleifera* are dimeric cationic proteins. However Okuda *et al.* [25] and Sanghi *et al*. [26] suggested that the coagulation active agent in the extract of *M. oleifera* was neither a protein, nor a polysaccharide, nor a lipid, but an organic polyelectrolyte.

In the present study protein concentrations in extracts

Table 1. Phytochemical screening of seed extracts of *T. foenum-graecum* and *C. cyminum*.

Phytochemicals	*T. foenum-graecum*	*C. cyminum*
Alkaloids	–	–
Flavonoids	+	+
Tannins and Phenolics	+	+
Amino acids	+	+
Carbohydrates	+	+
Cardioglycerides	+	+
Saponins	+	+
Terpenoids	–	–
Sterols and Steroids	+	–
Oils and Fats	–	–

"–" indicates the absence of phytochemical and "+" indicates the presence of phytochemical, *n* = 3.

of the different samples were determined. The efficiency of protein extraction from seeds of *T. foenum-graecum* and *C. cyminum* by distilled water, 0.5 M or 1 M NaCl solutions was done (**Figure 1**). Results showed the water extracts obtained from different samples has the highest values of protein concentration compared to 0.5 M and 1.0 M NaCl extracts. Water extract of *T. foenum-graecum* showed the highest (2.14 mg/ml) concentration of protein. The coagulating active agents were extracted by distilled water and two different salt concentrations (0.5 M and 1.0 M NaCl). 1.0 M NaCl extract of *T. foenum-graecum* showed highest coagulation efficiency (~81%) compared to 0.5 M NaCl (~25%) and water (~8%) extracts on pond water (**Figure 2**). *C. cyminum* also showed highest coagulation efficiency in 1.0 M NaCl extract (~16%). This coagulation efficiency of *C. cyminum* was negligible compared to that of *T. foenum-graecum* and hence the latter was used for further studies.

Figure 3 shows the coagulation efficiency of *T. foenum-graecum* along with 3 standard natural coagulants and 1 synthetic coagulant used in the study. The coagulation efficiency was maximum for synthetic coagulant $Al_2(SO_4)_3$ followed by *S. potatorum*, *T. foenum-graecum* and *M. oleifera* seed extracts. To verify that coagulation active components contributed to the coagulation efficiency, the authors performed coagulation test with NaCl alone (no added extracts). No significant changes were observed in the turbidity levels of control pond water and NaCl alone treated pond water (data not shown) even after 48 hr. This observation suggests the presence of coagulation active compounds in the seed extract of *T. foenum-graecum*, which is responsible for the coagulation of turbid pond water and not by other mechanisms [25].

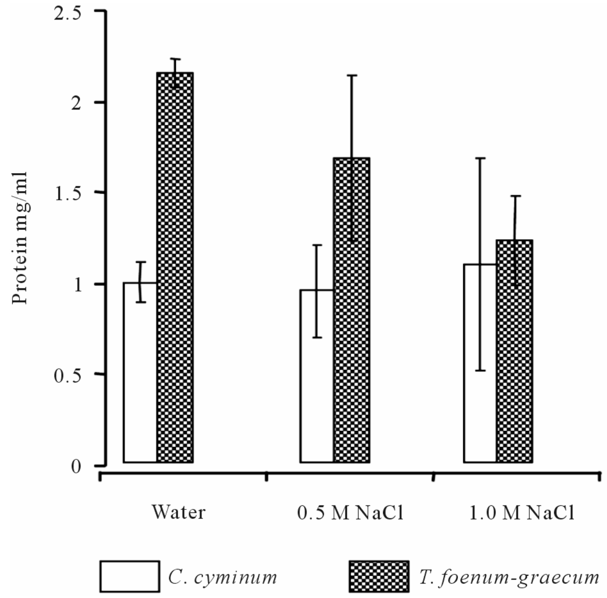

Figure 1. Protein concentration in extracts obtained from seeds of *T. foenum-graecum* and *C. cyminum* with distilled water or NaCl solution (sample: 1 g/100 ml; $n = 3$).

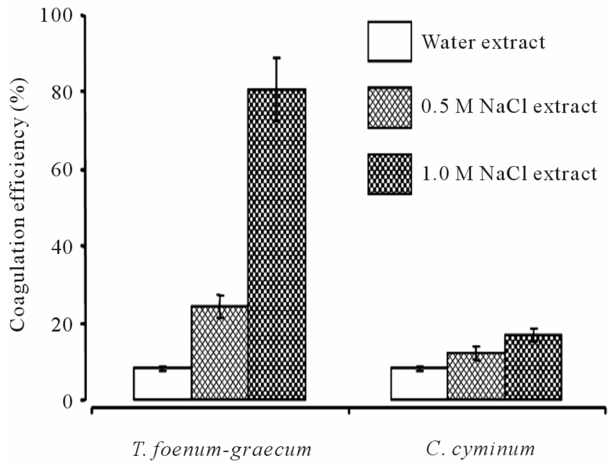

Figure 2. Coagulation efficiency of extracts obtained from seeds of *T. foenum-graecum* and *C. cyminum*. (coagulant dose 10 ml/L of pond water; pH 7.0; at room temperature; at 6 hr; $n = 3$).

3.2. Properties of Treated and Untreated Water

Table 2 shows a few essential properties of the tap water, untreated and treated pond water with different seed extracts. Comparison between tap and pond water shows higher values of alkalinity, turbidity, permanganate demand (presence of organic matter) and total coliform. These marked differences suggest the increased impurity and turbidity of pond water. When comparing the above properties between pond and treated water, there is a significant decrease in *T. foenum-graecum* and other seed extracts treated water (**Table 2**). To our surprise the total coliform is higher in *T. foenum-graecum* treated sample when compared to tap water, however it is low when compared with pond water (untreated); this may be due to the presence of organic and inorganic matter that promotes the microbial growth. The organic matter in water might cause color, odor, taste as well as microbial changes during storage of treated water. It might consume additional chlorine in the water treatment plant and can act as a precursor of byproducts during the disinfection process. It can be noticed from **Table 2** that extract treatment decreased organic matter concentration (4 ± 0.8 mg $KMnO_4$/L) when compared to pond water (7 ± 1 mg $KMnO_4$/L). The decrease in organic matter of treated water is due to the formation of flocks that settle during the coagulation process [25,27]. However, the authors are unable to explain, almost equal amount of organic matter content (it is anticipated more) in pond water and *M. oleifera* treated samples. Electrical conductivity observed in water is the result of ions of mineral salts and carbon dioxide dissolved in it. Pond water has low electrical

Table 2. Properties of untreated and treated water with different seed extracts.

Particulars	Tap Water	Pond Water	*T. foenum-graecum*	*M. oleifera*	*S. potatorum*	$Al_2(SO_4)_3$
pH	~7.0	~7.12	~7.0	~6.8	~7.0	~7.2
Conductivity ($\mu S \cdot m^{-1}$)	250 ± 18	71 ± 8	261 ± 28	270 ± 32	284 ± 32	200 ± 20
Alkalinity (mg of $CaCO_3$/L)	62 ± 8	126 ± 16	74 ± 9	112 ± 15	72 ± 8	11 ± 2
Calcium (mg/L)	44 ± 5	32 ± 4	34 ± 4	33 ± 2.8	34 ± 3.8	35 ± 4.5
Sodium (mg/L)	7 ± 0.6	0.7 ± 0.08	2.2 ± 0.2	2 ± 0.26	4 ± 0.8	3 ± 0.8
Potassium (mg/L)	0.4 ± 0.06	3.6 ± 0.7	4 ± 0.44	8 ± 2.9	4 ± 0.98	6 ± 0.5
Turbidity (NTU)	1 ± 0.96	228 ± 34	8 ± 1	15 ± 3	5 ± 1	2 ± 0.3
Permanganate demand (mg $KMnO_4$/L)	2 ± 0.84	7 ± 1	4 ± 0.8	6 ± 1	2 ± 0.3	2 ± 0.4
Total coliform (MPN-index/100 ml)	7 ± 1	111 ± 25	55 ± 8	4 ± 0.9	14 ± 2	Clear

Values are expressed as mean ± S.D.; $n = 3$.

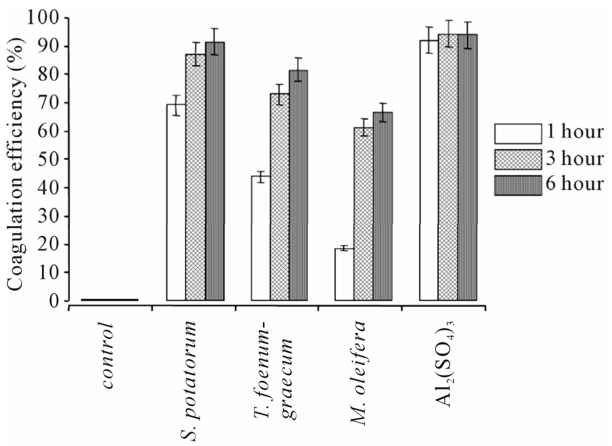

Figure 3. Turbidity removal of extracts obtained from seeds of *S. potatorum*, *T. foenum-graecum* (1 g/100 ml of water or NaCl solution; coagulant dose 10 ml/L of pond water; pH 7.0) and *M. oleifera* (5 g/100 ml of water or NaCl; coagulant dose 50 ml/L of pond water; pH 7.0) and $Al_2(SO_4)_3$ (coagulant dose 5 g/L of pond water; pH 7.0) at room temperature, $n = 3$.

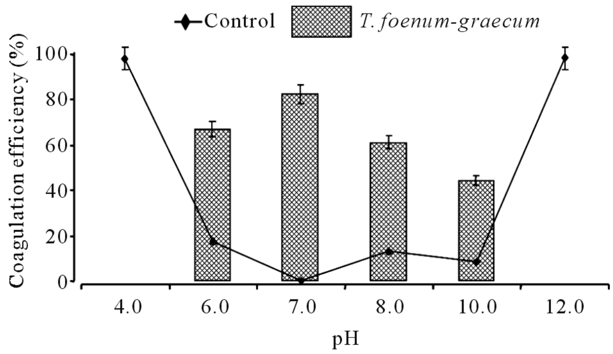

Figure 4. Coagulation efficiency of extracts obtained from seeds of *T. foenum-graecum* as a function of pH (coagulant dose 10 ml/L of pond water; at room temperature; at 6 hr; $n = 3$).

conductivity when compared to tap water, where as different extract treated pond water has more or less similar electrical conductivity to that of the tap water.

3.3. Effect of pH, Temperature and Coagulant Dose on Coagulation

Many parameters may have an influence on coagulation process, which includes nature and composition of coagulant, coagulant dose, composition of water, initial water turbidity, pH, temperature, jar test conditions etc. Since pH is a very important parameter with respect to the process of coagulation and protein charge, the influence of pH on coagulant efficiency was investigated. **Figure 4** shows the effect of different pH on the coagulation efficiency of *T. foenum-graecum*. The pond water (~200 NTU initial turbidity) was adjusted to the desired pH range (4.0 - 12.0) and seed extract of coagulant dose 10.0 ml/L of pond water was added and the coagulation efficiency was calculated. Results showed that the most appropriate pH to perform coagulation for *T. foenum-graecum* was 7.0. Also the extract showed good coagulation efficiency in the pH range of 6.0 to 10.0. Further increase (above pH 10.0) or decrease (below pH 6.0) in pH of pond water made it coagulated without an added natural coagulant. These results are not in accordance with reports, that higher pH values are optimal for other natural coagulants derived from *M. oleifera* [25], *P. juliflora* and *C. latifaria* [28], *C. angustifolia* [26], and common bean [29]. However, *T. foenum-graecum* extract can be used as natural coagulant within the recommended pH range for tap water, which is from 6.5 to 8.5 [30]. It was found that the pH of clarified water in our experiment was around 7.0.

The seed extract of *T. foenum-graecum* being an excellent natural coagulant, the authors tried to establish the stability of this coagulation active component for temperature and pH change. **Table 3** shows the stability of coagulation active components in the extract. Temperature stability was assessed by heating the extract at 50˚C and 100˚C for 10 min and then cooled to room temperature. pH stability was also assessed by changing the pH of extract from 4.0 to 10.0 using 1 N HCl or 1 N NaOH, incubated for 10 min and then neutralized (pH 7.0). It is evident from the results (**Table 3**) that change in pH or increase in temperature of the extract does not alter the stability of the coagulation active components in the extract and thereby the coagulation efficiency.

The other interesting factor that affects the coagulation efficiency is the dose of the natural coagulant. The extract with initial turbidity of around 200 NTU, pH 7.0 was investigated with range (coagulant dose) from 0.5 ml to 12.0 ml /L of pond water and the results are presented in **Figure 5**. Increase in coagulant dose (0.5, 1.0, 2.0, 4.0, 8.0 and 12.0 ml/L of pond water) and incubation time (1, 3, 6, 12 and 24 hr) resulted in increase of coagulation efficiency. The extract at a dose of 12.0 ml/L of pond water produced clear water at 6 hr. When compared to other natural coagulants [6,25-27] *T. foenum-graecum* extract showed coagulation property at very low concentration. Generally, it can be concluded that lower doses of investigated natural coagulants are better than higher doses. Lower doses are not only quite economic, but also low organic matter load in the processed water results in reduced microbial growth.

4. CONCLUSION

Seeds of *T. foenum-graecum* contain materials that can act as effective natural coagulant. The natural coagulant present in *T. foenum-graecum* shows its coagulation efficiency at neutral pH. Further it was found that this natural coagulant is temperature and pH stable. It may not produce total coliform-free water. It is to be noted that even with well-designed and maintained systems it is unable to produce zero total coliform without chlorination. The present study shows obvious presence of natural water coagulant in seed of *T. foenum-graecum*. However, further studies pertaining to the precise mechanism of action of water coagulant is warranted.

Table 3. Effect of temperature and pH on coagulant of *T. foenum-graecum* seed extract. Extract was kept at 50˚C and 100˚C (water bath) for 10 min and then cooled. The extract was adjusted to pH 4.0, 6.0, 8.0 & 10.0 by using 1.0 N HCl or 1.0 N NaOH, waited for 10 min then neutralized (coagulant dose 10 ml/L of pond water; at room temperature; at 6 hr).

Particulars	Coagulation efficiency (%)
Control (Pond water)	0
Extract treated	81 ± 4
Extract (Heated for 50˚C) treated	80 ± 2
Extract (Heated for 100˚C) treated	78 ± 3
Extract (Adjusted to pH 4.0 and neutralized) treated	78 ± 5
Extract (Adjusted to pH 6.0 and neutralized) treated	78 ± 4
Extract (Adjusted to pH 8.0 and neutralized) treated	80 ± 2
Extract (Adjusted to pH 10.0 and neutralized) treated	78 ± 5

Values are expressed as mean ± S.D.; $n = 3$.

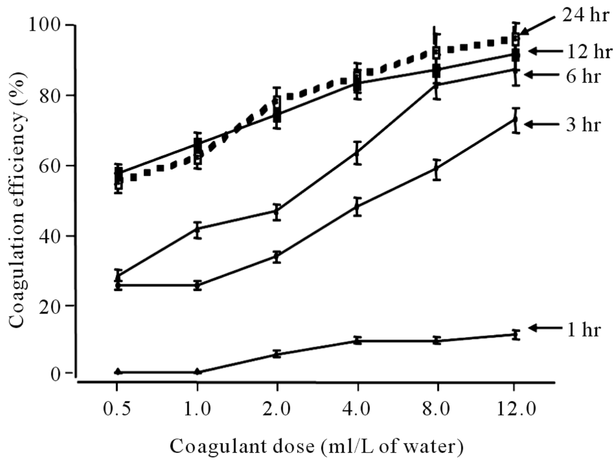

Figure 5. Effect of coagulant dose on coagulation efficiency of extracts obtained from seeds of *T. foenum-graecum* (coagulant dose 0.5 ml to 12 ml/L of pond water; pH 7.0; at room temperature; $n = 3$).

5. ACKNOWLEDGEMENTS

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