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Germination and growth promoting activity of crude extract and extracted chitosan of *Rastrelliger kanagurta* and *Eubleekeria splendens* on mung beans

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ABSTRACT

Indian mackerel, *Rastrelliger kanagurta* is pelagic and schooling fishes, widely distributed in the Indo-west Pacific region. *Eubleekeria splendens* is also known as slimy, belong *Leiognathidae* family. They inhabit marine and brackish waters in the Indian and West Pacific Oceans. Aqueous crude extract was prepared from dried fish and chitosan was extracted from the respective fish scales. The germination and growth promoting activity of the crude extracts and extracted chitosan was studied using mung bean. The prepared samples were prepared in different concentration and the seeds were soaked and germination rate was determined after 24hr. 20ppm of *E.splendens* chitosan was found to be the lowest optimum concentration. the soaking time required for seed germination was studied and it was found that Chitosan gave good germination rate at 4-6hr soaking. Stem height, total numbers of leaves, average leaf length and width were noted after one week of growing the seed in the extracts and extracted chitosan. *E.splendens* Chitosan showed higher growth promoting activity than *R.kanagurtachitosan*. the study confirmed that just like shrimp and crab chitosan, extracted chitosan from fish scales also shows good germination and growth promoting activity.

Keywords: *Rastrelligerkanagurta*, *Eubleekeriasplendens*, beans, chitosan, germination, growth promoting

INTRODUCTION

Seer fish is a subfamily of the *Scombridae* or Mackerel family. These include many species *Rastrelliger kanagurta*, *Scomberomorus guttatus*, *S.lineolatus*, *S. commerson*, *Scomberomorus koreanus* and *Acanthocybium solandri*. In India, it is well known as neimeen in Tamil [1].The Indian mackerel, *Rastrelligerkanagurta* is pelagic and schooling fishes, widely distributed in the Indo-west Pacific region. It is a major marine fishery resources of India and perishes easily.

Leiognathidae comprises nine genera namely *Gazza*, *Leiognathus*, *Secutor*, *Pho-topectoralis*, *Nuchequula*, *Eubleekeria*, *Equulites* and *Aurigequ-ula* [2,3]. Pony fish (*Eubleekeria splendens*) is also known as slimys, are under family of *Leiognathidae* and the order of *Perciforms*. They inhabit marine and brackish waters in the Indian and West Pacific Oceans. Pony fishes are small and literally compressed in shape, with a bland, silvery colouration. They are distinguished by highly extensible mouths, and presence of a mechanism for locking the spines in the dorsal and anal fins. They also possess a luminous organ in their throats, which projects light through the animal's underside. They are located in the ocean on the depth of 10 – 110 m. The length of pony fish is up to 28.0cm. Their locations around the world are Indo-West Pacific: Red sea, Persian Gulf and East Africa.

Chitosan is widely used in agriculture due to its positive effects on plants growth and development. Chitosan, is a biopolymer derivative of chitin, is mostly found in the exoskeleton of arthropods and crustaceans [4].Chitosan treatments have plant growth promoting effects, resulting in improved yields and plant health in numerous crops and

fruits. The activation of protective mechanisms in plant tissues with chitosan inhibited the growth of taxonomically different pathogens [5]. It has been considered as an alternative to chemical fungicides [6].

The present study aims at analysing the optimum concentration, soaking time and growth promoting activity of crude fish extracts and Fish chitosan.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

The dried fish samples and the fish scales of *Rastrelliger kanagurta* and *Eubleekeria splendens* were purchased from kamaraj Market, Tuticorin. These were then washed with deionised water, dried and the ground into fine powder. These were stored in air-tight container and used for further extraction process.

Extraction of Aqueous extract

50g of dried fish powder was extracted with 50ml of solvent. The extracts were dried in a desiccator and re-suspended in the solvent. 5mg/ml was used as stock solution.

Extraction of Chitosan

Chitosan was extracted from the dried fish scales of *Rastrelliger kanagurta* and *Eubleekeria splendens* by a method described by with minor modification.

The process mainly involved in the following steps:

- i. *Demineralization of fish scales*: The dried fish scales were demineralized with 0.5M hydrochloric acid at room temperature with constant stirring for overnight. These were then rinsed with distilled water to remove acid and salt. The decalcified product was dried at 60 °C in an oven overnight.
- ii. *Deproteinisation of fish scales*: Deproteinisation was carried out by adding 2.0M sodium hydroxide solution to the demineralised fish scales. The temperature of the reaction mixture was maintained at 60 °C with constant stirring for 5 hours. The residue was then collected and washed with distilled water until the pH become neutral. The final product was obtained as chitin.
- iii. *Preparation of chitosan from isolated chitin*: Isolated chitin was slowly added into a flask containing 40% sodium hydroxide solution with stirred continuously for 2 hours. The residue was washed with distilled water until the pH become neutral. Thus, the purified chitosan was prepared. 5mg of chitosan was dissolved in 100ml of 1% acetic acid and used for further studies.

Optimization of concentration of sample and soaking time on germination

Different concentration (10ppm, 20ppm, 50ppm, 100ppm and 200ppm) of extracts were prepared. 15 seeds of mung bean was placed in a cotton bed and 10ml of the prepared solutions were added. Germination of seeds were noted after 24 hr. The optimum concentration was then used to determine soaking time. 10 mung bean seeds were soaked in 10 ml of optimum concentration solution for 4,6,8,10,12 and 15 hrs and were then placed in cotton bed. Seed germination efficiency was noted after 15 hours.

Analysis of the growth promoting activity of the extracts

Pre-soaked seeds were sowed in pots containing sterile soil to a height of 10cm. 10ml of the optimum concentration solution was added daily along with normal watering. The plant growth was noted daily for one week. The stem height, number of leaves and total wet mass of the plant after a week was also noted.

RESULTS AND DISCUSSION

The prepared samples were prepared in different concentration and the seeds were soaked and germination rate was determined after 24hr. The results are tabulated in table 1. 20ppm of *E.splendens* chitosan was found to be the lowest optimum concentration followed by *R.kanagurta* chitosan (50ppm). The crude extracts gave the maximum germination at 200ppm.

Table 1: optimization of concentration

	10ppm	20ppm	50ppm	100ppm	200ppm
<i>R.kanagurta</i> extract	3	5	6	7	9
<i>E.splendens</i> extract	4	4	5	6	8
<i>R.kanagurta</i> chitosan	7	8	10	8	7
<i>E.splendens</i> chitosan	6	10	8	7	9

The optimum concentration was used to study the soaking time required for seed germination. The soaked seeds were placed in cotton bed and was wetted by water. The germination was noted after 24 hours. The results are tabulated in table 2. Chitosan gave good germination rate at 4-6hrs wherein the crude extracts gave maximum germination at 10-12 hrs.

Table 2: optimization of soaking time

	4hr	6hr	8hr	10hr	12hr	15hr
<i>R.kanagurta</i> extract	3	3	4	6	8	8
<i>E.splendens</i> extract	4	6	7	8	7	8
<i>R.kanagurta</i> chitosan	7	9	8	9	8	9
<i>E.splendens</i> chitosan	9	8	9	9	8	9

Stem height, total numbers of leaves, average leaf length and width were noted after one week of growing the seed in the extracts and extracted chitosan. The results of chitosan were satisfactory and more than their respective crude extracts. *E.splendens* Chitosan showed higher growth promoting activity than *R.kanagurta* chitosan.

Table 3: analysis of growth of mung bean

	Stem height (cm)	No. of leaves	Average leaf length (cm)	Average leaf width (cm)	Wet mass (g)
<i>R.kanagurta</i> extract	18	12	5.24	2.32	1.81
<i>E.splendens</i> extract	17	12	5.76	2.44	1.54
<i>R.kanagurta</i> chitosan	20.7	24	6.23	2.64	1.92
<i>E.splendens</i> chitosan	23.4	27	6.15	2.69	2.04

CONCLUSION

The prepared samples were prepared in different concentration and the seeds were soaked and germination rate was determined after 24hr. 20ppm of *E.splendens* chitosan was found to be the lowest optimum concentration. the soaking time required for seed germination was studied and it was found that Chitosan gave good germination rate at 4-6hr soaking. Stem height, total numbers of leaves, average leaf length and width were noted after one week of growing the seed in the extracts and extracted chitosan. *E.splendens* chitosan showed higher growth promoting activity than *R.kanagurta* chitosan.

Our study confirmed that just like shrimp and crab chitosan, extracted chitosan from fish scales also shows good germination and growth promoting activity.

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