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Anthoceros extract as Growth Promotor and Biochemical Stimulant in Okra Plants

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ABSTRACT: Bryophytes are the amphibians of the plant kingdom. In fact, the work on the bryophyte as biofertilizer is less. They are seasonal also. In the present study the effect of *Anthoceros* extract on growth performance and biochemical properties of *Abelmoschus esculentus* was studied and its antimicrobial effect was also detected. For this study germination rate of the seeds of the bhindi plants were recorded at various concentrations of the *Anthoceros* extract like 20%, 40%, 60%, 80% and 100%. The one without any extract was regarded as control. The length of roots, shoots, length and width of leaf, etc were also examined in the concentrations 60%, 80% and 100%. In addition to this the biochemical analysis for detection of carbohydrates and protein was also performed. Antimicrobial activity against *Staphylococcus aureus* was detected. Of the various concentrations studied, the 60% showed maximum seed germination within minimum days as compared to the others, and the length of roots, shoots, length and width of leaf, etc. were also high in this concentration. The root length was 4.5cm, shoot length was 13.2 cm, leaf length was 3.3cm and leaf width was 3.1. In this concentration the highest value of protein and carbohydrate was also recorded. The control showed lowest rate of germination, root length, shoot length, leaf length and leaf width. The protein content and amount of carbohydrate also less in control. It shows the influence of *Anthoceros* extract in seed germination and growth of bhindi plant. The extract of *Anthoceros* showed antibacterial property towards *Staphylococcus aureus.* This may be due to its symbiotic association of blue green algae *Anaebena*. Through this study it was made clear that the Anthoceros like bryophytes are good source of biofertilizers with antibacterial potential, and we have to explore it. More studies has to be conducted to isolate the compounds present in Anthoceros and to find out the effects of these compounds.

Keywords: Anthoceros, Biochemical analysis, Antimicrobial activity, symbiotic, germination.

INTRODUCTION

Bryophytes are non-vascular thalloid forms well adapted in their physiology and organization. They can grow in harsh conditions where other plants cannot grow. They have the ecological and evolutionary significant as they are the first group of plants migrated from water to land. They play major role in mineral cycling and also trap the nutrients in the soil so they can be considered as nutrient filters. Some of the bryophytes are the encrust the nitrogen fixing bacteria and so increase the fertility of the soil. Our rice fields are the good habitats of the bryophytes and help in retaining the water holding capacity of the soil there. Fungicidal, bactericidal and insecticidal activity of the bryophytes are well known (Asakawa *et al*., 1980; Ando and Matsuo 1984).

Anthoceros is one of the important bryophyte having the cyanobacteria as symbiont and is seen widely in the rice fields of our state. They are improving the texture of the soil by Increasing the pores of the soil by their filamentous structure and producing some adhesive substances , Excreting growth promoting substances, such as hormones (auxin, gibberlin), vitamins, aminoacids etc (Roger and Renaud 1982; Rodriguez *et al*., 2006), they are also responsible for Increasing the water holding capacity through their jelly structure (Roger and Reyanud 1982), soil biomass is getting enhanced after their death and decomposition, Decrease in soil salinity, Preventing weed growth and also Increase in soil phosphate by excretion of organic acids. They can also be used as biomonitors of metal in order to get an idea about metal precipitation.

Bryophytes are rich sources of oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, phenyl quinones and aromatic and phenolic compounds but few studies have been made between any medical effects and specific bryophyte species or compounds. They have been used as medicines in China, India and Americans from the ancient time onwards. Bryophytes like *Sphagnum* and *Rhodobryum* sps. Are well known for their antibiotic properties. They are contributing much towards carbon balance of the nature (Bisbee *et al*., 2001; Gowar *et al.,* 1997).

In horticulture, soil additives are prepared from the bryophytes. Some bryophytes are used against *Phytophthora infestans* or *Alternaria solani* etc. They can also be used for decorative purposes, land scaping, insect repellent, mounting medium for epiphytes etc.

Comparatively less works were conducted on the effect of Anthoceros extract or extract from any other bryophytes. There is good symbiotic relation between the two. In India about 25 species have been reported by various workers. *Anthoceros himalayensis, A. erectus* and *A. chambensis* are the common the three common Himalayan species.

There are some works on the effects of bryophytes on plant growth. Matsuo *et al*. (1981, 1984a, 1984b) studied the structures of various substances from liverworts which are having plant growth inhibitory activities. Huneck and Meinunger (1990) tested 52 species of mosses and 29 species of liverworts on growth regulation activity and found out that the bryophytes have the wonderful capacity to enhance the shoot and root growth. The studies of Mishra *et al.* (2014) showed that the bryophytes have good amount of flavonoids, terpenoids, glycosides and sterols in high amount which can have positive effect on growth of plants. Here an attempt was made to study the effect of Anthoceros extract on the growth performance and biochemical aspects of okra plant. Anthoceros sps are good host to the blue green algae Nostoc.

In the field of aquatics and fuel the bryophytes can be act as bio and radioactive indicators respectively (Saxena and Harinder 2004; Glime, 2007).

In addition to this they have a number of active constituents which demonstrates a variety of activities like antimicrobial, antifungal, cytotoxic, antitumor, and insecticidal properties (Asakawa 2008; Üçüncü *et al.,* 2010). These property of bryophytes can also be used in the field of agricultural and medicinal process.

Large number of bryophytes have secondary metabolites which can be used as potential A significant pharmacological, economic, or biotechnological sources. Potential plant-protection agents, enzyme inhibitors, anti-cancerous compounds, neurotrophic compounds, and compounds that relax muscles and strengthen the heart etc are some of the biologically active compounds that can be obtained from bryophytes (Asakawa, 2007).

In the high altitude areas the bryophytes control the ecosystem functioning by controlling the carbon and nitrogen cycles (Koranda and Michelsen 2020).

Some mosses are associated with N_2 fixing cyanobacteria and provide which may provide high N input in high latitude ecosystems (DeLuca *et al.,* 2002; Lindo *et al.,* 2013; Rousk *et al.,* 2013). They also serve as substrate for green roofs as such or in combination with some other plants as they are good colonizers and desiccation tolerant (Anderson *et al.,* 2010).

Mosses are useful as moss garden which are more common in Japan, UK, US, Canada (Glime 2017; Martin 2015). These moss gardens as they do not require fertilizer, and they form habitat for beneficial insect, salamanders, and other organism which traditional lawns do not support. More than this due to naturally occurring secondary metabolites in them, the need for herbicides and pesticides is lower; water usage is also less as compared to lawns; and there is no need of machine powered garden tools .Some species of bryophytes are also used as bioindicators like source of cadmium pollution (Donovan *et al*., 2016). Bryophytes have good allelopathic effects too (Meiners *et al.,* 2012). Mosses influence microorganisms that fix nitrogen by regulating soil temperature and moisture especially in artic systems (Gornall *et al.,* 2007).

Different biologically active compounds like Neomarchantins A and B, and Marchantin C have been found in bryophytes (Commisso *et al*., 2021). In this experiment also we found that Anthoceros have antibacterial effect. The work on biofertilizer activity of Anthoceros or any other bryophyte is very rare.

MATERIALS AND METHODS

Collection of bryophyte. The bryophyte *Anthoceros* has been selected to study their effect on germination behaviour of *Abelmoschus esculentus,* as they contain the blue green algae in symbiotic association. The bryophyte was collected by regular and repeated local field trips at different localities of Mala. The plants were collected in the first week of August. Fresh plants, devoid of dead tissue were collected. The plants were freed from contaminant parts of other plants, if present, and were carefully scooped out. Plants thus collected were kept in separate polyethylene bags and sealed immediately. The collected plant material taken to the laboratory.

Preparation of *Anthoceros* extract. The bryophyte material was washed thoroughly to remove adhering soil particles and blotted. As specimens were small and were not collected in large amounts due to conservation viewpoint, extracts were prepared from entire green part of the thallus. Water was used as extracting solvent. For preparing extract 100 g fresh material of bryophyte was ground with a pinch of sand in mortar to yield a pulp and dissolve in 100 ml of water and shaked well and filtered with Whatman No. 1 filter paper. Final volume of the extract was made upto 100 ml by adding respective solvent and considered as full concentration (100%). Then this extract was diluted to 20%, 40%, 60%, 80% concentration.

Collection of *Ablemoschus esculentus* seeds. The seeds of *Ablemoschus esculentus* were collected from Kerala agricultural University, Thrissur. The variety was *Arka anamika*. Seeds were brought to the laboratory, air dried and healthy seeds were sorted out.

Preparation of potting mixture. Potting mixture was prepared by using coir pith, sand in 1: 1 ratio after sterilization.

Experimental design. In special trays germination test was conducted for the six treatments namely C, T_1, T_2, T_3, T_4, T_5, T_6 with control, 20%, 40%, 60%, 80% and 100% concentration of extract. In a germination tray 4 rows were selected each with five wells. They were filled with potting mixture and one control and three treatments were selected. Seeds were sown in each row. First row was control which was without any extract. Distilled water was used in this. Before sowing the seeds

were soaked in respective concentrations of extract for 12 hours. The experiments were done at room temperature (30-33°C) and were carried out for 15 days. The seeds were considered germinated if the radical exceeded 3 mm in length. After the germination test the best three were selected.

Percentage of seed germination. Germination test was conducted in germination trays. The temperature of 25 ± 10°C was maintained during the germination test. The first and final germination counts were recorded on fifth and eighth days of germination test respectively for normal seedlings and germination was expressed in percentage.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds put for germination}} \times 100$$

Measurement of growth performance. Growth performance was studied by measuring root length, shoot length, leaf length and leaf width.

Biochemical analysis. After the germination performance the 60%, 80% and 100% was again grown for biochemical analysis. After 20 days of growth performance the seedlings are dried and taken for the biochemical analysis. For analysis of protein lowry's method was done for carbohydrate analysisphenol – sulphuric acid assay was performed.

Antimicrobial Activity. The acetone, methanol, ethanol, hot and cold aqueous *Anthoceros* extracts were used for evaluation of the antimicrobial activity by the agar well diffusion method (Ahmad and Beg 2001; Aneja *et al*., 2009).

In this method, pure isolate of microbe *Staphylococcus aureus* was subcultured on the agar media plates at 37ºC for 24 h. One plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Microbial suspension used as the inoculum for performing agar well diffusion assay. Inoculum of test organism was spread onto the agar plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells of 8mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. The dried extracts were reconstituted in 20% dimethylsulphoxide (DMSO) for the bioassay analysis (Rajasekaran *et al*., 2008). A 100µl volume of extract was propelled directly into the wells (in triplicates) of the inoculated agar plates for test organism. The plates were allowed to stand for 1hr for diffusion of the extract into the agar and incubated at 37ºC for 24h (Okeke *et al.,* 2001; Rios *et al.,*1980). Sterile DMSO (20%) served as the negative control.

RESULTS AND DISCUSSION

In the present study the rate of germination was high in 60 % concentration in the first 4 days followed by the 80% and 100%. Lowest rate of germination was in control. Then in the next 3 days there was full germination in all except the 20%, 40%. In control 5 seeds were germinated. 20% concentration showed the germination of 7 seeds. 6 seeds were germinated in 40 concentration. 10, 9, and 7 seeds were germinated in 60%, 80% and 100% concentrations respectively.

The root length and shoot length, leaf length, breadth etc were also measured. It was found that in 60% there is more increase in the length of root, shoot, leaf length, breadth, etc. It was followed by the 80% and 100%. The root length, shoot length, leaf length and leaf width were 4.5, 13.2, 3.3 and 3.1 respectively in 60% concentration. In 80% concentration 3.8 cm root length, 11.3cm shoot length, 2.7cm leaf length and 2.8 cm leaf width were observed. 100% shows 3 cm root length, 11 cm shoot length, 2.4 cm leaf length and 2.6 cm leaf width. The least was observed in control. In control 3.2 cm root length, 5.4 cm shoot length, 2.1 cm leaf length and 1.8 cm leaf width were seen. Percentage of seed germination, root and shoot length, leaf length, breadth on each concentration was shown in Graph 1.

The biochemical analysis was conducted. It was observed that the biochemical contents were high in 60% concentration as compared to the control and other 80% and 100%. The 60% concentration shows 1.50 µg/ml protein and 0.90 mg/g carbohydrate. 1.40 µg/ml protein and 0.85 mg/g carbohydrate observed in 80% concentration. 1.20 µg/ml protein and 0.82mg/g carbohydrate were detected in 100%. The results of biochemical analysis was given in Graph 2.

Antimicrobial activity was also observed for the microbe used. *Anthoceros* extract showed antibacterial effect on *Staphylococcus aureus.*

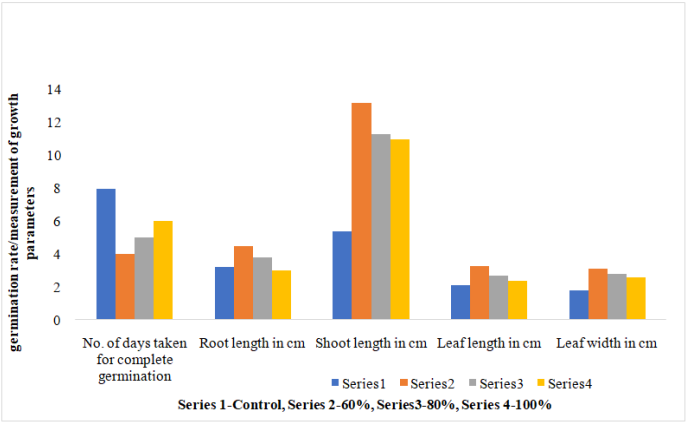

Fig. 1. Effect of Anthoceros extract on germination rate and growth parameters of Okra plant.

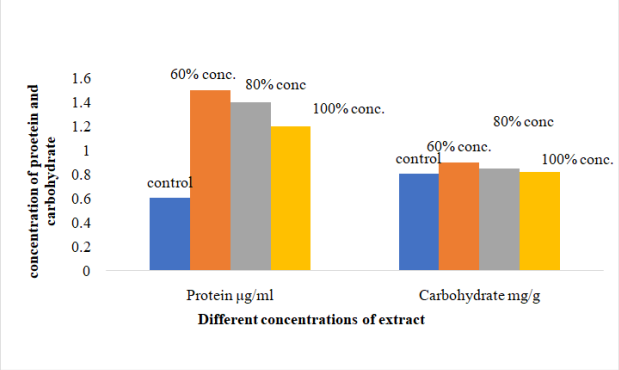

Fig. 2. Biochemical analysis of the seedlings treated with Anthoceros extract.

In this experiment in bhindi plant fastest seed germination was observed by 60% treated plant (4 days) and minimum by control, 20% and 40% concentrations. In all case 100 percentage germination rate was there but there was variation in number of days taken for full germination.

The maximum root length, shoot length, leaf length, leaf width etc was noted for the 60% concentration followed by 80%, and 100%. The value of root length for 60% was 4.5, shoot length was 13.2, leaf breadth and length was 3.3 and 3.1respectively. In 80% concentration the root length, shoot length, leaf length and leaf width were 3.8, 11.3, 3.3 and 3.1 respectively. 100% shown 3 cm root length, 11 cm shoot length, 2.4 cm leaf length and 2.6 cm leaf width. The lowest root length, shoot length, leaf length, leaf width were observed in control. These results suggest that the better germination and growth of bhindi seed was seen in 60 % concentration and also shown that the *Anthoceros* extract have some influence in seed germination and plant growth.

Biochemical analysis showed the maximum value for 60% for protein and carbohydrate. It was 1.50 and 0.90 respectively. It was followed by 80% and 100%. Lowest for control.

Many bryophytes like *Sphgnum* are considered as ecological engineers, as they modify their environment to create habitat thereby helping to modify the environment to maintain biodiversity (Jassey *et al*., 2013). In this study also the extract of bryophyte is performing well for plant growth.In addition to this antibacterial activity is observed by about 93% of bryophytes (Zhu *et al*., 2006)

The extract at 60% concentration increased leaf area and root,shoot length content to maximum when compared to control and other treatments. Protein synthesis turnover in growing plants is a basic component of metabolic regulation which provides a way for varying the enzymatic complement during the response to environmental conditions (Huffaker and Peterson 1974). Protein and carbohydrate content increased at all treatment compared to the control. It showed maximum content at T_1 treatment as compared to control and other treatments. According to Das *et al*. (2007), biomass increased progressively irrespective of treatments over control. However, the total fresh biomass production was recorded highest with combined application of biofertilizer when compared to sole application.

The aqueous extracts of bryophytes germination of *B,biternata* seeds was completed between 10 and 4 days (*Marchantia* and *Targionia*) in liverworts and between 3 (*Plagiomnium*) and 9 (*Rhodobryum*) days in mosses (Alka Sharma 2009). Total time taken for the completion of germination of seeds varied according to the type of the extracts. Variation in total germination period appears to be independent of the concentration of the moss extract. Some other factors like size of the seeds may be responsible for delaying the completion of germination in certain extracts. Among liverworts, maximum time was taken by *Marchantia* (10 days in 20 percent aqueous extract). Several species of bryophytes sowed antibacterial activity against *Staphylococcus aureus* (Vizma *et al*., 2012). The antibiotically active substances of *Atrichum* and *Dicranum* spp. are considered to be polyphenolic compounds (Mc Clery *et al.,* 1966). In particular, flavonoids, including phenolic acids, are the main group of phenols obtained from mosses (Mc Clery *et al.,* 1966). From the present study it is clear that *Anthoceros* extract has significant effect on the seed germination, growth performance, biochemical parameters of the bhindi plant. It also shows the antibacterial property. This fact can lighten the work in case if any of the extracts would be introduced in future practice.Bryophytes contains numerous secondary metabolites and their extract have significant antioxidant properties (Klavina, 2015).

CONCLUSIONS

From this study it was clear that the extract of bryophyte especially *Anthoceros* sps is having very good effect on the seed germination and also for having good biochemical constitution. It is also showing good antimicrobial activity against *Staphylococcus aureus.* All these points out towards the need of conservation of the bryophytes and their utilization in various medicinal, biofertilizer aspects.

In short we can say that the biofertilizers like Anthoceros, or any other bryophytes are low cost and can be used by farmers, they have no side effects, can reduce pollution, thereby increases the soil fertility. The blue green algae inhabiting inside the bryophyte will secrete various growth promoting substances like vitamins, protein etc, there by improving the texture, pH and water holding capacity of the soil. The extract of the bryophytes speed up the plant growth and will provide

quick results. So thay can be used in the coming days popularly as biofertilizers.

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Conflict of Interest. None.

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