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Received: August 23, 2020

Accepted: January 15, 2021

Published: January 25, 2021

Citation: Jha Y. Macrophytes as a potential tool for crop production by providing nutrient as well as protection against common phyto pathogen. 2021 Jan 25;4:bs202103

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Data Availability Statement: All relevant data are within the paper and supplementary materials.

Funding: The authors have no support or funding to report.

Competing interests: The authors declare that they have no competing interests.

Macrophytes as a potential tool for crop production by providing nutrient as well as protection against common phyto pathogen

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Abstract

Applications of synthetic chemical fertilizers and pesticides lead to several environmental hazards, causing damages to entire ecosystem. To reduce damage caused by such chemical inputs in agriculture and environment required a serious attention for replacement of chemicals input with eco-friendly options. In this study decaying macrophytes were selected as an option for organic agriculture, by analyzing its ability to provide important mineral nutrient to the maize crop grown in low nutrient soil as well as for providing resistant towards many common phyto-pathogens to enhance yield. The results of the study showed that decaying macrophytes have high concentration of stored important mineral nutrient in their body mass, which get released in the soil during its decay and to be used by the maize plant. The decaying macrophytes leaf extract have considerable amount of phenolic and flavonoids also having antimicrobial activity. The antimicrobial activity of the leaf extract has been analyzed against the common phyto-pathogen *Pseudomonas aeruginosa*, and *S. aureus* by agar disc method and the formation of clear zone indicate its potential as bio-control agent. So under intensive agricultural practices, application of such biological waste is of particular importance for enhancing soil fertility without chemical input, to ensure sustainable agriculture.

Keywords: Macrophytes, Phytochemicals, Phenolic, Flavonoids, Antimicrobial activity, Mineral nutrient.

Introduction

Aquatic plants are adapted to live in aquatic environments and are referred to as hydrophytes or macrophytes. These plants require special adaptations for living and can only grow in water or soil that is perfectly saturated with water. Aquatic plants generate one of the most productive ecosystems of the world and are essential life supporting systems, which provide a wide array of benefits to the aquatic life and human kind. However, these ecosystems are today's fast declining and rapidly deteriorating ecosystems in many parts of the world. Ignoring the economic and ecological significance of aquatic plants is often regarded menace [1]. Due to continuous pollution of water bodies and urbanization resulted in eutrophication, which disturbed the aquatic ecosystem. So to maintain the aquatic ecosystem, cleaning of water bodies and dumping of macrophytes is a global problem.

Global climate change causes a series of extremities like frost, salinity or drought, which adversely affect the plant nutrient dynamics as well as world food requirement. The availability of nutrients in the soil and its acquisition, assimilation, distribution within the plants or in the crop fields are directly disturbed by climatic stress factors [2]. Low soil nutrient has deleterious effect on nutrient dynamics within the plant root as well as in shoot, including transportation of nutrient to reproductive structures via xylem and phloem, or mobilization of nutrient from senescing leaves. Water fluxes, assimilatory activities, and the redistribution of inorganic nutrients are also affected by environmental stress. Macrophytes have the ability to accumulate large amount of water and nutrient in their leaves, can serve as a good option for the plant nutrient under stress/low soil nutrient condition. Macrophyte decomposition is important for carbon and nutrient cycling in lake ecosystems or in the low nutrient soil [3].

It act as a important source of essential nutrient like phosphorus, carbon, potassium, zinc and nitrogen in the form of ammonia etc., due to the ability of macrophyte plant to absorbs and accumulate these nutrients from soils or water in their body, which ultimately disposes during its decomposition in the ecosystem.

Such nutrient rich macrophytes plants can be used efficiently in organic agriculture/bio-composting that can be applied to obtain required mineral nutrient effectively and successfully for the proper growth of plant, results in increased plant growth and yield. As bio-composting is an economically and ecologically important necessity in resent agriculture, which in return increases the soil quality and crop yield. Biological agents are nowadays drawing attention because of its increased organic efficiency in terms of physical and chemical characteristics of the soil [4].

In nature, plants species grow together and interact with each other by inhibiting or stimulating the growth and development through various interactions. A special form of competition among plants is known as allelopathy, also shown by aquatic plants. Such interaction is a biological phenomenon in which an organism produces many bio-chemical compounds that influence the growth, and reproduction of other organisms. Such interactions are primarily based on the synthesis and release of secondary metabolites, known as allelochemicals or allelotoxins [5]. Such allelochemicals initiate a wide array of biochemical reactions and induce several biological changes, which inhibit the growth of target pathogenic organism, which is not possible solely by physical or chemical agents. The macrophytes also have ability to overcome stress arise due to multiple symbiotic or pathogenic interaction. The aim of the work is analyze the effect of decaying macrophytes on the plant nutrient status, growth and its ability for the production of antibiotics and related secondary metabolites, to protect host maize plant against the potential phyto-pathogens.

Materials and Methods

Aquatic plants collection and preparation of samples

Macrophyte plants were collected from nearby lake and ponds from Anand-Tarapur district of Gujarat. It was immediately packed in polyethylene bags to prevent the degradation of bioactive compounds of the collected plant samples and was brought to the laboratory for further study. The healthy and disease free plants were used for further study. The collected leaf samples were thoroughly washed with water to eradicate surface dust and foreign materials and used for sample preparation. The washed leaves were properly dried at room temperature for 7 days under shade and were ground into a powder with the help of grinder.

Estimation of mineral nutrient concentrations of soil, hydrophytes and maize leaves

The physio-chemical property of the soil, hydrophytes and maize leaves were analyzed by saturated water extraction method in SICART lab. The leaves powder sample were digested for 6 hours in nitric-perchloric acid (5:3) and used to estimate the

concentration of phosphorus by colorimetric. Similarly the concentration of nitrogen was estimated after the Kjeldahl digestion by colorimetry and 2g of plant material were digested in mixture of sulphuric acid, nitric acid and perchloric acid (tri-acid) in the ratio of 9:3:1 and were used to determine the concentration of N, P, K, Zn and Ca. The aliquots of digested filtered plant material were analyzed on digital flame photometry by using specific filter.

Preparation of leaf extract

The leaf extract was prepared by soaking 10g of leaves material powder in 50 ml of distilled water for 24 hours under shaking condition at 130-140 rpm at room temperature. After that the extract was filtered from eight layers of muslin cloth and filtrate was collected in petri-dish. The obtained filtered extracts were dried at room temperature in petri-dish, and was scraped, transferred to tubes for further use. Similarly, plant material was re-extracted with 50 ml of ethyl acetate, and methanol also. Finally obtained extracts were dried under reduced pressure and were stored in a refrigerator at 4 °C until use.

Qualitative analysis of phyto-chemical screening

The phyto-chemical tests to detect the presence of alkaloid, saponins, quinones, steroids, phenols, flavonoids, tannins, coumarins and reducteurs compound were performed. The visual observations like formation of a precipitate or change in color after the addition of specific reagents were used to determine the presence of specific phyto-chemical in the plant extract.

Quantitative analysis on phytochemicals constituents

Total phenolic content

The total phenolic content of leaf extracts was measured using colorimetric Folin–Ciocalteu method [6]. The leaf extract (1ml) was mixed with 5ml of distilled water and 250 µl of 1N folin-ciocalteu reagent. The mixture was covered and allowed it to stand for 3min at 25°C. In this mixture, 1ml of saturated Na₂CO₃ and 1ml of distilled water were added. The mixture was incubated for 1 hour at 25°C for color development and measured at 725 nm using spectrophotometer. Standard graph was prepared by using different concentration of phenol crystals.

Total flavonoid content

The colorimetric assay was used to estimate total flavonoid content in presence of aluminum chloride [7]. In 10 ml volumetric flask 1ml of leaf extract, 4 ml of distilled water and 0.3 ml of 5% NaNO₂ was added. After proper mixing for 5 minutes 0.3 ml of 10% AlCl₃ and 2 ml of 1 M NaOH was added in it. Finally 3.7 ml of distilled water was added to made up total 10 ml volume. The obtained mixed solution was used estimate total flavonoid content by taking absorbance at 510 nm against blank and calculated by the help of standard graph of gallic acid.

Phyto-pathogenic microorganism

Two most common plant pathogenic microorganisms *Pseudomonas aeruginosa* and *S. aureus* has been selected to analyze

the antibacterial potential of the leaf extract. These isolates were obtained from a biotechnology research lab of biosciences department SP University.

Antimicrobial activity assay

The Agar disc diffusion method was used for this analysis. The culture suspension was spread all over plate by placing 0.1ml of culture at center of nutrient agar plate and spreading uniformly with sterile spreader. Then, 6 mm in diameter filter paper discs containing the different types (water, ethyl acetate, and methanol) of leaf extract at a concentration of 100µl were used with distilled water as control. And 4 filter paper discs were placed on the nutrient agar medium surface for the diffusion of leaf extract in the nutrient agar. The Petri discs were then incubated in incubator at 37 °C for 24 h, plates were observed for zone of inhibition by measuring the colony diameter. A 100 % DMSO was used as control.

Effect of decaying macrophytes on minerals concentration under low soil nutrient condition in maize leaves

For this analysis, the maize seedlings were planted in the field with low soil nutrient supplemented with decaying macrophytes without chemical fertilizer for 5 weeks. After 5 weeks plant sample were used determined the foliar phosphorus and nitrogen content by colorimeter, after digesting the plant sample with nitric-perchloric acid (5:3) for 6h Kjeldahl digestion respectively. The foliar potassium, calcium and zinc content were estimated by digesting 1 g of plant sample with tri-acid mixture, which contain mixture of sulphuric acid, nitric acid and perchloric acid in the ratio of 9:3:1. The filtrates of digested material were used for the further analysis by using specific filter on digital flame photometry.

Effect of decaying macrophytes on plant growth parameters

Macrophytes were collected from the polluted water bodies and dumped at a site to initiate its decaying process. After 3 days of collection 10 cm thick bed was prepared on the crop field and was masked by 5 cm soil layer in a low soil nutrient field. After 10 days maize seed were sown in this field to analyze the effect of decaying macrophytes on plant growth parameters. Then seedlings were collected carefully with root after 35 days of sowing to measure the shoot and root length and dry weight. The plants were dried in an oven at 80°C for 72 hrs for dry weight.

Statistical Analysis

All experiments described were performed three times independently, with measurements taken from three (for all other measurements) or ten (for dry weight measurements) different plants for each treatment in each of the three independent experiments. One-way analysis of variance (ANOVA) test was used to analyze all data and mean (of three independent experiments) was compared at 5 % level of significance.

Results and Discussion

Soil hardly ever supports crop production at their full potential yield due to lack of sufficient nutrient. So use of chemical fertilizers is a regular mechanism to achieve the yield potential. But low use of chemical fertilizers may be results in reduced yield and excessive application pollute the environment including water bodies, if applied without prior information about the nutrient content of the soil. At the same time pollution of water bodies resulted in overgrowth of macrophytes having harmful affect on water life, so such macrophytes are problem for water bodies. Therefore use of such macrophytes as a source of nutrient in low nutrient soil status is the best option to overcome the problem, as it accumulates extra nutrient and water in their bodies. So in the present study, the macrophytes have been used as a source of supplement nutrient in the low nutrient soil in place of chemical fertilizer. So the initial major mineral nutrients (N, P, K, Zn and Ca) has been analyzed in the soil used for maize cultivation, control maize leaves and decaying macrophytes leaves and result of the study, showed significant variation in the initial mineral nutrient concentration in these three. Mineral nutrient concentration was considerably high in decaying macrophyte as shown in **Table 1**, in compare to control maize leaves as well as soil under consideration. The decomposition of macrophytes release higher concentration of phosphorus and nitrogen and its assimilation was also higher in plants as reported by Lu et al. [8].

The synthesis of the phenolic acids, flavonoids, quinones, flavonols, phenols, coumarins and tannins like aromatic secondary metabolites in plant takes place to acquire resistant against the adverse environmental stress [9]. And in the present study, such compounds present in significantly higher amount in macrophytes leaves. Phytochemical constituents of the macrophytes showed presence of different phytochemical constituents as alkaloid, saponins, cardiac glycosides, steroids, phenols and flavonoides (**Table 2**), but there was considerable variation in its constituents in fresh and decaying macrophytes. Phenolics having broad spectrum biochemical activity like antimicrobial, anti-tumors, antioxidant and also has the ability to alter the expression of related gene in plant under stress. The majority of the antioxidant activities in plants have been carried out by phenolics, which is a largest group of phytochemical [10].

Flavonoids are type of phenolic compounds naturally produced in the plant either in free or as glycosides have numerous biological functions like protein kinase inhibition, antioxidants, antimicrobial and mitochondrial adhesion inhibition etc. With the development of resistant towards the pesticides, there is requirement for ecologically sustainable natural alternatives or plants derivatives as a choice to enhanced crop production. Plant induced phyto-chemicals as well as plant extracts have antimicrobial properties against different phytopathogen including bacteria. Plants interact with its changing surrounding environment efficiently due to production of secondary metabolites, which helps in acquiring tolerance towards harsh environment for sustainable adaptation. Biotic stress in plants resulted in retarded

Table 1. Concentration of minerals nutrient concentration in soil, hydrophytes and maize leaves (n=5).

S.No	Sample	N (mgkg ⁻¹)	P (mgkg ⁻¹)	K (mgkg ⁻¹)	Zn (mgkg ⁻¹)	Ca (mg kg ⁻¹)
1	Soil	19.5±0.12	952.1±1.12	26246±0.11	285.4±1.27	11782±0.16
2	Decaying Hydrophytes	32.6±1.3	1372.4±2.11	58362±0.23	429.2±1.32	14467±1.37
3	Maize leaves	27.9±1.21	1068.2±0.16	31583±0.21	363.3±2.01	12947±0.13

Values are mean of three replications (p≤0.05; LSD test).

Table 2. Qualitative analysis of phytochemicals in fresh (A) and decaying (B) macrophytes.

Phytochemicals	A	B
Alkaloid	+	+
Saponins	+	+
Quinones	-	-
Steroids	-	+
Phenols	+	+
Flavonoides	+	+
Tannins	-	+
Coumarines	+	+
Reducteurs	+	-

+: Present and - : Absent.

plant growth with enhanced secondary metabolites production as phenolic compounds to defend biotic stress [11]. Phenolic and flavonoids metabolites of plants are organic compounds having aromatic rings with one or many hydroxyl groups. In this study, the phytochemicals constitutes as total phenolic and total flavonoid contents were quantitatively analyzed in the macrophyte and result showed that, the total phenolics content was higher in decaying macrophyte, while total flavonoids content was higher in fresh macrophyte (Table 3), and play major role in attaining maximum yield potential by providing nitrogenous product in normal state as well as in low soil nutrient. Also presence of significant amount of phenolic and flavonoids provide protection against wide range of phytopathogen.

Table 3. Quantitative analysis of phytochemicals in in fresh (A) and decaying (B) macrophytes.

Phytochemicals	A	B
Total Phenolic content (mg/g of extract)	3.34±0.01	4.76±0.12
Total Flavonoid content (mg/g of extract)	6.83±0.02	5.37±0.02

For each Parameter, values are mean of three replications (p≤ 0.05; LSD test).

The use of synthetic pesticides is a quick and effective tool to manage plant diseases. But nonstop and arbitrary use of such synthetic pesticides in the crop field has several deleterious effects on human health and ecosystem, due to its incorporation in food chain and residual toxicity. Use of synthetic pesticides attributes to long degradation periods, elevated acute toxicity, and gathering in the food chain. So the use of biological agents is an economically viable and safe option, to reduce the use of chemicals application. In the present study, the antibacterial ac-

tivity of the decaying macrophytes showed significant effect on the both *Pseudomonas aeruginosa* and *S. aureus* potential phytopathogenic bacteria observed during agar disc diffusion method, by formation of clear zone around the disc having different leave extract (Figure 1).

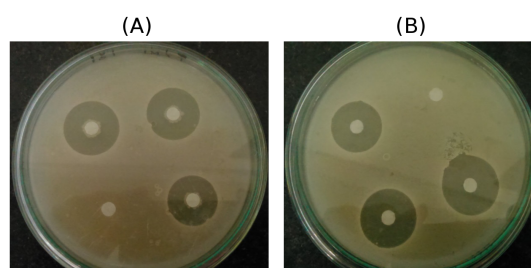


Figure 1. Effect of macrophytes plant extract (water, ethyl acetate, and methanol extract) with distill water as control on the bacterial pathogen on Nutrient Agar plate as Zone of Inhibition (ZOI). Where A for *Pseudomonas aeruginosa* and B for *S. aureus*.

Plant growth is a complex process and the plant production depends on availability of sunlight, chemical energy, carbon source, mineral nutrients and water from the soil. Many mineral nutrients in proper proportion are necessary for plant growth to attain its full strength yield. The plant having limited above ground resources like sunlight, and carbon source, then plant enhance the biomass allocation to the shoots and when there is limited below ground resources like mineral nutrients and water, then plant enhance the biomass allocation to the roots resulted in shunted growth according to functional balance theory[12].

So for proper plant growth the main nutrients required are potassium, phosphorus and nitrogen. These primary nutrients are accountable for limiting crop growth and among them nitrogen, is most intensively used element. Nitrogen is often unavailable in the correct form to be utilized by the plant.

In the present study, concentration of important mineral nutrients was analyzed in maize leaves grown in different condition. In this study, the nutrient content of the maize leaves has been assayed and result showed that concentration of mineral nutrient was significantly high in the maize leaves grown in low soil nutrient state supplemented with decaying macrophytes (Table 4).

So, use of decaying macrophytes as under soil bed has ability to enhance the nutrient content of maize for its the growth and development in low nutrient soil. Such organic agriculture plays an important role in minimizing the use of inorganic fertilizers

Table 4. Effect of decaying macrophytes on minerals concentration under low soil nutrient condition in maize leaves (n=5).

S.No	Sample	N (mgkg ⁻¹)	P (mgkg ⁻¹)	K (mgkg ⁻¹)	Zn (mgkg ⁻¹)	Ca (mg kg ⁻¹)
1	Maize in low nutrient soil	26.3±1.02	2383.1±1.02	73246±0.21	724.4±1.04	13782±1.43
2	Maize in low nutrient soil + Decaying Macrophytes	32.6±0.11	3272.4±0.78	78362±0.64	729.2±2.11	14467±0.87
3	Maize in low nutrient soil + Chemical fertilizer	28.9±0.21	2668.2±0.65	76583±0.73	733.3±1.87	13947±1.23

Values are mean of three replications (p≤ 0.05; LSD test).

and consequently minimizes potential pollution and improves soil quality. It is important to develop new applications which are sustainable both agronomically and economically, as well as environmentally to produce safe food for human consumption. So the use of biological based compounds of plant extracts is considered successful and environment friendly option for sustainable agriculture.

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