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**Research Communication**

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# Allelopathy of *Plantago ovata* on the same and different Plants

***Abstract*: Allelopathy is an ecological plant communication in response to biotic and abiotic conditions. *Plantago ovata* is rich in polyphenols and it is interesting to know how a medicinally important plant can affect its own and different species in their growth and yield. This new investigation is to study the impact of *Plantago ovata*, on the same and a different plant species *Vigna radiata*, in morphological characters, biochemical contents, and antioxidant potentials whose soil types, seasons and overall habitat are different.**

**Keywords:** *Plantago ovata, Vigna radiata*, Allelochemicals, Polyphenols, Sustainable development.

Hans Molisch, coined the term allelopathy and he is recognized as the father of allelopathy[1]. Allelopathy is a natural ecological phenomenon where an organism communicates with its surroundings by changing the chemical environment. It is known in different groups of plants like algae, lichens, crops, annual and perennial weeds[2]. It is an intricate trait that is known to be the effect of multiple genes[3]. Biotic and abiotic variations in natural soils greatly influence the chemical effects of such interactions[4]. In the case of plants, allelopathy is the chain of plant communications that shows different types of plant-plant interactions such as competition, mutualism, parasitism and more[5,6]. These communications are held by a special type of phytochemicals that play the different chemical interactions between plants, called allelochemicals. Plants release a variety of allelochemicals[7]. Majorly the plant communications can be divided into positive and negative types of interactions[3,8]. Positive interaction is necessary for plant diversity whereas negative interaction is necessary for plant species' survival based on the available resources that have been found among the tropical rainforest tree species, and it is suggested that the species are with protection[5,9]. Plants may induce resistance in the neighbouring plants to protect from unwanted pathogens[10]. Plants initiate a defensive response by chemical detection of their neighbouring plants[11]. Parasitic dodder plant shows a parasitic type of interaction with cranberry[12]. In arid and semi-arid plant neighbourhoods'plant-plant communications are important processes[5]. Allelopathy plays an important role in farming systems, control of diseases, weeds, and insects. It is a good weed management tool for the production of weed-resistant crops[13,14]. It will also help us to understand the dynamics of soil ecology, growth enhancement of plants in monoculture farms, resistance against abiotic stress and more. Allelochemicals are environmentally friendly and can lead to herbicides, fungicides, insecticides and plant growth regulators, and can bring a new generation to sustainable agriculture[15]. In-depth studies on signaling pathways of such polyphenols will show us new ideas for the prevention of plant diseases and an understanding of their resistance mechanisms and how they coordinate various signal components. It is required to set the knowledge on the structures of allelochemicals to design new biofertilizers or pesticides, both quantitatively and qualitatively[13,15,16,17].

***Allelochemicals and Polyphenols:*** Allelochemicals are the communicators of allelopathic communications. These are non-nutritional chemicals and signaling phytochemicals that get released into the environment and regulate their interactions with the surroundings[2]. Allelochemicals have been found to release in stress conditions both as positive and negative[5]. They are protective against microbial, herbivore attacks and competitors[16]. Beneficial allelochemicals enable to increase the plant fitness[18,19]. Studies have shown that the major allelochemicals are secondary metabolites and these are chemically diverse, and most of them are represented by polyphenols such as phenolic compounds, terpenoids and alkaloids[1,16]. Screening the allelopathic plants from medicinal plants is a good option since they are rich in metabolic compounds. Secretion of such plant secondary metabolites depends on genetic drift, physiological conditions, seasonal and harvesting time[20]. It has been found that the phenols, flavonoids, tannins and alkaloids have allelopathic reactions. Polyphenols belong to the common class of

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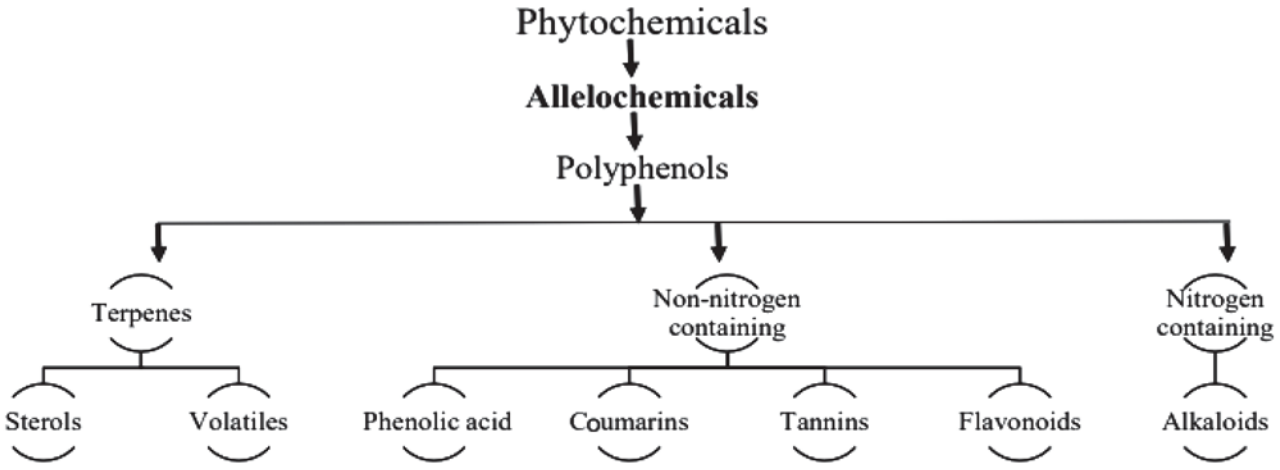


**Fig. 1:** Classification of polyphenolic allelochemicals.

plant allelochemicals and these are classified into different categories based on their structure and function[21] (Figure 1). Phenolic compounds are produced from the pentose-phosphate pathway[1]. There are also some other types of allelochemicals that show peroxidase and superoxide dismutase activities[17].

Allelochemicals are released from the donor plants through the soil as phytochemicals or as volatiles through the air and enter the target or the receiver plant(s) through the root system with the flow of water and nutrients or by absorbing the volatiles[16,22]. A signal exchange and recognition occur during such communication which signals the plant immune network[10,23]. The release of allelochemicals is dependent on the habits and habitats of the plants[4]. Stress conditions have been found to stimulate these allelopathic properties.

Soil on the other hand plays an essential role in the synthesis of allelopathic substances. Phenolic compounds accumulate in the rhizosphere soil and influence the accumulation and availability of soil nutrients as well as the rate of nutrient cycling[1]. The release of allelochemicals is also dependent on environmental factors like nutrition, humidity, radiation, temperature, pests as well as diseases[17]. Soil pH alteration and changes in the soil microbial activity, help the invasive plants to modify the nutrient uptake of the native plants[24]. During communication for space and role in their habitat, these allelochemicals can affect plant's growth signaling, showing the changes in mineral uptake, stomatal closure and water stress, and influence on respiration, photosynthesis, protein synthesis and enzyme activities, also changes in oxidative stress and in membrane permeability and some other changes in the general cell functioning[15]. Plant communications are also influenced by allelopathic non-pathogenic microorganisms in their root zone, on the root surface and on root hairs. Plant growth-promoting rhizobacteria and deleterious rhizobacteria have been shown to affect the growth of higher plants and are also related to soil toxicity. The rhizosphere soil microbes also contribute positively to the plants, they can even alter the forms and nature of allelochemicals[15,25,26].

***Plantago ovate* Forsk. and *Vigna radiata* (L.) Wilczek:** *Plantago ovata* Forsk. belongs to the family Plantaginaceae, a dicot annual herb, usually grown in arid conditions in the states of Gujarat and Rajasthan. So far it is known that for the Purulia and Bakura districts of West Bengal the soil and climate conditions may favour their cultivation. It has medicinal and economic importance. It is useful mainly in the pharmaceutical, agricultural and food industries. Once it was also cultivated in the medicinal plant garden, Ramakrishna Mission Ashrama, Narendrapur, Kolkata. The genus *Plantago* comprises 275 species all over the world. It has been documented to contain several phytochemicals. It is commonly called in English as psyllium and it is known for its rich source of polyphenolic compounds. The investigation of the phytochemicals present in this plant has led to the discovery of different types of polysaccharides, phenylpropanoid glycosides, alkaloids, triterpenes, flavonoids, and phenolic acids as the main bioactive compounds in their aerial parts and the antioxidant potential of its compounds have been studied in various species of *Plantago*[27,28]. Talukdar *et al., (2016)*[21] have worked on the antioxidant activity and HPLC analysis of the phenolic compounds of *Plantago ovata* and the effect of their exogenous additives on the accumulation of phenolic compounds where they have found different kinds of polyphenols[21]. Kundu *et al., (2018)*[29] have shown their work on Chromium (VI) – induced stress response in *Plantago ovata, in vitro* where

they have also found new kinds of major polyphenols[29]. The different types of polyphenols found to be present in *Plantago ovata* are enlisted in Table 1. *Vigna radiata (*L.*)* Wilczek belongs to the family Fabaceae commonly known as mung bean or green gram, grows in the warm season, is capable of growing in different soil types and is known as an important pulse crop, rich in protein and grown majorly in tropical, South and Southeast Asia and as introduced in Sundarban, West Bengal[30,31].

**Table1: Different types of polyphenols present in *Plantago ovata*.**

| Non-nitrogen containing polyphenols found in present study in case of *Plantago ovata* | |
|---|---|
| **Phenolic acids** | **Flavonoids** |
| • Gallic acid | • Rutin |
| • Trans-cinnamic acid | • Quercetin |
| • Vanillic acid | • Luteolin-7-o-β-D-glucoside |
| | • ±Catechin |

***Materials and Methods:*** **Procurement of Seeds, Imbibition, Sterilization and Seed Transfer:** The seeds of *P.ovata* were procured from Gujarat Agricultural University, Anand, Gujarat, India and *V.radiata* seeds were procured from the local market in Kolkata, India (Figure 2). Seeds were imbibed, sterilized, and then transferred for germination on the agar-sucrose medium under standard laboratory conditions as described by Pal and Raychaudhuri, *(2001)*[32].

***Plantago Ovata Extract Preparation and Addition:*** The seedlings of 7 days-old *P.ovata* were taken to make the extract, with 50% ethanol as the solvent. Mixtures were ultrasonicated and then centrifuged at 10,000g for 5 minutes, different concentrations of the extract were prepared (20mg/ml and 40mg/ml) and added to the test plants *P.ovata* and *V.radiata* on their 7$^{th}$ day. Then on the 11$^{th}$ day, the plants were harvested and their roots and shoots lengths were recorded.

**Total Polyphenol Content:** Total polyphenol content was determined using Folin-Ciocalteu Reagent (FCR) as described by Singleton *et al., (1999)*[33] slightly modified in our laboratory[33]. The absorbance was then recorded at 760nm using a Shimadzu UV spectrophotometer. Total polyphenol content was expressed as g Gallic Acid Equivalent (GAE)/kg of fresh weight (FW) of each sample by comparing with the gallic acid standard curve which was prepared with a range of standard gallic acid concentrations from 0-100µg/ml.

**DPPH Radical Scavenging Activity:** DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical scavenging activity was determined by the method of Brand-Williams *et al., (1995)*[34] with some modifications[34]. Absorbance was taken at 517nm and their antioxidant capacity was calculated.

***Statistical Analysis:*** All the experimental results were represented as mean ± SEM (standard error of the mean) and means were compared using Student's t-test using KyPlot (version 6.0). All the experiments were triplicated.

***Results and Discussion:*** Based on the preliminary data, in the case of the same plant *Plantago ovata*, it was observed that the root-shoot length of the *Plantago ovata* has decreased at 20mg/ml *P.ovata* ethanolic extract concentration. Total polyphenol content has majorly increased, at 20mg/ml concentration of *P.ovata* extract whereas, DPPH free radical scavenging activity has significantly increased with the increase in the concentration of the *P.ovata* extract, from which it can be concluded that there might be other types of plant antioxidants present other than polyphenols which are responsible for the scavenging activity of the DPPH free radical. In many different plants allelopathic components have shown activities of peroxidase and superoxide dismutase in their roots, but no direct relationship has been found with total phenolic content and antioxidant activity[17]. Overall, the sample

**Fig. 2:** Pictures of seeds of *Plantago ovata* (Left) and *Vigna radiata* (Right).

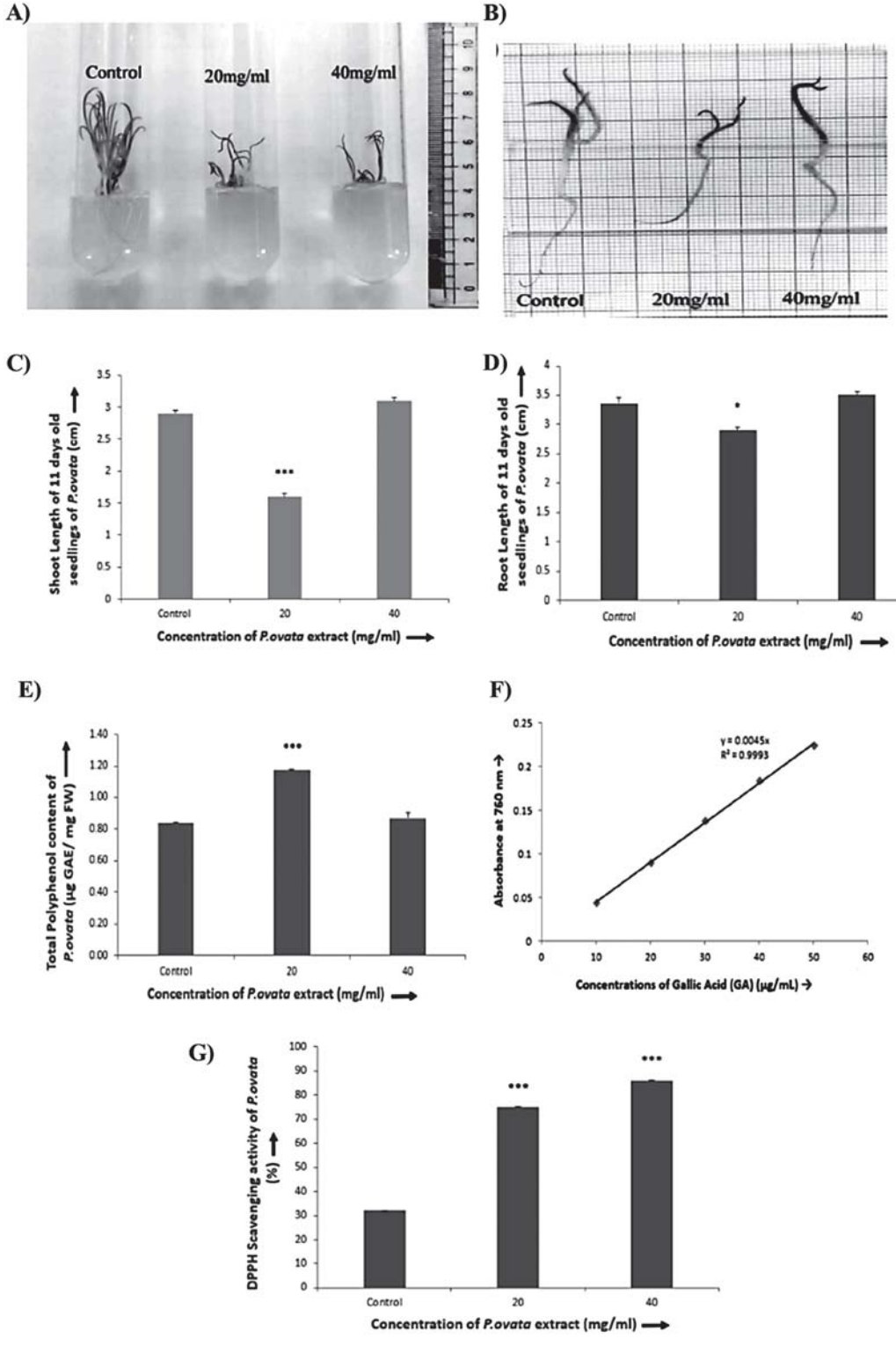


**Fig. 3:** (A) and (B) The 11 days old seedlings of Plantago ovata grown in agar-sucrose medium and on the scale of centimetres respectively, the aforementioned concentrations of 50% ethanolic extracts of 7 days old Plantago ovata seedlings was given on the 7$^{th}$ day.(C) and (D) alteration in the root and shoot length of 11 days old seedlings of *Plantago ovata* respectively, the aforementioned concentrations of 50% ethanolic extracts of 7 days *Plantago ovata* seedlings was given on the 7$^{th}$ day. (E) Total polyphenol content of 50% ethanolic extracts of 11 days old *Plantago ovata* seedlings treated with increasing concentrations of *P.ovata* extract. (F) Standard curve of gallic acid (GA) from which the unknown concentrations of total polyphenols of the ethanolic plant extracts are calculated. (G) DPPH free radical scavenging activity of 50% ethanolic extracts of 11 days old *Plantago ovata* seedlings treated with increasing concentrations of *P.ovata* extract. The data are represented as mean ± standard error of mean (SEM) (n = 3). Asterisks denote the level of significance; *p ≤0.05, **p ≤0.01, ***p ≤0.001.

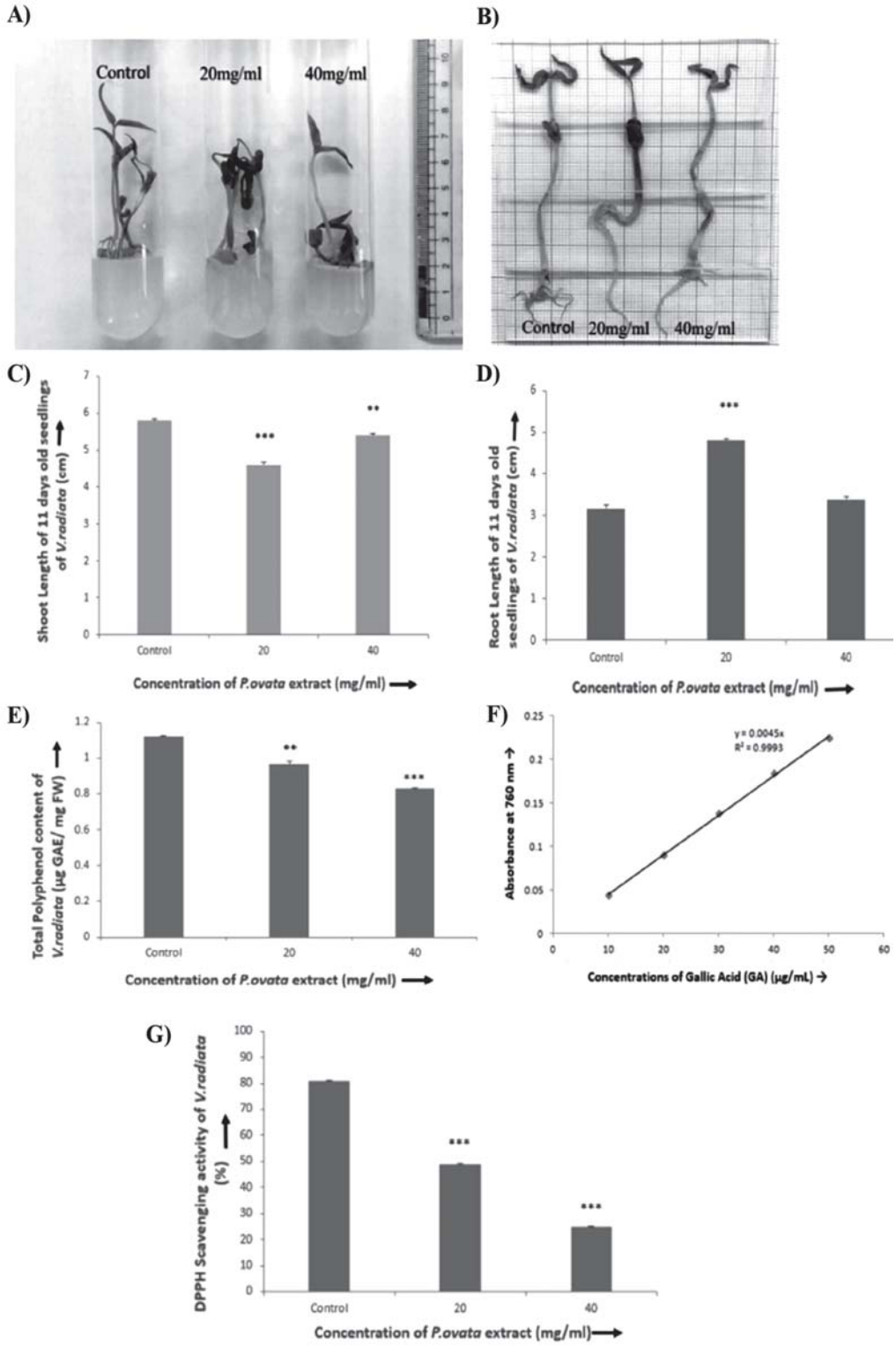


**Fig. 4:** (A) and (B) The 11 days old seedlings of *Vigna radiata* grown in agar-sucrose medium and on the scale of centimetres respectively, the aforementioned concentrations of 50% ethanolic extracts of 7 days old Plantago ovata seedlings was given on the 7th day. (C) and (D) alteration in the root and shoot length of 11 days old seedlings of *Vigna radiata*, the aforementioned concentrations of 50% ethanolic extracts of 7 days old *Plantago ovata* seedlings was given on the 7$^{th}$ day. (E) Total polyphenol content of 50% ethanolic extracts of 11 days old *Vigna radiata* seedlings treated with increasing concentrations of *P.ovata* extract. (F) Standard curve of gallic acid (GA) from which the unknown concentrations of total polyphenols of the ethanolic plant extracts are calculated. (G) DPPH free radical scavenging activity of 50% ethanolic extracts of 11 days old *Vigna radiata* seedlings treated with increasing concentrations of *P.ovata* extract. The data are represented as mean ± standard error of mean (SEM) (n = 3). Asterisks denote the level of significance; *p ≤0.05, **p ≤0.01, ***p ≤0.001.

containing 20mg/ml concentration of *P.ovata* extract has shown a significant inhibitory impact on the overall plant yield which might be considered autotoxicity among the same species (Figure 3). In the case of *Vigna radiata* (mungbean) it is observed that the sample containing 20mg/ml concentration of *P.ovata* extract has an inhibitory impact on the shoot length but a stimulatory impact on the root length whereas, the sample containing 40mg/ml concentration of *P.ovata* extract has a significant inhibitory impact on the shoot length only. Total polyphenolic content and DPPH free radical scavenging activity both have significantly decreased with the increase in the concentration of the *P.ovata* extract. In this case, polyphenols are more likely to be responsible for the DPPH free radical scavenging activity as found in the previous studies of our laboratory experiment[21]. Other studies like walnut leaf extract has also been shown to inhibit the seedling growth of weeds like *Amaranthus retroflexus* and *Chenopodium album*[35]. Overall, the samples have shown an inhibitory impact on the overall yield of the plant (Figure 4). Based on this preliminary data no such significant difference in the yield of the two different plants was observed, but further fieldwork experiments are required to support these laboratory experiments and the time of sowing of both *Plantago ovata* and *Vigna radiata* is an important aspect for the experimental design to analyze their germination percentage based on their natural cultivators.

***Conclusions:*** A proper methodology is necessary for the understanding and utilizing the knowledge of plant allelopathy and to integrate such chemical interactions into ecological theory. It further requires research on the proper identification and characterization of the allelochemicals. To perform such investigations computational, laboratory and fieldwork experiments must go hand-in-hand for more accurate and stronger allelopathic applications for a sustainable agricultural and environmental prospect.


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**SRIJITA GANGULY**
**SAPTAPARNA DUTTA**
**SARMISTHA RAYCHAUDHURI**

Plant Molecular Biology Laboratory
Department of Biophysics, Molecular Biology and Bioinformatics, University of Calcutta,
92, A.P.C. Road, Kolkata- 700009, India.

Co-authors: srijita2834@gmail.com, saptaparnadutta.09@gmail.com, sarmistharc@gmail.com



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