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EVALUATION OF PLANTS LEACHATES AS PHYTO-STIMULATORS THROUGH SEED PRIMING

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ABSTRACT

Phytoleachates obtained from culinary items exhibits the phyto stimulatory activity in enhancing the seed germination in paddy, sunflower and green gram seed samples. Out of the selected culinary items rose, fava bean, avacado, apple and manihot plants extract favoured the seed germinaiton followed by increased seedling growth. Apart from these parameters, the same plants items leachates also showed high activity of alpha amylase in the germlings of all the selected crop species (paddy, sunflower and green gram). The promising phytoleachates also resulted in the high phenolics accumulation proved the resistance to the seed borne pathogenic fungi. The seed borne pathogens like *Alternaria padwikii, Fusarium oxysporum, F. moniliforme,* in paddy, *Alternaria zinniae, Macrophomina phaseolina* in sunflower in sunflower and *M. phaseolina* in green gram compared to their control. The incidence of these fungal species was diminished to a greater extent over control; hence possibly strengthen the seedlings growth. The IAA level was also found to be more in seedlings of respected phyto leachates, which might be the probable role of phyto leachates in triggering the early growth leads to escape from the action of pathogens.

INTRODUCTION

Phyto-leachates are the substances leach from the cut portion of the plant materials in to the surrounding medium, which include certain nutrients like sugars, aminoacids, phytohormones even some secondary metabolites. They play a role in nourishing the plants helps in their growth. Some of them favour the growing plants through stimulation. Component wise stimulation of the growing seedlings is depending on the type of phyto-leachates, which is concentration dependant. Their synergistic effect might have played an important role as nutrients during their early growth stages.

Plant hormones are known as phytohormones in botanical terms. They are chemicals just like animal hormones that help in the growth, development, and functioning of plants. Like animals, plants too are living organisms that function as a unit. They carry out vital biochemical reactions that are required to survive. These biochemical reactions require hormones also known as 'plant growth substances'. These hormones help in the formation of leaves, flowers, stems, fruit, etc. They also help in determining the sex of the flowers, the color of the fruits, and leaves. They help in formation of tissues, respiration, energy production, and even plant longevity and death. Just as hormones are necessary for an animal body to function without any glitches, they too help the green living beings to survive normally. In the present study, leachates of culinary items (mint, murraya, lemon, ginger, garlic, spinach leaves/fruits/roots), pooja items (bilva, kondamavu, artimisia, beetal leaves), seeds (black pepper, capsicum, curcuma, faba bean, cowpea field bean, pea), flowers (kanakambaram, sevanthi, marigold, tuberose, rose, jasmine), rhizomes (manihot, suvarna gedde, muli, kesavu), fruits (cucumber, bitter gourd, bhendi, mango, sapota, avocado, water melon), fruit peels (apple, papaya, ananas), plants and weeds leaves (papaya, muththuga, elegalli, toddalia, coriander, ocimum, garike, konnari, pullampacchi, cymbopogan, phyllanthus), were utilized to evaluate their stimulatory/ regulatory effect on the seed germination, seedling growth, growth stimulating energy enzymes (amylase) and hormones (IAA), mycoflora of three representative crop species viz., paddy, sunflower and green gram.

MATERIALS AND METHODS

Considering all the above importance of the phyto-stimulators for the crop improvement, in the present study many plant species which are being used in our daily life have been used to get the leachates from them. The leachates obtained from different plant components of different species have been used to treat the seeds of different crop species *in vitro*.

For this purpose the components of different plant species preferred as culinary additives includes coriander leaves (*Coriandrum sativum*-Apiaceae), Mint leaves (*Mentha arvensis*-Lamiaceae), Onion bulb (*Allium cepa*-

Amaryllidaceae), Garlic bulb (*Allium sativum*-Amaryllidaceae), Ginger rhizome (*Zingiber officinale*-Zingiberaceae), Mustard seeds (*Brassica juncea*-Brassicaceae), Green chilli fruits (*Capsicum annuum*-Solanaceae), Palak leaves (*Spenacia olaracea*-Amaranthaceae), Lemon grass leaves (*Cymbopogon citratratus*-Poaceae); daily pooja items includes Tulasi (*Ocimum tenuiflorum*- Lamiaceae), Indian bael/Bilva (*Aegle marmelos*-Rutaceae), Hill mango/Kondamavu (*Commiphora caudata*-Burseraceae), Davana (*Artemisia vulgaris*-Asteraceae), Betel leaves (*Piper betel*-Piperaceae); dry seed leachates includes Jeera (Cuminum cyminum-Apiaceae), Termeric (Curcuma longa-Zingiberaceae), Black pepper (Piper nigrum-Piperaceae), Fava beans (Vicia faba- Fabaceae), Mustard (Brassica juncea-Brassicaceae), Dalchini (Cinnamomum zeylanicum-Lauraceae); raw seeds leachates includes.

In all the above cases, 100 g of each plant species component as mentioned was freshly collected from the available source, cut it to pieces of 1 cm size with the help of fine fresh scientific blade in to series of beaker contained 100ml distilled water. Such prepared samples were incubated for a period of 18h at 28+/- 2C. Then each sample leachate was decanted from the plant components by filtering through filter paper. Finally the filtrate was passed through Whatman No. 1 filter paper cones taken on a glass funnel. The filtrates were separately collected in a series of cleaned 100ml glass beakers and stored at 5^0C in the refrigerator for further use. Each filtrate was further used for seed treatment. For this purpose, 100ml of each filtrate of respective plant components was used to soak 1000 seeds of selected agricultural crop species, such as sorghum, ragi, paddy, maize, pea nut, cow pea, green gram, sunflower, etc were separately soaked for overnight (16-18h) at room temperature. Such seeds were further separated from the filtrate by filtering through the de-starched muslin cloth. Further, the treated seeds were spread on the blotter paper sheets for air drying for 72h. Same treated seeds after air drying were subjected to re-soaking in the same plant leachates as mentioned elsewhere. In the previous manner the soaked seeds were again air dried till reaches the original moisture. Such primed seeds of each treatment were further evaluated for their germinability based on the standard procedures.

For the purpose of germination test, in all the cases, 100 treated seeds of each selected representative crop species were subjected to blotter test in paddy, ragdoll test in sunflower and green gram *in vitro* and incubated for a period of 8 days as per the ISTA rules under standard conditions of temperature and relative humidity maintained in the incubation room (Anon., 1996). The incubated seed were then evaluated for the per cent occurrence of the normal seedlings and accordingly the per cent germination was calculated for each sample and treatment. The seeds not treated with leachates but incubated under similar conditions were maintained as control. The data was recorded and tabulated based on the 3 replicates for each treatment. In each cases, ten normal seedlings were randomly selected, their root and shoot length were recorded. Using these values the seedling vigor for each treatment and in each sample was tabulated.

Seeds of similar treatment which showed stimulated germnation and growth were further subjected to assess the phenolic content, alpha amylase content and IAA content following the standard procedures described here under pertaining to each test. In each case, the results were recorded and tabulated in comparison with their respective control.

Determination of total phenolics in the seedlings of plant leachate treated plants

Secondary metabolites accumulated in plants includes phenols, saponines, flavonins, flavones, flavonoids, tannins etc. are known to helpful for the plants defense against herbivores, insects and microbes. Their content varies in different plant species depended on the variable ecological parameters. Polyphenols present in various plant parts including roots. Once they are antimicrobial in function, they are highly protective to the plants against disease causing microbes. They may involve in defense mechanisms physically as well physiologically in host plants. Based on this, in the present study, seedlings of selected crop speices subjected to the phyto-leachates showed stimulated growth were used for the extraction and estimation of the total phenols. For this purpose, 1g of the seedlings of improved growth was taken in to pestle and mortar in grind in to paste using 10 ml 80% ethanol. The homogenate was centrifuged at 10000 rpm for 20min. Supernatant was collected and the residue was re-extracted with 5ml of 80% ethanol. The supernatants were pooled and evaporated to dry in the watch glass. The residue was then dissolved in 5ml of distilled water. This was pipette out in the series of test tubes at 02 to 2ml in the serially increased order. The volume in each tube was made up to 3ml using water and 0.5ml of Folin-Ciocalteau reagent was added. After 3min., 2ml of 20% Na2Co3 solution was added to each tube. The content in each test tube was mixed thoroughly by gentle shaking, manually. Further each tube was placed in a boiling water bath for 1min. Then cooled and the absorbance was measured at 650nm against a reagent blank. The standard curve was prepared using different concentrations of catechol. Using the standard curve the concentration of phenols in each test sample was estimated and it was expressed as mg phenols/100g sample, as per the procedures described by Malick and Singh (1980). The results were recorded and tabulated.

Estimation of alpha amylase in the stimulated seedings of plant leachates treatment

Starch degrading enzymes universally distributed in seedlings, act on glycogen and related polysaccharides. Alpha amylase causes endo-cleavage of substrates and hydrolyses alpha 1, 4 linkages in a random manner. At the time of seed germination, alpha amylase activity

increases and cleaves the starch molecules to simpler glucose. This is easily absorbed by the growing germlings and serves as the initial energy source.

The reducing sugars produced by the action of alpha and /or beta amylase react with dinitrosalicylic acid and reduce it to a brown colored product, nitroaminosalicylic acid. Considering these aspects in the present study, the seedlings of different crop species showing stimulated growth due to plant leachates treatment were subjected to the extraction and estimation of alpha amylase in comparison with the corresponding control, where the seedlings of untreated seeds were served as control.

For this purpose, 1g of seedlings of each crop and treatment were extracted in 10ml of ice cold 10 mM calcium chloride solutions overnight at 4^0C for 20min. The supernatant is used as enzyme source. Then 1ml of starch solution and 1ml of diluted enzyme was taken in a test tube and incubated for 15minutes at 27^0C. The reaction was stopped by adding of 2ml of dinitrosalicyclic acid reagent. The solution was then heated for 5min in a boiling water bath. While the tubes are warm 1ml of potassium sodium tartrate solution was added, then cooled in running water. The volume was made up 10ml by addition of 6ml water. The absorbance was measured at 560nm. In order to facilitate the estimation of unknown samples, a standard graph was prepared with 1-100ug maltose. Unit of alpha amylase was expressed as mg of maltose produced during 5minutes incubation with 1% starch, following the producers of Niku-Paavola and Enari (1972); Kruger (1972).

Estimation of IAA in the seedlings of plant leachates treated seeds

Growth stimulation in plants is usually operated by the influence of inbuilt *phytohormones* like IAA, NAA etc. The IAA in plants is known to influence the cell elongation and hence increase the internodal distance, apart from this they are known to reduce senescence and enhance the self-life of the plants. Considering these aspects of influence of hormones on plant growth, in the present study, samples showing stimulated seedling growth due to phyto leachates treatment were subjected for the extraction of IAA *in vitro*.

For this purpose, 5g of seedlings of stimulated treatment was freeze in liquid nitrogen and ground in to find powder using pestle and mortar. Further grinding was continued with 10ml methanol to a fine suspension. The homogenate was filtered using G4 glass seitz filter under suction into a 100ml flask. This was extracted twice by adding 10ml methanol and then once with 5ml. The filtrate was collected and evaporated in a rotary evaporator at 30C to an aqueous residue. To this 10ml of cold 0.5 M K2HPO4 solutions was added so that pH was reached to 8.5. This extract was transferred to a separatory funnel and was shaken with 10ml diethyl ether and each time the lipid fraction was discarded. The pH of the aqueous layer was adjusted to 3 by adding 3ml of 2l.8M phosphoric acid. Then IAA was extracted from this sample using 10ml of diethyl ether. Then it was extracted with 10ml cold 50mM K2HPO4 solution. The pH of this solution was adjusted to 3 with phosphoric acid (>28M) and the IAA was passed into 10ml diethyl ether finally. The ether was then evaporated under reduced pressure. The residue obtained was dissolved in 5ml of cold redistilled methanol.

In order to calculate the IAA in the unknown samples 1ml of the above methanolic extract or each was pipette out in to different test tubes. To each tube 1ml of methanol containing 1, 10, 20 or 30ng of IAA was added, respectively. The content in each tube was dried completely under reduced pressure and cooled at 0C. In each tube 0.2ml of ice cold trifluroacetic acid-acetic anhydride reagent was added and mixed. These tubes were then placed on ice for exactly for 15min to ensure the complete conversion of IAA in to indole alpha pyrone. The reaction was stopped by adding 3ml of water. A blank was prepared by adding first 3ml water to one of four aliquots and 0.2ml reagent was added after 15min. The reading was taken using a spectrophotofluorimeter using and excitation at 440mn and emission at 490 nm for low concentration samples. Then the amount of IAA in unknown was calculated as per the procedures followed by Stoessl and Venis (1970).

Evaluation of the effect of crop seed treatment on the incidence of seed borne fungi

In all the cases of selected crop species, seeds of respective test species were subjected to the soaking in the combined stimulatory plant leachates in the equal ratio (10ml each of 100% concentration). Accordingly, for each species, 400 seeds were separately soaked in the 100ml combined plant leachates at 28+/-2 C in a series of clean petri plates for 16 hours. Such soaked seeds of each target crop species were plated equidistantly on a series of 3 layers of wet blotter discs taken in the Perspex plates. The seeds in the plates were then incubated at 22+/-2C for a period of one week under standard conditions of 12/12h light and darkness. Then the incubated seeds of each crop species were examined carefully for the occurrence of fungi, microscopically. The per cent incidence of seedborne pathogenic fungi was recorded and tabulated. In each case, the seeds of respective crop species soaked in water alone and incubated in the similar manner were considered as corresponding control for comparison.

RESULTS AND DISCUSSION

Table 1: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Culinary items	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Coriander	**98**	**89**	**93**
Mint	92	86	86
Murraya	90	83	80
Lemon	83	80	79
Ginger	86	83	78
Garlic	80	80	76
Spinach	92	84	80
Control	**92**	**81**	**85**

Among the leachates of culinary items coriander leachate treatment showed enhanced seed germination in all the crop species tested. In which paddy showed more germination percentage over its control. In paddy, sunflower and green gram which represents cereal, oil crop and legumes, there was increased germination by 6, 8 and 8 per cent, respectively.

Table 2: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Pooja items	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Ocimum	**99**	**93**	**95**
Bilva	90	90	90
Kondamavu	88	89	90
Artimisia	88	88	89
Beetal	90	93	93
Control	**92**	**81**	**86**

Plants used as pooja items used as leachates showed increased germination percentage in paddy, sunflower and green gram, compared to their respective control. Comapatively, ocimum leachate treatment improved germination by 7 in paddy, 12 in sunflower 9% in green gram. Next to this beetle leaves leachates resulted in increased germination percentage over control.

Table 3: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Dry seed leachates	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Cuminum	**92**	**90**	**92**
Black pepper	89	79	80
Capsicum	85	80	85
Curcuma	86	78	78
Control	**92**	**81**	**88**

Dry seed leachates of cuminum resulted in increased percentage of seed germination in paddy, sunflower and green gram compared to the remaining treatment. In sunflower 9% increase was recorded, whereas paddy the percentage germination was not affected.

Table 4: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Raw seed leachates	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Faba beans	**98**	**96**	**92**
Pea	90	92	90
Cow pea	89	92	90
Field bean	88	86	89
Control	**92**	**80**	**86**

Seed sprouts of a few legumes also enhanced the seed germination in the entire sample tested. Among them Faba beans sprout leachates was found to enhance the seed germination from 6 to 16 per cent, which was followed by pea seed sprout leachates.

Table 5: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Flowers leachates	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Kanakambarm	96	96	90
Sevanthi	94	94	90
Marigold	94	90	89
Tuberose	96	94	94
Rose	**98**	**96**	**96**
Jasmine	96	95	96
Control	**92**	**80**	**86**

Table revealed the stimulated effect of rose petal leachates in all the test species. Compared to kanakambaram and tube rose, rose petal leachates was proved better in enhanced germination in the crop species selected for evaluation (Table 5). The seed germination was enhanced by 6, 16 and 10 per cent in paddy, sunflower, green gram, respectively.

Table 6: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Rhizomes leachates	Crop seeds used for phytoleachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Manihot	**96**	**94**	**95**
Suvarna gedde	90	89	88
Muli	92	90	90
Kesavu	92	90	92
Control	**92**	**80**	**86**

Among the leachates of rhizomatous plants rhizome leachates of manihot has increased the germination of all the test crop species. Though many of the rhizome leachates showed stimulatory effect, suvarna gedde leachates slightly affected the seed germination, compared its control (Table 6).

Table 7: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Fruits peel leachates	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Ridge gourd	92	89	90
Apple	**96**	**86**	**88**
Papaya	80	79	79
Ananas	80	78	76
Control	**92**	**83**	**86**

Fruit peel leachates treatment showed increased germination in all the test samples. Among the fruit peel leachates apple peel leachates showed enhanced seed germination over control and other treatment. In which papaya and ananas peel leachates reduced the seed germination compared to its control (Table 7).

Table 8: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Fruits leachates	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Cucumber	90	80	86
Bhendi	92	80	88
Bitter gourd	89	78	79
Mango	89	78	78
Sapota	86	6	76
Avacado	**96**	**92**	**94**
Watermelon	90	90	90
Control	**92**	**83**	**86**

The data represented in table 9 indicated the stimulatory effect of avocado fruit leachates in the entire crop species used. In which the seed germination was increased by 4, 9 and 8% with respect to paddy, sunflower and green gram (Table 9).

Table 9: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Weeds leaves leachates	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Garike	94	86	85
Konnari	90	83	85
Pullampacchi	89	83	89
Cymbopogan	78	80	79
Phyllanthus	**94**	**92**	**92**
Control	**92**	**83**	**86**

The data in Table 9 revealed the increased germination in all the test species due to phyllanthus leaf leachates over any other treatment. In which sunflower showed 9 per cent increase over its control (Table 9).

Table 10: Evaluation of the plant leachates for their effect on the stimulation of seed germination.

Plant Leaves leachates	Crop seeds used for phyto leachates treatment to asses % seed germination		
	Paddy	Sunflower	Green gram
Papaya	90	80	80
Muththga	94	85	89
Elegalli	89	80	78
Toddalia	**94**	**94**	**94**
Control	**92**	**83**	**86**

Table 10 revealed the data with respect to enhanced percentage of germination over control due to toddalia leachate treatment (Table 10). All the test species there was increased germination due to toddalia leachates, which was 2, 11and 8 per cent with respect to paddy, sunflower and green gram. Next to this muttuga leaf leachate treatment favored the germination all the test samples.

Table 11: Estimation of enhanced seedling growth due to plant leachates treatment.

Plants sources for leachates	Stimulated seedling growth of crops species subjected to phyto leachates								
	Paddy			Sunflower			Green gram		
	MRL+/-S.E. (cm)	MSL+/-S.E. (cm)	VI	MRL+/-S.E. (cm)	MSL+/-S.E. (cm)	VI	MRL+/-S.E.(cm)	MSL+/-S.E.(cm)	VI
Coriander	6+/-0.1	6+/-0.2	**780**	6+/-0.1	8+/-0.1	**789**	7+/-0.1	6+/-0.1	**892**
Ocimum	5+/-0.1	6+/-0.1	**768**	4+/-0.2	6+/-0.1	**698**	5+/-0.0	5+/-0.1	**568**
Cuminum	4+/-0.1	6+/-0.1	**760**	4+/-0.1	4+/-0.2	**569**	4+/-0.1	5+/-0.1	**609**
Faba beans	8+/-0.2	8+/-0.1	**876**	7+/-0.0	6+/-0.1	**890**	7+/-0.1	8+/-0.1	**898**
Rose	8+/-0.0	9+/-0.1	**890**	8+/-0.1	6+/-0.3	**892**	8+/-0.1	8+/-0.1	**908**
Manihot	6+/-0.1	6+/-0.2	**880**	6+/-0.1	7+/-0.2	**860**	6+/-0.1	6+/-0.1	**890**
Apple	7+/-0.1	7+/-0.1	**880**	8+/-0.1	7+/-0.1	**880**	8+/-0.1	7+/-0.2	**899**
Avacado	8+/-0.1	8+/-0.2	**906**	8+/-0.1	8+/-0.2	**890**	8+/-0.1	9+/-0.1	**920**
Phyllanthus	8+/-0.1	7+/-0.1	**900**	8+/-0.2	8+/-0.1	**908**	8+/-0.3	7+/-0.1	**900**
Toddalia	7+/-0.0	7+/-0.3	**880**	7+/-0.2	6+/-0.1	**900**	7+/-0.3	7+/-0.1	**896**
Control	**5**+/-0.3	**4**+/-0.1	**740**	**4**+/-0.1	**5**+/-0.2	**720**	**3**+/-0.1	**4**+/-0.1	**780**

MRL=Mean Root Length, MSL=Mean Shoot Length, S.E.=Standard Error
Data based on the average of 10 seedlings of 4 replicates

Table 11 revealed the data with respect to the seedling growth in all the test crop species. As per the information provided in this table, phytoleachates which stimulated germination over their respective control, normal seedlings indicated the enhanced root and shoot growth to a higher extent compared to their control. Rose, Phyllanthus, avocado and manihot leachates were stimulated the seedling growth and their vigor to a greater extent, comparatively. Paddy showed equal growth of root and shoot with respect to treatment with the leachates of faba beans, toddalia and avocado. Avacado and rose leachates proved better over other

treatment in enhancing the seedling growth. Vigor index of all the test species was also enhanced in avocado, phyllanthus, rose and faba beans leachates treatment (Table 11).

The increase in the seedling growth is perhaps due to the nutritive factors present in the phyto-leachates, which may vary in their concentration depending up on the plant species. Which is normally regulated by the ecological factors like RH, temperature, altitude, species variability, aerosols, light, edaphic factors, down pour etc. The stimulated growth may also due to secondary metabolites like phenols, favonoids, saponins, which are released into the leachates involved in the prevention of adverse effect created by the biotic stress due to microbes. Hence, the seedlings showed stimulated growth, in which there may be the avoidance of pathogenic effect of the microbes as well less competition to the nutrition. It is also possible that the antioxidants may occur in the leachates, which might have played a role in mitigating the effect of free radicals and hence stimulated the cell division and ultimately the growth. Apart from these it is also possible to claim that the phyto leachates might have also contained the plant growth hormones like IAA or its allied stimulatory factors, hence there was increased plant growth in all the test samples. One more possibility is that in all the treatment crop species, the seeds on imbibitions of moisture through leachates, alpha amylases get activated and might have provided the initial energy to the growing seedlings, and hence triggered the germination and vigor. This might be the additional thought for quoting the suitable reason for the enhanced seed germination and seedling growth. All these factors might have operated together and resulted the enhanced germination and seedling vigor.

Table 12: Estimation of A-amylase activity in the stimulated crop seedlings due to plant leachates treatment.

Plants sources for leachates	A-amylase activity in crop seedlings subjected to phyto leachates (IU/g fresh wt.)		
	Paddy	Sunflower	Green gram
Coriander	0.24	0.12	0.13
Ocimum	0.13	0.14	0.11
Cuminum	0.10	0.12	0.13
Faba beans	**0.78**	**0.49**	**0.91**
Rose	0.73	0.60	0.82
Manihot	0.62	0.44	0.61
Apple	0.60	0.40	0.88
Avacado	0.53	0.65	0.63
Phyllanthus	0.24	0.34	0.43
Toddalia	0.13	0.15	0.32
Control	**0.02**	**0.02**	**0.12**

In case of Faba bean, rose, apple and avocado leachates treatment all the test species showed the high activitiy of alpha amylase, which is more than threefold compared to their respective control (Table 12). In which fava bean leachates treatment stood superior, hence there was triggered growth of seedlings in the early stages itself. So this may be further used in combination with other stimulatory plants extract, which may result in the improved plant growth to yield productive plants in acting as phyto-tonics.

Table 13: Estimation of total phenols in the stimulated crop seedlings due to plant leachates treatment.

Plants sources for leachates	Total phenolics in crop seedlings subjected to phytoleachates (mg GAE/100g)		
	Paddy	Sunflower	Green gram
Coriander	16.90	48.42	41.90
Ocimum	18.77	45.01	43.63
Cuminum	8.98	23.56	30.90
Faba beans	15.90	87.58	90.80
Rose	34.82	68.05	84.50
Manihot	53.74	79.00	79.98
Apple	65.54	89.08	79.09
Avacado	73.98	93.02	91.07
Phyllanthus	78.00	87.00	87.86
Toddalia	79.35	82.34	76.89
Control	9.01	15.34	9.90

Data in table 13 indicated the possible extent of phytostimulatory activity of plant leachates (Table 13). Though there is variability in the content of phenols in the test species subjected to phytoleachates treatment, avocado stood superior in phenol content. This might have suppressed the colonization of the pathogenic fungi,

and hence there was no reduction in growth, instead there was enhanced seedling growth.

Table 14: Estimation of IAA in the stimulated crop seedlings due to plant leachates treatment.

Plants sources for leachates	Amount of IAA in crop seedlings subjected to phytoleachates (ug/g)		
	Paddy	Sunflower	Green gram
Coriander	0.03	0.05	0.05
Ocimum	0.04	0.06	0.05
Cuminum	0.06	0.12	0.76
Faba beans	1.40	2.00	1.90
Rose	2.90	1.65	1.98
Manihot	1.65	1.97	1.24
Apple	2.01	1.98	2.12
Avacado	**2.82**	**2.00**	**2.45**
Phyllanthus	1.88	2.07	1.92
Toddalia	1.22	2.01	1.89
Control	**0.02**	**0.03**	**0.12**

Since, auxins play a role in the cell growth, cell division and cell transformation, in the present study the seedlings of test species of stimulated growth were subjected to the IAA extraction and its content was determined following the available standard procedures as mentioned elsewhere. Accordingly the data pertaining to this was tabulated in the table 14. In all the cases, there was more content of IAA was recorded compared to their control (Table 14). It is interesting to note that there was parallel information to that of other stimulated aspects as shown in representative tables above. Rose, fava beans, phyllanthus, apple and avocado leachates showed increased content of IAA in the seedlings of selected crop species. In which comparatively more content of IAA was recorded in the seedlings of all target crop species, which is in correlation with that of other features of growth stimulation.

This is probably due to triggering of the precursor of IAA during the metabolic faces occur via growth stages of the seedlings. Along with this many more enzymatic pathway also involved in this process, which might be the cause of growth stimulation. The high level of hormone during the plant growth might also play a role in hindering the microbial growth both fungi and bactria which usually interfere with the plant metabolism through the production of secondary metabolites from them. IAA as it occurs in the root and shoot meristem at the earlier developmental stages of seedlings might have triggered the cell elongation and multiplication than in the control. It is also possible that the more development of root initially results in the robust vigorous seedlings ultimately the productive plants in the field.

Table 15: Effect of phytoleachates on the incidence of seedborne fungi of selected crop species.

Fungal species	% incidence of seed borne fungi in crop seed samples*					
	Paddy		Sunflower		Green gram	
	Control	Treatment	Control	Treatment	Control	Treatment
Alternaria padwikii	12	2	-	-	-	-
Alternaria zinnia	-	-	5	1	-	-
Drechslera oryzae	15	4	-	-	-	-
Fusarium moniliforme	6	4	2	1	5	2
Fusarium oxysporum	3	2	5	3	6	2
Macrophomina phaseolina	-	-	3	1	5	2

*Data based on the consideration of 400 seeds

Data represented in table 15 indicated the variable incidence of target seedborne fungi in the selected samples of different treatment. The effect of combined treating agents indicated the depleted level of target fungi namely *Alternaria padwikii*, *Drechslera oryzae*, *Fusarium moniliforme*, *F. oxysporum* in paddy; *Alternaria zinnia*, *Fusarium moniliforme*, *F. oxysporum* and *Macrophomina phaseolina* in sunflower; *F. moniliforme*, *F. oxysporum* and *M. phaseolina* in green gram, respectively. In all the cases of treatment with respect to test crop species, the incidence of target fungi was found to reduce to a considerable extent compared to their respective control.

The reduction in the incidence of fungi in all the test species is probably due to combined effect of phenols, antioxidants and other metabolites, irrespective of their type and concentration, which might have played a role in the prevention of their sporulation. They might have reduced the vegetative stage itself as the result there was

reduction in their incidence. Variable occurrence in the test species is may be due the variation in the permeability to the ambient solution during incubation.

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