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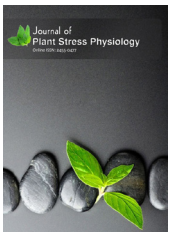

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Elucidation of seed dormancy and phytohormones by germination after exogenous foliage whey application

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ABSTRACT

A study was conducted with an aim of achieving 100% seed germination and to evaluate the rate of dormancy upon exogenous application of the deproteinised leaf juice (DPJ) in various leguminous and non leguminous seeds. DPJ if inducing retardation of dormancy, the activity of enzyme was analysed to prove the presence of phytohormones. DPJ from the three non leguminous foliages of brinjal, radddish and dasheen were prepared by green crop fractionation. The seed germination results obtained by the paper towel method and the activity of enzyme protease in DPJ of radish and *Colocasia* leaves separately. There was variation in germination rate by different DPJ influence. Some seeds showed fast germination while some germinated late. Few seeds were not germinated by control. In some seeds, DPJ decreased the rate of germination while on the contrary there was rapid growth of seedlings by the potentiality of DPJ. DPJ found mutagenic by its influencing inhibitory expression in seedling growth in some cases. The enzyme protease released by phytohormone gibberellin during the seed germination. Positive glyoxlic test indicated presence of auxins in whey.

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INTRODUCTION

The yields of extractable protein from leaves taken at the time of harvest of the edible part (root) from brassicas, radish ranged between 76 to 170 kg/ha [1, 2]. The nutritive value of LPC prepared from leaves of radish is superior to that prepared from lucerne [3]. In Leaf protein research [4], the green crop is fractionated to pulp and squeezed. Therefore, the juice and fibres are obtained. The juice and pressed crop are the by-products of GCF (Green crop fractionation). The liquid residue obtained by juice filtration called as deproteinised juice (DPJ) is another by-product. The enzymes present in it are denatured after heating at 90°C. During this process, the DPJ is thrown randomly. Therefore, proper employment of DPJ is necessary. When the juice is heated, majority of the nutrients are disposed in the deproteinised juice including enzymes and hormones. Few amino acids retains in DPJ during juice filtration.

During present investigation, the exogenous soaking effect of the DPJ was experimented on seed germination. In earlier findings, the effect of the DPJ made up of various forages viz., Lucerne and *Eichhornia crassipes*, was used for seed germination. Previously it was observed that at appreciable concentration of DPJ, the number of leaves, length of the plants, dry weight of the crop and the protein content gets enhanced [5]. Therefore, for the purpose to analyse the factors responsible for the induction of seed germination and the seedling growth, the presence enzymes protease in whey present was taken into consideration. These enzymes are activated by the phytohormones in the germinating seeds. The phytohormones also overcome the seed dormancy. However, the enzymes present in DPJ is studied by the cup plate assay. The effect of hydrolytic enzymes on seed germination of cotton hybrids was experimented [6]. *Rhizobium* bacteria also was grown successfully on DPJ indicated the potentiality of nitrogen fixation [7]. Yeast fermentation by foliage DPJ also stimulate production of the metabolite protease, amylase, cellulase and lipase enzymes [8]. Protease activity per gram of protein nitrogen increases in the leaves of wheat during the transformation of the shoot apex from vegetative into reproductive state. The potentiality of *Moringa* leaves because of the presence of cytokinin hormone, the extract of it when applied to wheat plants, there was the stimulation in salinity stress tolerance [9]. Among the three monocotyledonous crops viz., wheat, jowar and bajra, there was the decrease in the proline content and stomatal size effecting transpirational reduction due to the application of various DPJ made up of leguminous and non leguminous forage crops.

Protease degrade reserve proteins during germination of seeds. Plant proteases are involved in playing key roles in the generation of signaling molecules and regulates the essential cellular processes such as cell division and metabolism. Gibberellic acid increases the enzymes amylases, proteases and phosphatises. Salicylic acid effect on the proteases and ureases was studied in Norway Spruce stands [10]. Salicylic acid plant hormone also found inducing protease activity during programmed cell death in *Lycopersicon* plants [11].

It was already investigated that DPJ when used as a culture medium to grow the fungi, it induces more mycelial cell proliferation as compared with usual Glucose nitrate (GN) medium and even induces comparatively more protease and amylase enzyme activities [12, 13]. On other hand, protease enzyme was inhibited by onion DPJ during *Trichoderma* growth [14]. Juice of cauliflower leaves when fermented for various periods of hours also secretes the enzymes protease and amylase by which protein gets reduced by breakdown. It is because of the presence of bacterial lactic acid consumtion of carbohydrates and proteins in the fermentation of leaf juice [15]. The enzyme amylase gets activated during seed germination from the embryonic axis to the cotyledons because of the presence of the hormone auxin [16]. Therefore it is the prediction that DPJ consists of the hormone auxin as there is the presence of enzyme amylase. Amylase is the isozyme and the proteases actually degrades the superoxide dismutases. The presence of enzyme proteases are studied in the leaf extracts of ethnomedicinal plants [17]. Leaf proteases also could be exploited for various industrial, food and pharmaceutical applications.

In the present investigation, DPJ prepared after the GCF, when applied after soaking exogenously to the seeds, the rate of germination and seedling growth is expected to boost because of the presence of the hormones and the enzyme present in it. To overcome the dormancy of the seeds and to optimize seedling growth, the deproteinised leaf extracts prepared from *Colocasia*, *Raphanus* and brinjal were considered for treatment. Seeds of different leguminous, non leguminous and some monocots were taken into consideration for the observation of parameters like dormant seeds and rate of germination.

MATERIALS AND METHODS

Percent Seed Germination and DPJ Preparation

Twenty two (22) seeds were taken for the germination viz, sunflower (*Helianthus annus*. L), chilli (*Capsicum annum* L), lady's finger (*Abelmoschus esculentus* L), *Linseed* (*Linum usitassimum* L), snake gourd (*Trichsanthes cucumerina* L), fenugreek (*Trigonella foenicum graceum* L.), horse gram (*Macrotyloma uniflora* L), Pumpkin (*Cucurbita maxima* L), Mustard (*Brassica campestris* L), Castor (*Ricinus communis*. L), Coriander (*Coriandrum sativum* L), cucumber (*Cucumis sativus* L), ragi (*Eleusine coracana* L), sesame (*Sesamum indicum* L), bitter gourd (*Momordica charantia* L), bajra (*Pennysetum typhoides* L), *basil* (*Ocimum basilicum* L), Fennel (*Foenicum vulgare* L.), gardencress pepperweed (*Lepidium sativum* L), cumin (*Cuminum cyminum* L) and cantaloupe (*Cucurbita pepo* L.).

Foliage juice is prepared by squeezing of the pulp obtained by GCF. This juice heated to 90°C and filtered after the cooling. The supernatant is called as deproteinised juice and the precipitated residue obtained settled at the bottom is called as Leaf protein concentrate (LPC). LPC is composed of amino acids and vitamins [18, 19, 20].

The above mentioned seeds were soaked in water (control) and DPJ for 24 hours after the treatment of mercuric chloride to prevent contamination. Then by following the paper towel method, the filter paper used and folded after the seeds are arranged in sequence on it [21]. The folded papers were kept at room temperature in the laboratory for 15 days in the water. There was the continuous absorption flow of the water to provide moisture to the soaked seeds to germinate and grow. After 15 days the folded papers opened and the germinated seeds counted to calculate the percentage and recorded.

The percent germination of all seeds were recorded. When the rate of germination found less the seeds were treated with the foliage DPJ from brinal (*Solanum melongena*. L.), dasheen (*Colocasia esculentus* L) and radish (*Raphanus sativus* L).

Study of DPJ Enzyme Assay

The fresh deproteinised juice (DPJ) was prepared by the process of GCF. The pulp obtained is pressed, gives rise to fibre and juice. After the pulp squeezing, the juice is obtained and heated only at 50°C so that the enzymes will not be denatured. The proteins in the juice gets coagulated. The supernatant is considered as DPJ. The precipitate is called as Leaf protein concentrate (LPC). The agar "cup plate" diffusion assay of Dingle et al [22] was used to quantify the activity of a variety of enzymes in DPJ. The gel was developed after incubation by flooding the assay plate. DPJ samples were pipetted into diameters of wells (mm) punched in the agarose with a cork borer. It is incubated at 32°C.

Leaf DPJ Protease Assay

The basal medium composed of 2 % agar, 4 % gelatin, 1 % peptone, 1 % casein and pH was adjusted to 6.8 *in vitro*. In this assay, casein acts as a substrate. The poured medium after solidification was pippeted by DPJ in the cavity.

Auxin Precursor Tryptophan Test in DPJ

The glyoxylic test was performed by taking acetic acid (2 ml) in test tube. The fresh DPJ 2 ml was added to it. Again 2 ml of Conc. H_2SO_4 was added by the sides inside test tube. It gives violet colouration and formation of ring when tryptophan is present. DPJ made up of Spinach (*Spinacia oleracia*) and Fenugreek (*Trigonella foenicum graceum*) were taken into consideration for IAA test.

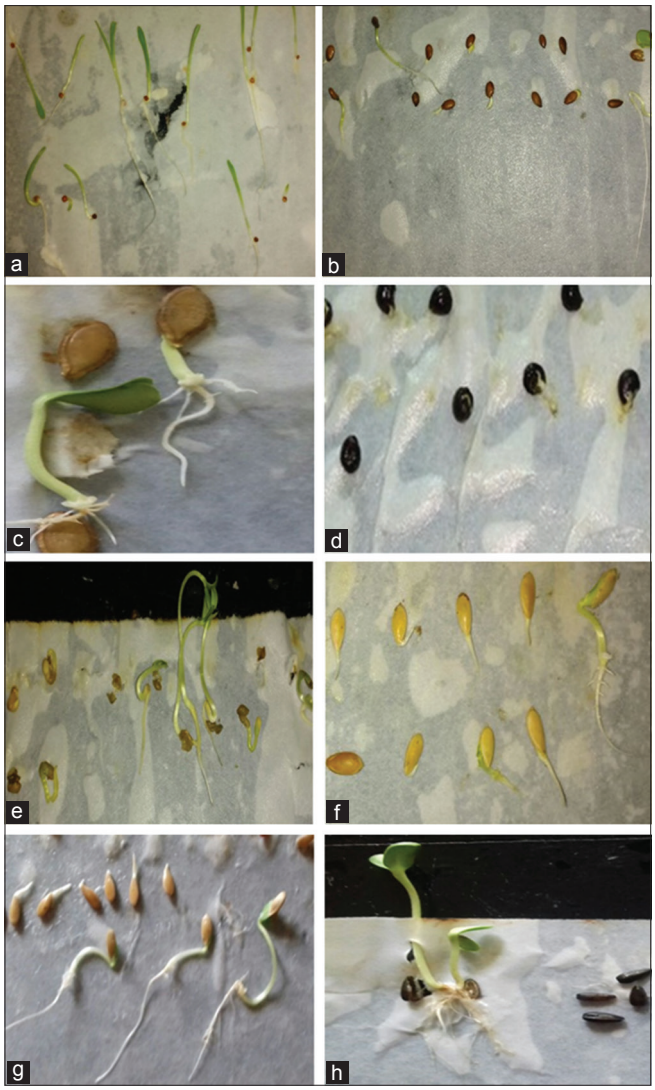

Figure 1: Illustration of Seed germination experiment of following crops by the influence of the fresh concentrated DPJ (a) *Eleusine coracana* (b) Linseed (*Linum usitassimum*) (c) Pumpkin (*Cucurbitamaxima*) (d) *Abelmoschus esculentus.L* (e) Fenugreek (*Trigonella foenicum graceum*) (f) Cantaloupe (*Cucurbita pepo*) (g) Cucumber (*Cucumis sativus*) (h) Sunflower (*Helianthus annus*)

Table 1: a. Illustration of the seeds germinated, inviable seeds or dormant seeds without any treatment

No	Seeds germinated (control)	% germination
1	Sunflower (*Helianthus annus* L)	95
2	*Capsicum annum* L	33
3	*Abelmoschus esculentus* L	100
4	*Linum usitassimum* L	98
5	Snake gourd	24
6	Fenugreek	77
7	*Macrotyloma uniflora* L	100
8	Pumpkin	98
9	Mustard	28
10	Castor	26
11	Coriander	37
12	*Cucumis sativus* L	100
13	*Eleusine coracana*	100
14	*Sesame indicum*	46
15	Cantaloupe	98
	Mean	71

Table 1: b. List of inviable or the dormant seeds and effect of DPJ on germination

Seeds inviable or dormant	Mutagenic effect by DPJ
Bitter gourd	Pumpkin
Pennysetum typhoides. L	Fenugreek
Basil	Cantaloupe
Fennel	Sunflower
Lepidium sativum. L	Sesame
Cumin	Linseed

Table 2: Illustrationtion of the effect of DPJ on the number of germination within 15 seeds of each crop when 20 seeds were arranged for germination

No	Seeds	Seeds germinated by effect of DPJ			
		Brinjal	Radish	*Colocasia*	Mean
1	Pumpkin	1	4	9	5
2	Fenugreek	1	9	18	6
3	Horse gram	9	9	9	10
4	Ragi	15	15	13	14
5	Cantaloupe	5	10	8	8
6	Sunflower	7	7	7	7
7	Sesame	3	7	7	6
8	Linseed	7	7	9	8
	Mean	6	8.5	9.1	
	S. D.	4.65	3.20	2.23	

RESULTS

Eight different seeds were taken into consideration for germination by the treatment of Deproteinised Juice (DPJ). There was poor germination of some seeds in the month of February at 29°C of room temperature by paper towel method. Some seeds found inviable or dormant. Therefore to overcome this dormancy, treatment of the natural leaf extract, which was deproteinised, used. So when the seeds were soaked in different deproteinised leaf extracts and allowed to germinate by paper towel method, there was found the increased growth rate in germination by majority of the seeds.

The list of the germinated seeds and the percent germination is illustrated in Table 1 a, while Table b shows the non viable seeds which were not germinated and dormant. Total 15 seeds from various species were found germinated and 6 seeds showed no response of the germination. The calculated percent of germination was 71.42. Table 1 b shows the dormant seeds and influence of DPJ as mutagenic on the specified seeds after the application on germination. In earlier finding, dry DPJ form lucerne at higher concentrations (1% and 0.5 %) caused chromosomal aberrations in the root tips of onion. In view of this, fresh liquid DPJ directly was considered to treat the seeds to initiate the germination. The result of the number of seeds germinated by the treatment of DPJ and the statistical elucidation is showed in Table 2. Seeds of ragi, horsegram and cantaloupe showed very positive effect of DPJ (Figure 1 F). The poorly germinated seeds were *Capsicum*, mustard, castor, and coriander. These seeds were also not germinated by DPJ

treatment. Research of these seeds in soil germination in progress. *Capsicum* germinates soon in soil. Therefore presently the DPJ was found mutagenic on these seeds. The paper towel method might not found feasible for them. Seeds mentioned in table 2 showed low rate of germination in control, therefore were taken into consideration for the exogenous DPJ application by soaking in it and number of seeds germinated were tested. There was the variation in the effect of different DPJ on germination. Ladyfinger, snakegourd and cucumber seeds germination thrieved well by control (Figure 1 D and G). Table 2 indicates the lowest rate of germination by pumpkin seeds by aubergine DPJ application as compared with radish and dasheen. DPJ found mutagenic on this climber crop (Figure 1 C). While DPJ induced rate of germination i.e., more than 50% in ragi and horsegram crops by three DPJ made from foliage of *Colocasia*, radish and aubergine (Figure 1 A).

Illustration of the comparison of seed germinaton by the influence of various DPJ by the error bars of standard deviation is depicted in Figure 2. The graph shows more germination of horse gram and ragi seeds by radish DPJ.

Table 2 gives the data of the effect of different deproteinised leaf extracts on the seedling growth of various plants. In control, all the seeds showed germination. But in case of all DPJ comparatively, germination was found more than control. Brinjal DPJ was also not found suitable for fenugreek seed germination, while it was found that the *Colocasia* and radish DPJ were suitable (Figure 1 E). On an average, the yield of fenugreek green vegetation is 10,000 kg/ha [23]. Fenugreek when cultivated during monsoon of 1971, on this farm, the crop yielded 100 kg leaf protein (LP) per hectare in 37 days. The yields of extractable protein from 15 varieties of fenugreek were maximum when the foliage was harvested 40 days after sowing. Phytohormone Indole acetic acid modifies the protein encoded by the gene in the plants [24]. Jadhav [25] observed that fenugreek contains 12.4% of dry matter of fresh crop, 4% of Nitrogen, 13.75 % of ash, 0.43% of calcium and 0.20 % of phosphorus. It was found more germination of ragi and horsegram because of the treatment of all *Colocasia*, brinjal and radish DPJ. On the other hand in control, there was no proper germination of horsegram. Therefore it indicates that all the three DPJ found suitable for ragi and *Macrotyloma uniflora* seed germination.

Cantaloupe seeds also germinated fastly because of the treatment of DPJ from radish plants. In case of sunflower also, all the DPJ of radish, dasheen and brinjal were found suitable for germination. It showed equal rate of germination. The treatment by these three DPJ seeds showed no significant difference on germination (Figure 1 h). However, in sesame and linseed, there was the positive effect of *Colocasia* DPJ to induce the seed germination (Figure 1 b).

Earlier we reported, from the dry matter of 583 g of fresh radish foliage crop, 4 % of dry matter of DPJ, it contains 5 of Nitrogen, 31.5 % ash, 1.21 % of calcium and 0.20 % of phosphorus. Nitrogen is important mineral controlling plant growth. Phytohormones have been considered as signalling substances of different pathways in plants [26]. 2.05 % of nitrogen in radish DPJ indicates presence of auxins cytokinins and abscissic acid. Although numerous Calcium dependent protein kinases (CDPKs) have been exhaustively studied, a comprehensive overview of the manner in which CDPKs participate in phytohormone signalling pathways, has not yet been undertaken [27]. 1.21 % of calcium in radish foliage DPJ indicates presence of auxins and other hormones.

Jadhav also observed that, in 340 ml of *Dolichos* Juice, 1.33 g of LPC, 9.47 % of nitrogen and 30 mg of protein. In 417 ml of cowpea leaf juice, there was 1.61 g of LPC, 8.33 % of Nitrogen

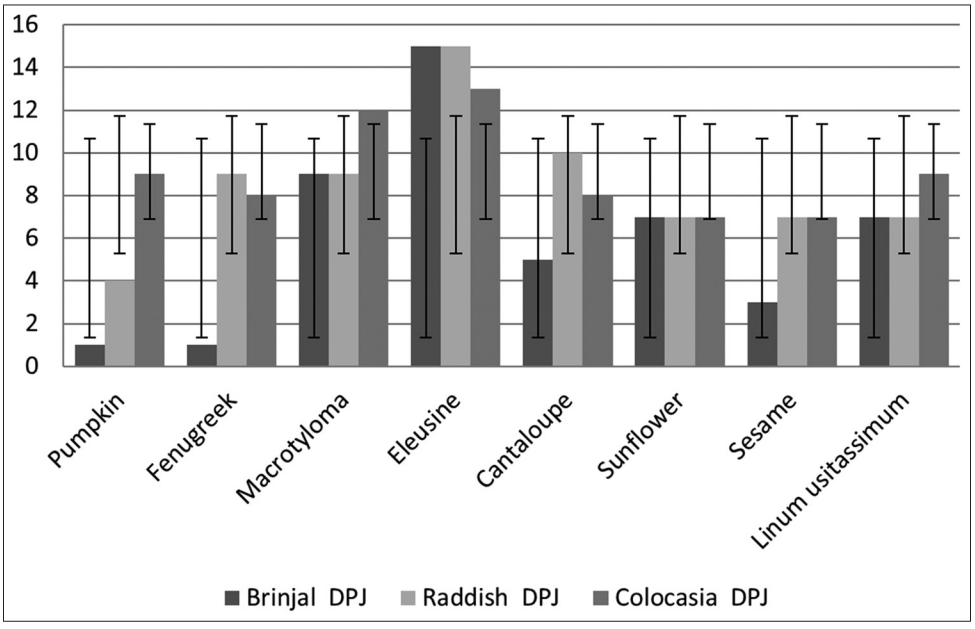

Figure 2: Graphical Illustration of number of the plants germinated after the treatment of brinjal, radish and dasheen DPJ by error bars of standard deviation

and 37 mg of protein respectively. There was decrease in nitrate reductase in *Eleusine coracana* because of weed plant *Cassia tora* DPJ application during growth despite it was of leguminoseae [28]. During extraction of leaf protein, the phytohormones also gets disposed into the deproteinised juice. Therefore this is the reason why in earlier studies lucerne and other plant DPJ showed favourable growth of crops. There were favourable results of the effect of the Lucerne DPJ on growth of cowpea and other monocotyledonous crops of jowar, maize and bajra [29]. The above results indicates that when the seeds are less viable for germination, DPJ induce and allow the seedlings to grow fastly due to its nutrients and hormones contents. There were variations of the effects of different DPJ on different seeds. In this experiment, radish DPJ induced the germination in pumpkin and sunflower seeds. *Colocasia* DPJ induced the fenugreek, *Macrotyloma*, *Eleucine*, sesame and linseed seeds. While brinjal DPJ enhanced cantaloupe seed germination. It was investigated that, when pumpkin DPJ was fermented by yeast, there was 1.300 g of single cell protein, 1.680 g of yeast by fermentation of bitter gourd DPJ and 1.680 g of yeast by cantaloupe forage DPJ.

Hence the aquatic *Colocasia* foliage DPJ performed more germination in seeds as compared with *Raphanus* and brinjal DPJ. Studies shows that protein denatures after heating at 100°C. Lokuruka MNI [30] studied that very few amino acids, proteins, enzymes and nutrients gets denatured at 90°C of temperature. In contrast to the protease enzyme activity illustrated in Table 3, DPJ of *Colocasia*, triggered the conspicuous zone of 4 mm by cup plate assay (Figure 3 a). DPJ of *Raphanus* also showed the protease enzyme activity of 3 mm by reacting with the substrate casein. It was less than that of *Cocolasia*. There was no significant difference among the two, as the protease activity was optimum by the DPJ of *Colocasia* and *Raphanus*. Figure 3 b illustrates the statistical error bars of the enzyme protease from DPJ. Therefore the experiment proves the presence of enzymes in the deproteinised juice, since during green crop fractionation the juice is heated at 50° C. At 50°C when juice was heated, the enzymes in it were not denatured [31]. However the presence of enzyme in DPJ indicates the presence of phytohormone gibberellin which stimulates the hydrolytic enzymes by exogenous application to the seeds. Hence this reason is considered for the proper growth of the seedlings as compared with the seedlings grown as control. Control (untreated) seedlings also showed the seedling growth but lesser than that of the DPJ treated. There can be the proportion variation of enzyme activity when various deproteinised leaf extracts were taken into consideration by process of GCF for successful evaluation of its byproducts [32-34]. Phenolic compounds in DPJ indicates the presence of plant hormone salicylic acid.

The Hopkins-Cole test is specific for tryptophan, the only amino acid containing an indole group. Both the DPJ from Fenugreek and Spinach showed the tryptophan presence. Content of tryptophan showed variation in colouration as per the various DPJ utilised for the tests. There was formation of violet coloured ring in both the test tubes consisting DPJ of spinach and fenugreek as shown in Figure 4 (A) and (B).

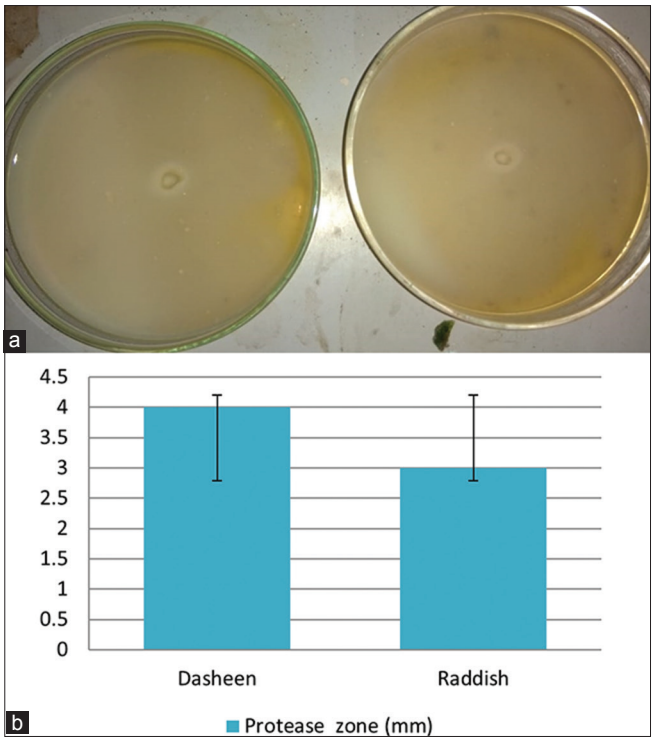

Figure 3: (a) *In vitro* activity of enzyme protease (central zones in mm) by gibberellin from DPJ of *Colocasia* and *Raphanus sativus* by cup plate assay. (b) Protease enzyme zone (mm) induced by gibberellins in *Colocasia* and *Raphanus* DPJ by *in vitro* cup plates essay method standard deviation error bars

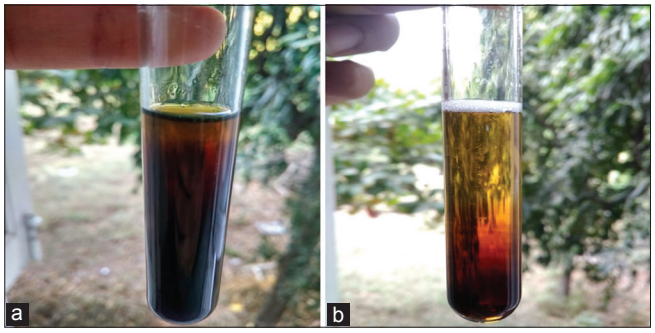

Figure 4: Tryptophan, a precursor of auxin, the ring and violet colour by glyoxylic acid test in (a) Fenugreek DPJ (b) Spinach DPJ

Fenugreek DPJ contains more violet colouration along with ring as compared with Spinach whey as the test figures shows. This result reveals the presence of auxins in the DPJ which was responsible for initiation of plumule and radicle of the seedlings. Hence it reveals that tryptophan presence in DPJ was responsible to overcome the dormancy of seeds. Auxins and gibberellins presence in Lucerne DPJ favoured the plant growth in earlier research when cowpea and pea were grown. These phytohormones also favoured the germination of seeds by the Lucerne DPJ influence [35].

There was muatagenic influence in Celosia argentia plants by the lethal dose of Eicchornia crassipes DPJ which induced chromosomal aberrations mostly in prophases and metaphases

[36]. The blue colour precipitate by ferric chloride phenolic test in the extract of Eichhornia indicates the presence of growth hormone salicylic acid [37]. Salicylic acid is the phenolic compound. Cytokinins modulates cell division and differentiation in presence of glucose. Positive Benedicts test indicates presence of glucose in cruciferous foliage DPJ [38].

CONCLUSION

During present investigation, it was found that there was the dormant behavior of the seeds. To overcome the dormancy and to initiate rapid germination, the treatment of DPJ prepared from *Colocasia* and radish given to crops, showed efficacy in optimization in the rate of germination by breaking the dormancy in specific crops viz., horsegram and ragi. While the DPJ from *Raphanus* was found suitable for the rapid germination of sunflower seeds. The inviability can also be controlled by DPJ. DPJ can be a mutagenic agent to induce changes in morphology.

The factor responsible to enhance the seedling growth and overcoming dormancy are phytohormones, without of which the hydrolytic enzymes cannot be stimulated in the seed cotyledons from the embryo. Activity of enzyme protease and the positive glyoxylate test indicates the presence of growth hormones gibberellin and auxin in DPJ.

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