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Gibberellin Induced α-amylase and Protein Optimization in the Seedlings by the Influence of Deproteinised Leafy Whey from Selected Crops

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Earlier findings showed the presence of phytohormones in deproteinised leaf extracts of cabbage, cauliflower, *Colocasia* and fenugreek and the expression of enzyme protease due to it. The presence of enzyme amylase was investigated by the treatment of hormone gibberellin to the cowpea and wheat seedlings. This was compared with the seedlings treated with deproteinised juice (DPJ) or whey of cabbage and *Colocasia*. There was striking influence of DPJ, optimizing the content of enzyme in the wheat and cowpea seedlings. The another DPJ prepared from beet foliage showed increment in the protein content in the seedlings of fenugreek, when treated exogenously by soaking the seeds in DPJ at various concentrations from 0.25 to 1 %. DPJ of beet showed significant seed germination result of bajra crop. The pH of lucerne DPJ is found slightly alkaline at various concentrations.

Key words: fractionation, beet, Colocasia, Pennysetum, germination, pH, enzyme

The green crop fractionation process results in three types of products : i) the pressed crop residue (PC) left after the extraction of juice (Raymond and Harris, 1957), which is suitable for animal nutrition ; ii) the leaf protein concentrate (LPC), suitable for human nutrition, and iii) the deproteinised juice (DPJ), which is a byproduct (Guha, 1960). The deproteinised juice (DPJ) also known as whey or liquor, is a good source for irrigation as it has some value of nutrient source and soil conditioner.

In earlier research, deproteinised leaf extract from cabbage was analysed with the nitrogen content. Major part of dry matter and nitrogen were lost in deproteinised juice during green crop fractionationin other fractions. The DPJ, a nutrient rich by product should be used carefully in order to gain economic advantages. DPJ from *Colocasia*, lucerne, radish when was treated to various seeds, it showed both enhancement and decrease in rate of seed germination as well as protein content. There was positive xanthoproteic test in *Colocasia* DPJ which indicates gibberellins presence. In earlier studies, gibberellins present in the DPJ of *Colocasia* induced the secretion and activity of enzyme protease, analysed by cup plate method after the seedling growth influenced by DPJ and also retarded the dormancy (Jadhav et al., 2019). *Colocasia* DPJ also investigated with the presence of triangular and spherical silver nanoparticles. DPJ enhanced the process of nitrogen fixation as it increased the nodulation as well as protein content in cowpea plants when treated with lucerne DPJ which is enriched with nitrogen, calcium and phosphorus. DPJ found increasing the enzyme nitrate reductase in the plant body after its treatment to the growing seedlings (Jadhav and Mungikar, 1998). DPJ also found retarding the proline content in plants by reducing the stress process of transpiration (Jadhav et. al., 2018). As DPJ enhances the protein content after its treatment, 14-3-3 proteins physically interact with many protein clients involved in the biosynthesis or signaling pathways of the main plant hormones (Camoni et al., 2018). DPJ enhanced the mycelial growth when it is used as liquid broth and secretes citric and oxalic organic acids. However high concentration of DPJ causes the phytotoxicity during plant growth due to chromosomal aberrations (Jadhav, 2009 a). High concentrations of DPJ from Lucerne and *Eicchornia* caused chromosomal aberrations like micronuclei and restitutions of the prophases in *Allium cepa, A. sativum* and *Celosia argentia*. L plants (Jadhav, 2009 b). Positive amino acids tests like tryptophan (Indole test) in brassicaceae conspicuously revealed presence of phytohormones in DPJ. When the brown juice of cauliflower was fermented, the yield of LPC was declined due to the activities of the enzymes amylases and proteases. DPJ when utilized as the broth medium for the growth of economically important fungi (Jadhav, 2019), it enhanced rate of molecular hydrolytic enzymes like amylase, protease, cellulase and lipase. DPJ from *Allium cepa* found inhibitory in the activity of enzyme protease (Jadhav and Mestry, 2019).

The yields of extractable protein from leaves taken at the time of harvest of the edible part root, tuber) from brassicas, beet root, turnip and radish ranged between 76 to 170 kg/ha. The DPJ of lucerne left after the precipitation of protein from juice, was found to be rich in non protein nitrogen, soluble carbohydrates, calcium and potassium. The presence of enzyme protease in DPJ indicates the presence of soluble protein.

Phytohormones are signalling substances coordinate the responses in plant bodies during growth and development to the element like nitrogen. Plants organs have different functions and nutritional requirements and they rely on hormonal signalling. Plants have to adjust in lack of element nitrogen. It is signalled by auxin, cytokinins and abscissic acid (Kiba et al., 2011).

Gibberellic acid effects on amylase in Seeds. The ability of gibberellic acid to stimulate amylase activity in cereal seeds has been well documented by many workers. However, in one of the few studies on dicot seeds, the most extensively studied system for the stimulation of amylase activity by gibberellic acid has been endosperm and, more specifically, the aleurone cells. Among the earlier workers, Paleg (1960) found that pretreatment of endosperms, gibberellic acid increased the water soluble amylase activity. He hypothesized that in vivo the embryo secreted a gibberellin which activated the amylases and other enzymes in the endosperm. Briggs (1963) confirmed the stimulation of amylase and other hydrolytic enzyme

activity by gibberellic acid in endosperm tissue. Gibberellic acid appeared to function by stimulating the living aleurone layer to synthesize enzymes de novo, rather than by activation of preformed enzymes or precursors.

Other studies of gibberellic acid effects in cereal seeds were made by Rowsell and Goad (1964 a, b). In their studies, gibberellic acid promoted the appearance of alpha amylase from endosperms and from isolated aleurone layers. In addition gibberellic acid also promoted the release of beta amylase from aleurone layers. Boothby and Wright (1962) showed that gibberellic acid increased the production of reducing sugars by seed halves. Wright (1963) also found that treatment of excised seed coleoptiles with gibberellic acid resulted in degradation of starch in the tissue.

The beet root leaves (Chenopodioideae) contains significant levels of protein and lipids in all developmental stages. The Fe content decreased during the developmental stages, while the content of K increased. With regard to fatty acid composition, linolenic acid was present in the greatest quantity (Biondo et al., 2014). *Colocasia esculenta* (family Araceae) leaves possess micronutrients, viz. iron, copper, magnesium, potassium and zinc are present in high amounts. The ratio of sodium to potassium in the leaves add specifically to the antihypertensive properties (Gupta et al., 2019).

Seed germination is the process of growth of the dormant embryo initiated by water imbibitions. The phase of growth is dependent on energy produced by metabolic conversion of food resources of seed endosperm/ cotyledon. During germination of the seed, various physical and chemical changes take place. Specifically variety of enzyme system get activated. One of the most important enzyme involved in the digestion of seed reserves is amylase which hydrolyse the reserve starch into sugar, and gibberellic acid, which is well known to break dormancy and induce germination. Endogenous GA bring about activation of amylase and promotes the rate of respiration. During present investigation, attempts were made to study the DPJ influence at various concentrations on seed germination, especially dicots and monocots and study of the enzyme amylase activity by preparing reaction mixtures to invigorate the gibberellin hormone implication. It is also the objective of the research to evaluate pH of the DPJ.

MATERIALS AND METHODS

Green crop fractionation technology and DPJ

In this technology, the green crop of beet (*Beta vulgaris* .L), Elephant ear (*Colocasia esculenta* L), cabbage (*Brassica oleracia* L. var capitata) and lucerne (*Medicago sativa* L.) were fractionated in IBP pulper to obtain pulp in the leaf protein research laboratory. This pulp was squeezed by pressing machine so as to obtain the juice. The juice heated to 90°C to get the proteins coagulate so that proteins gets precipitated. The supernatant is called as deproteinised juice (DPJ) which was filtered by whatman paper to obtain Leaf protein concentrate (LPC). The DPJ is thrown randomly. But during present investigation it is advocated to use it as the substrate for seed germination and seedling growth. It contains minerals, vitamins, growth regulators, soluble and insoluble proteins, lipids etc.

Seed germination

The seeds were soaked in beet DPJ for 24 hours in room temperature at 31°C. The seeds were germinated by the paper towel method. The filter paper was immersed in sterilised distilled water and the soaked seeds were arranged on the paper and folded. The folded paper towels incubated at room temperature for 10 days for plumule emergence. The seeds used were pearl millet *(Pennysetum typhoides L.),* fenugreek *(Trigonella foenicum graceum L.),* cowpea *(Vigna unguiculata L. walp),* to compare the influences of DPJ on dicot and monocot crops.

Four replications of each species of seed per treatment were sown on paper towel in the roll **system**. The paper towels were moistened using a water quantity equivalent to 2.5 times their weight and placed in a germination chamber previously set at 30ºC. Assessments of normal seedlings were made 7 and 14 days after sowing, according to the rules, for seed analysis (Brasil, 1992). Analysis of variance was calculated to observe the difference.

Protein estimation by microkjeldahls method

The beet leaves DPJ was dried in an oven at 95 ± 5°C for the determination of dry matter (DM). The dried samples were ground to a fine powder and stored in plastic containers for further analysis. Nitrogen (N) content was determined in duplicate by microKjeldahls method which involved digestion with sulphuric acid (H_2SO_4) in presence of catalyst and titration of ammonia liberated during distillation. Protein was calculated by N x 6.25, where N is denoted by Nitrogen.

Germinating seed extract for amylase

For the purpose to study the activity of enzyme amylase by GA_3 influence, soak wheat (*Triticum aestivum*. L.), cowpea [(*Vigna unguiculata.*L.) Walp] seeds in sterilized water and GA_3 (10 ppm) for 8 hrs. 1.0 g cotyledons of germinating wheat seedlings were taken and crushed them in mortar and pestle. 10 ml of D.W. was added to make the slurry. It was filtered through muslin cloth. The mixture was centrifuged for 10 minutes at 5,000 rpm. The supernanent was taken into consideration for making the volume by 10 ml with chilled D.W. This was stored in cool place for using it as enzyme source.

Activity of enzyme amylase by GA_3 treatment

Gibberellic acid solution was taken of 10 ppm. Starch solution 0.5 %, acetate buffer of pH 5.5/6.5, alkaline copper sulphate and phosphomolybdic acid reagent. Amylase activity expressed as OD/g fresh tissue. Enzyme blank and substrate blank was taken for control and treated is reaction mixture.

The amylase activity hydrolyses starch into the reducing sugar which is the final product of the activity. When sugars gets added with the alkaline copper sulphate it gives cupric and cuprous salts. It reacts with phosphomolybdic reagent to give precipitate of Cu^{++} $^{+}$which gets dissolved and forms blue coloured complex. It is read at 420 nm. Amylase activity is calculated by formula

Δ O. D. × 10 ÷ 1.0 (Volume of enzyme) = Δ O.D. / gm fresh tissue = Δ O. D. x 10 ÷ 1.0 × 1

Measurement of the pH of DPJ

The pH of fresh DPJ of lucerne was measured at various concentrations. These concentrations were utilised for the purpose of the growth *of Allium cepa* L. root tips to study further analysis of its meristematic cell division.

Statistical Analysis

The average values obtained were subjected to one – way analysis of variance using the ANOVA module to determine significant differences between the mean values.

RESULTS AND DISCUSSION

There was no influence of beet DPJ at various concentrations on rate of fenugreek seed germination, shoot and root length by paper towel method. It also effected slightly adverse on the reduction in fresh and dry weight illustrated in table 1 after calculating its arithmetic average as compared with untreated control. This might be because of the fatty chemical composition of the beet leaves DPJ. 0.4 % concentration of DPJ found feasible to increase the root and shoot lengths, as well as fresh weight of the seedlings.

But on the other hand, there was significant result of nitrogen and protein content enhancement of the seedlings of fenugreek by the effect of beet DPJ. This indicates favourable effect of non leguminous beet DPJ on leguminous crop fenugreek after calculating the arithmetic mean of the DPJ treated parameters illustrated in table 2. In previous experiments, lucerne DPJ found enhancing the plant growth and leaf protein, if poured exogenously on the soil for roots, where the seedlings raised after 10 days.

At high concentration of DPJ i.e. at 1 % level, the nitrogen and protein content found optimized as compared with untreated control depicted in Figure 1.

In case of bajra seed germination, there was favourable effect of beet DPJ on the shoot and root length as well as fresh and dry weights of the seedlings as compared with the untreated control. Therefore beet DPJ at various concentrations found suitable in enhancing the yield of bajra crop. DPJ at 1 % level found more appropriate for the growth. The root growth of bajra was higher at 0.3 % level illustrated in table 3.

There was the variations in the results of seed germination by the effect of DPJ. If DPJ average of various concentrations was taken into consideration, it

enhanced the root as well as shoot length. Table 5 indicates that the beet DPJ was not favourable for the seed germination at 1 % level. As compared with the leguminous dicotyledonous crop of fenugreek, the monocotyledonous crop of jowar which is non leguminous, the same non leguminous DPJ was not found significant illustrated in table 4. The dry weights of the seedlings got reduced due to DPJ treatment. The influence of different DPJ utilised on different species for seed germination, effects variously. In earlier investigation, the DPJ from *Colocasia* as well as radish which was prepared fresh without any concentration induced the rate of seed germination in *Macrotyloma*. Therefore the various concentrations of dry DPJ if is prepared and utilised it, influences the retardation of germination of the seedlings, like in the case of *Sorghum vulgare*. 0.2 % concentration of DPJ found feasible for the root, shoot growth and the fresh weight of the seedlings of jowar.

The overall influence of DPJ from beet on the dry matter content of the seedlings shown significant result on fenugreek and bajra. While jowar seedlings not showed the satisfactory influence, depicted in figure 2.

Table 1. Effect of beet foliage DPJ on fenugreek seed germination

Concentration Of DPJ (%)	No. of plants germinated	Mean Shoot length (cm)	Mean root length (cm)	Mean fresh weight of seedling (g)	Mean dry weight of seedling (g)
0 (Control)	5	3.9	3.5	0.790	0.300
0.1	5	3.9	2.7	0.780	0.170
0.2	5	3.9	3.2	0.485	0.200
0.3	5	4.2	2.4	0.740	0.170
0.4	5	4.6	4.5	0.860	0.170
0.5	5	4.3	2.6	0.825	0.170
1.0	3	2.6	2.7	0.450	0.120
Average (DPJ)	5	3.9	3.0	0.690	0.166
Variance	0.101667	0.666667	0.485667	0.03147	0.000667
F Crit	2.533555				
P-value	6.27E-16				

Table 2. Effect of beet foliage DPJ on percent nitrogen and protein content of fenugreek seedlings

Concentration Of DPJ (%)	Nitrogen content (%)	Protein content (%)
0 (Control)	0.34	2.12
0.1	0.56	3.5
0.2	0.34	2.12
0.3	0.45	2.81
0.4	0.54	3.37
0.5	0.60	3.75
1.0	0.67	4.18
Average (DPJ)	0.52	3.28
Variance	0.013587	0.530697
F Crit	4.964603	
P-value	3.5E-06	

Table 3. Effect of beet foliage DPJ on bajra seed germination.

Concentration Of DPJ (%)	No. of plants germinated	Mean Shoot length (cm)	Mean root length (cm)	Mean fresh weight of seedling (g)	Mean dry weight of seedling (g)
0 (Control)	4	0.1	0.1	0.030	0.015
0.1	3	2.0	5.0	0.050	0.050
0.2	5	3.0	7.0	0.075	0.050
0.3	2	1.0	10.5	0.100	0.010
0.4	2	3.3	4.8	0.320	0.100
0.5	2	3.2	1.0	0.100	0.050
1.0	1	2.9	3.9	0.420	0.150
Average (DPJ)	3	2.5	5.36	0.177	0.068
Variance	1.9	0.802667	10.13867	0.023578	0.002417
F Crit	2.75871				
P-value	2.77E-05				

Table 4. Effect of beet foliage DPJ on jowar seed germination

Concentration Of DPJ (%)	No. of plants germinated	Shoot length (cm)	Root length (cm)	Fresh weight of seedling (g)	Mean dry weight of seedling (g)
0 (Control)	4	1.5	0.8	0.300	0.150
0.1	0	0.00	0.00	0.000	0.000
0.2	3	5.7	9.1	0.400	0.040
0.3	2	5.5	6.0	0.120	0.010
0.4	0	0.00	0.00	0.000	0.000
0.5	2	4.0	12.0	0.200	0.050
1.0	0	0.00	0.00	0.000	0.000
Average (DPJ)	1.16	2.53	4.51	0.120	0.016
Variance	1.766667	8.046667	28.08167	0.0256	0.000507
F Crit	2.75871				
P-value	0.046714				

Table 5. Effect of GA3 and cabbage (*Cruceifereae*) foliage DPJ on activity of the enzyme amylase on the seedlings of wheat (*Triticum aestivum.* L)

Treatment	Enzyme Blank (EB)	Substrate Blank (SB)	Reaction Mixture (RM)	Fresh tissue / g = RM- (SB +EB)
Wheat seedlings (untreated)	0.06	0.22	0.73	0.45
GA_3 treated wheat seedlings	-0.02	0.34	1.78	1.46
DPJ treated Wheat seedlings	0.06	0.16	1.16	0.94

Table 6. Effect of GA3 and *Colocasia* (*Araceae*) DPJ on activity of the enzyme amylase on the seedlings of cowpea [(*Vigna unguiculata*. L.) walp]

Treatment	Enzyme Blank (EB)	Substrate Blank (SB)	Reaction Mixture (RM)	Fresh tissue/ g = RM- (SB +EB)
Cowpea seedlings (untreated)	0.05	0.50	0.29	- 0.26
GA_3 treated Cowpea seedlings	0.03	0.63	0.41	- 0.25
DPJ treated Cowpea seedlings	0.71	1.18	0.40	-1.49

Table 7. Effect of pH on onion root tips during growth on the lucerne DPJ at various concentrations

Concentration Of DPJ (%)	pH of DPJ at various concentrations.	pH of DPJ at various concentrations after 48 hours.
0 (Control)	7	5.66
0.01	6.72	6.07
0.03	6.86	6.40
0.06	6.35	6.40
0.12	6.15	6.08
0.25	6.10	5.85
0.50	6.02	6.22
1.00	6.64	6.45
Arithmatic Mean (DPJ)	6.40	6.21
Variance	0.121347	0.04576
F Crit	4.964603	
P-value	0.2659	

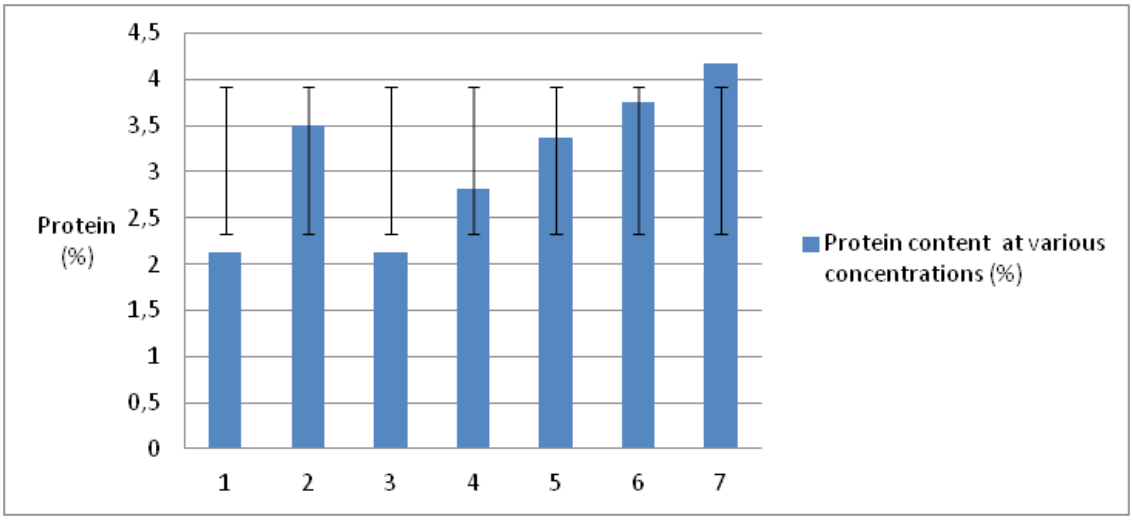

Figure 1. Graphical illustration of the percent protein content of the seedlings of fenugreek grown on various concentrations of lucerne DPJ calculated by nitrogen content (Standard deviation error bars)

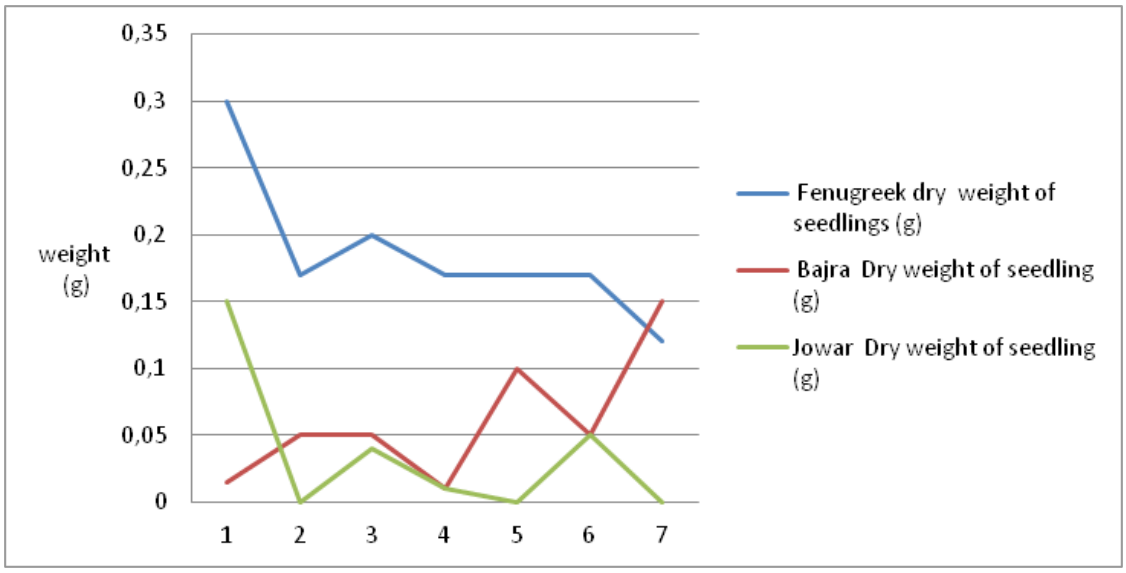

Figure 2. Graphical illustration of the comparative dry matter of the seedlings of fenugreek, bajra and jowar by the treatment of lucerne and beet foliage DPJ.

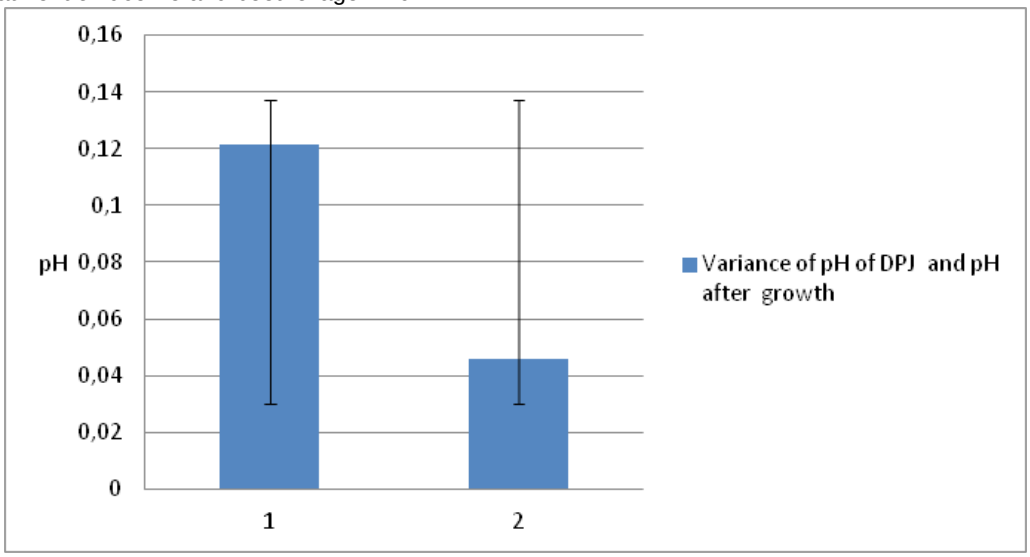

Figure 3. Graphical presentation by calculating statistical variance among the pH of fresh DPJ as compared with the pH of DPJ after the growth of Allium cepa root tips immersed in it (Standard deviation error bars)

The GA3 treatment at 10 ppm concentration to wheat seedlings found enhancing the enzyme amylase. As compared with control i.e 0.45, cabbage foliage DPJ application to wheat seeds prior to germinate found optimizing the activity of enzyme amylase i.e. 0.94. This indicates the presence of growth regulator gibberellin illustrated in table 5.

The source of GA3 was found getting expressed in the *Colocasia* DPJ to induce the amylase activity in cowpea seedlings to enhance as compared with the GA3 treatment at 10 ppm concentration. It was by influencing on the optical density by spectrophotometric analysis shown in table 6. It showed increment in the optical density as compared with untreated blank. This reveals the concentration of gibberellic acid in *Colocasia* DPJ was found in appreciable amount.

The crop of lucerne was selected for the following experiment, as it was utilised as the fertilizer for leguminous and non leguminous crops in earlier research. Table 7 indicates that when onion root tips grown on water (control), after 48 hours, the pH of water becomes very slightly acidic. The pH of fresh lucerne DPJ also decreases at various concentrations. While during the growth of *Allium* root tips at various concentrations of DPJ, the pH again slightly decreases. Very slightly the pH changes to acidic. It was not gone below pH 6. Therefore DPJ retains the alkalinity of the soil if is applied for the purpose of root growth of onion. It can keep the soil alkaline, which can be convenient for the plant growth. Therefore table 7 reveals that the fresh DPJ of lucerne can retain the alkalinity of the soil if utilized as the fertilizer to the plants depicted in figure 3.

CONCLUSION

Therefore beet DPJ found having the potentiality in optimizing the protein content in fenugreek seedlings as it consists of the presence of phytohormone gibberellin. It promoted the length and weight of monocotyledonous plants of bajra. But beet leaves DPJ found inhibitory in case of *Sorghum* seedlings growth. It might be perhaps because of the presence of nanaoparticles in it and its fat content. DPJ from *Colocasia* triggered the enzyme amylase activity in cowpea crop. Therefore this result reveals that the DPJ from *Colocasia* as well as cabbage naturally contains hormone gibberellins. The experiment of the paper towel method for seed germination was not found feasible as compared with the soil seed germination.

The pH of lucerne DPJ was found slightly alkaline for the root growth of onion bulbs at various concentrations reveals the suitability of it as the fertilizer.

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