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# Evaluation and detection of Brassicaceae leafy whey phytohormones efficacy for exploiting as a broth medium obtaining optimistic culture of fungi *in vitro*

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## ABSTRACT

The DPJ (Deproteinised Juice) or whey constituents were responsible for the induction of growth optimization of plants, various fungi including yeast, *Rhizobium* reported by earlier workers. In previous experiments, DPJ maximised the growth of plants and seed germination. During present investigation the carbohydrates, amino acids and protein tests were taken into the consideration. All the tests found positive. Despite, the extract is deproteinised, still there was persistence of few proteins and amino acids. The collection of mycelia grown on DPJ was filtered and the culture filtrates recommended to use *in vitro* for the industrial purpose for biomass and secondary metabolites. Experimental DPJ is compared with the glucose nitrate medium as control. These positive tests revealed the suitability of DPJ to be used as the medium for the growth of fungi. Positive amino acid tests conspicuously revealed presence of phytohormones in members of Brassicaceae DPJ and hence advisable to be utilised for the plant growth *in vivo*, plant callus growth and cell proliferation of mycelia *in vitro*.

**KEYWORDS:** DPJ, carbohydrates, amino acids, protein, cabbage, broth, mycelia, phytohormone

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## INTRODUCTION

In the earlier research, DPJ as the medium was compared with Hansens medium, Potato Dextrose Broth (PDB) and Glucose nitrate (GN) medium. Radish DPJ was found optimizing the rate of germination of seeds and retarding dormancy. Cabbage and cauliflower DPJ was found increasing the microbial biomass of yeast by fermentation and their enzymes. DPJ of *Eichhornia* found mutagenic to cause chromosomal aberrations in root meristems of *Celosia*. There was variation in the growth of fungi when the DPJ consumed prepared from different weeds like *Celosia* [1]. Majority of amino acids gets disposed off to the leaf protein concentrate (LPC) during Green crop fractionation (GCF) during the filtration of juice when heated to 90°C [2]. Therefore few amino acids remains in DPJ.

Deproteinized leaf juice or whey is the by-product of the process called Green Crop Fractionation. Deproteinized leaf juice is the fraction of the juice extracted from the green foliages that remains after the precipitation of the LPC. DPJ is rich in the water soluble nutrients like carbohydrates, vitamins, minerals and unidentified growth factors [3]. Deproteinized leaf juice of various plants have been used for the preparation of culture media for cultivation of many useful bacteria, fungi, and actinomycetes especially species of *Saccharomyces*, *Streptomyces*, *Rhizobium*, *Penicillium*, etc. [4]. Several attempts have been done by scholar on the production of fungal biomass and their secondary metabolites on the deproteinized leaf juice showing that suitability of deproteinized leaf juice for the growth and production of microorganisms like fungi [5]. Deproteinized leaf juice used as a novel medium for rhizogenesis *in vitro* [6]. The process of green crop fractionation results into three major fractions i.e. pressed crop residue (PCR), leaf protein concentrate and deproteinized juice. The leaf juice expressed during fractionating of green foliage is employed for the preparation of food grade leaf protein concentrate. The LPC is a source of protein and vitamin A in human and poultry diet. About 50% of fresh weight from green foliage contributes the DPJ [7]. This product, with 4 to 5% solids, is generally rich in nitrogen and phosphorus [8]. In addition, earlier study [9] observed that this fraction may contain various nutrients. The data on the chemical composition of DPJ obtained from 10 crops has been given by [10]. It is evident from the data that the percent DM and nutrient composition of this fraction varies from species to species.

The culture filtrates of fungi as well as yeast grown on Lucerne DPJ was collected to examine the enzyme activities like protease, amylase, cellulase and lipase. The activity of the enzymes proteases and amylases from the culture filtrate reveals the presence of the hormone gibberellins. DPJ was responsible to reduce the enzyme invertase as compared with Hansens media [11, 12]. For the purpose of sporulation and the spore germination of the fungi, detection of amino acids tests were carried out in DPJ for the purpose to maximise the mycelia, as amino acids induce spore germination [13]. The culture filtrates of *Trichoderma* grown on DPJ can be the good source of hormone Indole acetic acid (IAA) for plant growth or for the treatment to seeds to germinate optimistically [14]. Amino acid tryptophan is the precursor of IAA. Tryptophan test in DPJ was performed by glyoxylic test. Iodine and Molischs tests indicate the presence of starch or carbohydrate in the DPJ. Starch when added to DPJ, it found maximising the mycelial weight. Carboxymethyl cellulose (CMC) also enhances or shows variation of the growth of fungi when added to DPJ. It secretes enzyme cellulase appropriately. Casein substrate was added to DPJ to induce enzyme protease. Fungi enhances organic acids when grown on DPJ [15, 16]. DPJ has the efficacy in retarding the proline and reducing physiological rate of transpiration [17]. High concentration treatment of DPJ caused chromosomal abberations in *Celosia* meristematic root cells [18].

Benedicts tests indicates the presence of glucose in DPJ. In earlier finding, glucose when added to DPJ there was variation in the yeast as well as other fungal biomass. It depends on the appropriate plant DPJ to induce the proper growth. In few cases, the DPJ from some cucurbitaceous plants alone enhanced the yeast single cell protein without glucose. Therefore glucose benedicts test was taken into consideration. For the purpose to provide the extra carbohydrate source for the mycelial proliferation in addition to the DPJ medium, it supported the result.

### Phytochemistry of Brassicaceae Species Foliage DPJ

*Cabbage*

From 6.3 to 21 % of total nitrogen in crop was removed in deroteinised juice (DPJ). In cabbage, only 3.5 % of total calcium got incorporated in LPC. Major part of the inorganic constituents were however lost in deproteinised juice (DPJ). 258 g DPJ from of fresh 1 kg cabbage crop contained 4.20 % of Dry Matter (DM). From 4.20 % of DM of DPJ, 3.90 % of Nitrogen, 35.5 % of Ash, 1.17 % of calcium and 0.24 % of phosphorus [19]. [20] reported that the LPC prepared from the byproduct leaves of cabbage contain 70 % protein, 10 % lipid and 2.6 % starch and found that this product is suitable as a protein supplement for non ruminants.

*Cauliflower*

483 g of DPJ from fresh 1 kg foliage of cauliflower contained 4.15 % of DM of DPJ. From that 4.15 % of DM of DPJ, 1.38 % of N, 13.3 % of ash, 1.16 % of calcium and 0.18 % of phosphorus was found in cauliflower leaves [21]. The good feeding value of the residual pressed crop from cauliflower leaves had been shown.

*Radish*

In 1 kg of fresh leaves of radish, 583 g of DM of DPJ was found. In that, 4 % of DPJ was found. This 4 % of DPJ contained 2.05 % of nitrogen, 31.5 % of ash, 1.21 % of Calcium and 0.20 % of phosphorus. The yields of extractable protein from leaves taken at the time of harvest of edible part (root tuber) from brassicas, beet root, turnip and radish ranged between 76 to 170 kg/ha [22]. The nutritive value of LPC prepared from byproduct leaves of radish is superior to that prepared from Lucerne [23].

Considering the above nutrients in DPJ, the yield of *Penicillium chrysogenum* when grown on cabbage DPJ was 168 mg on 20 ml of DPJ while it was on 91 mg in control GN medium [24].

## MATERIALS AND METHODS

### Preparation of Leafy DPJ from Brassicaceae

Green foliages from 3 Brassicaceae plants were employed for GCF. Leaves of Cabbage (*Brassica oleracia var* botrytis. L), cauliflower (*Brassica oleracia var capitata* L), and raddish (*Raphanus sativus* L.) were washed with water and crushed in mortar and pestle to get pulp. Pulp was put on muslin cloth and squeezed and the juice was collected in container. 200 ml of juice was allowed to boil between 90-95°C. The protein gets separated by coagulation and the precipitation settles at bottom and the brown liquor was DPJ or whey becomes the supernatant. The residue then filtered through whatman filter paper and the LPC was recovered. The brown liquid obtained is the DPJ, was taken in three replicates of conical flasks, each 50 ml.

### Preparation of Culture Media

The fungi *(Aspergillus niger)* was cultivated on synthetic GN medium as well as on aqueous solution of the DPJ. The GN medium was prepared by dissolving glucose 10 gm, $KNO_3$ 2.5 gm, $KH2PO_4$ 1 gm and $MgSO_4$ 0.5 gm in one litre of distilled water. This process is used for other two fungi also as control for growth of the fungus.

Sterilization and inoculation were done as explained early [32].

### Collection of Microbial Biomass

After each incubation period (7 to 10 days) the fungal biomass mat of *Curvularia*, *Aspergillus* and *Trichoderma* grown on DPJ from cabbage, cauliflower and radish foliages, each three replicates was harvested by filtration through pre-weighed whatman No. 1 filter paper shown in Figure 1. In all experiments, each treatment was replicated for 3 times. At each experiment a control flask was also kept simultaneously. The mycelia of all three fungi viz., *Aspergillus niger, Curvularia lunata* and *Trichoderma viride* considered for the preparation of slides and used under the light microscopes for


**Figure 1:** Filteration of mycelia through whatman filter paper after the growth on three members of Brassicaceae DPJ

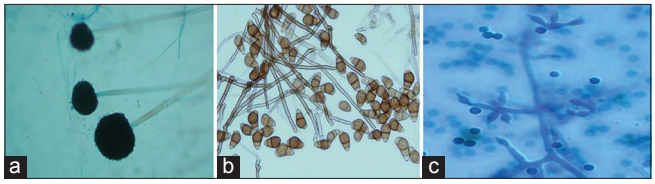


**Figure 2:** Slides of the fungi grown on DPJ (a) *Aspergillus niger* (b) *Curvularia* (c) *Trichoderma viridie*

the observation of the morphology and the sporulation. All the three fungi slides shown in the Figure 2, A, B and C.

*Aspergillus niger* belongs to order moniliaes is a haploid filamentous fungi and is a very essential microorganism in the field of biology.

*Curvularia* is a hyphomycete (mold) fungus which is a facultative pathogen of many plant species and common in soil. It belongs to form class Deutromycetes. *Trichoderma viride* is a type of fungi that present in all soil, where they were most prevalent, culturable fungi. They have a rapid growth rate, sporulated abundantly. It produce greenish colonies when cultured. It belongs to division amastigomycota.

## Test for Detection of Constituents in Leafy DPJ of Brassicaceae

*Test for Carbohydrates in Raphanus, Brassica oleracia and Brassica botrytis*

Molisch 's Test, Iodine Test and Benedict's Test were done by following standard methods as explained earlier [33].

*Test for Amino acid and Proteins in Raphanus, Brassica oleracia and Brassica botrytis*

**Ninhydrin test**

Take 4 ml of the DPJ and add 1 ml of freshly prepared ninhydrin solution. Mix the content and boil for couple of minutes, allow to cool. Most of the amino acids, except proline, are hydrolyzed and react with ninhydrin.

**Glyoxylic reaction**

Take 2 ml of glacial acid and 2 ml of DPJ then add about 2 ml of concentrated $H_2SO_4$ carefully down the sides of the test tube. Observe the colour change. Add at the junction of the two liquids.

**Sulphur test**

Take 2 ml of the DPJ, add 2 ml of 40 % NaOH and 10 drops of 2 % lead acetate solution. Boil for a minute and cool.

## RESULT AND DISCUSSION

The fungus *Aspergillus niger, Trichoderma viride, Curvularia* grew well on all DPJ samples. The growth of the fungus was evaluated on the basis of the amount of MDW per 25 ml of DPJ obtained after filtration. When the yield of MDW on DPJ was compared to that obtained on GN medium, it was observed that, on the DPJ of almost all plants the weight of MDW was more than that obtained on GN medium.

The fungal growth among 3 plants under investigation was almost similar for all 3 DPJ studied. The overall results obtained during present investigations supports the findings reported by the earlier workers. The overall results indicated suitability of DPJ as medium for growth of *Aspergillus niger, Curvularia* and *Trichoderma*.

Before the growth of the fungi, the DPJ obtained from Brassicaceae family species viz., *Raphanus*, *Brassica oleracia* and *Brassica botrytis* by green crop fractionation were utilized for the detection of carbohydrates, amino acids and proteins. After the harvesting of the fungi, the culture filtrates were collected for the study of secondary metabolites. However the enzyme protease from fungi *Trichoderma* found inhibited when DPJ from onion was utilized for growth [25].

The harvested fungi in filter paper showed in Figure 3 A, B and C. In earlier studies the medium used for the growth of fungi were potato dextrose broth and glucose nitrate medium. For the yeast fermentation as the control the medium used was glucose nitrate and Hansens media as control for the comparison with the DPJ. PDB, Hansens and glucose nitrate media contains all nutrients which favours growth of microorganisms. During present investigation attempts have been done to prove Brassicaceae DPJ separately as the source of all the nutrients required for the fungal growth. Therefore various tests were carried out.

## Test for Carbohydrates in Fresh DPJ from *Raphanus, Brassica oleracia* and *Brassica botrytis* Medium Broth

*Molisch 's test*

A red-cum violet ring appears at the junction of two liquid in DPJ showed in Figure 4, A. This test indicates the presence of carbohydrate starch in DPJ. In earlier research starch when added at low concentration to DPJ, it favoured fungal growth and enzyme amylase. Gibberelin is the hormone which stimulates

enzyme amylase. The effect of gibberellic acid (GA) on microbial growth and spore germination of some fungi was studied. The results indicate that gibberellic acid stimulates growth of the investigated organisms with the exception of Penicillium sp. and Alternaria sp. However, with higher concentrations of GA the growth-promoting action is reduced [26]

*Iodine test*

Appearance of deep blue colour in Brassicaceae DPJ showed in Figure 4, B. This positive test indicates the presence of starch in DPJ which is required to enzyme amylase as the substrate. The positive amylase activity in the culture filtrate of the fungi grown on DPJ indicates the presence of hormone gibberellin. This hormone activity or the synthesis becomes rapid if fungi grown on DPJ.

**iii. Benedict's Test:** Formation of red, yellow or green colour precipitated in Brasicaceae DPJ showed in Figure 4, C. The positive Benedicts test indicates the presence of glucose in the DPJ. Therefore DPJ is enriched with the glucose to induce the mycelia growth. Therefore in the earlier research, DPJ from various species like Lucerne, coriander, beet leaves, *Anathum graveolens* favoured the mycelial growth and the secondary metabolites. DPJ fulfills the need of the glucose source for the growth of fungi. Cytokinins modulates cell division and differentiation in presence of glucose.

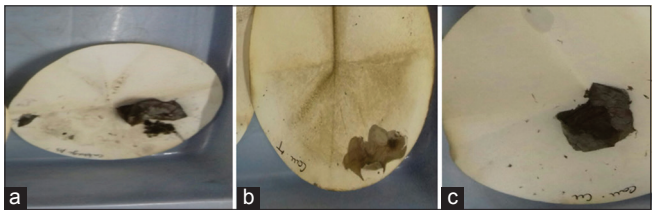


**Figure 3:** Harvesting of the MDW of the fungi after the growth on DPJ by whatman filter paper (a) Mycelia of *Aspergilus niger* (b) Mycelia of *Curvularia* (c) Mycelia of *Trichoderma viridie*

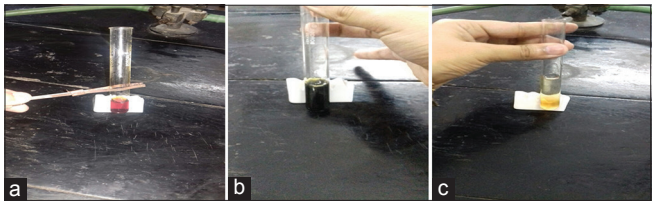


**Figure 4:** Different Carbohydrate tests in DPJ (a) Molischs Test (b) Iodine Test (c) Benedicts Test

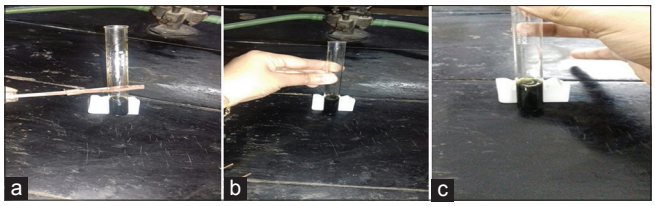


**Figure 5:** Different tests for amino acids and proteins in DPJ from *Raphanus, Brassica oleracia* and *Brassica botrytis* (a) Ninhydrin test (b) Glyoxylic reaction test (c) Sulphur test

## Test for Amino acid and Proteins from *Raphanus*, *Brassica oleracia* and *Brassica botrytis*

*Ninhydrin test*

Violet or purple colour indicates the presence of amino acids showed in Figure 5, A. The amino acids enhanced the favourable sporulation in all the three fungi. Besides amino acids, other complex structures such as peptides, peptones and proteins also react positively when subjected to the ninhydrin reaction. The tests were positive in all three DPJ. Amino acids influence its effect on optimising some fungal growth [27, 28].

*Glyoxylic reaction*

Violet ring is formed at the junction showed in Figure 5, B. This positive reaction indicates the presence of the amino acid tryptophan which is the precursor of Indole acetic acid (IAA). Therefore The DPJ contains the hormone auxin which is necessary for the callus growth, root and shoot initiation, vegetative reproduction and fungal cell proliferation. In earlier research, the DPJ from Lucerne was utilized for plant growth like cowpea, wheat etc. DPJ induced the plant growth as well as nodulation [29]. During present investigation, due to tryptophan content in all the Brassicaceae members used for DPJ, there was enhancement of the mycelia of *Aspergillus niger, Curvularia* and *Trichoderma viridie*.

*Sulphur test*

Black precipitate in Brassicaceae DPJ showed in Figure 5, C. The positive sulphur test indicates the presence of proteins and amino acid cysteine in DPJ. Cysteine regulates morphogenesis and mitochondrial activity in the dimorphic fungus [30]. This positive protein test indicates the presence of enzyme protease. Protease enhanced activity was found by the fungi in the culture broth of DPJ. Therefore it is revealed that brassicaceae DPJ Cysteine Protease has a Dual function as a Regulator step in ethylene biosynthesis in higher plants [31].

## CONCLUSION

It is evident that all the carbohydrates, amino acids and protein tests found positive in DPJ obtained from Brassicaceae viz., *Raphanus*, *Brassica oleracia* and *Brassica botrytis*. Tryptophan is the precursor of IAA. It proves that tryptophan positive test i. e. glyoxylic test indicates presence of plant growth regulator IAA in it. Gibberellins induce enzyme amylase. The presence of amylase by cup plate assay indicates phytohormone gibberellin in the culture filtrate of the fungi grown on DPJ in earlier findings. The positive benedicts test of glucose indicates the interaction with the cytokinin as the fungi grows favourable by cell division and differentiation. Therefore DPJ can also favour the callus growth during rhizogenesis or tissue culture of different plants. As the three different fungi grown on three Brassicaceae DPJ, it boosts or maximizes the synthesis of phytohormones like auxins, gibberellins, cytokinins and ethylene in the culture broth. However the work of DPJ effect on tissue culture, plant growth, fungal cultivation are in progress.

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## CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

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