International Journal of Basic and Applied Chemical Sciences ISSN: 2277-2073 (Online)
An Online International Journal Available at http://www.cibtech.org/jcs.htm
2013 Vol. 3 (4) October-December, pp. 48-61/Sayyed
Research Article

PRODUCTION OF α-AMYLASE ON DPJ OF FOUR DIFFERENT PLANTS

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ABSTRACT
It was interesting to note that with the increase in incubation period, the mycelial dry weight increased. When *Aspergillus niger* and other seven fungi were cultivated on DPJ prepared from lucerne, the increase in mycelial dry weight was upto 171 ± 28.2 mg on 12th day. For *Spinacia oleracea* the increase in mycelial dry weight was 146.3 ± 48.9 mg, *Anethum graveolens it was* 111.5 ± 21.5 mg, *Trigonella foenium graecum* 128 ± 15.9 mg, Maximum mycelial dry weight was achieved with *Aspergillus. niger* cultivated on DPJ of *Spinacia oleracea* after 12th day of inoculation. During present investigation positive correlation between incubation time on growth of fungi and mycelial dry weight was significant. Maximum production of ∝-amylase was recorded on seventh day of inoculation (264.6 ±177.95 U/ml) among which *A.niger* recorded 606 U/ml ∝-amylase activity when this fungi was grown on DPJ of lucerne. During present course of investigation it was concluded that *A. niger* produced maximum enzyme ∝-amylase on 7th day of inoculation (606 U/ml) on the DPJ of lucerne.

Key Words: *α-amylase, LPC, Protein, Lucerne*

INTRODUCTION
During the process of Green Crop Fractionation (GCF), mechanical operations (grinding and pressing) separates fresh green leafy foliage into two fractions: (1) Protein and moisture rich juice and (2) fibrous Pressed Crop Residue (PCR). The juice or leaf extract, expressed due to the pressing of green foliage is usually employed to prepare Leaf Protein Concentrate (LPC). For this purpose, it is either heated or acidified for the coagulation of proteins, which results into a curd called as leaf protein concentrate (LPC). The LPC is then separated from the remaining portion of the juice – Deproteinised Juice (DPJ) – by filtration through cotton cloth. Thus, the process of GCF results into three fractions. 1) Pressed Crop Residue (PCR) left after the extraction of juice, which can be used as a feed for cattle (Raymond and Harris, 1957; Bryant *et al.*, 1983; Walker *et al.*, 1983) Leaf protein concentrate (LPC) obtained due to the precipitation of proteins in juice which can be used as a protein – vitamin – mineral supplement in poultry, calf (Joshi *et al.*, 1983) and human nutrition (Pirie, 1978; Shah, 1983) and A tobacco leaf protein concentrate (TLPC) from bidi tobacco leaves was prepared by a heat coagulation method which contained 52% protein and was free from nicotine and polyphenols (Parameswaran *et al.*, 1988). Highest yields per acre of essential amino acids were calculated from forages which could be processed into leaf protein concentrates. Alfalfa produced the highest yield per acre of essential amino acids of the twenty major crops; soybean seed was second (Walter and Stahmann, 1966). Leaf protein concentrates were prepared by heat coagulation of lucerne 'brown juice'. A fraction of the phenolics was completely extractable by organic solvents. Coumestrol (which occurs in lucerne leaf proteins) was estimated after progressive elution with solvents and fluorimetry in solution. Significant reductions of both coumestrol content and total flavonoîdes were observed after washing of the concentrates. (Rambourg and Monties, 1983). LPC of twenty plants was prepared. Cyanocobalamine (B12), ascorbic acid (vitamin C) and folic acid (vitamin B9) was estimated from LPC. Higher yield of vitamin B12 (cynocobalamine) in wet LPC was recorded in Bennicasa hispida, Coccina grandis and Vigna mungo. The maximum yield of vitamin C (Ascorbic acid) and folic acid was recorded in Vigna mungo (Badar and Sayyed, 2010a).Thiamine, riboflavin and pyridoxine were estimated from leaf protein concentrate (LCP) of twenty plants viz *Adhatoda vasica, Benincasa hispida, Brassica oleracea V. botrytis, Brassica oleracea V. Capitata, Cassia tora, Centella*

Asiatica, Coccinia grandis, Coriandrum sativum, Cucusmis sativus, Eclipta alba, Erythrina varegata, Medicago sativa, Moringa oleofera, Phaseolus valgaris, Raphanus sativus, Sesbania grandiflora, Solanum nigrum, Trigonella foenum graecum and Vigna mungo. Higher yield of thiamine was recorded in LPC of *Brassica oleracea V. botrytis, Brassica oleoracea V. capitata, Coriandrum sativum, Raphanus sativus* and *Vigna mungo*. LPC of *Adhatoda vasica and Trigonella foenum graecum* yielded maximum riboflavin while more pyridoxine was estimated from *Brassica oleracea, V. botrytis* and *Bennicasa hispida* (Badar and Sayyed 2010b). It was observed that the heat coagulation method was most suitable for commercial production of LPC as it is simple, less expensive and gives a product of desirable value (Sayyed, 2011) Deproteinised juice (DPJ) left behind after isolating LPC from the heated juice which is considered as a by-product of GCF system.

The DPJ is rich in water soluble nutrients present in the leaves. It contributes to more than 50% of the fresh weight from green foliage which is fractionated for the production of pressed crop residue (PCR) and leaf protein concentrate (LPC). It is rich in water soluble carbohydrates, free amino acids, minerals, lipids and vitamins. It also contains small fraction of protein. This product, with 4 to 5% solids, contain large proportion of nitrogen and phosphorus (Ream *et al.,* 1983). Festenstein (1961) observed that the DPJ may contain from 11 to 47 g dry matter (DM), 0.25 – 1.20 g nitrogen (N) and 2.5 to 22.0 g carbohydrates per litre.

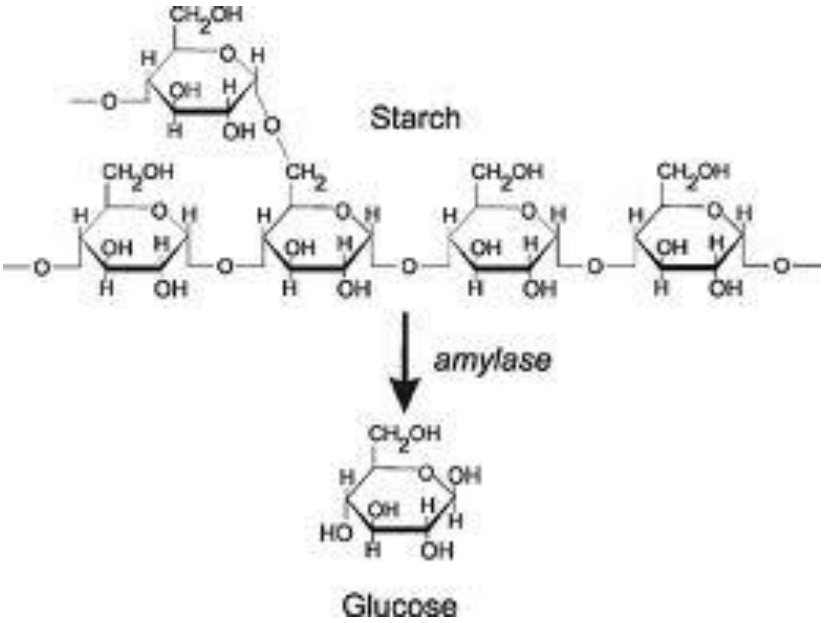
The dry matter (DM) and nutrient composition of DPJ varies from species to species. At Rothemsted Experimental Station in U.K., the DM content in this fraction was found to be between 1.2 to 4.0%. Pirie (1971) stated that, on an average, the N and carbohydrate content in DM of DPJ are 3 and 40% respectively. The dominant monosaccharides in DPJ are glucose and fructose. However, the contents of these reducing sugars in DPJ is subject to a great change, depending upon the species used for GCF and maturity of the plants used (Bekeries *et al.,* 1983). The contents of nitrogenous substances in the DPJ also vary widely. Pirie (1942) suggested that this by-product should be disposed properly in order to avoid local environmental bio-pollution.

Davys (1973) suggested the use of DPJ for irrigation as a source of nutrients and as a soil conditioner. When the DPJ is added to soil, growth of gas forming bacteria increases with subsequent improvement in the structure of intractable soil (Arkcoll, 1973). Research on the suitability of DPJ as a culture medium for growth of micro-organisms started in Uppsala during 1955 (Pirie, 1971).

Jonsson (1962) cultivated seven types of micro-organisms on DPJ from pea vines and other leafy materials. He observed that the DPJ supports growth of *Rhizobium maliloti, Penicillium chrysogenum* and *Aspergillus niger* Worgan and Wilkins (1977) evaluated the growth of the *Trichoderma* and *Aspergillus* on DPJ of lucerne. Pirie (1971) also stressed that uncoagulable material i.e. the DPJ is suitable for the cultivation of micro-organisms.

At the Indian Statistical Institute, Calcutta, Chanda *et al.,* (1984) reported that some important micro-organisms can grow on DPJ samples obtained from various crops. Amylase production by various fungi cultivated on DPJ of lucerne under various environmental conditions was studied by Sayyed and Mungikar (2000) and they reported that the DPJ could be employed for enzyme production by cultivating fungi.

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain much starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because amylase turns some of their starch into sugar in the mouth. The pancreas also makes amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria also produce amylase. As *diastase*, amylase was the first enzyme to be discovered and isolated (Robert and Joseph, 1970; Richard, 2002; Jochanan, 1998 and Robert, 1995). Specific amylase proteins are designated by different Greek letters. All amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds.



During present investigation attempts have been made to study microbiological aspects of DPJ in view of its use as a medium to cultivate fungi. For this purpose the samples of DPJ obtained from lucerne and other plants species were employed for cultivation of different fungi. Experiments were also undertaken to explore the possibility of growing fungi efficiently by correcting nutrient status of deproteinised juice. Attempts were also made during present investigation to evaluate the use of DPJ as a medium for growing micro-organisms and production of ∝-amylase by fungi grown on DPJ.

MATERIALS AND METHODS

Preparation of DPJ

The green foliages from *Medicago sativa, Anethum graveolens, Trigonella foenium graecum,* and *Spinacia oleracea* were used for fractionation. The juice released during fractionation was employed for the preparation of LPC by heat coagulation and the DPJ released after precipitation and isolation of proteins in juice was collected. The samples of DPJ were dried in hot air oven at 65^{o}C. The dry DPJ was stored in sealed glass jar until used. Sufficient care was taken to minimise absorption of moisture by the DPJ samples.

Preparation of Culture Media

The conventional GN medium was prepared by dissolving glucose 10 g, KNo_3 2.5 g, KH_2 PO_4 1g and $MgSo_4$ 0.5g in one litre of distilled water. Simultaneously, the dry DPJ was dissolved in distilled water at 2% concentrations and used as a medium for growing fungi.

Sterilization

Twenty five ml of either GN medium or the aqueous solution of DPJ was poured into 250 ml conical flask. The flask were then plugged with non absorbent cotton and autoclaved at 15 lbs for 30 minutes.

Inoculation

The autoclaved flasks were transferred to the inoculation room for inoculation with fungi. The stock cultures of the fungi used during present study were collected from the Departmental culture collection wherein the fungi were maintained on Potato Dextrose Agar (PDA) medium. The inoculation was always done in UV chamber under aseptic condition. The inoculum in the form of spore suspension was prepared by adding 10 ml sterile distilled water to six day old slope culture of the fungi. The medium, either GN or DPJ, was inoculated with 5 drops of the spore suspension which contained 5 x 10^2 spores per microscopic field. The inoculated flasks were incubated at room temperature.

Collection of Microbial Biomass

The flasks were inoculated for 8 to 12 days after inoculation. The fungal biomass was harvested by filtration through Whatman filter paper. The mycelial biomass was dried along with the filter paper in an oven at 65±5°C till constant weight. The yield of mycelial dry weight (MDW) was then recorded by substracting the weight of filter paper from the weight recorded for dry mycelium.

A blank or control flask was also processed simultaneously, during all experiments wherein flasks containing either GN or DPJ medium remained uninoculated. The MDW was corrected each time by substracting the dry weight obtained from uninoculated flasks.

During all experiments, the culture filtrate was collected after harvesting the microbial biomass, centrifuged at 10,000 rpm for 15 minutes at 0°C and supernatant was used as the crude enzyme extract as source of ∝ -amylase.

Assay of ∝-amylase

Amylase activity was determined using modified Bern Field's (1955) method. The starch solution (1%) for which was prepared by adding a paste of soluble starch to boiling phosphate buffer (20 mM) and stirring constantly with glass rod. This solution was filtered with Whatman paper No.1 before use.

Starch solution (1 ml) was taken in a glass tube and placed in water bath at 40°C for temperature equilibration. 1 ml properly diluted crude enzyme extract (either from GN media or DPJ) was added to preincubated starch. The reaction mixture was incubated at 40°C for 20 minutes and the reaction was terminated by adding 0.4 ml DNS reagent prepared by adding 1 g of 3,5 dinitrosalysilic acid to 20 ml of 2 N NaoH and 30 g sodium potassium tartarate. The blank was prepared similarly but without substrate i.e. starch. The tubes were kept in boiling water bath after addition of DNS reagent for 5 minutes, cooled immediately under tap water and reaction mixture was diluted by adding 3 ml distilled water and the amylase activity was determined by measuring the optical density (O.D) at 460 nm and comparing it with the calibration curve. The calibration curve was established with maltose (0.2 to 2 mg/2 ml water) and was used to convert the optical density (O.D.) into the amount of maltose released. Amylae activity was expressed in terms of mg of maltose liberated within one minute when the reaction mixture was incubated for 20 minutes at 40°C with 1 ml of the crude enzyme extract.

The unit of enzyme activity (U) was defined as the number of micromoles of ∝-1-4 glycosidic bonds hydrolyzed per minute and was calculated as described by John and Rosalie (1987) using following equation:

$$\text{Unit (U)} = \frac{\mu\text{g of maltose equivalents produced/min/ml of digest}}{342}$$

Where, 342 is the molecular weight of maltose.

Production of ∝- amylase

During the first experiment, *Aspergillus niger, A. flavus, Helminthosporium oryzae, Fusarium oxysporum, Phytophthora infestans* and *Curvularia lunata* were grown on the DPJ expressed from *Medicago sativa, Anethum graveolens, Spinacia oleracea and Trigonella foenium graecum* and also on conventional GN medium to evaluate the suitability of DPJ as a medium for fungal growth and subsequent production of enzyme ∝-amylase. The culture filtrates left after isolating the mycelium after 7 days of growth of these fungi were employed to assay ∝-amylase production as described above.

Effect of Incubation Period on the Production of ∝-amylase

Experiment IV gives information about the effect of incubation period on ∝-amylase production. For this experiment eight different fungi were cultivated on the DPJ samples expressed from *Medicago sativa, Spinacia oleracea, Anethum graveolens* and *Trigonella foenium graecum.* The microbial biomass obtained on these DPJ samples was harvested after the incubation period of 1 to 12 days as described earlier. In order to find out the optimum incubation period for ∝-amylase production, the culture filtrates from the flasks wherein the fungi were incubated for 1 to 12 days were tested.

RESULTS AND DISCUSSION

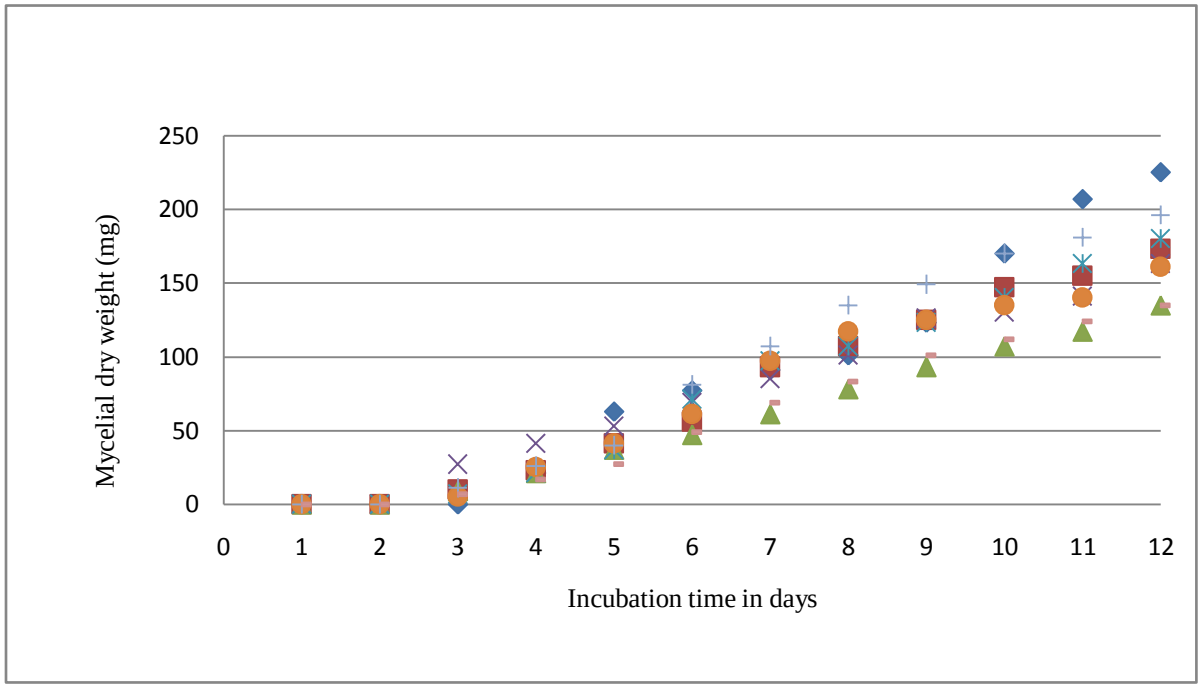


Figure 1: Effect of incubation time on growth of fungi on DPJ prepared from *Medicago sativa* L

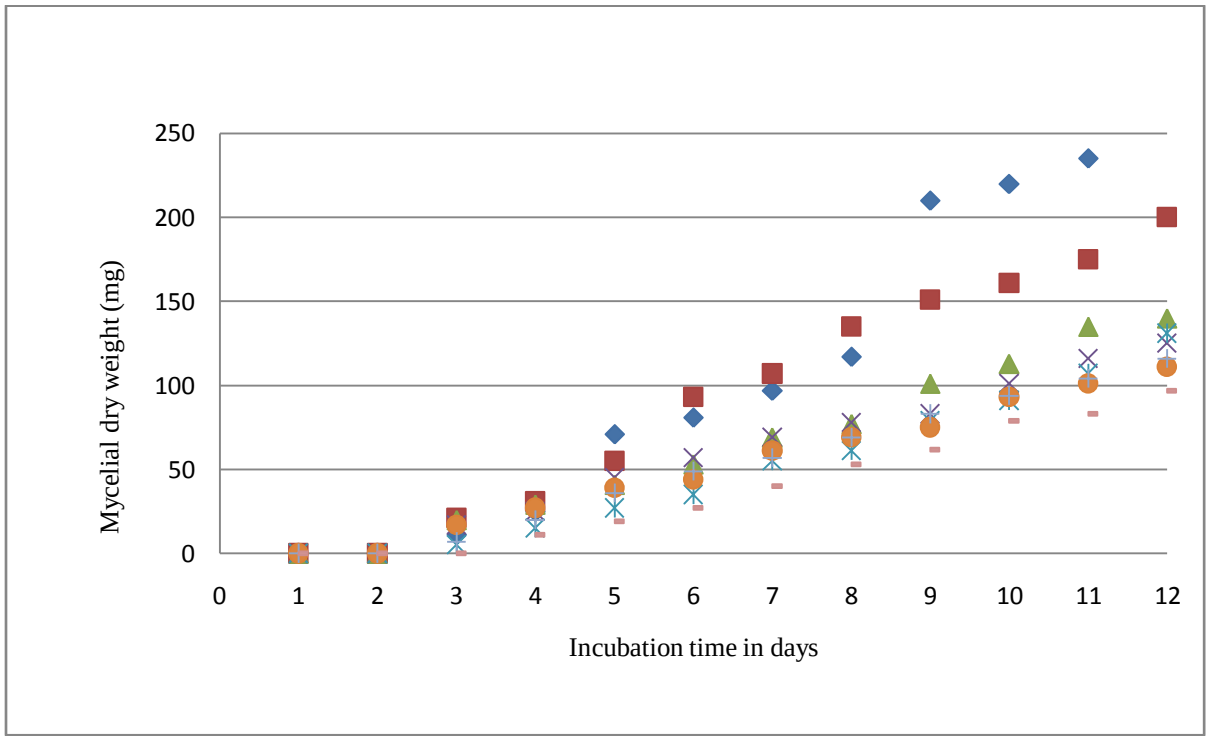


Figure 2: Effect of incubation time on growth of fungi on DPJ prepared from *Spinacia oleracea*

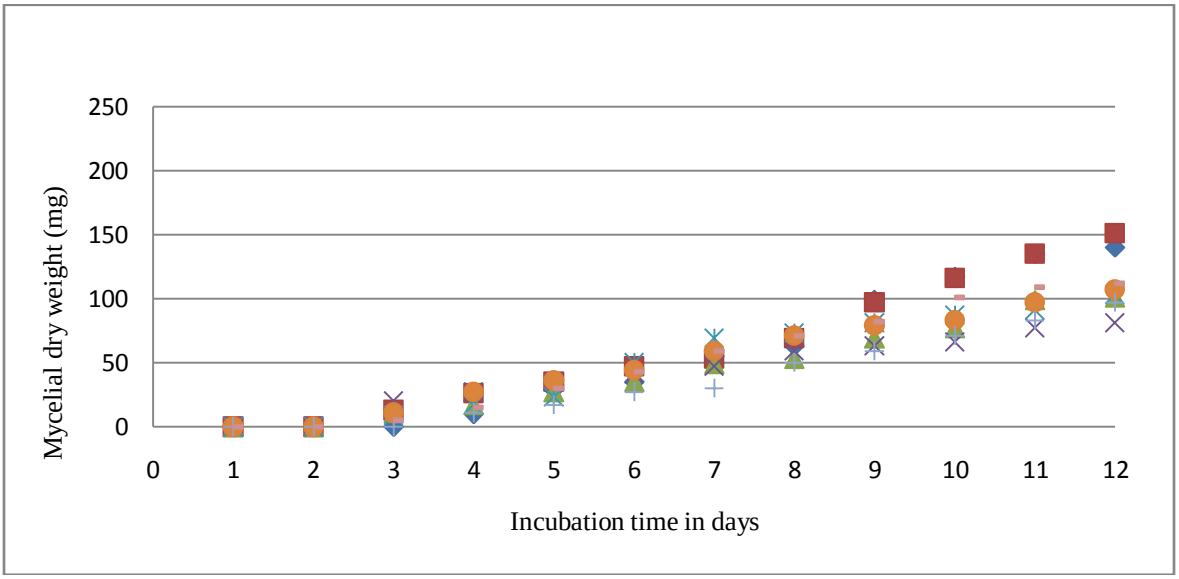


Figure 3: Effect of incubation time on growth of fungi on DPJ prepared from *Anethum graveolens*

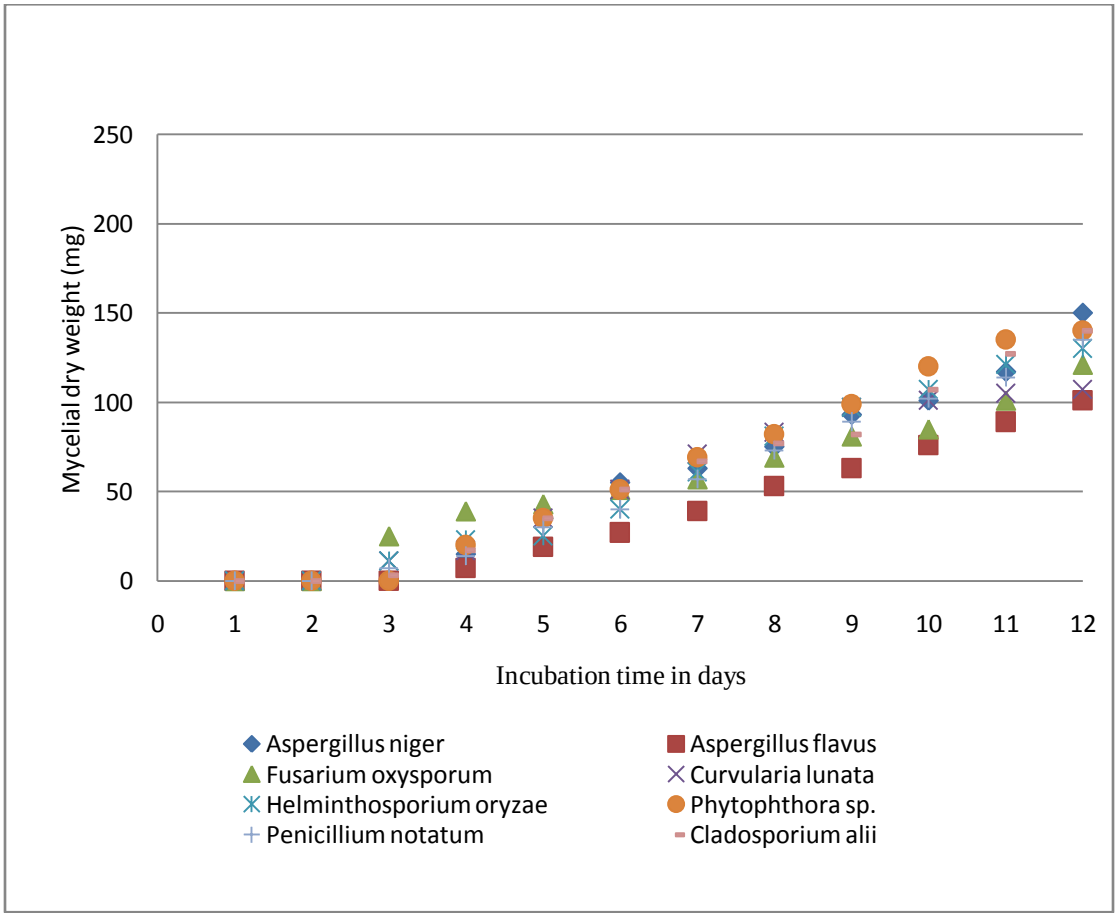


Figure 4: Effect of incubation time on growth of fungi on DPJ prepared from *Trigonella foenium graecum*

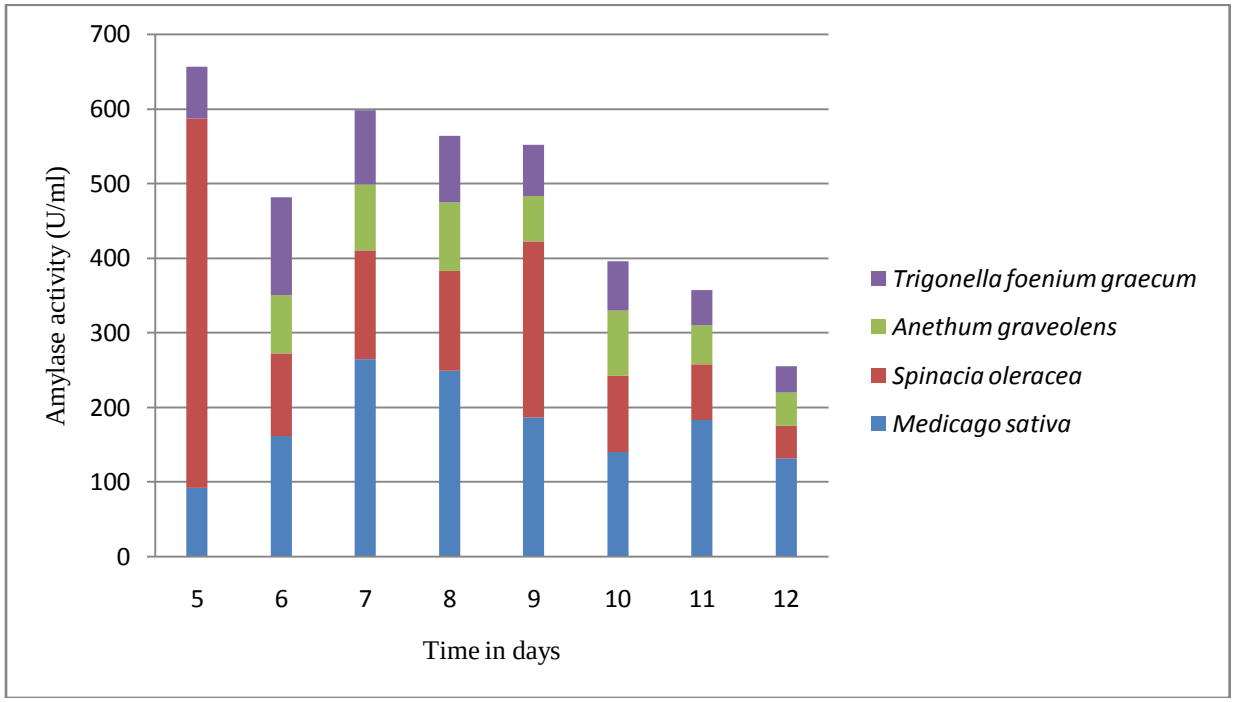


Figure 5: Effect of incubation period on production of amylase by various fungi grown on DPJ

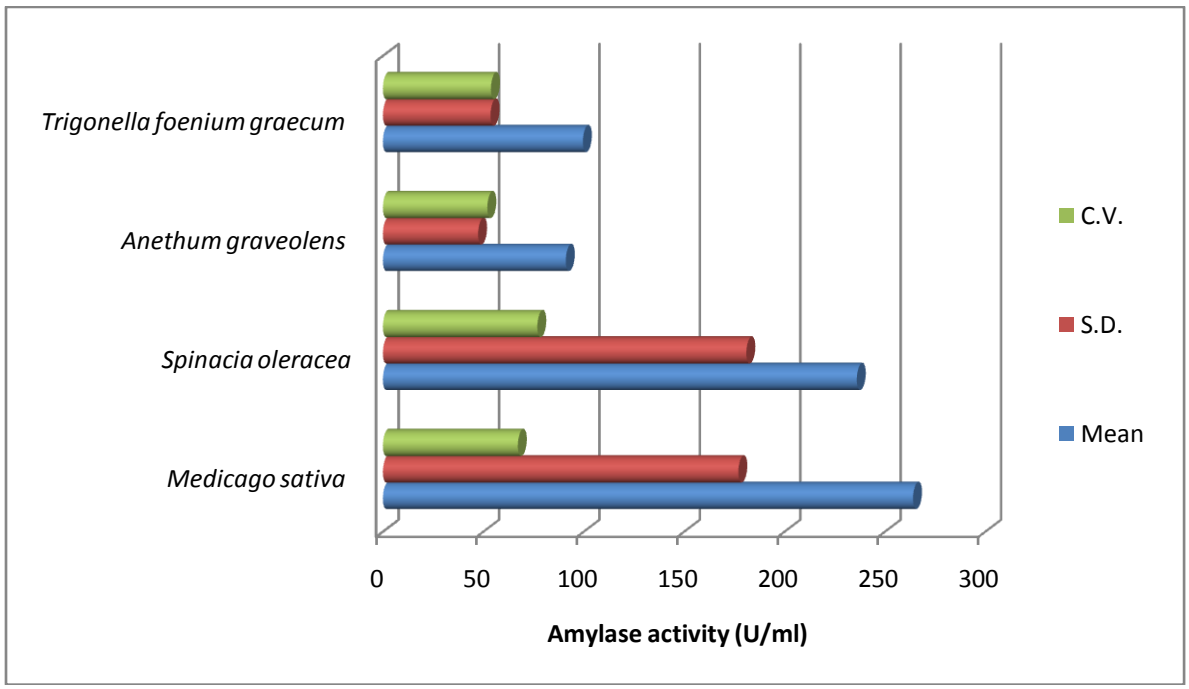


Figure 6: Effect of incubation period on production of amylase by various fungi grown on DPJ

Table 1: Effect of incubation time on growth of fungi on DPJ prepared from *Medicago sativa* L

Fungi	Mycelial dry weight (mg) Incubation time in days												r
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Aspergillus niger	-	-	-	021	063	077	091	101	123	170	207	225	0.873
Aspergillus flavus	-	-	010	023	041	056	093	107	125	147	155	173	0.902
Fusarium oxysporum	-	-	009	021	037	047	061	078	093	107	117	135	0.907
Curvularia lunata	-	-	027	041	053	069	085	101	126	130	141	163	0.938
Helminthosporium oryzae	-	-	007	021	037	071	097	107	123	140	163	180	0.902
Phytophthora sp.	-	-	005	025	041	061	097	117	125	135	140	161	0.914
Penicillium notatum	-	-	011	026	040	081	107	135	149	170	181	196	0.907
Cladosporium alii	-	-	007	017	027	049	069	083	101	112	124	135	0.900
Mean	-	-	10.8	24.3	42.3	63.8	87.5	103.6	120.6	138.8	153.5	171	0.905
S.D.	-	-	6.8	6.7	10.2	11.8	14.3	16.8	15.9	21.8	27.9	28.2	0.016
C.V.	-	-	63.1	27.9	24.2	18.5	16.4	16.2	13.2	15.7	18.2	16.5	1.850

Table 2: Effect of incubation time on growth of fungi on DPJ prepared from *Spinacia oleracea*

Fungi	Mycelial dry weight (mg) Incubation time in days												r
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Aspergillus niger	-	-	011	025	071	081	097	117	210	220	235	251	0.874
Aspergillus flavus	-	-	021	031	055	093	107	135	151	161	175	200	0.926
Fusarium oxysporum	-	-	020	029	041	053	069	077	101	113	135	140	0.915
Curvularia lunata	-	-	017	025	045	057	069	078	083	101	116	125	0.935
Helminthosporium oryzae	-	-	005	015	027	035	055	061	079	091	107	131	0.866
Phytophthora sp.	-	-	017	027	039	044	061	069	075	093	101	111	0.889
Penicillium notatum	-	-	007	020	036	049	057	069	083	094	104	116	0.920
Cladosporium alii	-	-	-	011	019	027	040	053	062	079	083	097	0.883
Mean	-	-	14	22.8	41.6	54.8	69.3	82.3	105.5	119	132	146.3	0.901
S.D.	-	-	5.8	6.5	15.0	20.8	20.8	26.6	46.8	44.7	46.7	48.9	0.024
C.V.	-	-	41.9	28.5	36.2	37.9	30.0	26.6	44.3	37.5	35.4	33.4	2.707

Table 3: Effect of incubation time on growth of fungi on DPJ prepared from *Anethum graveolens*

Fungi	Mycelial dry weight (mg) Incubation time in days												r
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Aspergillus niger	-	-	-	010	029	035	055	063	099	117	135	140	0.857
Aspergillus flavus	-	-	013	026	035	047	053	069	097	116	135	151	0.879
Fusarium oxysporum	-	-	011	017	027	035	049	053	069	077	099	101	0.900
Curvularia lunata	-	-	020	027	035	041	047	059	063	066	077	081	0.921
Helminthosporium oryzae	-	-	009	017	023	050	069	073	081	087	091	103	0.921
Phytophthora sp.	-	-	011	027	036	044	059	071	079	083	097	107	0.936
Penicillium notatum	-	-	-	011	017	027	030	050	059	071	083	097	0.863
Cladosporium alii	-	-	005	015	030	043	059	071	082	101	109	112	0.906
Mean	-	-	11.5	18.7	29.0	40.2	52.6	63.6	78.6	89.7	103.2	111.5	0.897
S.D.	-	-	4.5	6.5	6.1	7.01	10.6	8.2	13.6	18.3	20.4	21.5	0.027
C.V.	-	-	39.4	35.0	21.3	17.4	20.2	12.9	17.3	20.4	19.8	19.3	3.011

Table 4: Effect of incubation time on growth of fungi on DPJ prepared from *Trigonella foenium graecum*

Fungi	Mycelial dry weight (mg) Incubation time in days												r
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Aspergillus niger	-	-	-	015	035	055	063	075	093	101	117	150	0.889
Aspergillus flavus	-	-	-	007	019	027	039	053	063	076	089	101	0.868
Fusarium oxysporum	-	-	025	039	043	051	057	069	081	085	101	121	0.938
Curvularia lunata	-	-	011	017	035	051	071	083	097	101	105	107	0.927
Helminthosporium oryzae	-	-	011	023	025	040	061	081	097	107	121	130	0.895
Phytophthora sp.	-	-	-	020	035	051	069	082	099	120	135	140	0.898
Penicillium notatum	-	-	007	014	030	040	057	073	089	102	114	135	0.892
Cladosporium alii	-	-	003	017	035	051	067	077	082	107	127	140	0.895
Mean	-	-	11.4	19	32.1	45.7	60.5	74.1	87.6	99.8	113.6	128	0.900
S.D.	-	-	7.4	8.7	6.8	8.7	9.4	9.1	11.2	12.7	13.8	15.9	0.020
C.V.	-	-	65.0	45.9	21.4	19.1	15.6	12.3	12.8	12.7	12.2	12.4	2.302

Table 5: Effect of incubation period on production of amylase by various fungi grown on DPJ of *Medicago sativa* L.

Fungi	Amylase activity (U/ml) Time in days							
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Aspergillus niger	-	209	606	552	500	446	345	146
Aspergillus flavus	92	128	182	135	92	87	539	45
Fusarium oxysporum	-	148	216	268	227	142	-	-
Curvularia lunata	-	-	-	-	155	92	76	42
Helminthosporium oryzae	-	-	232	220	133	78	67	56
Phytophthora sp.	-	-	-	-	83	87	90	95
Cladosporium alii	-	-	-	-	142	137	110	85
Penicillium notatum	-	-	87	74	60	56	56	539
Mean	92	161	264	249	186	140	183	131
S.D.	0	34.4	177	165	163	118	172	157
C.V.	0	21.3	67.2	66.1	87.7	84.2	74.0	120

Table 6: Effect of incubation period on production of amylase by various fungi grown on DPJ of *Spinacia oleracea* L

Fungi	Amylase activity (U/ml) Time in days							
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Aspergillus niger	60	87	363	400	542	69	29	6
Aspergillus flavus	-	67	87	142	142	137	99	4
Fusarium oxysporum	-	-	51	65	148	142	114	96
Curvularia lunata	-	-	-	99	495	67	49	31
Helminthosporium oryzae	-	146	123	87	87	56	24	6
Phytophthora sp.	-	-	-	187	175	142	119	92
Cladosporium alii	-	76	92	81	67	-	-	-
Penicillium notatum	-	180	164	146	135	101	84	76
Mean	495	111	146	134	236	102	74	44.4
S.D.	0	44.0	102	70.0	181	35.5	36.8	39.0
C.V.	0	39.6	70.0	52.2	76.9	34.8	49.7	87.9

Table 7: Effect of incubation period on production of amylase by various fungi grown on DPJ of *Anethum graveolens* L

Fungi	Amylase activity (U/ml) Time in days							
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Aspergillus niger	-	69	76	187	100	74	69	51
Aspergillus flavus	-	87	128	119	99	-	-	-
Fusarium oxysporum	-	-	76	69	22	-	-	-
Curvularia lunata	-	-	56	56	49	47	42	-
Helminthosporium oryzae	-	-	-	29	29	202	-	-
Phytophthora sp.	-	-	108	128	130	126	96	74
Cladosporium alii	-	-	-	60	62	51	33	29
Penicillium notatum	-	-	87	81	62	24	22	24
Mean	-	78	88.5	91.1	60.7	87.3	52.4	44.5
S.D	-	9	23.4	47.4	34.7	60.2	26.7	19.8
C.V.	-	11.5	26.5	52.0	57.2	68.9	51.0	44.5

Table 8: Effect of incubation period on production of amylase by various fungi grown on DPJ of Trigonella foenium graecum L

Fungi	Amylase activity (U/ml) Time in days							
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Aspergillus niger	69	262	205	164	47	-	-	-
Aspergillus flavus	-	40	40	31	29	-	-	-
Fusarium oxysporum	-	-	-	78	74	-	56	24
Curvularia lunata	-	-	110	78	67	-	49	49
Helminthosporium oryzae	-	-	112	123	142	81	31	33
Phytophthora sp.	-	-	-	74	67	55	-	-
Cladosporium alii	-	-	62	74	51	42	38	15
Penicillium notatum	-	92	69	92	74	353	62	53
Mean	69	131	99.6	89.2	68.8	66.3	47.2	34.8
S.D.	0	94.8	53.6	36.8	31.2	112	11.3	14.4
C.V.	0	72.3	53.8	41.2	45.3	169	24.0	41.5

The effect of incubation period on amylase production by eight fungi is given in Tables 5-8. On an average, maximum production of ∝-amylase was recorded on seventh day of inoculation (264.6 ±177.95 U/ml) among which *A.niger* recorded 606 U/ml ∝-amylase activity when this fungi was grown on DPJ of lucerne (Table 5). When this fungi was cultivated on DPJ of *Spinacia oleracea*, the production of ∝-amylase was maximum on 9th day of inoculation 236.5±181.9 U/ml). Out of the fungi cultivated, *A. niger* produced 542 U/ml ∝-amylase on the 9th day of inoculation (Table 6). In case of *Anethum graveolens*, the maximum production of ∝-amylase by fungi was recorded on eighth day of inoculation (91.1 ± 47.4 U/ml), whereas *A. niger* produced maximum ∝-amylase activity (187 U/ml) on 8th day of inoculation (Table 7). These fungi, when inoculated on DPJ of *Trigonella foenium graecum* showed maximum ∝-amylase production on seventh day of inoculation (99.6 ± 53.6 U/ml) among which *A. niger* produced 262 U/ml ∝-amylase activity on sixth day of inoculation (Table 8).

From results obtained during present study it was concluded that *A. niger* produced maximum enzyme ∝-amylase on 7th day of inoculation (606 U/ml) on the DPJ of lucerne (Figures 1-6).

Conclusion

The results obtained during the experiments undertaken by author on DPJ for its potential as microbial growth medium indicated suitability of lucerne DPJ for cultivating *Aspregillus niger*. It was thus concluded from the experiments undertaken during present investigation that for maximum production of ∝–amylase take place by *Aspergillus niger*. From the results, it can be concluded that *Aspergillus niger* produced maximum enzyme ∝–amylase on 7th day of incubation period on the DPJ of lucerne. Production of ∝-amylase will be helpful in the field of microbial biotechnology.

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