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Production of Lovastatin by *Aspergillus fischeri* NCIM-509 using horse gram, grape pomace and sweet potato under Solid state fermentation

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ABSTRACT

Production of lovastatin was carried out by Aspergillus fischeri NCIM-509 using agro-industrial residues like horse gram,grape pomace and sweet potato,as substrates under solid state fermentation. Lovastatin is a potent hypercholesterolemic drug used for lowering blood cholesterol. In recent years, lovastatin has also been reported as a potential therapeutic agent for the treatment of various types of tumors and also play a tremendous role in the regulation of the inflammatory and immune response, coagulation process, bone marrow, neovascularization, vascular tone, and arterial pressure. Process parameters like Incubation time (5-days), Temperature (30^{o}C), Moisture content (60%), Inoculum age (4 days), Inoculum volume (30% v/w),pH of the medium(6.0), carbon source (1.5% Lactose) and nitrogen source(1.5% Ammonium Sulphate) were optimized and gave an overall yield of 13.5mg/gds of lovastatin after optimization.

Keywords: Lovastatin, *Aspergillus fischeri*, horse gram, grape pomace, sweet potato, Solid state fermentation, Process parameters, Optimization.

INTRODUCTION

Lovastatin is a member of the drug class of statins, used for lowering cholesterol (hypolipidemic agent) in those withhypercholesterolemia and so preventing cardiovascular disease. It is a potent hypercholesterolemic drug used forlowering blood cholesterol. Lovastatin was the first statin approved by the US FDA [1,2].

Lovastatin, also known as Monacolin K, is a kind of fungal metabolite, serving as a competitive inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMG-CoA), the rate-limiting enzyme in cholesterol biosynthesis[3].Lovastatin not only inhibits cholesterol biosynthesis but also reduces blood cholesterol level in both humans andanimals. In addition to this, lovastatin has a clear evidence to benefit on stroke and it also shows in vivo tumorsuppression by inhibiting the synthesis of non-sterol isoprenoid compounds [4,5].

In addition, lovastatin has been used in the biomedical applications such as in treating coronary heart diseases, renaldiseases, Alzheimer's disease and bone fractions[6] Several fungal genera including *Aspergillus*, *Penicillium*,*Monascus*,

Paecilomyces, *Trichoderma*, *Scopolariopsis*, *Doratomyces*, *Phoma*, *Phythium*, *Gymnoascus*, *Hypomyces*and *Pleurotus* have been reported to be able to produce lovastatin [7].

Currently, lovastatin is mainly produced by submerged fermentation (SmF), a very few works are carried on solidstate fermentation (SSF). In recent years, researchers have shown an increasing interest in SSF as a potentialalternative of submerged fermentation because it uses economical substrates (agricultural residues), requires fewerprocessing and down-streaming stages, utilizes lesser power and generates lesser effluent It provides an opportunity toexploit agro-industrial residues for the production of value added products [8]. Moreover, SSF has higher product yieldand offers better product stability [9].

In the present investigation, we evaluate the feasibility of solid state fermentation process for the production oflovastatin by the fungal strain *Aspergillus fischeri* using horse gram,grape pomace and sweet potato as the substrates and also to optimize the process parameters (both physico-chemical and nutritional) that maximize the lovastatin yield in a step-wise manner**.**

MATERIALS AND METHODS

Microorganism: The fungal strain *Aspergillus fischeri* NCIM-509 used in this study was procured from the culture collection centre of National Collection of Industrial Microorganisms (NCIM), Pune, India. The microbial strain was maintained on Potato Dextrose Agar (PDA) medium. Inoculated slants were grown in an incubator at 28°C for five days. After that the slants were preserved at 4°C and sub-cultured once every four weeks (monthly).

Inoculums' preparation: Inoculum was prepared by adding 10.00 mL of sterilized 0.1% Tween 80 solution to awell-sporulated *Aspergillus fischeri* slant. The spore surface was scrapped with an inoculating loop to suspend the spores in an above solution and the obtained spore suspension was used as the inoculum for the fermentation process.

Solid state fermentation (SSF): Horse gram,grape pomace and sweet potato procured from the local market of Visakhapatnam, Andhra Pradesh were used as the substrates in the present investigation. Ten grams of of each dried powdered substrate was taken in a 250 mL Erlenmeyer flask and moistened with distilled water (moistening media) and autoclaved at 121ºC (15lb) for 15-20 min, cooled to room temperature and inoculated with 1.00 mL of the 5 day spore suspension (spore concentration of about 10^7-10^8 per mL) of *A.fischeris* under aseptic conditions. The contents of the inoculated flasks were mixed thoroughly and incubated at desired temperature in an incubator for desired length of fermentation time.

Optimization of Process Parameters: Various process parameters that influence the lovastatin production duringSSF were optimized over a wide range. The strategy adopted for standardization of fermentation parameters is toevaluate the effect of an individual parameter and incorporate it at the standard level before standardizing the nextparameter. The process parameters optimized in the present study include fermentation time (1-7days), incubationtemerature (26-34°C), initial moisture content (40-90% v/w), inoculum age (2-7days), inoculum volume (10-60 %v/w), initial pH (3-9), carbon sources (1% w/w) and nitrogen sources (1% w/w). All the experiments were donein triplicate and the mean values of the lovastatin yield was reported.

Extraction of lovastatin: After the incubation period, lovastatin from the fermented substrate was extracted usingorganic solvent acetonitrile. 50.00 mL of acetonitrile was added to fermented substrate. The flasks were kept in anorbital shaking incubator at 180 rpm for 2 hrs. The residue was filtered with filter paper. The solids were removed bycentrifuging the homogenate at 3000 X at 4ºC for 8 min and the resultant clear supernatant was used for analyticalstudies.

Quantitative analysis of lovastatin[10]: To 1.00 mL of Supernatant, 1.00 mL of 1% trifluroacetic acid was added andincubated for 10 min (lactonization of hydroxyl acid form of lovastatin).From the above solution 1.00 mL was takenand diluted to 10 times and further from that solution 1.00 mL was taken and diluted to 10 times with acetonitrile. The
absorbance was read at 238 nm by using UV-Visible Spectrophotometer.

RESULTS AND DISCUSSION

Selection of solid substrates in SSF for lovastatin production: The solid substrates used in solid state fermentationare generally insoluble in water and play a dual role of supply of nutrients to the microbial culture growing andanchorage for the growing cells. In SSF, the selection of a suitable substrate for a fermentation process is a critical factor and thus involves the screening of a number of agro-residual wastes for microbial growth and product formation. In the present study, three different substrates, viz. horse gram,grape pomace,and sweet potato were screened for production of lovastatin. The maximum yield of 2.7 mg/gds of lovastatin **(Fig.1)** was achieved in a medium containing horse gram as the substrate.

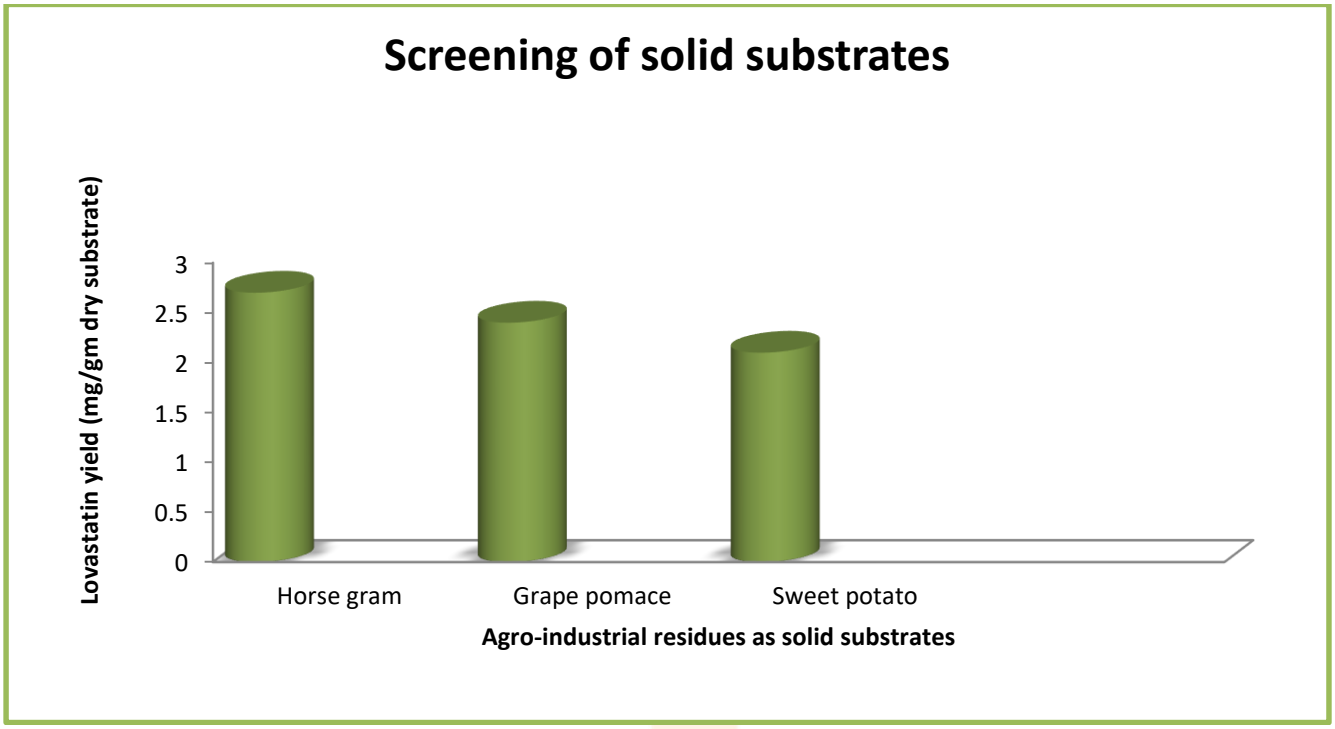

Fig. 1: Screening of solid substrates

Effect of Fermentation time: The effect of fermentation time on lovastatin production was studied by incubating thefermentation flasks from 3-7days. The maximum lovastatin yield of 3.9 mg/gds was achieved at 5days offermentation time **(Fig.2).** Lovastatin is an intracellular product, its accumulation in mycelia is growth related andsimltaneously increased as cell grows[11] .Decrease of yield after five days maybe due to onset of death phase oforganism and due to nutrient depletion. [10]Pei-Lian *et al*., reported highest yield of lovastatin at 11 days with*Aspergillus terreus* using rice powder as substrate under SSF .[9]Valera *et al*., reported maximum yield of lovastatin at6th day by*Aspergillus flavipes* using wheat bran under SSF.

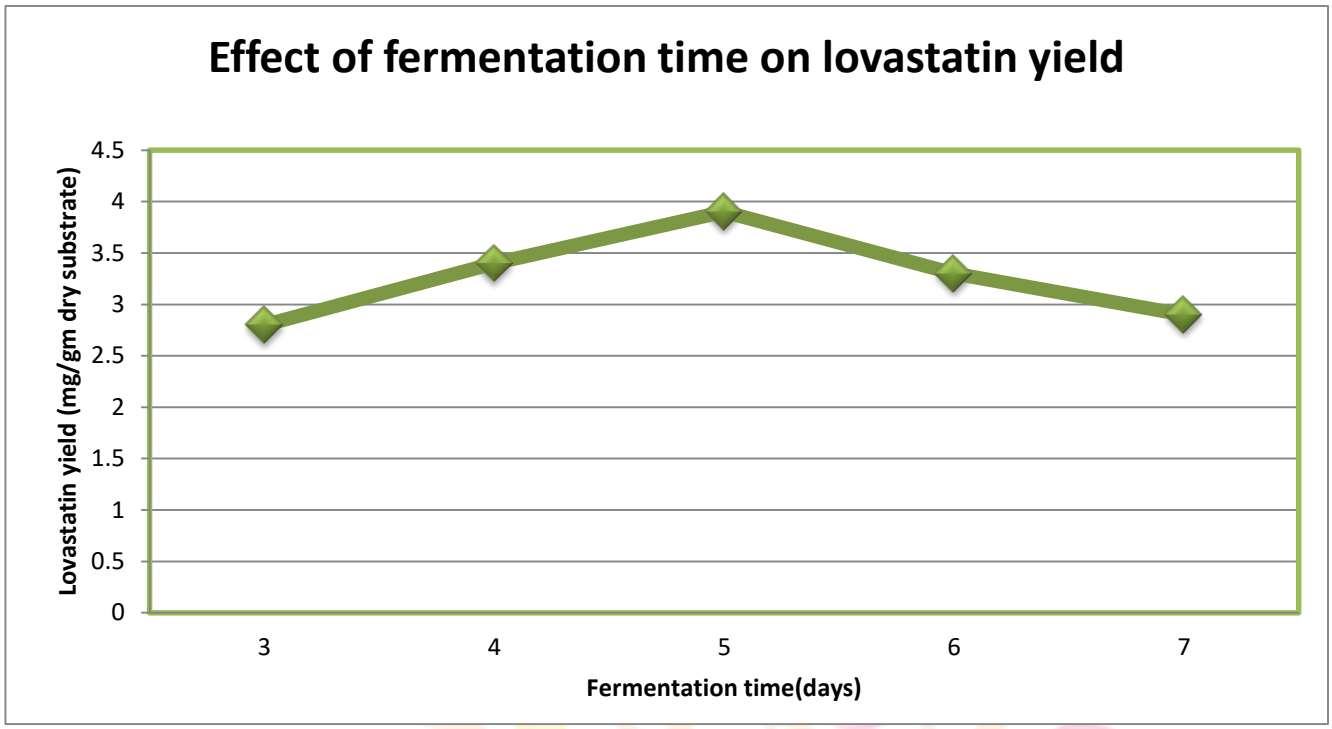

Fig. 2: Effect of fermentation time on lovastatin production

Effect of temperature on lovastatin production: Fermentation was carried out at various temperatures such as 26, 28, 30, 32, 34°C to study their effect on lovastatin production. Maximum yield of 4.1 mg/gds was observed at 30°C temperature**(Fig. 3)** and yield decreased with further increase in temperature.

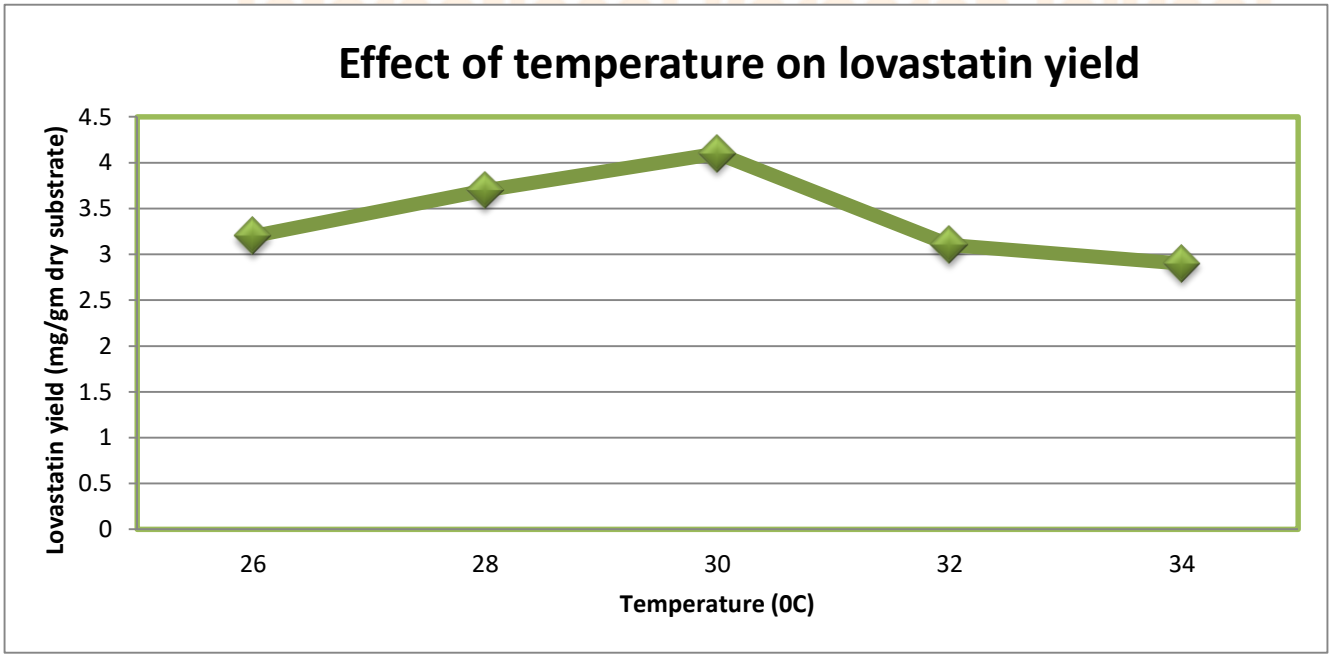

Fig. 3: Effect of temperature on lovastatin production

This decrease in yield may be due to the heat that accumulates in the medium during mesophilic aerobic SSF and poorheat dissipation which could lead to a further drop in the oxygen level and thereby reducing the growth of testorganism as lovastatin is growth related product.

Similar observation was also reported by [11]Pei-Lian *et al*., for lovastatin yield with *Aspergillus terreus* whereas[12]Panda *et al*., reported highest yield of lovastatin at 30^{o}C using co-culture of *Monascus pupereus and Monascus ruber* under SSF.

Effect of initial moisture content on lovastatin production: To investigate the influence of initial moisture contentof the substrate, fermentation was carried out with various initial moisture levels (40, 50, 60, 70, 80, 90 (% v/w)) ofhorse gram, adjusted with moistening media. Maximum lovastatin yield (4.5mg/gds) was achieved at 60%(v/w) intial moisture content **(Fig.4).**

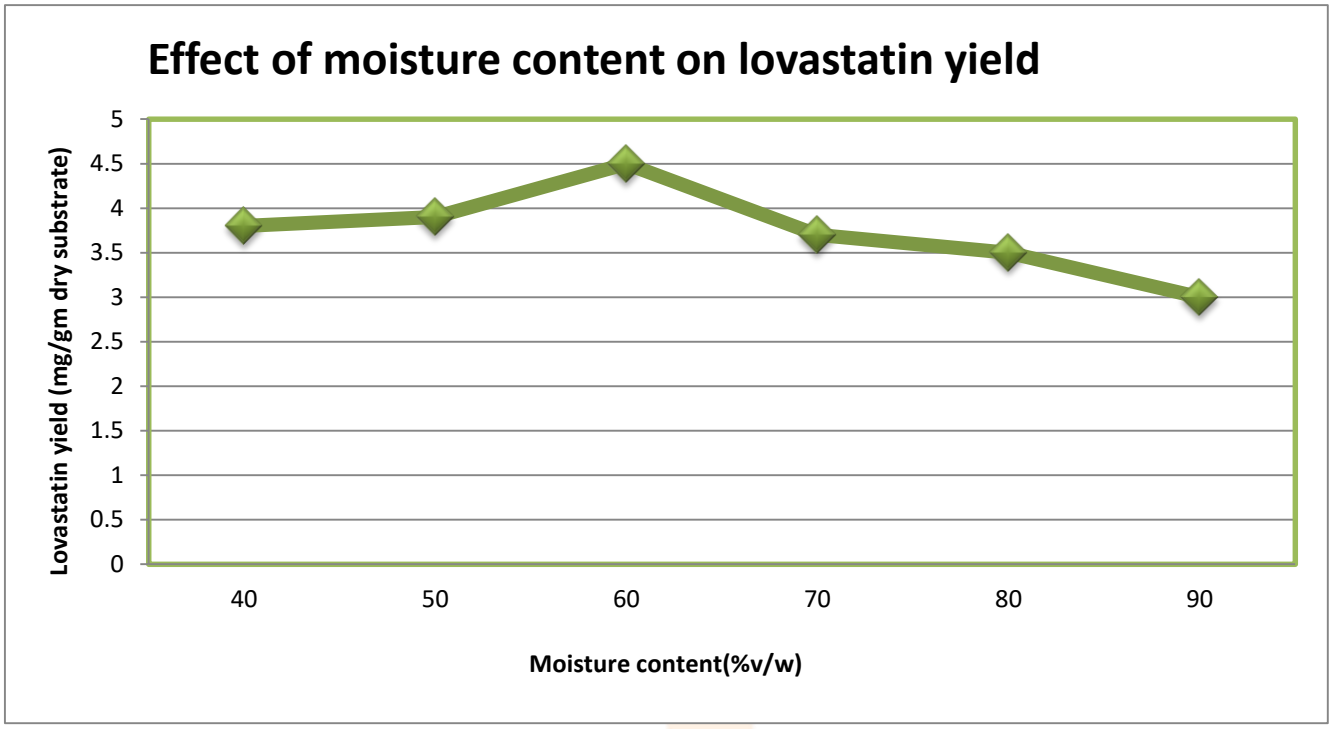

Fig. 4: Effect of moisture content on lovastatin production

As the moisture content increase the air present in the void volume decreases, resulting in poor oxygen availability inthe processes without forced aeration. With low moisture content, the available oxygen is sufficient, but the watercontent is not enough to support good metabolic activity and dissipation of heat generated. This may account for lowerlovastatin production and biomass[13].

[9]Valera *et al*., reported highest lovastatin production with *Aspergillus flavipes* at 60% moisture content under SSF and[11]Pei–Lian *et al*., also reported maximum yield of lovastatin with *Aspergillus terreus* at 60% moisture content underSSF.

Effect of inoculum age on lovastatin production: Experiments were carried out to determine the optimum age ofinoculum for the production of lovastatin by varying the age of inoculum from 2nd day to 7th day. The maximumyield of lovastatin of 5.2 mg/gds was obtained with 4th day old culture **(Fig. 5.).**

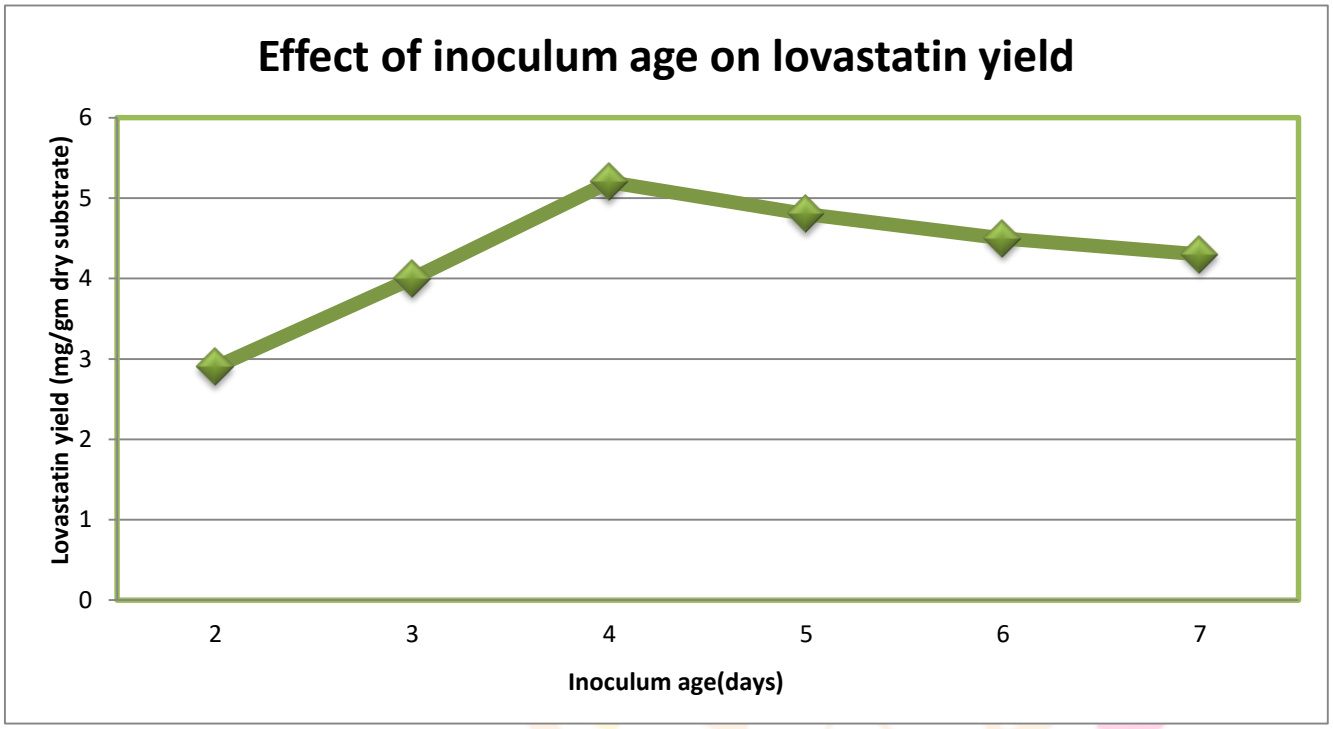

Fig. 5: Effect of inoculums age on lovastatin production

The production of biomass and lovastatin by spore initiated solid or submerged fermentation of fungi was shown to depend on the age of the spores used for inoculation. Control of spore age at inoculation of fermentation is required for obtaining optimal production of lovastatin[14].

[14]Porcel *et al*., reported highest yield of lovastatin by *Aspergillus terreus* with spore age of 16 days.

Effect of inoculum volume on lovastatin production: Fermentation was carried out with different inoculumvolume(10–60 %v/w) of five day old *A.fischeri* culture for a period of 5 days to study its effect on lovastatinproduction. Maximum yield of 6.3 mg/gds was obtained with 30 % v/w inoculum concentration **(Fig. 6).** With furtherincrease in inoculum volume/concentration , the yield decreased due to depletion of nutrients in the production medium. The free excess liquid present in an unabsorbed form will give rise to a diffusional barrier imposed by the solid nature ofthe substrate leading to the poor mycelia growth and product formation. With low inoculum volume, the yield is alsolow due to insufficient microbes to form mycelia and accumulate lovastatin (Pansuriya and Singhal. 201).[13]Pansuriya and Singhal., reported 20%v/w to be optimum for lovastatin production. [12]Panda *et al*., reported highestyield of lovastatin with inoculum volume 5.10 mL using co-culture of *Monascus purpureus* and *Monascus ruber*under SSF.

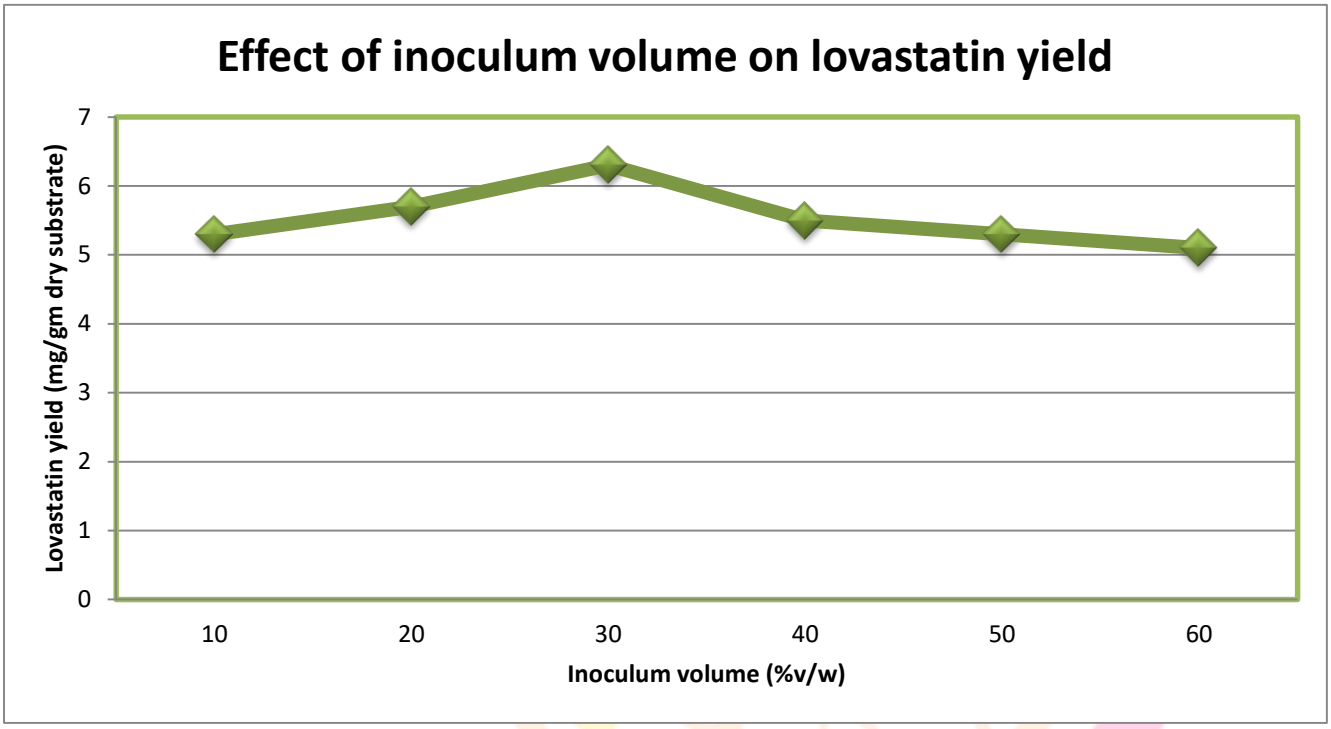

Fig. 6: Effect of inoculum concentration on lovastatin production

Effect of initial pH on lovastatin production: In order to maintain the favorable conditions for increased lovastatinproduction pH was optimized. This was established by carrying out the fermentation by varying the pH from 3-9(adjusted with 1N HCl or 1N NaOH). The maximum yield of 7.4 mg/gds was obtained at pH 6.0 **(Fig.7).**Most of the fungi grow actively at acidic pH which is necessary for transport of various components across the cell membrane thus strongly influencing the cell growth and secondary metabolites production.

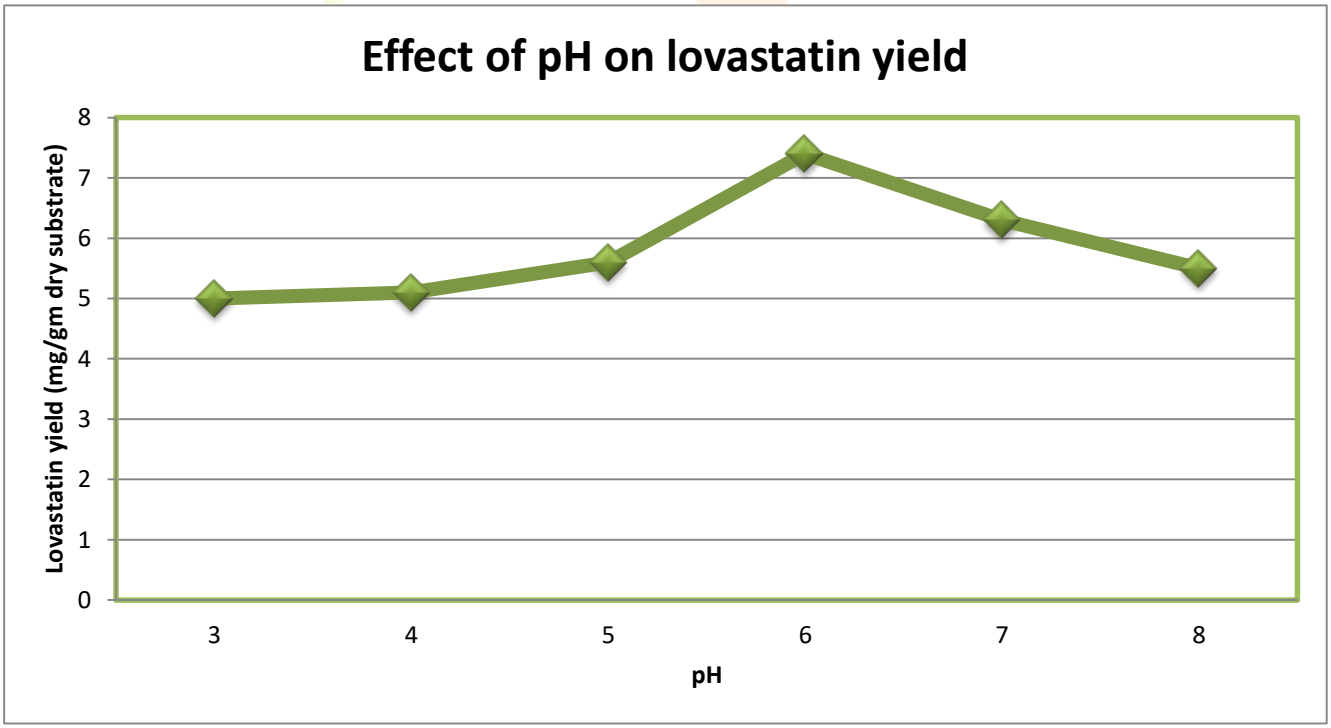

Fig. 7: Effect of pH on lovastatin production

These findings were similar with those reported by [12]Panda *et al*., for maximum lovastatin production at pH-6.0 using co-culture of *Monascus purpureus* and *Monascus ruber* under SSF where as[9]Valera *et al*., reported highest yield of lovastatin production with *Aspergillus flavipes* at pH 5.0 under SSF.

Effect of carbon supplementation on lovastatin production: To determine the effect of carbon sources on lovastatinyield, different carbon sources such as starch, sucrose, lactose, fructose, maltose, glucose, at a concentration of 1%w/w was supplemented to the basal solid state fermentative medium. Among different carbon sources tested,lactose enhanced lovastatin yield to 8.1 mg/gds when compared to other carbon sources **(Fig. 8).**

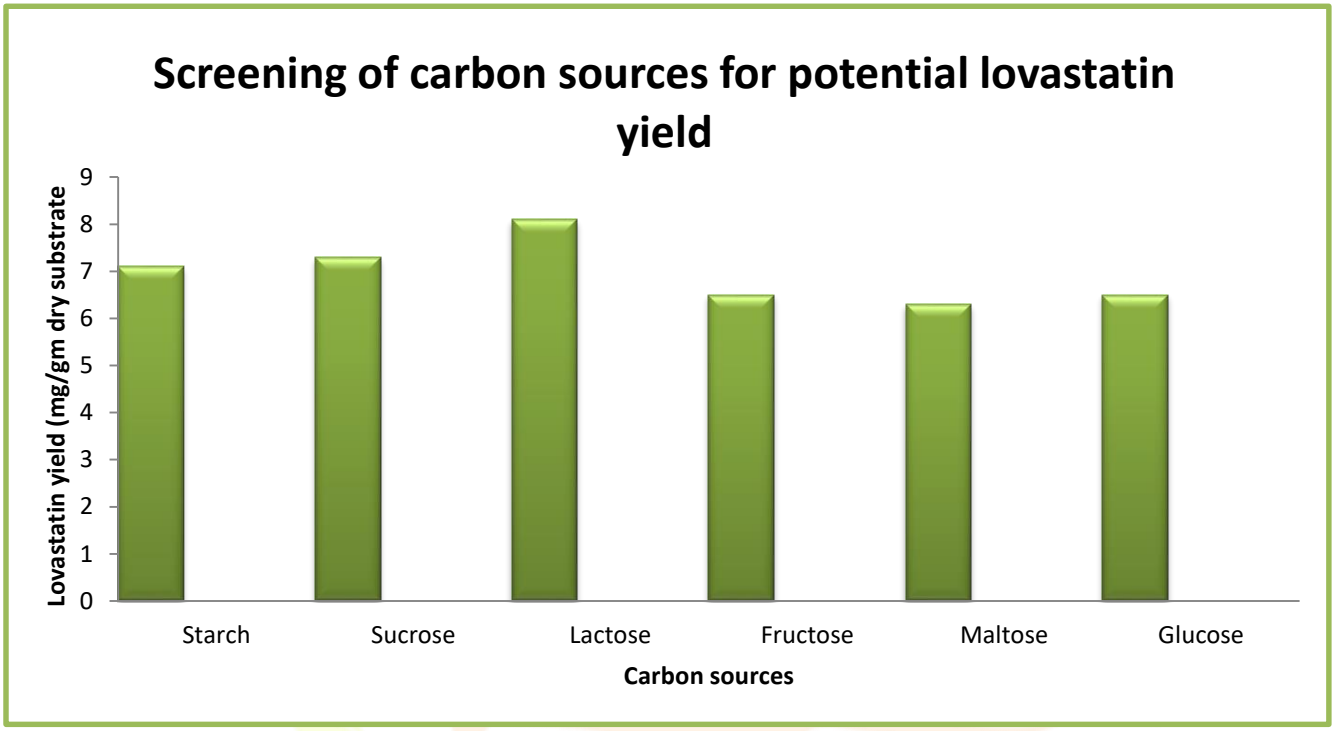

Fig. 8: Effect of carbon supplementation on] lovastatin production

Similar observations were reported by [15] Szakacs*et al*., as lactose was a favorable carbon source for the production oflovastatin with *Aspergillus terreus* under SmF. [16]Xu *et al*., reported significant increase in lovastatin production usingsucrose and glycerol as carbon supplement and glucose as repressor for lovastatin production with *Monascus ruber* using steamed rice under SSF

Effect of lactose concentration: In order to investigate the effect of lactose concentration on the fermentationmedium, SSF was carried out with different lactose concentrations varying from 0.5-3.0% (w/w). Maximum yield of lovastatin yield of 8.5 mg/gds was obtained at a concentration of 1.5 %w/w **(Fig. 9).**

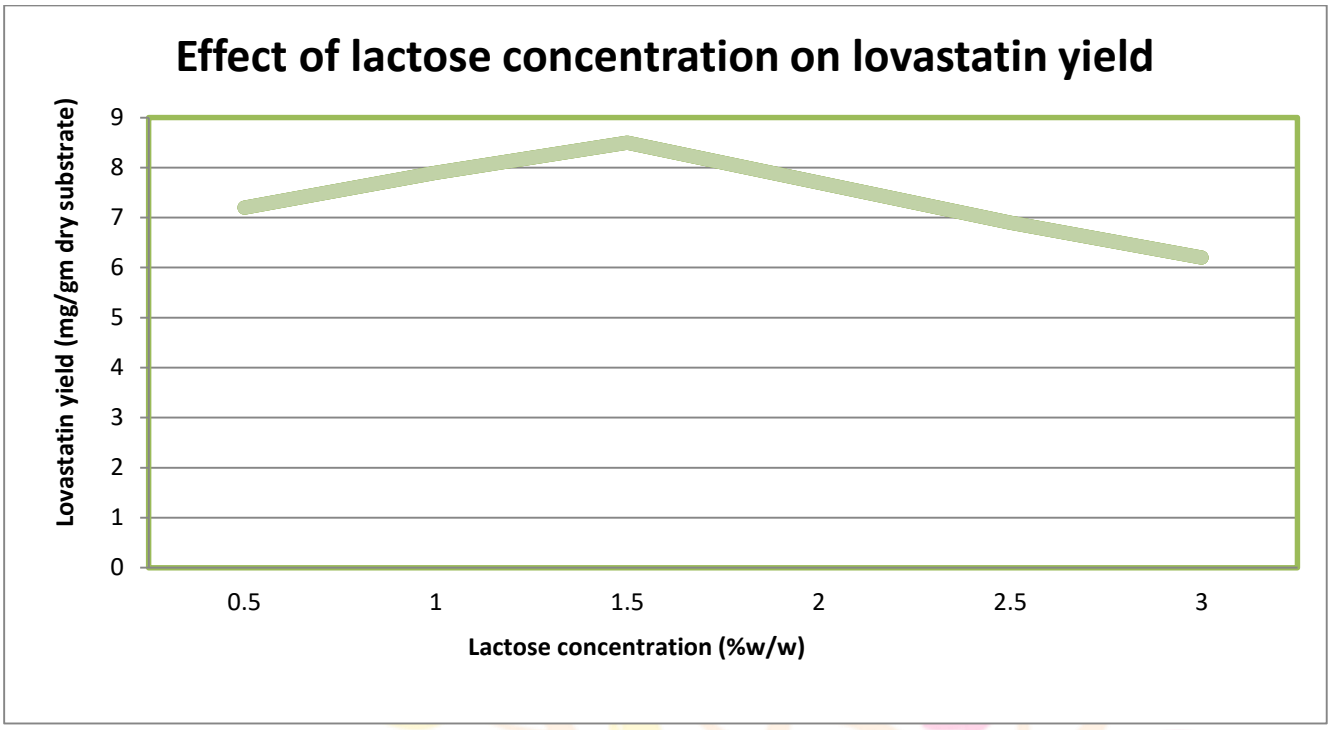

Fig 9: Effect of lactose concentration size on lovastatin production

Effect of nitrogen supplementation on lovastatin production: To determine the effect of nitrogen sources onlovastatin production, different organic nitrogen sources such as peptone, beef extract, yeast extract, peptone, maltextract and the inorganic nitrogen sources such as urea, ammonium sulphate, ammonium nitrate, sodium nitrate weresupplemented at a concentration of 1 %w/w to the basal solid state fermentative medium.Among different nitrogen sources, with ammonium sulphate high lovastatin yield of 7.5mg/gds was obtained when compared to other nitrogen sources **(Fig. 10).**

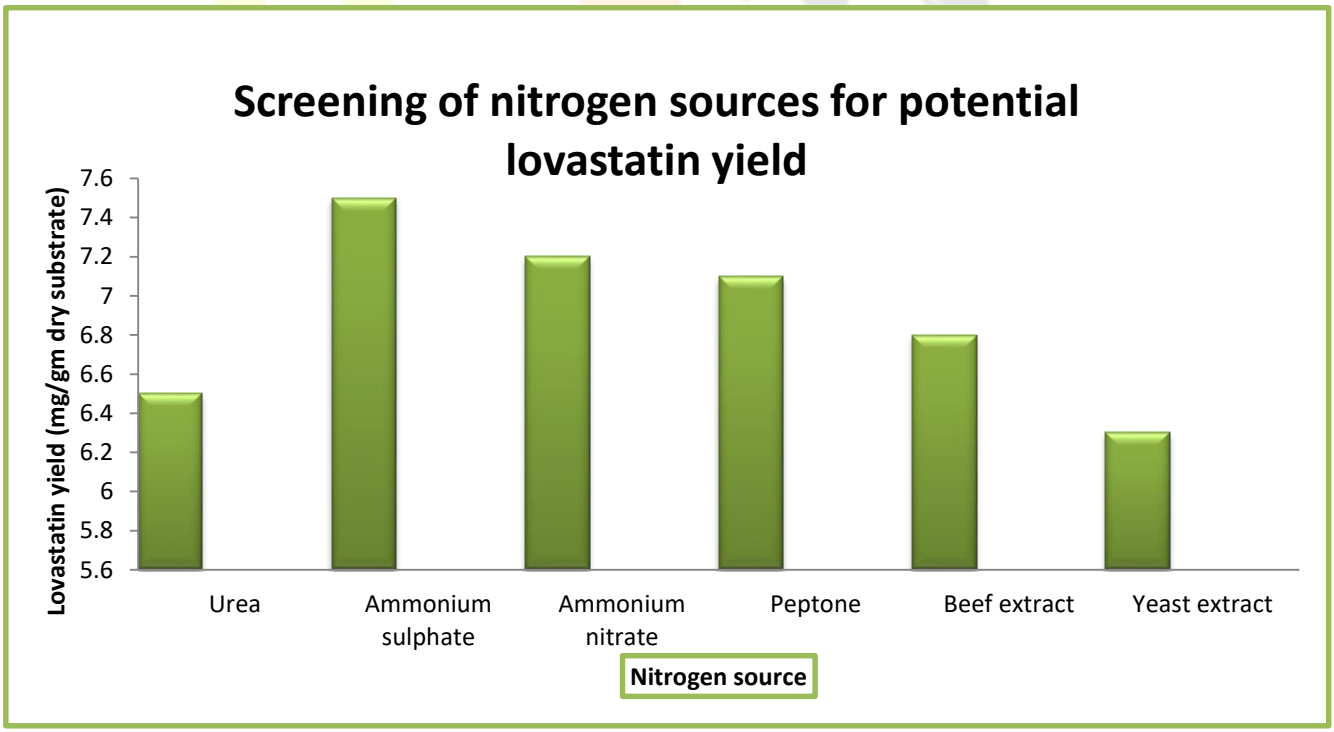

Fig. 10: Effect of nitrogen supplementation on lovastatin production

[17]Atalla *et al*., reported that ammonium sulphate was used to enhance the lovastatin yield. [16]Xu *et al*., reported significant increase in lovastatin production using yeast extract as organic nitrogen source and sodium nitrate as inorganic

nitrogen source with *Monascus ruber* using steamed rice under SSF.

Effect of ammonium sulphate concentration: In order to evaluate the effect of ammonium sulphate concentration on the fermentation medium, SSF was carried out with different concentrations of ammonium sulphate varying from 0.5-3.0% (w/w). Maximum yield of lovastatin yield of 8.3 mg/gds was obtained at a concentration of 1.5 %w/w **(Fig. 11).**

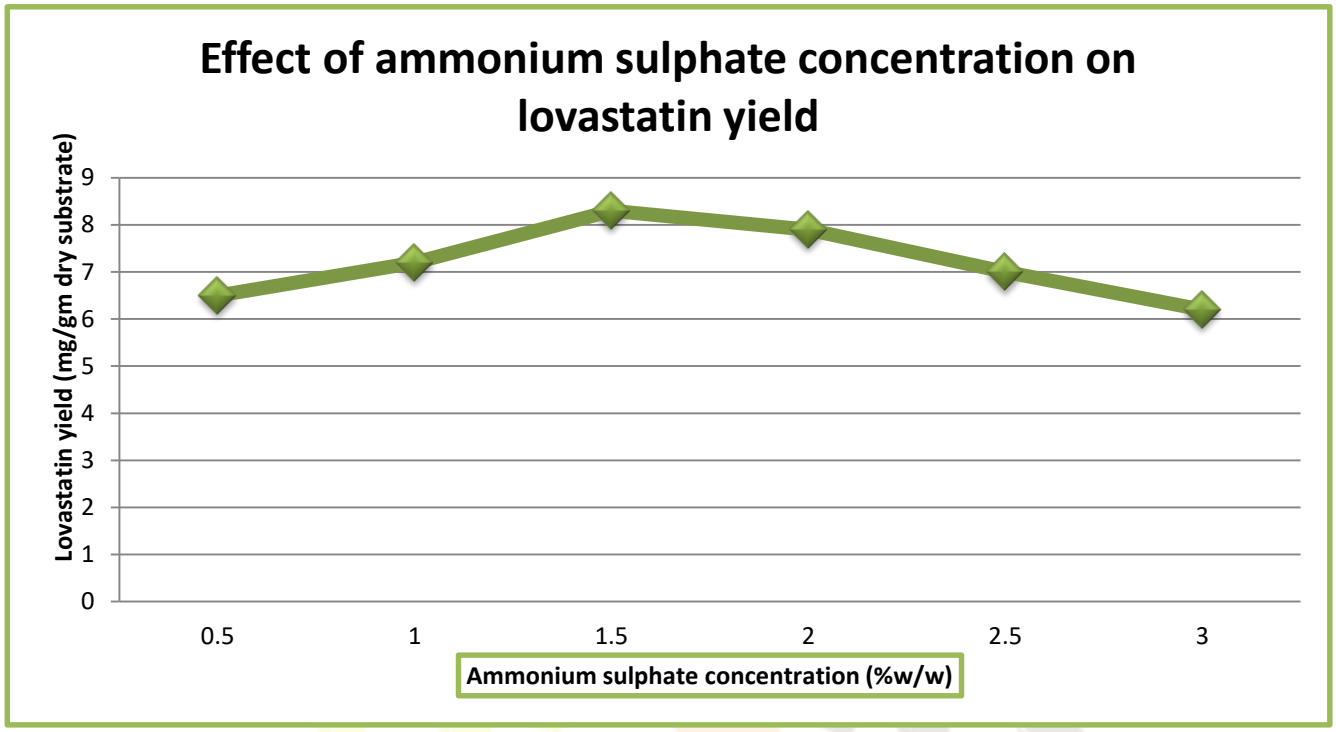

Fig. 11: Effect of ammonium sulphate concentration on lovastatin production

Finally with all the optimized parameters, of incubation time5days, incubation temperature 30ºC, initial moisturecontent 60 %(v/w), inoculum age 4 days, inoculum volume 30 %v/w , pH 6 .0,carbon source and its concentration 1.5%(w/w)lactose, nitrogen source and its concentration 1.5% w/w ammonium sulphate, maximum lovastatin yield of 13.5
mg/gds was obtained.

CONCLUSION

The present study shows that ***Aspergillus fischeri*** **NCIM-509** can produce an important secondary metabolite -lovastatin with relatively good yield from horse gram which is an agro-industrial residue, easily available andeconomical. The significant improvement in lovastatin yield was recorded when the basal medium wassupplemented with different carbon sources. This study indicates that horse gram from agro-industrial residues can be exploited from their commercial use in various industries.

REFERENCES

1. A. Endo, *J. Lipid Research, 1982*, **33:** 1569.
2. J.A.Tobert, *Nature Reviews Drug Discover.2003,* **2:** 517.
3. Alberts A.W., Chen J., Kuron G., Hunt V., Huff J., et al. , *Proc. Natl. Acad. Sci.* 1980,**77:** 3957.
4. K.D.Jones , W.T. Couldwell , D.R.Hinton ,Y. H. Su . D. K. He, L. Anker, R.E. Law, *Biochem. Biophys. Res. Commun , 1994,* 205, 1681.
5. A.Newman, R.D.Clutterbuck, R.L.Powles ,J.L.Millar , *Leukemia,1994,* **8,** 2022..
6. R. Aravindan, A. Seenivasan, S. SubhagarViruthagiri, *Ind. J. Pharm. Sci., 2008,* **70:** 701.
7. M.S.Samiee, N. Moazami, S. Haghighin, F.A. Mohseni, S. Mirdamiadi,M.R. Bakhtiari, *Iran Biomed*,003, 7: 29.8. S. R.Couto, *Biotechnol.J*.,2008, **3 :** 859.
9. H.R.Valera, J. Gomes, S. Lakshmi, R.Gurujara, S. Suyanarayan, D. Kumar, *Enz. Microbiol. Technol*, 2005, **37:** 521.
10. J.Mielcarek, M. Naskrent, P. Grobelny, *J. Thermal Anal. and Chemistry*. 2009, **96**: 301.

11. W.E.I.Pie-Lian, X.U.Zhi-nan, C.E.N. Pei-Lin, *J. ZheijanUniv, 2007.* **8:** 21.
12. B.P.Panda, S. Javed, M. Ali, *Food Bioprocess Techno,2008,*. **53:** 342.
13. R.C.Pansuriya and R.S. Singhal, *Brazilian J. Microbiol.* , 2010, **41:**164.
14. R.E.Porcel, C.J.L. Lopez, V.M.A. Ferron, S.J.A. Perez, G.J.L. Sanchez,Y. Chisti, *Bioprocess Biosyst. Eng.*,2006, **29:** 1.
15. G.Szakacs, G. Morovjan, R.P. Tengerdy, *Biotechnol. Let., 1998,* **20:** 411.
16. B.J.Xu,Q.J. Wang, X.Q. Jia, C.K. Sung, *Biotechnol and Bioprocess Eng*.,2005,**10:**78.
17. M.M.Atalla, E.R. Hamed and A.R.El-Shami,*Malaysian Journal of Microbiology*.,2008, **4:**6.