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Screening of Inhibitory Activity against α -Amylase of Fungal Endophytes Isolated from Medicinal Plants in Uzbekistan

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ABSTRACT

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Diabetes is a common metabolic disorder characterized by abnormally increased plasma glucose levels. Postprandial hyperglycemia plays an essential role in development of type 2 diabetes. Inhibitors of carbohydrate-hydrolyzing enzymes such as α -amylase retard the liberation of glucose from dietary complex carbohydrates and delay the absorption of glucose. The purpose of the study was to isolate and select α -amylase inhibitor-producing endophytic fungi from well-known medicinal plants used in the treatment for diabetes and inhabiting in Uzbekistan. The obtained data showed that 100 fungal endophytes isolated from 16 local antidiabetic plants produce metabolites with potent inhibiting activity in vitro (60-82%) and are expected to be a source for therapeutic agents in treatment of diabetes.

Introduction

Diabetes mellitus (DM) is a metabolic disorder caused by insulin deficiency and/or its action promoting chronic hyperglycemia, dislipidemia and protein metabolism disturbance. Complications of diabetes mellitus include increased risk of cardiovascular disease, retinopathy, nephropathy, neuropathy (Kumar *et al.*, 2012; Laar *et al.*, 2008; Mitra *et al.*, 2012). According to data of World Health Organization currently more than 180 million people worldwide have diabetes and it is likely to double by 2030 (Federation, 2011; Mitra *et al.*, 2012). Despite huge advances in modern medicine, the availability of insulin and synthetic hypoglycemic agents, their inability to restore normoglycemia without

side effects necessitating alternative therapy (Fujisawa *et al.*, 2005; Mitra *et al.*, 2012).

One of the treatment strategies of diabetes type 2 is reducing of postprandial blood glucose. This may be caused by delayed absorption of glucose via inhibition of the breakdown of oligo- and polysaccharides to mono- and disaccharides by α -amylase and α -glucosidase in the intestine (Funke *et al.*, 2006; Kumar *et al.*, 2012; Laar *et al.*, 2008; Mentreddy *et al.*, 2007; Sales *et al.*, 2012). Well-known inhibitors of these enzymes such as acarbose and voglibose prolong the total time of digestion of carbohydrates, which delay absorption of glucose with subsequent prevention from postprandial hyperglycemia.

However, these inhibitors have serious side effects - abdominal pain, bloating, diarrhea, kidney cancer, liver damage and acute hepatitis (Fujisawa *et al.*, 2005; Laar *et al.*, 2008). In this regard, a search of inhibitors of intestinal pancreatic α -amylase and α -glucosidase, free from major side effects is requested. Studies show that some secondary metabolites of the antidiabetic plants successfully demonstrate the properties of inhibitors of enzymes of carbohydrate degradation, and consequently, may serve to control type 2 diabetes (Bailey *et al.*, 1989; Funke *et al.*, 2006; Kumar *et al.*, 2012; Mentreddy *et al.*, 2007; Sales *et al.*, 2012).

At the same time endophytes inhabiting plants is known as unique class of microorganisms characterized by the production of a large variety of substances contributing to resistance of host plants under various biotic and abiotic stresses. Moreover, they are able to synthesize the characteristic metabolites of the host plants (Gutierrez *et al.*, 2012; Padhi *et al.*, 2013).

Having the ability to synthesize the same secondary metabolites as that of the host plant, endophytes relatively quickly grow on ordinary nutrient media. That's why, currently, endophytes are viewed as an alternative source of useful known and new metabolites including hypoglycemic compounds (Gutierrez *et al.*, 2012; Padhi *et al.*, 2013).

According to the database NAPRALERT (NAtural PRoducts ALERT) and ethnobotanical literature there are about 800 species of antidiabetic plants (Sales *et al.*, 2012; Zubek *et al.*, 2011). many of these plants grow and successfully used in folk medicine of Uzbekistan. In this context the aim of this work was the isolation and study of the endophytes of some antidiabetic plants as potential producers of α -amylase inhibitors.

Materials and Methods

Study area and material sampling

Plant material was collected in spring 2013 and 2014 on the territory of Tashkent region (Uzbekistan). Plant samples were identified and stored in a herbarium.

Isolation of endophytic fungi

Endophytic fungi were isolated by the method as described previously by Hazalin *et al.*, (Hazalin *et al.*, 2009). Roots, stems and leaves were respectively washed in tap water, sterilized in 70% ethanol for 1 min followed by 0.1% HgCl₂ for 7 min, rinsed three times in de-ionized water, cut into segments approximately 5 mm in diameter and placed in 90 mm Petri dishes containing Czapek-Dox agarized medium with 50 mg/ml chlortetracycline and 250 mg/ml streptomycin sulfate to inhibit bacterial growth. The plates were incubated for 7-14 days at 28 °C. Different mycelia growing out of the segments were sub-cultured and individually maintained on antibiotics-free Czapek-Dox-agar medium. Colony morphology and growth and spore formation of the isolates were then studied on Potato-Dextrose-agar medium.

Endophytic fungi identification

Isolated strains were identified by classical methods on the basis of morphology using pertinent monographs (Litvinov, 1967). Isolated strains were deposited at the Institute of Microbiology of the Uzbekistan Academy of Sciences where they were maintained at +4 °C.

Fermentation

To accumulate biomass for further extraction and determination of biological activity,

endophytes were grown by submerged fermentation in 500 ml flasks containing 100 ml of Chapek-Dox liquid medium for 5 days at 26 °C.

The extraction of secondary metabolites of endophytic fungi

For extraction of secondary metabolites 5 g of biomass of each isolate was milled in a Potter homogenizer, transferred to a cone flask containing 50 ml of ethyl acetate, and left for 24 hours on the rotary shaker at room temperature. The mixture was filtered through filter paper (Whatman #1) and Na₂SO₄ (40 µg/ml) was added. After the filtration, the extract was striped to dryness on a rotary evaporator and mixed with 1 ml of dimethyl sulfoxide (DMSO). The resulting extract was used as a stock solution and stored at 4 °C.

Determination of α-amylase activity

Determination of α-amylase activity was carried out by the modified method used for measurements in the plant extracts (Picot *et al.*, 2014). For this purpose a solution of starch as a substrate was prepared at the rate of 1 g /10 ml water, boiled for 2 minutes, adjusted to 100ml with distilled water and used within 2 - 3days. To 2 ml of the prepared starch solution 100 µl pancreatic α-amylase (13 u/ml in 0.1 M Na-acetate buffer pH4,7), 100 µl of the extract endophyte (20 mg/ml), 2 ml of acetate buffer were added and incubated for 10 minutes at 30⁰C. In contrast to the test sample the extract was not added to a control . After incubation the reaction was terminated by adding 10 ml of iodine reagent and the absorbance was measured on spectrophotometer SPECOL -1300 at a wavelength of 630 nm. For the preparation of iodine reagent 0.5 g of crystalline iodine, 5 g of potassium iodide were dissolved in 250 ml of water; to obtain a working solution of 2 ml of this reagent was adjusted to 100 ml by 0.1 M HCL.

All the assays were carried out in triplicates and average percent inhibition of enzymes by the fungal extract was calculated using the following formula:(A0 –At)/A0x100%, where A0 - absorption of control sample, At is the absorption of test sample, respectively.

Results and Discussion

The study of lipid-lowering and anti-diabetic activity of various fractions of extracts of the mycelium of 17 endophytic fungi isolated from *Salvadora oleoides Decne* (*Salvadoraceae*) showed that the extracts of two endophytic fungi pp. *Aspergillus* and *Phoma* significantly decrease blood glucose level (Dhankhar *et al.*, 2013). It was found that the basic substance of these active extracts are presented by phenolic compounds. It was also shown that all investigated extracts are safe and have no lethal effect on animals at doses up to 1000 mg / kg of body weight, and safe dose for human erythrocytes is up to 500 µg/ml. Positively active as inhibitors of α- amylase and α-glucosidase were extracts of 9 from 22 fungal endophytes isolated from the Indian anti-diabetic plants *Momordica charantia* and *Trigonella foenum-graceum*. In the experimental conditions *in vitro* IC₅₀ of two isolates were lower than the control acarbose. The active strains were identified as *Trichoderma atroviride* and *Stemphylium globuliferum* (Pavithra *et al.*, 2014).

According to research of antidiabetic activity of *Syncephalastrum racemosum*, isolated from red algae *Gracilaria corticata*, its acetone and methanol extracts inhibited amylase activity up to 19,4 and 23.7%, respectively (Ushari *et al.*, 2015).

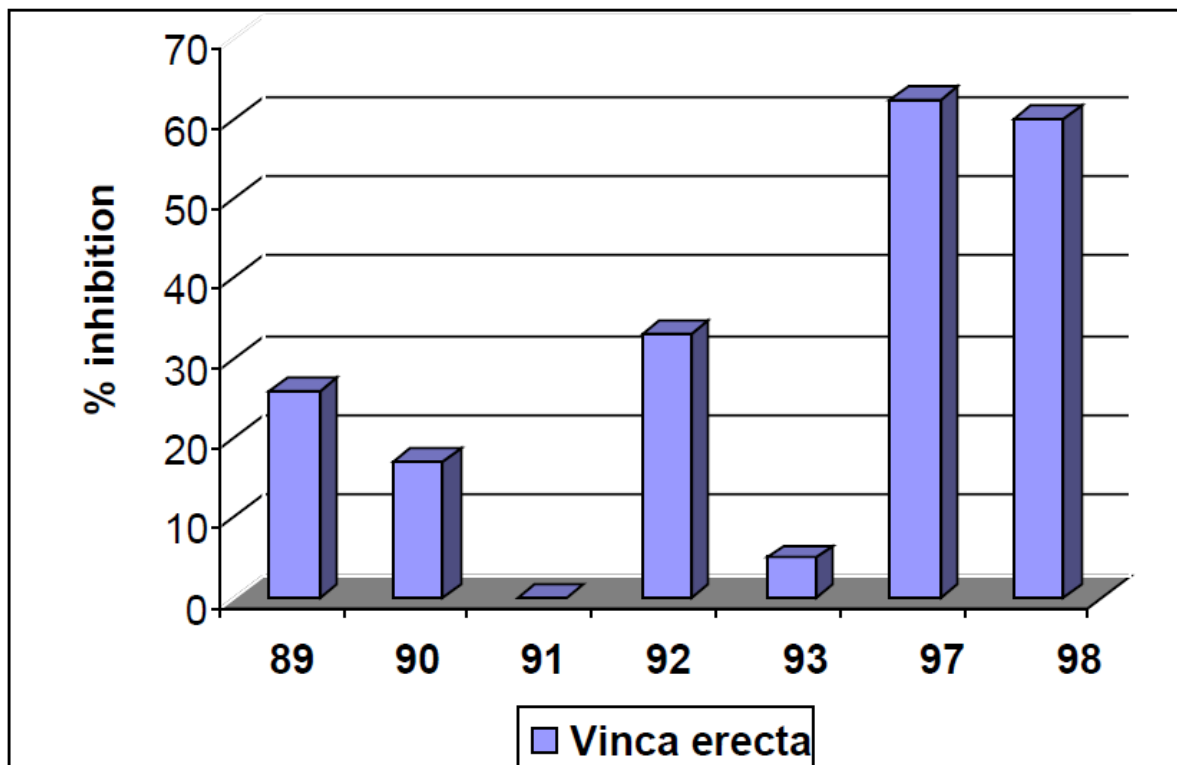
From the roots, stems and leaves of 16 plants growing in Uzbekistan, we obtained 100 endophytic fungal isolates related to *Acremonium*, *Alternaria*, *Aspergillus*,

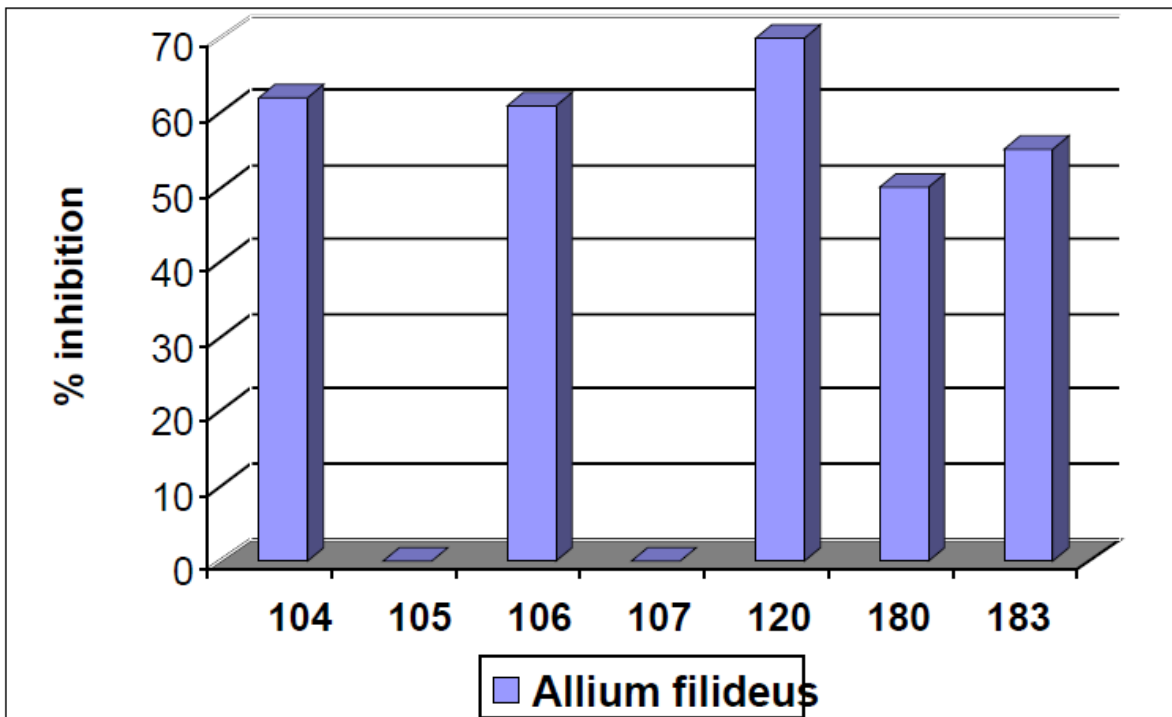
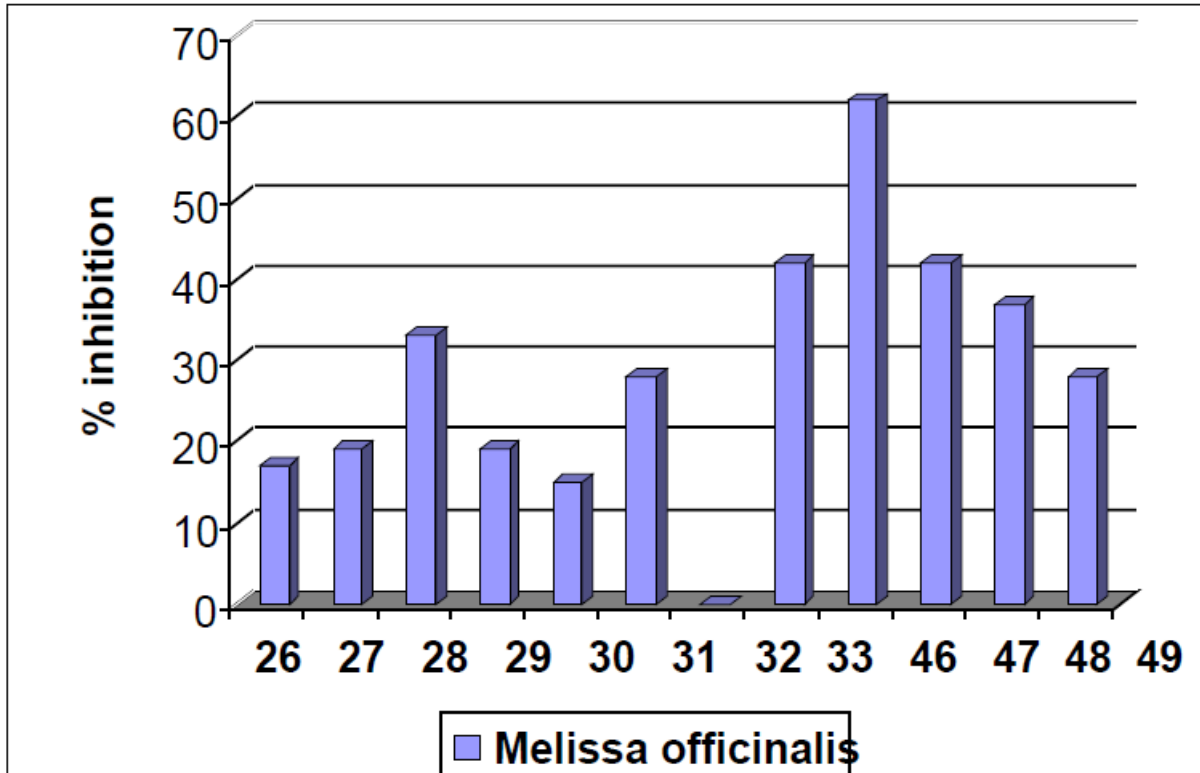
Gliocladium, *Fusarium*, *Penicillium*, *Trichoderma* and *Ulocladium* genera. Antidiabetic properties of their ethyl acetate extracts were carried out *in vitro* with using

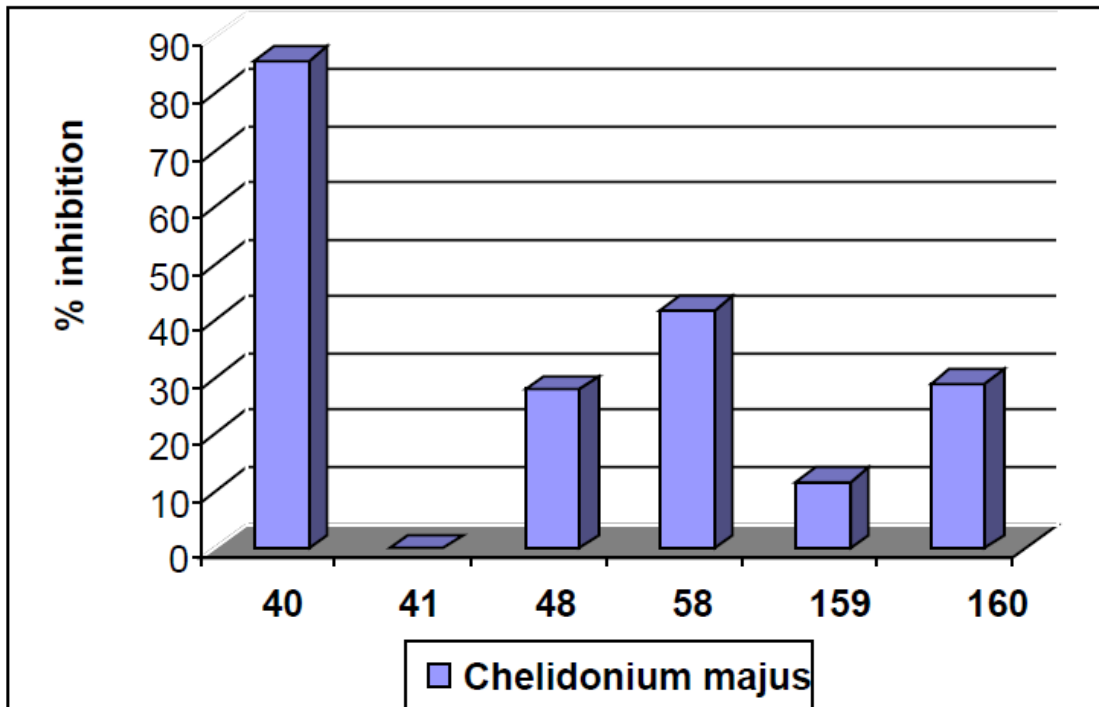
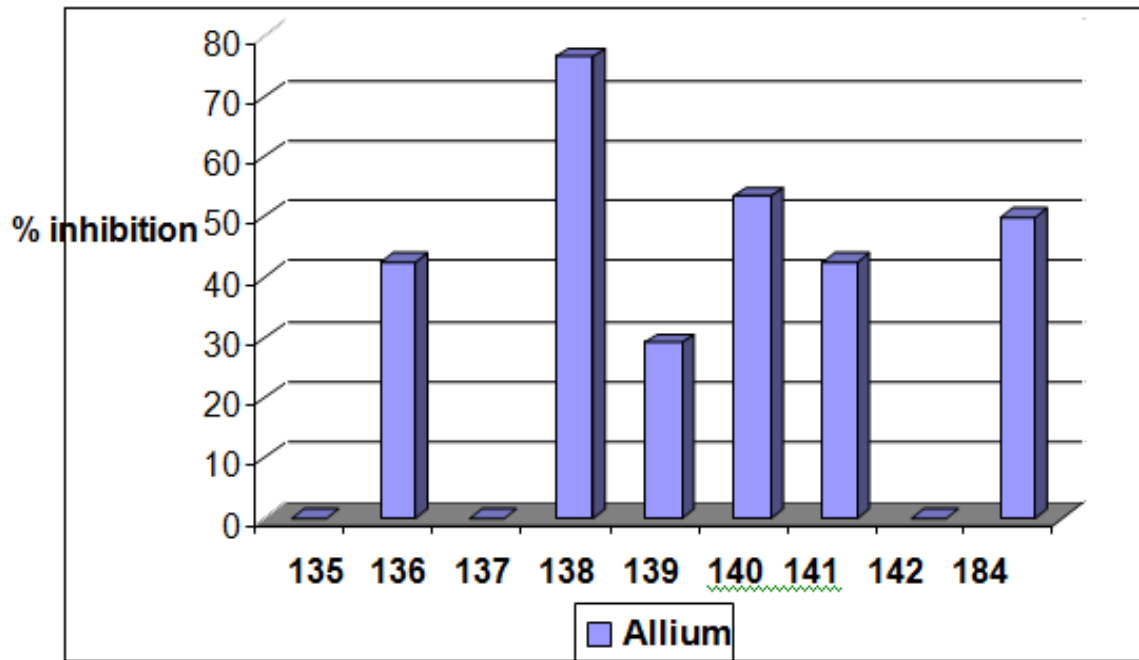
pancreatic α -amylase and starch as a substrate, the concentration of the extracts was 100 μ g/ml.

Table.1 α -Amylase inhibition activity of isolated endophytes

#	Endophyte	% inhibition
1	<i>Alternaria sp.</i> – VE98L	60,0
2	<i>Aspergillusterreus</i> - AF104S	60,0
3	<i>Penicillium sp.</i> - AF106	60,0
4	<i>Mycelia sterilia</i> - MO46L	62,0
5	<i>Eupenicilliumbrefeldianum</i> - VE97R	62,5
6	<i>Aspergillusterreus</i> - FS114	64,0
7	<i>Acremoniumroseum</i> - HT164S	64,0
8	<i>Aspergillusflavus</i> - HP131L	66,0
9	<i>Penicillium sp.</i> - AF120	70,0
10	<i>Aspergillusterreus</i> - 138	76,05
11	<i>Aspergillus sp.</i> - HT162S	81,0
12	<i>Penicillium sp.</i> - HT165S	81,0
13	<i>Aspergillusegypticus</i> - HT166S	82,0
14	<i>Aspergillusterreus</i> – CM40L	82,0







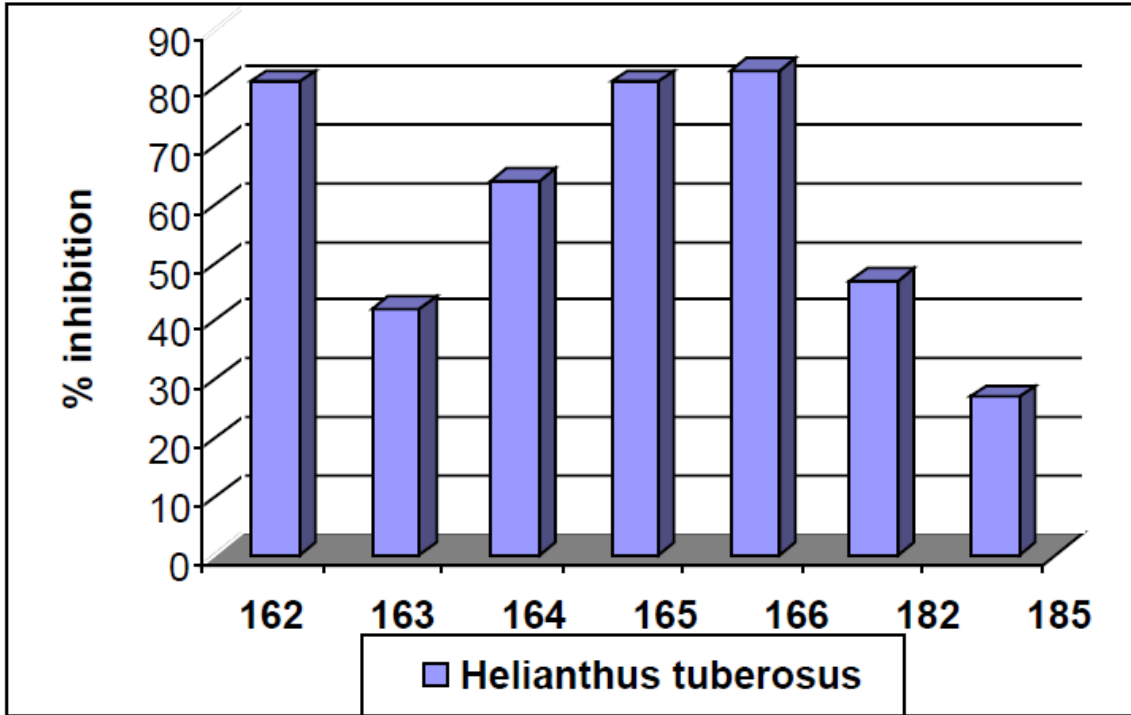
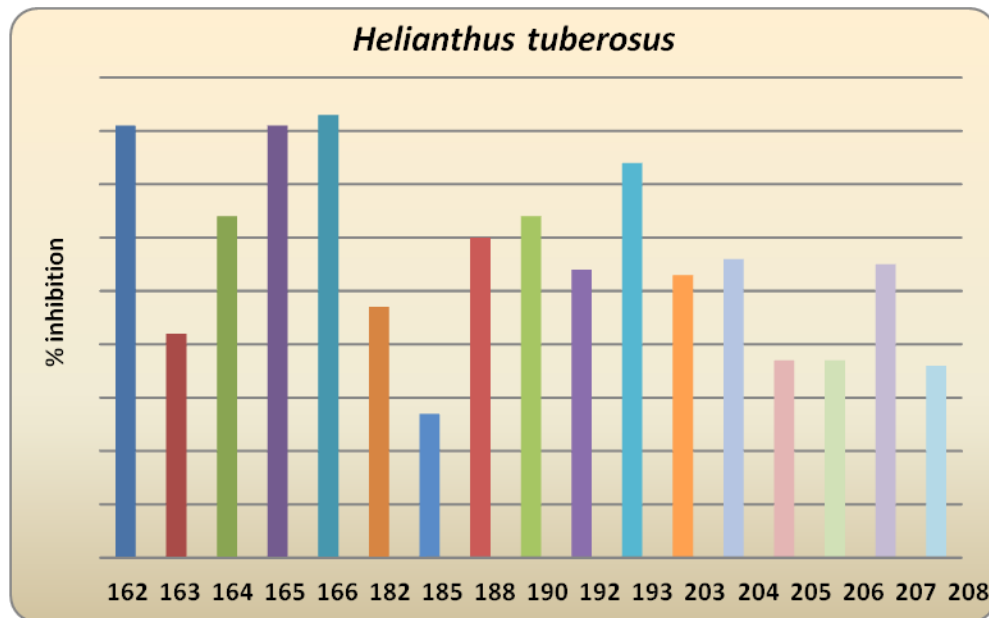


Fig.1 Inhibitory activity of fungal endophytes



The results of this experiment showed the presence of inhibitory activity in 67% of studied endophytes. The low activity (20-40%) showed 27% of strains, medium (40-60%) – 28%, and only 15% inhibited the activity of α -amylase by more than 60%.

Thus, endophytic fungi isolated from *Ocimum basilicum*, *Ephedra strobilacea*, *Eremurus*, *Crocus sativus*, *Lagochilus olga*, *Peganum harmala*, *Vinca rosea* showed low inhibitory activity. It should be noted that the distribution and the magnitude of the

inhibitory activity of endophytic fungi are quite different even within a single host plant (Figure 1.).

For example, the most endophytes selected from two kinds of periwinkle (*Vinca minor* and *Vinca erecta*) inhibit the action of the enzyme but a high activity is shown only by two strains: *Alternaria sp.* - VE98L (60%) and *Eupenicillium brefeldianum* - VE97R (62,5%). Three selected fungal isolates of peppermint (*Mentha piperita*) show the inhibition of α -amylase in the range of 46-50%. In 11 out of 12 fungi isolated from different organs of balm (*Melissa officinalis*) it is observed inhibitory activity from 19 to 62%. The highest inhibitory activity detected in a strain *Mycelia sterilia* - MO46L (62%).

High activity of inhibition of the enzyme was found in 3 of the 6 extracts of endophytic fungi from bulbs of *Allium filideus*: *Aspergillus terreus* - AF104S (62%), *Penicillium sp.* - AF106 (61%) and *Penicillium sp.* - AF120 (70%). In the other plant of the genus *Allium longicuspis* highest inhibitory activity at the level of 76.5% exhibited by endophyte *Aspergillus terreus* - 138. Among endophytes of endemic plants *Haloxylon persicum* and *Ferula sumbul* only one strain inhibited alpha-amylase by 66% (*Aspergillus flavus* - HP131L) and 64% (*Aspergillus terreus* - FS114), respectively.

Finally, the highest inhibitory effect on α -amylase activity showed three strains isolated from *Helianthus tuberosus*: *Aspergillus sp.* - HT162S (81%), *Penicillium sp.* - HT165S (81%), *Aspergillus egypticus* - HT166S (82%), one strain isolated from *Chelidonium majus* - *Aspergillus terreus* - CM40L (82%), and one strain isolated from *Celosia cristata* - *Penicillium sp.* - CC200 (80%).

It should be noted that acarbose used as a reference drug, at a concentration of 100

$\mu\text{g/ml}$, inhibited the enzyme activity by 82% (Table1).

Analysis of the total data shows that the endophytes related to genus *Aspergillus* and of *Penicillium* isolated from the sunflower native plants (*Helianthus tuberosus*) and (*Chelidonium majus*) synthesize metabolites which have great inhibition potential against pancreatic α -amylase comparable with commercial inhibitor acarbose (over 80%). Thus, the results of the study are a prerequisite for the study of metabolites of these strains of endophytic fungi as perspective source of new native inhibitors of pancreatic α -amylase.

References

- Bailey C.J., Day C. 1989. Traditional plant medicines as treatments for diabetes. *Diabetes Care*. 12: 553-564.1. Mitra A., Dewanjee D., Dey B. 2012. Mechanistic studies of lifestyle interventions in type 2 diabetes. *World J Diabetes*, 3: 201-207.
- Dhankhar S.1, Dhankhar S., Yadav J.P. 2013. Investigations towards new antidiabetic drugs from fungal endophytes associated with *Salvadora oleoides* Decne. *Med Chem. Jun 1*, 9 (4): 624-629.
- Federation I.D. 2011.IDF diabetes atlas: International Diabetes Federation, Executive Office.
- Fujisawa H.I., K. Inoue, Y. Kawabata and T. Ogihara. 2005. Effect of two alpha-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. *Metabolism.*, 54: 387-3903.
- Funke I., Melzing M.F. 2006. Traditionally used plants in diabetes therapy - phytotherapeutics as inhibitors of α - amylase activity. *Rev Bras*

- Farmacogn.*, 16: 1-5.
- Gutierrez R.M.P., Gonzalez A.M.N. and Ramirez A.M. 2012. Compounds Derived from Endophytes: A Review of Phytochemistry and Pharmacology. *Curr Med Chem*, 19: 2992-3030.
- Hazalin N.A., Ramasamy K., Lim S.M., Wahab I.A., Cole A.Lj, Majeed A.A. 2009. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. *BMC Complementary and alternative medicine*, 9: 46.
- Kumar S., Kumar V., Rana M., Kumar D. 2012. Enzyme inhibitors from plants: An alternate approach to treat diabetes. *Pharmacog Communi*, 2: 18-33.
- Laar F.A., Lucassen PL.BJ., Akkermans R.P., Lisdonk E.H., Rutten GEHM, Weel C. 2008. Alpha-glucosidase inhibitors for type 2 diabetes mellitus (Cochrane Review). *The Cochrane Library*.
- Litvinov M.A. 1967. Identification guide for microscopic fungi. *Nauka, Leningrad*.
- Mentreddy S.R. 2007. Medicinal plant species with potential antidiabetic properties. *J. Sci. Food Agric.*, 87: 743-750.
- Mitra A., Dewanjee D., Dey B. Mechanistic studies of lifestyle interventions in type 2 diabetes. 2012. *World J Diabetes*, 3: 201-207.
- Padhi I., MohandaY.K., Panda S.K. 2013. Endophytic fungi with great promises: A Review *J. Adv. Pharm. Edu. & Res.*, 3 (3): 152-170.
- Pavithra N., Sathish L., Babu N., Venkatarathanamma V., Pushpalatha H., Reddy G.B., Ananda K. 2014 Evaluation of α -amylase, α -glucosidase and aldose reductase inhibitors in ethyl acetate extracts of endophytic fungi isolated from antidiabetic medicinal plants. *Int. J. Pharm Sci Res*, 5 (12): 5334-5341.
- Picot C.M.N., Subratty H., Mahomoodally F. 2014 Inhibitory potential of five native antidiabetic medicinal plants on α -amylase, α -glucosidase, glucose entrapment, and amylosis kinetics *in vitro*. *Adv.Pharm.Sci*, V., 1-7.
- Sales P.M., Souza P.M., Simeoni L.A., Magalhães P.O., Silveira D. 2012. α -Amylase Inhibitors: A Review of Raw Material and Isolated Compounds from Plant Source. *J. Pharm. Pharmaceut Sci.*, 15 (1): 141-183.
- Ushari R., Anusha R. 2015. *In vitro* anti-diabetic activity of ethanolic and acetone extracts of endophytic fungi *Syncephalastrum racemosum* isolated from the seaweed *Gracilaria corticata* by α -amylase inhibition assay method. *Int. J. Curr. Microbiol. App. Sci.*, 4 (1): 254-259.9.
- Zubek S., Nobis M., Blaszkowski J., Mleczko P., Nowak A. 2011. Fungal root endophyte associations of plants endemic to the Pamir Alay Mountains of Central Asia. *Symbiosis*, 54: 139-149.

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