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02

EFFECT OF GUMS ON AMYLASE ACTIVITY COLLECTED FROM AJANTA FOREST

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Abstract: Gum is naturally occurring chemical substance in the plant. Gums are exudates naturally from the stem or from the wounds of the plants. Gums are characterized by ability to dissolve in water forming viscid solution by absorbing water to form gelatinous paste. Production of amylase(s) was studied by growing the fungi in liquid medium. Determination of amylase activity was done with the help of cup-plate method. Effect of gum 1% concentration on amylase activity of *Alternariaalternata*, *Aspergillusniger*, *Curvularialunata*, *Curvulariapellecence*, *Fusariumequiseti*, *Fusariumoxysporum*, *Macrophominaphaseolina*, *Penicilliumdigitatum*, *Penicillium chrysogenum* and *Rhizopusstolonifer* were studied. Maximum activity against all the fungi under investigation was recorded with gums collected from *A. indica* (Average activity: 12.80); while minimum with gums collected from *A. Arabica* (Average activity: 12.80). Gums collected from Ajanta forest have effect on amylase activity exhibited by the fungi under investigation.

Key words: Gums, Amylase activity, Ajanta Forest, Hydrolytic enzymes

Introduction

Gum is a group of plant products resembling carbohydrates. Gums are characterized by ability to dissolve in water forming viscid solution by absorbing water to form gelatinous paste. In some cases the production of gum has been attributed to fungi attacking the plant, these fungi being responsible for enzymes that penetrate the tissues and transform the celluloses and hemicelluloses of the cell wall into gum. Bio deterioration has been attributed mainly to the efficiency of seed moulds to produce various types of hydrolytic enzymes like amylase for the degradation of starch (Vidhyasekaran and Govindswamy, 1968), lipase for the degradation of oil (Goodman and Christensen, 1952) and Lalithakumari *et al.*, (1971) protease for the degradation of protein (Sinha and Prasad, 1977) and Chauhan and Magar (1979) reported that cellulolytic and pectolytic for the degradation of cellulose and pectin. Hydrolytic enzymes such as amylase, cellulase, pectinase, protease and lipase etc. in case of fungi have been studied by many workers. These enzymes are found to be helpful during invasion and colonization by various plant pathogens. The *Alternariaalternata*, *Fusariumsolani*, f. sp. Minus, *Pleospora infectoria* and *Alternariasolani* were capable of producing pectinase, amylase, cellulase and protease types of enzymes which results into seed bio deterioration.

Among various group of fungal pathogens which are reported to be amylolytic in nature are species of *Penicillium* and *Aspergillus* (Le Menseet *et al.*, 1947), *Alternariatenuis*, *Fusariumcoeruleum* and *Curvularialunata* (Tondon, 1949), Somethermophilic fungi (Cooney and Emerson, 1964), Actimonycetes (Emerson 1968), *Aspergillusflavus*, *Aspergilluscandidus*, *Curvularialunata* and *Curvulariapellecence* (Prasad, 1979). Fashimet *et al.*, (1985) found that *Aspergillusflavus* as most efficient amylase producing fungus which was followed by *Alternariaalternata* and *Aspergillusniger*. During the present investigation effect of gums on amylase activity collected from Ajanta forest was studied.

Material and Methods

Plant gums were regularly collected in all the seasons from various parts of Ajanta forest. It was done by using axe, sterilized blade. Fine cut was made at different parts of the plant, like root,

stem, leaves, flower and fruits. Later on at 30, 45 and 60 days exudates gums were collected in pre-sterilized plastic bags, kept in laboratory condition until it was used.

Preparation of fine powder of Gum

The powder was prepared from collected dry gums and kept in clean glass pots. It was used for the further study of various chemical properties like crude fibre, crude fat, protein and nitrogen content etc. These chemical properties were studied by using following methods:

Study of hydrolytic enzymes

i) Production of amylase

Production of amylase(s) was studied by growing the fungi in liquid medium containing starch 1%, KNO₃, 0.25%, KH₂PO₄, 0.1. % and MgSO₄.7H₂O, pH of the medium was adjusted at 5.5. 25 ml of the medium was poured in 100ml conical flasks autoclaved and inoculated separately with 01 ml spore suspension of the fungi which were grown for 7 days on PDA slants. Unless otherwise stated, the flasks were incubated for 6 days at 25 ± 1°C with diurnal periodicity of light. On 7th day, the flasks were harvested by filtering the contents through Whatman filter No.1. The filtrates were collected in presterilized bottles and termed as crude enzyme preparation (Badar, 2011).

Composition of media used for Amylase production

i) Starch nitrate medium

Soluble starch 10gm, KNO₃ 2.5gm, KH₂PO₄ 1gm, MgSO₄.7H₂O 0.5 gm, dissolved in 1000 ml distilled water.

ii) Glucose nitrate medium:

Glucose 10g, KNO₃ 2.5gm, KH₂PO₄ 1.0gm, and MgSO₄.7H₂O 0.5 gm, dissolved in 1000 ml distilled water.

Assay method for amylase enzymes (Cup-plate method)

Determination of amylase activity was done with the help of cup-plate method which was adopted by Singh and Saxena (1982), where 20ml of starch agar assay medium (soluble starch – 10gm, Na₂HPO₄ – 2.84gm, NaCl – 0.35gm, Agar agar 20gm, distilled water 1000ml and pH 6.9) was poured in each petriplate. On solidification of the medium, a cavity (08 mm diameter) was made in the centre with the help of a cork borer (No.4) and was filled with 1ml culture filtrates (crude enzyme preparation) of the test fungi. The plates were incubated at 28°C for 24 hours, and then they were flooded with Lugol's iodine solution as an indicator. A clear, non-blue, circular zone obtained surrounding the central cavity; diameter of the zone was measured (mm) as the amylase activity zone. Similar procedure was followed for the control except pouring of culture filtrates in the central cavity instead of the activity enzyme.

Fashimet *al.*, (1985) reported that *Alternariaflavus*, *Alternariaalternata* and *A. niger* found to be most efficient amylase producing fungi, while Khairnar (1987) reported that amylase production in the species of *Alternaria*, *Curvularia* and *Helminthosporium* was stimulatory in the presence of substrate.

RESULTS AND DISCUSSION

Effect of gum on amylase activity

Effect of gum 1% concentration on amylase activity of *Alternariaalternata*, *Aspergillusniger*, *Curvularialunata*, *Curvulariapellecence*, *Fusariumequiseti*, *Fusariumoxysporum*, *Macrophominaphaseolina*, *Penicilliumdigitatum*, *Penicilliumchrysogenum* and *Rhizopusstolonifer* were studied and results are given in table 1. It was found that *Acacia Arabica* gum inhibited the amylase activity of all tested fungi viz., *Alternariaalternata*, *Aspergillusniger*, *Curvularialunata*, *Curvulariapellecence*, *Fusariumequiseti*, *Fusariumoxysporum*, *Macrophominaphaseolina*, *Penicilliumdigitatum*, *Penicilliumchrysogenum* and *Rhizopusstolonifer*. *Acacia chundragum* inhibited the amylase production of *Alternariaalternata*, *Aspergillusniger*, *Fusariumequiseti*, *Macrophominaphaseolina*, *Penicilliumdigitatum*, *Penicilliumchry sogenum* and *Rhizopusstolonifer*. While it induced the same of *Curvulariapellecence* and *Fusariumoxysporum*. Gum of *Azadirachtaindica* increased the amylase action of *Alternariaalternata*, *Aspergillusniger*,

Curvularialunata, *Curvulariapellecence*, *Fusariumoxysporum*, *Penicilliumdigitatum* and *Penicilliumchrysogenum*. Whereas, it retarded the amylase production of *Curvulari apellecence* and *Rhizopusstolonifer*.

Amylase production of *Alternariaalternata*, *Curvularialunata*, *Penicilliumdigitatum*, *Penicilliumchrysogenum* and *Rhizopusstolonifer* increased by *Boswelliaserratagum* while that of *Fusariumequiseti*, *Fusariumoxysporum* and *Macrophominaphaseolina* was retarded by *Boswelliaserrata*. *Buteamonospermagum* activated the amylase producing all tested fungi except *Macrophominaphaseolina*.

Amylase production of *Alternariaalternata*, *Curvularialunata*, *Fusariumequiseti*, *Fusariumoxysporum* and *Macrophominaphaseolina* was retarded by *Cassinealbengum*. *Mangiferaindica* and *Moringaoleiferagum* decreased the amylase activity of *Alternariaalternata*, *Aspergillusniger*, *Curvularialunata*, *Curvulariapellecence*, *Fusariumequiseti*, *Fusariumoxysporum* and *Macrophominaphaseolina*. While *Sterculiaurens* gum induced the activity of all fungi except *Fusariumoxysporum* and *Macrophominaphaseolina*. Amylase production of all tested fungi was retarded by *Terminaliaarjuna*. Maximum activity against all the fungi under investigation was recorded with gums collected from *A. indica* (Average activity: 12.80); while minimum with gums collected from *A. Arabica* (Average activity: 12.80). Mehrnouseh *et al.*, (2014) reported microencapsulation of purified amylase enzyme from Pitaya (*Hylocereuspolyrhizus*) peel in Arabic gum-chitosan using freeze drying method.

Table 1: Effect of Gum on Amylase Activity

Sr. No.	Name of Gum plant	No. of Fungi										Average Activity
		Aa	An	Cl	Cp	Fe	Fo	Mp	Pn	Pc	Rs	
01	<i>Acacia arabica</i>	09	11	11	11	10	09	8	09	10	11	9.90
02	<i>Acacia chundra</i>	10	11	12	12	11	13	10	11	09	12	11.10
03	<i>Azadirachtaindica</i>	14	13	13	12	12	13	14	12	13	12	12.80
04	<i>Boswelliaserrata</i>	12	10	14	13	9	9	10	12	12	13	11.40
05	<i>Buteamonosperma</i>	13	11	13	12	14	12	11	13	12	12	12.30
06	<i>Cassinealbans</i>	9	11	11	11	09	09	12	12	13	12	10.90
07	<i>Mangiferaindica</i>	10	10	09	09	10	11	13	12	11	12	10.70
08	<i>Moringaoleofera</i>	10	09	09	09	11	09	12	12	11	13	10.50
09	<i>Sterculiaurens</i>	12	13	12	12	12	11	13	14	12	13	12.40
10	<i>Terminaliaarjuna</i>	9	11	9	9	12	11	13	13	11	12	11.00
11	Control	11	10	12	11	13	12	14	11	10	13	11.70

Aa-*Alternariaalternate*

As-*Aspergillusniger*

Cl-*Curvularialunata*

Cp-*Curvulariapellecence*

Fe-*Fusarium equisetum*

Fo-*Fusariumoxysporum*

Mp-*Macrophominaphaseolina*

Pn-*Penicilliumdigitatum*

Pc-*Penicilliumchrysogenum*

Rs-*Rhizopusstolonifer*

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