steep medium. The tests were carried out after 12 days of cultivation. As a substrate 1% carboxy methyl cellulose solution was used. Ten ml of the filtrate of culture media were mixed with 20 ml of carboxy methyl cellulose solution and immediately, the viscosity was measured. Similar procedure was carried out after 24 hours of incubation at 27°C.

Individual results were calculated according to the formula :

$$\frac{TO - TE}{TO} \times 100$$

where

TO—Initial reading

TE—Final reading after 24 hours.

The fungi were cultivated on the synthetic medium with cellulose or carboxy methyl cellulose as alternative carbon sources. Concentration of cellulose was 10 g per litre of media. The mycelial growth was measured by dry weight estimation, in cultures grown on medium with carboxy methyl cellulose and in the case of cultures with cellulose, mycelial growth was determined by estimating the quantity of cell protein. Protein estimation was done by Follin's reagent method[4] in the culture which was treated overnight in 1 N NaOH. One per cent cellulose suspension in acetate buffer (pH 4·0) was overnight incubated with the filtrate of the fermentation medium at 37°C and reducing sugars were estimated according to method of Miller[5] (Table I).

From 25 species tested, 22 cultures grew well on the medium with carboxy methyl cellulose and 18 on the medium with cellulose. The quantitative difference in growth was compared with the cellulose decomposing activity of the cultures grown on medium with cellulose as carbon source. The results obtained in the studies of cellulolytic activity of Pyrenomycetes by different methods are not well correlated. However, most of the fungal species tested exhibit cellulolytic activity. *Anthostoma turgidum* was the most active species, because it showed the highest activity, determined by all the mothods used.

TRB is thankful to UNESCO for the fellowship granted for this work. Thanks are also due to Dr. K. Prasil for supply of cultures used in the present investigations.

June 30, 1980.

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INHIBITION OF MONOAMINE OXIDATION OF GUINEA PIG LIVER MITOCHONDRIA BY EXTRACT OF *LATHYRUS SATIVUS* SEEDS

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THE consumption of *Lathyrus sativus* seeds as the principal dietary constituent causes a neuropathological disease called 'lathyrism' among the rural population in certain parts of Central India[1]. *β-n*-oxalyl-L-*α*, *β*-diaminopropionic acid, the toxin present in these seeds, is believed to be the cause of neurolathyrism in adult human[2–4]. The amino acid is neurotoxic to many experimental animals[5–7] and causes certain biochemical changes[8,9]. As yet there is no report regarding the effect of this toxin on amine metabolism. Biogenic amines in the brain, heart, kidney, liver, lungs and intestine are metabolized by the action of monoamine oxidase (MAO)[10–12] and also by the action of amine tetrazolium reductase (MADH)[13,14]. *In vitro* effects of *Lathyrus sativus* extract on MAO and MADH systems of guinea pig liver mitochondria were studied, an account of which is presented in this communication.

Materials and Methods

Lathyrus sativus seed concentrate (L.S.C.) was prepared according to the method of Adiga *et al.*[15]. The seed meal was refluxed with 75% aqueous ethanol (400 g seed/600 ml ethanol) for 90 minutes and the extraction repeated thrice with fresh portions of the solvent. The pooled extract (crude) was concentrated *in vacuo* (40–45°C) to one-sixth the volume and shaken with equal volume of chloroform to remove lipids and pigments. L.S.C. after treatment with chloroform is described as partially purified. Guinea pig liver mitochondria was prepared according to the method of Schneider and Hogeboom[16].

The standard assay system for MAO contained 0·02 M PO_4 buffer, pH 7·0. 0·01 M tyramine, 0·0125 M semicarbazide, 20 mg mitochondria (1 mg protein) and 0·4 ml of different L.S.C. preparations (crude–7·8 protein/ml, partially purified–4·5 mg protein/ml) in a total volume of 2 ml. The incubation mixture was preincubated for 10 min followed by 15 min incubation period at 37°C. The aldehyde formed was measured at 420 nm[17].

The standard assay system for MADH contained 0·025 M PO_4 buffer, pH 7·5, 0·01 M tryptamine, 0·5 mg neo-tetrazolium chloride (NTC), 50 mg mitochondria (2·5 mg protein) and 0·4 ml of different L.S.C. preparations in a total volume of 2 ml. The assay mixture was preincubated for 10 min followed by 8 min incubation period. Diformazan produced

TABLE I

Effect of Lathyrus sativus *extract on monoamine oxidase activity in guinea pig liver mitochondria*

Fraction	Conc. in mg (protein/ml)		Micromoles of aldehyde formed/ 15 min/20 mg mitochondria	Per cent inhibition	Student's '*t*'
Crude *Lathyrus sativus* extract	7·85	Control (6)	0·377±0·033	29·4	7·62
		Expt. (6)	0·266±0·018		($P < 0·001$)
Partially purified *Lathyrus sativus* extract	4·55	Control (6)	0·325±0·008	17·5	6·36
		Expt. (6)	0·268±0·022		($P < 0·001$)

Results are expressed as micromoles of aldehyde formed/15 min/20 mg tissue (mean ± S.E.). Figures within parentheses indicate the number of experiments performed.

TABLE II

Effect of Lathyrus sativus *extract on amine tetrazolium reductase activity in guinea pig liver mitochondria*

Fraction	Conc. in mg (protein/ml)		micromoles of diformazan formed/ 8 min/50 mg mitochondria	Per cent inhibition	Student's '*t*'
Crude *Lathyrus sativus* extract	7·85	Control (5)	0·165±0·005	31·5	14·44
			0·113±0·007		($P < 0·001$)
Partially purified *Lathyrus sativus* extract	4·55	Control (6)	0·158±0·007	20·2	6·34
		Expt. (6)	0·126±0·010		($P < 0·100$)

Results are expressed as micromoles of diformazan formed/8 min/50 mg tissue (mean ± S.E.). Figures within parentheses indicate the number of observations performed.

was measured at 520 nm[18]. Protein was estimated according to the method of Lowry *et al.*[19].

Results

The results presented in Tables I and II indicate that the respective inhibition of MAO with crude and partially purified L.S. extract are 29·4 and 17·5% while that of MADH are 31·5 and 20·2. The decrease in inhibitory effect on MAO and MADH by L.S.C. upon purification, indicates, that during purification some of the toxic principle possibly get lost or inactivated.

However further work is necessary before explaining the neurotoxicity of the amino acid as a function of its effects on these enzymes of monoamine oxidation.

October 17, 1980.

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PATHOGENESIS OF *RHIZOPUS ORYZAE* WENT AND GEERLINGS IN FRUIT-ROT DISEASE OF BRINJAL (*SOLANUM MELONGENA* L.)

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Rhizopus oryzae Went and Geerlings have been reported to cause rot diseases of apple, banana, mango, potato, tomato[9], garlic[5], and sugarbeet[7] in storage and fields in various parts of India. During a survey of vegetable fields around Gwalior (M.P.) from November to March (1977 and 1978) a soft rot of brinjal fruits was observed. The incidence of disease was recorded on both young and mature fruits during this period. However, the disease incidence was relatively low on younger fruits. Isolation from the diseased lesions showed *Rhizopus oryzae* Went and Geerlings (IMI No. 233341) to be the causal organism of this fruit-rot.

In nature the disease starts as a soft watery rot accompanied by tissue decoloration of the brinjal fruits, which later on develop browning, and the symptoms progress rapidly in the healthy tissues. Later the pathogen covers the fruit surface with effuse growth and the entire fruit rots in about 15 20 days. Pathogenicity experiments, using Granger and Horne's[8] method of inoculation, proved the Koch's postulates. The organism was found to be a wound pathogen as the infections of fruits were mainly caused through the surface wounds (Fig. 1). Since, the rot syndrome

Fig. 1. *R. oryzae* infected fruit.

exhibited much involvement of macerating enzymes in producing the disease, the enzyme complex *in vivo* was also investigated. For these studies, extracts from the diseased tissues were obtained at different periods of incubation for the assays of enzymes of the pectolytic and cellulolytic moieties. The preparation of extracts and assay procedures followed were similar to those reported elsewhere[1,2,4].

The results of these studies (Table I) showed that the pectolytic enzyme moiety, of the pathogen *Rhizopus oryzae*, consisted of polygalacturonase (PG), pectin methylgalacturonase (PMG), and pectinmethylesterase (PME) whereas, cellulase (C_1 and C_x) represented the cellulolytic moiety. The quantitative production of PG and PMG was relatively high at 5 and 10-day stages of the disease than that of PME. Quantities of cellulase were higher at 10 and 15-day stages

Table I

Periodic levels of pectolytic and cellulolytic enzymes produced in vivo by R. oryzae

Incubation period (days)	Relative enzyme activity (REA)			
	PG	PMG	PME	Cellulase
5	67	63	12	36
10	50	59	14	77
15	21	27	10	59
20	20	14	10	27