particle morphology and physical properties but serological relationship of present isolate with other polyviruses including carnation vein mottle virus has to be confirmed. To our knowledge, it is the first report of a virus causing a disease in carnations, in India.

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Fungus and insect spoiled maize for ethanol production by *Saccharomyces cerevisiae* var *ellipsoideus* and *Zymomonas mobilis*

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The alcohol yield (w/v) from maize of mould-infected, insect-infested and mould-insect affected grains using *Saccharomyces cerevisiae* var *ellipsoideus* were 7.67, 7.32 and 6.85; and by *Zymomonas mobilis* were 7.26, 6.92 and 6.49 respectively. The fermentation efficiencies (%) by *S. cerevisiae* var *ellipsoideus* were 97.58, 97.08 and 97.49; and by *Z. mobilis* were 92.36, 92.44 and 92.31 respectively. The present observations using spoiled maize grains are promising.

OUR studies using maize (*Zea mays*) Ganga-5 (hybrid variety) as substrate, fermented by *Saccharomyces cerevisiae* var *ellipsoideus* and *Zymomonas mobilis* revealed that maize could be used as a potential biomass for ethanol production[1,2]. Often ill storage and improper harvesting processes lead to fungal and insect infestations of maize and so disqualify this grain for human consumption. Such spoiled grains are used as animal feed or manure. Very scanty literature is available on the use of spoiled grains for alcohol production. Calleja *et al*[3], suggested that spoiled grains and roots could well serve as raw materials for production of ethanol. A few studies carried out on the fate of mycotoxin-contaminated grains, used as substrate for the fermentative alcohol, have shown no toxin in the distilled alcohol[4–8]. Therefore, an attempt is made here to use spoiled maize grains for production of an industrially valuable product such as ethanol.

Samples of spoiled maize grains of Ganga-5 (hybrid variety), were randomly collected from different warehouses, storehouses and grain shops in clean polythene bags. These samples were analysed to find out causative agent/s for spoilage. The causes were found to be infection by mould, primarily being *Aspergillus niger* and infestation by Coleopteran storage pest, *Sitophilus oryzae* (Linne).

Such grains were assorted into three sets, comprising grains of fungus-infected (Set I), grains of insect-infested (Set II) and third set as grains spoiled by both the agents (Set III). Another set (Set IV) using unaffected, healthy maize grains was also processed similarly as control. These samples were further cleaned and washed separately to free them from contaminants. Each set of grains were pulverised (30 mesh) separately to prepare 20% solids' slurry.

The slurry was prepared out of 30 mesh flour, on dry weight basis by using clean tap water. Liquefaction of the slurry was carried out by enzyme process by adjusting pH of the slurry to 6.0 using 2.5 N HCl. Bacterial alpha-amylase was added at the concentration of 3 g/kg of flour. An amount of 0.022% $CaCl_2$ and 0.014% NaCl were added to the slurry for the satisfactory activity of the enzyme as described by Srikanta *et al*[9]. Then the whole slurry was cooked in a water bath at 80–85°C for 30 min.

The slurry thus hydrolysed was subjected to saccharification by lowering the temperature of the slurry to 60°C under running tap water. The pH of the slurry was adjusted to 4.5 by using 2.5 N HCl. The slurry was then treated with fungal amyloglucosidase (glucozyme) enzyme at the concentration of 5 g/kg of flour and was maintained at 60°C for 48 h in a thermostatically controlled device, as suggested by Srikanta *et al*[9].

The reducing sugars of the saccharified mash were determined by Shaffer-Hartmans' micromethod[10], which were considered as initial sugars for fermentation. Fermentation was carried out using *S. cerevisiae* var *ellipsoideus* and *Z. mobilis*. The ethanol content was determined by A.O.A.C. method[11].

The initial and final reducing sugar, ethanol yield

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Table 1. Ethanol yield and fermentation efficiency from spoiled and healthy maize grains.

Type of Infection	Organsim	Reducing Sugar (g%) 0 h	Reducing Sugar (g%) 72 h	Alcohol yield w/v	Fermentation efficiency %
Set-I					
Fungus-infected (70.64%)	*S. cerevisiae* var *ellipsoideus*	15.42	0.39	7.67	97.58
	Z. mobilis	15.42	0.39	7.26	92.36
Set-II					
Insect-infected (68.50%)	*S. cerevisiae* var *ellipsoideus*	14.97	0.11	7.32	97.08
	Z. mobilis	14.97	0.18	6.92	92.44
Set-III					
Fungus-insect affected (64.60%)	*S. cerevisiae* var *ellipsoideus*	13.78	0.12	6.85	97.49
	Z. mobilis	13.78	0.19	6.49	92.31
Set-IV					
Healthy grains (78.40%)	*S. cerevisiae* var *ellipsoideus*	16.42	0.10	8.18	97.73
	Z. mobilis	16.42	0.09	7.79	93.97

Figures in parenthesis indicate starch content.

(w/v) and fermentation efficiency (%) at 72 h duration of fermentation of three sets of affected grains are presented in Table 1. The alcohol yield and fermentation efficiency by *S. cerevisiae* var *ellipsoideus* with grains of sets I–III were 7.67, 7.32, and 6.85 respectively and the fermentation efficiency were 97.58, 97.08 and 97.49 respectively. The same by *Z. mobilis* were 7.26, 6.92 and 6.49; and 92.36, 92.44 and 92.31 respectively. Thus the study revealed that *S. cerevisiae* var *ellipsoideus* was more efficient than *Z. mobilis* in bioconversion of spoiled maize grains to alcohol.

The alcohol yield and fermentation efficiency of mould infected grains (Set-I) by *S. cerevisiae* var *ellipsoideus* and *Z. mobilis* was respectively 7.67 and 7.26 (w/v) and 97.58 and 92.36(%). The same with insect infected grains (Set II) was 7.32, 6.92 (w/v) and 97.08, 92.44(%) respectively and the grains affected by both agents (fungus and insect) revealed the values as 6.85, 6.49 (w/v) and 97.49, 92.31(%) respectively. The results revealed that, while the ethanol yield with combined affected grains (Set III) (6.85, 6.49 w/v), was marginally reduced when compared to that of 7.67, 7.26 (w/v) grains infected by mould and that of 7.32, 6.92 (w/v) grains infested by insects; whereas the fermentation efficiency with all the three categories of affected grains remained more or less same.

The results revealing alcohol yield and fermentation efficiency with unspoiled, healthy maize grains are also presented in Table 1 (Set IV). The alcohol yield and fermentation efficiency with healthy grains were 8.18 (w/v) and 97.73(%) by *S. cerevisiae* var *ellipsoideus* and 7.79 and 93.97 by *Z. mobilis*, respectively.

The observations using spoiled grains for ethanol production by fermentation were encouraging. The fermentation efficiency which ranged from 97.08 to 97.58% by *S. cerevisiae* var *ellipsoideus* and 92.31 to 92.44% by *Z. mobilis* with spoiled grains when compared to the same by using unspoiled, healthy maize grains which was 97.73 and 93.97% respectively, were found almost similar.

From the above observations it could be derived that fungus, insect and fungus-insect affected maize grains can be better utilized as substrates for effective ethanol production, instead of using them as animal feed or manure. Therefore such an attempt of using spoiled grains for ethanol production would be a wiser concept in the developing countries, and paves the way for a more economical method of using waste to produce valuable products.

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Increase in NADH-glutamate synthase activity in bean leaf segments by light

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Light increased NADH-glutamate synthase activity in excised leaf segments from dark grown bean seedlings both in the absence as well as presence of ammonium nitrate. The enzyme activity in excised leaves in the presence of ammonium nitrate increased linearly after a lag of 1 h upto 12 h in light, although there was only a slight increase in enzyme activity in dark. The light-induced increase in enzyme activity was inhibited by cycloheximide and DNP and was unaffected by lincomycin and DCMU. Sucrose and glucose supply had differential effects on dark and light-induced enzymes. It is suggested that light induces *de novo* synthesis of the enzyme in leaf segments.

PYRIDINE nucleotide dependent glutamate synthase (glutamate: NAD^+ oxidoreductase (transaminating) EC 1.4.1.14) plays an important role in ammonium assimilation in young seedlings[1,2], and it coexists with the ferredoxin-dependent enzyme in non-green tissues[3]. The effect of light on glutamate synthase activity has been studied only in a few cases and the results seem to be variable. Exposure of etiolated pea and barley shoots to light increases ferredoxin-dependent glutamate synthase, but has no effect on NADH-enzyme[4,5]. Further, upon greening of tobacco cultured cells and callus of *Bovardia ternifolia*, an increased Fd-glutamate synthase activity and unaltered NAD(P)H-dependent activities have been reported[6,7]. However, Fd-glutamate synthase does not change in etiolated maize leaves during their greening[8]. On the other hand, NADH-glutamate synthase is described as a light-regulated enzyme in spinach leaves[9]. In the present investigation, the effect of light/dark changes on levels of NADH-glutamate synthase was studied with a view to find out the possible mechanism.

Seeds of *Phaseolus vulgaris* L.cv. Rajmah were surface sterilized with 0.1% $HgCl_2$ for 30 sec and then washed thoroughly with distilled water. Seedlings were raised in plastic pots containing washed sand in darkness at $25 \pm 2°C$. They were watered on alternate days with $\frac{1}{2}$ strength Hoagland's solution containing no nitrogen. Primary leaves from 7-day old uniformly grown seedlings were used for the described treatments at pH 6.0 either in continuous light of about 65 Wm^{-2} radiant flux density or in darkness at $25 \pm 2°C$.

All the operations for enzymie extraction were carried out at 1–4°C. The enzyme was extracted from the fresh material with pestle and mortar using a pinch of acid washed sand in an extraction medium consisting of 0.2 M phosphate buffer pH 7.5, 2 mM EDTA, 100 mM KCl, 12.8 mM mercaptoethanol and 0.5% Triton X-100. The ratio of leaf material to extraction medium was 1:4 (g:ml). The extract was centrifuged at 20,000 × g for 10 min. The supernatant obtained was saturated to 30% by adding solid ammonium sulphate. The precipitated protein was removed by centrifugation and solid ammonium sulphate was added to the supernatant to bring it upto 55% saturation. The precipitated protein was separated again by centrifugation at 20,000 × g for 10 min and dissolved in one ml of 0.025 M phosphate buffer (pH 7.5) containing 1 mM EDTA, 50 mM KCl, and 12.8 mM mercaptoethanol. The protein solution was then filtered through a sephadex G-75 column (25 × 1 cm) and eluted with the same buffer. Two ml fractions were collected and 4th and 5th fractions with maximum activity were pooled for enzyme assay.

The enzyme activity was assayed spectrophotometrically by observing decrease in absorbance at 340 nm, by a method slightly modified from that of Matoh *et al*[3]. The assay mixture consisted of 10 mM phosphate buffer (pH 7.5) containing 0.4 mM EDTA and 20 mM KCl, 0.2 mM NADH, 0.4 ml enzyme preparation, 0.67 mM 2-oxoglutarate and 2.67 mM glutamine in a total volume of 3.0 ml. The reaction was started by the addition of glutamine and the absorbance was noted after 5 min. Control sets did not include glutamine. One enzyme unit was expressed as nmole NADH oxidized h^{-1}.

Protein in the extract was estimated by Folin phenol reagent by Lowry's[10] method, after precipitation with TCA.

The data presented are average values of at least three replicates with ± S.E. in the tables.

Supply of 5 mM ammonium nitrate to excised leaves from dark grown seedlings increased total and specific enzyme activities both in dark as well as in light (Table 1). The enzyme activities in the leaf segments from dark grown seedlings were higher in light than in dark both in the absence as well as presence of ammonium nitrate.

In the dark, the enzyme activity in the leaf segments increased slowly upto 12 h after a considerable lag of