Antifungal Activity

The complexes were dissolved in dimethyl formamide to get 1% conc Cup diffusion[7] method was followed for determining antifungal activity. Sabroud's agar media was used for culturing the organism. The melted media was aseptically poured into petridishes and then 2 ml inoculum was added to the dish. After settling, 4 cups of 6 mm dia. were cut by sterile cork borer and these cups were filled by test solution. A control was also run. These inoculated plates were then incubated for 72 hours at 24° ± 1. The inhibitory effect was noted against the test organism by measuring the diameter of zones of inhibition appearing around the cups. The experiments were performed in triplicate and the mean values were recorded in Table II.

Discussion

A perusal of Table I indicates that the mixed ligand metallic complexes of salicylic acid as primary ligand and pyridine, β-picoline, 8-hydroxy quinoline and nicotinic acid as secondary ligands using Cu, Ni, Co, Fe as metals had the expected chemical composition. The anti-fungal activity was compared with simple metallic complexes of salicylic acid which is reported as an anti-fungal agent and as a standard for the purpose for various experiments. The mixed ligand complexes were found to possess higher activity against some organisms and hence it can be presumed that the mixed ligands potentiate the activity to a considerable extent against *T. eqningia, T. viride, A. flavus*; the former two being the cause of many dermal infections. The complexes were, however, active against *C. fulcatum A. solani, C. neoformans* also.

Copper complexes were found to be comparatively more active while those of iron were least active.

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A SOFT FRUIT ROT OF *AEGLE MARMELOS*

FOLLOWING a cyclonic storm, quite a good number of raw fruits of *Aegle marmelos* were found damaged while still attached to the tree. The rotten fruits were brought to the laboratory and the following studies were made.

Small bits of surface sterilized rotten fruits were plated on PDA and Czapek's media and incubated at 28° C (± 2° C). The fungus showing consistent growth on the plated bits was isolated, purified and maintained on PDA. It was identified as *Fusarium solani* (Mart.) Sacc.

The pathogenicity tests were conducted under aseptic conditions following the knife injury method of Tandon and Mishra[2]. Inoculations were done on injured and uninjured raw fruit surfaces and incubated at 28° C for 15 days to observe the symptoms and for making enzymatic studies. Respective controls were also maintained.

The results revealed that no rotting occurred in intact uninjured fruits while the inoculated injured fruits developed the brownish soft rot with three distinct zonations of colour: (i) the severely macerated black regions near and at the site of injury containing white mycelia and spores, (ii) inner to it was dark-brown and considerably macerated tissue showing brownish watery secretions from which the mycelia were occasionally isolated and (iii) innermost dull brown regions which were visibly affected and sharply distinct from the yellowish apparently healthy tissue. The pathogen was reisolated from outer two zones only.

The seeds were yellowish-brown and pulpy in less effected regions while completely transformed into watery substances in severely infected dark-black region. The rotting advanced more quickly in tissue of the hard pericarp (3/4 area blackened within 15 days) as compared to its advancement in depth (1·6 cm out of 5·5 cm fruit diameter) within the same period. The rotten tissue emitted pungent odour.

Since the tissue maceration and release of watery substances were noticed well ahead of mycelial growth, certain hydrolysing enzymes were determined for their possible role in present host-pathogen interaction. Two pectolytic, *viz.*, Polymethylgalacturonase (PMG) and Polygalacturonase (PG) and one cellulolytic, *viz.*, Cellulase (Cx) were taken into consideration as

they are known to degrade the pectic and cellulosic substances of the cell wall[1].

For making enzymes preparations, 5·0 gm of above rotten portion was ground thoroughly in 15 ml sterilized distilled water and 15 ml of 0·5 N NaCl solution. The resulting extract was squeezed through 3–4 layers of muslin cloth and finally centrifuged at 4,000 rpm for 20 minutes. The clear supernatant was used as enzyme sample. Similar enzyme samples were also prepared from "control fruits". The activity of these enzymes was assayed by measuring the loss in viscosity using Oswald-Fanske Viscometer. The viscosity of different reaction mixtures was measured immediately (Vo) and then after 120 minutes (Vt). The viscosity of distilled water was also measured (Vw). Boiled samples of crude enzyme extract was used as control. These estimations were made at 28° C in a water-bath. The percentage of enzyme activity was calculated by the formula given below:

$$\frac{Vo - Vt}{Vo - Vw} \times 100.$$

The composition of reaction mixtures used for different enzymes was as follows: PMG [Pectin 5·0 ml (1% solution, pH 5·5), enzyme sample 2·0 ml, 1·5 ml phosphate citrate buffer (pH 5·5) and 1 ml distilled water]; PG [5 ml Sodium polypectate (1% solution, pH 4·5), 2 ml enzyme sample, 1·5 ml phosphate citrate buffer (pH 4·5) and 1 ml distilled water]; and Cx [Carboxymethyl cellulose (1% solution, pH 5·5) 2 ml enzyme sample, 1·5 ml phosphate citrate buffer (pH 5·5) and 1 ml distilled water].

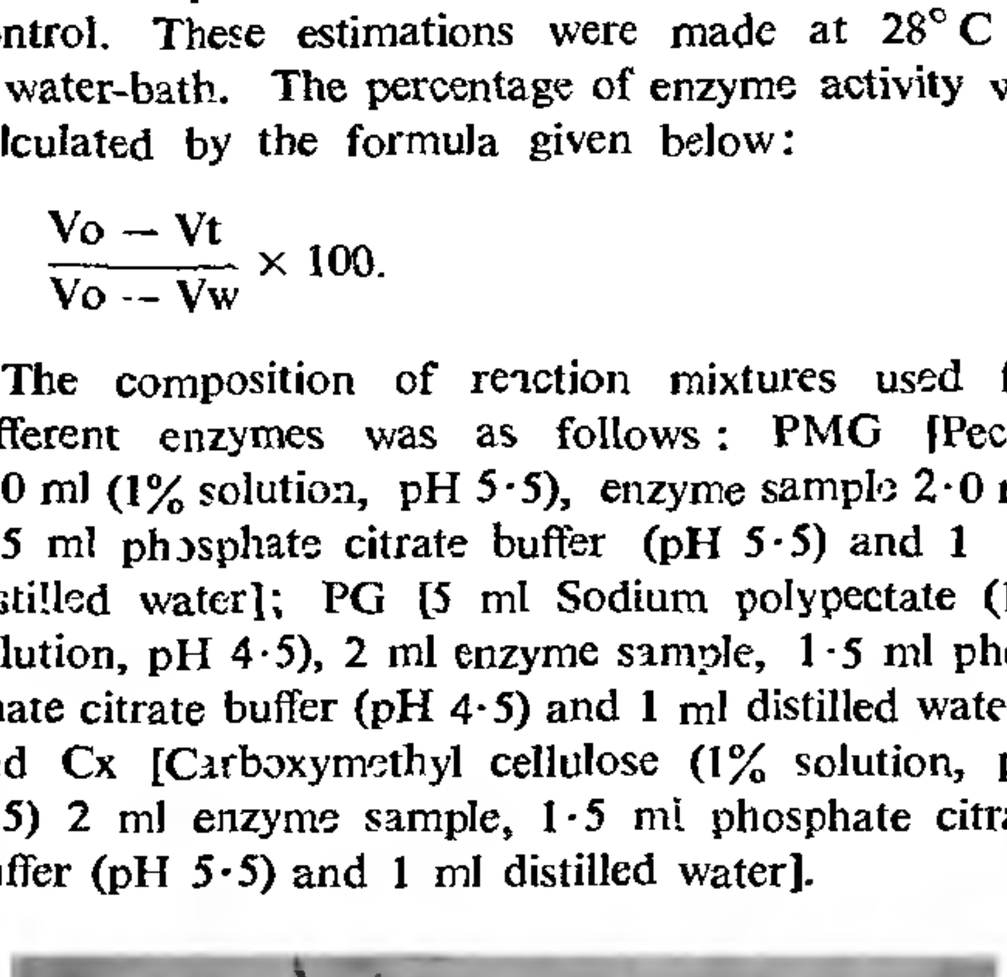

FIG. 1

The studies demonstrated the production of PMG, PG and Cx enzymes in the diseased tissues of Bhel fruits. Since these enzymes were not present in fruits used as control, it is apparent that these were produced by the pathogen, *Fusarium solani*. Among the pectolytic enzymes, PMG was most active (86·9%) whereas PG was moderately active (50%). This shows that PMG was the main enzyme responsible for degradation of pectic substances present in the form of incrusting and sheathing substances of plant cell wall including the middle lamella. These enzymes therefore facilitated the entry of the pathogen inside the fruit tissue. The *in vivo* production of cellulase with an activity of 57·5% indicates that the pathogen is fairly capable of killing the cells and hence the rotting.

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EFFECT OF HORMONES ON THE GROWTH AND CHLOROPHYLL CONTENT OF *WESTIELLOPSIS PROLIFICA* JANET

THE effect of Indole-3-acetic acid on the growth of blue-green algae has been reported previously[1–3]. From ecophysiological consideration the effect of indole-3-acetic acid on various physiological aspects of micro-organisms is important as the environment contains a considerable amount of excreted organic compounds and growth regulators[4,5]. The present communication describes the effect of kinetin, indole-3-acetic acid (I.A.A.) and gibberelic acid (G.A.) on the growth and chlorophyll content of *Westiellopsis prolifica* Janet, a blue-green alga.

Pure strain of the alga was grown in nitrogen-free Allen and Arnon's medium (1955) with the micronutrients modified by Fogg (1949). The cultures were maintained following the methods adopted earlier[6,7] and were grown at 24 ± 2° C in a culture room under continuous light. The hormones kinetin, indole-3-acetic acid and gibberelic acid were added to the culture media to obtain the concentration 1 μM each. Cultures were harvested at 5 day intervals upto 20 days of incubation. Growth of the algal sample was determined by dry weight method[8]. Chlorophyll contents were estimated colorimetrically after extracting in 80% acetone.

An analysis of the data clearly indicates the stimulating action of all the three hormones for algal growth in terms of dry weight (Fig. 1). The alga cultured with exogenously applied kinetin and indole-3-acetic acid exhibited significant increase in their growth rates over the controls (cultured without hormones).