

similarity in the production of sclerotia but they differ in their mycelial characteristics. In *M. phaseoli*, the mycelium is coarse, dark coloured and thick walled; but in *S. rolfii*, it is fine, hyaline and thin walled. The differential behaviours of these two fungi in response to soil pH and soil texture may be due to the differences in the morphology of the mycelium.

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December 29, 1976.

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INHIBITORY EFFECT OF LIGHT ON THE PRODUCTION OF CITRIC ACID BY *ASPERGILLUS NIGER*

INFLUENCE of light of the visible range on growth, sporulation, etc., in microorganisms had been reported earlier¹⁻³. Kamal *et al.*⁴ had shown that light is necessary for good growth and formation of conidiophores, sterigmata, etc., in *Aspergillus niveus*. Little attention, however, was given on the effect of light on biosynthesis and accumulation of citric acid by fungal organisms.

Materials and Methods

The strain used for this study was *Aspergillus niger* 6N3 isolated from the soil of Naihati, West Bengal. Erlenmeyer flasks (100 ml) containing 25 ml of Shu and Johnson's medium (Shu and Johnson, 1948)⁵ were inoculated with 0.1 ml of the conidial suspension (10×10^6 conidia/ml) and incubated at 30°C under light, darkness and alternate light and dark conditions. Cultures under light condition were kept in an incubator fitted with fluorescent lamps for continuous illumination. The intensity of illumination falling on the cultures was measured with a lux meter and the value adjusted at

2,200 lux units equivalent to 205 candle power approximately. The dark condition was created inside a chamber covered with black cloth. Alternate light and dark conditions were given to two sets of flasks in the following way:

1. 12 hrs. darkness followed by 12 hrs. light (Dark-Light).
2. 12 hrs. light followed by 12 hrs. darkness (Light-Dark).

Control cultures were kept in an incubator at 30°C, and opened only for casual observation. Observations were made on the 9th day of incubation. Mycelial dry weight was taken on previously weighed filter papers by drying them at 60°C for 24 hours. Total acidity was estimated by titrating the culture filtrates against 0.1 N NaOH solution to the phenolphthalein end point. Citric acid content was estimated following the methods of Marrier and Boulet (1958)⁶. Different organic acids accumulated in the culture filtrates were extracted in ether and detected by the thin layer chromatographic methods⁷.

Results and Discussion

Mycelial dry weight remained almost the same in the cultures grown under the different conditions. This clearly indicates that light or dark condition is not having any remarkable effect on the vegetative growth of mycelium. Even though the total acid production had not varied much under light or dark conditions when given separately, it showed a slight increase when light and dark conditions were given alternately. A profound decrease in the citric acid production had been noticed in those cultures grown under light condition, while dark condition slightly increased the citric acid production (Fig. 1). Cultures grown under illumination

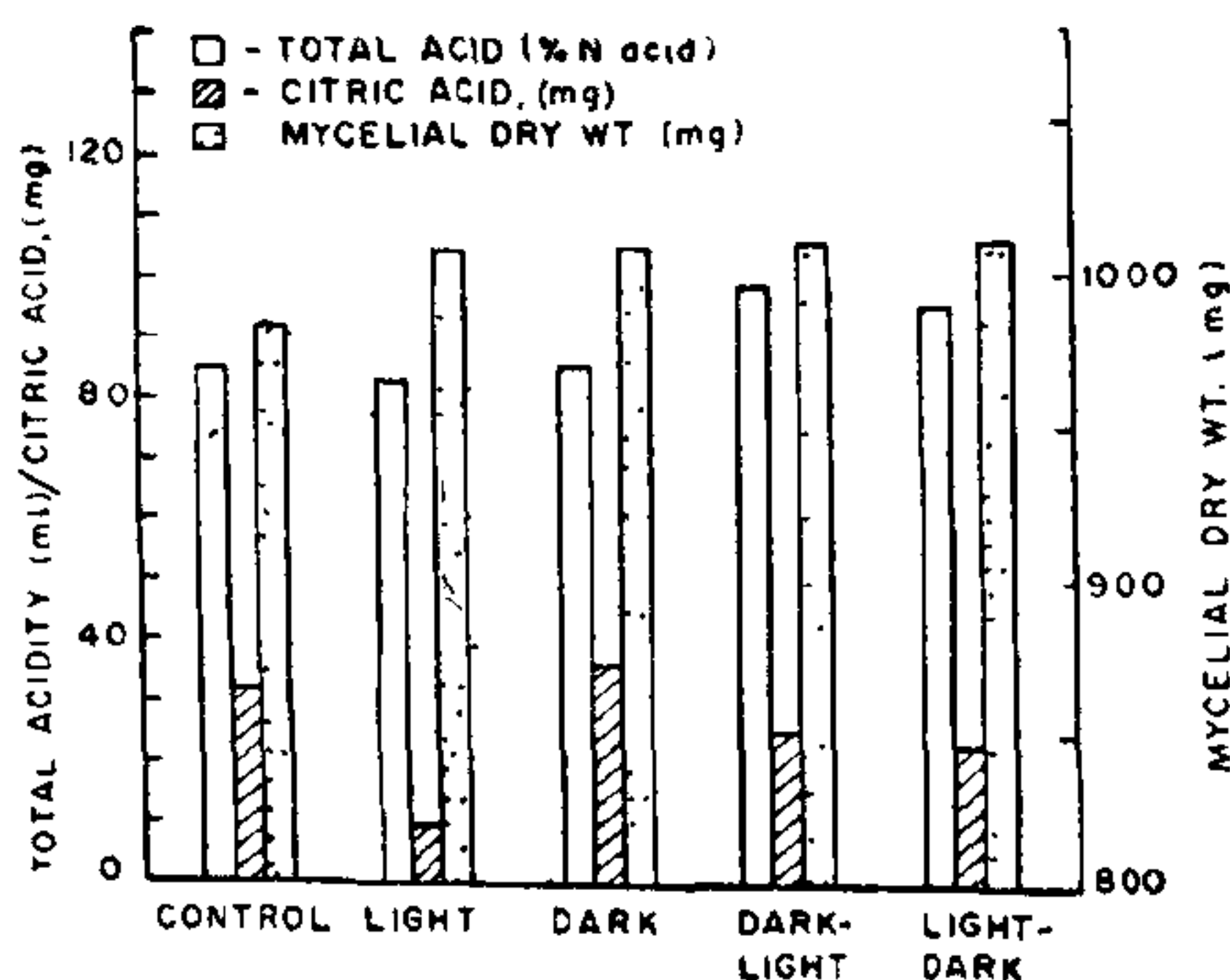


FIG. 1. Effect of light, dark and alternate light and dark conditions on mycelial growth and acid production of *A. niger* 6N3.

had shown the accumulation of glycolic and α -ketoglutaric acids in addition to the other acids (Table I).

TABLE I

Different organic acids accumulated in the culture filtrates of cultures grown under light, dark and alternate and dark conditions for 9 days at 30°C

Control (Normal incubation)	Light	Dark	Light- dark	Dark- light
Oxalic	Oxalic		Oxalic	Oxalic
Citric	Citric	Citric	Citric	Citric
Isocitric	Isocitric Succinic α -Keto- glutaric Glycolic	Succinic Malic	Succinic	Succinic

Legend:

Light-dark: Light treatment followed by dark treatment.

Dark-light: Dark treatment followed by light treatment.

It has been found that light plays a significant role on the biosynthesis and accumulation of citric acid in the culture of *A. niger* 6N3. Generally, the synthesis and accumulation of citric acid occurs in mold cultures through the Krebs tricarboxylic acid cycle⁸⁻⁹⁻¹⁰. Presence of succinic, α -ketoglutaric, isocitric and glycolic acids in the culture filtrate of cultures grown under light condition probably suggest the presence of some alternate pathways like S.K.I. cycle² (Succinate- α -ketoglutarate-isocitrate). Therefore, the low citric acid yield may be due to depletion of the intermediates of TCA cycle to the S.K.I. cycle, necessary for the normal functioning of the TCA cycle. Production of citric acid by fermentation can be kept more or less constant if the fermentation is carried out in the dark as there should be very little chance under such a condition for a deviation in the normal pathway through which citric acid is produced.

The authors wish to express their gratitude to the International Atomic Energy Agency, Vienna, Austria, for financial assistance.

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REEVALUATION OF α -GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITY IN SKELETAL MUSCLE FIBRE TYPES

CONFLICTING reports have appeared in the literature about the histochemical localization and distribution of lactate dehydrogenase (LDH) and NAD⁺-linked α -glycerophosphate dehydrogenase (GPD). When assayed *in vitro* white muscle fibres show higher GPD activity, but show lower enzyme activity than the red muscle fibres in histochemical tests¹⁻⁴. It has since been demonstrated, particularly for LDH⁵, that when a sequential staining technique involving the use of phenazine methosulfate (PMS) is used, the white muscle fibres show higher enzyme activity than the red ones. This has led to the suggestion that PMS should be used for the histochemical demonstration of dehydrogenases's activity in locations where diaphorases are a limiting factor³⁻¹⁰.

It has also been demonstrated⁶ that LDH and possibly GPD also survive formalin fixation, provided the muscle sections have been fixed in formalin vapour. This step is important since without prior fixation, considerable leakage of these two dehydrogenases may take place particularly from white muscle fibres^{1,6}. Therefore, using PMS (0.4 mg per ml of the incubation medium⁶) and formalin vapour fixation of the muscle sections, we have reevaluated the histochemical profile of GPD activity in mixed skeletal muscle fibre types. The diaphragm of the rhesus monkey (*Macaca mulatta*) was used in the present study. Formalin vapour fixed sections, 10 μ thick, were processed for the histochemical demonstration of GPD activity¹¹. By varying the concentration of PMS in the incubation medium it was possible to elicit varying histochemical profiles, with higher or