Figure 1. Photograph of Male sterile plant (MS–2).

Male sterile-2 (*MS–2*): In figure 1 male sterile plant is shown. This was recovered in F_3 family of a cross between yellow sarson (as female parent) and brown sarson (as male parent). This produced cleistogamous flowers with highly elongated pistil protruding outside of flower. Empty non-dehising pollen sac with small filament remaining inside the unopened petals renders the plant to get open pollinated. Plant stature, branching pattern and flowering time resembles with male fertile. It is interesting to note that pistil length is almost double to that of anther in MF–2. Decreased flower parts, small curved-seedless fruits (figure 2) resulted by self-pollination (by bagging) and poor seed set under open pollination are some other characteristic features of the plant. The comparative morphological features are given in table 1.

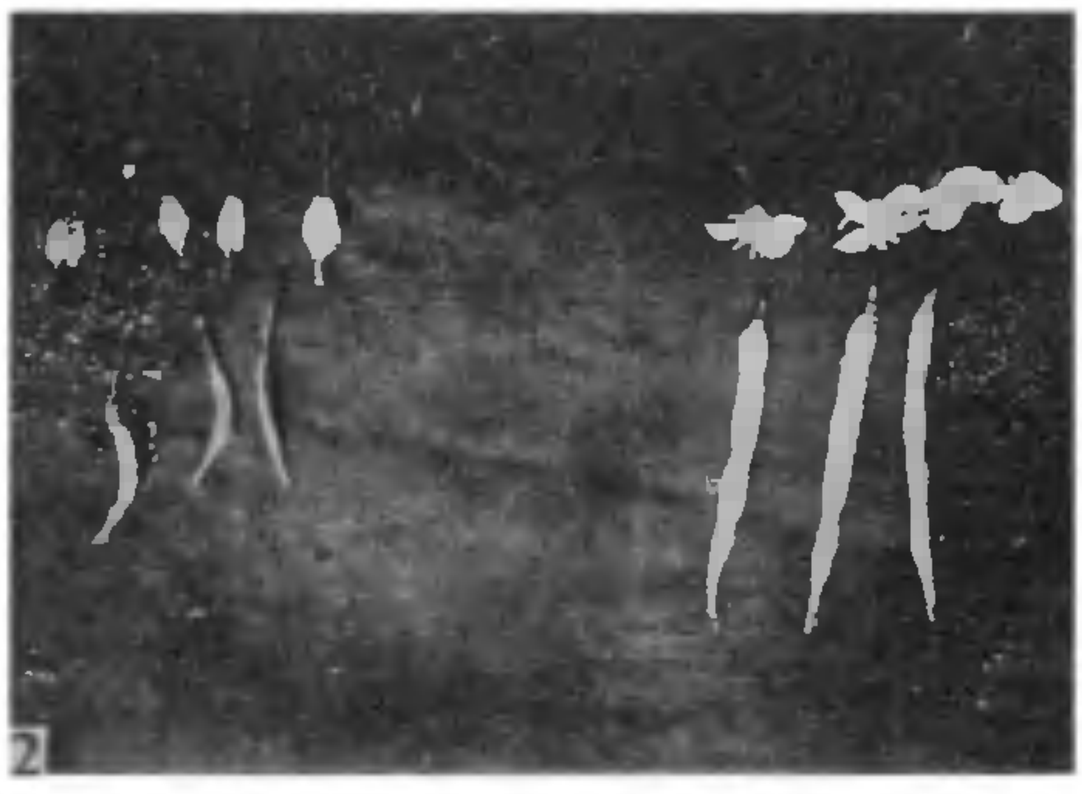

Figure 2. comparison of flowers and fruits of male fertile and male sterile plants, left hand side-flowers and pods from male fertile, right hand side-flowers and pods from male sterile plant (MS-2).

The male sterile plants have been crossed to a number of lines including their parents to study the genetic behaviour and explore the possibility of maintenance of sterility and restoration of fertility for aiming successful heterosis breeding in this crop.

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ACUTE TOXICITY OF STERIGMATOCYSTIN IN CHICKS

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ASPERGILLUS versicolor has been detected as natural contaminant in grains, bread, cheese and similar edible products[1]. Sterigmatocystin, a secondary metabolite of *A. versicolor*[2]. bears a structural relationship to aflatoxins[3-5]. Preliminary studies indicated that it was a hepatotoxin inducing tumours in lungs[6]. It was, therefore, of interest to study the acute toxicity of this mytoxin in chicks.

The medium of Rabie *et al*[1] was used for growing *A. versicolor*. *A. Versicolor* strain and was obtained

from IARI, New Delhi. Sterigmatocystin was extracte by the method of Vorster and Purchase[7] and purifieo using preparative TLC. It was crystallized using acetone and the product was compared with the authentic sample supplied by Robert M. Eppley, Food and Drug Administration, Washington, USA.

The mycotoxin sterigmatocystin was administered orally in 1% gelatin to one day old chicks weighing approximately 35 g. In one trial, 4 different concentrations of sterigmatocystin (0,1,1,10 and 100 mcg) were administered to 4 different batches of birds. Conentrations of the toxin excepting that which contained 100 mcg proved fatal on the second day of adminstration itself.

In a second trial higher concentrations of the toxin 50, 100, 250 and 500 mcg each was given as a single dore and the mortalities were accumulated and noted on the 1st, 2nd, 3rd and 7th day respectively. In the third trial (table 1) high concentrations (50, 100, 150, 200 and 300 mcg) were given as a single dose in one day. Body weights of the surviving birds were recorded after which the birds were killed and gross lesions if any in organs like liver, kidney, heart and brain were examined in the 3 trial of experiment, the LD_{50} was determined using the formula of Reed and Muench[8] which states

$$\text{Proportionate distance} = \frac{\text{Mortality above 50\%} - 50}{\text{Mortality above 50\}} - \text{Mortality below 50\%}$$

Negative logarithm of LD_{50} titre = Negative logarithm of dilution above 50% mortality + Proportionate distance = LD_{50}.

Birds given a dosage of 100 mcg of sterigmatocystin died on the second day. But the birds given 10 mcg or less survived. Administering different doses of the toxin nothing the mortality on each day as well as the weight gain using the mathematical derivation to determine LD_{50} it was found that the seven day LD_{50} was approximately. 3.4 mg/kg body weight at lower concentrations and 4 mg/kg body weight at higher concentrations of the toxin (table 1.)

In the case of birds treated with mycotoxin of higher concentrations, distributed to different days as well as birds given higher concentration of the toxin as a single dose it was found that the sub lethal doses (LD_{50}) produced growth suppression (table 1). In such animals, histopathological studies on liver, kidney, heart and brain presented an interesting picture. In the case of liver, the total necrosis was observed with mild fatty changes, periportal fibrosis, fatty vacuolation in addition to hepatic lesions. In kidney cells, tubular nephrosis, fatty changes in distal tubules and glomerular endothelium and fragmentation of glomerular capillaries were observed. The epithelial cells of nephron showed hyalin degeneration fatty changes and necrosis. In the case of heart tissue, congestion and hyperaemia with pericarditis, and in the case of brain tissue the degeneration of neurons with vacuolation and occasional lesions were observed.

It is interesting to note in detail the effect of acute oral toxicity of sterigmatocystin in chicks and this observation is very similar to that observed in the case of chicks 9-14 suffering from aflatoxicosis B_1. A precursor of a mycotoxin can be more powerful than the product is seen in the case of sterigmatocystin which is a precursor of aflatoxin B_1.

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TABLE 1

Mortalities and body weight changes in one-day old chicks give single oral doses of sterigmatocystin: Trial 3

Toxin	Dose in mcg per chick	Accumulated mortalities one day 1	2	3	4	Weight gain of survivers at one week (gms)
Sterigmatocystin	300	2/5	4/5	5/5	5/5	—
	200	1/5	3/5	4/5	5/5	—
	150	0/5	2/5	3/5	4/5	42
	125	0/5	0/5	0/5	1/5	53
	100	0/5	0/5	1/5	1/5	73

(a) Ratio of deaths to the number of birds in the group.
(b) Mean weight gains of chicks in controls groups was 39.3 gm
(c) Seven day LD_{50} for sterigmatocystin at 3.4 mg per kg of body weight, in trial 2.
(d) Seven day LD_{50} for sterigmatocystin estimated at 4 mg per kg of body weight in trial 3.

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PHOSPHATE MEDIATED BIOCHEMICAL CHANGES IN *NEUROSPORA CRASSA*

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PHOSPHATE was found to influence the morphology of *Neurospora crassa*. High phosphate was found to stimulate growth, sugar uptake and polyphosphate content of the culture. Carotenoids were found to be inhibited under high phosphate conditions. Glucose 6P dehydrogenase, mitochondrial and cytosolic malate dehydrogenase and invertase showed considerable decrease in the activity while amylase showed an increase in the activity under high phosphate conditions as compared to low phosphate conditions. These data demonstrate a significant regulatory role of phosphate in primary as well as secondary metabolism.

Though the regulation of secondary metabolism by inorganic phosphate is well documented[1,2] information on the role of inorganic phosphate in the regulation of enzymes of primary metabolism leading to the formation of secondary metabolites is meagre. Increase in the production of a variety of commercially important and useful secondary metabolites can be achieved by adjusting carefully the phosphate concentration in the growth medium. An understanding of the control of enzymes of primary metabolism by phosphate could boost the production of secondary metabolites. The present study demonstrates the increased production of invertase and carotenoids by controlling the phosphate in the growth medium, which controls several enzymes of primary metabolism.

The carotenogenic strain of *N. crassa* (wild type) was maintained on Saboraud's agar slants. The composition of the synthetic medium employed was the same as described earlier[3]. The culture was grown in 100 ml liquid medium in 250 ml Erlenmeyer flasks on a rotary shaker (180 rpm) at 30° C for 6 days. The mycelia were harvested and stored at −5° C before use. For growth measurement the mycelia were dried at 50° C to a constant weight. The growth was expressed as dry mat weight per 100 ml.

All studies were carried out using 5% glucose as carbon source. For study of amylase and invertase, 1% starch and 2.5% sucrose respectively were added as carbon sources in the growth medium.

'Low phosphate' indicates the addition of 0.01% KH_2PO_4 while 'high phosphate' means the addition of 1% KH_2PO_4 to a synthetic medium devoid of phosphate. These concentrations were selected since they were used earlier in this laboratory[4]. The pH after growth was not affected under these conditions.

Protein, carotenoids and polyphosphates were estimated according to the methods of Warburg and other workers[5-7].

For intracellular enzyme assays, a cell-free extract for cytosolic as well as mitochondrial enzyme was prepared as described earlier[8].

The assay methods used for malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37) and glucose-6P dehydrogenase (D-glucose 6-phosphate: NADP oxidoreductase, EC 1.1.1.49) were the same as described by Ochoa[9] and Kornberg and Horecker[10] respectively. Units are defined as the amount of enzyme which causes 0.001 change in optical density at 30° C per min.

Amylase (1,4 α D glucan maltohydrolase, EC 3.2.1.2) was assayed according to the method of Bernfeld[11]. Invertase (B fructofuranoside fructohydrolase, EC 3.2.1.26) activity was measured by estimating the reducing sugars released by Bernfeld method[11]. Unit of amylase and invertase are defined as the amount of enzyme which cause the liberation of 1 μ mole of maltose and reducing sugars respectively at 37° C hr.