This preliminary study shows that the method used may be of great help in determining the sex of the fetus at the early stage of pregnancy. It is a test which can be performed in less than one hour.

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RELATION OF SPORULATION TO FUSARIC ACID PRODUCTION IN MUSKMELON WILT PATHOGEN

FUSARIC acid (FA), a wilt toxin, is produced by many fusaria both *in vitro* and *in vivo*. From this laboratory Bhaskaran and Prasad[1] reported the *in vitro* production of FA by the muskmelon wilt pathogen [*Fusarium oxysporum* f. *melonis* (Leach and Currence) Snyd. and Hans.]. Interestingly the toxin production was more in media which favoured good sporulation[2]. Studies on the relation between sporulation and fusaric acid production were made and the results are reported here.

Hundred ml aliquot of sterilized Czapek's medium in 250 ml conical flasks were inoculated with 8 mm disc of the actively growing fungus and incubated at room temperature ($28 \pm 2°$ C). At the end of incubation period, the flasks were shaken in a rotary shaker for 30 min and the spore load was estimated by Haemoaglometer counts. The content was passed through a double layered cheese cloth, washed with distilled water. The mycelial mat was separated from cheese cloth, blotted and weighed. The filtrate was centrifuged to separate spores from culture filtrate. The fusaric acid in culture filtrate, mycelium and spores was detected following the respective methods[3,4]. The presence of FA was confirmed by paper chromatography. The influence of certain media, *viz.*, Czapek's, Coon's, Horne and Mittar's and Park's suggested for Fusarium[5] was tried for assessing growth, sporulation and FA level.

The effect of incubation periods on growth, sporulation and FA production is shown in Table I and the effect of different media on growth and FA production is presented in Table II. Among the media tested, Czapek's medium favoured relatively higher sporulation and FA production. Growth studies in Czapek's broth indicated maximum sporulation and FA production on 20th day and growth on 30th day. Thus a parallelism existed between sporulation and FA production by this pathogen. Sandhu[6] demonstrated that FA is a product of active metabolism, probably ultimately connected with tricarboxylic acid cycle. The accelerated metabolism during sporulation may be the cause of more FA production. The toxin level in mycelium and conidia showed a marked variation. FA in mycelium and conidia was first reported[2] in *Fusarium oxysporum* f. *vasinfectum*. Toxin in mycelium and

TABLE 1

Effect of incubation period on growth, sporulation and fusaric acid (FA) production by* Fusarium oxysporum *f.* melonis, *when grown in Czapek's medium*

Incubation period in days	Mycelial dry weight mg/ 100 ml	FA in mycelium	Number of spores/ ml	FA in spores	FA in culture filtrate
10	175	274·5	15,000	310·0	610·0
15	227	334·0	38,000	615·0	928·3
20	232	330·0	53,000	980 0	1240·0
25	240	332·0	52,000	950·0	1220·0
30	245	335·0	49,000	890·5	1195·0

* FA in inhibition annules (mm^2).

TABLE II

Effect of different media on growth, sporulation and FA production by* Fusarium oxysporum *f.* melonis

Media used	Mycelial dry weight (mg/ 100 ml)	FA in mycelium	Number of spores/ ml	FA in spores	FA in culture filtrate
Coon's medium	247	285·0	42,000	612·0	1130·2
Czapek's medium	238	280·0	51,000	845·0	1230·8
Horne and Mittar's medium	312	315·0	28,000	394·5	829·5
Park's medium	215	225·0	32,000	480·0	890·5

* FA in inhibition annules (mm^2).

conidia was estimated for the first time in this pathogen. Toxin content of conidia was more than that of the mycelium produced per unit quantity of medium. These results further substantiate that there is a definite positive correlation between sporulation and toxin production.

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A BIOLOGICAL APPROACH TO THE CONTROL OF MAIZE BORER *CHILO ZONELLUS* (SWINHOE)

ISOLATIONS made from the body surface of the borer, *Chilo zonellus* (Swinhoe), its frass deposits and rotted maize stalk in the vicinity of borer tunnels gave the following fungi : *Cephalosporium acremonium, Fusarium moniliforme, Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Trichoderma* sp., *Botrytis* sp. and a *Fusarium* sp., Among the bacterial forms were *Pseudomonas* sp., and *Bacillus* sp.

A species of *Fusarium* which was identified as *Fusarium aleyrodis* Petch was found to be pathogenic on this insect, others were not.

For confirmation of the pathogenicity of *F. aleyrodis*, originally isolated from the insect body surface, field collected larvae ranging from 2nd to 4th instar were individually immersed in fungal spore suspension. A control was maintained. The larvae were fed with finely chopped pieces of maize stem. Whitish fungal mat appeared gradually on the insect's body. Single spore isolation from this hyphal mat yielded *F. aleyrodis* Petch. Repeated pathogenicity tests also confirmed it. The larvae during fungal colonisation became sluggish, stopped feeding, turned brown and succumbed within 2–3 days of infection. Toxicity of the fungus is believed to be responsible for pathogenic action on the insects[2,3,6]. Mechanism of fungal action could be ascertained by observing separately the effect of fungal spore spray and its crude toxin on the insect larvae.

Spore spraying showed that by the 7th day the insects were killed (Table I). Fungal mat appeared on the 8th day and on the 9th day the entire insect body was covered with sporulating hyphae.

TABLE I

Effect of fungal spore spray on larvae of Chilo zonellus (*20 larvae were used in each replication*)

Days	Number of larvae dead			
	Replications			
	1	2	3	4
1	..	..	..	..
2	..	..	..	..
3	..	..	..	..
4	..	..	..	1
5	5	7	6	6
6	8	8	9	7
7	All	All	All	All
8	Fungal mat seen on the insect			
9	Fungal mat as well as spores found on all the insects.			

Crude toxin obtained from *F. aleyrodis* culture grown in Richard's liquid medium, when applied topically on the larvae, killed them. The toxin-treated insects stopped taking food, showed tetanic reaction and subsequently died. The larvae started dying right from the very beginning reaching the maximum on the 13th day (Table II). Mortality rate progressed with number of days of incubation.

Chilo zonellus has been found to be attacked and pathogenically colonized by another fungus *Beauveria densa* Link.[4,5]. *Chilo partellus* was effected by *Aspergillus flavus* and a *Fusarium* sp.[1]. The pathogenic attack of *Chilo zonellus* by *Fusarium aleyrodis* Petch, adds to the list of fungi parasitic on insects. Spraying of the insect with spore suspension or treatment with crude toxin of this fungus could be of biological control value.