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Cytogenetic Activities of *Fusarium spp.* Metabolites on *A. cepa*

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Abstract. The effect of four mycotoxins on the chromosomal arrangement during mitotic cell division of *Allium cepa* was examined. Results indicate the presence of certain changes in mitotic stages index (M.S.I) as well as some chromosomal anomalies which reflect toxic activity due to the exposure of the tested bulbs to the four fungal species. *F. equiseti* gave the highest deviation of chromosomal index from the normal chromosome behaviour followed by *F. solani*, *F. moniliforum* and *F. culmorum*.

Introduction

Onion is one of the main agricultural crops in Egypt. Intensive onion cultivations are concentrated in Upper Egypt, particularly in Sohag, Minia, Assuit and Qena governorates. The onion crop is usually attacked by many diseases among which are the white rot caused by *Sclerotium cepivorum* and the neck rot caused by *Botrytes sp.*

Fusarium species are among the common soil fungi. They are the main causative agents of damping off disease for many crops. Recently, many of *Fusarium* species are known to be harmful due to certain metabolites such as Zearalenone and Trichothecens. These toxic metabolites were reported as cell division inhibitors by affecting DNA synthesis [1]. Some fungal metabolites (Trichothecenes) are potent inhibitors of protein and DNA synthesis in eukaryotic cells [2]. Smith and Sullivan [3], Taylor *et al.*[4] and Abdou *et al.* [5] suggested that some fungal metabolites are acting as enzymatic inhibitors for enzyme system requirement for the chain reaction of DNA synthesis.

The present study aims at studying the possible mutagenic effect of metabolites of four *Fusarium* species on root meristematic tip cells of *A. cepa* bulbs.

Materials and Methods

Fungal species used

Four species of Fusarium, namely: *F. equiseti* (Corda) Sacc., *F. moniliforum* (Scheldon), *F. culmorum* (Schelecht ex. Fr.) and *F. solani* (Mart.) sacc. were locally isolated and identified.

Culture medium

Shredded wheat medium (S.W.) [6] and Yeast Extract Sucrose (Y.E.S.) [7] were used.

Procedure

Spores from heavily spore cultures were harvested and washed off with sterile 0.05% Tween 80. Flask cultures were inoculated with 1.0 ml spore suspension containing approximately 10^4 spore ml^{-1} and incubated at 28 °C for 10 days as static culture. Toxin extraction was carried out according to Abdou *et al.* [5]

Cytological examinations

Allium cepa bulbs were injected with 1 ml of tested fungal metabolites using a sterile syringe. The inoculated bulbs were left to grow in sterile tap water for 48 hours at 28 °C. Roots 1 – 2 cm long were excised from bulbs and fixed in 1:3 (v/v) glacial acetic acid-ethanol for overnight. The cytological preparations were made according to Darlington and La Cour's [8] Feulgen squash technique. All cytological observations were based on the examinations of at least 10 root tips from each treatment.

Results and Discussion

Effect on mitotic stages

Fusarium spp. were found to induce a wide range of mitotic abnormalities in meristematic cells of *A. cepa* root tips. Their frequencies were found to vary according to fungus species (Table 1 and Fig. 1). The least effective fungus was *F. culmorum*, while the most effective was *F. equiseti* where the abnormalities were ranged between 0.86 – 7.81%, 3.47 – 10.94% and 4.00 – 7.81% in case of prophase, metaphase and ana-telophase stages, respectively. In certain treatments, the mitotic index for *Allium cepa* was reduced due to *Fusarium* infection as in case of *F. solani* and *F. culmorum*. The inhibition of cell division by fungal metabolites may be attributed to the effect of these compounds on DNA synthesis, an explanation which is supported by the findings reached by Vielemetter and Schuster [1], and Beu *et al.* [9].

Table 1. Mitotic Index (M.I.) and Mitotic Stage Indices (M.S.I.) for *Allium cepa* root tips after treating bulbs with different *fusarium spp.* for 48 hours.

Fus. sp.	Total cells	No. of inter-	Prophase						Metaphase						Anaphase and Telophase						Total	
			Normal		Abnormal		Total		Normal		Abnormal		Total		Normal		Abnormal		Total			
	examined	phase	No.	%	No.	%	No.	M.S.I.	No.	%	No.	%	No.	M.S.I.	No.	%	No.	%	No.	M.S.I.	mitosis	M.I.
Fusarium solani	10060	9673	178	45.995	21	5.426	199	1.978	64	16.537	20	5.168	84	0.835	89	22.997	15	3.876	104	1.034	387	3.847
Fusarium culmorum	9363	9013	167	47.712	3	0.857	170	1.816	54	15.428	12	3.472	66	0.705	100	28.571	14	4.000	114	1.218	350	3.738
Fusarium moniliforum	4619	4260	190	52.925	7	1.950	197	4.265	48	13.370	20	5.571	68	1.472	78	21.727	16	4.457	94	2.035	359	7.772
Fusarium equiseti	1225	1661	27	42.188	5	7.813	32	2.612	8	12.500	7	10.937	15	1.224	12	18.750	5	7.812	17	1.388	64	5.224
Control	1197	1136	33	54.098	—	—	33	2.757	9	14.754	1	1.639	10	0.835	17	27.869	1	1.639	18	1.504	61	5.096

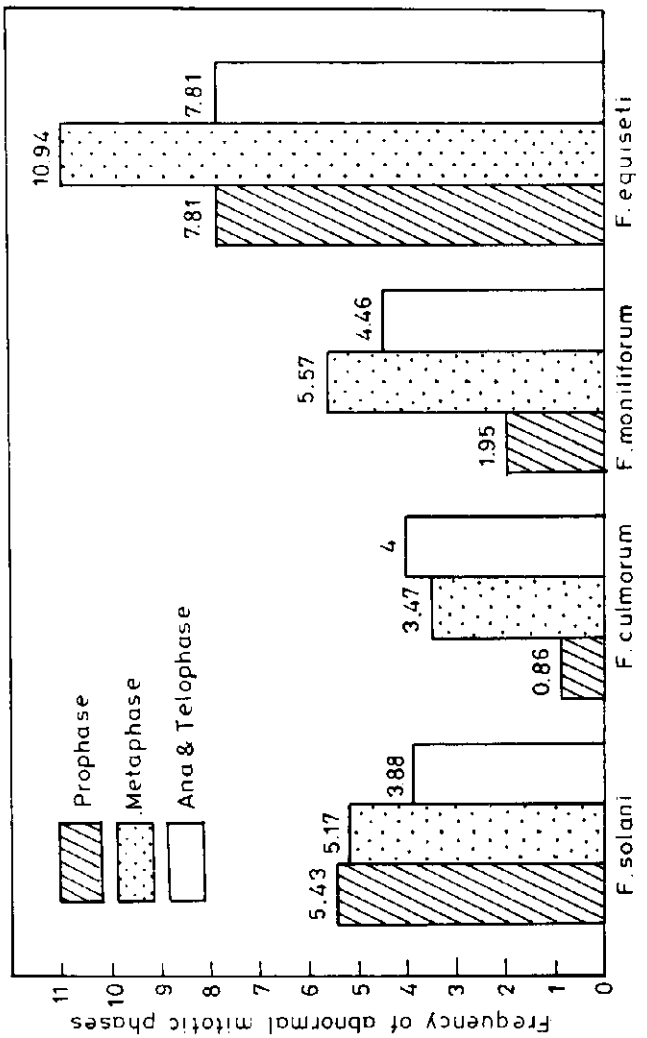

Fig.1. Frequency of abnormal mitotic phases after treating *Allium cepa* root tips with different *Fusarium sp*

It may also be assumed that these compounds are acting as enzymatic inhibitors of the enzyme system required for the chain reaction of DNA synthesis [3] and [4]. A slight increase was observed in mitotic index of roots belonging to bulbs treated with *F. equiseti* and a considerable increase in case of *F. moniliforum*. This increase may be due to the changes in the frequencies of the different mitotic stages (Table 1 and Fig. 2). This indicates that the tested *Fusarium spp.* could affect the relative duration of each stage. These results are in agreement with those obtained after treating *A. cepa* root cells with the herbicide Dual [10], Garlon-4 [11] and Sencor [12].

Stickiness was manifested in prophase, anaphase and interphase stages (Figs 3, 4 and 5). Stickiness was reported to be a result of depolymerization of DNA [13], partial dissolution of nucleoproteins [14]. Similar results were obtained after treatment with Rotenone [16] and Glean [17].

A considerable percentage of cells showed scattered chromosomes in the cytoplasm giving the C-metaphase (Figs 6 and 7). This type of abnormality was produced as a result of complete inhibition of spindle fiber formation similar to the effect of colchicine [18; 19]. C-anaphase was resulted as a consequence of the C-metaphase (Figs. 8 and 9).

Non-congression was noticed in metaphase stage (Fig. 10) as well as free and lagging chromatid in anaphase stage (Figs 11 and 12). Several pesticides such as 2 – 4 D, Gramoxone and Hyvarx [20], Metamidophos [21] and Dual [10] were reported to induce the same effect.

Chromatid-bridges were also noticed during anaphase stage (Figs 13 – 16). The observed chromatid bridges may be due to the effect of fungal metabolites in producing stickiness of chromosomes or due to breakage and reunion of terminal breaks (Figs 17 – 20). These results are in accordance with those obtained by Umeda *et al.* [22] and Abdou *et al.* [5]

It was noticed that there were some disturbed anaphases (Figs 21 – 23) due to the spindle fibers disturbance, as well as star-anaphase cells (Fig 24). These results are supported by those obtained by abdou *et al.* [5] and Megalla *et al.* [23].

Binucleate cells were also produced (Figs 25 and 26) as a result of *Fusarium* metabolites which may delay or inhibit the cytokinases.

These findings indicate that the exposure of certain plants, as represented by *A. cepa,* to fungal infection induce mitotic variation which can be seen in chromosomal arrangement in all mitotic phases.

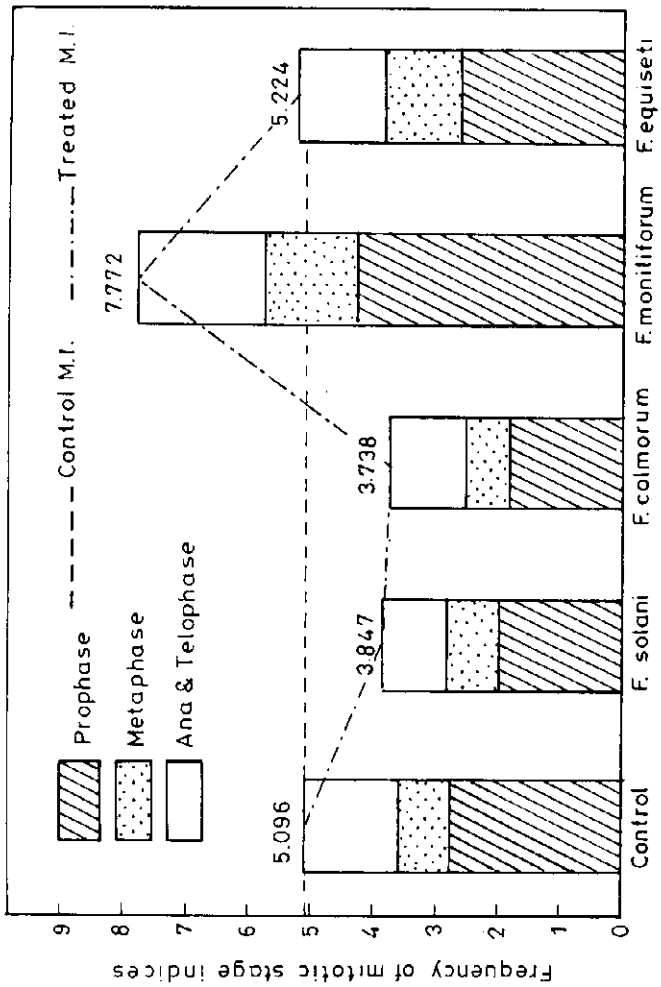

Fig.2. Changes in the mitotic stages indices (M.S.I) and mitotic index (M.I) after treating *Allium cepa* root tips with different *Fusarium spp.*

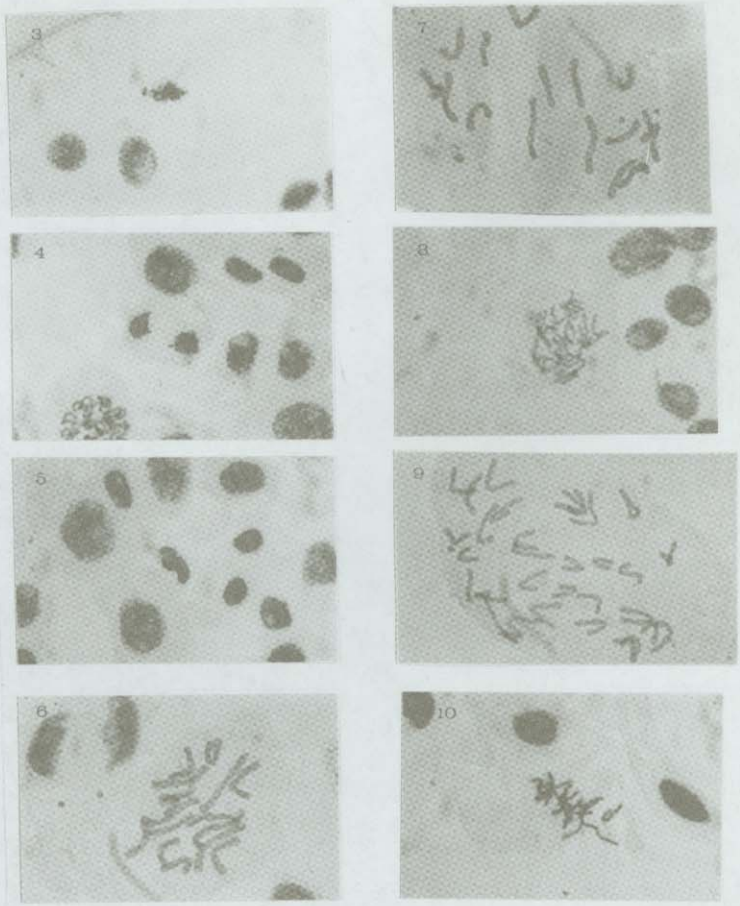

Plate 1. Effect of different *Fusarium spp.* metabolites on *A. cepa* root meristematic cells: (x=250).

(Fig. 3) of *F. equiseti*
(Fig. 4) of *F. solani*
(Fig. 5) of *F. solani*
(Fig. 6) of *F. moniliforum*
(Fig. 7) of *F. solani*
(Fig. 8) of *F. equiseti*
(Fig. 9) of *F. culmorum*
(Fig. 10) of *F. solani*

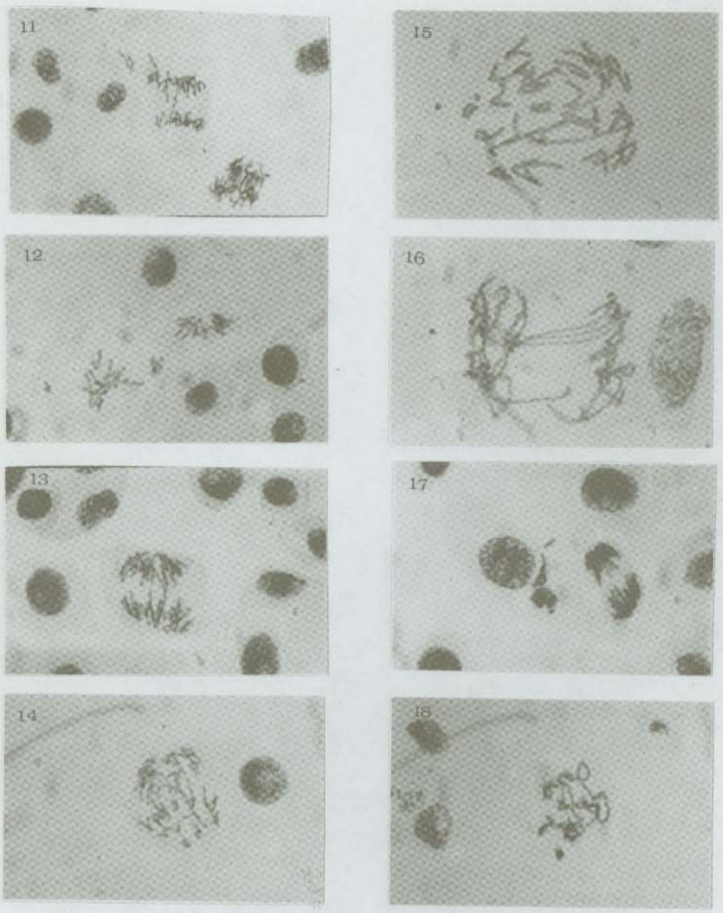

Plate 2. Effect of different *Fusarium spp.* metabolites on *A. cepa* root meristematic cells: (x=250).

(Fig. 11) of *F. solani*
(Fig. 12) of *F. equiseti*
(Fig. 13) of *F. solani*
(Fig. 14) of *F. equiseti*
(Fig. 15) of *F. solani*
(Fig. 16) of *F. moniliforum*
(Fig. 17) of *F. moniliforum*
(Fig. 18) of *F. equisete*

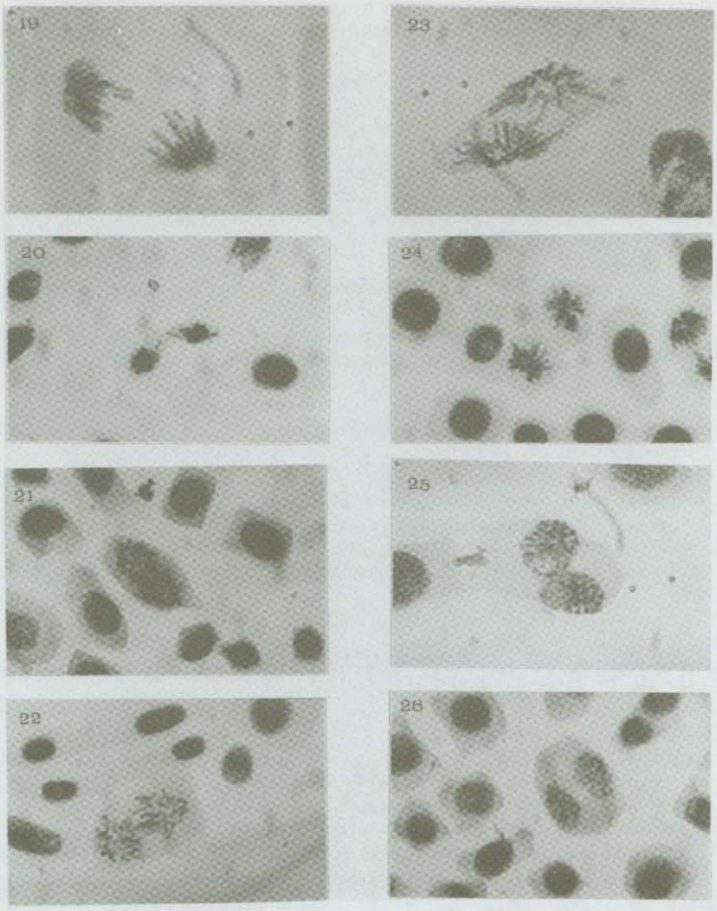

Plate 3. Effect of different *Fusarium spp.* metabolites on *A. cepa* root meristematic cells: (x=250).

(Fig. 19) of *F. solani*
(Fig. 20) of *F. solani*
(Fig. 21) of *F. culmorum*
(Fig. 22) of *F. moniliforum*
(Fig. 23) of *F. culmorum*
(Fig. 24) of *F. moniliforum*
(Fig. 25) of *F. solani*
(Fig. 26) of *F. culmorum*

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النشاط الخلوي الوراثي لنواتج أيض فطر الفيوزاريوم على نبات البصل (اليوم سيبا)

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ملخص البحث. تمّ فحص تأثير أربع سموم فطرية على الترتيب الصبغي وذلك خلال مراحل الانقسام الخلوي غير المباشر لنبات البصل. وقد دلّت النتائج على وجود بعض التغيرات في معدّل خطوات الانقسام غير المباشر وكذلك الاستدلال على وجود الشذوذ الصبغي والذي يعكس نشاط سُمي يُعزى إلى تعرض الأبصال المختبرة إلى أنواع الفطر الأربعة.

أعطت فطرة فيوزاريوم اكويزيتي أعلى معدّل شذوذ صبغي بالمقارنة بالسلوك الصبغي الطبيعي، استتبع بفطر فيوزاريوم سولاني، فيوزاريوم مونيليفورم، وفيوزاريوم كولومورم.