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EFFECT OF THE METABOLITE OF SEEDBORNE FUNGI OF BENGAL GRAM ON THE GERMINATION OF SEEDS AND GROWTH OF THE SEEDLINGS

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Per cent germination and the rate of germination of Bengal gram seeds and vigour index were considerably suppressed due to the metabolite of *Aspergillus flavus*, *A. niger* and *Fusarium moniliforme* in Richards solution. The length and dry weight of the seedlings faced the same fate. The rate of elongation of the radicles due to the metabolite was clearly sluggish. The mitotic index of the radicle tip was low. In all these cases the deleterious effect on the growth parameters was maximum due to *A. flavus* and minimum due to *F. moniliforme*. The diameter of the nucleus in the cells of radicle tip was more due to *A. niger* (9.78 µm), and less due to *F. moniliforme* (5.40 µm) than the control (8.81 µm) while due to *A. flavus* it was 7.62 µm. Considerable per cent of the radicle were curved and very short while negligible per cent were straight due to the metablite. The radicles produced by the control seeds were all straight.

Keywords: Bengal gram seed; Germination of seed; Growth of seedlings; Metabolite; Seedborne fungi.

Introduction

The deterioration of seeds due to storage fungi has been assessed in terms of loss in germination[1,2], retarded growth of the seedlings[3], high value of the electrical conductivity of the seed leachate and root exudate of the seedlings containing cations, anions, pentose and hexose sugars and amino acids[4-6], stimulated respiration of seeds and seedlings[7,8] and the activity of respiratory enzymes and oxidases[9], damage of the plasmamembrane[10] and ultrastructural changes[11,12]. Deficiency of photosynthetic pigments, total soluble sugars and total free amino acid were reported in the seedlings raised from mycodeteriorated seeds[8,13] besides sluggish activity of nitrate reductase and urease[9,14] and enzymes related with amino acid metabolism[9]. The present paper deals with soaking of Bengal gram seeds in metabolite of common seedborne fungi and observation of their germination and the growth of the seedlings and the related aspects.

Material and Methods

Preparation of the metabolite: Seedborne storage fungi of local variety of Bengal gram (*Cicer arietinum* L) were isolated adopting Blotter technique[15], and based on the highest frequency, *Aspergillus flavus* Link ex Fries, *A. niger* Van Tieghem and *Fusarium moniliforme* Sheldon were selected to observe the effect of their metabolite in Richards solution. The fungi were cultured on Czapek Dox Agar medium for 7 days at 28±1°C. 150 ml of Richards solution was autoclaved taking in conical flasks of 250 ml capacity. On cooling, two bits of the culture of the noted fungi were cut with sterilized cork borer of 4 mm diameter and transferred aseptically to Richards solution. The culture was incubated for 10 days at 28±1°C. After the expiry of incubation period, the mat produced as a result of fungal growth was removed and the metabolite was filtered on Whatman No. 1 filter paper using Buchner funnel. The filtered metabolite was centrifuged at 10,000 rpm for 10 min. This metabolite was used for soaking the seeds.

Soaking of the seeds: Two hundred seeds of Bengal gram, possessing 98% germinability, were surface sterilized with 0.1% mercuric chloride for 1 min and washed four times with autoclaved tap water and finally with distilled water. The water adherent to the seed was removed by soaking with sterilized dry blotting sheets. This seedlot was soaked in 80 ml of the metabolite of the fungi separately for 18 hr at 30°C maintaining the control of the seeds soaked in Richards solution only. The metabolite soaked seeds were set for germination in autoclaved moist blotters of 10 cm

diameter taking 10 seeds nearly at equal distance in twenty replicates. Moist blotters were incubated at 30°C for 5 days. The germination of seed was recorded (Table 1).

Calculation of the rate of germination: The rate of germination was calculated[16] as follows and recorded in Table 1.

$$\frac{A_1 + A_2 + \ldots\ldots\ldots\ldots + A_n}{A_1T_1 + A_2T_2 + \ldots\ldots + A_nT_n} \times 100$$

where A = number of seedlings emerged, T = Number of days after sowing corresponding to A. The suffix 1,2 etc. = Number of days.

Determination of vigour index: It was determined[17] by multiplying mean seedling length with mean germination percentage and noted in Table 1.

Estimation of length of the seedlings: For this purpose, the seeds soaked as above were grown in garden soil autoclaved at 20 psi for 15 min on two consecutive days. The soil was taken in earthen pots of 20 cm top diameter, 15 cm base diameter and 15 cm depth. 20 seeds each in five pots for one sp of the fungus were sown 1 cm deep in the soil and moistened lightly and covered with 5 mm thick sterilized cotton wool, and shifted in the garden. The cotton wool was removed after 6 days of sowing and the seedlings were raised for 15 days after the date of sowing and watering every alternate day lightly. The length of the seedlings was measured in mm (Table 2) after randomly taking out 10 seedlings from each pot cautiously to avoid damage of the root. The length was recorded as mean of 50 seedlings (10 seedlings each pot x 5 pots).

The radicle of the seeds germinated in moist blotter shows distinct abnormality, therefore, the rate of growth of the radicle, mitotic index in the cells of its tip and the size of the nucleus were measured. The total length in mm of the radicles emerged by 20 seeds after 5 days of setting for germination was first of all measured and the mean was recorded. The length was again measured every day for total period of next four days (Table 2). Radicles were categorised into per cent normal (straight), curved and extremely short based on 100 germinating seeds in triplicate in moist blotters. For observing mitotic index, the radicle tip of 20 germinating seeds were cut at 11.00 AM and fixed in aceto-alcohol (1 part glacial acetic acid + 3 parts absolute alcohol) for 12 hr and mitotic division was observed on softening and staining the tissue by warming in 2% acetocarmin and preparing smear. The dividing cells were counted besides the total number in five fields of high power (40x X 5x) of the compound microscope and the mean of 100 was recorded for each fungus and the control. The size of the nucleus in randomly focussed 25 resting cells was also measured as diameter in μm after camera lucida sketch (Table 3, Fig. 5).

Results and Discussion

It appears that the per cent germination of Bengal gram seeds, their rate of germination and vigour index were considerably suppressed due to soaking of the seeds in the metabolite of the fungi noted earlier. There is significant difference in the effect of storage fungi (Table 1, Fig. 1-3). The rate of elongation of the radicle due to the metabolite (Table 2, Fig. 4) indicates considerably slow growth which is again evinced in decreased length and dry weight of the seedlings and meagre value of mitotic index (Table 3). The data are statistically significant for the effect of the metabolite of these fungi. In all these cases, the adverse effect is caused to the maximum by *A. flavus* followed in succession by *A. niger* and *F. moniliforme*. The radicle of the normal seedlings produced by the control seed lot were straight while those produced by the seeds treated with the metabolite were observed considerably abnormal characterised by curved and extremely short ones (Table 3). Maximum per cent of the former type of radicles were due to *A. flavus* and latter type due to *A. niger*. It is surprising that the size of the diameter of the nuclei of the resting cells of the radicle tip was adversely affected. The minimum diameter (5.40μm) was due to *F. moniliforme* and maximum due to *A. niger* (9.78 μm). It was 7.62 μm due to the metabolite of *A. flavus* whereas the diameter of the nuclei of the control radicle tip was 8.81 μm (Table 3, Fig. 5).

The germination of the seeds of Bengal gram was observed adversely affected probably due to toxic metabolite produced in Richards solution. The suppression of seed germination has earlier been worked out by storage of crop seeds with seedborne fungi[2,18]. The observation of germination of the seeds due to the effect of storage fungi by incubating them with fungal spores as executed conventionally, is a bit time consuming and cumbersome process whereas the use of the metabolite as opted presently, seems easier and producing immediate result besides providing ample scope of detailed investigation of deterioration of seeds. The involvement of toxic principles produced by *Aspergillus ruber* in pea seeds has been held responsible in producing abnormal seedlings[19,20]. The notion of toxicity to the seed by storage fungi has also been confirmed earlier in case of lablab bean seed due to *A. niger*[21] and in paddy due to *Curvularia lunata* and *Memnoniella echinata*[22]. Also, ultrastructural change in wheat embryo due to storage fungi has been recorded[11].

The diminutive rate of germination of the seed in the present crop and the low vigour index, slow rate of

Table 1. Germination (%) and rate of germination of Bengal gram seeds treated with metabolite of seedborne fungi and vigour index of the seedlings.

Fungus spp	Germination (%)	Rate of germination	Vigour index
A. flavus	65	10.72	723.68
A. niger	76	11.63	1057.63
F. moniliforme	87	12.71	1521.34
Control	98	14.58	2196.77
CD value at 5%	10.432	1.355	193.923

* Figures were rounded to their whole number.

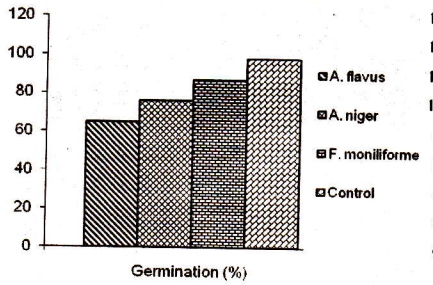

Fig.1. Germination (%)

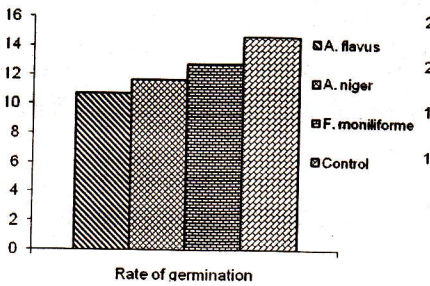

Fig.2. Rate of germination

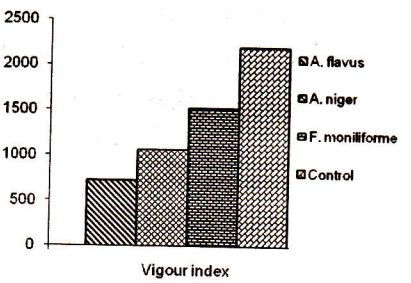

Fig.3. Vigour index

Table 2. Rate of growth of the radicle (measured in mm) of Bengal gram due to treatment of the seeds with metabolite of seedborne fungi for 8 days.

Fungus spp	Days after germination			
	5th	6th	7th	8th
A. flavus	3.2	4.3	5.6	6.4
A. niger	4.2	6.5	8.3	10.6
F. moniliforme	6.3	10.2	13.4	19.7
Control	11.6	16.7	27.9	38.8

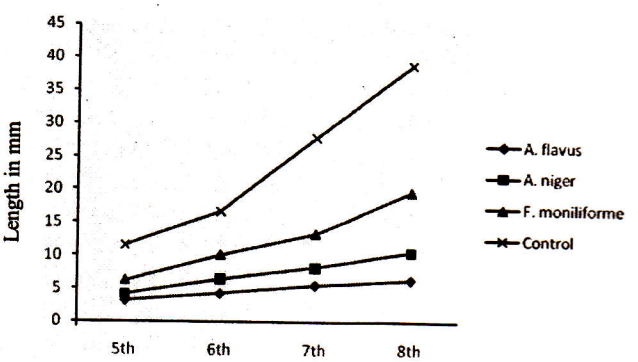

Fig.4.

Table 3. Length of the seedlings (in mm) of Bengal gram due to treatment of the seed with metabolite of seedborne fungi, abnormal seedlings (%), dry weight (in mg), mitotic index and diameter of the nuclei (in µm) in the radicle tip.

Particulars	Seedborne fungi				
	A. flavus	*A. niger*	*F. moniliforme*	Control	CD Value at 5%
Length of the seedlings	122.4	159.7	191.9	249.2	20.643
Abnormal seedling					
Straight	07	00	16	100	
Curved	36	32	20	000	
Extremely short	57	68	64	000	
Dry weight of seedlings	11.29	13.68	16.61	20.62	2.226
Mitotic index	41	50	59	87	
Diameter of nuclei	7.62	9.78	5.40	8.81	

growth of the radicle and other parameters opted for assessing the growth, point out the suppressive effect of the metabolite and this can be looked upon in the light of deleterious effect of the toxic principles secreted by the seedborne fungi. The assessment of the abnormal seedlings indicates that the effect of these fungi is highly toxic which is also reflected in the abnormal size of the nucleus, the explanation of which needs meticulous investigation.

The shortening of the seedlings might result due to suppressed cell division as evidenced by low mitotic division and adverse effect on other growth physiology besides excited activity of IAA oxidase lessening the quantity of IAA involved in cell division, as observed in lady's finger due to storage fungi[23]. The meagre dry weight of the seedlings might result due to their shortening and loss of cations, anions, pentose and hexose sugars and amino acids as exudate from the root[6] and deficient amount of photosynthetic pigments, soluble sugars and free amino acids as observed in maize and mustard in comparable condition[13]. Stimulated oxidase, decarboxylase and non-oxidative deaminase of amino acids and sluggish activity of nitrate reductase and urease in wheat seedlings[14] and enhanced O_2 uptake observed earlier in mustard seedling[8] due to *Aspergillus flavus* might happen in the seedlings of Bengal gram also deranging their physiology and biochemistry and rendering them sick. Extreme toxicity by the seedborne fungus is further manifested in utter cessation of expansion of cotyledonary leaf of mustard due to *Aspergillus flavus*[24,25]. The derangement in the physiology and biochemistry of the seedlings of crop plants due to storage fungi has recently been pointed out[25].

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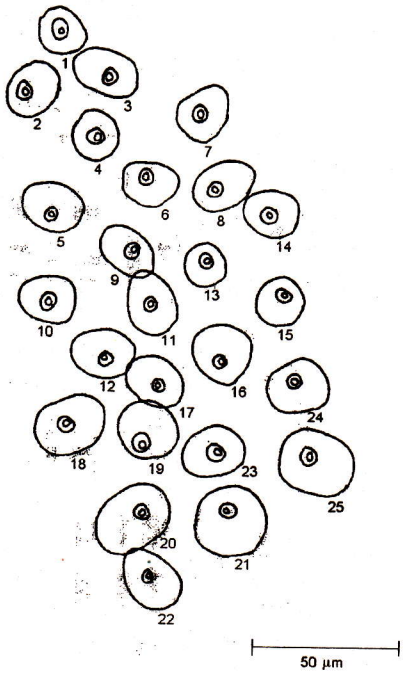
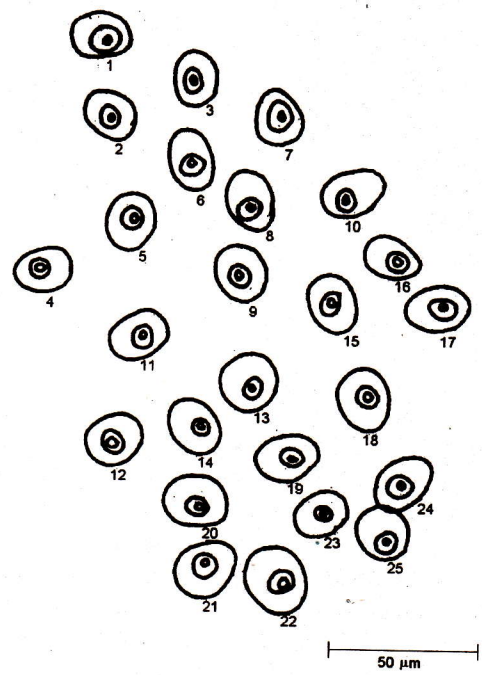
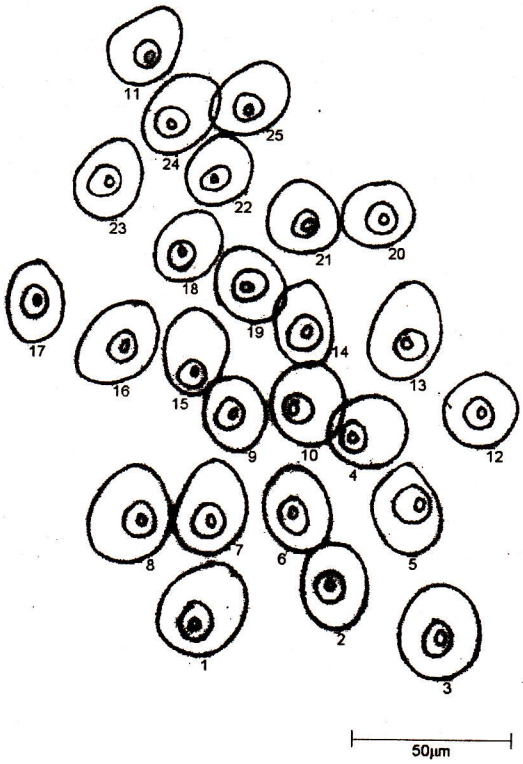
A. flavus



A. niger



F. moniliforme



Control

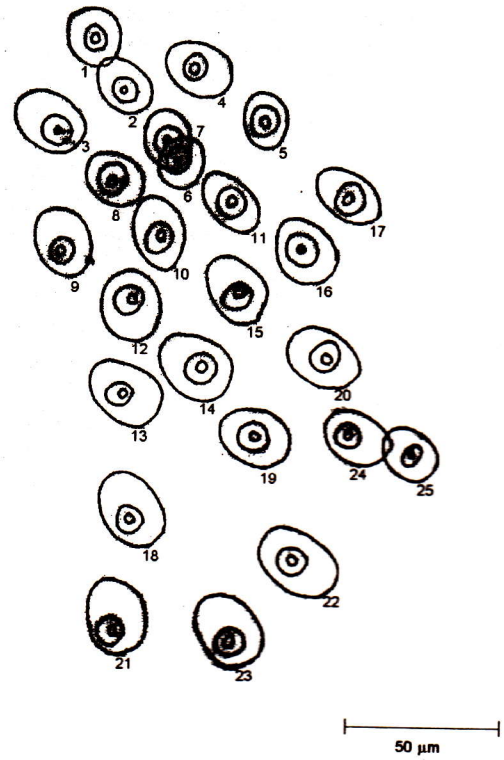


Fig. 5. Size of the nuclei due to the seedborne fungi and the control.

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