

EFFECT OF CARBON DISULPHIDE, ACRYLONITRILE AND FORMALDEHYDE ON EGYPTIAN PADDY GRAIN-BORNE FUNGI

By

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ABSTRACT

Fumigation, with various doses of carbon disulphide, acrylonitrile and formaldehyde for 8, 16 and 32 hours, of paddy grains indicate that formaldehyde was more toxic to the fungi than carbon disulphide and acrylonitrile. The most tolerant fungi to the three fumigants were *Alternaria alternata* and *Cladosporium cladosporioides*, in addition to *Epicoccum purpurascens* to acrylonitrile, and *Penicillium chrysogenum* and *E. purpurascens* to carbon disulphide. But, all the preceding fungi were eradicated at the highest doses of the three fumigants.

Formaldehyde and acrylonitrile seriously reduced the germinability of paddy grains, whereas carbon disulphide was less deleterious.

INTRODUCTION

Fumigation of seeds and grains has been extensively used to eliminate insects and mites. Also, several investigations have been carried out to control the fungi of paddy and rice using different forms of pesticides (Roy and Grewal, 1963; Kameswara, 1967; Kaul, 1973; Srinivasa, 1973; Kannaiyan *et al.*, 1977; Tyagi *et al.*, 1981; Bernaux, 1982).

In Egypt, there is no information about the influence of fumigants on Egyptian paddy grain-borne fungi. The present investigation focussed on the effect of three fumigants on germinability and paddy grain-borne fungi.

MATERIALS AND METHODS

Samples of Egyptian paddy grains (rice in the husk) of 1983 crop were collected from different Governorates in Egypt, characterized by rice cultivation, and carried to the laboratory and thoroughly mixed under aseptic conditions. The moisture content of mixed sample was 6.2% and the germinability was 98%.

Fumigation of paddy grains

Fifty grams of paddy grains were placed in 250 ml capacity conical flasks. Three fumigants were used and these were carbon disulphide (0.1, 0.4, 0.8, 1.6 and 2.4 ml per 250 ml container capacity), acrylonitrile (0.1, 0.4, 0.8 and 1.6 ml) and formaldehyde (0.01, 0.04, 0.08 and 0.16 ml). The fumigants were injected within the grains at the center of conical flasks. Immediately the mouths of the flasks were tightly stoppered with rubber bungs and secured by binding with "cellotape". Duplicate conical flasks were used for each dosage. They were incubated at 28°C for 8, 16 and 32 hours, thereafter, the rubber bungs were replaced by cotton wool plugs to allow the fumigant to escape. Isolation of the surviving fungi were made by transferring the fumigated grains to the surface of sterile glucose (10 g/l)-Czapek's agar + rose benghal (1/15000) as a bacteriostatic agent (Smith & Dawson, 1944). Five plates (4 grains for each plate) were used for each sample and the plates were incubated at 27°C for 7 - 10 days and the developing fungi were identified, counted and calculated per 20 grains.

The least significant difference (L.S.D.) was employed for statistical analysis of the results.

Germinability of paddy grains

The grains were incubated at 25°C over a pad moist sterile filter paper placed in a sterile Petri-dish for 7 days which the grains with healthy roots and plumules were counted and expressed as percentage.

RESULTS

A: Effect of fumigation on mycoflora of paddy grains (recovered at 28°C)

1. Effect of carbon disulphide

The results in Table 1 show clearly that the total count of fungi was significantly decreased with the increase of fumigants dose and exposure period until a nil value at 2.4 ml after 32 hours.

Aspergillus was the most common genus in the control sample. Its count was significantly reduced to half the control count at 0.1 and 0.4 ml after the three periods (8, 16 and 32 hours), but sharply declined at the remaining doses until completely eradicated after 16 and 32 hours at 2.4 ml. *A. niger* count was

Table 1

Counts (calculated per 20 grains) of common fungal genera and species (recovered at 28°C) of paddy grains after fumigation with various doses of carbon disulphide (0.1, 0.4, 0.8, 1.6 and 2.4 ml per 250 ml container capacity) for 8,16 and 32 hours.

Species	Control	Doses (ml)														
		0.1			0.4			0.8			1.6			2.4		
		Hours of exposure														
		8	16	32	8	16	32	8	16	32	8	16	32	8	16	32
Total count	46	27*	26*	26*	27*	26*	25*	24*	22*	16*	22*	15*	11*	19*	12*	0*
<i>Aspergillus</i>	12	6*	6*	6*	6*	6*	6*	3*	1*	1*	3*	2*	1*	1*	0*	0*
<i>A. niger</i>	5	4*	3*	4*	4*	4*	2*	1*	1*	1*	2*	2*	1*	1*	0*	0*
<i>A. flavus</i>	4	1*	1*	2*	1*	1*	2*	0*	0*	0*	1*	0*	0*	0*	0*	0*
<i>A. sydowi</i>	1	1	1	0*	0*	0*	1*	0*	0*	0*	0*	0*	0*	0*	0*	0*
<i>A. versicolor</i>	0	0	0	0	1 ⁰	1 ⁰	0	0	0	0	0	0	0	0	0	0
<i>Penicillium chrysogenum</i>	2	2	2	1*	2	1*	1*	1*	1*	1*	1*	1*	0*	1*	0*	0*
<i>Alternaria alternata</i>	9	5*	5*	4*	5*	4*	3*	4*	3*	2*	5*	2*	2*	4*	2*	0*
<i>Cladosporium cladosporioides</i>	6	4*	3*	3*	3*	2*	2*	2*	2*	2*	1*	2*	1*	2*	2*	0*
<i>Epicoccum purpurascens</i>	5	2*	0*	2*	3*	1*	3*	3*	4*	2*	3*	3*	1*	2*	2*	0*
<i>Fusarium oxysporum</i>	5	3*	3*	2*	3*	2*	2*	3*	2*	2*	1*	0*	1*	1*	0*	0*
<i>Acremonium strictum</i>	1	2 ⁰	1	3 ⁰	1	1	2 ⁰	0*	0	1	0*	0*	0*	0*	0*	0*
<i>Stachybotrys chartarum</i>	0	0	0	0	0	0	0	3 ⁰	1 ⁰	2 ⁰	1 ⁰	1 ⁰	3 ⁰	4 ⁰	3 ⁰	0
<i>Rhizopus stolonifer</i>	1	1	1	1	2 ⁰	0*	0*	2 ⁰	0*	1	0*	0*	0*	0*	0*	0*
<i>Trichoderma viride</i>	1	1	2 ⁰	0*	0*	3 ⁰	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*

N.B. * means significant decrease of count, comparable with the control count.

⁰ means significant increase of count, comparable with the control count.

significantly decreased at different treatments after all experimental periods, but the decrease did not show any regular trend. *A. flavus* count was significantly decreased at 0.1 and 0.4 ml. and completely eradicated, except at 1.6 ml after 8 hours, at the other treatments after all periods. *A. sydowi* was tolerant to the fumigant at 0.1 and 0.4 ml after 8 and 16 hours; and 32 hours respectively, whereas it was completely reduced at the remaining treatments. *A. versicolor* was absent in the control sample, but it was recovered in significant counts at 0.4 ml after 8 and 16 hours.

Penicillium chrysogenum was tolerant to CS₂ at 0.1 ml and 0.4 ml after 8 and 16 hours; and 8 hours, respectively, but it was significantly decreased or completely missed at the remaining treatments.

Alternaria alternata, *Cladosporium cladosporioides*, *Epicoccum purpurascens* and *Fusarium oxysporum* counts were significantly decreased or completely eradicated at all treatments after the three periods.

Acremonium strictum was significantly flourished at 0.1 ml after 8 and 16 hours and at 0.4 ml after 32 hours. At the higher doses (1.6 and 2.4 ml), it was completely eliminated after the three periods.

Stachybotrys chartarum was zero in the control sample, but it was significantly flourished at 0.8, 1.6 and 2.4 ml after the three experimental periods.

Rhizopus stolonifer and *Trichoderma viride* were significantly increased at two treatments (0.4 and 0.8 ml after 8 hours; and at 0.1 and 0.4 ml after 16 hours, respectively), but they were completely eradicated at almost the remaining treatments after the three periods.

2. Effect of acrylonitrile

Table 2 reveals that the total count of fungi was significantly decreased with the increase of acrylonitrile dose and period of exposure until equal zero at 1.6 ml after 32 hours.

Aspergillus count was sharply decreased at all treatments after all periods of exposure. *A. niger* and *A. flavus* were the most common *Aspergillus* species in the control sample, but their counts were significantly decreased at all treatments after the three experimental periods.

Penicillium chrysogenum was tolerant to acrylonitrile dose at 0.1 ml after 8 and 16 hours, but at the remaining doses (0.4, 0.8 and 1.6 ml) its count was significantly decreased or completely eradicated.

Cladosporium cladosporioides, *Alternaria alternata*, *Epicoccum purpurascens* and *Fusarium oxysporum* were common in the control sample, but their counts were

Table 2

Counts (calculated per 20 grains) of common fungal genera and species (recovered at 28°C) of paddy grains after fumigation with various doses of acrylonitrile (0.1, 0.4, 0.8 and 1.6 ml per 250 ml container) for 8, 16 and 32 hours.

Species	Control	Doses (ml)											
		0.1			0.4			0.8			1.6		
		Hours of exposure											
	8	16	32	8	16	32	8	16	32	8	16	32	
Total count	46	29*	28*	21*	25*	20*	9*	22*	18*	5*	16*	14*	0*
<i>Aspergillus</i>	12	4*	5*	5*	1*	3*	0*	3*	1*	2*	0*	1*	0*
<i>A. niger</i>	5	3*	3*	3*	0*	2*	0*	2*	1*	1*	0*	1*	0*
<i>A. flavus</i>	4	1*	0*	1*	1*	1*	0*	0*	0*	0*	0*	0*	0*
<i>Penicillium chrysogenum</i>	2	2	2	0*	0*	0*	0*	1*	0*	0*	1*	0*	0*
<i>Cladosporium cladosporioides</i>	6	4*	4*	3*	4*	3*	3*	4*	3*	1*	3*	2*	0*
<i>Alternaria alternata</i>	9	7*	4*	4*	6*	5*	0*	6*	5*	0*	3*	2*	0*
<i>Epicoccum purpurascens</i>	5	3*	2*	0*	3*	3*	0*	3*	2*	0*	3*	2*	0*
<i>Fusarium oxysporum</i>	5	4*	3*	0*	1*	2*	0*	1*	2*	0*	0*	2*	0*
<i>Stachybotrys chartarum</i>	0	0	0	1 ⁰	2 ⁰	0	1 ⁰	1 ⁰	1 ⁰	0	2 ⁰	1 ⁰	0
<i>Drechslera halodes</i>	0	0	1 ⁰	0	1 ⁰	0	0	0	1 ⁰	0	1 ⁰	0	0

N.B. * means significant decrease of count, comparable with the control count.

⁰ means significant increase of count, comparable with the control count.

significantly decreased or completely missed at all treatments after the three periods of exposure.

Stachybotrys chartarum and *Drechslera halodes* were zero in the control sample, but their counts were significantly increased at 0.1 ml after 32 hours, 0.4 ml after 8 and 32 hours, and 0.8 and 1.6 ml after 8 and 16 hours; and at 0.1 and 0.8 ml after 16 hours and at 0.4 and 1.6 ml after 8 hours, respectively.

3. Effect of formaldehyde

The results in Table 3 clearly show that formaldehyde was very toxic to the associated fungi of paddy grains. Therefore, the numbers of genera and species collected from treated grains (3 genera and 4 species) were very low if compared with those obtained from untreated grains (10 genera and 18 species).

The total count of filamentous fungi was sharply decreased at all doses and after the three exposure periods and became zero at 0.04 ml after 32 hours and at 0.08 and 0.16 ml after 16 and 32 hours.

Aspergillus decreased sharply at all doses and after the three exposure periods and was completely eliminated at 0.04 ml after 16 hours, and 0.08 and 0.16 ml after 8 hours. *A. flavus* and *A. niger* were recovered with very low counts in two and one treatments (0.01 ml after 8 and 32 hours and 0.04 ml after 8 hours; and 0.01 ml after 8 and 16 hours, respectively) and they were completely eradicated at the remaining treatments after the three exposure periods.

Cladosporium cladosporioides and *Alternaria alternata* were sharply and significantly decreased at all doses after the three periods and were completely eliminated at 0.04 ml after 32 hours and 0.08 and 0.16 ml after 16 and 32 hours; and at all treatments after 16 and 32 hours, respectively.

B: Effect of fumigation on germination of paddy grains

The results in Table (4) show clearly that the eradication of paddy grain-borne fungi using formaldehyde, carbon disulphide and acrylonitrile was accompanied by reducing the viability of the grains. Formaldehyde seriously reduced the germinability of the grains with the increase of doses used (0.04 to 0.16 ml) and the mortality reached 100% at 0.04, 0.08 and 0.16 ml after 32 h. Acrylonitrile was also toxic to embryo of the grains at the high doses (0.4 to 1.6 ml) and complete mortality was obtained at 0.8 and 1.6 ml after 32 h. But, at the lowest dose (0.1 ml) slight loss of germinability (10%) was recorded after 32 h. Carbon disulphide was less deleterious to paddy grains viability at 0.1 to 1.6 ml after 32 h (4-14% mortality). But, at the highest dose (2.4 ml) the germinability was reduced to reach 44% after 32 h.

Table 3

Counts (calculated per 20 grains) of common fungal genera and species (recovered at 28°C) of paddy grains after fumigation with various doses of formaldehyde (0.01, 0.04, 0.08 and 0.16 ml per 250 ml container capacity) for 8, 16 and 32 hours.

Species	Control	Doses (ml)											
		0.01			0.04			0.08			0.16		
		Hours of exposure											
		8	16	32	8	16	32	8	16	32	8	16	32
Total count	46	6*	5*	2*	4*	1*	0*	3*	0*	0*	3*	0*	0*
<i>Aspergillus</i>	12	2*	1*	1*	1*	0*	0*	0*	0*	0*	0*	0*	0*
<i>A. flavus</i>	4	1*	0*	1*	1*	0*	0*	0*	0*	0*	0*	0*	0*
<i>A. niger</i>	5	1*	1*	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*
<i>Cladosporium cladosporioides</i>	6	1*	1*	1*	1*	1*	0*	1*	0*	0*	1*	0*	0*
<i>Alternaria alternata</i>	9	2*	0*	0*	1*	0*	0*	1*	0*	0*	1*	0*	0*

N.B. * means significant decrease of count, comparable with the control count.

Table 4

Effect of different doses of carbon disulphide, acrylonitrile and formaldehyde on percentage germination of paddy grains after 8, 16 and 32 hours.

Fumigant	Doses ml/250 ml container capacity	Hours of fumigation		
		8	16	32
Carbon disulphide	0.1	98	98	96
	0.4	96	94	94
	0.8	94	88	86
	1.6	94	86	86
	2.4	92	82	44
Acrylonitrile	0.1	98	96	90
	0.4	96	58	20
	0.8	96	39	0
	1.6	90	22	0
Formaldehyde	0.01	96	86	25
	0.04	92	32	0
	0.08	86	30	0
	0.16	32	8	0

Initial percentage germination 98%.

DISCUSSION

Parasitic or saprophytic fungi may get into the flower and become attached to the surfaces of seeds and grains as external contaminants or may penetrate into their external covers inducing "transitional infections" (Gaumann, 1950). These fungi interfere with the viability of the embryos only under favourable conditions (Christensen and Kaufman, 1965) and some fungi could seriously damage the grains under the proper environmental conditions. Therefore, in the present investigation three fumigants were used to eliminate the paddy grain-borne fungi. Fungi and viability of the grains exhibited various degrees of sensitivity to the three fumigants. Formaldehyde was generally more toxic to the paddy grains and the grain-borne fungi than carbon disulphide or acrylonitrile and this is in agreement with the results obtained by Moubasher *et al* (1974). Also, Kaul (1973), Srinivasa (1973), Das and Chandrika (1974), Chiu and Liu (1975) and Roy and Jana (1976) found that the pesticides reduced the germination of paddy grains. Among the fungi infesting paddy grains, *Aspergillus niger*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum purpurascens* and *Fusarium oxysporum* could survive the high dose (1.6 ml) of carbon disulphide and acrylonitrile, respectively, for 32 and 16 h; none was tolerant to the low dose of formalin (0.04 ml). Therefore, carbon disulphide and acrylonitrile cannot be recommended as an adequate

fumigant. On the other hand, formaldehyde when applied at the low dose (0.01 ml) for 8 h, could eradicate about 90% of paddy grain-borne fungi and appeared without inducing any deleterious effect on the viability of grains.

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تأثير ثنائي كبريتيد الكربون ، الاكريلونيتريل والفورمالدهيد على الفطريات المحمولة على حبوب الأرز المصري

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أظهرت دراسة التدخين على الفطريات المحمولة على حبوب الأرز بتركيزات مختلفة من ثنائي كبريتيد الكربون ، الاكريلونيتريل والفورمالدهيد لمدة ٨ ، ١٦ ، ٣٢ ساعة على التوالي أن الفورمالدهيد كان أكثر سمية على الفطريات المحمولة على حبوب الأرز من ثنائي كبريتيد الكربون والاكريلونيتريل . ولقد كانت أكثر الفطريات مقاومة للمركبات المستخدمة في التدخين هي الترناريا الترنااتا ، كلادوسبوريم كلادوسبورويدز بالاضافة إلى الايبكوكم بيرابايوسينس في حالة مركب الاكريلونيتريل والبنسليوم كريزوجيتم والايكوكم بيرابايوسينس في حالة مركب ثنائي كبريتيد الكربون . ولقد تأثرت بوضوح كل الفطريات الباقية باستخدام تركيزات مرتفعة للمركبات الثلاثة . ولقد ثبت أن الفورمالدهيد الاكريلونيتريل يؤثر بوضوح على قدرة حبوب الأرز على الأنبات بينما في حالة ثنائي كبريتيد الكربون يكون التأثير أقل .