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Toxicity of Agricultural Dust Extract Using Microtox™

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Single acute toxicity laboratory tests help evaluate the nature and degree of harmful effects produced on living species by toxicants. Thus, these tests provide necessary information for implementing environmental protection. Due to the increasing concern regarding acute and chronic effects of an ever-increasing number of chemicals in the environment, there is a need for a simple, inexpensive, rapid and sensitive test for toxicity screening. The use of microorganisms, particularly bacteria, as the sensor organism offers an opportunity to meet these challenges. Of all of the available the bacterial test systems, Microtox™ is the most popular because of its rapidity, sensitivity, reproducibility as well as being cost effective (Kwan and Dutka, 1990). No studies have been conducted to determine the toxicity end point of the agricultural dusts, which are extremely complex in nature. In addition to organic and inorganic matter, grain dusts are also harbour microorganisms. A large quantity of dust is generated into the environment from grain processing industries when converting agricultural commodities into an edible form for human consumption thus causing a potential health risk to workers due to inhalation. There are few reports, which deal with exposure to airborne aflatoxin through inhalation (Karen and Cole, 1993; Nigam et al. 1994; Wilson et al. 1990). Residue levels of airborne aflatoxin were detected from agricultural dust samples by various workers (Burg and Shotwall, 1984; Burg et al. 1981) and reported that the respiratory system's pneumocyte like the hepatocytes was capable of metabolizing aflatoxin B_1 (AFB_1) to its ultimate carcinogenic form. Too, cases of pulmonary interstitial fibrosis have been reported due to occupational exposure to aflatoxin (Dvorachova and Pichova, 1986).

Reports are available on the presence of aflatoxigenic strains of *Aspergillus flavus* in the dust samples of rice mill from this subcontinent (Ghosh et al. 1977; Desai and Ghosh 1989). Thus, the present investigation has been designed to determine the toxicity of airborne vegetable dust, to compare it with the toxicity of aflatoxin and to determine if the toxicity is caused due to presence of alfatoxin.

MATERIALS AND METHODS

The study was carried out in two different grain processing industries – one was

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rice mill located at Bawla town situated about 35 kms away from Ahmedabad city and the other was a maize processing plant situated within the city. Three sample sites were selected in the rice mill – work place, store and office (control). Six different sites were selected from the maize processing plant. including Loading-unloading facility, elevator, oil mill, starch processing, store and office (control).

Dust samples were collected from the breathing zone of the workers using a high volume cone sampler at a flow rate of 28 L/min for 2 hrs. as per the instruction of the manufacturer. Samples were collected from the working environment. At least 5 samples were collected from each site except for the control sites where the number was increased to obtain desirable quantity of dust for extraction. Dust concentrations were measured by gravimetric method. After collection, dust samples were extracted with dimethyl sulphoxide (DMSO) in the ratio of 1:10 (W/V) as described by Kwan and Dutka (1990) for a sediment extraction procedure with slight modification. Instead of each sample, pooled sample (1g each time) collected from each site was processed for extraction.

Toxicity screening of the sample was carried out by using a Microtox^{TM} analyzer (Beckman Inc., Carlsbad, CA). The instrument is equipped with a 15 well temperature controlled incubator and a reaction chamber. The basic procedure for this approach employs duplicates of a non-toxic control (reagent blank) and four serial dilutions of original sample (45.0, 22.5, 11.3 and 5.6% concentration in the final assay). Reagent blank is used to normalize the duplicate responses of the four test concentrations of sample when the test results are reduced. A portion of (10μL) the rehydrated luminescent bacteria, *Photobacterium phosphoreum* was placed into the cuvette that contained 0.5 mL Microtox diluent equilibrated to 15°C. For initial light output measurement, each of the cuvette was cycled three times through the turret in ascending order of diluted sample. Aliquots (0.5 mL) of sample and control were then added to individual cuvette and light output were measured after the incubation periods. Blank was run simultaneously.

Data reduction was worked out using a Sharp Model (Japan) EL-5100 pre-loaded programmable calculator supplied by the manufacturer. The normalized gamma (Γ) effect was calculated using following formula:

$$\Gamma\,(t,T)^{*} = \frac{\text{Mean blank ratio (Rt)} \times \text{Initial light reading at zero time (I(0)}}{\text{Final light reading for the corresponding test cuvette at time (I(t)}} \quad -1$$

* t = exposure time, T= temperature

RESULTS AND DISCUSSION

Tables 1 and 2 show the environmental total dust concentrations in rice and maize processing industries. It was found that DMSO extracts of the samples elicited either of the three types of responses (Table 3). Control samples of rice and maize processing industries did not show adverse effects on the sensor organism, *Photobacterium phosphoreum,* while some of the sample extracts obtained from

Table 1. Gravimetric analysis of airborne total dust concentration in different sites of a rice mill (Mean ± SEM).

Site	Dust concentration (mg/m^3)
Work place	78.6±11.0**
Store	1.6±0.2*
Office (Control)	0.6±0.1

** $p<0.01$ in comparison to control * $p<0.05$ in comparison to control

Table 2. Gravimetric analysis of airborne total dust concentrations in different sites of a maize processing plant (Mean ± SEM).

Site	Dust concentration (mg/m^3)
Elevator	72.4±2.9**
Starch Processing	31.9±1.7**
Loading – Unloading Facility	30.1±3.5**
Oil Mill	3.0±0.2**
Store	1.9±0.3
Office (Control)	1.0±0.1

** $p<0.01$ in comparison to control

departments in the work place and store of the rice mill, and store, loading-unloading facility and starch processing of the maize processing plant demonstrated only slight inhibition in the light output of the glowing bacteria (Table 3A). Since the response was confined only to Δ% decrement, EC_{50} values of these samples could not be determined. Therefore, these samples were regarded as less toxic. On the other hand, extracts of dust samples obtained from the sites of elevator and oil mill from the maize processing plant caused significant reduction in the luminescence, which enabled the quantitation of EC_{50} values. This category is regarded as the toxic group.

EC_{50} values of these two dust extracts (elevator and oil mill) showed variations in toxicity manifestation on the bacteria at different temperatures and incubation periods (Tables 4 and 5). Variation in temperature caused considerable differences in the toxicity expression for each of dust sample (Table 4) and a sharp increase was noticed when the incubating temperature was increased from 10°C to 15°C. The increase was less between 15°C and 20°C. In the case of elevator dust, a marked increase was noticed after 5 min. incubation period. A more pronounced increment was observed with the dust extract from the oil mill at a 5 min. incubation period from 10°C (390 μg/mL) to 15°C (550 μg/mL); afterwards the increase was less. Tables 4 and 5 also show that the EC_{50} of dust samples was time dependant and at each incubation temperature, the increase in the EC_{50} values increased with increasing incubation period.

However, the increase between 15 and 30 min. of incubation period was not so remarkable as that observed between 5 and 15 min. incubation time. Between the

Table 3. Microtox response to dust extract.

Degree of toxicity	Microtox response	Type of dust extract
None (non toxic)	Sensor organism, *Photobacterium phosphoreum* did not show any alteration in its light output	Dust samples collected from control sites of rice and maize processing plants.
Slight (less toxic)	Decreased luminescence by <50% (only Δ% decreased, not possible to detect EC_{50}).	Dust samples collected from the sites of work place and store of rice mill and from store, loading – unloading and starch of maize processing plants.
Significant (toxic)	Luminescence decreased by >50% (significant reduction in Δ% possible to determine (EC_{50}),	Dust samples of elevator and oil mill of maize processing plant

Table 3A. Luminescence produced by the quantity of dust wood for extraction (Mean ± SEM)

Site of dust collection	Luminescence			
	Dust amount (μg)			
Oil Mill [1]	8.3±2.7	17.2±5.3	38.9±8.9	53.4±7.1
Elevator [1]	5.7±0.8	14.3±2.1	36.8±3.2	67.0±8.5
Loading Unloading facility [1]	52.9±4.1	64.0±3.9	67.8±4.6	68.8±4.0
Starch processing [1]	39.7±6.1	46.4±7.3	54.3±5.8	63.3±8.8
Store [1]	35.3±4.7	47.0±9.2	50.9±8.6	59.4±9.7
Work place [2]	46.5±7.3	53.3±6.2	61.4±9.1	66.1±5.0
Store [2]	38.5±4.9	46.0±5.2	57.9±8.2	68.2±7.2

1 = Maize processing plant, 2 = Rice mill

two types of dust extracts, effects were more pronounced with the oil mill dust at 15 min. incubation time. The increment was 540 μg/mL at 10°C to 650 μg/mL at 20°C.

Table 6 shows the EC_{50} values of AFB_1 at different temperatures at different incubation times. It is seen that the EC_{50} value of AFB_1 varied inversely with increasing temperature, but the EC_{50} values increased progressively with the increase of incubation time. At 15 min. of incubation the EC_{50} values of AFB_1 were 21.8 μg/ml at 10°C, 18.0 μg/mL at 15°C and 16.5 μg/mL at 20°C. Table 7 shows the EC_{50} values of dust extracts collected from the elevator and oil mill sites, and AFB_1 at 15°C and 15 min. incubation time. The EC_{50} value of dust samples obtained from the elevator site was 580 μg/mL while that of the oil mill was 600 μg/mL. The EC_{50} value of AFB_1 was 18.0 μg/mL (Fig. 1).

Table 4. Effect of temperature and incubation period on EC_{50} values (μg/mL) of environmental dust extract obtained from the site of the elevator of a maize processing plant (Mean ± SEM).

Temperature (°C)	Incubation time (min)		
	5	15	30
10	350±14.7	470±13.4	520±9.3
15	490±7.3	580±3.0	640±6.1
20	520±5.4	610±7.3	660±15.4

Table 5. Effect of temperature and incubation period on EC_{50} values (μg/mL) of environmental dust extract obtained from the site of oil mill of maize processing plant (Mean ± SEM).

Temperature (°C)	Incubation time (min)		
	5	15	30
10	390±10.2	540±11.4	560±10.6
15	550±17.8	600±15.4	620±14.7
20	590±12.7	650±16.3	680±8.2

Table 6. Effect of temperature and incubation period on EC_{50} values (μg/ml) of AFB_1 (Mean ± SEM)

Temperature (°C)	Incubation time (min)		
	5	15	30
10	19.1±0.2	21.6±0.2	22.0±0.5
15	17.8±0.5	18.0±0.4	19.3±0.4
20	16.1±0.1	16.4±0.2	17.3±0.2

Among the two factors temperature and incubation period, temperature played an important role in the toxicity expression for dust extract and AFB_1. An increase of temperature during the same incubation time caused a highly significant difference in EC_{50} values ($p<0.01$). On the contrary, a change of incubation period did not show any significant changes in AFB_1 at 10°C (between 15 min. and 30 min. incubation period), and at 15°C and 20°C (between 5 min and 15 min.). In other Mycotoxins including AFB1 analysed by them required a unique cases, it was found to be highly significant ($p<0.01$) except for oil mill dust exposed at 15°C between 5 min. and 15 min. ($p<0.05$) and AFB_1 at 20°C between 5 min. and 30 min ($p<0.05$).In this investigation, the results of AFB_1 agree closely with the results obtained by Yates and Porter (1984). combination of physical conditions like pH, temperature and incubation period to determine toxicity. Temperature and incubation period were regarded as the most important physical factors, which affected maximum in the toxicity expression of any chemical. However, in the case of AFB_1, temperature was the primary factor contributing maximally in its toxicity expression as observed in this study. Increasing the period of incubation did not significantly alter the EC_{50} values of AFB_1. On the contrary,

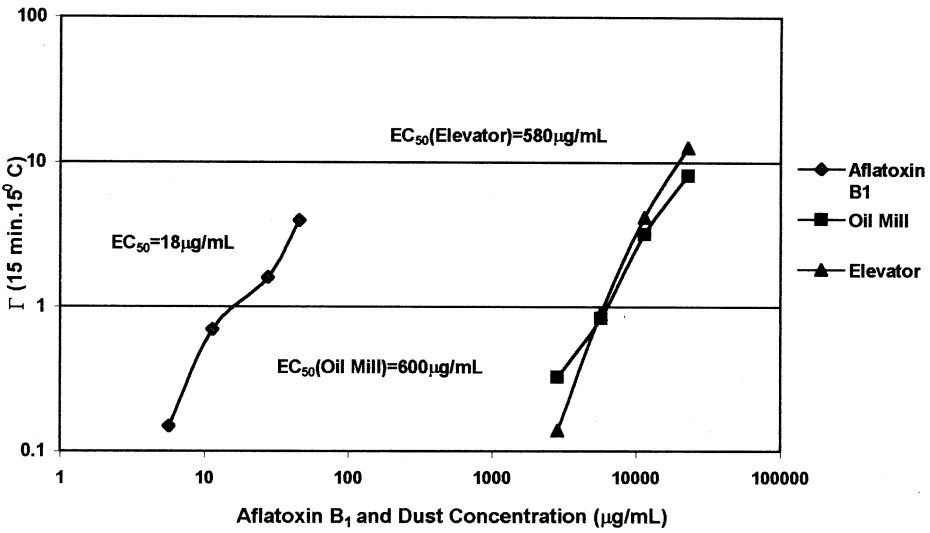

Figure 1. EC_{50} values ($T_{15°C}$ $t_{15\ min}$) of AFB_1 and dusts.

Table 7. EC_{50} values of dust and AFB_1 at optimum temperature and incubation period ($T_{15°C}t_{15min}$) (Mean ± SEM)

Sample	EC_{50} values (μg/ml)
Elevator dust extract	580±3.0
Oil mill dust extract	600±15.4
AFB_1	18±0.4

the toxicity expression of dust samples were dependent on both temperature and incubation time. This differential kinetic behavior between the dust samples and AFB_1 cannot be explained. The environmental dust samples of both the sites responded in similar manner. It is well known that vegetable dust contains various types of organic and inorganic matters. Besides mycotoxins, lectins, endotoxins etc. are also present. Therefore, further work on this line only can bring out the useful information, which is required for drawing a conclusion whether the effect is additive, synergistic or antagonistic in nature or if it is caused due to some other agent. However, this is the first report of application of Microtox™ for toxicity estimation of agricultural dusts.

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