carbonatization. However, studies on the analyses of gonnardites from other places reveal that gonnardite of Korati is deficient in alumina and soda with excessive silica [Number of ions on the anhydrous basis of 80 (0)], deserves an explanation. After the emplacement of carbonatites, the percolation of postmagmatic solution caused the enrichment of acidic components which stipulated the formation of silicarich gonnardites and subsequent increase in alkalinity caused the precipitation of calcites. This feature again points out the improbability of the presence of extensive fenitization at the contacts of Tirupattur carbonatites and alkaline rocks.

October 27, 1980.


**MYCOTOXINS IN STORED RICE**

S. GANGOPADHYAY AND N. K. CHAKRABARTI
Central Rice Research Institute,
Cutack 753006, India

Over the past 20 years, a number of mycotoxins have been detected in cereal grains. Cereals consumed directly or used as ingredients must meet higher standards regarding microbial quality and must be free of
mycotoxins if a safe and wholesome food is to be consumed. Karki\textsuperscript{6} evaluated 17 cereal samples from Nepal and pointed out aflatoxin contamination in raw and parboiled rice. Christensen\textsuperscript{7} described a wide variety of fungi that invade cereal grains from field fungi, storage fungi and advanced decay fungi. More than 10,000 tons of rice imported into Japan during 1947–1954 were found unfit for human consumption because of fungal damage.

In the eastern part of India single and double parboiled rice are consumed by the majority of the people, whereas, in northern India raw rice is preferred. Due to faulty drying and improper storage, a large quantity of rice mixed with discoloured grains are observed. Accordingly a study was initiated to acquire the information on the presence of toxin producing fungi rice on samples collected from villages in Cuttack and Puri districts of Orissa.

Eight month old, raw, single and double parboiled rice samples were collected from different villages of Cuttack and Puri districts of Orissa namely, Biritati, Alapur, Sharpara, Gobindapur, Kantapara, Bhadimal and Cuttack College Square markets. Moisture content was read in OSW automatic universal moisture meter with 30 g rice sample. After surface sterilization with 1\% sodium hypochlorite for 5 min, 100 rice grains from each locality sample were put on Czapec\'s Dox Agar and incubated for 10 days at 25°C.

As the sample obtained from the village Kantapara of Puri district yielded high degree of Aspergillus flavus and Fusarium moniliforme, only 500 g of Kantapara rice were dried to 12\% moisture level, autoclaved for 25 minutes and the moisture level was increased to 22-25\% by adding spore suspension of A. flavus at 80,000 spores/ml sterilised water and incubated in glass stoppered conical flasks, for prevention of moisture evaporation for 15 days by vigorous hand shaking 3 times a day.

Inoculated rice was blended for 5 min in waring blender with sterilized distilled water. The aqueous slurry was acidified to pH 5-7 with 6 N HCl. After standing for 20 minutes, acetonitrile and chloroform (1:6) were added to the aqueous slurry which was mixed for an additional 5 min, filtered through Whatman No. 1 and No. 42 filter papers, the protein was precipitated with anhydrous sodium sulphate and partitioned with ice-chilled acetone following the method of Sorenson et al.\textsuperscript{11}. Partial purification was done by passing through 5 x 200 mm column having alumina c.e. (20 mm), silica gel c.e. (20 mm), washed sand (20 mm), amberlite (20 mm), quartz sand (20 mm) and glass wool (20 mm) following the method described in the first action of the Association of Official Analytical Chemists.\textsuperscript{3} Quantities of aflatoxin present in the extracts were determined on thin layer chromatographic plates coated with 0.5 mm silica gel. The plates were run in the closed glass chamber at 36°C in the solvent system of chloroform: acetone : water (88:12:1.5 V/V) and the spots were read under UV lamp. The zones were scrapped, diluted with 5 ml distilled water, measured in UV spectrophotometer at 325 nm emission and compared with pure B\textsubscript{1} aflatoxin.\textsuperscript{6}

Kantapara rice samples were similarly inoculated with F. moniliforme conidia (50,000 conidia/ml water). The crude extract was prepared following the method of Schroeder et al.\textsuperscript{8} using hexane and diethyl-ether for partitioning. The bulk extracts were absorbed on silica gel (particle size 0.05-0.20 mm) in 25 mm x 10 cm column and eluted first with hexane followed by diethyl-ether. The diethyl fraction (containing toxin) was again passed through silica gel column (particle size 0.063 mm) and washed with 3\% chilled acetone in chloroform. The eluted fractions were evaporated to dryness, and dissolved in methylene chloride. Hexane was added to the incipient precipitation points and allowed to crystallize. The extract was compared by t.l.c. method as stated earlier with Rf value of authentic zearalenone.

One month old female Belgium white rabbits, 1.5-5 kg in body wt, were injected (intravenous) with the help of borosilicate hypodermic syringe, needle No. 26 in the ear with 2 ml N/100 sterile saline solution having aflatoxin B\textsubscript{1} of about 50 ppm and 2 ml N/100 sterile saline solution having Fusarium toxin of about 50 ppm zearalenone equivalent. The animals were fed with 150 g Bengal gram and green grass daily. Similar control set was maintained without toxin treatment. After 4 days the animals were kept without food for 24 hr and bled from the ear vein and differential counts were taken from blood slides.

Double parboiled rice contained more moisture (20.5 to 22.5\%) than those of single parboiled (13.5–19.5\%) and raw rice (12.3-17.5\%). A. flavus and F. moniliforme isolated were (a) 22-4-29.2, and 23.8-32.5\%, (b) 17.9-24.5\% and 22.5-30.8\%, (c) 12.9-19.5 and 19.8-30.5\% from (i) double parboiled, (ii) single parboiled and (iii) raw rice samples respectively. Kantapara sample had moisture content of 22.5, 19.5 and 17.5 and A. flavus and F. moniliforme colonies of (a) 23.8 and 32.5\%, (b) 24.5 and 30.8\% and (c) 21.3 and 30.5\% in (i) double parboiled, (ii) single parboiled and (iii) raw rice respectively.

From the spectrophotometric study of rice infected with A. flavus presence of aflatoxin B\textsubscript{1} could be traced yielding peaks at UV absorption 265 nm and 363 nm and the absorption wave numbers were 13410 and 21608 respectively, and maximum fluorescence emission was 425 nm. Rf value on silica gel plates was 0.36,
TABLE I
Symptoms on rabbits after intravenous injection with aflatoxin and toxin from F. moniliforme and feeding rice contaminated with A. flavus and F. moniliforme

<table>
<thead>
<tr>
<th>Items</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Control fed with Bengal gram and green grass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Injected with*</td>
<td>Fed with**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aflatoxin</td>
<td>F. moniliforme toxin</td>
</tr>
<tr>
<td>Body wt. (kg)</td>
<td>1.5</td>
<td>1.485</td>
<td>1.389</td>
</tr>
<tr>
<td>Body temp. (C)</td>
<td>37.5</td>
<td>37.5</td>
<td>37.5</td>
</tr>
<tr>
<td>Heart beat</td>
<td>75</td>
<td>80</td>
<td>82</td>
</tr>
<tr>
<td>Other symptoms</td>
<td>Normal</td>
<td>Drooping of ears, dragging of hind legs, normal running nose and eyes in all the treated ones.</td>
<td></td>
</tr>
</tbody>
</table>

Blood differential count

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>42.1</td>
<td>33.1</td>
<td>32.8</td>
<td>32.5</td>
<td>32.8</td>
<td>42.0</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>40.2</td>
<td>49.1</td>
<td>48.9</td>
<td>49.8</td>
<td>49.8</td>
<td>40.0</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>7.8</td>
<td>15.2</td>
<td>15.5</td>
<td>15.5</td>
<td>15.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Monocyte</td>
<td>1.5</td>
<td>1.1</td>
<td>1.2</td>
<td>1.0</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Basophil</td>
<td>0.8</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Stool

<table>
<thead>
<tr>
<th>Colour</th>
<th>Yellow</th>
<th>Black</th>
<th>Black</th>
<th>Black</th>
<th>Black</th>
<th>Yellow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>125 g</td>
<td>5 g</td>
<td>4.8 g</td>
<td>5 g</td>
<td>4.6 g</td>
<td>150 g</td>
</tr>
</tbody>
</table>

* 2 ml saline solution having 50 ppm toxins injected intravenous fed with 150 g Bengal gram and green grass.

** 150 g fed every day along with green grass.

Toxin extracted from rice contaminated with F. moniliforme yielded light yellow crystals. In the scanning spectrophotometer, the peaks were observed between 227 nm and 323 nm, absorptions were 47250 and 58358 and maximum fluorescence emission was 450 nm and Rf value on silica gel plates was 0.68. This unidentified toxin seemed to be very close to zearalenone though not exactly the same.

Rabbits injected with N/100 saline solution having aflatoxin B₁ and toxin isolated from F. moniliforme and rabbits fed for 30 days with rice contaminated with A. flavus and F. moniliforme (Table I) showed similar symptoms: decrease in body weight, increase in heart beat, increase in blood eosinophil, lymphocyte, drooping of ears followed by shot holes in both ears, dragging of hind legs, reduction in stool weight along with deep black colour were observed. Occurrence of black stool might be an indication of intestinal haemorrhage.

Rambo et al. explored the effects of moisture, temperature, and two species of the A. flavus group on the production of bright greenish yellow fluorescence on aflatoxin production in corn. Mirocha et al. have enumerated about estrogenic mycotoxin from Fusarium sp. on corn but no report is available about the toxin production from F. moniliforme on rice. This is the first report of production of toxin close to zearalenone by F. moniliforme in rice. Schroeder et al. isolated and identified zearalenone from contaminated grain sorghum. Kobayashi et al. observed fibrosis, bile duct hyperplasia and molecular hyperplasia of hepatic cells of rats fed with rice contaminated with Penicillium islandicum. Decrease in body weight, increase in eosinophil, lymphocyte in blood and black stool of rabbit after feeding rice contaminated with A. flavus and F. moniliforme provide additional information on the harmful effect of consuming contaminated rice. Further studies on toxin purification, etc., are in progress.

The authors are grateful to Ford Foundation for providing partial grants to carry out the investigations.
They are also thankful to Dr. H. K. Pande, Director, for his keen interest and encouragement, to Sri N. Mishra and Dr. S. K. Ghosh for assistance.

January 8, 1980.


EFFECT OF GIBBERELLCIC ACID SPRAYING ON BANANA FRUIT DEVELOPMENT

S. D. Misra, B. M. Desai and B. K. Gaur
Biology and Agriculture Division
Bhabha Atomic Research Centre, Trombay Bombay 400 085, India

In the recent past, crop yield has been maximized by employing appropriate genetic methods and increasing the agricultural inputs. Further increase in the crop yield may be possible by physiological manoeuvring in terms of application of plant growth regulators at an appropriate time and using effective concentrations. These chemicals, by controlling internal metabolism induce plant to produce more than it would under the best of conditions without them. Depending upon the time of application and concentration, gibberellic acid (GA) is able to produce an astonishing diversity of responses on the majority of crop plants. Schwabe and Goldwin reported increase in yield and size in apple and stone fruits. Similarly, a marked increase in fruit weight of Cucumis sativa has been observed by GA application directly to the fruit initials. The present paper deals with the response of intact banana (Musa paradisiaca) fruits of different ages to GA application.

Two fruit bunches (35 day and 55 day old from the date of fruit initiation) were selected on two different plants. The pendant of these bunches was removed. The individual bunches were divided into two equal longitudinal halves with the help of a glazed cardboard paper. While one side of the bunch was sprayed on alternate days with GA3 (10-4 M) solution, the other side (control) was sprayed with distilled water. This technique offered the advantage of having fingers of about the same chronological age on the two sides. Tween-20 (0.1% V/V) was used as surfactant. Spraying was repeated thrice. After final spraying, the fingers on the treated side were marked and the separator removed. Forty-five days after the first spraying, the bunches were detached from the plant and data on weight and volume of individual fingers were recorded. Mean of five representative fingers, in each of the three hands, viz., 1st, 3rd and 5th and an overall grand mean of about 50 fingers per treated bunch are presented in Table I and Fig. 1 respectively.

An examination of response to the hormonal spray, given in Fig. 1, clearly indicates the GA-induced improvement of weight and volume per finger in both young and old bunches. While, the increase in weight was more than in volume in the fruits of same age, the younger bunch respond more favourably than the old bunch. It is evident from Table I that the weight of the fingers from the first hand of 35 day old bunch increased most favourably followed by that from third and fifth hands. There was an inverse relationship between weight and age of the fingers in 55 day old bunch. The maximal response to GA observed in the first hand of 35 day old bunch and also in the fifth one of the 55 day old bunch may be attributed to the closeness in their chronological ages. Thus, younger the fruits better was the response to GA treatment both inter- and intra-bunchwise.

Similar to our observations, wherein we have obtained increase in yield, Lockard has earlier reported increase in weight, volume and length of the finger by spraying