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Destruction of Toxic Fungi with Low Concentrations of Methyl Bromide

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Aspergillus parasiticus and *Penicillium rubrum* spores at the level of 10^4 to 10^5/g were completely killed by prolonged exposure to 30 to 45 mg of methyl bromide per liter.

Methyl bromide (CH_3Br) is widely used to control soil fungi (4) and insects in grains (3). No practical method to eliminate toxic fungi from crops exists. Therefore, it is of interest to explore the fungicidal effect of CH_3Br at concentrations within the established bromide residue tolerance for grains and oilseeds (1).

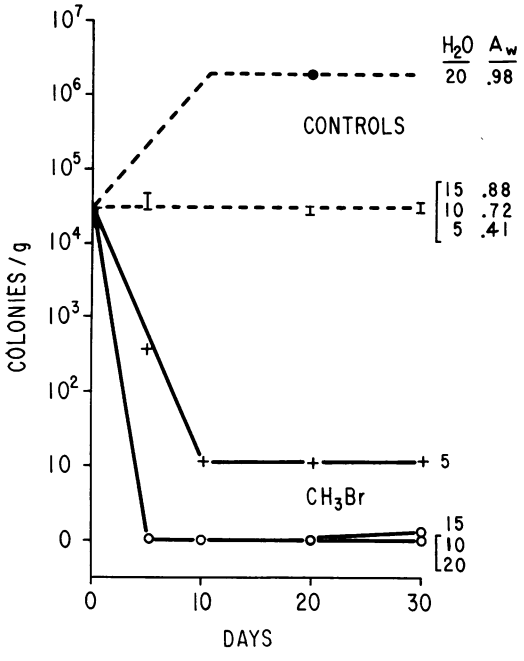

FIG. 1. *Destruction of Aspergillus parasiticus spores in 10-g rice samples by using 30 or 45 mg of CH_3Br per liter with identical results (solid line). Controls without CH_3Br are indicated by the dashed line.*

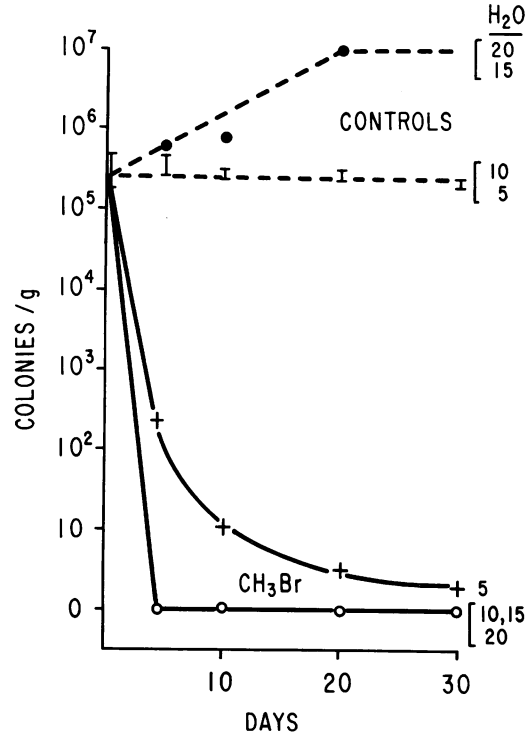

FIG. 2. *Destruction of Penicillum rubrum in 10-g rice samples by using 30 or 45 mg of CH_3Br per liter with identical results (solid line). Controls without CH_3Br are indicated by the dashed line.*

Aspergillus parasiticus (NRRL 2999) and *Penicillium rubrum* (NRRL 3290) spores were grown and also plated in triplicate on Difco Yeast Malt Extract Agar. Fungal spores and water were added to lyophilized rice to adjust the moisture content. The few naturally occurring fungi and bacteria in the rice never interfered with the plate counts. Inoculated rice samples were fumigated in quart-size mason jars at 28 C. CH_3Br was metered into the jars with a 10-ml Hamilton (Whittier, Calif.) back-fill and gas-tight syringe with ±5% accuracy. Water ac-

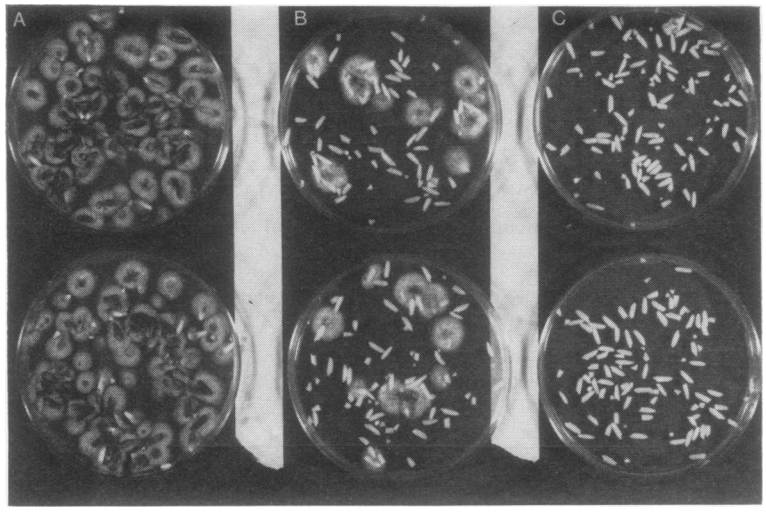

FIG. 3. *Plated rice samples from the 500-g lots (15% water and 9 × 10^4 Aspergillus parasiticus spores per g) which have been treated with (A) 0, (B) 30, or (C) 45 mg of CH_3Br per liter for 180 days.*

tivity (A_w) was determined by the method of Landrock and Proctor (2). Aflatoxins were extracted by the method of Yin (5) and detected on two-dimensional thin-layer chromatography with toluene-ethyl acetate-formic acid (5:4:1) as the first solvent. The plates were dried in a vacuum and then developed in chloroform-acetone (9:1) solvent.

Initially, 10 g of inoculated rice was treated with 0, 15, 30, and 45 mg of CH_3Br per liter. Except for a few fungal spores which persisted in the 5% moisture samples, $\sim 10^4$ *A. parasiticus* spores per g (Fig. 1) and $\sim 10^5$ *P. rubrum* spores per g (Fig. 2) were destroyed by 30 or 45 mg of CH_3Br per liter in 5 days. Figures 1 and 2 represent more than 10 separate control samples and 24 successfully treated samples, fumigated at various times, moistures, and CH_3Br concentrations. Aflatoxins were detected in the control sample with 20% moisture but not in the samples treated with CH_3Br.

Next, 500-g samples were tested. Bacteria were resistant to low concentrations of CH_3Br and spoiled the rice with 20% moisture. Bacteria did not grow in the rice with 15% moisture, and $\sim 10^5$ *A. parasiticus* spores per g were completely killed by 45 mg of CH_3Br per 500 g of rice after 180 days (Fig. 3). Aflatoxins B_1 and G_1 were detected in all of the 500-g samples and were shown to be carried by the spore inoculum. Aflatoxins were not destroyed by the CH_3Br treatment. Residual bromide analysis by Dow Chemical Co. showed that 90% of the CH_3Br added was recovered from the rice and confirmed the accuracy of the CH_3Br addition.

The above results showed that it is possible to eliminate fungi with low concentrations of CH_3Br, but more work is necessary to develop a practical system. Bacterial spoilage occurred at high moisture levels, and such high-moisture materials are best preserved by ensilaging. In contrast to the 10-g control samples, the spore count of the 500-g control sample (Fig. 3) decreased from $\sim 10^5$ to 400/g in 180 days. Thus, scaling-up may favor the destruction of fungi. Samples of three to six bushels can be tested in ordinary steel barrels.

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