Table 2. Accumulation ratio of Phosvel in adipose tissue of hens*

| Duration of dose (days) | Dose (mg/kg/day) 5 | 10 | 20 |
|---|---|---|---|
| 7 | 1.00 | 2.26 | 3.43 |
| 14 | 1.00 | 2.79 | 4.15 |
| 21 | 1.00 | 2.03 | 3.60 |
| 28 | 1.00 | 2.88 | 9.04** |

* Each value was calculated as the ratio of mean concentration of Phosvel found in fat in the same test period. ** The samples were taken at 26 days after the begining the test.

Although the reason is obscure, this finding may reflect the effect of death. No contamination occurred in fat of the control hens. The concentration of Phosvel in fat of 2 hens given 10 and 20 mg/kg for 25 and 28 days respectively was not estimated, because the birds were depleted of body fat. Except for the case dosed 20 mg/kg for 25 days, the figures of the accumulation ratio show that the level of Phosvel in fat was proportional to the size of the daily dose (table 2). Although this study was of relatively short duration, it clearly indicates that the organophosphorus pesticide, Phosvel, accumulates in the adipose tissue of hens after daily oral administration of small doses.

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## Effect of 3 polyphenolic compounds against ear-rot of corn incited by *Fusarium moniliforme* Sheld*

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*Summary.* Anti-*F. moniliforme* activity of 3 non-toxic polyphenolic compounds, viz. mangiferin, 1,3,6,7-tetrahydroxyxanthone, and 2,2',4-trihydroxybenzophenone, was evaluated. The mycotoxin-producing fungus incited ear-rot in sweet corn in fields and during storage. The test compounds provided different degrees of protection to sweet corn from the ingress of the fungus. Additionally, noteworthy postinfectional curative action was observed in the case of the trihydroxybenzophenone.

We have recently reported[1] the incidence of *Fusarium moniliforme* Sheld. (CMI-IMI 204057)-induced ear-rot of Indian corn (*Zea mays* L., sweet corn, local variety) while growing in the valley of the Ganges, in Varanasi District of Uttar Pradesh. A market survey in the area also revealed that about 30% of the corn, collected at the preharvest stage for table use, were badly infested with the fungus. 3 mycotoxins, viz. zearalenone, diacetoxyscirpenol, and T-2 toxin, were isolated and characterized from the diseased corn[1]. The presence and prevalence of the mycotoxin-producing fungus in sweet corn in this area could be a recurring phenomenon and is, therefore, a cause for alarm from a public health view point. Application of the conventional fungicides to control the pathogen would be risky, because sweet corn is consumed in semi-baked form, or as such, in substantial amounts, in India. This fact necessitated the search for non-toxic agents to control this food-destructive mold.

Phenolic substances have been reported[2] to offer general resistance for higher plants towards bacteria and fungi. We have recently reported[3,4] significant antifungal actions of mangiferin, a naturally occurring glucosylxanthone (1,3,6,7-tetrahydroxy-$C_2$-$\beta$-D-glucosylxanthone) and of 2,2',4-trihydroxybenzophenone against *F. oxysporum* f.sp. *carthami*, the causal organism for the wilting of safflower. The toxicity of the 2 compounds, in animal testing, was found to be very low[3,5]. It was therefore thought worthwhile to evaluate the potential of the 2 compounds, and also of a simpler analogue of mangiferin, viz. 1,3,6,7-tetrahydroxyxanthone, against *F. moniliforme*-induced ear-rot of corn. The details of these findings constitute the subject of this communication.

*Materials and methods.* At the early milk-stage of sweet corn, growing in a field in the valley of the Ganges in Varanasi (July 1977, temperature 33±8 °C, relative humidity 46–78%), a dense spore suspension (about $5 \times 10^5$/ml) of *F. moniliforme* Sheld. (CMI-IMI 204057) was uniformly sprayed over the silk and husks of the developing ears. After 3 days, the 3 test compounds ($1 \times 10^{-4}$ M), in aqueous

Table 1. Effect of polyphenolic compounds against ear-rot of corn incited by *Fusarium moniliforme* Sheld. in the fields

| Test compound | Symptoms |
|---|---|
| Mangiferin | [a] 4 kernels showed pinkish stain over the surface, bleached lesions (2–14 × 1–4 cm) upto 10th husk, grain formation normal. [b] Similar to those observed in the control. |
| 1,3,6,7-tetrahydroxy-xanthone | [a] Brownish discolouration of epicarp of 3 kernels, bleached lesions (2–6 × 1–2 cm) upto 4th husk, grain formation normal. [b] Tip turned black, lesions (4–6 × 1–2 cm) upto 10th husk, grain formation observed only at the basal portion. |
| 2,2',4-trihydroxy-benzophenone | [a] No sign of infection on grains, ears well developed. [b] Brownish discolouration of 3 kernels, lesions (2 × 1 cm) upto 4th husk, ears well developed. |
| Control | 10 kernels from the tip turned brown and shrunken, bleached lesions (2–14 × 1–5 cm) upto 14th husk, grain formation irregular and only partial. |

Test compound sprayed. [a] before inoculation; [b] after inoculation of the fungus.

sodium carbonate (2%) solution, were uniformly sprayed over the inoculated areas. In another set of experiments, the order of spraying of the fungal spores and of the test compounds was reversed. In the control, similar number of ears was sprayed only with the fungal spores. The cobs were then covered with polythene bags. The treated and the control ears were harvested after a 2-week period. For each experiment, 10 replicates were maintained.

To determine the effect of the 3 test compounds on the fungal growth in vitro, each of the test compounds ($1 \times 10^{-4}$ M) was taken in sterilized Richard's solution (50 ml in a 250 ml Erlenmeyer flask) to which the fungus was inoculated. The mixture was incubated (temperature 18 °C) for 7 days. The fungal mat was collected by filtration and its dry weight was noted. For each test compound, 5 replicates were maintained.

*Results and discussion.* The ears inoculated with the fungal spores showed the disease symptoms (table 1), within 72 h, similar to those observed in the natural infection. In the control, the pathogen first damaged the kernels at the tip and then proceeded downward and into the layers of the husks. Pretreatment of the ears with 2,2',4-trihydroxybenzophenone provided significant protection from the ingress of the fungal hyphae. Also, considerable recovery of the host from the fungal infection was observed when the benzophenone was sprayed 3 days after the spray of the fungal spores. Pretreatment of the ears with mangiferin also provided some degree of protection from the fungus invasion as was revealed from the considerably diminished number of infected kernels and from the normal formation of the grains. However, mangiferin did not produce any curative action of the infected grains or husks. The potency of antifungal action of 1,3,6,7-tetrahydroxyxanthone was of intermediate order of the other 2 test compounds (table 1).

In vitro experiments with the 3 test compounds resulted, in each case, in the lysis of hyphal cells and considerably reduced growth of the fungus. The results are recorded in table 2.

In view of the well-documented monoamine oxidase inhibitor activity of mangiferin[6], it would seem likely that this could be the mode of the antifungal action of the 3 test compounds against *F. moniliforme* Sheld. This study has assumed additional significance since toxicity of natural products from *Fusarium* has received wide attention in recent years[7-9] because of the greater incidence of the fusarial toxins and perhaps of their greater importance than aflatoxins[10].

Table 2. Effect of polyphenolic compounds on the growth of mycelium of *Fusarium moniliforme* Sheld

| Test compound | Dry weight and nature of fungal mat |
|---|---|
| Mangiferin | 0.348 g; lysis of the mycelia, the mat became black, the protoplast of the hyphae became contracted and collected in the centre or at the corner of the hyphal cells. |
| 1,3,6,7-tetrahydroxy-xanthone | 0.335 g; appreciable blackening of the mat and lysis of the hyphae. |
| 2,2',4-trihydroxy-benzophenone | 0.10 g; no mycelial growth upto 72 h, subsequently only feeble growth followed by lysis of the hyphae. |
| Control | 0.90 g; normal light pink coloured mycelia. |

* Part 9 in the series 'Toxic substances produced by *Fusarium*'. For part 8 see S. Ghosal, K. Biswas and B.K. Chattopadhyay, Phytochemistry *17*, 689 (1978).

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## Studies of the mechanism of action of the carotenoid crocetin

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*Summary.* The carotenoid crocetin was found to be taken up by normal rat muscle cells grown in vitro, and was found in the ribosomal-microsomal fraction, supporting the hypothesis that crocetin affects cell division enzymatic processes.

Crocetin is a carotenoid compound which appears to enhance oxygen diffusivity in vitro[2,3] and in vivo[4,5]. Based on several theories which support the hypothesis that deficiency of oxygen (hypoxia) is the initiator of diseases[6], the application of crocetin to several of these diseases has shown to be effective[7,8]. An example is the radio-sensitization of Walker 256 carcinoma in vivo as well as in vitro[9]. As tumor cells are known to be hypoxic and therefore radio resistant, by administering crocetin the tumor could be cured using lower radiation dosages[9,10]. Oxygen therapy also results in tumor cures at lower radiation dosages. Further, crocetin lowered the cholesterol and triglyceride level of plasma, and the severity of atherosclerotic lesions in the aorta of high-cholesterol diet fed rabbits[5,6]. The action of crocetin in this case may be due to 2 effects: 1stly, increasing oxygen transport and therefore decreasing the severity of the initiation of the sclerotic region, and 2ndly, presumably decreasing the cholesterol adsorption from the gastrointestinal tract. Increased oxygen environments have been shown previously to have the same effects on atherosclerosis in rabbits[5,6].

Crocetin is a carotenoid as is vitamin A, the precurser of ocular rhodopsin. Using rats as experimental animals, it has been found that the amount of ocular rhodopsin in the