Effects of citrinin, a mycotoxin, on behaviour of cockroach

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Citrinin, a secreted toxin obtained from cultures of *Penicillium citrinum* Thom. isolated from the pseudoscorpion *Oratemnus indicus* With. when injected intracoelomically into cockroach, brought about behavioural changes in preening, agility, posture, locomotion, ventilation and reversal reflex. It also induced tremor and ultimately killed the insect. Citrinin brings about these effects by acting on the central nervous system of the insect.

CITRININ is a potent mycotoxin produced by *Penicillium citrinum*, a hyphomycete fungus. It is a nephrotoxin and is known to cause kidney damage in mouse[1], rat and rabbit[2], pig[3], dog[4] and guinea pig[5]. However, investigations of the effects of mycotoxins, including citrinin, on invertebrates are meagre and have been carried out only to study their insecticidal properties[6,7]. We report here the effect of citrinin on some behavioural patterns of the common cockroach *Periplaneta americana*.

P. citrinum isolated from pseudoscorpion[8] (*Oratemnus indicus*) was grown in liquid Czapek-Dox medium (pH 6.5) for 14 days at $30 \pm 2°C$. Citrinin was precipitated from the culture filtrate by adding 3 N HCl and reducing the pH to 2. The yellow precipitate was shaken repeatedly with chloroform to extract all the citrinin. The extracts were pooled and evaporated to obtain yellow crystals of citrinin. The chemical nature and purity of citrinin were confirmed by TLC[9] and UV absorption data[10].

A preliminary study showed that a minimum dose of 5 μg of citrinin was necessary to bring about behavioural changes and death of cockroach.

Adult cockroaches, each weighing about 1 g, were used. Citrinin solution (0.1 ml, containing 5 μg of citrinin in 0.2% Na_2CO_3) was injected intracoelomically. The injection was given ventro-laterally between the 2nd and 3rd thoracic segments using a Hamilton microsyringe. Insects injected with 0.1 ml of 0.2% Na_2CO_3 solution served as control. Eighteen insects were used in each group. The treated and control insects were kept separately in rectangular boxes ($60 \times 30 \times 15$ cm) containing moistened bread as food.

There was 100% mortality in the group of insects treated with citrinin between 40 and 48 h of treatment. Dead insects had a characteristic appearance in that the body turned black and brittle; legs, antenna, wings and palps were disjointed (Figure 1). Before death, treated insects showed many behavioural changes, which are described below. The control insects behaved normally.

Feeding. One of the first behavioural changes observed was feed refusal. This was noticed from 2 h after treatment till death.

Agility. Within 2 to 3 h of treatment, the insect became listless and remained stationary, and lacked the inclination to move about freely.

Posture. The bearing of the body in cockroach is characteristic in that the thorax and abdomen are held horizontal to the substratum while the head is held at right angles to the body (hypognathous). However, within 3 h of citrinin injection, the insect inclined its body at an angle of about 45° with the substratum, resulting in the trailing of the anal segment (Figure 2).

Antennal and leg preening. Preening of antennae and legs ceased about 3 h after treatment. The infundibular and sweeping movements of the antennae were affected and the antennae fell flat and trailed on the substratum (Figure 3).

Locomotion. Citrinin affected coordination of leg movements. The normal pattern of leg movements changed at about 4 h after treatment. The relative periods of protraction and retraction of legs became erratic, suggesting that the toxin affected the promotion and remotion of the coxa[11]. The hind legs were stretched far back fully and remained inflexible during movement. This resulted in loss of balance and the insect swerved to its side, and in the process turned upside down.

Reversal reflex. Toxin-treated insects turned upside down owing to lack of coordination of leg movements. Such insects were significantly slow in reattaining normal posture. Control insects, however, when turned upside down, flipped back to normal posture instantly (Table 1).

Ventilation. The ventilatory activity of the treated insect became apparent about 9 h after treatment. While treated insects showed rapid abdominal contractions (10–12) in 20 sec, control insects did not show any even after 60 sec.

Tremor. About 18 h after treatment, the treated insects showed trembling of the body followed by irregular quiescent periods.

Studies on the effects of mycotoxins on insects are concerned with their insecticidal properties rather than the behavioural effects[6,7]. This report appears to be the first of mycotoxin-induced behavioural changes in an insect.

We presume that the toxin acts on the nervous system. This presumption stems from the fact that citrinin inhibited antennal and leg preening—a strong reflex response deep-seated in the nervous system[12]. The abnormal posture of the insect and the delay in reversal also indicate that the toxin affected the nervous

Figure 1. Citrinin-treated cockroaches showing *a*, disjointed body parts 48 h after treatment; *b*, inclined body posture; *c*, uncharacteristic positioning of antennae.

system as orientation of the insect is due to stimulation of proprioceptive organs. The lack of agility and coordination in leg movements may also be attributed to disturbances in the normal functioning of the nervous system. The rapid ventilatory activity in treated

Table 1. Effect of citrinin on reversal mechanism of cockroach.

Time after toxin treatment (h)	Time taken for reversal* (sec±SD, $n = 18$)	
	Control	Toxin-treated
0	1.5±0.15	1.88±0.22
4	1.5±0.10	2.7±0.29
6	1.8±0.18	7.4±1.36
8	1.6±0.10	76.1±2.5

insects and the fact that this activity is controlled by ganglions[13] further substantiate our belief. Toxins from some fungi cause tremors in Gypsy moth and wax moths[14] and such toxins are currently used in understanding the functions of the central nervous system[15].

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