INSECTICIDAL ACTIVITY OF THE PETROLEUM ETHER EXTRACT OF AGERATUM conyzoides L.

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RESUMEN

Se estableció que el extracto de éter de petróleo (pe 40-60°) de *Ageratum conyzoides* L. presenta fuerte actividad insecticida contra larvas de tercer estadío de *Musca domestica* (Diptera), contra larvas de tercer, cuarto y quinto estadío de *Cynthia carye* (Lepidoptera) y contra individuos adultos de *Acanthoscelides obtectus* (Coleoptera). En esta acción insecticida podrían estar involucrados dos flavonoides conocidos que se aislaron de este extracto: eupalestina y lucidina dimetiléter. También se aisló prococeno II, cromeno éste que demostró ser altamente tóxico contra larvas de *M. domestica* cuando las pruebas de contacto se hicieron irradiando con luz solar. En cambio, no mostró ningún efecto cuando los ensayos se hicieron en la oscuridad o bajo irradiación de luz UV.

ABSTRACT

We have determined the insecticidal activity of the petroleum ether (bp 40-60°C) extract of *Ageratum conyzoides* L. towards *Musca domestica* (Diptera) third instar larvae and *Cynthia carye* (Lepidoptera) third, fourth and fifth instar larvae, being this extract also active against *Acanthoscelides obtectus* (Coleoptera) adults. We have isolated the known chromene precocene II, from this extract which is highly toxic to *M. domestica* third instar larvae under sunlight exposure, while no larvicidal effect was shown under U.V. irradiation or in dark. We have also identified two flavonoids: eupalestin and lucidin dimethyl ether, which insecticidal role in this extract has not been determinated.

INTRODUCCION

The biological activity of natural secondary compounds of plants, has received special attention in recent decades because of their possible use as natural pesticides against phytophagous insects. (1)

In Colombia, among other places plants have tradicionally been used to protect crops from insect attack because of their insecticidal, repellent or antifeedant attributes. In fact, Pérez Arbeláez (2) lists more than fifty plants suitable for the control of various species of insects or other pests. Among these plants, *Ageratum conyzoides,* a widespread weed in Colombia and in other countries, has been used in folk medicine to cure several diseases and also as an insecticide. We have selected this plant for a bioassay-conducted chemical screening based on its insecticidal activity mentioned above.

Besides precocenes I and II several chromenes and benzofurans have been isolated from many plants of the genus *Ageratum.* These compounds have a well known insecticidal activity when topically applied or incorporated into artificial diets. These products promote physiological changes in the insects that include precocious metamorphosis, sterilization, inhibition of sex pheromone production, embryogenetic damage, interrupted circadian feeding rhythms or diapause induction (3). Although these compounds are currently being screened for phototoxic activity (4), to our knowledge no photoactivity has been reported for precocene II.

EXPERIMENTAL

Plant material. Aerial parts of *A. conyzoides* plant were collected during the last week of July 1989 at an altitude of 1,200 m,100 Km NW of Bogotá, Colombia. After discarding flowers,the leaves were air-dried and crushed. The material (1.5 Kg) was Soxhlet extracted with petroleum ether (bp 40-60^{o}C). The extract was defatted by treatment with methanol, filtered and concentrated under reduced pressure. Upon biossay of this crude extract it was found to have insecticidal properties. A portion (25 g) of the concentrated petroleum ether extract was further fractionated using column chromatography into 4 fractions: 1: petroleum ether; 2: petroleum ether-chloroform (9:1); 3: petroleum ether-chloroform (8:2); 4: petroleum ether-chloform (6:4).

Fraction 1 (1.2 g) contained waxes; fraction 2 (5 g) consisted of a mixture of terpenes; fraction 3 (4 g) was a mixture of chromenes and fraction 4 (1.8 g) was Precocene II. Except fraction 1, which was expected not to have insecticidal activity, all other fractions were bioassayed.

Insects. *Musca domestica* (Diptera) larvae (third instar) were obtained from a colony maintained at INIA, Estación Experimental La Cruz, Chile. *Cynthia carye* (Lepidoptera) larvae (3rd, 4th and 5th instar) and *Acanthoscelides obtectus* (Coleoptera) adults were obtained from colonies established in the University of Chile.

Bioassay

Forced-contact test. A stock solution of crude extract and of each fraction obtained from CC was made by dissolving the dried sample in acetone. For crude extract, concentration of 1.0, 0.5, 0.25 and 0.1% were used, while the concentrations of fractions eluted from column were adjusted according to the weight relative to the crude extract. An aliquot (1 ml) of each solution was withdrawn and placed on the bottom of a Petri dish (9 cm diameter). After the solvent had evaporated, 25 *M. domestica* larvae were introduced into the dish and allowed to have contact with the substances. There were four replicates for each concentration. Bioassays lasted for at least 120 hours. Responses were quantified by counting the number of specimens which had died after different time intervals. Experiments with *A. obtectus* were conducted indentically.

Topical Assays. *C. carye* larvae (ten of each instar) were treated topically on the protorax with Precocene II (500 μg) in 1 μL of acetone and reared on *Malva nicaensis* leaves, one of the hosts of *C. carye*. Survivor larvae were allowed to pupate.

Phototoxicity: Aliquots (1mL) of the stock solution of precocene II were used and test were performed in duplicate as described above for the forced-contact test. After one hour of contact, the Petri dishes containing the treated larvae were placed under sunlight for 15 min. The larvae survivorships were monitored for 48 h.

Identification. Both proton and carbon-13 NMR spectra were recorded with a Varian XL-300 apparatus; Infrared spectra were obtained either from liquid films or KBr pellets using a Perkin Elmer model 467 spectrophotometer; mass spectra were run on a JMS-051G-2 JEOL spectrometer.

RESULTS AND DISCUSSION

In our studies of the biological activity of *A. conyzoides* L. we have demostrated that the petroleum ether extract of the plant had a strong larvicidal action towards *M. domestica* third instar larvae and *C. carye* third and fourth instar larvae, as well as insecticidal action against *A. obtectus* adults. As shown in figure 1, *M. domestica* larvae survival rates were negatively affected by increasing concentrations of the *A. conyzoides* crude extract, reaching a 100% or a 40% mortality when either 1.0% or 0.5% extract concentrations were used. The major mean mortality rates were observed during the first 72 hours. On the other hand, crude extract concentrations below 0.25% were not sufficiently toxic to larvae which continued development and pupated successfully. We observed a mutagenic activity since some houseflies emerged with atrophied wings. Similar mortality rates were observed when the same concentrations of crude petroleum ether extract were assayed on adults of *A. obtectus* as shown in figure 2.

Once the insecticidal activity of the crude extract was established, column chromatography of 25 g of the extract eluting with petroleum ether containing increasing amounts of chloroform gave fractions 1 to 4, in order of elution.

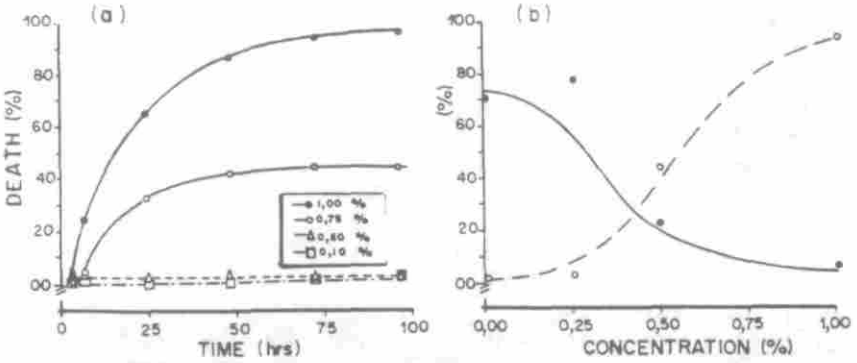

FIGURE 1. a) Larvicidal effect of the petroleum ether extract of A. conyzoides on M. domestica larvae as a function of time. b) Death larvae (solid – line) and pupae (dotted – line) 72 hrs. after exposure.

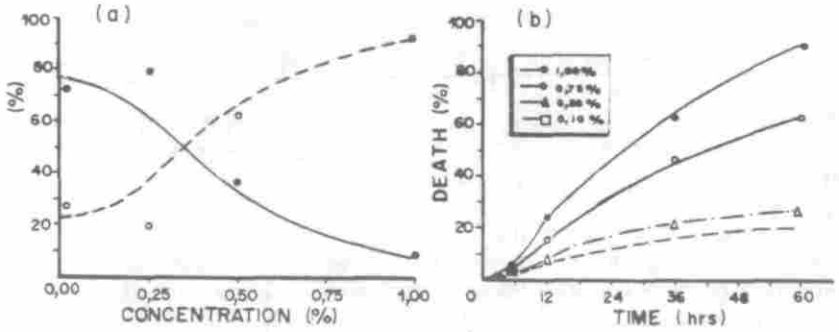

FIGURE 2. a) Death of specimens (solid line) and survival (dotted line) 60 hrs. after treatment. b) Insecticidal activity of the petroleum ether extract of A. conyzoides towards A. obtectus adults.

The IR spectrum of the least polar fraction clearly indicated the presence of waxes and was not therefore bioassayed.

The second fraction afforded a mixture of terpenes as revealed by proton NMR. No insecticidal activity was observed for this fraction.

The third fraction corresponded to a mixture of chromenes (results on the separation and identification of individual chromenes will be published elsewhere). At the concentrations used, no larvicidal action of this fraction was observed when tested towards *M. domestica* third instar larvae. It is clear that under our experimental conditions the petroleum ether extract is much more toxic to *M. domestica* larvae than the chromenes fraction. We can conclude from these results and of the inactivity of precocene II against *M. domestica* (see below) that the crude extract contains other compounds which are probably responsible for the increased insecticidal activity. On this assumption, we suggest

that these results are related with the presence of eupalestin and lucidin dimethyl ether. We isolated have these two known (5) highly oxygenated flavones from the petroleum ehter extract and it has been described (6) that some flavonoids of this type exhibit toxic effects towards insects.

Finally, the proton NMR spectrum of the most polar fraction was closely related to that of the known Precocene II. It showed two six proton singlets at δ 3.78 and δ 1.40 indicating the presence of two methoxyl groups and a gem-dimethyl group respectively. The C-3 olefinic proton appeared as a doublet δ 5.25 (J = 10 Hz) and the C-4 olefinic proton absorbed as a doublet at δ 6.18 (J = 10 Hz). Finally, the C-5 and C-8 aromatic protons appear as two one proton singlets at δ 6.38 and δ 6.51 respectively.

This last fraction, showed potent insecticidal action when tested against 3rd, 4th and 5th instar larvae of *C. carye,* (figure 3). Most (80%) of the 3rd and 4th instar larvae died on the 100 h period following the topical aplication while 5th instar larvae survived (100%) and pupated. Adults emerged normally. This is very interesting, considering the biological action of this type of compounds (2, 4,7,8) that usually led to precocious metamorphosis giving juvenile or moribund miniature adults (9). Further research is necessary to establish possible sterilization or other damages on adult insects.

On other hand, the forced contact test of *M domestica* larvae with precocene II gave no mortality. Interestingly enough the survivorship was affected by sun light. In figure 4 we show the mortality rates of larvae exposed to chromenes and sunlight. Related experiments to establish this unexpected phototoxic action are currently in progress.

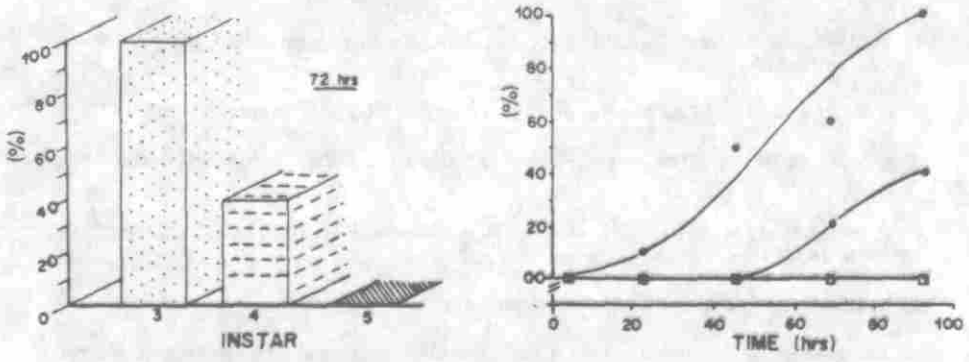

FIGURE 3. a) Topical assay with Precocene 2 on C. carye (Lepidoptera) larvae. b) Death of C. carye larvae: third instar (closed cirles ●), fourth instar (open circles ○) and fifth instar (open squares □). Details in experimental.

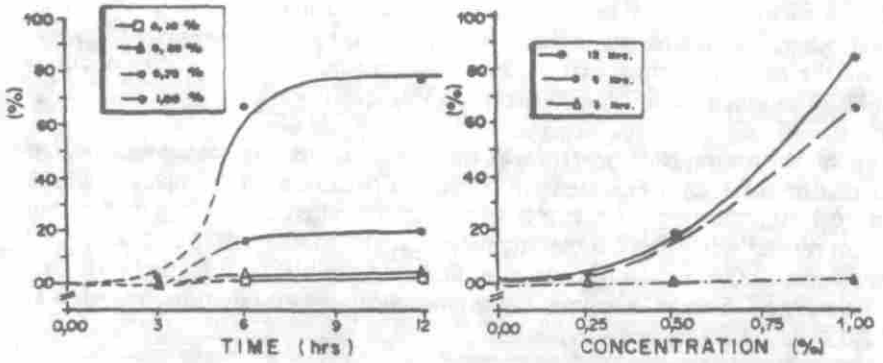

FIGURE 4. a) Phototoxicity of Precocene 2 against M. domestica larvae before (dotted line) and after (solid line) sunlight irradiation. b) Details in experimental.

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