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Laboratory assessment of pesticide toxicity to bumblebees

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Abstract

The toxicity towards *Bombus terrestris* (L.) of the insecticides acephate, buprofezin, carbaryl, cartap hydrochloride, chlorpyrifos-methyl, cyfluthrin, cyromazine, dimethoate, heptenophos, imidacloprid, lamda-cyhalothrin, methomyl, phosalone, pirimicarb, quinalphos, rotenone, and teflubenzuron, of the acaricides fenazaquin, fenpyroximate, hexythiazox, propargite, and tebufenpyrad and of the insecticide-acaricides abamectin and amitraz was tested in the laboratory. Oral, topic contact, and indirect contact trials were carried out for each pesticide, employing formulated compounds dispersed in water at the highest field dose marked on the label. The pesticides that caused a mortality higher than the untreated controls were tested also at decreasing concentrations until the mortality was statistically insignificant in comparison with that of the control; also the acute oral and topic contact LD_{50}, the indirect contact LC_{50} and the related hazard ratios were calculated for these pesticides.

Key words: *Bombus terrestris,* insecticides, acaricides, oral toxicity, topic contact toxicity, indirect contact toxicity, hazard ratio.

Introduction

In the last years the use of *Bombus terrestris* (L.) colonies is more and more widespread in the pollination of protected crops. During their foraging behaviour, bumblebees are exposed to the risk of poisoning due to pesticide treatments. Therefore it is necessary to assess the possible dangerousness of pesticides. Such a research was begun in some European countries and permitted to prepare experimental protocols and to assess the toxicity of some of the most commonly used active compounds; the results have been recently reviewed and discussed by Thompson and Hunt (1999), Thompson (2001), and van der Steen (2001).

In Italy, the absence of similar data stirred the onset of a joint research programme between researchers of the University of Ferrara and of Turin. The present paper deals with some methodological aspects and refers on the toxicity of various pesticides widely used on protected crops in Italy.

Materials and methods

Bumblebee origin and management

The experimentation was carried out on workers of *B. terrestris* coming from families purchased on the market and reared in the laboratory.

All handlings were made under red light and for all the test the bumblebees were kept in a climatized chamber in the dark at a temperature of 28°C and a relative humidity of 60%.

Disposable transparent polystyrene containers with a volume of about 600 ml and a moveable bottom of 120 cm^2 were used as cages; in these containers holes of 2 mm of diameter were drilled for air exchange and a hole of 14 mm of diameter was made so to introduce a feeder, i.e. a polypropylene test tube drilled near the base and containing a solution of about 1 ml of a water and honey 1:1.

In each cage five medium size *B. terrestris* workers were introduced; they were collected from the same colony reared in the laboratory. All trials were performed testing bumblebees from two different colonies at the same time, so to verify possible differences in the response to the toxic action of the tested products.

Products tested

The insecticides acephate, buprofezin, carbaryl, cartap hydrochloride, chlorpyrifos-methyl, cyfluthrin, cyromazine, dimethoate, heptenophos, imidacloprid, lamda-cyhalothrin, methomyl, phosalone, pirimicarb, quinalphos, rotenone, and teflubenzuron, the acaricides fenazaquin, fenpyroximate, hexythiazox, propargite, and tebufenpyrad and the insecticide-acaricides abamectin and amitraz were tested.

Commercial products were employed as dispersion in water and in syrup. They were tested at the highest dose advised on the label; when a significantly higher mortality than the controls was found, the products were tested at progressive dilutions until this difference disappeared.

Acute oral toxicity tests

For the trials by ingestion in which the bumblebees were fed not *ad libitum*, but with individual doses (Schaefer *et al*., 1996), the workers were placed singly in cylindrical containers (diameter of 30 mm and height of 50 mm) of black high density polyethylene in which a hole of 2 mm in diameter was made in the side wall near the bottom; after a starving period of 3 hours, a droplet of 10 µl of the dispersion to be tested was left near the hole so that the insect could reach it with its tongue. Only the bumblebees that consumed the whole dose within 15 minutes were transferred into the cages and checked to evaluate their mortality.

Acute contact toxicity tests

In order to carry out these trials, plastic containers of 30-35 dm^3 were prepared with the sides 20-25 cm high,

on the bottom of which a layer of 2 cm of dry ice was placed and covered with an insulating panel. In a short time the containers were saturated with carbon dioxide and the temperature inside them stabilized around 12°C; then the workers were introduced. On the individuals, that fell anesthetized after a few minutes, a droplet of 10 µl of the dispersion to test, or just of water for the control, was laid between the coxae by means of an automatic pipette. After the necessary time for a complete evaporation the still anesthetized bumblebees were put into the cages and checked to evaluate their mortality.

Indirect contact toxicity tests

For the indirect contact tests, the bottom of the cages were sprayed, by means of a normal volume pump, with the tested products mixed with water; on the average 1.1 ml/bottom were sprayed. After the complete evaporation of the water in the shade, the bottoms of the containers were mounted with the upper parts. Then the bumblebees were introduced and the feeding solution was immediately supplied; after 3 hours the treated bottoms were replaced with clean ones. The controls were always kept in cages with untreated bottoms.

Observations

The mortality counts were made 3, 6, 24, 48, and 72 hours after having supplied the products by ingestion and topical contact, or having exposed the bumblebees to indirect contact.

In all kinds of tests for each product and for each concentration, repetitions were carried out using different colonies. The repetitions in which over one control individual had died were not considered. The number of repetitions ranged from two to seven so to obtain significant mortality values, after 72 hours, with respect to those of the controls.

Statistical evaluations

The comparisons between the number of dead bumblebees in treatments and controls were made employing Fisher's exact test.

From the mortality data the relative LC_{50} were calculated by means of probit analysis. From the LC_{50} values the LD_{50} were derived for the oral and topical contact trials, for which the individual dose applied to each bumblebee was known. Subsequently, the hazard ratios were calculated taking into account the highest dose suggested on the label of the formulated products.

The response to treatments of the pairs of families employed at the same time for the experimentation was compared by means of the χ^2 test, or, when the data were few, by means of Fisher's exact test; the cases in which mortality was null or total for both families were excluded from calculations.

Results

For the trials by ingestion, 10 of the 24 tested products did not cause an appreciable mortality; for the remaining 14 their LD_{50} was defined and the hazard ratio was calculated (table 1). Of these 14 pesticides, nine resulted to be toxic also by topical contact at the initial tested dose, but in table 2 the LD_{50} values and the hazard ratio is given only for eight products, since the data concerning quinalphos need further study.

Table 1. Acute oral LD_{50} (µg/bumblebee) calculated at 24 and 72 hours since the beginning of trials and the relative hazard ratio at 72 hours of the pesticides that caused a higher mortality than in controls at the concentrations advised for field use.

Pesticides	LD_{50}		Hazard ratio
	24h	72h	
abamectin	n.c.	0.07	193
acephate	8.36	7.37	86
carbaryl	3.92	3.84	309
cartap hydrochloride	2.44	1.98	453
chlorpyrifos-methyl	0.38	0.36	2478
cyfluthrin	0.13	0.11	455
dimethoate	0.44	0.33	1848
heptenophos	0.53	0.53	1038
imidacloprid	0.04	0.02	8870
lambda-cyhalothrin	0.21	0.16	249
methomyl	3.46	3.30	271
phosalone	3.98	3.98	176
quinalphos	0.19	0.17	2882
rotenone	0.97	0.68	275

n.c.: not calculated

Table 2. Topical contact LD_{50} (µg/bumblebee) calculated at 24 and 72 hours since the beginning of trials and the relative hazard ratio at 72 hours of the pesticides that caused a higher mortality than in controls at the concentrations advised for field use.

Pesticides	LD_{50}		Hazard ratio
	24h	72h	
abamectin	n.c.	0.14	96
chlorpyrifos-methyl	0.09	0.09	10098
cyfluthrin	0.56	0.36	139
dimethoate	1.55	0.94	761
heptenophos	3.16	2.19	251
imidacloprid	n.c.	0.02	6769
lambda-cyhalothrin	0.22	0.11	369
phosalone	5.98	4.39	159

n.c.: not calculated

In the trials by indirect contact, only six products caused a mortality higher than in the control and their LC_{50} has been determined (table 3).

The comparison of the mortalities checked in the pairs of families employed for the different trials put in evidence some differences; in five couples out of the 30 used complexively, those differences were significant statistically at a probability level of $P = 0.05$.

Table 3. Indirect contact LC_{50} (ppm) calculated at 72 hours since the beginning of trials of the pesticides that caused a higher mortality than in controls at the concentrations advised for field use.

Pesticides	LC_{50}
acephate	996.33
chlorpyrifos-methyl	16.90
dimethoate	27.14
heptenophos	400.84
lambda-cyhalothrin	3.05
quinalphos	10.13

Discussion and conclusions

The results obtained in the trials by ingestion and by topical contact confirm a modest decrease of LD_{50} in the interval between 24 and 72 hours after the treatment as generally reported in the literature; such a trend was evidenced also for acephate on the contrary of what was observed by Drescher and Geusen-Pfister (1991). The values relating to dimethoate and phosalone appear neatly lower than what was indicated in the literature (Gretenkord and Drescher, 1993; van der Steen, 1994; Thompson, 2001). Concerning pirimicarb, the doses employed in our trials, calculated with the reference to the concentrations advised for field use, resulted to be remarkably lower than the oral LD_{50}, expressed in µg/bumblebee, determined by Gretenkord and Drescher (1993) and therefore it is clear that in our trials no appreciable mortality was checked.

The hazard ratio values permit only a comparative evaluation between the different active compounds we tested, referring to the different concentrations actually used in the field, but as far as we know it is not possible to indicate for *B. terrestris* any limits of dangerousness as for *Apis mellifera* L. (EPPO, 1993).

The trials by indirect contact supplied results that are difficultly comparable with those obtained in the trials by topical contact and, altogether, they appeared less significant. This may depend in part from methodologic problems (van der Steen, 2001), as it is not possible to determine the real quantity of active compound uptaken by each individual; moreover the exposure time to the tested products may be not so realistic, even if we referred to a similar method, developed on honeybees, in which this time was fixed arbitrarily (Arzone and Vidano, 1980).

The differences in sensibility towards the same pesticide showed in a low, but not neglectable, number of cases by *B. terrestris* workers coming from different families suggest the opportunity to use in every test individuals from at least two families.

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References

ARZONE A., VIDANO C., 1980.- Methods for testing toxicity to honey bees.- *Bollettino del Laboratorio di Entomologia agraria "Filippo Silvestri"*, Portici, 37: 161-165.

DRESCHER W., GEUSEN-PFISTER H., 1991.- Comparative testing of the oral toxicity of Acephate, Dimethoate and Methomyl to honeybees, bumblebees and Syrphidae.- *Acta Horticulturae*, 288: 133-138.

EPPO, 1993.- Decision making scheme for the environmental risk assessment of plant protection products-honeybees.- *Bulletin OEPP/EPPO Bulletin*, 23: 151-165.

GRETENKORD C., DRESCHER W., 1993.- Die Auswirkungen von vier Planzenschutzmitteln (Decis, Metasystox, Pirimor, Rubitox) auf die Dunkle Erdhummel *Bombus terrestris* L.: Bestimmung der oralen LD_{50} und erste Erfahrungen mit Zeltversuchen.- *Apidologie*, 24: 519-521.

SCHAEFER A., GRETENKORD C., VAN DER STEEN J. J. M., 1996.- Methode zur Bestimmung der akuten oralen LD_{50} von Planzenschutzmitteln an Hummeln (*Bombus terrestris* L.).- *Apidologie*, 27: 275-277.

STEEN J. J. M. VAN DER, 1994.- Entwicklung einer Methode zur Bestimmung der Kontakt LD_{50} von Planzenschutzmitteln bei Hummeln (*Bombus terrestris* L.).- *Apidologie*, 25: 463-465.

STEEN J. J. M. VAN DER, 2001.- Review of the methods to determine the hazard and toxicity of pesticides to bumblebees.- *Apidologie*, 32: 399-406.

THOMPSON H. M., 2001.- Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.).- *Apidologie*, 32: 305-321.

THOMPSON H. M., HUNT L. V., 1999.- Extrapolating from honeybees to bumblebees in pesticide risk assessment.- *Ecotoxicology*, 8: 147-166.

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