Toxicity to Honeybees of Water Guttation and Dew Collected from Winter Rape Treated with Nurelle D®

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Abstract

Shawki M.A.-A., Titěra D., Kazda J., Kohoutková J., Táborský V. (2006): **Toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle D®**. Plant Protect. Sci., **42**: 9–14.

The acute and chronic toxicity to honeybees, *Apis mellifera* L. of water guttation and dew collected from winter rape plants treated with the insecticide Nurelle D® (a.i. chlorpyriphos + cypermethrin) was investigated. Caged bees were fed on sugar syrup containing water guttation and dew for 24 h (acute toxicity test) and for 10 days (chronic toxicity test). Bee mortality and food consumption were determined daily. A contact toxicity test was performed within 24 h on bees kept in Petri dishes (10 bees per dish) lined with filter paper saturated with the test solution. The acute Nurelle D® contact and oral toxicity tests showed that the mortality of bees treated with water guttation and dew collected from the treated plants did not exceed 10%. A chronic toxicity test showed that adding contaminated water guttation and dew to the syrup caused an insignificant increase in bee mortality and reduced the syrup consumption significantly. The chlorpyriphos residue found in contaminated water guttation and dew were below the limit of detection (0.8 µg/kg) and the cypermethrin residue was below the detection levels.

Keywords: *Apis mellifera*; mortality; chlorpyriphos residue

It is known that honeybees (*Apis mellifera* L.) play an important role in the pollination of winter rape. Beekeepers usually move beehives near rape plants before flowering. Honeybees need much water during early spring. During this period foraging bees visit rape plants not to collect nectar or pollen but to collect water guttation and dew. Nurelle D® is applied on winter rape crop in Growth Stages DC 21-51, that is from the end of March till the end of April in the Czech Republic (Kazda *et al*. 2003). If foraging bees collect this contaminated guttation and dew water and carry it back to their hives, not only foragers visiting the crop, but also the bees and larvae in the hives are exposed to the pesticide. This means that life stages of honeybees can be exposed, with the exception of eggs.

Guttation is the appearance of drops of water on the edge of leaves of certain vascular plants. On humid and windless nights, when atmospheric

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6046070901.

conditions are not conducive to transpiration, root pressure may be so strong that the plant cannot rid itself of water fast enough. In that event, the water will push out through the ends of the veins in the leaves, forming drops of water. Dew is the term for the small water droplets or waterfilm that appear on thin objects in the morning. It results from atmospheric moisture that condenses after a warm day and appears during the night as small drops on cool surfaces.

Water is collected by bees and used primarily as diluents for thick honey, to maintain optimum humidity within the hive, and to maintain appropriate temperatures in the brood area. The amount of water required and collected by a colony generally correlates with the outside air temperature and the relative humidity, the strength of the colony, and the amount of brood rearing in progress. Bees need much water to dissolve the honey in the combs, and to dissolve pollen to feed the brood of bees. They collect water from damp dew on leaves, from puddles and from damp earth.

Nurelle D® is an insecticide which contains two active ingredients (chlorpyriphos + cypermethrin) in an emulsifiable concentrate. Chlorpyriphos is a non-systemic insecticide with contact, stomach and respiratory action. Chlorpyriphos acts as a cholinesterase inhibitor (Tomlin 2000). Cypermethrin is a non-systemic insecticide with contact action. This insecticide controls of the rape stem weevil (*Ceutorhynchus napi*) and of the cabbage stem weevil (*Ceutorhynchus pallidactylus*) pests of winter rape very well.

In recent years, many cases of intoxication of bee colonies have been found after the treatment of winter rape against weevils. The treatment of this crop was based on application of the insecticide Nurelle D® (Kamler *et al*. 2003). Generally the application rate was 0.6 l of product per hectare. Reports by some Czech winter rape breeders on suspected impacts of water guttation and dew of winter rape treated with Nurelle D® on honeybees made some beekeepers believe that there might be a link between their bee losses and the use of this insecticide on winter rape. In response to this concern, investigation was conducted to examine whether or not such a link exists.

The aim of this study is to examine whether or not water guttation and dew of winter rape treated with Nurelle D® has an impact on honeybees, *Apis mellifera* L.

MATERIALS AND METHODS

The experiments were carried out in the apiary and in the laboratories of the Bee Research Institute at Dol near Prague. Chemical analysis was carried out at the Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Prague. These studies were carried out in accordance with to the international testing guidelines EPPO No. 170 (EPPO 1992; ICBB 1985).

Collection of water guttation and dew. Nurelle D® contains chlorpyriphos (500 g/l) and cypermethrin (50 g/l) in an emulsifiable concentrate. A Nurelle D® emulsion concentrate equal to the recommended field rate (0.6 l in 500 l of water per ha) was applied to a 15-m^2 plot of winter rape using a hand-held sprayer (1 l capacity). The treatment plot was sprayed on April 18, 2004 (7–9 days before flowering began). There were three replicates of three 15 m^2 treated plots, plus three control plots without treatment.

Samples of water guttation and dew were collected daily until 10 days after treatment. Each day, about 20 ml of water guttation and dew were collected randomly using micropipettes at different levels on the plant. The samples were taken directly to the laboratory and filtered using pieces of cotton; they were then tested on the same day. Unused samples were stored immediately at –18°C.

To increase the quantity of water guttation and dew and to avoid wastage, the following points were done: (1) The plants were irrigated before being covered; (2) Adequate plastic covers were used to cover the plants at night to induce higher production of water guttation and dew; (3) Samples were collected before or early after sunrise; (4) In order to prevent it from falling down, the water guttation was collected before the dew samples.

Bees. To minimise the genetic variations much as possible, only one healthy honeybee's colony with a single queen was used. Honeybee workers needed for laboratory tests were collected from the peripheral combs of the colony.

Acute oral toxicity test. The worker bees were slightly anaesthetised with CO_2 and placed into plastic pots (10 bees/pot) volume 200 ml with unlimited air access. After one-hour starvation the bees were fed on 50% sugar solution mixed with water guttation or dew (1:1 v/v) for 24 h, using the glass feeding device (50 mm long and 10 mm wide with the open end narrowed 2 mm in diameter). The control workers were fed on sugar syrup only. Three replicates were used for

each treatment. The bees were kept at laboratory temperature 20–22°C and honeybee mortality was determined after 24 h.

Acute contact toxicity test. The contact toxicity test was performed on bees kept in Petri dishes covered with nylon mesh (10 bees per dish) with unlimited air access. 2 ml of water guttation or dew collected from treated and untreated plants were placed on filter paper on the dish bottom. The control workers were treated with water only. Three replicates were used for each treatment. The bees were kept at laboratory temperature and honeybee mortality was determined after 24 h.

Chronic toxicity test. To test chronic toxicity, the worker bees were brushed from brood combs and carefully transferred to laboratory cages, 140 × 140 × 70 mm. They were provided with 50% sugar syrup mixed with water guttation or dew (1:1 v/v) collected from treated and untreated plants for 10 days. The control workers were fed on sugar syrup only.

Fumagillin DCH (a.i. fumigillinum dicyclohexylammonium) was added to the syrup at 8 mg/100 g to prevent infection with nosema. The syrup was offered in glass bottles with small holes in the cap. The experimental design consisted of five groups (four treatments + one control) with four cages per group and 25 bees/cage. Bee mortality and intake of food were determined for 10 days after treatment. From these data we computed for each cage: (a) Total mortality (%); (b) Correction mortality = (% mortality for treatment group × % mortality for control)/(100% mortality for control) × 100 (Abbott 1925); (c) Daily food consumption/bee (mg) = food consumption per cage in 24 h period/mean number of living bees for the period (mean of number alive at the beginning and number at the end of the period); (d) Average daily food consumption/bee (mg).

The bees were kept at laboratory temperature. Duncan's multiple-range test was used to compare the means for total mortality after 10 days and average daily food consumption.

Analytical method of the quantification of the active substance:

1. The samples of water guttation, dew and nectar were analysed by method involving following steps: (*i*) microliquid-liquid extraction by toluene, (*ii*) identification/quantification of target analyte by high-resolution gas chromatography employing mass spectrometric detector (HRGC/MS).

2. The samples of pollen were analysed by method involving following steps: (*i*) extraction by ethyl acetate, (*ii*) clean-up by high performance gel permeation chromatography (HPGPC), (*iii*) identification/quantification of target analyte by high-resolution gas chromatography employing mass spectrometric detector (HRGC/MS).

3. The samples of honey bee workers were analysed after homogenisation (20 bodies) by method involving following steps: (*i*) extraction by acetonitrile, (*ii*) identification/quantification of target analyte by high resolution gas chromatography employing mass spectrometric detector (HRGC/MS) (Hajšlová & Kohoutková 2004).

RESULTS AND DISCUSSION

Acute contact and oral toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle D®

The data presented in Tables 1 and 2 shows that the mortality in bees fed with syrup contaminated

Table 1. Acute oral toxicity to honeybees of water guttation and dew water collected from winter rape treated with Nurelle D®

Treatments	Mortality on indicated days after treatment (%)				
	1	2	3	5	7
Control	0.0	3.3	0.0	3.3	0.0
Untreated guttation	3.3	0.0	3.3	0.0	0.0
Treated guttation	6.7	6.7	3.3	0.0	0.0
Untreated dew	3.3	3.3	0.0	3.3	0.0
Treated dew	10.0	10.0	6.7	3.3	0.0

% mortality is the mean of three replicates

Table 2. Acute contact toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle D®

Treatments	Mortality on indicated days after treatment (%)				
	1	2	3	5	7
Control	0.0	0.0	3.3	0.0	0.0
Untreated guttation	3.3	3.3	0.0	0.0	0.0
Treated guttation	10.0	10.0	6.7	3.3	0.0
Untreated dew	3.3	0.0	3.3	0.0	3.3
Treated dew	10.0	6.7	10.0	3.3	0.0

% mortality is the mean of three replicates

with water guttation and dew collected from treated plants did not exceed 10.0% on any day. The mortality in the control bees and in the groups fed syrup containing water guttation and dew collected from untreated plants did not exceed 3.3% on any one day. The residual toxicity of chlorpyriphos in water guttation and dew declined with time to a low level on the 5th day after treatment.

The Council of the European Union (CEU 1991) classifies plant protection products as safe to honeybees if the quotient between the highest proposed application rate and LD_{50} dose (so-called hazard quotient HQ) is less than 50. Under the conditions of our study we cannot calculate the HQ for chlorpyriphos because residue levels of this active substance was below the limit of detection (0.8 µg/kg), also the mortality percentage did not exceed 10%, so we cannot generate the LD_{50} of chlorpyriphos in water guttation and dew.

Chronic toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle D®

The show in Table 3 indicates data that adding water guttation and dew collected from winter rape treated with Nurelle D® to sugar syrup caused an insignificant increase in bee mortality by 14.8% and 16.6%, respectively. In bees fed on water guttation and dew collected from untreated plants the increase in mortality was 0.0 and 4.1%, respectively. There were no significant differences between the means for total mortality after 10 days Duncan's multiple-range test ($P < 0.05$).

Chronic intoxication may be explained by some kind of accumulation of chlorpyriphos residues that may occur if daily intake exceeds the detoxification capacity of the bee. While detoxification is an unknown value, the cumulative intake of

Table 3. Cumulative mortalities for water guttation and dew collected from winter rape treated with Nurelle D® supplied in sugar syrup to caged honey bees

Treatments	Cumulative mortality on indicated days from exposure (%)					CM*
	2	4	6	8	10	
Control	0.0	5.0	8.8	15.5	20.0	
Untreated guttation	3.3	3.3	7.5	12.5	17.5	0.0
Treated guttation	6.7	12.5	19.3	22.3	31.8	14.8
Untreated dew	3.3	8.8	12.5	17.5	23.3	4.1
Treated dew	10.0	14.5	16.8	25.5	33.3	16.6

% mortality is the mean of four replicates

*Cumulative mortalities (CM) = % mortality after 10 days corrected by Abbott's formula

There were no significant differences between the means for total mortality after 10 days Duncan's multiple-range test ($P < 0.05$)

Table 4. Average daily consumption/bee (mg) of sugar syrup contaminated with water guttation and dew collected from rape plants treated with Nurelle D® offered simultaneously to caged honeybees for 10 days

Treatments	Daily food consumption/bee (mg)						
	2	4	6	8	10	mean	% reduction
Control	55.0	47.5	45.5	45.0	42.5	47.1 a	
Untreated guttation	53.3	55.0	43.3	42.0	35.0	45.7 a	3.0
Treated guttation	43.5	45.5	40.0	32.3	33.3	38.9 b	17.4
Untreated dew	47.5	44.3	45.5	40.0	37.5	42.9 a	8.9
Treated dew	38.5	40.0	37.5	36.8	32.3	37.0 b	21.4

Means followed by the same letter are not significantly different by Duncan's multiple-range test ($P < 0.05$)

insecticide may be calculated (Fiedler 1987). Under the conditions of our study we cannot calculate the cumulative intake of the insecticide, because residue levels of chlorpyriphos in water guttation and dew were below the limit of detection (0.8 µg/kg). The cumulative insecticide consumption for low concentrations of chlorpyriphos and cypermethrin in water guttation and dew collected from treated rape plants was lower than the LD_{50} of these substances chlorpyriphos LD_{50} (oral) 360 ng/bee, (contact) 70 ng/bee, cypermethrin LD_{50} (oral) 35 ng/bee, (contact) 20 ng/bee (Tomlin 2000).

It is known that collecting of water by bees is up to take of distance 50 m from the bee colony, so that the beehives must be outside this distance. In addition, we can present honeybees from seeking these two types of water by feeding them with sugar solution in the hives during early spring.

Effect of Nurelle D® in water guttation and dew collected from treated rape plants on the food consumption of honeybees

The shown in Table 4 indicates data that the food consumption of all test groups generally decreased during the experiment. Food consumption was significantly reduced by 17.4% and 21.4% in comparison with that of the control group, when the workers were fed on sugar syrup containing water guttation and dew collected from treated plants, respectively. In case of workers fed on sugar syrup containing water guttation and dew collected from untreated plants the reduction in food consumption fell by 3.0% and 8.9%, respectively.

Residue of the active ingredients

Residue levels of chlorpyriphos in water guttation and dew collected from treated and untreated rape plants are shown in Table 5. Data showed that no chlorpyriphos was found (detection limit 0.8 µg/kg) in guttation samples on the following 5 days. This may be due to chlorpyriphos as a non-systemic insecticide cannot translocate and penetrate the plant tissues. In case of dew samples residue levels were below the limit of detection (0.8 µg/kg) till the 3rd day after spraying, after that residue levels were 3.72 and 1.5 µg/kg on the 4th day and on the 5th day after spraying, respectively. This may be because Nurelle D® is an emulsion and after spraying the water evaporated and the active ingredient took a solid form. Our results are in partial agreement with those of Kamler *et al.* (2003), who found that leaves of rape plants

Table 5. Residues of chlorpyriphos in water guttation and dew collected from treated rape plants

Time after spraying	Untreated rape		Treated rape	
	guttation	dew	guttation	dew
1st day	< 0.8	< 0.8	< 0.8	< 0.8
2nd day	< 0.8	< 0.8	< 0.8	< 0.8
3rd day	< 0.8	< 0.8	< 0.8	< 0.8
4th day	< 0.8	< 0.8	< 0.8	3.7
5th day	< 0.8	< 0.8	< 0.8	1.5

The limit of detection in water guttation and dew was 0.8 µg/kg (Hajšlová & Kohoutková 2004)

treated with Nurelle D® indicated toxicity up to the 8th day after treatment. Residue active ingredient "cypermethrin"was below of detection levels. Futhermore, Kamler *et al*. (2003) added that the content of chlorpyriphos in the leaf samples reduced slowly.

CONCLUSION

In conclusion, our result show that water guttation and dew collected from winter rape plants after treatment with Nurelle D® were only slightly harmful to honeybees. Further studies are needed to investigate whether water guttation and dew of rape treated with Nurelle D® has an impact on small or standard-size honeybee colonies under field conditions.

Acknowledgements. The authors would like to thank Assoc. Prof. Dr. Pavel Ryšánek, Head of the Department of Plant Protection, for support encouragement.

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Received for publication June 15, 2005
Accepted after corrections September 20, 2005

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