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Original Research

Occurrence of Residues of Warfarin after Its Application to Cereal Crops

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Abstract

Environmental hazards posed by unintentional and excessive penetration of chemical substances into water, air and soil is noticeable in its harmful effects on plants, free range animals, quality of environment components and biodiversity in general.

The chemicals used in agricultural practices enter the food chain most frequently through their application to soil or crops. Regarding the employment of pest-control preparations, rodenticides play an important role in controlling harmful rodents.

The present paper reports the investigation of residues fromKumatox Z rodenticide preparation with an active ingredient – warfarin – applied to cereal crops. Kumatox Z is a rodenticide bait containing the active ingredient warfarin in 0.05% concentration. The preparation was employed in the form of cereal baits to soil surface. The research results showed the presence of warfarin residues in the treated soil and cereals grown over there. Determination of residues was carried out by HPLC; interestingly, they were found in the soil even after three years.

Keywords: warfarin, soil, barley, wheat

Introduction

Field crops, particularly cereals, may get infested with harmful rodents. The most common agricultural pests prove to be field mice (*Microtus arvalis*) and hamsters (*Cricetus cricetus*). To reduce damage caused by pest rodents to crops, they are controlled by means of rodenticide preparations. In addition to acute rodenticides, including rodenticide baits with zinc phosphide or scilirozide, chronical rodenticidal preparations interfering with blood coagulation mechanism, i.e. anticoagulants, are also used. Uptake of rodenticide baits results in pest death as soon as a couple of days following its administration.

The general effectiveness of anticoagulant rodenticides is high [1]. However, according to the available results, the preparation residues left over in the environment raise serious concerns. This issue was addressed in 1990 by Smith and Cox [2]. as well as Eason and Spurr [3] and other numerous authors.

Although the professional literature dealing with intoxication of mammals or the treatment of affected non-target species is extensive, the ecological impact related to the residues of chronic, crop-applied residues has not yet been studied sufficiently.

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The aim of the present study was to examine quantitatively both soil and cereals for the presence of residues of warfarin employed as commercial preparation KUMATOX Z. Soil and crops were examined for residues at the end of the growing season and the soil was additionally analyzed after 3 years.

Experimental Procedures

KUMATOX Z belongs to the group of first generation-anticoagulant rodenticides [4, 5]. We refer to treated wheat grains of blue colour containing 0.025% warfarin.

Determination of Warfarin Residues

The samples for examination (10 g) were homogenized and extracted twice with 25 ml of a chloroform-acetone mixture (1:1) for 15 min. Each time the solvent was decanted through a filter paper.

The combined extracts were evaporated in a rotating vacuum evaporator at 40ºC. The residuum was dissolved in 1 ml of methanol followed by the addition of 1 ml of a mobile phase (0.1 M sodium acetate and 0.01 M acetic acid) to remove the co-extracted proteins.

Afterwards, the solution was placed in a centrifuge tube with n-hexane and centrifuged for 10 min at 3,500 r.p.m. The clear bottom layer was filtered and used for HPLC chromatography.

HPLC chromatography was performed on a column LiChrospher® 100 RP-18 (5 µm) using acetonitrile (A) and acetate buffer pH 4.6 (B) (50+50) as a mobile phase, at a flow rate of 1 ml·min^{-1}, the volume injected - 10 µl. UV detection was conducted at 265 and 310 nm.

Statistical Evaluation

Statistical evaluation was carried out using software Graph Pad-Test Anova. We determined the significance of differences between the samples obtained from the treated soil and control. The P threshold value for statistical significance was set up at $P< 0.001$.

Results

Table 1 summarizes the mean levels and standard deviations of warfarin residues in the investigated cereal crops.

The samples of soil and above-ground parts of barley and wheat were collected at harvest in the harvest-ripe stage, e.g. early August. The total cereal crop served as one sample at individual application. A barley sample No. 1 was taken from the crop treated with rodenticide at a ratio of 100 g·m^{-2}, while sample No. 2 corresponded to the treatment with 500 g·m^{-2}. Similarly, two wheat samples were collected for which the rodenticide doses were the same, i.e. 100 g·m^{-2} and 500 g·m^{-2}.

Mean levels and standard deviation of warfarin residues in soil are presented in Table 2.

Table 1. Mean levels and standard deviation of warfarin residues in the investigated cereal crops.

Sample No.	Rate of KUMATOX Z application (g·m^{-2})	Crop	Warfarin content $\overline{x} \pm$ SD (ppm)
1	100	Barley	0.016 ± 0.0021
2	500	Barley	0.036 ± 0.0023
3	100	Wheat	0.037 ± 0.0020
4	500	Wheat	0.065 ± 0.0021

Table 2. Mean levels and standard deviation of warfarin residues in soil.

Sample No.	Rate of KUMATOX Z application (g·m^{-2})	Material	Warfarin content $\overline{x} \pm$ SD (ppm)
1	100	Soil	0.020 ± 0.0023
2	500	Soil	0.074 ± 0.0025
3	after 3 years 500	Soil	0.072 ± 0.0021

For each exposure, the control crops (no exposure to rodenticide) were sampled and served as controls.

At the end of the growing seasons, 2 samples were taken from the soil. Sample No. 1 was a representative (pooled) sample of soils treated with 100 g·m^{-2} KUMATOX Z, while sample No. 2 corresponded to the exposure of 500 g·m^{-2} (Table 2).

After a 3-year period, the samples were taken from the soil treated with 500 g·m^{-2} of KUMATOX Z (Sample No. 3) and from the control.

Subject to rodenticide quantity applied, lower residue levels were established in sample No. 1, sown with barley and No. 3, sown with wheat, in which warfarin levels reached 0.016 ppm and 0.037 ppm, respectively.The samples of both soils treated with rodenticide at a dose of 100 g·m^{-2} were analyzed as a composite sample No. 1,where a rodenticide residue content was equal to 0.020 ppm.

At the rate of 500 g·m^{-2} of rodenticide, residues in sample No. 2 sown with barley and in No. 4 sown with wheat appeared to have 0.036 ppm and 0.065 ppm levels, respectively. The samples of both soils treated with rodenticide at a dose of 500 g·m^{-2} were studied again as a composite sample No. 2, where a rodenticide residue level was shown to be 0.074 ppm.

The samples taken 3 years later from the soils treated with rodenticide at a dose of 500 g·m^{-2} were shown to contain 0.072 ppm of warfarin.

Statistical evaluation of the results obtained from the study on samples treated with different doses of warfarin and control ones, i.e not exposed to warfarin, revealed significant differences with the exception to the residues in barley and soil at a dose of 100 g·m^{-2} KUMATOX Z and warfarin residues in soil in the first year and after 3 years at a dose of 500 g·m^{-2} KUMATOX Z.

Discussion

Economical losses caused by rodent damage to cereal crops come to millions. Stenseth et al. [6] reports that pest rodents contaminate and destroy enough food in China each year to feed 200 million people. In Indonesia, rodents damage approximately 15% of all rice produced.

The present research indicated periodical infestation with field mice, namely every 3-5 years, and its close relation to temperature and snow-cover in the winter period. This area is considered overinfested when rodent numbers per 1 ha amount to 3,000 field mice. Fig. 1 illustrates the situation of field mice overinfestation.

According to the list of registered preparations for protection of crops and other preparations [7], the following rodenticide preparations are permitted for use in free space: BROMADIC H - based on brodifacoum; COMMANDO, RODENTIC BLESK and STUTOX – based on zinc phosphide; and REDENTIN 75 – based on chlorophacinone [8]. Earlier, warfarin could also be used to control harmful rats, which is why it was evaluated.

Out of acute rodenticides, bait formulation containing zinc phosphide, e.g. commercial preparation STUTOX, has been recommended as the rodenticide of choice to reduce field mouse numbers. The bait contains 4% zinc phosphide and is of yellow-green colour to make it non-attractive to birds. Staples et al. [9] found that the uptake of baits with zinc phosphide by rodents is generally good, the baits are relatively safe as they do not develop true secondary poisoning in natural predators and leave no significant residues in cereals, soil, water and the atmosphere.

Voles and field mice are also controlled by anticoagulant rodenticides. The currently used anticoagulant preparations are the largest group of pesticides which is divided into 3 subgroups: first, second and third-generation, subject to the active ingredient concentration in a bait as well as bait quantity needed to achieve lethal effects [4, 5].

In the present study, the focus has been on determining residues of warfarin, a first-generation rodenticide with 0.05% active ingredient content in bait. The tested preparation KUMATOX Z comprised 0.025% warfarin. Warfarin was registered as a rodenticide in the USA in 1947-48, and since 1952 has been used in human medicine to treat and prevent thrombo-embolic diseases [10]. Coumarin is found naturally in many plants, notably in goose-grass (*Galium odoratum*), at lower levels in lavender (*Lavandula officinalis*), white and common melilot (*Meliotus albus* and *officinalis*), and in other species. The results obtained from the cereals and soil studies point unambiguously to the formation of residues. The residues in barley crop appear to be lower compared to wheat, yet they correlated with a dose of KUMATOX Z applied to the soil (Figs. 2-5).

The correlation with amount of rodenticide employed was observed in the soil samples as well. It is important that soil sampled 3 years after KUMATOX Z application contained residues of this rodenticide. A difference in residue levels in the soil analyzed at the end of the vegetation period and three years later was shown to be minimal (0.074 ppm and 0.072 ppm, resp.), as Table 2 and Fig. 6 illustrate.

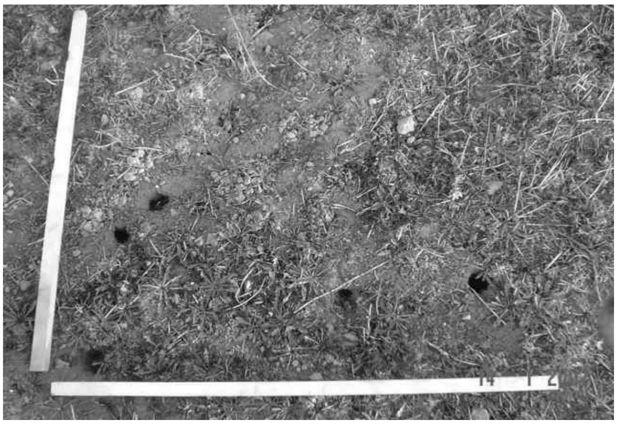

Fig. 1. Field mouse burrows - area of 1 m^2.

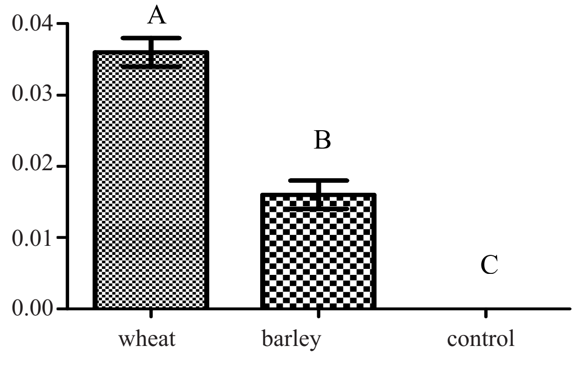

Fig. 2. Mean levels and standard deviations of warfarin residues in investigated cereal crops at warfarin application at a dose of 100g·m^{-2}.
A-B, A-C, B-C, - statistical significant difference, $P < 0.001$.

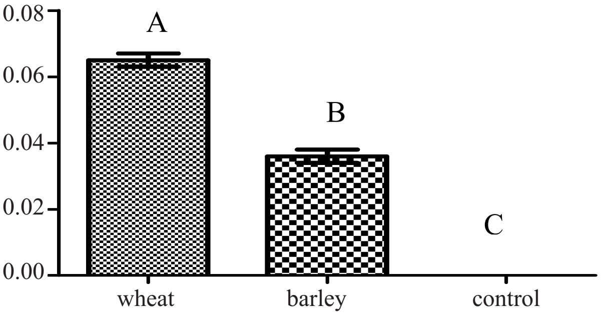

Fig. 3. Mean levels and standard deviations of warfarin residues in the investigated cereal crops at warfarin application at a dose of 500g·m^{-2}.
A-B, A-C, B-C, - statistically significant difference, $P < 0.001$.

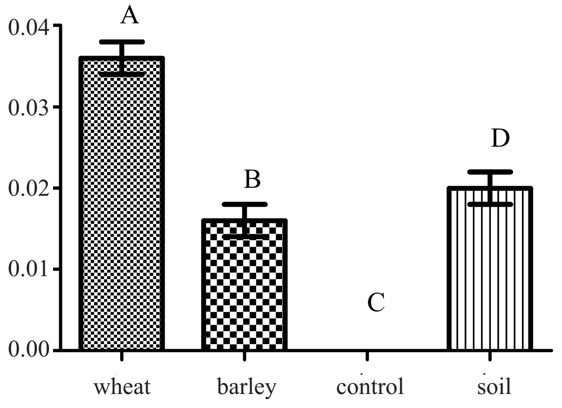

Fig. 4. Mean levels and standard deviations of warfarin residues in the investigated cereal crops and soil at warfarin application at a dose of 100g·m^{-2}.
A-B, A-C, B-C, - statistically significant difference, P < 0.001.

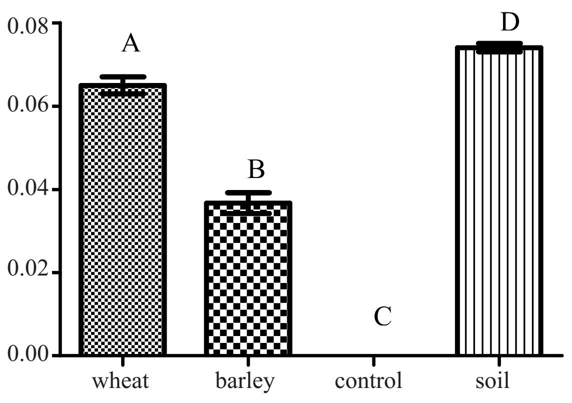

Fig. 5. Mean levels and standard deviations of warfarin residues in the investigated cereal crops and soil at warfarin application at a dose of 500g·m^{-2}.
A-B, A-C, A-D, B-C, B-D, C-D - statistically significant difference, P < 0.001.

With a melting point of 159-165ºC or 318-322ºF, Warfarin is practically insoluble in water [1,4]. It is readily soluble in acetone, chloroform and dioxanes [11, 12]. As its degradation rate is relatively low, warfarin residues persist in soil for a long time, so it is a challenge to improve warfarin-contaminated soil. Kunc [13] carried out the microbiological study and isolated 139 bacterial strains from soil, which induced coumarin breakdown. Out of them, 25 belong to the genus Pseudomonas, 7 to Cellulomonas and 7 to Achromobacteria.

According to the current legislative standards, rodenticide concentrates are listed as top dangerous poisons which, however, does not apply to rodenticide baits. With regard to their high toxicity to animals, current investigations focus on their influence on non-target species of animals. Booth et al. [14] described acute toxicity of brodifacoum to invertebrates, such as snails and terrestrial species of crabs.

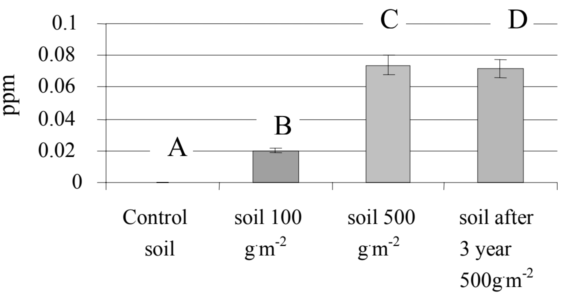

Fig. 6. Mean levels and standard deviations of warfarin residues in soil.
A-B, A-C, A-D, B-C, B-D, C-D - statistically significant difference, P < 0.001.

Eason et al. [15] investigated residues of brodifacoum in poisoned wild pigs and goats. Booth et al. [16] observed the toxic effects of brodifacoum on earthworms, which points to the need of observation and evaluation of the influence of anticoagulant rodenticides on invertebrates, but also investigation of the presence of their residues in plants after their application in free nature.

Conclusion

Our study has shown that the application of KUMATOX Z to field crops for the purpose of controlling harmful rodents is associated with the preparation residues left over not only in soil but in crops as well.

The residue content in barley ranged between 0.016-0.036 ppm, and in wheat between 0.036-0.065 ppm, depending on the rodenticide dose employed.

The residues in soil were found within 0.020-0.074 ppm and correlated with a dose of KUMATOXU Z. Even after 3 years following its application, residue content in soil reached 0.072 ppm.

Acknowledgements

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