PLANT PROTECTION PRODUCTS WITH REDUCED IMPACT FOR AQUATIC ENVIRONMENT USED IN POTATO CROP

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Abstract. Water protection is a priority on the list of public concerns about the environment and is recognized as one of the fundamental elements necessary for the existence of life on the planet. Proper use of pesticides is continuously supported in a sustainable and productive agriculture. In order to determine the plant protection products impact on aquatic organisms, the carp (*Cyprinus carpio*), a species found on the list of species recommended by European legislation and a species of unicellular green algae (*Selenastrum capricornutum*), were used as a biological material. The plant protection products for which the fish and algal growth impact was determined under laboratory conditions, were the Nuprid 200 SC insecticide, the Sencor 600 SC herbicide and the Consento 450 SC fungicide, tested at concentrations of 1 ml / l water, 2 ml and 5 ml / l water and respectively 50 µl water. During the 96-hour observations period, registered fish mortality was zero, except for the concentration of 5 ml Consento 450 SC / l water, which caused the death of the entire batch of fish. Regarding algae, for treated variants the growth factor was 16, and for the control variant was 18. Researches have shown that the tested chemicals under laboratory conditions do not pose a risk to the aquatic environment, and have with a low impact on *C. carpio* and *S. capricornutum*. Under field conditions, the products were applied according to technology, in a potato crop, located near the Tarlung River, Braşov County. The water samples which were collected at 30 and 60 days after treatment were brought to the laboratory and were made observations on the behavior of *C. carpio* species and the studied algal growth rate. Due to their very high selectivity, these plant protection products can be recommended in potato crop pest control schemes, by applying the aquatic organisms risk management measures, along with respecting distances to surface waters and water shadows, ensuring properly environmental protection, and also obtaining healthy crops.

Key words: *plant protection products, aquatic environment, Cyprinus carpio, Selenastrum capricornutum;*

INTRODUCTION

The contamination of groundwater and surface water is a consequence of the use of pesticides in the agriculture and out of the agriculture. Their significance for human health and the environment largely depends on the quantity applied annually within an area, the toxicological and ecotoxicological properties of the pesticides and the persistence in the environment (Beitz et al., 1994). For a risk assessment of surface and ground water contamination, the occurrence and fate of these chemicals in aquatic environments is to be considered. It requires detailed knowledge of the flow regime and of the geochemical behaviour of the pesticides in water and soil with respect to the physical, chemical and microbial processes controlling their persistence and transport in the different aquatic systems (Matthess, 1994).

Aquatic ecosystems may be contaminated with PPPs as a result of spray-drift, leaching, runoff, and/or accidental spills, and because aquatic ecosystems contain species related to the target organisms of PPPs, undesirable side effects may occur. Therefore, governmental authorities have set criteria to protect aquatic life from pesticide stress. These criteria, however, often are debated because of the high economic consequences of too strict - and the high ecological consequences of too weak - environmental risk assessment procedures.

Consequently, the ecological relevance of estimated risk levels is an important item in recent ecotoxicological research with PPPs (Brock et al., 2006).

Water protection is an essential concern of the European Plant Protection Association (ECPA) and the pesticide industry, materialized by developing projects that support the proper use of pesticides in a sustainable and productive agriculture.

Pesticides can reach surface waters in two ways, with two major sources of pollution: point sources mainly represented by pesticide manipulation within agricultural farms and diffuse sources due to rainwater leakage, soil erosion, unfavorable weather conditions or the phenomenon of drift.

Under Directive 128 on the sustainable use of pesticides, research programs aimed at assessing the impact of pesticide use on human health and the environment, including studies on the most exposed groups, should be promoted at European and national level.

The aquatic environment is particularly sensitive to pesticides. The average permissible drinking water concentration for chemical pesticides applied to potato culture is between 0.1 μg / l and 0.5 μg / l, depending on the level of pesticide toxicity.

This paper presents the study impact of chemical plant protection products on the *Cyprinus carpio* fish species and *Selenastrum capricornutum*, an algae species, both in laboratory and field conditions.

MATERIALS AND METHODS

In the first step, the toxicity of chemical phytosanitary products to fish, *C. carpio* species and algae, *S. capricornutum*, was determined under laboratory conditions. Testing activities of selected products were conducted against the two aquatic species, species for which plant protection legislation requires the determination of the influence of chemical phytosanitary products. Carp (*C. carpio*) is a species that is part of the list of species recommended for this test, according to Method C1 of Regulation (EC) No. 440/2008. To determine toxicity to fish, a European Guideline (OECD 203, 1984) procedure has been used, a procedure which establishes the methodology for determining acute lethal toxicity of a substance.

The chemical plant protection products tested in order to determine their impact on aquatic species were Nuprid 200 SC, Sencor 600 SC and Consento 450 SC. Chemicals have been tested at concentrations 10 times lower than the recommended dose. They were diluted, considering that the concentration that reaches the surface waters or groundwater is much lower compared to the one given under production conditions.

The fish were purchased from the Nucet Fish Research Development Resort where they had controlled growth conditions. They belonged to a single batch, corresponded in terms of health, being approximately the same age and no visible malformations. During the acclimatization period, the fish were fed on a daily basis with standardized feed, with a 24-hour break before testing, with the daily ration administered representing 2% of the weight of the fish batch.

Inside the LECO test facility, for the maintenance of fish in the laboratory, including for testing is used reconstituted water produced by automated equipment. Reconstituted water is obtained by adding to the deionized water a specific quantity of reagents with an analytical grading recognized in accordance with the requirements of Appendix 1 to Method C1 of Regulation (EC) No. 440/2008.

The adaptation period was 7 days, the fish were kept in the reconstituted water at a temperature appropriate for the tested species, with a lighting time of 12-16 hours per day; the dissolved oxygen concentration was kept at least 80% of the saturation value in the air and the

pH of the water was between: 6.0-8.5. Fish that showed atypical swimming behavior were isolated from the rest of the lot and were not used for the test. These, as well as surviving fish at the end of the test period were euthanized according to a specific procedure and disposed of in accordance with specific requirements. The fish were exposed to the water-solubilized test substance in a series of concentrations over a period of 96 hours. The mortality was recorded at 24, 48, 72 and 96 hours and the median lethal concentration causing 50% death of the fish group was calculated, if any, being considered dead if the touch of the caudal peduncle produces no reaction, and no breathing movements are visible.

To test substance toxicity to fish, the Static Test method was used in which the test solution was not replaced, so the solutions remained unchanged throughout the test. For the preparation of the test solutions, appropriate quantities of the test substance were solubilized in reconstituted water, fish test aquariums having a capacity of 20 liters.

To determine the influence of chemical plant protection products on algal growth (*S. capricornutum)*, algae growth inhibition test was performed. Unicellular green algae species are best suited for testing, due to their fast growing, so that relatively short trials can determine the possible effects of a chemical over several generations.

The procedure is used in the Ecotoxicological Testing Laboratory of the ICDPP to determine the effects of substances on algal growth using as reference document SR EN ISO / CEI 17025: 2005 - General Requirements for the Competence of Testing and Calibration Laboratories and the OECD Guidelines for Testing of chemical products no. 201 /23.03.2006.

The test organism is subjected to various concentrations of the test substance for 72 hours. The system's response consists in reducing the growth of algal culture and is evaluated according to the concentration of the test substance compared to an untreated control. In order to have a complete system response to toxic as much possible, algae-toxic-treated culture is maintained in a nutrient-enhancing environment to ensure adequate growth under continuous illumination conditions.

Growth and inhibition of growth are quantified and recorded by counting the cells at the beginning of the test and then at 24, 48 and 72 hours using Thoma blade. For the test to be valid, the growth factor on the untreated control should be at least 16, after 72 hours.
Algae have been grown using the growing medium according to OECD 201/2006 consisting of:

Stock solution	Nutrients	Stock concentration
Stock solutions no 1 Macronutrients	NH_4Cl	1,5 g/l
	$MgCL_2.6H_2O$	1,2 g/l
	$CaCl_2.2H_2O$	1,8 g/l
	$MgSO_4.7H_2O$	1,5 g/l
	KH_2PO_4	0,16 g/l
Stock solution no 2 -iron	$FeCl_3. 6H_2O$	64 mg/l
	$Na_2EDTA. 2H_2O$	100 mg/l
Stock solutions no 3	H_3BO_3	185 mg/l
	$MnCl_2. 4H_2O$	415 mg/l
	$ZnCl_2$	3 mg/l
	$CoCl_2. 6H_2O$	1.5 mg/l
	$CuCl_2. 2H_2O$	0.01 mg/l
	$Na_2MoO_4. 2H_2O$	7 mg/l
Stock solutions no 4	$NaHCO_3$	50 mg/l

The growth medium was inoculated with the species *S. capricornutum* and incubated under controlled conditions of light and temperature. For each concentration were used three repetitions, including the untreated control. The initial concentration of cells in the test cultures was about 104/ml.

In order to determine the relationship between concentration and effect, it was used comparing the areas method. To determine the effect of chemically active substances on algae growth, the green algae culture with a cell concentration of about 10 mg/ml was exposed to different concentrations of the test substance. The algae culture, prepared in conical flasks, was treated with the test solution, then maintained under controlled conditions: 240C and a brightness of approx. 8000 lux.

Figure 1. Aspects during laboratory test

Further, the experimental model was carried out in field conditions to determine the toxicity of the products to the same species: *C. carpio* and *S. capricornutum*.

The research was conducted under field conditions, crop potato experience being located in Brasov, near the river Tarlung, potato cultivation favorable area. It was intended to place the experience near awater in an area characterized by the presence of water shadows (irrigation channels, ponds), thus ensuring that the plant protection products used are leached in these waters.

To determine the influence of phytosanitary treatments on the aquatic organisms studied, the experimental chemical products were applied according to the potato culture maintenance technology, observing the application times, according to the pest development cycle.

To determine the impact of chemical plant protection products applied to the experimental model, water samples were collected at 30 and, respectively, 60 days after the treatment. Samples were collected in 20-liter plastic containers, each 100 liters for the two periods mentioned. Under the ecotoxicology laboratory testing conditions, the water samples were distributed in special vessels, in which the aquatic species, namely juveniles *C. carpio*, *S. capricornutum* in the concentration of 104/ml were distributed. Observations have been made on fish behavior and algal growth rate.

Figure 2. Potato crop plot; Biological material.

RESULTS AND DISCUSSIONS

The concentrations of the tested products were calculated based on the recommended use dose per hectare, considering that if the potato culture is located less than 10 m away from a water luster, there is the possibility of leaching the product in surface water. Thus, the concentrations used were 10 times lower, and in the case of the Consento 450 SC, 100 times

smaller. These concentrations have been established to demonstrate that the products used do not adversely affect aquatic flora and fauna.

After implementing both experimental models and testing the toxicity of chemicals for the two aquatic species, *C. carpio* and *S. capricornutum*, the following results were obtained:

Table 1. Results for impact of plant protection products on fish (*Cyprinus carpio*)

Plant protection product	Tested doses	Observations after :							
		24 h		48 h		72 h		96 h	
		alive	dead	alive	dead	alive	dead	alive	dead
Nuprid 200 SC (imidacloprid 200 g/l)	1 ml/ 10 l water	7	0	7	0	7	0	7	0
Sencor 600 SC (metribuzin 600 g/l)	2 ml/ 10 l water	7	0	7	0	7	0	7	0
Consento 450 SC (propamocarb hydrochloride 375 g/l + fenamidone 75 g/l)	5 ml/ 10 l water	0	7	0	7	0	7	0	7
	50 µl/ 10 l water	7	0	7	0	7	0	7	0
Untreated control	-	7	0	7	0	7	0	7	0

With regard to toxicity to *S. capricornutum* algae, the 3 products tested did not show toxicity. After 24, 48 and 72 hours, an increase in the number of cells / ml was determined, the growth factor being between 16 in the treated and 18 in the control variant. Observations on the viability of the tested species were performed at 7 and 21 days and demonstrated that the chemicals tested were at low risk for aquatic organisms. Their impact study on aquatic organisms in field conditions has shown that the aquatic ecosystems close to the potato culture in the Sacele-Brasov area have not been affected in terms of the viability of the two studied species.

CONCLUSIONS

The study shows that phytosanitary chemicals tested for impact on the *C. carpio* and *S. capricornutum* species do not pose a risk to the aquatic environment, with little impact on the tested organisms. Due to the very good selectivity, these plant protection products can be recommended in the potato crop pest control schemes, by applying risk management measures to aquatic organisms, observing distances to surface waters and water shadows, ensuring protecting the environment properly, while getting healthy crops.

In order to protect the aquatic ecosystem, it is recommended to use primarily pesticides that are not classified as dangerous for the aquatic environment, use the most efficient application techniques, and low diversion equipment, the use of mitigation measures to reduce the risk of external pollution caused by spray drift, drainage and leakage. These include the establishment of buffer zones of adequate size to protect aquatic organisms and protection areas for groundwater or surface water sources.

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REFERENCES

BEITZ H., SCHMIDT H., HERZEL F. (1994). Occurrence, Toxicological and Ecotoxicological Significance of Pesticides in Groundwater and Surface Water. In: Börner H. (eds) Pesticides in Ground and Surface Water. Chemistry of Plant Protection, 9. Springer, Berlin, Heidelberg;
BROCK T. C., ARTS G. H., MALTBY L., VAN DEN BRINK P.J. (2006). Aquatic risks of pesticides, ecological protection goals, and common aims in european union legislation. Integrated Environmental Assessment and Management, 2, e20–e46. doi:10.1002/ieam.5630020402.
DIRECTIVE 2009/128/EC of the European Parliament and of the Council.
MATTHESS G. (1994). Fate of Pesticides in Aquatic Environments. In: Börner H. (eds) Pesticides in Ground and Surface Water. Chemistry of Plant Protection, 9. Springer, Berlin, Heidelberg.
OECD 201 (2006). Freshwater Alga and Cyanobacteria, Growth Inhibition Test, 2-7.
OECD 203 (1984). Fish, Acute Toxicity Tests, 2-9.