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Occurrence of fungal and pesticides contamination in rapeseeds depending on the cultivars and systems of farming

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ABSTRACT

The aim of this study was to compare the incidence of pathogens and pesticide residues in rapeseed samples depending on integrated and organic cultivation systems and cultivars. No pesticide residue was detected in seeds coming from the organic production system. However, trace amounts of pesticide residues admissible in rapeseed protection were detected for samples from integrated pest management. Seeds from both cultivation systems were most frequently infested by fungi *Alternaria brassicicola* and *A. alternata*. The greatest number of *Leptosphaeria* spp. cultures was obtained from seeds from organic cultivation.

Keywords: chemical contamination; fungal infestation; *Brassica napus*; oilseed rape; organic farming

The diversification of farming systems primarily consists in the selection and application manner of production means (i.e. soil management, cultivars selection, use of biocontrol products etc.). Crop rotation is one of the very basic building blocks of organic farming systems. The crop rotation in organic farming must provide the soil fertility required for maintaining productivity and it must prevent problems with weeds, pests and diseases. This is obtained through a proper sequence of crops in time and space and through the use of N fixing crops and cover crops (Lampkin 1990). This is contrary to conventional or integrated farming systems, where lack of soil fertility can be ameliorated by use of artificial fertilizers and weeds, pests and diseases can be controlled through the use of agrochemicals (Olesen 1999). Integrated pest management (IPM), aims at limiting the use of synthetic plant protection products (PPPs), chemical fertilizers and plant growth regulators and replace them by applying alternative methods, e.g. biological, physical and agronomical practices (Jajor and Mrówczyński 2013). Adoption of this system ensures safe food production and guarantees that permissible pesticide residue limits are not exceeded in agricultural produces. Organic farming (OF) is a production system which does not permit using synthetic means of agricultural production. It also promotes the introduction into agriculture of organic methods, which are environmentally friendly and treat the farm as a holistic system including plant and animal production. This fact is connected with the legal requirements of the Commission Regulation (EC) No. 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No. 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. The characteristics of organic agricultural systems are their biodiversity at soil, crop, field, whole rotation or polyculture, and landscape level and the greater focus on integration of crop and livestock production systems on the farm compared with conventional farming systems (Mäder et al. 2002).

In both production systems an important element is connected with an adequate selection of sowing material. In organic plant production

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the ban of applying chemical seed dressing may lead to problems with plant diseases in the first weeks of their vegetation period. Additionally, natural colonisation by harmful microorganisms of seeds may have a negative effect on plant germination and health status of seedlings (Maude and Humperson-Jones 1980).

Recently in Poland we have been observing increased interest in OF rape (*Brassica napus* L.), despite its considerable requirements and required plant protection measures. Oilseed rape is considered as a species that is very difficult to cultivate in the organic farming. It is due to the requirements associated with rape nutrition and its protection against harmful organisms (Weiher et al. 2007). *Trichoderma* spp. are beneficial microorganisms, which are able to compete with several fungal pathogens and have been used in protection of many crops, including oilseed rape (Harman et al. 2004, Neri et al. 2008). Therefore, *Trichoderma asperellum* was included in the scheme of this study. Selection of this biocontrol agent was made taking into account that the scope of its effectiveness is very wide and it can be used in the form of the trade product available in Poland.

The aim of this study was to compare the incidence of pathogens in samples of rape seeds depending on the adopted cultivation system, i.e. integrated vs. organic. Although the general public opinion assumes that undamaged and older seed pods provide a natural, mechanical barrier against oviposit (Pavela et al. 2007). Science authors did not find in the literature data on the potential protective role against chemical or microbiological pods contamination, so it was decided to prepare an experiment about this issue.

MATERIAL AND METHODS

Cultivation of winter rape in the organic system. In the years 2012–2013 winter rape was grown at the Institute of Plant Protection – National Research Institute (IPP-NRI) Field Experimental Station at Winna Góra, Poland. Every year the same cultivation schedule was repeated as a large-area experiment (500 m^2 per plot, 4 replications in Latin square design). In the experiments with organic crop, a commercial product called Plantos (0-8-18) at a dose of 150 kg/ha with the content of particular elements was used as follows: P of 5.3 kg/ha, K of 22.4 kg/ha, Mg of 7.2 kg/ha and S of 18 kg/ha was applied. Plantos is in line with law regulation and it is qualified for organic plant production in Poland (Kowalska 2014). Additionally, organic part of field was fertilized with cattle slurry at a dose of 160 L/ha and diluted with 140 L/ha water (equivalence of 80 kg of nitrogen), applied by spraying. The cattle slurry was obtained from an extensive farm, and the products not allowed in organic farming were not used in animal nutrition.

Undressed seeds of winter rape cvs. Californium and Bogart were used. In order to stimulate plant development *T. asperellum* was applied (5×10^8 of conidia per gram of commercial product contain isolate T1 NCAIM 68/2006). Foliar sprays were performed twice at 500 L water/ha at the BBCH 61 and BBCH 67 phases, applying product at 200 g/ha. Two spinosad treatments were applied against *Meligethes aeneus* Fab. at the BBCH 53–59 phase as foliar sprays of Biospin 120 SC at 24 g a.s. (active substance)/ha.

Cultivation of winter rape in the IPM system. In the years 2012–2013 winter rape was grown at the IPP-NRI Field Experimental Station in Winna Góra. Prior to rapeseed sowing mineral fertilization was applied over the entire experimental area using a commercial product Amofoska at 450 kg/ha, which supplied nutrients at N 18 kg/ha, P 23.8 kg/ha, K 41.5 kg/ha, Ca 25.6 kg/ha and S 18 kg/ha. Additionally, in the spring seasons ammonium nitrate enriched with micronutrients was applied twice, introducing a total of 130 kg N/ha.

Dressed seeds of winter rape cvs. Californium, Bogart, Monolit, Pamela and Visby were used for the experiment. Treatments with chemical PPPs were applied twice in autumn at the early plant development phase and three times in spring using commercial preparations containing acetamiprid, boscalid, chlopyrifos, clomazone, dimethenamid, metazachlor, metconazole, prochloraz and tebuconazole.

Collection of material for laboratory analyses. Seed samples were collected during harvest time from both organic and integrated cultivation systems from three different locations of each plot. Rapeseed was harvested using a Wintersteiger Classic plot harvester (Ried, Austria). Seeds were used for mycological and pesticide residue analyses.

Mycological analysis. Seeds of winter rape were sterilised for 4 min in 5% sodium hypochlorite. The test is carried out on a working sample of 400 seeds of each cultivar. One sample was divided to four

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subsamples with 100 seeds and each one hundred was treated as 1 replication. Seeds were placed on Petri dishes with potato medium (PDA pH 6.5) (ISTA 2015). *Alternaria* was identified using the CW medium which consists of 30 g galactose, 3 g calcium nitrate, 1 g dipotassium hydrogenphosphate and magnesium sulfate, 20 g agar, as well as 100 ppm benomyl and chloramphenicol per liter (Wu and Chen 1999), while OA (oatmeal agar) medium was used for *Leptoshaeria* spp. (Boerema et al. 2004). Moreover, the identification was also made based on morphological characteristics of fungi. Results are presented in the form of the mean percentage of obtained species.

Pesticide residues analysis. Analyses were performed using the procedure of pesticides fast extraction with acetonitrile in the presence of a buffer mixture, followed by purification by dispersive solid phase extraction. Finally, the extracted compounds were identified using liquid chromatography mass spectrometry (Drożdżyński and Kowalska 2009).

Statistical analysis. Fungi analyses were conducted using the Statistica 8.0 programme (StatSoft, Poland) followed by the Tukey's test at the level of significance $P < 0.05$. Data expressed as percentages were arcsine transformed before being analysed.

RESULTS

The seeds derived from cv. Californium OF were the most infected by fungi. Infestation rate in seeds of this cultivar was 3% lower in IPM compared to OF. Seeds of remaining cultivars were less colonised than cv. Californium. In case of cv. Bogart a similar colonisation rate by fungi was recorded in both cultivation systems. Seeds of cvs. Visby and Monolit from IPM system were least colonised by fungi (Figure 1).

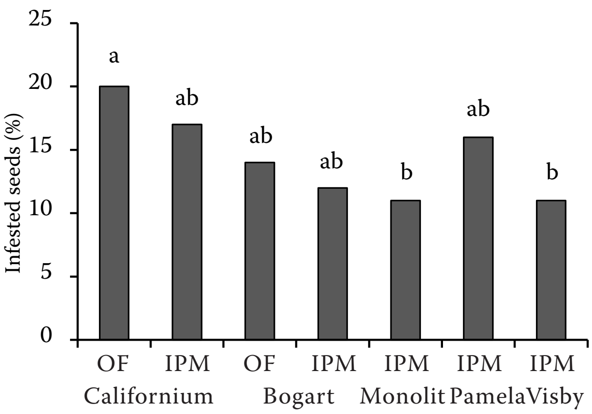

Figure 1. Mean percentage of seeds infested by fungi. IPM – intergrated pest management; OF – organic farming

Among the genus *Alternaria*, species of *A. brassicae*, *A. brassicicola* and *A. alternata* were isolated the most numerous, regardless of cultivar. It was found that the pathogen *A. brassicea* more frequently infected seeds of cvs. Californium, Pamela, Bogart and Monolit. A similar phenomenon was also reported in the case of *A. alternata* that infected the most seeds of cv. Bogart in both systems. Only in case of *A. brassicicola* was observed that seeds delivered from organic cultivated Californium and Bogart cultivars were less contaminated compared to seeds received from integrated system (Figure 2a).

The highest numbers of *L. maculans* strains were isolated from seeds of cv. Californium OF as well as cv. Bogart OF and IPM. Seeds of cv. Californium IPM were significantly less colonised than seeds of cv. Californium OF. Also seeds of cv. Bogart OF were less infected by this pathogen. *L. biglobosa* ranked second among the isolated species of genera *Leptosphaeria*. The most colonised were seeds of cv. Californium OF. When comparing cv. Californium from both cultivation systems, seeds from IPM were significantly less infected compared to OF (Figure 2b).

The conducted analysis for the presence of pesticide residues in samples coming from the IPM detected three fungicides permitted in integrated culture of winter rape. They were the following active substances: metconazole, prochloraz and tebuconazole. Concentrations of all the compounds were close to the lower determination limit of 0.01–0.02 mg/kg, calculated based on validation data for the applied testing method. Seed samples from OF (cv. Californium OF and cv. Bogart OF) and for cv. Bogart IPM did not show the presence of chemical pesticide residues above their chromatographic detection limits (Table 1).

DISCUSSION

Colonisation of rapeseed plants by main pests and possibilities of control them in the organic system was discussed in the study of Kowalska (2014). Microbiological treatments based on

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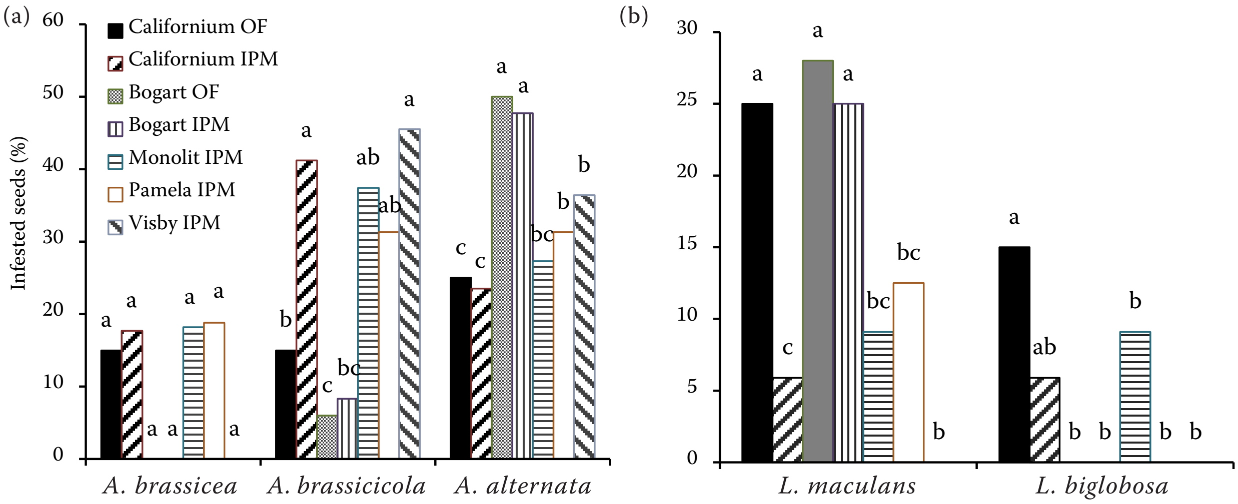

Figure 2. Mean percentage of seeds infected by (a) fungi from the genus *Alternaria*, and (b) *Leptospiroza maculans* and *L. biglobosa*. OF – organic farming; IPM – integrated pest management

T. asperellum performed in organic field can be useful in reducing disease incidence in different crops, a significant decrease in dry rot of cabbage and gray mould symptoms were observed (Kowalska and Remlein-Starosta 2011).

Cultivation systems can have a significant effect on the occurrence of specific contaminations of produced yield, included seeds. Apart from the cultivation system, the incidence of diseases and quality of yield are influenced by many factors such as cultivar characteristics (resistance), crop rotation, date of sowing, sowing rate, applied fertilization and tillage (Söchting and Verreet 2004, Rathke et al. 2006). Obtained results suggest that for seeds delivered from cvs. Californium and Bogart from both farming systems no differences in the intensity of fungal colonisation on seeds were observed. According to Bugge (2000) crop rotation with four years break in cultivation of oilseed rape and other cruciferous plant could reduce serious diseases problem. In case of both cultivation systems due to its rules of rotation could be important factor reducing seed fungal colonisation. Based on the obtained results it was

Table 1. Pesticide residues (mg/kg) detected in winter rape seeds in 2013

Investigated compounds (mode of action)	OF		IPM				
	Bogart	Californium	Bogart	Californium	Monolith	Pamela	Visby
Acetamiprid (I)	–	–	ND	ND	ND	ND	ND
Boscalid (F)	–	–	ND	ND	ND	ND	ND
Chlorpyrifos (I)	–	–	ND	ND	ND	ND	ND
Clomazone (H)	–	–	ND	ND	ND	ND	ND
Dimethenamid-P (H)	–	–	ND	ND	ND	ND	ND
Metazachlor (H)	–	–	ND	ND	ND	ND	ND
Metconazole (F)	–	–	ND	0.010	ND	ND	0.016
Prochloraz (F)	–	–	ND	0.014	0.020	0.021	0.022
Spinosad (I)	ND	ND	–	–	–	–	–
Tebuconazole (F)	–	–	ND	0.013	0.016	0.016	0.024

ND – below detection limit; F – fungicide; H – herbicide; I – insecticide; OF – organic farming; IPM – integrated pest management

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found that both the seeds from IPM and OF are infected by the fungus at a comparable level. This can imply that crop rotation is not significantly influencing the degree of microbiological purity of seeds. Probably physiological factors associated with cultivar or morphology of pods can be more important. This aspect of the research should be developed.

Rape seeds were colonised by 2 main groups of pathogens (*Alternaria* spp. and *Leptosphaeria* spp.). In our experiments only in case of *A. brassicicola* and *L. maculans* were observed differences between seed infestation in both cultivation systems. This observation could be connected with cultivation system but also it is possible that other characteristics connected of given cultivar may be of importance such as genetic resistance, response to weather condition or fertilization (Valantin-Morison and Meynard 2008). Our results confirm also variation in cultivar susceptibility to infestation and colonisation by individual fungal species. Brazauskiene et al. (2013) noted different reactions of cultivars to diseases. They observed the variability of response to fungicide treatment in tested cultivars. Nerad et al. (2009) described experiments with different rape cultivars grown in organic system and their response to diseases. They noted that the disease level (i.e. symptoms caused by *Leptosphaeria* spp.) was lower on cultivars with higher resistance. In investigation with IPM rape seeds treated and untreated with fungicides (Jajor et al. 2011) noticed the most frequent was *A. alternata*. This phenomenon is consistent with our observation, because both in IPM and OF systems seeds contaminated by *A. alternata* were found.

The level of colonization of the seeds by *Alternaria* fungi probably depends mainly on the weather conditions prevailing during the growing season and there were no significant differences between plots treated and untreated with fungicides.

Comparison of fungal infection of rapeseed coming from different cultivation systems (organic and IPM) is probably one of the first remarks. So far common opinions have existed that the seeds originating from organic crops can less germinate and plants can be heavily infested by pathogens at the first stage of development. These opinions were not scientifically confirmed. Our results have not confirmed stronger fungal infection in organic seeds compared with seeds received from integrated system. The authors did not find other papers discussing on incidence of harmful microorganisms on the seed surface, generally.

It may also be inferred that pesticide residues influence the microbial composition of colonising seeds. In the case of all investigated samples of rape seeds, in which PPPs residues were detected, a weaker colonisation by *L. maculans* and *L. biglobosa* was observed, while that caused by *A. brassicicola* was stronger. No correlation was found between the presence of pesticides residues and degree of fungal infestation based on the obtained results. It requires a broader research and should be continued. It could be explained by sensitivity of fungi from the genus *Leptosphaeria* to contamination with fungicide residues (West et al. 2002) and *A. brassicicola* may exhibit increased resistance to applied PPPs.

Analysing the results from the tested samples from the trials at the IPP-NRI Winna Góra Experimental Station we observed a lack of residue in samples of organic seeds and sporadically slight concentrations of pesticide residue in samples from the integrated system. In seeds coming from OF no residue was detected of the only insecticide used at later developmental stages of organic oilseed rape, i.e. spinosad. Spinosad is a contact active substance and it is mainly present on the surface of plants, where it may be washed or degraded under the influence of insolation and higher temperatures. It is of interest that in contrast to spinosad applied in the OF system, all substances detected in rape seeds come from systemic preparations and penetrated to seeds during the vegetation period through the root system and leaves, avoiding the protective action of rape pods. It also needs to be stressed that the compounds detected in samples were only fungicides, at the absence of residue of herbicides, the most numerous group of pesticide preparations. It may be assumed that this is directly connected with the application method of herbicide preparations, most frequently onto plants at an early stage of vegetation or to soil before sowing (Kierzek et al. 2011), while pesticides are analysed only in the final product, i.e. seeds, several months after the plant protection treatment. In the case of fungicides and insecticides it is the substances from these groups that are applied at practically all development phases of plants (also during storage) and their residues may still be present in seeds.

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