The influence of the maturity regulators on the number of selected microorganisms groups settling rapeseed's surface

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Abstract

The microorganisms are in the different of habitants, they are also on the surface of seeds of cultivated plants. Quantitive and generic composition of saprophagan air-plant microflora is very diversified and still it is not well known. Microbial populations of seeds depend on numerous factors. Chemical constitution of seeds and different abiotic environment factors have the significant influence on microflora populations of seeds. This factors, that is desiccant and plant growth regulator, also cause the changes in the microbial populations of rapeseeds. Diversity of microorganisms of seeds have the significant impact on the quality of food products. The toxinogenic fungi living on the surface of rapeseeds and their toxic metabolits are health hazard. The aim of this study was to evaluate of the influence of the desiccant (Reglone and Randap) and plant growth regulator (Harvarde and Spodnam) on the total number of bacteria, fungi and yeast, amylolytics mocroorganisms, proteolytic and lipolytic bacteria and fungi. The investigation was made on the rape seeds cultivar Californium. Composition of microbial populations and their activity can be indicate the accumulation of the applicated plant pesticides on the rapeseeds and their toxicity.

Key words: rapeseed, maturity regulators, microorganisms

Introduction

Microorganisms occur in variable environments and Their existence is connected with conditions in them. One of the natural environments of microorganisms' occurence saprophytic, phitopathogenic and pathogenic are seeds (Bielecka et. al., 1994; Majchrzak et al., 2002; Mrugas & Gwiazdowski, 1998). The seeds contain proteins, fats and mineral salts – variable nutritious substrats for phisiological different groups of organisms. Both bacteria and fungus have possibility to decompose organical compounds, contributing to losses accuring while seeds storage (Grabska et al., 1994). Technological moisture content is generally a barrier limitating bacteria's development. Fungus can still develop because of having reduced water level in the cell. That is the main cause of seeds' decay. Moreover some of the fungus can produce toxic metabolits – carcinogenic mykotoxins (Chełkowski, 1985; Korniłłowicz-Kowalska et al., 2000). The development of epifitical microorganisms is influenced not only by chemical composition of rapeseedss, but also by storage conditions applied during plants' vegetation and just before crops.

Material and Methods

The aim of conducted research was to define the number of specific groups of microorganisms growing on the surface of winter rapeseed 'Californium', in the cultivation of which following chemical protection agents were used: Reglone, Randap, Harvarde and Spodnam. Doses of individual agents were consistent with producents' recommendations.

Moreover the aim of the research was the genus and spiecies identification of fungus.

Following research objects were specified:

1. seeds cultivated without any chemical protection agents – control;
2. seeds cultivated with presence of "Reglone" disicant;
3. seeds cultivated with presence of "Roundup" desiccant;
4. seeds cultivated with presence of maturity regulator – 'Harvade";
5. seeds cultivated with presence of 'Spodnam" – siliques cracking limiting agent;

Samples were taken directly from fields during combine harvest. Microbiological research (bacteria and fungus) were conducted according to PN-ISO 7698 (2004) and on the basis of standard grounds for chosen physiological groups of microorganisms according to Burbianka (1983). They included the number of following microorganism groups: bacteria, fungi, proteolytic bacteria and fungi, lipolytic bacteria and fungi and amylolytic bacteria. Genus and species identification of fungus material was conducted on the basis of micro – and macromorfological observations with the use of systematical studies (Barnett & Hunter, 1987; Domsch & Gams, 1972; Marcinkowska, 2004).

Results and discussion

Conducted research confirmed that numerous microflora encompassing variable fisiological groups of bacteria and fungus occur on the rapeseeds' surface. The number of microorganisms present on rapeseeds depended on the kind of chemical protection agents from the group of desiccants and vegetation regulators used. The bacteria-contamination level of

rapeseeds was quite variable. The greatest number of these microorganisms was stated in the control object (1). The significant reduce of general number of bacteria on rapeseeds' surface, which were taken from the remaining experimental objects (2-5) indicates the restraining influence of the applied chemical agents on the tested microorganisms' development. The greatest reducement of bacteria number on the collected rapeseeds in relation to the control material was stated as an effect of the desiccant's Reglone action (object 2). However, only slight number reducement of these microorganisms occurred after using siliques cracking limiting agent "Spodnam" (object number 5). On the seeds collected from the remaining two objects (3 and 4) on which Roundup and Harvade were used, the number reducement on the similar level was stated (table 1).

Tabela 1. The number of different microorganisms groups settling rapeseed's surface in the different treatments (treatment 1 – rapeseeds control; treatment 2 – rapeseeds – Reglone, treatment 3 – rapeseeds – Randap, treatment 4 – rapeseeds – Harvade, treatment 5 – rapeseeds – Spodnam)

Numbers of selected microorganisms groups [CFU · g^{-1} seeds]	Treatments				
	1	2	3	4	5
Bacteria	$3{,}5 \times 10^5$	$7{,}8 \times 10^3$	$3{,}8 \times 10^4$	$3{,}3 \times 10^4$	$13{,}7 \times 10^4$
Fungi	$1{,}7 \times 10^4$	$1{,}5 \times 10^4$	$6{,}7 \times 10^4$	$3{,}5 \times 10^4$	$1{,}9 \times 10^4$
Proteolytic bacteria	$5{,}2 \times 10^4$	$7{,}6 \times 10^3$	$1{,}7 \times 10^4$	$1{,}5 \times 10^3$	$1{,}5 \times 10^3$
Proteolytic fungi	$3{,}2 \times 10^1$	$3{,}0 \times 10^1$	$3{,}5 \times 10^1$	$4{,}5 \times 10^1$	$5{,}6 \times 10^1$
Lipolytic bacteria	$1{,}9 \times 10^4$	$7{,}4 \times 10^2$	$3{,}5 \times 10^3$	$3{,}8 \times 10^3$	$9{,}1 \times 10^3$
Lipolytic fungi	$4{,}1 \times 10^2$	$4{,}6 \times 10^2$	$1{,}9 \times 10^2$	$6{,}9 \times 10^1$	$1{,}7 \times 10^2$
Amylolytic bacteria	$4{,}1 \times 10^2$	$6{,}5 \times 10^2$	$4{,}5 \times 10^2$	$4{,}3 \times 10^2$	$8{,}4 \times 10^1$

The data from table 1 indicate not significant, in comparison with the initial material (object 1), fungus number fluctuations on the seeds from objects exposed to the chemical protection agents' action. The fall of the fungus number was tatted only in the object 2, in which the seeds were exposed to the Reglone's action. Application of the remaining desiccants and rapeseeds vegetation regulators (objects 3-5) was conductive to the increase in fungus number. The greatest frequency of fungi were stated in he case of the rapeseeds from object 3, in which Roundup was applied.

Moreover, bacteria and proteolytic fungi were isolated from the rapeseeds' surface. Conducted research has proven that the examinated rapeseeds were settled in greater extent by bacteria than by fungus with enzimatical aptitude to the proteins decomposition. According to Bielecka's et al. research (1994) has proven, the high rapeseeds' moisture and degree of the damage influence the development of proteolytic microorganisms. From the data in table 1 follows, that chemical protection agents have in a large extent reduced the number of proteolytic bacteria occurring on the rapeseeds' surface (in comparison with the control material). The lowest number of examined microorganisms was isolated from the surface of rapeseeds which were exposed to the action of vegetation regulators Harvade and Spodnam (objects 4 and 5). Roundup has caused only slight decrease in the number of proteolytic bacteria. The number of fungi with proteolytical capabilities in objects 2 and 3 was exposed to only slight fluctuations in comparison with the initial material (object 1). The application of vegetation regulators Harvade and Spodnam was conductive to the increase in number of proteolitical fungus (objects 4 and 5).

Lipolytic bacteria and fungi were the next group of examined microorganisms. They deserve the extraordinary attention because development of these microorganisms can cause lipids' hyrdrolise – what is the reason of increase in lipidical acidity and in great extent enables the rapeseeds' damage by moulds (Grabska et al., 1994). Conducted research has proven that all the chemical protection agents applied lowered in a large extent the bacteria-settlement on the rapeseeds in comparison with the control material (object 1). In general the number of lipolitical fungus has also lowered, in exception with the object 2 (Reglone) in which only slight increase in the number of these microorganisms on rapeseeds was stated.

During research, also number of amylolytic bacteria was investigated. Research indicates that quantity of these microorganisms generally increased in comparison with control material. Only in case of object 5 (Spodnam) an increase of number of investigated microorganisms in comparison with control object was stated.

In this paper it was moreover stated that quantity changes of fungus which settle rapeseeds are accompanied with genus changes of these microorganisms (table 2). During conducted research occurrence of 160 isolates belonging to 10 species and 8 genuses was distinguished. Moreover it was proved that fungus' colonies settling rapeseeds' surface were characterised by similar genus composition in all objects that were investigated. Observed differences were mainly quantitive. Analysis of distinguished fungus' species composition revealed that in the control object (1) and in the object exposed to Randap agent action (3) mainly two species occurred. That were: *Alternaria alternata* and *Cladosporium cladosporoides*. In objects 2 and 4 *Alternaria alternata* and *Penicillium glabrum* and in object 5 *Alternaria alternata* fungus which are tipical rapeseeds' micloflore were observed (Mrugas & Gwiazdowski, 1998). On rapeseeds' surface in objects 1 and 2 toxical alergetive fungus appeared sporadically: *Aspergillus flavus* and *Penicillium citrinum*.

Conclusion

Research proved that in general chemical protection agents from the disicants and riping regulation agents group that were used significantly lowered quantity of investigated miroorganisms' groups which occur on rapeseeds' surface. Chemical protection agents which were used, signifiantly influenced changes in qualitative composition of investigated fungus' colonies. Generally on rapeseeds' surface fungus *Alternaria, Cladosporium* and *Penicillium* are settled.

Table 2. Lists of fungi isolated from rape seeds in the different treatments

Fungi	Number of isolats on the rapeseeds in the particular treatments				
	1	2	3	4	5
Acremonium strictum	0	0	3	1	0
Alternaria alternata	17	6	17	8	20
Alternaria brassicola	*0*	*0*	*0*	*2*	*0*
Aspergillus flavus	2	0	0	0	0
Cladosporium cladosporoides	13	1	19	4	0
Cladosporium herbarum	*3*	*1*	*2*	*0*	*6*
Mucor sp.	0	0	0	1	0
Penicillium citrinum	0	4	0	0	0
Penicillium expansum	2	1	0	0	0
Penicillium glabrum	0	7	1	14	2
Phoma glomerata	0	0	0	1	1
Rhodotorula	0	0	0	1	0
Total	37	20	42	32	29

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