

SUMMARY

The PhD thesis is structured in accordance with the norms, on two parts: first part- bibliographical study- is extended on 83 pages, represents 31% of the volume of the work, and the second- personal research- covers 182 pages, representing so 69% of the total of 265 pages of the paperwork. Within the thesis are exposed 78 figures and 33 tabels.

The first part, „The current state of knowledge”, which includes five chapters, is a brief overview of the main aspects of the literature relating to the means and methods of oilseed preservation, the bibliographic material being founded on the consultation of 214 scientific papers from the country and from abroad.

The first chapter, entitled „Oilseed quality assessment”, highlights the biological value of the three varieties of seed used in the context of this research- soy beans, sunflower seeds and pumpkin seeds, in this division being also mentioned some considerations in relation with technical analysis.

*The second chapter describes the main features of micromycetes, and the genera frequently involved in the contamination of seeds, the literature providing consistent studies on systematic categories of pathogens which colonize the vegetal substrates during the period of conservation. For this reason, the *Aspergillus*, *Penicillium* and *Fusarium* genera have received a wider exposure in this chapter, „Description of the pathogens”.*

In the third chapter are briefly presented the most effective antifungal substances used in vegetal substrates conservation, natural, produced or extracted from plants, and synthetic, insisting on the organic acids: acetic, lactic, propionic, benzoic and formic.

Because the vegetal material has been represented by seeds with high nutritional value, it was also studied the degree in which the incriminated pathogens- the fungi, and chemicals used as preservatives may influence the germination capacity of seeds and, in consequence, the production per hecatre, the analyzed data being synthesized and exhibited in the IVth chapter

(„Fungi action on seed germination capacity”) and the Vth („Influence of organic acids on seed germination capacity”).

The second part of the thesis consists of eight chapters, which include both the discussion and the interpretation of the results and also the general conclusions. To comply with the norms in force regarding the editing and the structuring ways of a doctoral thesis, this part begins with two chapters in which are presented the research goal and objectives, and the natural, organizational and institutional framework in which the research activity was conducted.

The purpose of the work has been the evaluation of the efficacy of lactic and acetic acids, used separately or in combination, for the conservation of oilseeds. The strategic objectives of these vast investigations were ordered in a logical sequence, whose conduct has imposed the following stages:

- the determination of the antifungal effect of lactic and acetic acids in soybean, sunflower seeds and pumpkin seeds conservation, which were artificially contaminated with an inoculum of fungi, the samples being maintained under laboratory conditions for five months;

- the assessment of the preservative capacity of lactic and acetic acids by quantitative and qualitative micological examinations, the oilseeds that have received this treatment being stored for ten months in production conditions, a confined space, arranged for that purpose, and a shed, which only provided protection against hydrometeors;

- each experiment started with an examination of the quality of the vegetal substrates, based on the organoleptic characteristics, physicochemical properties and germination capacity;

- the evaluation of the influence exerted by fungi and chemicals on the germination capacity of soybeans, sunflower seeds and pumpkin seeds in distinct experiments.

The first stage of the research was carried out in the Laboratory of Mycology and Micotoxicology within the Faculty of Veterinary Medicine, from Iași, a place that has adequate space, necessary equipment and materials for the performance in optimal conditions of the research activity. In the pilot phase, the experiments have been carried out in a commercial society, whose object of activity is represented by the production and the export of seeds for sowing and consumption. The necessary research to determine the degree of purity, moisture and seed germination capacity was made in the laboratory of analysis within the Territorial inspectorate for the quality of seeds and seedling, in Iași.

The assessment of the antifungal activity of lactic and acetic acids is described in detail in the eighth chapter of the thesis, entitled “The efficiency of organic acids evaluation in oilseed conservation in laboratory conditions”. To select only those concentrations of acids that exhibit a powerful antifungal effect in order to use them in seed treatment that are to be stored for a

period of ten months, as is natural, the antifungal capacity of the inferior fatty acids was tested in the lab, the chemical agents being dispersed in different concentrations over the samples, which were first contaminated with a suspension of fungi belonging to Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium and Stachybotris genera.

As a result of quantitative and qualitative mycological investigations performed on a monthly basis, it was found that all chemical formulations have expressed a high antifungal effect, the most effective substances in soybean conservation proved to be the acetic acid and the mixture in equal parts between the two organic acids. The fungal species constantly identified in the samples chemically treated belonged to the genera Penicillium, Cladosporium and Aspergillus. The microflora which characterizes the blanks was dominated by fungi belonging to the genera Aspergillus (A. niger, A. flavus), Penicillium and Cladosporium. In the case of sunflower seed, the results were similar to those recorded on soya beans, the acetic acid and the mixture of acids, particularly in concentrations of 50%, 40% and 25%, showing a higher antifungal effect than lactic acid. Throughout the period of preservation, the dominant species of filamentous fungi isolated from this assortment have belonged to the taxonomic genera Penicillium, Alternaria, Aspergillus, Fusarium and Cladosporium. The pumpkin seeds behaved differently than the other two types of seeds, the efficiency of the chemical formulations in this category of seeds conservation being significantly lower, compared with the results observed on soy beans and sunflower seeds. In the case of this type of seeds, the mixture of 40% showed a surprising and superior antifungal effect than the other two substances, maintaining its values over 96% throughout the period of conservation. The pumpkin seed microflora was made up of inferior fungi, which incidentally had the highest incidence, being observed continuously throughout the experimental period on all samples. Of the filamentous fungi introduced in the inoculum stood up A. niger, A. flavus and species of the genera Penicillium and Cladosporium.

In chapter IX are presented the results regarding the action of micromycetes on oiled germination capacity, the research of this preliminary test having a role in establishing with greater certainty if the low germination percentage are due to the action of organic acids or pathogens. Of the three kinds of seeds, soy beans were most sensitive to the action of fungi, the seed germination ability of the contaminated sample being with 24 per cent lower than that of the witness. The fungi that have had the greatest impact on these seeds were A. flavus, A. niger and the species of the genera Penicillium. The micromycetes of A. flavus had a lush development on husked grains, but also on the cotyledons, thus severely affecting the viability of the seedlings. A. niger have been observed in the form of a thistle, rarefied mycelium, situated exclusively on the root system and on the cotyledons of seedlings. Penicillium sp. and A. niger have produced the most reduced harmful effects on the development and viability of seedlings, compared to the

strains of A. flavus and the colonies of the inferior fungi, which proved much more aggressive and with the most severe effects. The viability of seedlings was affected both chemically, highlighted by an area of necrosis of tissues at the place of contact between the pathogen and the nutrient substrate and mechanically, due to their development over the entire surface of the seedlings.

Compared with the blank, the sunflower seeds contaminated with the artificial fungal suspension, had a lower germination capacity with six percent, 81%, the fungal species identified on this assortment (A. flavus, Penicillium sp., and the inferior fungi) colonizing, in particular, the protective envelope of seeds, without affecting the seedlings and their viability. In the case of the contaminated pumpkin seeds, the germination capacity was 78%, with 13 per cent lower than that of the blank. The fungi have been developed mainly on the sprouting seeds and on those in the early stages of development, which probably had the mechanisms of defence cancelled or greatly reduced.

The action of organic acids on oilseed germination ability was studied over a period of three months in the year 2010. The results highlights the sensitivity of the soy bean at the action of chemical agents, in particular of lactic acid. The effect of acids on this vegetal assortment may be due to the morphostructural particularities of the protective membrane, because the same substances have boosted the germination of sunflower seeds and pumpkin seeds. In consequence, these acids are not recommended for the conservation of soybeans intended for sowing, only for those intended for consumption. On the sunflower seeds, exclusively, the chemicals have had a temporary slowing effect on seed germination, the seedlings from the samples treated with acids being smaller than those in the control group. Although the acids influenced the germination energy, they stimulated further the development of the seedlings, who were healthier and less affected by fungi. Acids scattered on pumpkin seeds have not influenced negatively their germination capacity, on the contrary, the samples treated with lactic acid and with the mixture of acids have recorded higher values of the percentages of seed germination versus that of the reference sample.

In chapter XI, entitled "Elaboration of some effective methods of oilseed preservation in production circumstances using organic acids", are presented the results of the extensive investigations that have targeted the evaluation of antifungal ability of organic acids as efficient alternatives, both in terms of sanitary-hygiene, as well as economically, in the chemical treatment of seeds stored in a warehouse, in bulk, and in a shed, in raffia bags, for a period of ten months. During this period, the main parameters of microclimate, temperature and humidity, were monitored from the both spaces of seed storage. In the warehouse, from November to May, the mean values of temperature have vacillated between 13°C and 16°C, in the last months,

corresponding to the summer season, rising from 18°C to 22°C. The relative humidity of the storage depot has varied between 47% and 62%, depending on the season.

The parameters of microclimate of the shed presented significant differences compared to those from the warehouse. The mean values of temperatures were between -5°C and 29,5°C, being negative during the winter months and quite high in the summer. The atmospheric humidity varied between 62,8%, and 85%, being influenced by the seasonal climate changes.

Regarding the effectiveness of lactic and acetic acid in soy bean conservation, it was observed that, in different storage conditions, the two chemicals, concentration of 10%, alone or in mixture spread on the surface of the grains can be used successfully in the treatment of this type of seed, when the lots are stored in a space where the microclimate factors varies little throughout the storage period.

*The fungal categories which showed an increased resistance to the organic acids were represented by the species classified in the genera that usually colonize the vegetal substrates during the period of storage: *Penicillium*, *Cladosporium* and *Alternaria*. From May, the micromycetes of the genus *Penicillium* were developed almost exclusively on culture media, regardless of the examined sample.*

On sunflower seeds, the chemicals have revealed a variable antifungal efficiency, but strong enough for the entire period of storage. In consequence, when the seeds are stored for a long period of time, either in bulk or in raffia bags, for a good conservation it is recommended to use 25% acetic acid or the mixture in equal parts between the acid and lactic acid, both with 25% active substance. The results of the qualitative mycological investigations showed that the micromycetes identified on sunflower seeds were represented by the same genera as on soya beans.

The fungal load of the pumpkin seeds from the blank sample has maintained throughout the period of storage over the data recorded on the groups chemically treated, which demonstrates unequivocally that organic acids can represent an effective alternative to preserve this type of vegetal substrate. Of the three chemical formulations used in the conservation of pumpkin seed stored in raffia bags, in a shed, it can be successfully used the acetic acid or the mixtures of acids, in concentrations of 30%.

*The fungal species isolated and identified on pumpkin seeds were represented by the inferior fungi, the genera *Penicillium*, *Cladosporium*, and frequently by yeasts.*