HELIA, **36**, Nr. 59, p.p. 59-70, (2013) UDC 633.854.78:632.581.2
DOI: 10.2298/HEL1359059L

DETECTION OF *Rhizomucor pusillus* ON SUNFLOWER SEED

Lević, J.*, Ivanović, D., Stanković, S., Milivojević, M., Vukadinović, R. and Stepanić, A.

Maize Research Institute, Zemun Polje, S. Bajića 1, 11185 Belgrade, Republic of Serbia

Received: August 20, 2013
Accepted: December 05, 2013

SUMMARY

The accelerated ageing test method (AA), agar plate method (A) and blotter method (B) have been used to detect the *Rhizomucor pusillus* and other mycobita on 24 samples of sunflower seed. Sterilised and unsterilised sunflower seeds have been incubated at 25°C and 42°C in the dark for 72 and 144 hours. The fungus was not detected in any sample at 25°C, not even after 144 h incubation of seeds. The fungal frequency ranged from 58.3 (B method) to 75.0% (A method) and from 4.2% (B method) to 16.7% (AA method) after 72 h incubation of unsterilised and sterilised samples at 42°C, respectively. The fungal incidence was 25.5% (AA method), 21.9% (A method) and 20.3% (B method) after 72 h incubation of unsterilised seed, and 2% on sterilised seed in all three applied methods. By extended incubation of unsterilised and sterilised seeds up to 144 h at 42°C the frequency and incidence of the fungus did not significantly change. The results of the present research show that the AA test method, widely applied in seed longevity testing, can be used as a simple and efficient method for the detection of *R. pusillus* as well as other mycobiota on sunflower seed. Furthermore, these are the first data on determining the presence of *R. pusillus* in Serbia.

Key words: ***Rhizomucor pusillus*, methods, incidence, sunflower seed**

INTRODUCTION

Rhizomucor pusillus (Lindt) Schipper (syn. *Mucor pusillus* Lindt) is globally widespread and has been found in the UK, Chad, former Czechoslovakia, South Africa, Indonesia, India, Japan, the USA, Nigeria and Australia (Salar and Aneja, 2007). The fungus has been mainly detected on various types of seeds of cacao, barley, oat, maize, wheat, soya bean, rape, lupine and flax, groundnuts, nuts, and on fruits, different types of compost, municipal waste, horse dung, guayule, hay, herbs, bird's nests, air, water, submerged mud and soil (Nedzinskiene and Asakaviciute,

* Corresponding author: Fax: +381 11 37 56 707; e-mail: jlevic@mrizp.rs

2011; Salar and Aneja, 2007; Lugauskas *et al.*, 2006a; Kačergius *et al.*, 2005; Řezáčová and Kubátová, 2005; Adebajo and Popoola, 2003; Straatsma *et al.*, 1994; El-Hissy *et al.*, 1990; Niles, 1981). There are little data in available literature on the occurrence of *R. pusillus* in sunflower seed (Tansey, 1984).

According to Kačergius *et al.* (2005) the fungus is predominant in the air of stored grain, fruit storehouses and processed products. Furthermore, it is one of the major micobiota of stored vegetables (Adebanjo and Shopeju, 1993). The incidence of the fungus increased from the third (3-5%) to the ninth month (7-11%) in kola nuts during storage (Adebajo and Popoola, 2003). *R. pusillus* prevailed in one or more types of different ingredients of poultry feed (soya bean meal, ground maize, cotton-seed cake, wheat bran and fish meal) (Moharram *et al.*, 1989), in various foodstuff of floral origin (Lugauskas *et al.*, 2006b) or in buckwheat mass during different technological stages of groats production (Lugauskas *et al.*, 2006a).

R. pusillus is the only *Rhizomucor* species that causes diseases in humans (Chayakulkeeree *et al.*, 2006). Human infections caused by this fungus have been most frequently reported in the European Union and the United States, then in Canada, and one case in Australia, Brazil and Japan (Gomes *at al.*, 2011). It has been reported in cases of pulmonary, disseminated and cutaneous types of infection (Ellis *et al.*, 2007). Moreover, it has been more often associated with animal diseases, especially bovine abortion (Ellis *et al.*, 2007).

R. pusillus is a beneficial fungal species because certain enzymes produced by it are extensively used in the production of rennet in cheese manufacture (Neelakantan *et al.*, 1999). In addition, the fungus has been used in retting of flax for manufacturing natural fibres in the United States and Europe (Henriksson *et al.*, 1997). Generally, *Rhizomucor* species might be a prime candidate for the glycerol and biomass production (Millatia *et al.*, 2005).

There are no data on the detection of *R. pusillus* in papers printed in scientific journals in Serbia. However, we have detected an intensive development of this fungus on one sample in our previous tests on sunflower seed longevity by the accelerated ageing (AA) test method. Considering the above facts the aim of the investigation was to study some aspects of test methods used in detecting this fungus and to estimate its frequency and incidence on sunflower seed in Serbia.

MATERIAL AND METHODS

After the 2011 harvest, 24 samples of sunflower seeds were collected in 17 locations in Serbia. Samples were packed in paper bags and stored at 16ºC until tested for the presence of *R. pusillus*.

The detection of *R. pusillus* by the accelerated ageing (AA) test method (Hampton and TeKrony, 1995), widely used in seed longevity studies, was compared with

conventional seed test methods such as the agar plate medium (A) and the blotter (B) method (Mathur and Kongsdal, 2003) with some modifications.

According to the description of the AA test given by Hampton and TeKrony (1995) seeds were placed on the wire mesh screen tray, which was then inserted into the inner chamber - a plastic box (Hoffman Manufacturing Jefferson, OR, USA) containing 50 ml of distilled water (Figure 1a-c). Twenty five seeds per box were placed apart at a uniform distance to avoid contamination or fungal spread from infected to uninfected seeds (Figure 1e-f).

Malt extract agar (MEA) containing 20 g of malt extract, 20 g of agar and 1000 ml of distilled water adjusted to pH 6.5 was used for the agar plate method (Smith and Onions, 1983). Five seeds were placed in 90 mm Petri dish on the agar surface.

The initial preparation of the modified blotter method involves making sets of two and one layer of Whatman filter paper grade 1 of the appropriate size to be placed on the bottom and the lid of Petri dishes, respectively. The filter papers on the bottom of each 90 mm Petri dish were soaked with 5 ml and the filter paper on the lid with 2 ml of sterile water. Five seeds were distributed on the well water-soaked filter paper. At the first screening after 72 h incubation, sterile water was added if it was necessary to maintain filter paper moistened.

Fifty seeds each with and without pre-treatment at the incubation temperature of 25°C and 42°C were used for the fungal detection in each of the applied test methods. All samples with pre-treatments were surface-sterilised in 1% sodium hypochlorite for 3 min, rinsed three times with distilled water and then dried between two layers of soft paper. In all cases, the seeds were incubated in the dark for 144 h.

The first screening of the fungal presence was done after the 72 h and the final after the 144 h incubation. The preliminary identification of the *R. pusillus* on incubated seeds was done with a stereomicroscope (× 15-25) according to formed sporangiophores and heads with spores (Figure 2g). In order to reliably identify the fungus, the fragments of colonies developed on incubated seed (Figure 1g) were transferred to potato dextrose agar (PDA) and incubated under chamber conditions at 25°±1°C in the dark. Fungal subcultivation on carnation leaf agar (CLA) and incubation at 36±1°C and 42±1°C was used for fungal morphological studies. Media PDA and CLA were prepared according to Burgess *et al.* (1994). The fungus was identified after seven days as described by Ellis *et al.* (2007).

Beside *R. pusillus*, some other fungal species were identified on incubated sunflower seeds according to the description given by von Arx (1981).

The fungal frequency (F) and incidence (I) were estimated by following equations:

$$F(\%) = \frac{\text{number of seed samples in which a species occurred}}{\text{total number of seed samples}} \times 100$$

$$I(\%) = \frac{\text{number of seeds in which a species occurred}}{\text{total number of seeds)}} \times 100$$

The incidence rate of *R. Pusillus* in seed samples was classified as low (0 to ≤20), moderate (>21 to ≤50) and high (over 50%) (Lević *et al.*, 2012).

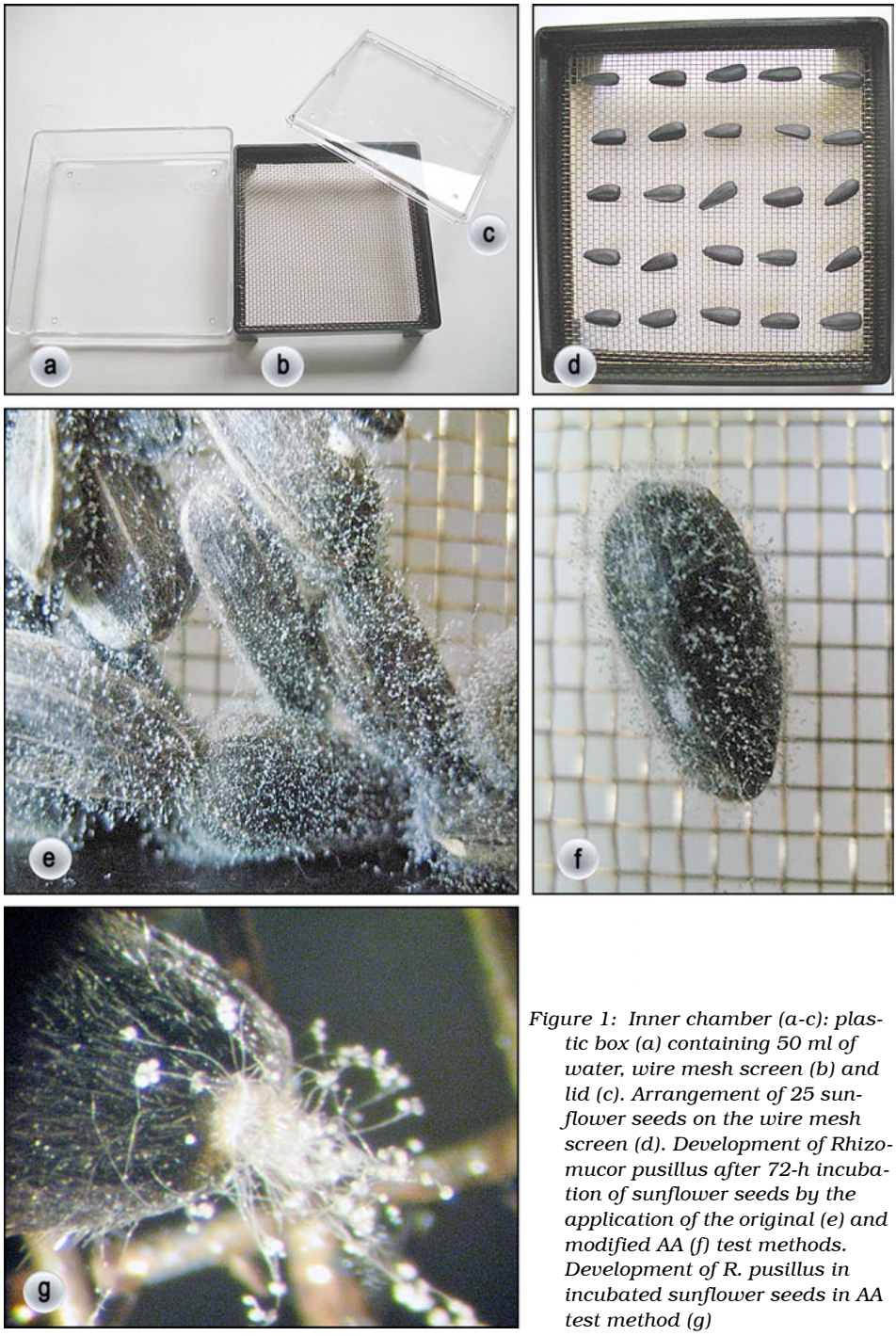

Figure 1: Inner chamber (a-c): plastic box (a) containing 50 ml of water, wire mesh screen (b) and lid (c). Arrangement of 25 sunflower seeds on the wire mesh screen (d). Development of Rhizomucor pusillus after 72-h incubation of sunflower seeds by the application of the original (e) and modified AA (f) test methods. Development of R. pusillus in incubated sunflower seeds in AA test method (g)

The correlation between the incidences of *R. pusillis* determined by three different test methods was calculated using the Spearman's rank correlation coefficient (Zar, 1972). The rank correlation coefficient (r_s) is expressed as: $r_s = 1 - 6 \Sigma d^2 / (n^3 - n)$, where n is the number of measurements in each of the two variates in correlation, $\Sigma d^2 = \Sigma_{i=1}^{n} d_i^2$, and d_i is the ranked difference between the i^{th} measurement for the two varieties. Student's t test was used for testing significance of r_s, as: $t = r_s / \sqrt{(1 - r_s^2)/(n-2)}$, with $n-2$ of freedom.

RESULTS

Detection of *R. pusillus*

The fungus isolated from colonies developed on incubated seed (Figure 1g) formed sympodially branched sporangiophores, globose sporangia with oval to pear-shaped columellae, hyaline, smooth-walled, globose to subglobose sporangiospores and rarely rhizoids on CLA at 36±1°C and 42±1°C. The size of sporangiospores increased several times before germination.

R. pusillus was identified in 22 and 21 out of 24 samples after 72 and 144 h seed incubation at 42°C, respectively (Table 1). However, the fungus was not detected in any sample when seed was incubated at 25°C (data are not presented).

AA method. The frequency of *R. pusillus* determined on unsterilised seeds by the AA method was 66.7% and 70.8% after 72 and 144 h incubation, respectively (Table 1). The incidence of the fungus was on average 25.5% after 72 h and somewhat higher (32.6%) after 144 h incubation. Depending on a sunflower seed sample, the fungal incidence varied from 2 to 100% after both 72 and 144 h incubation. In the majority of samples (11-13 samples) of unsterilised seeds the fungal incidence after 72h was low (≤20%), while it was moderate (21-50%) in up to two samples and high (>50%) in up to three samples. After 144 h incubation of unsterilised seed at 42°C the number of seed samples with a low fungal incidence reduced (from 11 to seven samples), while the number of samples with moderate and high incidence increased (from two to six and from three to four, respectively, Burgess *et al.,* 1994).

A method. The fungal frequency determined by the A method was at the same level (75.9%) after 72 and 144 h incubation of unsterilised seeds (Table 1). The variation range of incidence was not significantly changed after 144 h incubation (2-100%) in relation to 72 h incubation (2-98%). On average, the incidence of the fungus after 72 and 144 h incubation determined by the A method amounted to 21.9% and 30.9%, respectively.

B method. The application of the B method showed that the *R. pusillus* frequency on unsterilised sunflower seeds after 72 h incubation was somewhat greater than after 144 h incubation (58.3% *vs*. 54.2%) (Table 1). Similarly, the variation of the fungal incidence was fairly greater after 72 h incubation than after 144 h incu-

bation (2-100% *vs*. 2-98%). However, the incidence determined by the B method was, on average, greater after 144 h (24.6%) than after 72 h incubation (20.3%).

Table 1: Frequency and incidence of R. pusillus determined by three different test methods after 72 and 144 h incubation of unsterilised and sterilised sunflower seeds at 42°C

Sample No.	Location	*R. pusillus* incidence (%)											
		Unsterilised seed						Sterilised seed					
		72 h			144 h			72 h			144 h		
		AA	A	B	AA	A	B	AA	A	B	AA	A	B
2216	B. Palanka	8	16	12	26	8	8		2				
2187	Begeč		2			2							
2225	Čoka	46	48	18	42	82	42			2			4
2227	Čoka	50	24	38	60	84	36				2		
2229	Čoka	100	98	100	96	100	98						
2185	Feketić												
2218	Futog			4									
2219	Futog	2	2		28	8		2	2			2	
2223	Ilinci	8	4	2	30	4	2						
2224	Ilinci		6	2		10	4						
2182	Irig	2	4	4	10	20	2				2		2
2220	Jamena	6	4		4	4	8		2				
2221	Jamena		2	2		8	10						
2172	Kovačica	10	2		8	18					2		
2230	Kovilj	64	82	72	72	94	82						2
2184	Lugovo												
2174	Samoš	4		2	10		2						
2222	Šid	4	4		34	12		2			8		
2190	Sirig	14	8	2	36	10							
2189	Sivac		2			4							
2188	Srbobran	4			16			2			2		
2217	Srbobran	12	18	8	8	6	8	2					
2231	Srbobran	74	68	18	68	82	18						
2183	Vrbas				6								
Incidence (%)*		25.5	21.9	20.3	32.6	30.9	24.6	2.0	2.0	2.0	3.2	2.0	2.7
Frequency (%)		66.7	75.0	58.3	70.8	75.0	54.2	16.7	12.5	4.2	20.8	4.2	12.5

AA – Accelerated ageing test method
A – Agar plate method
B – Blotter method
* – Average of positive samples

Both, frequency and incidence of *R. pusillus* were drastically decreased on sunflower seeds when seeds had been sterilised (Table 1). The presence of the fungus on sterilised seeds was mostly determined by the AA method (up to 20.8% after 144 h incubation) in relation to remaining two methods (up to 12.5%). On average, the

fungal incidence determined on sterilised seeds did not significantly varied (2.0-3.2%) depending on either applied method or incubation period.

Table 2: Frequency and incidence of fungi other than *R. pusillus* and bacteria determined by three different methods after 72 and 144 h incubation of unsterilised and sterilised sunflower seeds at 25°C and 42°C

Species	IP (h)	Fungal incidence (%)											
		Unsterilised seed						Sterilised seed					
		25°C			42°C			25°C			42°C		
		AA	A	B	AA	A	B	AA	A	B	AA	A	B
Alternaria spp.	72	83.6	82.3	76.2				59.3	65.2	56.4			
	144	84.5	87.7	83.4				69.5	66.5	69.0			
A. flavus	72	15.3	12.5	4.0	14.7	16.0	4.8	6.8	7.0	3.2	11.0	12.1	3.0
	144	14.5	6.4	3.6	22.5	12.2	6.9	8.3	4.6	7.2	16.4	8.5	3.0
A. niger	72	8.0	3.0	6.0	6.0	9.3	2.3	2.0	5.0	4.8	4.9	7.0	2.0
	144	5.5	5.3	6.8	12.6	14.0	6.6	4.0	4.9	4.0	12.7	6.2	3.1
Aspergillus spp.	72	24.0	28.0	6.0	4.9	3.0	3.0	2.0	2.0		2.0		2.0
	144	21.0	3.4	10.0	7.2	6.8	4.0	7.6	3.0	2.0	6.7	3.0	3.3
Cladosporium spp.	72	17.6	29.9	31.2				9.4	14.9	15.7			
	144	20.1	31.4	17.8				9.2	16.4	5.8			
Penicillium spp.	72	4.0	6.0	3.3	4.6	4.5	4.0	4.0	2.5		3.2	2.0	
	144	10.9	3.0	8.5	39.5	14.3	4.6	6.0	2.7		15.1	5.0	4.5
Rhizopus spp.	72	17.4	26.0	22.9		4.9		2.0	3.6	4.0			
	144	25.0	26.7	19.3		4.7		2.0	5.4	3.5			
Other fungi	72	4.0	5.0	3.3	18.6	13.8	9.4		2.0	2.4	13.0	3.5	3.3
	144	6.0	6.0	8.0	27.5	16.1	9.6	2.0	4.3	8.5	14.2	2.0	3.0
Bacteria	72			2.0		12.1	9.7			2.0		7.5	4.9
	144		2.5	24.0		11.6	3.8			2.0		6.0	1.7
Incidence (%)*													
	72	21.7	24.1	17.2	9.8	9.1	5.5	12.2	12.8	12.6	6.8	6.4	3.0
	144	23.4	19.2	20.2	21.9	11.4	5.9	13.6	13.5	12.8	13.0	5.1	3.1
Frequency (%)**													
Min	72	4.2	8.3	12.5	37.5	4.2	20.8	4.2	4.2	4.2	4.2	8.3	8.3
	144	33.3	8.3	8.3	45.8	29.2	33.3	8.3	12.5	4.2	8.3	4.2	12.5
Max	72	100.0	100.0	100.0	79.2	79.2	54.2	100.0	91.7	100.0	29.2	25.0	25.0
	144	100.0	100.0	100.0	91.7	87.5	90.0	100.0	100.0	100.0	54.2	45.8	33.3
Average	72	38.5	47.4	40.7	62.5	52.4	37.5	29.2	21.4	29.2	18.3	16.7	17.5
	144	46.9	45.8	38.1	74.9	63.1	54.6	34.4	37.5	27.1	34.2	27.1	22.2

IP (h) – Incubation period (hour)
AA – Accelerated ageing test method
A – Agar plate method
B – Blotter method
* – Average of positive samples
** – Minimum, maximum and average frequency of fungi and bacteria

Detection of fungi other than *R. pusillus*

Aside from *R. pusillus*, the frequency and incidence of other fungi, as well as bacteria, were determined in the present study. Generally, the frequency and inci-

dence of fungi and bacteria were not more significantly changed by prolonged incubation of unsterilised and sterilised seeds. The majority had already reached their maximum after 72 h incubation (Table 2).

The frequency of fungi other than *R. pusillus* determined by AA, A and B methods on unsterilised seeds after 72 h incubation at 25°C ranged from 4.2-12.5 to 100% or on average from 38.5% to 47.4% (Table 2). In addition, the frequency of this group of fungi significantly changed at 42°C, hence minimum, maximum and average values amounted to 37.5, 79.2 and 62.5%, respectively. Furthermore, the frequency of these on sterilised seeds incubated at 25°C did not change significantly in comparison to unsterilised seeds. However, these changes were significant when sterilised seeds were incubated at 42°C, as minimum, maximum and average values were lower.

Table 3: Spearman's rank correlation coefficient calculated between incidence of *R. pusillus*[a] and fungi other than *R. pusillus* and bacteria[b] determined by three different methods under various temperature conditions and incubation periods of unsterilised seed

Fungi	Method	Incubation period					
		72 h			144 h		
		AA	A	B	AA	A	B
		Temperature 25°C					
R. pusillus[a]	AA	-	-	-	-	-	-
	A		-	-	-	-	-
	B			-	-	-	-
Other fungi and bacteria[b]	AA	-	0.842**	0.633**	-	0.825**	0.916**
	A		-	0.691**		-	0.800**
	B			-			-
		Temperature 42°C					
R. pusillus[a]	AA	-	0.807**	0.690**	-	0.710**	0.591**
	A		-	0.883**		-	0.691**
	B			-			-
Other fungi and bacteria[b]	AA	-	0.825**	0.687**	-	0.702**	0.820**
	A		-	0.841**		-	0.833**
	B			-			-

[a]The correlation was calculated on the basis of data presented in Table 1
[b]The correlation was calculated on the basis of data presented in Table 2
AA – Accelerated ageing test method
A – Agar plate method
B – Blotter method
** – Significant at $P < 0.01$

In regard to all fungi other than *R. pusillus*, the greatest incidence (76.2-83.6%) was identified for the *Alternaria* species on unsterilised sunflower seeds after just 72 h incubation at 25°C (Table 2). Under such conditions of incubation of unsterilised seeds the mean incidence was determined in species of the genera *Aspergillus*, *Cladosporium* and *Rhizopus*. The incidence of the majority of fungi incubated at 42°C was low with the exception of species of the genera *Aspergillus* and *Penicil-*

lium. Furthermore, the incidence of other fungal species but *Alternaria* spp. was significantly reduced on sterilised seeds, particularly on those incubated at 42°C. Bacteria developed more intensively on both unsterilised and sterilised seeds when the incubation temperature was 42°C.

Correlation

The Spearman's rank correlation coefficient indicated a significant positive correlation between the incidence of *R. pusillus* determined by the AA, A or B methods (Table 3). The high rank correlation coefficient also confirmed consent of results for the incidence of fungi other than *R. pusillus* determined by different methods applied.

DISCUSSION

Obtained results indicate that the *R. pusillus* is a widespread species in sunflower seeds in Serbia. These results were confirmed by the application of three different methods (AA, A and B) under laboratory conditions. In addition to advantages in the detection of *R. pusillus*, each of these methods had certain disadvantages. Correlation coefficients showed that the most similar results had been gained between AA and A test methods, while in some cases the greater difference in results had been obtained by AA and B test methods.

There are several advantages in the application of the accelerated ageing test methods (AA) in the detection of *R. pusillus* in relation to remaining two methods. This method is easy to apply because it is not necessary to prepare the medium or filter paper, then it provides high moisture throughout the incubation period and prevents both spreading of the fungus from one to another seed and the development of bacteria (Table 2). Based on the specific development on incubated seeds (Figure 1g), this fungus was easily detectable at a low magnifying of the stereomicroscope, even in the case when it was associated with other fungal species. The fungus mostly developed on the upper part of the sunflower seed.

The advantages stated for the accelerated ageing test method in the detection of *R. pusillus* were disadvantages for the agar plate test method (A). Spreading of the fungus from one to another seed was a particular disadvantage of the A method. The consequence of this could be that positive results were significantly higher than the actual one. The advantage of the A method was in the fact that fungi on this medium formed colonies specific for each of them and that they was visually recognisable.

The advantage of the blotter test method (B) was in the fact that fungi, particularly *R. pusillus*, did not spread from a seed to a seed and that in the majority of cases they form colonies specific for each of them. The disadvantage of this test method was that it took a long time to prepare filter paper and to maintain required moisture during incubation. Although, filter paper was moistened with the same

amount of water in all cases, in some Petri dishes it was necessary additionally to moisten filter paper after 72 h.

Surface sterilisation of seeds with 1% sodium hypochlorite for 3 minutes significantly reduced the frequency and incidence of *R. pusillus* (Table 1), but this did not happen with some other fungi (Tables 2). In certain cases, the frequency, *i.e.*, incidence of *R. pusillus*, depending on the applied test method and the duration of the incubation period, was decreased from 75.0% to 4.2%, *i.e.*, from 30.9% to 2.0%, respectively. These results indicate that the fungus predominantly developed on the surface of sunflower seeds, but they have to be proven by further detailed studies.

Furthermore, gained results show that the temperature of seed incubation was a more significant factor in the detection of *R. pusillus* than the applied test method or the duration of the incubation period were. Namely, the fungus was detected on sunflower seeds at the incubated at the temperature of 42°C. The frequency and incidence of the fungus detected after 72 h of seed incubation at this temperature did not significantly differ from the corresponding values recorded after 144 h incubation.

The fact that *R. pusillus* did not develop on sunflower seeds incubated at 25°C can be explained by the competition of other species of fungi. Namely, other fungal species developed at this temperature, particularly fungi of the genus *Alternara* that formed a very dense mycelium. In contrast to these fungal species, *R. pusillus* developed very slowly at this temperature, while its mycelium was very sparse.

According to the literature data, *R. pusillus* has been reported to be a thermophilic and hydrophilic zygomycete (Tansey, 1984; Johri *et al.*, 1999; Ellis *et al.*, 2007). Our results confirmed thermophilic properties of *R. pusillus* because the fungus had been determined when the sunflower seeds had been incubated at 42°C, but not at 25°C. Furthermore, the morphological properties of the fungus isolated from incubated sunflower seeds corresponded to *R. pusillus* described Ellis *et al.* (2007).

The obtained results show that the AA test method is suitable for the rapid and efficient determination of the *R. pusillus* presence in sunflower seeds under laboratory conditions. The application of this test method offered data on the differences between samples and the maximum value in the fungal incidence in a very short time (72h) on both unsterilised and sterilised seeds. Furthermore, this test method simultaneously provided the necessary conditions for the development of *R. Pusillus.* The incidence, as well as, the frequency of the fungus indicated that the *R. pusillus* was a widespread species in sunflower seeds in Serbia. These were the first results on the occurrence of this fungus in Serbia. According to our opinion and experience, this test method was also effective for the identification of this fungus in seeds of other plant species, but this have to be confirmed in further research.

In conclusion, the obtained results indicate that the presence of *R. pusillus* on sunflower seeds can easily be detected if some of the basic environmental requirements for fungal growth indoors are fulfilled. First of all, it refers to the temperature that has to be 42°C, while the environmental moisture is the second important requirement. The fungus is mostly eliminated by surface sterilisation of seeds. Nevertheless, the fungus may be important from the sanitary point of view if fresh sunflower seeds are used, or during storage under extreme conditions or in certain cases in food processing.

ACKNOWLEDGEMENTS

The study is a part of the research performed within the scope of the Project No. TR-31023 financially supported by the Ministry of Education and Science of the Republic of Serbia.

REFERENCES

Adebajo, L.O. and Popoola, O.J., 2003. Mycoflora and mycotoxins in kolanuts during storage. African Journal of Biotechnology 2(10): 365-368.

Adebanjo, A. and Shopeju, E., 1993. Sources and mycoflora associated with some sundried vegetables in storage. International Biodeterioration and Biodegradation 31: 255-263.

Arx, J.A. von, 1981. The Genera of Fungi Sporulating in Pure Culture. J. Cramer, Germany, pp. 424.

Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P. and Backhouse, D., 1994. Laboratory Manual for Fusarium Research. Sydney: University of Sydney and Royal Botanic Gardens, Australia.

Chayakulkeeree, M., Ghannoum, M.A. and Perfect, J.R., 2006. Zygomycosis: the re-emerging fungal infection. European Journal of Clinic Microbiology and Infectious Disease 25: 215-229.

El-Hissy, F.T., Moharram, A.M. and El-Zayat, S.A., 1990. Studies on the mycoflora of Aswan High Dam Lake, Egypt: monthly variations. Journal of Basic Microbiology 30: 81-94.

Ellis, D., Davis, S., Alexiou, H., Handke, R. and Bartley, R., 2007. Descriptions of Medical Fungi. (2nd ed.). Adelaide: Bibliography, Australia, pp. 204.

Gomes, M.Z.R., Russell, E.L. and Kontoyiannis, D.P., 2011. Mucormycosis caused by unusual mucormycetes, Non-*Rhizopus*, -*Mucor*, and -*Lichtheimia* species. Clinical Microbiology Reviews 24: 411-445.

Hampton, J.G. and TeKrony, D.M., 1995. Handbook of Vigour Test Methods. (3rd ed.). Zürich: The International Seed Testing Association, Switzeland, pp. 117.

Henriksson, G., Akin, D., Hanlin, R.T., Rodriguez, C., Archibald, D.D., Rigsby, L.L. and Eriksson, K-E.L., 1997. Identification and retting efficiencies of fungi isolated from dew-retted flax in the United States and Europe. Applied and Environmental Microbiology 63: 3950-3956.

Johri, B.N., Satyanarayana, T. and Olsen, J., 1999. Thermophilic Moulds in Biotechnology. Dirdrech: Kluwer Academic Publishers, The Netherlans, pp. 355.

Kačergius, A., Lugauskas, A., Levinskaité, L., Varbaité, R., Mankevičiené, A., Bakutis, B., Baliukoneiné V., Brūkštiené, D., 2005. Screening of micromycetes producing toxic substances under various conditions. Botanica Lithuanica Suppl. 7: 65-75.

Lević, J., Stanković, S., Krnjaja, V., Bočarov-Stančić, A. and Ivanović, D., 2012. Distribution frequency and incidence of seed-borne pathogens of some cereals and industrial crops in Serbia. Pesticide and Phytomedicine 27: 33-40.

Lugauskas, A., Raila, A., Railiene M. and Raudoniene V., 2006a. Toxic micromycetes in grain raw material during its processing. Annals of Agricultural and Environmental Medicine 13: 147-161.

Lugauskas, A., Raudonienė, V., Varnaitė, R., Dirginčiutė, V., Baliukonienė, V. and Bakutis, B., 2006b. Ecological and sanitary significance of micromycetes brought from abroad with various foodstuff of floral origin. Ekologija 3: 28-41.

Mathur, S.B. and Kongsdal, O., 2003. Common Laboratory Seed Testing Methods for Detecting Fungi. First edition, International Seed Testing Association, Bassersdorf, CH-Switzerland, pp. 425.

Millatia, R., Edebob, L. and Taherzadehc, M.J., 2005. Performance of *Rhizopus*, *Rhizomucor*, and *Mucor* in ethanol production from glucose, xylose, and wood hydrolyzates. Enzyme and Microbial Technology 36: 294-300.

Moharram, A.M., Abdel-Gawad, K.M., Megalla, S.E. and Mahmoud, A.L., 1989. Fungal flora of poultry feedstuff ingredients. Journal of Basic Microbiology 29: 491-499.

Nedzinskiene, L.T.R. and Asakaviciute, R., 2011. Development of fungi on *Lupinus angustifolius* L. and *Lupinus luteus* L. Research in Plant Biology 1: 20-29.

Neelakantan, S., Mohanty, A.K. and Kaushik, J.K., 1999. Production and use of microbial enzimes for dairy processing. Current Science 77: 143-148.

Niles, E.V., 1981. Microflora of imported cocoa beans. Journal of Stored Products Research 17: 147-150.

Řezáčová, V. and Kubátová, A., 2005. Saprobic microfungi in tea based on *Camellia sinensis* and on other dried herbs. Czech Mycology 57: 79–89.

Salar, R.K. and Aneja, K.R., 2007. Thermophilic fungi: taxonomy and biogeography. Journal of Agricultural Technology 3(1): 77-107.

Smith, D. and Onions, A.H.S., 1983. The Preservation and Maintenance of Living Fungi. Page Bros (Norwich) Ltd, Mile Cross Lane, Norwich, Norfolk, pp. 51.

Straatsma, G., Samson, R.A. Olijnsma, T.W., Op den Camp, H.J.M., Gerrits, J.P.G. and van Griensven, L.J., 1994. Ecology of thermophilic fungi in mushroom compost, with emphasis on *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. Applied and Environmental Microbiology 60: 454-458.

Tansey, M.R., 1984. Efficient isolation of thermophilic and thermotolerant mucoralean fungi. Mycopathologia 85: 3-42.

Zar, J.H., 1972. Significance of the Spearman rank correlation coefficient. Journal of American Statistical Association 67: 578-580.