BIO-ECOLOGICAL RELATIONS OF SUNFLOWER PATHOGENS – *MACROPHOMINA PHASEOLINA* AND *FUSARIUM* SPP. AND SUNFLOWER TOLERANCE TO THESE PATHOGENS

Sonja Tančić[1]*, Boško Dedić[1], Aleksandra Dimitrijević[1], Sreten Terzić[1], and Siniša Jocić[1]

Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia
*Corresponding author. E-mail: sonja.tancic@ifvcns.ns.ac.rs

ABSTRACT

Macrophomina phaseolina, *Fusarium solani* and *F. oxysporum* are soil borne species which infect root, stem and collar region of plant host and cause cortical and vascular discoloration. There is need for identification of these pathogens at genus, species and isolate level. *Fusarium* spp. are mainly cosmopolites while *M. phaseolina* is prevalent in arid regions, but can be found in moderate climates when high temperature and dry conditions occur. The genera *Macrophomina* and *Fusarium* have some common hosts, such as sunflower, and tend to form mixed infections under favourable weather conditions. Coexistence of these pathogens, defining their relationship-type in a community and possibility of their additive pathogen effect, as well as dynamic of necrosis development on sunflower seeds were the aim of this research. Isolates of *M. phaseolina* and *Fusarium* spp. obtained from seven localities in Vojvodina (Northern Serbia), were tested in confronted colonies test and in pathogenicity test on three experimental sunflower hybrids.

In the research, two types of interaction between *M. phaseolina* – *Fusarium* isolates were registered. Isolates of *M. phaseolina* in comparison with *Fusarium* isolates had faster growth, which resulted in significant growth inhibition of *Fusarium* isolates during confrontation (41.11-50.36%). Among *Fusarium* spp. isolates, the least pathogenic were isolates originated from Kuštin (41.92% and 40.06%), while the most pathogenic on sunflower seeds of all three hybrids tested was isolate from Pančevo (79.08%). *Fusarium* isolate from Pančevo was also the most aggressive in the confronted colonies test. The most pathogenic, on all three sunflower hybrids, of all *M. phaseolina* tested isolates, was isolate from Zrenjanin (68.14%), while the least pathogenic was isolate from Rimski Šančevi (47.37%). No additive pathogenic effect on sunflower seeds in a test with mixed pathogen suspensions was observed. Among three tested hybrids, NS-H-V4 was the most susceptible to *Fusarium* spp. and combinations of *Fusarium* + *M. phaseolina*, while NS-H-P3 was the most susceptible to *M. phaseolina*. Necrosis development dynamic was more dependent on resistance level of the hybrid than on the pathogen species.

Key words: *M. phaseolina*, *Fusarium* spp., sunflower.

INTRODUCTION

*M*acrophomina phaseolina (Tassi) Goid. is a well known pathogen of more than 500 cultivated and wild plant species (Khan, 2007). This fungus causes charcoal rot of sunflower and after a seedling infection stage, if the invaded plant survives from the seedling mortality, the fungus moves to the above ground parts. Temperatures near 30°C and dry conditions are optimal for *M. phaseolina* growth, which makes this pathogen prevalent in arid regions, such as Pakistan, China, India, but also in Uruguay, Spain, Russia and USA (Aćimović, 1998a). Generally, it is estimated that charcoal rot affects the crop throughout the world reducing seed yields by 20-36%

(Jimenez-Diaz et al., 1983). Charcoal rot of sunflower can also be found in moderate climates when high temperature and dry conditions occur, as it happened in 1981-1983 period in all European countries except Poland. This disease on oilseed sunflower in 1998 in western North Dakota was registered with incidence of 25%, according to Gulya et al. (2002). Recently, very high incidence and spreading of charcoal rot on sunflower was recorded in Slovakia (Bokor, 2007) and in the Czech Republic in 2007 (Veverka et al., 2008). Variation among *M. phaseolina* isolates may be related with geographical origin and source (Harlton and Levesque, 1995). The fungus has a host specific behaviour and a high degree of variation in its

morphological, cultural and pathological properties, even when it is isolated from different parts of the same plant (Khan, 2007).

Fusarium species are known as cosmopolites, which are pathogenic to great number of agricultural plants, such as sunflower (Aćimović, 1989b; Lević, 2008), maize (Leslie et al., 1990; Doko et al., 1995; Logrieco et al., 2002; Fandohan et al., 2003), wheat (Brizele et al., 2002; Logrieco et al., 2003; Furlong et al., 2005; Krnjaja et al., 2008), barley (Bottalico, 1998), oat (Bottalico and Perrone, 2002), hops (Stanković et al., 2008), sorghum (da Silva et al., 2006), asparagus (Moretti et al., 1997; Elmer et al., 1999), rice (Desjardins et al., 2000), bananas (Marasas et al., 1998; Burgess et al., 1994), mango (Britz et al., 2002), pineapple (Hidalgo et al., 1999), etc. They cause root, stem and fruit rot as well as whole plant wilting, with reductions in crop yields estimated between 10% and 30% in Europe (Logrieco et al., 2002). Yield losses caused by Fusarium wilt of sunflower, are low in moderate regions, but in India yield losses can reach 45% (Aćimović, 1989b).

The most common *Fusarium* species identified as sunflower pathogens were *F. oxysporum* Schlecht. Emend. Snyd & Hans., *F. solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen, *F. verticillioides* (Saccardo) Nirenberg, *F. equiseti* (Corda) Saccardo, *F. culmorum* (W.G. Smith) Saccardo, *F. semitectum* Berkeley & Ravenel etc. *F. oxysporum* is the most economically important species in the *Fusarium* genus, given its cosmopolitism and numerous hosts. Disease development caused by *F. oxysporum* is favoured by high temperatures and warm moist soils. Optimal temperature for the growth of *F. oxysporum* on artificial media is between 25-30˚C, while optimal soil temperature for root infection by this pathogen is 30˚C or above (Goss Russ, 1936). *F. solani* can be easily confused with *F. oxysporum* because of their overlaps in some aspects of morphology and ecological niches. *F. solani* also has a cosmopolitan distribution with a numerous host plants. Optimal growth temperature for *F. solani* is between 27 and 30˚C, and good growth generally is possible even at 37˚C (http://mycota-crcc.mnhn.fr/site/specie.php?idE=104#ancre11).

The genera *Macrophomina* and *Fusarium* have some common hosts, such as sunflower, and tend to form mixed infections under favourable weather conditions resulting in charcoal root rot and wilt (Bhatti and Kraft, 1992). Due to that, sunflower plants with charcoal rot symptoms in the field usually have not only *M. phaseolina* present but also *F. oxysporum* and/or *F. solani* in a stem, which many times contributed to sunflower plant wilting (de Barry, 1985).

Nahar (2002) registered these three pathogens on wilted sunflower seedling in a relation: *M. phaseolina* (29.4%), *F. oxysporum* (27.5%) and *F. solani* (24.6%). During the vegetation of 2009 in Serbia, weather conditions were optimal for charcoal development, and there were a lot of sunflower plants with mixed infection of *M. phaseolina, F. oxysporum* and/or *F. solani*. Coexistence of these pathogens, defining their relationship-type in a community and possibility of their additive pathogen effect, as well as dynamic of necrosis development on sunflower seeds, were the aim of this research.

MATERIAL AND METHODS

All tested isolates in this research, were isolated from sunflower medulla during 2009 at five localities in Vojvodina (Table 1).

Table 1. M. phaseolina and Fusarium spp. isolates origin, isolated in 2009 on Vojvodina territory

Species	Isolate code	Site of origin
M. phaseolina	RŠ-H16	Kuštin
	RŠ-H19	Deliblato
	RŠ-H13	Pančevo
	RŠ-H15	Zenjanin
	RŠ-H37	Rimski Šančevi
F. oxysporum	RŠ-H33	Kuštin
	RŠ-H29	Deliblato
F. solani	RŠ-H31	Pančevo
	RŠ-H34	Kuštin
	RŠ-H20	Zrenjanin
	RŠ-H55	Rimski Šančevi

For further research, those isolates were refined to single-spore *Fusarium* spp. isolates and single-hyphae *M. phaseolina* isolates. Identification of *Fusarium* spp. was done according to Leslie & Summerell (2006) and Burgess et al. (1994).

Growth dynamics. 5 mm^2 plug of 7 days old mycelia of each isolate was placed in the centre of the Petri dish (Ø 90 mm) on PDA and incubated on 28ºC in dark. Growth rate of isolates was measured 3rd, 5th and 7th day along median line. Growth dynamic test for each isolate was done in four replicates.

Interaction type of confronted colonies. Different interaction types were determined through observation of culture morphology at the interaction zone. Confronted isolate pairs were selected according to same locality of their origin: RŠ-H16 with RŠ-H33, RŠ-H16 with RŠ-H34, RŠ-H19 with RŠ-H29, RŠ-H13 with RŠ-H31, RŠ-H15 with RŠ-H20, and RŠ-H37 with RŠ-H55. Each *M. phaseolina* - *Fusarium* pair was isolated from the same sunflower plant on a different locality. Plugs of 5 mm^2 size were placed at the 50 mm distance on PDA in 90 mm Petri plates, in four replicates. After 7 days of incubation at 29°C in dark, culture morphology at the interaction zone was observed and different interaction types were determined according to Rodriguez et al. (2000) scale:

1. growth of one colony surrounding the other with contact between hiphae <3 mm;
2. growth of one colony surrounding the other with contact between hiphae >3 mm;
3. growth of one colony surrounding the other without contact between hiphae and d ≤2 mm;
4. unilateral inhibition at a distance d >2 mm.

Also according to these authors and based on the growth radius of confronted and control colonies, the Radial Growth Inhibition (RGI) was calculated according formula:

$$RGI = [(r_1 - r_2)/r_1] \times 100$$

where: r_1 = radius of the control colony and r_2 = radius of confronted colony.

Difference in pathogenicity of *M. phaseolina* and *Fusarium* spp. isolates on sunflower seeds. Pathogenicity of *M. phaseolina* and *Fusarium* spp. isolates was tested on seeds of 3 sunflower experimental hybrids which showed different level of resistance (NS-H-D1 and NS-H-V4) or susceptibility (NS-H-P3) to charcoal rot in the field inoculation test during the 2010 on Rimski Šančevi (unpublished data). One hundred superficially sterilized seeds of e ach hybrid were equally distributed on the sterilized filter paper in the Petri dish (25 x 4 replicates) and then soaked with the 10 ml of pathogen suspension. Suspension was made by mixing 100 ml sterilized distilled water and 7 days old mycelia scraped from PDA surface of one Petri dish. Concentration of *M. phaseolina* suspension was calculated according Day & MacDonald (1995), and it was adjusted on 320-340 cfu/ml. Number of *Fusarium* spp. conidia in suspension was measured by haemocytometer and concentration was adjusted on 1-2 x 10^5. Suspension for the pathogen synergism test was consisted of 5 ml *M. phaseolina* suspension and 5 ml of *Fusarium* spp. suspension above menti-oned concentrations. Mixed suspensions were made of paired isolates according to same site of origin. One hundred seeds of each hybrid, soaked in 10 ml of sterilized dis-tilled water, were used as a control. Seeds were incubated for 7 days at 29°C in the dark.

Necrosis development on sunflower seeds was measured on 3rd, 5th and 7th day according Day & MacDonald (1995) scale: 0 – zero necrosis; 1 – <20% host surface affected; 2 – 20-40% host surface affected; 3 – 40-60% host surface affected; 4 – 60-80% host surface affected; 5 – 80-100% necrosis. Disease intensity on sunflower seeds was calculated according to McKinney's formula.

Dynamic of necrosis development was calculated as a difference of disease intensity values between 3rd, 5th and 7th day of measurements.

RESULTS AND DISCUSSION

Growth dynamics of *M. phaseolina* and *Fusarium* spp. isolates and growth inhibition of confronted colonies. All isolates of *M. phaseolina*, with the exception of RŠ-H13 isolate, exceeded maximal growth radius (90 mm) after 5 days of incubation. What is more, the isolate RŠ-H15 exceeded maximal radius at 3rd day of incubation. Growth dynamic of tested isolates in this research, which exceeded maximal growth radius at 5th day of incubation, is in correlation with growth dynamic of Hungarian *M. phaseolina* isolates tested at optimal growth temperatures (Csönders et al., 2011). This can also be connected to dependence on the geographical region the fungus was isolated from. Manici et al. (1995) reported that isolates from four various climatic regions in Italy grew the best at temperatures close to those in soils from which they had been isolated. Similarity of climatic conditions in Vojvodina (north Serbia) and Hungary, from which *M. phaseolina* isolates were isolated, can explain the same growth dynamic of tested isolates in those two countries.

Contrary to *M. phaseolina* isolates, no colony of *Fusarium* spp. isolates exceeded maximal growth radius after 7 days of incubation (Table 2). *F. solani* isolate with the fastest growth rate was RŠ-H55. Among *F. oxysporum* isolates tested, RŠ-H29 had the faster growth than RŠ-H33 (Table 2). Lević (2008) measured growth of *F. oxysporum* isolates at 25°C, and average growth after 7 days on PDA was between 75-80 mm, which was the same as the growth of the *F. oxysporum* isolates in this research. According to data published by the same author for the growth rate of *F. solani* isolates (73-80 mm under the same conditions), only two isolates from our research - RŠ-H20 and RŠ-H31 had a little bit lower growth rate than the mentioned average.

Table 2. Growth dynamics and RGI of the *M. phaseolina* and *Fusarium* spp. isolates

	3 DAY R±SD (mm)		5 DAY R±SD (mm)		7 DAY R±SD (mm)		RGI (%)
	M. phaseolina						
	SINGLE	*CONFR*	*SINGLE*	*CONFR*	*SINGLE*	*CONFR*	
RŠ-H13	73.63±5.47	63.00±2.86	77.13±7.15	65.13±2.46	77.75±6.50	65.38±2.75	15.90
RŠ-H15	90.00±0.00	65.75±2.84	90.00±0.00	67.13±0.48	90.00±0.00	67.63±0.75	24.85
RŠ-H16 (33)	74.50±5.07	62.13±0.85	90.00±0.00	64.63±0.48	90.00±0.00	64.75±0.65	28.05
RŠ-H16(34)	74.50±5.07	63.25±1.19	90.00±0.00	65.50±0.71	90.00±0.00	65.75±0.65	26.94
RŠ-H19	82.38±3.15	64.00±2.74	90.00±0.00	66.50±0.71	90.00±0.00	66.63±0.63	25.97
RŠ-H37	88.63±2.75	65.75±0.87	90.00±0.00	67.38±0.25	90.00±0.00	67.38±0.25	25.13
Average	81.83±3.39	63.98±1.89	87.43±1.43	66.05±0.85	87.55±1.30	66.25±0.95	23.98
	F. solani						
	SINGLE	*CONFR*	*SINGLE*	*CONFR*	*SINGLE*	*CONFR*	
RŠ-H20	29.00±0.58	26.88±0.85	47.50±0.71	33.88±1.85	65.88±1.44	37.63±2.39	42.88
RŠ-H31	29.75±0.29	29.13±1.03	48.25±0.29	39.50±2.12	67.50±0.91	39.75±4.11	41.11
RŠ-H34	38. 05±1.58	36.50±0.91	61.88±2.14	45.13±2.29	82.75±3.93	46.50±1.78	43.81
RŠ-H55	34.75±0.65	31.00±1.93	59.63±0.48	45.00±4.42	83.38±1.65	45.25±5.17	45.73
Average	33.00±0.78	30.88±1.18	54.32±0.91	40.88±2.67	74.88±1.98	42.28±3.36	43.38
	F. oxysporum						
	SINGLE	*CONFR*	*SINGLE*	*CONFR*	*SINGLE*	*CONFR*	
RŠ-H29	38.88±0.63	36.38±1.37	62.38±0.95	41.38±1.89	87.13±2.75	43.25±2.25	50.36
RŠ-H33	35.38±0.85	37.86±0.75	55.75±1.55	44.38±1.31	74.00±0.71	48.25±1.55	34.80
Average	38.12±0.74	37.12±1.06	59.06±1.25	42.88±1.6	80.57±1.73	45.75±1.9	42.58

The growth radius of confronted and control (single) colonies was used to establish percentage of radial growth inhibition (RGI). The lowest RGI among *M. phaseolina* isolates was registered at RŠ-H13 isolate, while the highest growth inhibition was in RŠ-H16

isolate in RŠ-H16 vs. RŠ-H33 confrontation. Among *Fusarium* isolates, RŠ-H33 had the lowest RGI while RŠ-H29 had the highest RGI. Data in Table 2 shows the obvious domination of *M. phaseolina* isolates over the *Fusarium* spp. isolates according to their growth rate, which resulted in significant growth inhibition of *Fusarium* isolates during confrontation.
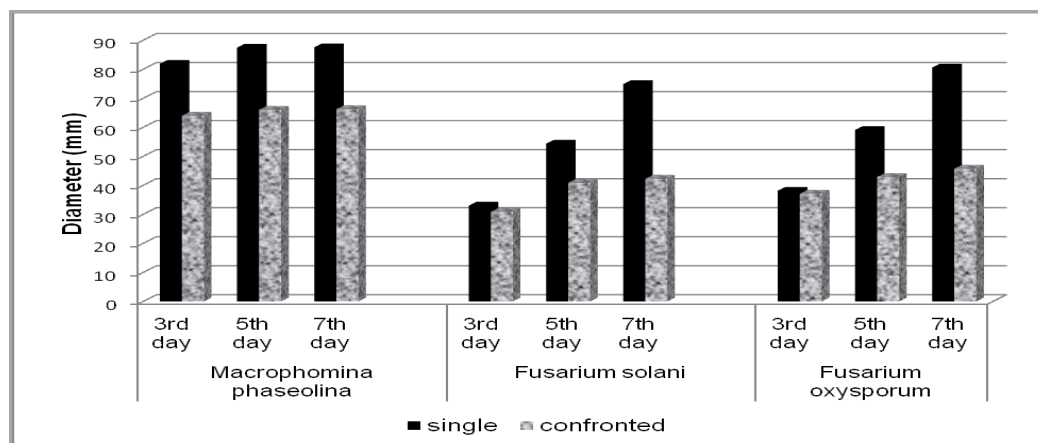

Figure 1. Average growth dynamics of *M. phaseolina*, *F. solani* and *F. oxysporum* isolates as single or confronted colonies

According to average growth rate of both single and confronted tested isolates (Figure 1), it was obvious that isolates of *M. phaseolina* were dominant in comparison with *Fusarium* isolates and had faster growth. Comparing *Fusarium* species, isolates of *F. oxysporum* had faster growth than *F. solani* isolates (Figure 1).

Interaction types of confronted colonies and isolates morphology. All *M. phaseolina* isolates had the same morphology on PDA with the exception of isolate RŠ-H13. All isolates had fast growth of mycelia, which was white or pale grey in the beginning and became black with microsclerotia formation (Figure 2a). Microslerotia formation started around 3rd day of incubation. Isolate RŠ-H13 had the only exception of colony shape, which was more star-like shaped instead of regular rounded shape (Figure 2b).
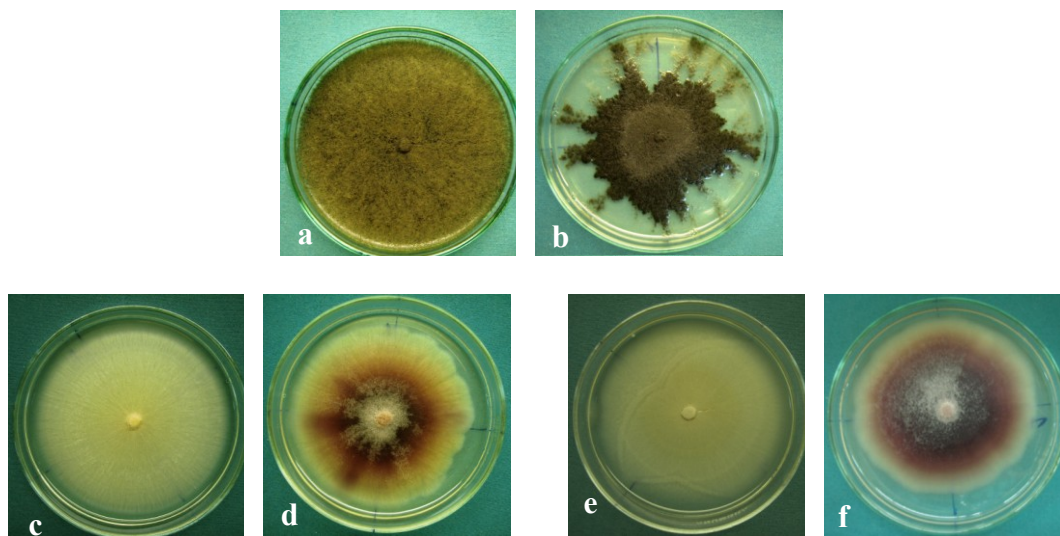

Figure 2. Morphology of some *M. phaseolina* and *Fusarium* isolates
M. phaseolina : a) RŠ-H37 and b) RŠ-H13;
F. solani : c) RŠ-H55 and d) RŠ-H34;
F. oxysporum : e) RŠ-H29 and f) RŠ-H33.

Both *F. oxysporum* and *F. solani* are known to form mycelia in range white to pale violet on PDA (Leslie and Summerell, 2006), and most of tested isolates formed characteristic sparse white area mycelium (Figure 1c and 1e). Exceptions were isolates RŠ-H33 and RŠ-H34 from Kuštin with violet pigment in the agar (Figure 2d and 2f).

All confronted pairs had the interaction of type 2, according to scale Rodriguez et al. (2000) (Figure 3a). Exceptionally, RŠ-H16 (*M. phaseolina*) vs. RŠ-H33 (*F. oxysporum*) and RŠ-H34 (*F. solani*) included interaction type 1 (Figure 3b).

In confrontation assay, all *M. phaseolina* isolates were dominant, had faster growth and formed microsclerotia over mycelia of *Fusarium* isolates in confrontation zones (Figure 4a). Exception was RŠ-H13 vs. RŠ-H31 where mycelia of RŠ-H31 (*F. oxysporum*) overgrew *M. phaseolina* in confrontation zone (Figure 4b).
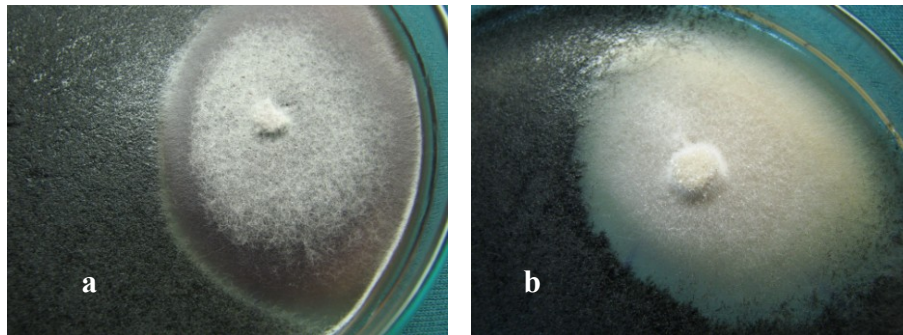

Figure 3. Interaction types – a) type 1 (RŠ-H16 with RŠ-H33) – one colony surrounds other with contact between hyphae <3 mm; b) type 2 (RŠ-H15 with RŠ-H20) – the growth of one colony surrounding the other with hyphae contact >3 mm
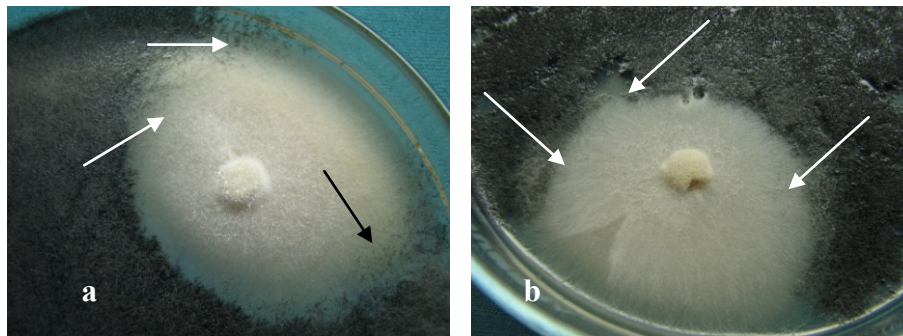

Figure 4. a) Interaction type 2 – overgrowth of *Macrophomina* over *Fusarium* isolate (RŠ-H15 with RŠ-H20); b) interaction type 2 – overgrowth of *Fusarium* over *Macrophomnina* isolate (RŠ-H13 with RŠ-H31)

Difference in pathogenicity of *M. phaseolina* and *Fusarium* spp. isolates on sunflower seeds. Difference in pathogenicity was assessed according intensity of necrosis developed on sunflower seeds, and was observed between isolates of the same species as well as between different species - *M. phaseolina*, *F. solani* and *F. oxysporum* (Table 3).

Among *M. phaseolina* isolates, according average necrosis intensity on all 3 hybrids tested, the most pathogenic was RŠ-H15 isolate from Zrenjanin, while the least pathogenic was RŠ-H37 from Rimski Šančevi. On seeds of NS-H-D1 and NS-H-V4, the most pathogenic was RŠ-H15, while on the seeds of NS-H-P3 that was RŠ-H19.

SONJA TANČIĆ ET AL.**:** BIO-ECOLOGICAL RELATIONS OF SUNFLOWER
PATHOGENS – *MACROPHOMINA PHASEOLINA* AND *FUSARIUM* SPP. AND SUNFLOWER
TOLERANCE TO THESE PATHOGENS

The isolates with a lowest pathogenic effect were different for all three hybrids - RŠ-H37 (NS-H-D1), RŠ-H16 (NS-H-P3) and RŠ-H19 (NS-H-V4).

Difference in virulence of *Macrophomina* isolates originated from Tamil Nandu area (India) on sunflower plants was tested by Suriachandraselvan et al. (2006). Those authors reported that virulence varied from 38.3-88.3% which is close to results of pathogenicity test in this research. Suriachandraselvan et al. (2005) also reported that all tested isolates from sunflower were cross-pathogenic and the most aggressive of all tested isolates from a different plant hosts.

Generally, among all *Fusarium* spp. isolates, according to average necrosis intensity on all 3 hybrids tested, the most pathogenic effect had RŠ-H31 isolated from Pančevo, while RŠ-H33 (*F. oxysporum*) and RŠ-H34 (*F. solani*), both originated from Kuštin, had the lowest pathogenic effect on seeds of NS-H-D1 and NS-H-P3. On NS-H-V4 seeds the most pathogenic was RŠ-H55, and RŠ-H20 was the least pathogenic isolate. Disease intensity caused by RŠ-H55 (80.00%) on NS-H-V4 was very close to pathogenicity of RŠ-H31 (79.26%), which turned out to be the most pathogenic on seeds of the other two tested hybrids.

Table 3. Individual and related pathogenic effect of *M. phaseolina*, *F. oxysporum* and *F. solani* isolates on sunflowers seeds

Isolates	Site of origin	McKinney index (%)			
		NS-H-D1	NS-H-P3	NS-H-V4	Average
M. phaseolina					
RŠ-H13	Pančevo	32.08	73.60	57.88	54.52
RŠ-H15	Zrenjanin	53.48	81.60	69.35	68.14
RŠ-H16	Kuštin	35.74	63.24	46.76	48.58
RŠ-H19	Deliblato	32.94	82.93	37.33	51.07
RŠ-H37	R. Šančevi	31.94	67.47	42.69	47.37
Min.	-	31.94	63.24	37.33	47.37
Max.	-	53.48	82.93	69.35	68.59
Average	-	37.24	73.77	50.80	53.94
F. solani					
RŠ-H20	Zrenjanin	71.43	54.37	48.94	58.25
RŠ-H31	Pančevo	82.16	75.83	79.26	79.08
RŠ-H34	Kuštin	23.57	48.00	54.18	41.92
RŠ-H55	R. Šančevi	48.57	71.34	80.00	66.64
Min.	-	23.57	48.00	48.94	41.92
Max.	-	82.16	75.83	80.00	79.08
Average	-	56.43	62.39	65.59	61.47
F. oxysporum					
RŠ-H29	Deliblato	63.08	63.33	70.81	65.74
RŠ-H33	Kuštin	22.04	44.80	53.33	40.06
Average	-	42.56	54.06	62.07	52.90
M. phaseolina + F. solani					
RŠ-H13 + RŠ-H31	Pančevo	74.55	69.33	69.03	70.97
RŠ-H 15 + RŠ-H20	Zrenjanin	32.43	55.20	27.00	38.21
RŠ-H16 + RŠ-H34	Kuštin	12.82	29.60	27.59	23.34
RŠ-H37 + RŠ-H55	R. Šančevi	50.30	30.00	65.83	48.71
Min.	-	12.82	29.60	27.00	23.34
Max.	-	74.55	69.33	69.03	70.97
Average	-	42.53	46.03	47.36	45.31
M. phaseolina + F. oxysporum					
RŠ-H19 + RŠ-H29	Deliblato	59.33	46.03	63.33	56.23
RŠ-H16 + RŠ-H33	Kuštin	21.60	31.35	25.61	26.19
Average	-	40.46	38.69	44.47	41.21
Control					
sdH$_2$O	-	0.00	0.80	0.30	0.40

Estimation of related pathogenic effect of mixed pathogens suspension on sunflower seeds showed that the most pathogenic was RŠ-H13+RŠ-H31, which can be related with RŠ-H31 domination among *Fusarium* isolates applied as a single pathogen suspension. On the opposite, RŠ-H16+RŠ-H34 and RŠ-H16+RŠ-H33 were the least pathogenic suspensions. *Fusarium* spp. isolates RŠ-H33 and RŠ-H34, expressed low pathogenic effect on all hybrid seeds, as a single pathogen suspension too. Mixed suspensions RŠ-H16+RŠ-H33 and RŠ-H16+RŠ-H34 caused lower necrosis on seeds of all three hybrids than in treatments with a single pathogen suspension of both pathogens (Table 2). The cause of this can be antagonism or high competitiveness between these isolates, which is in line with the results of confrontation assay – interaction type 1. Anyway, this relation cannot be utilized for the biological control, because all isolates expressed pathogenic effect on sunflower seeds. Additionally, low pathogenic effect of these isolates can be linked with their same site of origin. Absence of additive pathogenic effect was registered in all mixed suspensions, and necrosis intensity caused by mixed pathogens was, except those mentioned above, somewhere in between values of necrosis intensity caused by single pathogens.

Differences in a susceptibility to *M. phaseolina* among hybrids can be noticed according to average necrosis intensity caused by all tested pathogen isolates (Table 3). All *M. phaseolina* isolates, except RŠ-H16, caused lower necrosis development on hybrids NS-H-D1 and NS-H-V4 than their paired *Fusarium* spp. isolate as a single pathogen. This can be explained by higher resistance of these hybrids to charcoal rot caused by *M. phaseolina*, which was previously confirmed in the field inoculation tests during the 2010 (unpublished data). On the contrary, NS-H-P3 was susceptible to charcoal rot in the field in 2010 and in a glass house, which was confirmed in this laboratory test too – 73.77% of the average necrosis intensity (Table 3). All tested isolates of *M. phaseolina* caused higher necrosis development than

Fusarium spp. isolates on sunflower seeds of NS-H-P3. Exception were *M. phaseolina* isolates RŠ-H37 and RŠ-H13 which caused lower necrosis development than their paired *F. solani* isolates, provided that necrosis intensity caused by *M. phaseolina* and *F. solani* isolates were almost equal. This was expected because RŠ-H31 and RŠ-H55 were the most pathogenic among *F. solani* isolates, and NS-H-P3 is susceptible to *M. phaseolina* which caused almost equal pathogenic effects of *M. phaseolina* and *F. solani* isolates on its seeds.

Observing the average disease intensity caused by all tested isolates (Table 3), generally, NS-H-D1 and NS-H-V4 were more sensitive to *Fusarium* isolates than to *M. phaseolina,* while NS-H-P3 had the opposite reaction. Among three tested hybrids, NS-H-V4 was the most susceptible to *Fusarium* isolates and combinations of *Fusarium* + *M. phaseolina*, while NS-H-P3 was the most susceptible to *M. phaseolina*. The biggest difference in average disease intensity between hybrids was in *M. phaseolina* treatment and varied from 37.24–73.77% (Table 3), which reflects the difference in hybrids susceptibility to the pathogen. In other treatments hybrids were almost equally susceptible to the pathogen. Hybrid NS-H-D1 was the most tolerant to all pathogen treatments, with the exception of treatment *M. Phaseolina* + *F. oxysporum* (Table 3).

Necrosis development dynamic on sunflower seeds. Observation of necrosis development dynamics on sunflower seeds were based on the differences between McKinney index values recorded on 3rd, 5th and 7th day (Figure 5).

Necrosis development was faster on seeds of susceptible hybrids, as demonstrated by the fact that necrosis caused by *M. phaseolina* had a faster development in the first 3-5 days on NS-H-P3, as well as necrosis caused by *Fusarium* spp. and *M. phaseolina*+*Fusarium* spp. on NS-H-P3 and NS-H-V4, which were susceptible to this pathogen combinations (Figue 5). Hybrid NS-H-D1 was the most tolerant to the

all tested pathogens and their combinations. As seen in Figue 5, on seeds of this hybrid disease development was faster after 5th day of incubation.

It can be concluded that necrosis development dynamic was more dependent on level of hybrid resistance than on the pathogen species.
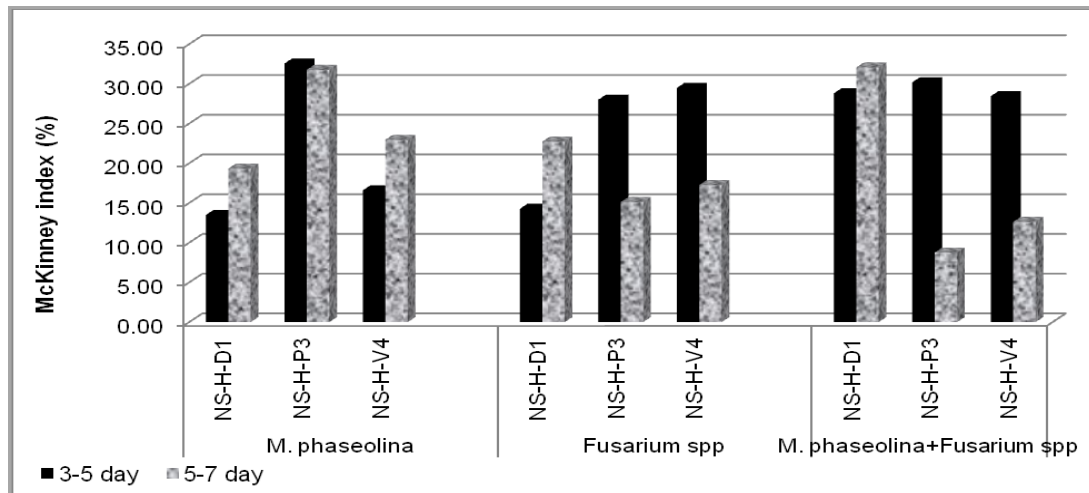

Figure 5. Necrosis development dynamics on sunflower seed

CONCLUSIONS

Isolates of *M. phaseolina* in comparison with *Fusarium* isolates had a faster growth, which resulted in significant growth inhibition of *Fusarium* isolates during confrontation. Comparing *Fusarium* species, *F. oxysporum* had the faster growth than *F. solani* isolates. Two types of interaction between *M. phaseolina* and *Fusarium* isolates were registered – interaction zone bigger or smaller than 3 mm. *Fusarium* isolates originated from Kuštin were the least pathogenic as a single pathogen and that affected the least pathogenic effect of their mixed suspensions on sunflower seeds of all three hybrids tested. *Fusarium* isolate with the most pathogenic effect on all three sunflower hybrids tested was the isolate from Pančevo. This contributed to the most pathogenic effect of mixed suspension on seeds of all three hybrids tested. Isolate RŠ-H31 was the most aggressive and the only one from *Fusarium* isolates which overgrew *M. phaseolina* mycelium in the confronted colonies test. The most pathogenic on all three sunflower hybrids of all *M. phaseolina* isolates, was the isolate from Zrenjanin, while the least pathogenic was from Rimski Šančevi. No additive pathogenic effect on sunflower seeds

in a test with a mixed pathogen suspensions was observed. Among the three tested hybrids, NS-H-V4 was the most susceptible to *Fusarium* spp. and combinations of *M. phaseolina* + *Fusarium*, while NS-H-P3 was the most susceptible to *M. phaseolina*. Necrosis development dynamic was more dependent on the resistance level of the hybrid than on the pathogen species.

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359

SONJA TANČIĆ ET AL.: BIO-ECOLOGICAL RELATIONS OF SUNFLOWER
PATHOGENS – *MACROPHOMINA PHASEOLINA* AND *FUSARIUM* SPP. AND SUNFLOWER
TOLERANCE TO THESE PATHOGENS

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