COMPARISON OF ANTIOXIDANT ACTIVITY BETWEEN TWO SWEET PEPPER GENOTYPES INFECTED WITH *ALTERNARIA ALTERNATA*

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Abstract
The resistance of two sweet pepper varieties (cultivar Anita and breeding line 82/16) to fruit root caused by fungus *Alternaria alternata* was evaluated. In order to determinated antioxidant capacity of methanol extracts of tested fruits as well as its relation with phenolic compounds three different antioxidant tests were used and content of total polyphenols and total flavonoids was measured. The results suggested that not only phenolic compounds might be involved in antioxidant activity of pepper fruits, but also carotenoids and vitamins C and E

Introduction
Sweet peppers (*Capsicum annuum* L.) are one of most worldwide cultivated vegetable, especially in China, Mexico and Turkey [1]. Apart from its economic importance, peppers have significant place in human diets because of its high amount of vitamin C, vitamin E, capsacinoids, carotenoids and phenolic compounds [2]. This valuable crop is mostly consumed as fresh fruits or in dried form used as a spice [1].
Many commercial varieties of pepper are susceptible to damage caused by different biotic factors (fungi, bacteria and insects) as well as abiotic factors (drought, metals in soils, ultraviolet radiation or in appropriate temperature). This could lead to significant losses of pepper crops so that is one of main reason why many researchers are focused on understanding the mechanisms of antioxidant protection [3].
Fungus *Alternaria alternata* can infects living plants and fresh fruits, especially after mechanical damage or insects injuries. This destructive fungus causes damage such as fruit rot, black spots on the fruit surface and internal mould of fruits. Fruit rot appears before or after harvest on mature pepper fruits more often than immature. One way to avoid fungal contamination is careful handling during harvesting, storing and other processes involved in pepper producing [1, 4, 5].
Because of pepper nutritional importance and widespread use, the aim of this study was to compare the resistance of two pepper cultivars on *Alternaria* infection. In this purpose were determined content of total polyphenols, total flavonoids and antioxidant activity measured by three different antioxidant tests.

Experimental
Genotype Anita and breeding line 82/16, used in this study, are sweet pepper cultivars with bell shaped fruits. Both varieties have light yellow color of fruits at technological maturity. These genotypes of pepper were grown in the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Pepper seeds for seedlings production were sown on 30th of March. Seedlings were transplanted into the open field was done on 3rd of June. The distance between plants in the row was 25 cm, while the space between rows was 70 cm. All fruits were collected at the technological maturity stage.
The inoculation of fruits was done with monohyfal isolate *Alternaria alternata* (K-93) as described by Fallik et al. [6]. Fruits of pepper were inoculated with 40 µl spore suspension per puncture (3 puncture in each of the fruits). Fruits were put into PVC bags and incubated for

10 days at 20 °C in thermostat. Evaluation of infection was performed according to a method by Frans et al. [7].

Methanol extracts of sweet pepper fruits were prepared by homogenization of 4 g fresh plant material with 10 ml of 70% methanol. After an overnight extraction, the methanol extracts were centrifuged 15 min at 5000 rpm and the obtained supernatants used in further biochemical analyses were kept at 4 °C. The total polyphenol content was measured by spectrophotometry using Folin-Ciocalteu method [8]. Determination of total flavonoid content was performed by aluminium chloride colorimetric method based on building metal-complexes between flavonoid compounds and aluminium chloride. [9]. The concentration of total polyphenols and flavonoids were read (mg/ml) on the standard calibration cruve were expressed as quercetin equivalents in mg per 100 gram of fresh weight (mg QE/100 g FW). The antioxidant capacity of fruits was measured by three the most widely used methods. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using the method described by Yazdizadeh Shotorbani et al. [10] with some modification. This spectrophotometric method is based on reduction DPPH radical by antioxidant compounds. The ABTS assay is spectrophotometric method based on reduction the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$), which has a dark blue color, by an antioxidant into colorless ABTS. ABTS antioxidant activity of the tested extracts was measured according to Zheleva-Dimitrova et al. [11] with slight modification. Ferric reducing antioxidant power (FRAP) is based on measuring the reduction of ferric ion (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants in extracts. The FRAP assay was carried out as described by Thaipong et al. [12]. Determination of antioxidant activity by DPPH, ABTS and FRAP method was calculated from the standard cruve constructed with Trolox. The results of DPPH, ABTS and FRAP were expressed as Trolox equivalents in mg per 100 g of fresh weight (mg Trolox/100 g FW).

All the assays were carried out in triplicate and the data were reported as mean ± standard deviation (Table 1). Statistic evaluation of data was analysed using software STATISTICA ver. 13.2 (StatSoft, Inc., USA). Analysis of variance (Factorial ANOVA) with the Bonferroni test was used to compare significant difference between the groups at the 5 % significance level ($p< 0.05$). The correlation coefficients were done by Spearman.

Results and discussion

The content of total polyphenols, total flavonoids and antioxidant capacity of sweet pepper extracts measured by DPPH, ABTS and FRAP tests is presented in Table 1. Polyphenols are a large group of secondary metabolites involved in non-enzymatic defence on damage caused by different biotic and abiotic agents [13]. In general, non-infected fruits of genotype Anita contained more phenolic compounds (150.20 mg QE/100 g FW) than non-infected fruits 82/16 cultivar (98.51 mg QE/100 g FW). Furthermore, infected fruits of both tested cultivars showed lower amount of polyphenols than non-infected fruits. Previous study of Kevers et al. [14] reported high concentration of polyphenols in fruits of peppers (from 215 to 296 mg CAE/100 g FW). However, these results cannot be compared with ours since these authors used different standard for expressing results.

The highest content of total flavonoids was obtained for non-infected fruits of Anita cultivar. The flavonoid content in our study was lower for both tested cultivars (from 8.61 to 13.96 mg QE/100 g FW for non-infected fruits) than previously reported by Medina-Juárez et al. (from 25.38 to 60.36 mg QE/100 g FW) [15], while the values obtained by Kevers et al. were even lower (from 2.1 to 4.8 mg QE/100 g FW) [14]. According to some researchers, this inconsistency in published results could be explained by the fact that concentration of polyphenols and flavonoids depends on varieties, the technological treatments and time of harvesting [15, 16].

Table 2. Phenolic compounds and antioxidant activity of methanolic extracts

Genotype	Anita		82/16	
Infection	Non-infected	Infected	Non-infected	Infected
Total polyphenols[1]	150.20 ± 4.28[a]	86.93 ± 2.84[b]	98.51 ± 6.55[c]	88.81 ±8.12[bd]
Total flavonoids[1]	13.96 ± 1.74 [a]	5.25 ± 0.67 [b]	8.61 ± 0.18 [c]	5.03 ± 0.39[bd]
DPPH[2]	57.16 ± 3.49[a]	46.21 ± 5.83[b]	35.94 ± 1.69[c]	57.18 ± 2.23[a]
ABTS[2]	43.30 ± 5.07[ac]	34.24 ± 3.25[b]	37.45 ± 1.5[ab]	45.26 ± 4.01[c]
FRAP[2]	127.32 ± 7.97[a]	116.05± 12.24[a]	95.35 ±7.10[b]	133.41± 13.71[a]

Value is a mean of three replicates ± standard deviation (SD)
[1]Expressed as mg Quercetin/100 g FW [2]Expressed as mg Trolox/100 g FW
Value without the same superscript within each column differ significantly at p <0.05 (Bonferroni *post hoc* test)

Taking into account the ANOVA, interaction between genotype and infection show statistically significant (p<0.05) influence on the all measured biochemical assays but genotype as a single factor do not have statistically significant influence on the values of ABTS and FRAP tests.
Infected Anita fruits expressed lower antioxidant activity than non-infected fruits. This could be in a close connection with the reduction of amount polyphenol compounds. Breeding line 82/12 was shown completely opposite results-–in the infected fruits antioxidant activity was higher than non-infected peppers. This indicates the possibility that some other non-phenolic compounds have the main role in the mechanisms of antioxidant protection of pepper fruits. According to some authors, carothenoids, capsacinoids, vitamins C and E have the most important role in the antioxidant activity [17, 18].

Table 3 Correlation coefficient between biochemical assays

	Total polyphenols[1]	Total flavonoids[1]	DPPH[2]	ABTS[2]	FRAP[2]
Total polyphenols[1]			0,099918	0,123991	0,012174
Total flavonoids[1]	0,756846*	1,000000	-0,065872	0,021361	0,001740

*Statistically significant at *p*< 0,05 (Spearman correlation)
[1]Expressed as mg Quercetin/100 g FW [2]Expressed as mg Trolox/100 g FW

Considering the correlation coefficients, polyphenols and flavonoids are in a very week correlation with all measured antioxidant tests in our study (Table 2). These results are in agreement with the report of Škrovánková et al [19].

Conclusion

The amount of measured polyphenols and flavonoids decreased in infected fruits of both tested genotypes. Taking into account the values of antioxidant tests, non-infected fruits of Anita cultivar show higher antioxidant activity than infected fruits. Even though antioxidant activity is higher in infected fruits than in non-infected fruits of breeding line 82/16, there is no correlation with phenols and flavonoids. The values of correlation coefficient between antioxidant tests and concentration of total polyphenols and flavonoids for both genotypes

might indicate that some other compounds have the principal role in antioxidant activity of tested pepper fruits.

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