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BIOCHEMICAL COMPOUNDS FROM APRICOT LEAVES INVOLVED IN RESISTANCE TO *MONILINIA* SPP.

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Abstract: Brown rot caused by *Monilinia laxa* is an important fungal disease of apricot *(Prunus armeniaca)* causing economical losses in different growing areas in Europe. *M. laxa* induces great damage in apricot, attacking flowers, leaves, stem, branches and fruits. The damage severity is strictly related to the climate conditions and several fungicide treatments are often necessary in order to limit the disease**.** Therefore, the aim of this study was to investigate the changes in the content of pigments (chlorophylls, carotenoids), total polyphenols, peroxidase, catalase, polyphenol oxidase in the leaves of the different apricot varieties. All these parameters were chosen taking in account their involvement in plant disease resistance. Concerning to chlorophylls and carotenoids level, less amounts were recorded in the leaves collected from the all symptomatic apricot varieties studied. Activities of peroxidase, catalase and polyphenol oxidase and total polyphenols were increased in the apricot samples affected by *M. laxa* compared with the healthy ones. Among the analyzed cultivars, Dacia, Litoral and Favorit showed a great tolerance to *M. laxa*. The genotypes like Carmela and Viorica showed different susceptibility.

Keywords: *antioxidant enzymes, phenols, carotenoids, infected leaves.*

INTRODUCTION

Monilinia spp. are a complex of pathogens which causes significant losses to pome fruits. *Monilinia laxa* (Aderhold and Ruhland) Honey and *M. fructicola* (G. Winter) Honey occur frequently on plum, cherry, apricot, peach, nectarine, cherry and sour cherry (Poniatowska et al., 2013; Rungjindamai et al., 2014; Martini & Mari, 2014). Brown rot caused by *M. laxa* is one of the most important diseases in *Prunus* species grown in commercially orchards, inducing blossom wilt and twig blight in stone fruits, causing significant losses in production capacity of infected trees (Mari et al., 2003; Lino et al., 2016).

Some endogenous chemical compounds (ascorbate, phenols, carotenoids, carbohydrates) and various enzymes (peroxidases, catalases, glutathion reductase, polyphenoloxidase) play an important role in plant tolerance and self-protection against biotic factors. Infections cause changes in secondary metabolism by activating self-protection processes and altering primary metabolism, affecting plant growth and development (Swarbrick et al., 2006; Szugyi & Sardi, 2018).

Numerous scientific reports suggest involvement of phenolic compounds and assimilation pigments in plant defense against different pathogens (Ivascu et al., 2002; Arun et al., 2010; Petrisor et al., 2012; Rasoulnia et al., 2018; Almatwari et al., 2020; Del Cueto et al., 2021). Previous work on resistance mechanisms to apple scab *Venturia inaequalis* (Cke.) Wint., has proven that the changes in phenolic compounds and enzymes between susceptible and resistant apple fruit cultivars are especially evident in the content of flavanols, phloridzin and hydroxycinnamic acids. Resistant apple cultivars have a higher content of hydroxycinnamic acids and flavanols compared with susceptible apple cultivars (Arici et al., 2014). A good correlation between level of phenolic in the leaves and resistance to pathogen attack was reported by Hassan et al. (2020).

Importance of antioxidant enzymes in resistance reactions have been reported for a range of crops and also in trees (Vanitha & Umesha, 2008; Debona et al., 2012; Khodadadi et al., 2016; Hassan et al., 2020; Obi et al., 2020). These enzymes participate not only in phenol metabolism but also the formation and accumulation of some metabolites such as lignin and phytoalexin. Similarly, it has been reported that the presence of some preformed antifungal compounds, such as catechin and epicatechin, proved to be responsible for resistance against gray mold *Botrytis cinerea* in strawberries (Terry et al., 2004).

Results of Szugyi & Sardi (2018) demonstrated that sour cherry genotypes with different levels of disease resistance could be clearly distinguished based on the monosaccharide (glucose + fructose) to sucrose ratio. The sucrose quantity had a significant effect on the level of resistance. Also, studies of Goncalves et al. (2005) showed that the carbohydrate contents in the leaves were increased in sugarcane infected with *Sugarcane yellow leaf virus*. They support that sucrose was the sugar that accumulated most in the leaves of infected plants, followed by total soluble sugars and reducing sugars.

The aim of this study was to investigate the photosynthetic pigments, total phenols and antioxidant enzymes involved in the defense mechanism of apricot leaves naturally affected by symptoms specific to brown rot.

MATERIALS AND METHODS

Leaves samples with natural symptoms of the brown rot from five apricot cultivars with early ripening (Dacia, Carmela and Viorica) and late ripening (Litoral and Favorit) were collected from the orchards of the Research-Development Station for Tree Fruit Growing Baneasa, Bucharest (Figure 1).

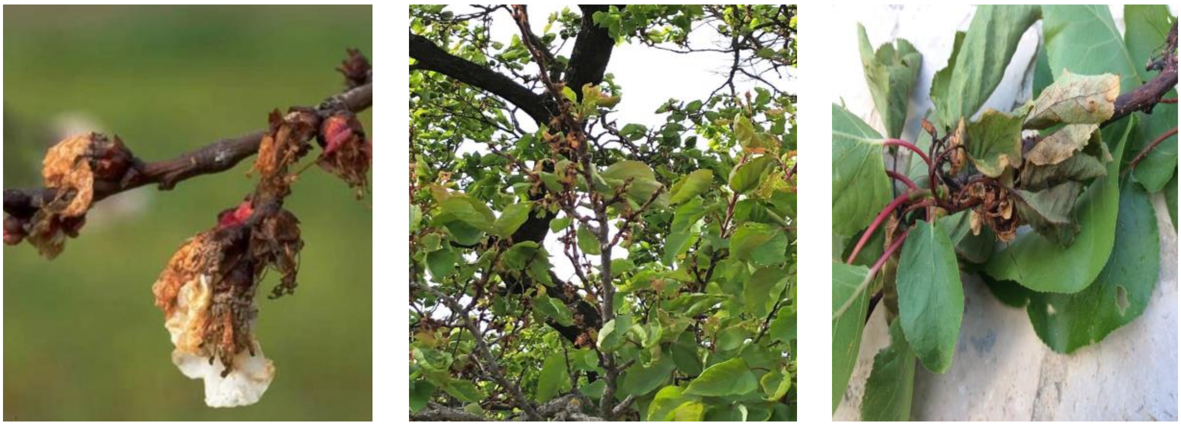

Figure 1. *Monilinia laxa* infection on apricot tree

Chlorophyll (Chl a, b*)* and total carotenoid (Car) were determined and calculated according to Lichtenthaler & Wellburn (1985) method. In brief, leaves' photosynthetic pigments were extracted with 80% (v/v) acetone in presence of calcium carbonate. Extracts were centrifuged at 5000 rpm for 15 minutes and the absorbance of the supernatant was measured at 663, 646 and 470 nm with a UV-VIS spectrophotometer. Chlorophyll and carotenoid contents were calculated by specific formulas and results were expressed as mg/L. Total phenolic content of extracts was determined using the Folin-Ciocalteu method (Singleton

& Rossi, 1965) with three replicates. The content of phenolic compounds was determined both in healthy and infected tissue with *Monilinia* sp. The absorbance was measured at 725 nm, with UV-VIS spectrophotometer. Results are expressed as GAE mg/g fresh weight [FW]).

To assay enzymes activity, fresh tissue from infected and healthy leaves was homogenized in an ice cooled mortar in 100 mM Potassium-phosphate buffer, pH 7.0 containing 1% soluble PVP and 1 mM EDTA. The homogenate was centrifuged at 10 000 g for 15 min and the supernatant was passed through a 0.45µm filter and used for assay of the antioxidant enzymes activity. Catalase (CAT) activity was measured according the method of Beers & Sizer (1952). The peroxidase (POX) activity was estimated according to the method of Hammerschmidt et al., (1982) using guaiacol as the substrate. Polyphenol oxidase (PPO) was determined according to the method described by Mayer et al. (1966).

RESULTS AND DISCUSSIONS

This study was conducted to investigate the changes in the antioxidant enzymes activity and non-enzymatic compounds content of five apricot varieties against *M. laxa* infection in order to understand the biochemical tolerance mechanism of apricot. Our results showed that apricot varieties respond differently to a fungal infection, based on their susceptibility or resistance to disease. Under stressful conditions, such as fungal infection produced by *M. laxa*, activation of biochemical parameters plays a vital role in the defense mechanism.

The concentration of the most abundant plant pigments, Chl a, Chl b, Chl a/Chl b ratio and Car are summarized in the Table 1. In all apricot cultivars leaves affected by *M. laxa* symptoms, there is a decrease in the content of total chlorophyll and total carotenoids, compared to unaffected varieties. In the most strongly affected varieties by brown rot (Viorica and Carmela), the chlorophyll content had a more pronounced decrease than the tolerant ones. Similar results have been reported by Ivascu et al., (2002). The Chl a/ Chl b ratio, which is used as stress indicator, was increased in symptomatic leaves for all apricot cultivars studied. Our results show that Car/Chl ratio increased in leaves affected by symptoms of all cultivars studied. These results are in agreement with the findings of Zafari et al., (2012).

Table 1**.** Variation of physiological parameters in apricot leaves - healthy and infected with *M. laxa*

Cultivar	Leaves status	Chl a (mg/L)	Chl b (mg/L)	Chl(a +b) (mg/L)	Chl a / Chl b	Car (mg/L)	Car/Chl (a+b)
Dacia	Healthy	46.89	10.80	67.9	4.34	13.20	0.19
	Infected	42.3	9.10	51.40	4.64	12.33	0.23
Viorica	Healthy	32.69	11.66	44.35	2.80	8.69	0.19
	Infected	27.3	7.9	35.2	3.45	7.95	0.22
Carmela	Healthy	54.40	11.34	65.74	4.80	14.55	0.22
	Infected	45.51	9.20	54.72	4.94	12.83	0.23
Litoral	Healthy	45.88	11.73	67.61	4.16	11.18	0.16
	Infected	43.39	10.25	53.64	4.23	9.42	0.17
Favorit	Healthy	29.50	10.90	40.40	2.70	8.40	0.20
	Infected	26.5	7.68	34.18	3.45	7.53	0.22

Infected plants show a high content of phenols, which could be due to both of the activation of the host defense mechanism and the pathogen attack mechanism (Arun et al., 2010). The results of the present study showed that higher level of total phenols was found in the infected apricot cultivars leaves compared to those of healthy cultivars. Also, the quantity of total phenols is in lower in uninfected tissue of Carmela and Viorica compared to Dacia, Litoral and Favorit that have a higher phenols content in healthy leaves. The increase in total phenols content in apricot after infection with *M. laxa* has also been demonstrated by Del Cueto et al., 2021 studies, which showed that phenols content is higher in apricot branches infected by *M. laxa* than in healthy ones. Also, other researchers have shown a significantly higher content of total phenols, total flavonoids and tannins in asymptomatic leaves of persimmom (Dyospiros kaki) cultivars affected by *Plurivorosphaerella nawae* fungus, compared with symptomatic leaves (Hassan et al., 2020). However, Obi et al., 2020 supported that besides the phenolics, neither ascorbate nor glutathione or carotenoids have ever been considered in conferring tolerance to brown rot in peach.

Phenolic compounds such as caffeic acid and chlorogenic acid, have been shown to inhibit the production of cell wall degrading enzymes (cutinases and polygalacturonases) by *M. fructicola*, and these may play a role in reducing the infectivity of *M. fructicola*. There is also some evidence (Lee & Bostock, 2007) that these phenolics may inhibit appressorial formation**.**

Table 2. Variation of antioxidant enzyme and total phenols in apricot leaves healthy and infected with brown rot

Cultivar	Leaves status	Peroxidase U/min/mg protein	Catalase U/min/mg protein	Polyphenol oxidase U/min/g F.W.	Total phenols mg/g F.W.
Dacia	Healthy	300	7.60	0.14	2.89
	Infected	545	14.6	0.30	3.90
Viorica	Healthy	88	6.6	0.16	1.55
	Infected	134	8.6	0.18	2.95
Carmela	Healthy	100	5.8	0.48	1.82
	Infected	167	10.7	1.35	3.73
Litoral	Healthy	115	9.5	0.20	2.97
	Infected	296	19.8	0.65	3.78
Favorit	Healthy	199	7.34	0.24	2.85
	Infected	421	22.6	0.65	3.95

Antioxidant enzymes are produced by host plant to promote cells protection from oxidative damage caused by pathogens and thus inducing resistance against pathogen (Hernandez et al., 2006). Our results showed that the activity of antioxidant enzymes such as polyphenol oxidase, peroxidase and catalase was increased in the leaves affected by brown rot in all apricot varieties studied (Table 2). However, the response to infection was different depending on cultivar and enzyme. The activity of peroxidase in the leaves affected by *M. laxa* was doubled, compared to the value of the control in Dacia, Litoral and Favorit cultivars.

Catalase activity increased 2-fold in three of the studied apricot varieties affected by brown rot except for Favorit, where the activity of this enzyme is much more intense compared to the healthy variety. Similar results have been reported by Siddique et al. (2014) which showed that PAL, POD, CAT and SOD have active roles in disease resistance against *Cotton Leaf Curl Burewala Virus* in different varieties of cotton. PPO may play an important role in defense and inhibition of disease in infected cultivars, because a higher PPO activity can enhance resistance to phytopathogen attack. Our results showed a little increase of PPO in leaves of infected apricot cultivars with *M. laxa*. In conclusion, the present results regarding antioxidant enzymes suggest that brown rot infection is accompanied by oxidative stress.

CONCLUSIONS

The amount of chlorophylls and carotenoids has been significantly reduced in the leaves of apricot cultivars with symptoms specific to brown rot, in both early maturation and late maturation ones.

The symptomatic leaves had a higher concentration of total phenols compared to the leaves of healthy cultivars.

The enzymatic activity of peroxidase, catalase and polyphenol oxidase was increased in apricot varieties showing symptoms of brown rot, compared to healthy varieties.

We can support that in the case of apricots, the content of total phenols and the activity of antioxidant enzymes in healthy varieties can be associated with resistance to brown rot.

Dacia, Litoral and Favorit cultivars showed a great resistance to *M. laxa*. The genotypes like Carmela and Viorica showed different susceptibility to *M. laxa.*

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