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EFFECT OF FARMING SYSTEMS ON PATHOGEN INFECTIONS AND CONTENT OF PHENOLIC COMPOUNDS IN CARROT (*DAUCUS CAROTA* L. SUBSP *SATIVUS* (HOFFM.) ROOTS

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ABSTRACT

The health benefits of carrots are determined by their nutritional value, including total phenolic content. Phenolic compounds are accumulated, among others, in response to biotic stress. The objective of this study was to evaluate the severity of black rot, crater rot, Sclerotinia rot and soft rot on the roots of carrots cv. *Koral* grown in plantations under integrated and organic farming systems. Chemical analyses were performed to determine the content of phenolic acids in harvested carrot roots. Carrots were grown in 2010-2012 in seven plantations located in Godki, Taraskowo, Tomaszkowo, Zgniłobłoty, Królikowo, Mielno and Rywociny (Poland). Carrot roots with disease symptoms were encountered more frequently in integrated plantations. Chemical analyses revealed higher concentrations of phenolic compounds in organically grown carrots, in comparison with the integrated farming system. The organic farming system seems preferable for growing carrots.

Key words: carrots, *Daucus carota*, roots, health status, pathogens, phenolic compounds.

INTRODUCTION

The most common pathogen species attacking carrots are *Alternaria dauci* (Kühn) Groves & Skolko, *A. radicina* Meier, Drechsler & Eddy, *Botrytis cinerea* Pers., *Cercospora carotae* /Pass./ Kazn. & Siemaszko, *Rhizoctonia carotae* Rader, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Thielaviopsis basicola* (Berk. & Broome) Ferraris (Coles and Wicks 2003; Farrar *et al*. 2004; Weber and Tribe 2004). The sources of infection caused by fungi of the genus *Alternaria* include seeds, nearby carrot fields, weeds and soil **(**Pryor *et al*. 2002; Strandberg, 2002). The more effective protection against pathogens during the growing season can improve the efficiency of crop yields. Alternatives to fungicides used in integrated production (Bounds *et al*., 2006) are the sowing of healthy seeds of varieties with increased resistance to pathogens, the use of agro-technical measures and biological control agents (Ratajkiewicz *et al*. 2011).

Plant protection products contribute to improving crop health and affect the nutritional value of vegetables. Carrots are used for preparing fresh juice and therefore their quality, including the content of biologically active and health-promoting compounds, is an important consideration (Ismail *et al* 2004; Nicolle *et al.* 2004). The most important antioxidants present in vegetables and fruits are vitamin C, carotenoids and phenolic compounds. The concentrations of phenolic compounds are up to five-fold higher in the roots of purple carrot varieties, compared with orange varieties (Leja *et al.* 2013). Agricultural practices may also contribute to the accumulation of phenolics in carrot roots. Smoleń and Sady (2009) demonstrated that foliar and soil application of nitrogen had no significant effect on the total phenolic content of carrot roots. In another study, the cited authors found that urea application increased the concentrations of phenolic compounds in carrots (Smoleń and Sady, 2010). Phenolic compounds are involved in the induction of defense mechanisms of plants exposed to biotic stress. Crops attacked by pathogens and pests synthesize phenolic compounds characterized by strong cytotoxic properties (Brandt and Molgaard 2001). Under stress conditions, phenolic compounds are also accumulated in the peel of carrot roots (Smoleń and Sady, 2010).

The objective of this study was to evaluate the severity of black rot, crater rot, Sclerotinia rot and soft rot on the roots of carrots cv. Koral grown in plantations under integrated and organic farming systems. Chemical analyses were performed to determine the content of phenolic acids in harvested carrot roots.

MATERIALS AND METHODS

Plant material: The experimental materials comprised carrot roots of the late cultivar *Koral*, grown in north-eastern Poland, in plantations with an area of 0.5 ha to 5 ha, under the integrated farming system (Journal of Laws of 2004. No. 11, item 94, as amended) – three plantations located in Królikowo, Mielno and Rywociny, and under the organic farming system (Journal of Laws of 2004,

No. 93, item 898, as amended) – four plantations located in Godki, Taraskowo, Tomaszkowo and Zgniłobłoty.

Phytopathological analyses: The severity of black rot (*Alternaria radicina, A. dauci*), crater rot (*Rhizoctonia carotae*), and Sclerotinia rot (*Sclerotinia sclerotiorum*) and soft rot (*Pectobacterium carotovorum*) analyzed together on carrots was evaluated at harvest. The rates of infection caused by fungi of the genus *Alternaria* were estimated on a four-point scale where: 1º – black lesions without root narrowing, 4º – black lesions with root narrowing >50% at discolored sites. The rates of infection caused by *Rhizoctonia carotae* were estimated on a nine-point scale where: 1º – no symptoms, 9º – more than 25% of affected root area. The evaluation was carried out on samples of 50 roots randomly collected at three sites in each plantation. The results were expressed as a percentage, in the form of infection index Ii. The severity of soft rot (*Pectobacterium carotovorum*) and Sclerotinia rot (*Sclerotinia sclerotiorum*) was expressed as a percentage of infected roots in 5 kg samples randomly collected from each plantation.

Extraction of phenolic compounds: After harvest, analytical samples (10 g) of carrots cv. Koral were diced, immersed in 100 ml of an 80% (v/v) aqueous solution of methanol, and homogenized for 3 minutes. The homogenate was quantitatively transferred to tightly sealed 250 ml bottles, which were placed in a shaking water bath (Julabo SW 22). Extraction was carried out for 15 minutes at 70ºC. The extract was cooled and filtered through Whatman 1 filter paper, and the residue was quantitatively transferred to a bottle. After the second extraction the procedure was repeated, and the third extraction was performed. The extracts were combined, methanol was distilled in a rotary evaporator (Büchi R-200) at 45ºC, and water was removed by lyophilization (Labconco freeze dryer). The lyophilizate was weighed to determine extraction efficiency.

Determination of phenolic compounds by reversed-phase high-performance liquid chromatography (RP-HPLC): Phenolic compounds were identified by RP-HPLC using the Shimadzu system comprised two LC-10AD pumps, an SPD-M10 photodiode array detector, and an SCL-10A controller. Samples (40 mg) were dissolved in 2 ml of 80% methanol and filtered through 0.45 µm filter. Samples were analyzed on a Luna C_{18} column (Phenomenex; 4 × 250 mm, 5 µm). A gradient of two solvents was generated: solvent A – a 5% (v/v) aqueous solution of acetonitrile + 0.1% trifluoroacetic acid (TFA), solvent B – a 60% (v/v) aqueous solution of acetonitrile + 0.1% TFA. The solvent B content of the mobile phase changed from 0% to 60% over 50 minutes, after which time columns were rinsed with solvent A for 10 minutes. Injection volume was 20 µl, mobile phase flow rate - 1 ml/min, detection at a wavelength of 320 nm. The standards used to determine phenolic compounds were chlorogenic acid and caffeic acid (Sigma).

Statistical analysis: All determinations were performed in this study in triplicate. Results are reported as mean and SD values. Analyses of variance and Dunkan's' test were performed at level of $P < 0.05$ to evaluate the significance of differences among mean values in phytopathological analyses. Mean values of HPLC results were compared by Student's t-test at significance level $P < 0.05$ (STATISTICA ® 9.0 2009-10).

RESULTS AND DISCUSSION

At harvest, the incidence of black rot, crater rot, Sclerotinia rot and soft rot on the roots of carrots was low in both farming systems (Tables 1, 2). Carrot roots were not infected by fungi of the genus *Alternaria* in 2010 (except for carrots harvested from plantations in Królikowo and Godki) and 2012. Black rot symptoms were observed on carrot roots in both farming systems in the second year of the study (except for carrots harvested from the plantation in Tomaszkowo), and the highest infection index (3%) was noted in Rywociny. The average values of infection index in all plantations did not exceed 1%.

Alternaria dauci and *A. radicina* are major pathogens of carrots and the causative agents of root rot (Farrar *et al.* 2004; Mazur and Nawrocki 2007a). *A. radicina* produces secondary metabolites which probably have no adverse effects on human health (Solfirizzo *et al.* 2005), but may negatively affect the germination capacity of carrot seeds (Tylkowska *et al.* 2003). *Alternaria* spp. fungi pose a serious threat in organic farms where chemical seed treatment is prohibited (Köhl *et al.* 2004). Alternative methods of carrot seed disinfection include treatment with hot water (45-55°C) with or without NaOCl (Nega *et al.* 2003). Jayaraj *et al.* (2008) noted lower rates of infection caused by *A. radicina* and *Botrytis cinerea* on carrot plants sprayed with *Ascophyllum nodosum* extract. According to Ratajkiewicz *et al.* (2011), *A. radicina* was sensitive to *Bacillus coagulans*, *Propionibacterium freudenreichii* ssp. *shermanii* and *Pythium oligandrum* strains, which inhibited the growth of this pathogen on carrot roots. Mazur and Nawrocki (2007b) reported that azoxystrobin and grapefruit extract were effective in preventing carrot yield loss.

The symptoms of infection caused by *Rhizoctonia carotae* were not observed on organic carrots during first growing season and on carrots harvested from integrated production plantations in the last season. Significantly higher infection rates were noted in the first two years of the study on carrots harvested in Rywociny, as compared with the other plantations, which was

confirmed by average values of the infection index determined for locations. Weaker symptoms of crater rot were observed on the roots of organically grown carrots, and the highest infection index (5.3%) was noted in Godki. *R. carotae, B. cinerea* and *S. sclerotiorum* attack carrot roots in the soil, and are transferred to storage facilities with soil particles attached to the roots. The above pathogens, in particular *S. sclerotiorum*, are responsible for considerable economic losses (Farrar *et al.* 2004; Vikram *et al.* 2005). According to Jensen *et al.* (2008), wild carrots are less susceptible to *S. sclerotiorum* than cultivated carrots, and may thus serve as a host of *S. sclerotiorum* and benefit from the uptake of resistance genes.

The symptoms of soft rot and Sclerotinia rot on carrot roots in the integrated farming system were observed during first two years of the experiment. The highest number of infected carrot roots were harvested in Królikowo. The rates of infection on organic carrots were similar in all plantations in the first year of the study and in some plantations in subsequent years (except for the plantation in Taraskowo in 2011 and the plantation in Tomaszkowo in 2011 and 2012).

Bourn and Prescott (2002) demonstrated that organically grown vegetables are characterized by higher levels of phenolic compounds than conventionally grown vegetables. Olsson and Stevenson (1997) observed a correlation between the presence of phenolic compounds in stored carrot roots and the incidence of infections caused by *A. alternata, R. carote* and *S. sclerotiorum*. The predominant phenolic compound in carrot roots is chlorogenic acid which accounts for 60-80% of all phenolic acids (Leja *et al.* 2013). Other phenolic acids (*p*-hydroxybenzoic acid, caffeic acid and cinnamic acid derivatives) were present at substantially lower concentrations in carrots. Polyphenols accumulate mostly in the periderm, their concentrations were lower in the xylem and lowest in the phloem (Sharma *et al.* 2012). Their levels vary during ripening and storage (Arscott and Tanumihardjo 2010).

The chromatograms of extracts obtained from carrot fields revealed the presence of four major peaks with retention times of ca.13.4 min. (compound 1), 15.2 min. (compound 2), 19.5 min. (compound 3) and 25.2 min. (compound 4) (Fig. 1). The UV absorption spectra of compounds 1, 2, 3 and 4 had their maximum intensities at wavelengths of 324 nm, 318 nm, 326 nm and 324, respectively (Fig. 2). Based on the retention time of the reference standard and the UV spectrum, compound 1 was identified as chlorogenic acid. Compounds 2, 3 and 4 were found to be derivatives of caffeic acid. Since the retention times of their peaks were higher than the retention time of caffeic acid, they were probably characterized by lower polarity than caffeic acid. They certainly were not glycosides of caffeic acid; most probably, they were esters of this phenolic acid. Based on the works of Alasalvar *et al.* (2001) and Surjadinata and Cisneros-Zevallos (2012), compound 4 was identified as 3,5-dicaffeoylquinic acid.

Table 3 showed the content of chlorogenic acid and compounds 2, 3 and 4 (expressed as caffeic acid equivalents) in carrots. Chlorogenic acid was the predominant compound in all samples. The second most abundant compound in carrot samples was compound 4, while compounds 2 and 3 were present at considerably lower concentrations. The data were characterized by high statistical dispersion (high values of the variation coefficient).

A statistical analysis revealed that in all years of the study, the content of chlorogenic acid and compounds 2 and 4 was higher in organically grown carrots, in comparison with the integrated system. In 2012, the concentrations of compound 2 were higher in organic carrot roots.

The time of harvest had an insignificant effect on the concentrations of phenolics in carrots. The chlorogenic acid content of carrots grown in the integrated system was higher in 2011 than in 2010. The content of compound 2 in organic carrots was higher in 2012 than in 2010. The content of compound 3 in organic carrots was higher in 2011 than in 2010 and 2012. No significant differences were observed in the content of compound 4 in carrots harvested from organic and integrated production plantations in 2010, 2011 and 2012.

The chlorogenic acid content of organically grown carrots, determined in our study, is similar to that reported by Alasalvar *et al.* (2001) for yellow carrots (4.41 mg/ 100 g). The cited authors noted higher concentrations of chlorogenic acid in orange carrots (8.50 mg/100 g). In a study by Surjadinata and Cisneros-Zevallos (2012), the chlorogenic acid content of carrots was slightly higher than that determined for organic carrots in our experiment, at 5.49, 5.29 and 6.30 mg/100 g. In a study by Leja *et al.* (2013), the total phenolic content of carrots ranged from 4.5 to 16.6 mg/100 g.

Most authors (Faller and Fialho 2009; Smoleń and Sady 2009; Mattila and Hellstrőm 2007; Søltoft *et al.* 2010; Sun *et al.* 2009; Jacobo-Velázquez *et al.* 2011; Leja *et al.* 2013; Kenny and O'Beirne 2010; Du *et al.* 2012) used the Folin-Ciocalteu reagent (FCR) for the colorimetric assays of total phenolics in carrots. The values obtained by the above method are generally higher than those obtained using HPLC, which makes it difficult to compare our findings with those of other authors.

It can be concluded that carrot roots showing rot symptoms were encountered more frequently in the integrated farming system than in organic plantations. The content of phenolic compounds was higher in organic carrots than in carrots grown in integrated production plantations. Thus, the organic farming system seems preferable for growing carrots.

Table 1. Disease severity on the roots of carrots at harvest – integrated farming system

Location	*Alternaria* spp. Infection index Ii (%)				*Rhizoctonia carotae* Infection index Ii (%)				*Sclerotinia sclerotiorum* *Pectobacterium carotovorum* % by weight of affected roots			
	2010	2011	2012	Mean	2010	2011	2012	Mean	2010	2011	2012	Mean
Królikowo	1.0b	0.5c	0d	0.5b	1.0d	2.0c	0e	1.0	4.2b	11.0a	0d	5.1
Mielno	0d	0.7bc	0d	0.2c	1.5cd	1.3cd	0e	0.9	0d	4.6b	0d	1.5
Rywociny	0d	3.0a	0d	1.0a	4.3b	10.7a	0e	5.0	2.2c	1.0d	0d	1.1
Mean	0.3	1.4	0		2.3	4.7	0		2.1	5.5	0	

Values followed by the same letters are not significantly different

Table 2. Disease severity on the roots of carrots at harvest – organic farming system

Location	*Alternaria* spp. Infection index Ii (%)				*Rhizoctonia carotae* Infection index Ii (%)				*Sclerotinia sclerotiorum* *Pectobacterium carotovorum* % by weight of affected roots			
	2010	2011	2012	Mean	2010	2011	2012	Mean	2010	2011	2012	Mean
Godki	0.5c	1.0b	0d	0.5	0e	1.3c	5.3a	2.2	2.0b	0c	0c	0.7
Taraskowo	0d	0.5c	0d	0.2	0e	0e	3.1b	1.0	2.2b	2.2b	4.0a	2.8
Tomaszkowo	0d	0d	0d	0	0e	0.7d	1.3 c	0.7	2.0b	0c	0c	0.7
Zgniłobłoty	0d	1.5a	0d	0.5	0e	1.3c	0.7d	0.7	2.5b	2.3b	0c	1.6
Mean	0.1	0.8	0		0	0.8	2.6		2.2	1.1	1.0	

Values followed by the same letters are not significantly different

Table 3. The content of phenolic acids in the roots of carrots cv. Koral grown in plantations under integrated (I) and organic (O) farming systems (mg/100 g)

Year	Plantation	Compound 1 (chlorogenic acid)	Compound 2	Compound 3	Compound 4
2010	I	1.914 ± 0.722^{aA}	0.408 ± 0.178^{aA}	0.322 ± 0.170^{aA}	1.031 ± 0.504^{aA}
	O	4.782 ± 1.627^{bA}	0.472 ± 0.217^{aA}	0.646 ± 0.287^{bA}	1.638 ± 0.658^{bA}
2011	I				
	O	2.241 ± 0.821^{aB}	0.423 ± 0.258^{aA}	0.301 ± 0.155^{aA}	1.321 ± 0.550^{aA}
2012	I	4.945 ± 1.756^{bA}	0.558 ± 0.302^{aAB}	0.820 ± 0.322^{bB}	1.845 ± 0.695^{bA}
	O				
		1.789 ± 0.654^{aAB}	0.452 ± 0.267^{aA}	0.280 ± 0.162^{aA}	1.178 ± 0.485^{aA}
		4.689 ± 1.552^{bA}	0.623 ± 0.238^{bB}	0.584 ± 0.234^{bAC}	1.789 ± 0.675^{bA}

I – integrated production plantation; O – organic plantation. Compounds 2, 3 and 4 are expressed as caffeic acid equivalents. Values followed by the same letters are not significantly different; small letters – comparison of means between plantations in the same year, capital letters – comparison of means between years in the same plantation.

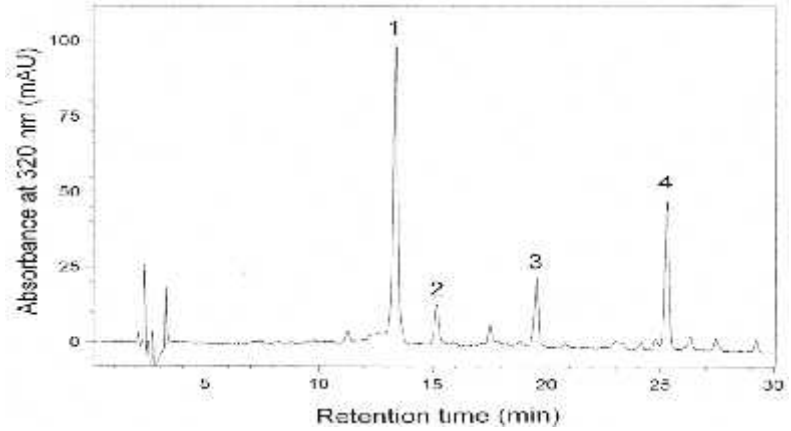

Figure 1. RP-HPLC chromatogram of phenolic compounds found in carrots

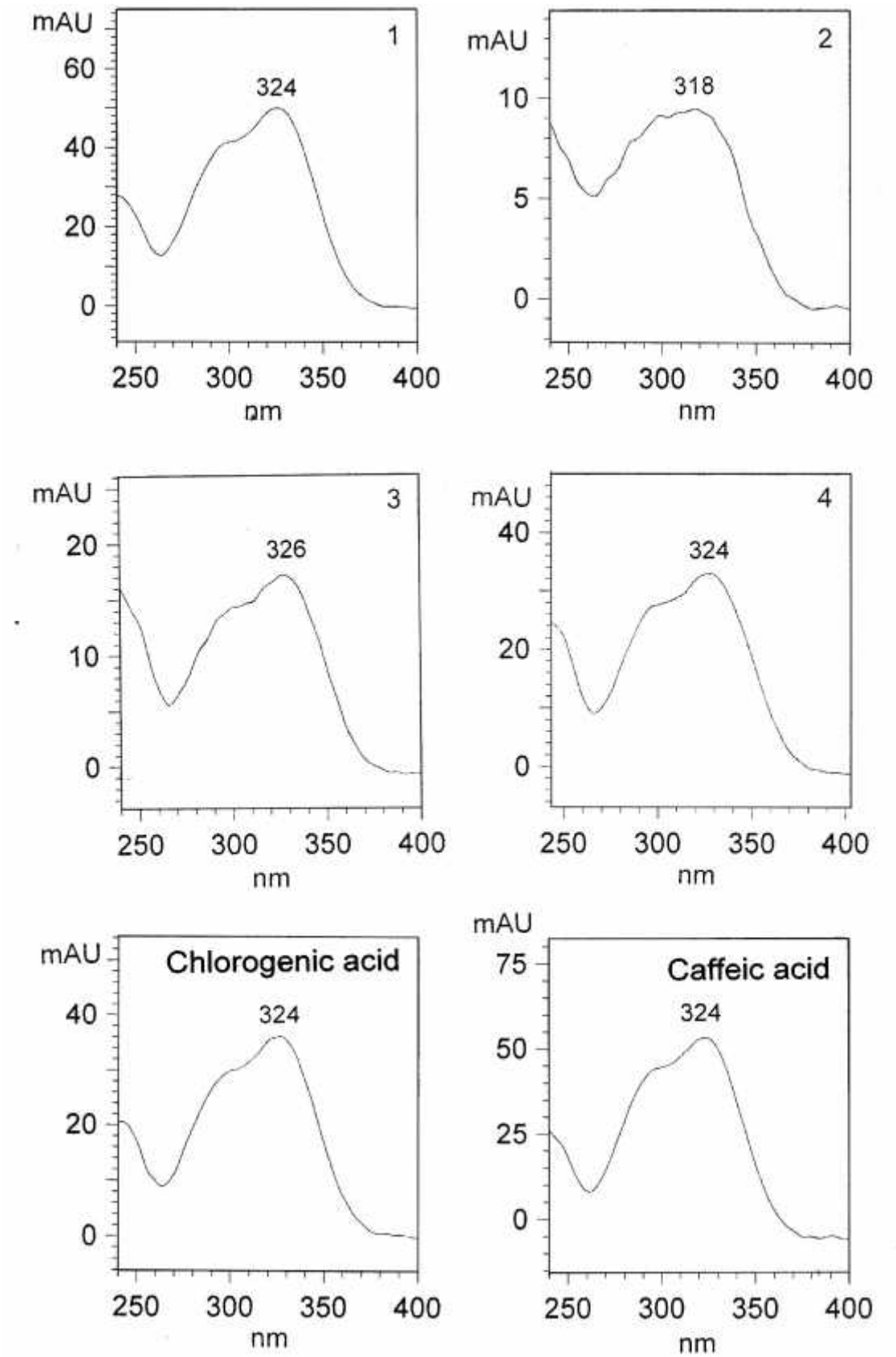

Figure 2. UV spectra of compounds 1, 2, 3, 4, and standard of chlorogenic and caffeic acids.

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