

DOI: 10.11931/guihaia.gxzw201406008

孙雪丹,何苗,罗友,等. 石菖蒲醇提物对番茄幼苗防御酶影响[J]. 广西植物, 2015, 35(4):597-602

Sun XD, He M, Luo Y, et al. Effects of alcoholic extracts from *Acorus tatarinowii* on defensive enzymes of tomato[J]. *Guihaia*, 2015, 35(4):597-602

石菖蒲醇提物对番茄幼苗防御酶影响

孙雪丹¹, 何苗¹, 罗友¹, 刘铁秋^{1,2}, 卢勇³, 曾宪垠^{1,2*}

(1. 四川农业大学 生命科学学院, 四川 雅安 625014; 2. 四川农业大学 原子能农业应用研究室, 四川 雅安 625014; 3. 四川玛思特农业开发有限公司, 四川 南充 637105)

摘要: 为了探讨石菖蒲醇提物作为一种中药杀菌剂对病菌寄主植物本身防御酶体系的影响, 该研究以番茄欧粉贝幼苗和川产道地中药石菖蒲为材料, 采用冷浸法制备石菖蒲醇提物, 经不同浓度石菖蒲醇提物对番茄幼苗进行处理, 用比色法测定多酚氧化酶(PPO)、苯丙氨酸解氨酶(PAL)和过氧化物酶(POD)的活性, 并利用GC-MS联用技术, 对石菖蒲醇提物的主要化学成分进行定性分析。结果表明: 第1天, 醇提物浓度在0.04~0.08 g·mL⁻¹时, PAL活性显著高于对照; 随后PAL活性随浓度升高降低; 当浓度在0.04~0.16 g·mL⁻¹时, POD活性与对照无显著差异; 浓度为0.32 g·mL⁻¹时, POD活性显著高于对照; 浓度为0.64 g·mL⁻¹时, POD活性显著低于对照; 当浓度在0.04~0.32 g·mL⁻¹时, PPO活性显著高于对照; 浓度为0.64 g·mL⁻¹时, PPO活性与对照无显著差异; 随着时间的延长, 三种酶各处理组之间的差异逐步减少。第4天时, 各处理组之间PAL的活性无显著差异; 当浓度为0.04 g·mL⁻¹和0.64 g·mL⁻¹时, PPO活性与对照无显著差异, 其余范围均显著高于对照; 当浓度在0.08~0.32 g·mL⁻¹时, POD活性数值上均高于对照。以上表明在一定浓度范围内醇提物可以增强防御酶活性, 未破坏植物的防御酶体系。因此, 该醇提物作为一种新型的中药杀菌剂有巨大的开发和利用潜力。利用GC-MS联用技术, 可从醇提物中鉴定出30种化学成分, 这些物质中多为苯丙素类物质, 如β-细辛醚、α-细辛醚和顺式甲基异丁香油酚等, 其中含量最高的物质为β-细辛醚(42.48%)。这提示在后续的研究中, 可针对β-细辛醚等醇提物的主要化学成分进行。

关键词: 石菖蒲; 番茄; 防御酶; GC-MS; β-细辛醚**中图分类号:** Q946; S482.2 **文献标识码:** A **文章编号:** 1000-3142(2015)04-0597-06

Effects of alcoholic extracts from *Acorus tatarinowii* on defensive enzymes of tomato

SUN Xue-Dan¹, HE Miao¹, LUO You¹, LIU Tie-Qiu^{1,2},
LU Yong³, ZENG Xian-Yin^{1,2*}

(1. College of Life Sciences, Sichuan Agricultural University, Ya'an 625014, China; 2. Isotope Research Laboratory, Sichuan Agricultural University, Ya'an 625014, China; 3. Sichuan Master Agricultural Development Co., Ltd., Nanchong 637105, China)

Abstract: This paper aims to explore *Acorus tatarinowii* alcohol extracts as a kind of traditional Chinese herbal medicine fungicide effects on the plant defense enzyme system of fungi host. The test materials were tomato seedlings and Sichuan authentic herbal medicine *A. tatarinowii*. *A. tatarinowii* alcohol extracts were prepared by cold-maceration. The main experimental methods were colorimetric method and gas chromatography-mass spectrometry. The activity of plant defensive enzymes were examined by colorimetric method after the treatment of tomato seedlings using dif-

收稿日期: 2014-10-29 **修回日期:** 2014-12-11**基金项目:** 四川省科技厅国际合作项目(2012HH0013); 四川农业大学双支计划项目(00770107)。**作者简介:** 孙雪丹(1993-), 男, 主要从事中药天然抗菌化合物研究, (E-mail) jijichenmo@163.com。**通讯作者:** 曾宪垠, 博士, 研究员, 主要从事天然药物筛选及其生物学功能研究, (E-mail) xyzen@sicau.edu.cn。

ferent concentrations of alcoholic extracts from *A. tatarinowii*. And the main chemical compositions of *A. tatarinowii* alcohol extracts were analyzed by gas chromatography-mass spectrometry. These results showed that when the concentration of *A. tatarinowii* alcohol extracts in $0.04\text{--}0.08\text{ g}\cdot\text{mL}^{-1}$, the activity of PAL was significantly higher than control groups on the first day. Then the activity of PAL reduced with the increase of the concentration of alcohol extract. And at the outset of the activity of POD had no significant difference on concentration condition of $0.04\text{--}0.16\text{ g}\cdot\text{mL}^{-1}$. When the concentration of *A. tatarinowii* alcohol extracts in $0.64\text{ g}\cdot\text{mL}^{-1}$, the activity of PAL was significantly lower than control groups. And the activity of PPO was significantly higher than control groups on concentration condition of $0.04\text{--}0.32\text{ g}\cdot\text{mL}^{-1}$. When in $0.64\text{ g}\cdot\text{mL}^{-1}$, the activity of PPO had no significant difference with control groups. But with the extension of time, the difference between each group of three enzymes was reduced. On the 4th day, the activity of PAL had no significant differences between treatment groups. When the concentration of alcohol extracts in $0.04\text{--}0.64\text{ g}\cdot\text{mL}^{-1}$, the activity of PPO had no significant differences with control groups. The activity of PPO was significantly higher than control groups on the scope of the other. When in $0.08\text{--}0.32\text{ g}\cdot\text{mL}^{-1}$, the activity of POD of values were higher than those in control groups. The results showed that *A. tatarinowii* alcohol extracts could influence the activity of plant defensive enzymes and a certain concentration of *A. tatarinowii* alcohol extracts could enhance it without damaging plants defense enzyme system. Therefore, *A. tatarinowii* alcohol extracts as a new type of traditional Chinese herbal medicine fungicide is a huge potential of development and utilization. And thirty chemical compositions were identified from *A. tatarinowii* alcoholic extracts by gas chromatography-mass spectrometry. The main chemical composition of *A. tatarinowii* alcohol extracts was phenylpropanoids such as β -asarone, α -asarone and cis-Methylisoeugenol. The most abundant chemical composition was β -asarone. And the content of β -asarone in *A. tatarinowii* alcohol extracts accounted for 42.48%. And in follow-up studies, we can study the main chemical composition of *A. tatarinowii* alcohol extracts like β -asarone.

Key words: *Acorus tatarinowii*; tomato; defensive enzymes; GC-MS; β -asarone

番茄(*Lycopersicon esculentum*)是我国重要的蔬菜之一,但危害番茄较为严重的病害就有 40 余种,每年给番茄种植业造成巨大损失(赵统敏等, 2011)。其中,番茄灰霉病菌(*Botrytis cinerea*)是严重影响番茄产量的重要病菌(Wang *et al.*, 2010),该病菌危害大,繁殖快,抗病育种困难(Decognet *et al.*, 2009),现主要依靠传统化学农药进行防治。随着传统化学农药所带来的环境和健康问题日益凸显,植物源农药因其环境友好型的特点成为了农药发展的新热点(单承莺等, 2011)。

石菖蒲(*Acorus tatarinowii*)为天南星科菖蒲属多年生草本植物,味辛性温,具有保护神经细胞、抗癌、抗惊厥、抗心律失常、抗病虫害等作用(Li *et al.*, 2010;王睿等, 2013;Kim *et al.*, 2003)。刘铁秋等(2013)发现石菖蒲醇提取物对番茄灰霉病菌有显著抑菌效果,这使得以石菖蒲为原料研发中药杀菌剂成为可能。但关于该醇提取物对植物防御酶体系影响的研究一直未见报道。鉴于此,本文测定经醇提取物处理后的番茄 3 种防御酶酶活并分析提取物的主要成分,以探讨其用于番茄灰霉病防治的实际意义,为石菖蒲中药杀菌剂的开发利用奠定科学的基础。

1 材料与方 法

1.1 材料

石菖蒲根茎(*Rhizoma Acori tatarinowii*),购自四川省成都市北京同仁堂总府路店;欧粉贝番茄幼苗,购自山东寿光旺盛种业总公司。

1.2 提取物制备

参考刘铁秋等(2014)的方法,制备原药浓度为 $1\text{ g}\cdot\text{mL}^{-1}$ 的醇提取物。

1.3 对植株防御酶的影响

选择生长旺盛且长势基本相同的欧粉贝番茄幼苗进行试验。用浓度为 0.04 、 0.08 、 0.16 、 0.32 、 $0.64\text{ g}\cdot\text{mL}^{-1}$ 的石菖蒲醇提取物均匀涂抹相同叶位的叶片正反面,无菌水作对照,3 次重复。处理后 1、2、3、4 d 进行取样测定。苯丙氨酸解氨酶(PAL)活性测定参考秦培文等(2011)的方法,以 1 min 内 OD_{290} 增加 0.01 为 PAL 的 1 个酶活力单位(U);多酚氧化酶(PPO)活性测定参考邹芳斌等(2008)的方法,以 1 min 内 OD_{398} 增加 0.01 为 PPO 的 1 个活力单位(U);过氧化物酶(POD)活性测定参考关丽杰等

(2008)的方法,以每 1 min OD₄₇₀ 值变化 0.01 为一个酶活性单位(U)。

1.4 GC-MS 条件

气相色谱条件:色谱柱为 Agilent DB-5 MS DG;进样温度为 250 °C;柱温 60 °C 保持 5 min,再以 20.0 °C · min⁻¹ 速率升温至 270 °C,保持 5 min;溶剂延迟为 3.5 min;载气为体积分数 99.999% 的高纯氦气;柱前压 60 kPa;进样方式为 GC 自动进样器;进样量为 0.2 μL,分流比为 30 : 1。质谱条件:EI 离子源温度为 280 °C;MS 四极杆温度为 150 °C;电子能量为 70 eV;接口温度为 270 °C;质量范围为 30 ~ 400 amu。

1.5 分析方法

1.5.1 统计分析 采用 PASW Statistics 18 软件进行,显著性分析采用 DMRT 法 ($P < 0.05$)。

1.5.2 GC-MS 分析 对总离子流图中的各峰进行质谱扫描,用色谱数据处理系统,以峰面积归一法确定各化学成分的相对含量。通过 NIST 05 质谱库检索和有关质谱资料查阅进行成分定性分析。

2 结果与分析

2.1 石菖蒲醇提物对番茄幼苗防御酶活性的影响

2.1.1 对苯丙氨酸解氨酶(PAL)活性影响 图 1 显示,第 1 天,当醇提物浓度为 0.04 ~ 0.08 g · mL⁻¹ 时,PAL 活性显著高于对照;浓度高于 0.08 g · mL⁻¹ 时,PAL 活性显著低于对照;第 2 天至第 3 天 PAL 活性变化规律同第 1 天,即浓度为 0.04 ~ 0.16 g · mL⁻¹ 时,PAL 活性高于对照,浓度高于 0.16 g · mL⁻¹ 时,PAL 活性显著低于对照;到第 4 天,各处理组 PAL 活性无显著差异仅数值上存在以上变化规律。这表明石菖蒲醇提物处理初期,低浓度处理可以激活 PAL,高浓度处理有抑制 PAL 作用,但随着时间的延迟 PAL 水平又恢复到正常水平。

2.1.2 对多酚氧化酶(PPO)活性的影响 由图 2 可知,第 1 天,当醇提物浓度为 0.04 ~ 0.32 g · mL⁻¹ 时,PPO 活性显著高于对照;当浓度为 0.64 g · mL⁻¹ 时,PPO 活性与对照无显著差异。第 2 天,当浓度为 0.04 g · mL⁻¹ 时,PPO 活性与对照无显著差异;当浓度为 0.08 ~ 0.32 g · mL⁻¹ 时,PPO 活性显著高于对照;当浓度为 0.64 g · mL⁻¹ 时,PPO 活性显著低于对照。第 3 天,当浓度为 0.04 ~ 0.08 g · mL⁻¹ 时,PPO 活性与对照无显著差异;当浓度为

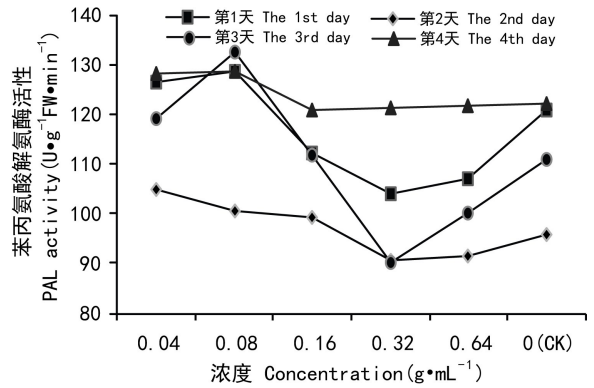


图 1 石菖蒲醇提物对番茄幼苗苯丙氨酸解氨酶活性影响

Fig.1 Influence of *A. tatarinowii* ethanol extracts on PAL activity of tomato seedlings

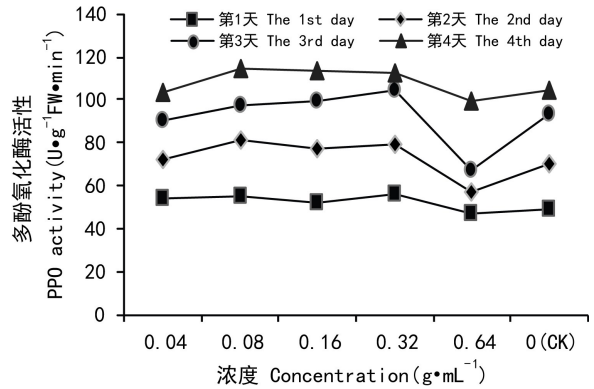


图 2 石菖蒲醇提物对番茄幼苗多酚氧化酶活性影响
Fig. 2 Influence of *A. tatarinowii* ethanol extracts on PPO activity of tomato seedlings

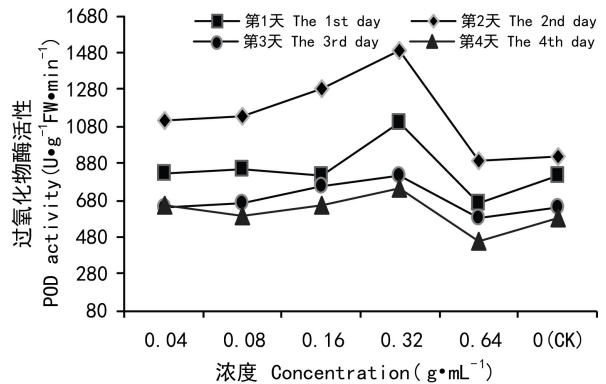


图 3 石菖蒲醇提物对番茄幼苗过氧化物酶活性影响
Fig. 3 Influence of *A. tatarinowii* ethanol extracts on POD activity of tomato seedlings

0.16 ~ 0.32 g · mL⁻¹ 时,PPO 活性显著高于对照;当浓度为 0.64 g · mL⁻¹ 时,PPO 活性显著低于对照。

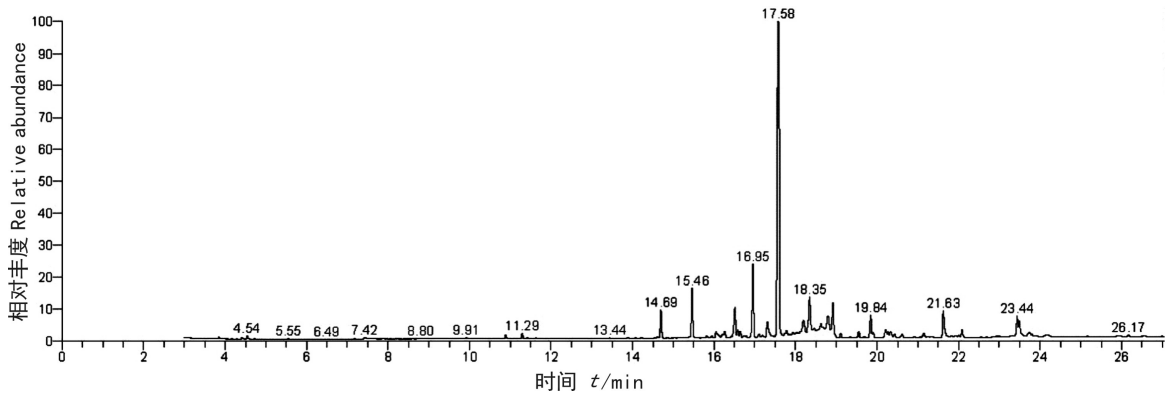


图 4 石菖蒲醇提物总离子流色谱图

Fig. 4 TIC of *A. tatarinowii* ethanol extracts

表 1 石菖蒲醇提物成分分析

Table 1 Analysis results of component of ethanol extracts from *A. tatarinowii*

序号 No.	保留时间 RT (min)	化合物 Compound	分子式 Molecular formula	分子量 Molecular weight	相对含量 Relative content(%)
1	3.85	乙缩醛 Acetal	$C_6H_{14}O_2$	118	0.10
2	4.42	(2S,3S)-(+) -2,3-丁二醇 (2S,3S)-(+) -2,3-Butanediol	$C_4H_{10}O_2$	90	0.10
3	4.54	丙三醇 1,2,3-Propanetriol	$C_4H_{10}O_2$	90	0.23
4	7.42	桉叶油醇 Cineole	$C_3H_8O_3$	92	0.15
5	8.80	芳樟醇 Linalool	$C_{10}H_{18}O$	154	0.02
6	9.91	左旋樟脑 l-Camphor	$C_{10}H_{18}O$	154	0.06
7	10.88	4-萜烯醇 Terpinen-4-ol	$C_{10}H_{16}O$	152	0.29
8	11.29	2-茨醇 Borneol	$C_{10}H_{18}O$	154	0.39
9	11.41	DL-异冰片醇 DL-Isoborneol	$C_{10}H_{18}O$	154	0.06
10	14.69	甲基丁香酚 Methyl eugenol	$C_{11}H_{14}O_2$	178	2.30
11	15.46	顺式甲基异丁香油酚 cis-Methylisoeugenol	$C_{11}H_{14}O_2$	178	4.59
12	16.05	反式甲基异丁香油酚 trans-Methylisoeugenol	$C_{11}H_{14}O_2$	178	0.84
13	16.26	β -石竹烯 β -caryophyllene	$C_{15}H_{24}$	204	0.87
14	16.51	d-杜松烯 d-cadinene	$C_{15}H_{24}$	204	3.31
15	16.58	喇叭茶萜醇 ledol	$C_{15}H_{26}O$	222	0.72
16	16.95	α -细辛醚 α -Asarone	$C_{12}H_{16}O_3$	208	7.04
17	17.31	吉玛烯 B germacrene B	$C_{15}H_{24}$	204	2.14
18	17.58	β -细辛醚 β -asarone	$C_{12}H_{16}O_3$	208	42.48
19	17.78	二羟基-异菖蒲二醇 dehydroxy-isocalamendiol	$C_{15}H_{24}O$	220	0.57
20	18.19	(2E,6E)-金合欢醇 (2E,6E)-farnesol	$C_{15}H_{26}O$	222	1.97
21	18.35	γ -细辛醚 γ -Asarone	$C_{12}H_{16}O_3$	208	4.05
22	18.63	tau-紫穗槐醇 tau-muurolol	$C_{15}H_{26}O$	222	1.04
23	18.80	tau-杜松醇 tau-cadinol	$C_{15}H_{26}O$	222	3.66
24	18.91	α -杜松醇 α -cadinol	$C_{15}H_{26}O$	222	3.79
25	19.84	菖蒲酮 shyobunone	$C_{15}H_{24}O$	220	2.58
26	20.27	柏木-9-酮 cedran-9-one	$C_{15}H_{24}O$	220	0.41
27	20.33	桉油烯醇(-)-Spathulenol	$C_{15}H_{24}O$	220	0.52
28	21.63	棕榈酸 Palmitic acid	$C_{16}H_{32}O_2$	256	3.01
29	23.44	亚油酸 Linoleic acid	$C_{18}H_{32}O_2$	280	1.65
30	26.17	己二酸二辛酯 Dioctyl adipate	$C_{22}H_{42}O_4$	370	0.15

第 4 天,当浓度在 $0.08 \sim 0.32 \text{ g} \cdot \text{mL}^{-1}$ 时,PPO 活性均显著高于对照;当浓度为 $0.04 \text{ g} \cdot \text{mL}^{-1}$ 和 0.64

$\text{g} \cdot \text{mL}^{-1}$ 时,PPO 活性与对照无显著差异。以上表明除第 1 天外低浓度醇提物处理对 PPO 活性无显

著影响,而高浓度醇提物处理在初期有抑制 PPO 作用,到了第 4 天抑制作用消失。

2.1.3 对过氧化物酶(POD)活性影响 由图 3 可知,第 1 天,当醇提物浓度为 $0.04 \sim 0.16 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性与对照无显著差异;当浓度为 $0.32 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性显著地高于对照;当浓度为 $0.64 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性显著低于对照。第 2 天,当浓度为 $0.04 \sim 0.32 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性显著高于对照;当浓度为 $0.64 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性与对照无显著差异。第 3 天,当浓度为 $0.04 \sim 0.08 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性与对照无显著差异;当浓度为 $0.16 \sim 0.32 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性显著高于对照;当浓度为 $0.64 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性显著低于对照。第 4 天,当浓度为 $0.04 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性显著高于对照;当浓度为 $0.08 \sim 0.32 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性数值上均高于对照;浓度为 $0.64 \text{ g} \cdot \text{mL}^{-1}$ 时,POD 活性显著低于对照。这表明高度醇提物处理有抑制 POD 的作用且抑制作用可持续,而低浓度处理后第 4 天仍可使 POD 活性保持在一个较高水平。

2.2 GC-MS 分析

对石菖蒲醇提物进行 GC-MS 测定,获得其总离子流色谱图(图 4)。从石菖蒲醇提物中共鉴定 30 种化学成分(表 4),占提取物总量的 89.09%。其中主要成分为 β -细辛醚(42.48%)、 α -细辛醚(7.04%)、顺式甲基异丁香油酚(4.59%)、 γ -细辛醚(4.05%)和 α -杜松醇(3.79%)等。

3 讨论

防御酶活性增强是诱导抗病性产生的重要机制之一(Latha *et al.*, 2009)。在病原微生物侵染过程中 POD、PPO、PAL 等防御酶都为保护植物体起着重要的作用。POD 主要在木质素生物合成过程中催化 H_2O_2 分解而发挥作用;PPO 主要参与酚的氧化,形成对病菌毒性较高的醌类物质,从而构成保护性屏蔽而使细胞免受病菌的侵害;PAL 则是木质素与植保素沿苯丙烷类代谢途径合成的关键调节酶(Grosskopf *et al.*, 1990;Liu *et al.*, 2005;刘姬艳等, 2010;张绍珊等, 2010)。各浓度的石菖蒲醇提物处理初期,3 种防御酶活总体表现为 Hormesis 效应(高抑低促)。但随着时间的推移,不同防御酶活性变化有很大差异。其中 PAL 和 PPO 这两种酶的大多数处理组都在第 4 天恢复至正常水平。但在第 4

天时,POD 活性依然表现为 Hormesis 效应。可见随时间延长石菖蒲醇提物对 PAL 和 PPO 并无明显影响但对 POD 有明显诱导或抑制作用,从而引起植株本身抗病性的变化。

本研究中石菖蒲醇提物主要化学成分为苯丙素类,如细辛醚、甲基异丁香油酚,其中相对含量最高的物质为 β -细辛醚。这与林双峰等(2004)的研究结果一致。除了苯丙素类物质外,石菖蒲醇提物还检出多种单萜类和倍半萜类物质,如石竹烯、杜松烯等。但石菖蒲中含量较少的成分类型,在不同文献中结果存在较大差异,这可能与中药的道地性有关(房敏峰等, 2009;刘春海等, 2006)。而石菖蒲醇提物中具体哪些物质影响番茄防御酶活性及其影响机制,目前仍有待进一步研究。

参考文献:

- Decognet V, Ravetti F, Martin C, *et al.* 2009. Improved leaf pruning reduces development of stem cankers caused by grey mould in greenhouse tomatoes[J]. *Agron Sust Dev*, **30**(2):465-472
- Fang MF(房敏峰), Zhang WJ(张文娟), Li YF(李云峰), *et al.* 2009. Analysis of fat-soluble components from drug matched pair of *Polygala tenuifolia* willd. and *Acorus tatarinowii* Schott. by GC-MS (GC-MS 分析药对远志-石菖蒲中的脂溶性成分)[J]. *Chin Pharm* (中国药业), **18**(23):3-5
- Grosskopf DG, Felix G, Boller T. 1990. K-252a inhibits the response of tomato cells to fungal elicitors *in vivo* and their microsomal protein kinase *in vitro*[J]. *FEBS Lett*, **275**(1):177-180
- Guan LJ(关丽杰), Bai K(白坤), Li HY(李海燕), *et al.* 2008. Effects of *Psoralea corylifolia* L. extract on the defensive enzymes of cucumber(补骨脂提取物对黄瓜体内防御酶系的影响)[J]. *Chin J Biol Contr* (中国生物防治), **24**(S1):71-75
- Kim S, Park C, Ohh M, *et al.* 2003. Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: anobiidae)[J]. *J Stor Prod Res*, **39**(1):11-19
- Kim S, Roh J, Kim D, *et al.* 2003. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*[J]. *J Stor Prod Res*, **39**(3):293-303
- Latha P, anand T, Ragupathi N, *et al.* 2009. antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and *Zimmu* leaf extract against *alternaria solani*[J]. *Biol Contr*, **50**(2):85-93
- Li C, Xing G, Dong M, *et al.* 2010. Beta-asarone protection against beta amyloid induced neurotoxicity in PC12 cells via JNK signaling and modulation of Bcl-2 family proteins[J]. *Eur J Pharmacol*, **635**(1):96-102
- Lin SF(林双峰), Wei G(魏刚), He B(何斌), *et al.* 2004. GC-MS Analysis of alcoholic extracts from *Acorus tatarinowii* (石菖蒲醇提液主要化学成分 GC-MS 分析)[J]. *Tradit Chin Drug Res & Clin Pharmacol* (中药新药与临床药理), **15**(2):116-118
- Liu CH(刘春海), Liu XJ(刘西京), Yang HS(杨华生). 2006.

- GC-MS analysis of essential oils from *Acorus tatarinowii* Schott. (石菖蒲挥发油的 GC-MS 分析)[J]. *Chin Arch Trad Chin Med* (中医药学刊), **24**(7):1 280—1 281
- Liu H, Jiang W, Bi Y, *et al.* 2005. Postharvest BTH treatment induces resistance of peach (*Prunus persica*) fruit to infection by Penicillium expansum and enhances activity of fruit defense mechanisms[J]. *Postharvest Biol Technol*, **35**(3):263—269
- Liu JY(刘姬艳), Liu RD(刘如东), Hu DH(胡定慧). 2010. Effects of water extract of *Sophora* on defensive key enzymes activities in wheat leaves(苦参提取物对小麦防御性关键酶活性的影响)[J]. *J Hangzhou Norm Univ; Nat Sci Ed* (杭州师范大学学报·自然科学版), **9**(5):369—371
- Liu TQ(刘铁秋), Sun XD(孙雪丹), He M(何苗), *et al.* 2013. Inhibitory effects of 20 species of Sichuan authentic herbal medicine on mycelial growth of *Botrytis cinerea* (20种川产道地药材对番茄灰霉病的抑菌作用研究)[J]. *Sci Technol Food Ind* (食品工业科技), **34**(24):114—119
- Liu TQ(刘铁秋), Sun XD(孙雪丹), Lu FR(卢发瑞), *et al.* 2014. Inhibitory effect on *Botrytis cinerea* and optimization of extraction procedure of antifungal substances from rhizome *acorus tatarinowii* (石菖蒲提取物的抑菌效果及其提取工艺的优化)[J]. *Chin J Biol* (中国生物制品学杂志), **27**(1):94—99
- Qin PW(秦培文), Song WD(宋文东), Li XF(李晓菲), *et al.* 2011. The inhibition of sea brine on *Alternaria solani* and its effects on defense enzymes of tomato leaves(海盐苦卤对番茄早疫病菌的抑制及其对番茄叶片防御酶系的影响)[J]. *Chin Agric Sci Bull* (中国农学通报), **27**(16):182—185
- Shan CY(单承莺), Ma SH(马世宏), Zhang WM(张卫明). 2011. Review on research and development of botanical pesticides in China(我国植物源农药研究进展)[J]. *Chin Wild Plant Resour* (中国野生植物资源), **30**(6):14—18
- Wang R(王睿), Fei HX(费洪新), Li XM(李晓明), *et al.* 2013. Research progress on chemical compositions and pharmacological actions of *Acorus tatarinowii* Schott(石菖蒲的化学成分及药理作用研究进展)[J]. *Chin Arch Trad Chin Med* (中华中医药学刊), **31**(07):1 606—1 610
- Wang YF, Yu T, X JD, *et al.* 2010. Biocontrol of postharvest gray mold of cherry tomatoes with the marine yeast *Rhodospodium paludigenum*[J]. *Biol Contr*, **53**(2):178—182
- Zhang SS(张绍珊), Chen JJ(陈娇娇), Yang XP(杨晓萍). 2013. Induction of *Phenylalanine ammonium Lyase*, *Polyphenol Oxidase* and *Peroxidase* in postharvest peach fruit by tea extract (茶叶提取物对采后桃果苯丙氨酸解氨酶、多酚氧化酶、过氧化物酶活性的诱导)[J]. *Food Sci* (食品科学), **34**(10):304—307
- Zhao TM(赵统敏), Yu WG(余文贵), Zhao LP(赵丽萍), *et al.* 2011. Research progress in breeding of tomato resistance to *Botrytis cinerea* (番茄抗灰霉病育种研究进展)[J]. *Jiangsu J Agric Sci* (江苏农业学报), **27**(5):1 141—1 147
- Zou FB(邹芳斌), Si LT(司龙亭), Li X(李新), *et al.* 2008. The relation between the resistance of the cucumber wilt and the activities of several enzyme in defense system(黄瓜枯萎病抗性与防御系统几种酶活性关系的研究)[J]. *Acta Agric Bor Sin* (华北农学报), **23**(03):181—184

(上接第 563 页 Continue from page 563)

- mic characters in sugarcane population in contrasting environment and different crop year[J]. *Sugarc Intern*, **4**:6—12
- Silva MDA, Jifon JL, Dasilva JAG, *et al.* 2012. Relationships between physiological traits and productivity of sugarcane in response to water deficit[J]. *J Agric Sci*, **7**:1—15
- Selvi A, Nair NV, Balasundaram N, *et al.* 2003. Evaluation of maize microsatellite markers for genetic diversity analysis and fingerprinting in sugarcane[J]. *Genome*, **46**:394—403
- Salem KFM, El-Zanaty AM, Esmail RM. 2008. Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellite markers[J]. *World J Agric Sci*, **4**(5):538—544
- Steel RGD, Torrie JH. 1980. Principles and Procedures of Statistics [M]. New York: McGraw Hill Book Co. Inc.
- Sajjad M, Khan FA. 2009. Genetic diversity among sugarcane cultivars in Pakistan. *American-Eurasian J* [J]. *Agric & Environ Sci*, **6**(6):730—736
- Soomro AF, Junejo S, Ahmed A, *et al.* 2006. Evaluation of different promising sugarcane varieties for some quantitative and qualitative attributes under Thatta (Pakistan) conditions[J]. *Intern J Agric & Biol*, **8**(2):195—197
- Smiullah FAK, Afzal A, Abdullah IU, *et al.* 2013. Genetic diversity assessment in sugarcane using principal component analysis (PCA)[J]. *Intern J Mod Agric*, **2**(1):34—38
- Verma SK, Sachan JN. 2000. Genetic divergence in Indian mustard *Brassica juncea* Czern and Coss[J]. *Crop Res (Hisar)*, **19**(2):271—276
- Waclawovsky AJ, Sato PM, Lembke CG, *et al.* 2010. Sugarcane for bioenergy production: an assessment of yield and regulation of sucrose content[J]. *Plant Biotechnol J*, **8**:263—276
- Wagih ME, Musa Y, Ala A. 2004. Fundamental botanical and agronomical characterization of sugarcane cultivars for clonal identification and monitoring genetic variations[J]. *Sugar Technol*, **6**(3):127—140
- Wagner GP, Schwenk K. 2000. Evolutionarily stable configurations: functional integration and the evolution of phenotypic stability[J]. *Evol Biol*, **31**:155—217
- Xue W. 2006. Data Analysis Based on SPSS[M]. Beijing: the Chinese People's University Press, **5**:359—385
- Yang LT, Mo FL, Zhu QZ, *et al.* 2012. Effect of vinasse application on sugarcane growth and yield[J]. *J Southern Agric*, **43**(1):18—21