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陕西周至杏树流胶病病原菌鉴定及其对 3 种药剂的敏感性

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[摘要] 【目的】分离纯化陕西周至地区杏树流胶病病原菌,筛选对其菌丝生长有显著抑制作用的杀菌剂,为其田间防控提供参考。【方法】采集陕西周至地区杏树流胶病发病枝条,表面消毒后用组织块分离法分离、纯化、培养病原菌,并进行回接试验,对能引起相同病症的病原菌菌株进行菌落形态和孢子形态观察,并利用 ITS 通用引物进行 PCR 扩增,测序后采用 MEGA 软件通过邻接法(Neighbor-joining)建立系统发育树,鉴定致病菌株的分类地位。采用生长速率法测定该病原菌对 70% 甲基硫菌灵可湿性粉剂、2.6% 靓果安水剂和 2% 溃腐灵水剂 3 种药剂的敏感性。【结果】陕西周至地区杏树流胶病病原菌分离物(编号为 SXZZ)培养初期为白色,随后逐渐变为黑色;菌丝无色有隔;分生孢子无色单孢纺锤形。对该病原菌的 ITS 序列进行 Blast 比对后发现,其与葡萄座腔菌的同源性高达 99%;结合形态学鉴定结果,确定陕西周至杏树流胶病病原菌为葡萄座腔菌(*Botryosphaeria dothidea*)。药剂敏感性测定结果表明,该病原菌对甲基硫菌灵非常敏感,1 $\mu\text{g}/\text{mL}$ 甲基硫菌灵即可明显抑制其菌丝生长, EC_{50} 为 2.8315 $\mu\text{g}/\text{mL}$;但其对靓果安和溃腐灵不敏感,二者在最低质量浓度分别为 86.7 和 20.83 $\mu\text{g}/\text{mL}$ 时,才能抑制其菌丝生长,且二者的 EC_{50} 非常高。【结论】陕西周至地区杏树流胶病病原菌为葡萄座腔菌,该菌株对甲基硫菌灵极其敏感,但对靓果安和溃腐灵不敏感。

[关键词] 杏树; 流胶病; 葡萄座腔菌; 病原鉴定; 陕西周至

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Identification of apricot gummosis pathogen in Zhouzhi, Shaanxi and its sensitivity to three fungicides

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Abstract: 【Objective】This study aimed to identify causal agent of apricot gummosis in orchards in Zhouzhi, Shaanxi and thereby apply effective chemical control methods. 【Method】Gummosis twigs were sampled and surface sterilized, followed by pathogen isolation using the tissue block isolation method. The pathogen was thereafter confirmed by its inoculation on healthy twigs and re-isolation from the inoculated twigs. To classify the isolated pathogen, its colony and conidia morphology was observed, combined with phylogenetic analysis by MEGA software using Neighbor-joining method after its ITS PCR amplification and sequencing. Moreover, three fungicides including 70% Thiophanate-Methyl wetable powder, 2.6% Liangguoan aqueous solution and 2% Kuifuling aqueous solution were respectively added into plates to culture the pathogen, followed by colony diameter evaluation and calculation of EC_{50} . 【Result】The colony of

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pathogen causing apricot gummosis in orchards in Zhouzhi, Shaanxi was white at the beginning of incubation and gradually turned into black afterwards. Its mycelium was colorless and septal, while its condium was fusiform and colorless. After amplification using PCR, its ITS sequence showed more than 99% homology towards *Botryosphaeria dothidea* by Blast. Thus, it was confirmed that the apricot pathogen was *Botryosphaeria dothidea*. Thiophanate-Methyl significantly depressed *Botryosphaeria dothidea* growth at the minimum concentration of 1 $\mu\text{g}/\text{mL}$, the EC₅₀ of Thiophanate-Methyl against our isolate was 2.8315 $\mu\text{g}/\text{mL}$. While Liangguoan and Kuifuling showed a weak inhibition at least at the concentration of 86.7 and 20.83 $\mu\text{g}/\text{mL}$, respectively, the EC₅₀ values of the two chemicals were very high. 【Conclusion】 *Botryosphaeria dothidea* induced apricot gummosis in Zhouzhi, Shaanxi and it was sensitive to Thiophanate-Methyl but not to Liangguoan and Kuifuling.

Key words: apricot tree; gummosis; *Botryosphaeria dothidea*; pathogen identification; Zhouzhi, Shaanxi

杏树是一种宜林宜果的果树,不仅能起到抵御风沙、保护农田、改善环境的生态作用,其果实还具有很大的经济价值。杏树在我国主要分布在东北、华北和西北地区,其中,陕西杏树栽培面积约有10.7万hm²,是全国第三大产区^[1-3]。

流胶病是杏树最主要的病害之一,流行范围广泛,轻者引起杏减产,重者可造成绝收,导致严重的经济损失^[4]。杏树流胶病可分为生理性流胶和侵染性流胶2种,其中生理性流胶主要由虫伤、切口等机械性伤口引起,而侵染性流胶则是由轮枝孢菌、葡萄座腔菌等真菌侵染造成^[4-6]。流胶主要发生在杏树的枝干上,在病害处流出透明的树胶,与空气接触后,树胶逐渐变成褐色,成为晶莹柔软的胶块,最后变成深褐色硬质胶块。此外,流胶处常呈肿胀状,病部皮层及木质部逐渐褐化腐朽,严重时可造成整株树死亡^[5,7]。其果实也会流胶,多在有虫伤的伤口处发生,树胶糊在果面上,导致果实停止生长。该病在春季到秋季均可发生,每年5月下旬至6月中旬和8月下旬至9月中旬出现发病高峰^[7]。流胶病的防治主要有以下方法:首先是农业防治,包括及时清理果园移走感染源,及时排涝防旱减少发病条件,合理施肥以改善杏树长势,适度修剪以减少伤口和病原侵入口^[8-9]。其次是生物防治,杨艳敏^[10]和Li等^[11]分别筛选出1株对桃树流胶病有较好防治效果的解淀粉芽孢杆菌;董施源等^[12]从健康桃树叶片中分离筛选到1株枯草芽孢杆菌,其对造成桃流胶病的可可毛色二孢(*Lasiodiplodia theobromae*)有较强的拮抗作用。再次,合理应用病毒也是潜在的生防手段,Yang等^[13]鉴定了一种可感染葡萄座腔菌的正义单链RNA病毒。最后是化学防治,将病部流胶和溃烂组织刮除干净后,用杀菌剂涂抹伤口,也可进行喷洒或灌根处理^[7-8]。但目前最有效、最直

接和最稳定的防治方法还是依赖于杀菌剂的化学防治。

近几年流胶病在陕西周至杏树果园广泛流行,对果农造成较大经济损失,但其病因尚不明确。因此,本研究通过对发病果园内杏树流胶病枝取样,用组织培养法进行病原菌的分离纯化,采用形态学和ITS序列分析法进行病原菌鉴定,再用菌丝生长速率法测定3种市面常用杀菌剂对该病菌的抑制作用,以期为该病的诊断和田间有效防治提供参考。

1 材料与方法

1.1 病原菌的分离与纯化

从陕西周至杏李研究所采集有流胶病症的杏树枝条带回实验室,自来水冲洗后用体积分数75%酒精擦拭表面,用灭菌解剖刀切成小块后置于PDA平板中心,25℃培养,定期观察菌落生长情况并及时转接新的PDA平板进行纯化,得到分离病原菌的纯培养。

1.2 病原菌的致病性测定

取幼嫩健康的杏树枝条,用体积分数75%酒精进行表面消毒后,切成9 cm枝条段,用无菌解剖刀小心刮掉表皮,露出木质层。用灭菌的直径9 mm打孔器从培养4 d的PDA平板上取带有新鲜菌丝的菌饼,有菌丝一面贴紧枝条伤口处,覆盖吸有无菌水的无菌脱脂棉,用封口膜固定。将接种后的枝条放入装有100 mL无菌水的组培瓶中,置于光照培养箱中(温度25℃,光照周期16 h/8 h,相对湿度70%)培养6 d,每天更换培养枝条用水,以无菌的纯PDA培养基琼脂块接种为对照,定期观察病症发展情况。

1.3 病原菌鉴定

1.3.1 形态学观察 将分离纯化的菌株接种于

PDA 平板中,置于 25 ℃恒温培养箱中培养,定期观察菌落颜色、表面纹饰、形态与质地,描述其培养特征。将菌株接种在水琼脂培养基上,光照条件下培养 2 周后在显微镜(Nikon)下观察其分生孢子形态。

1.3.2 ITS 序列分析 收集长满平板的新鲜菌丝,用天根公司植物全基因组抽提试剂盒提取总 DNA,然后使用通用引物 ITS1(5'-TCCGTAGGTGAAC-CTGCGG-3') 和 ITS4 (5'-TCCTCCGCTTATT-GATATGC-3') 进行 PCR 扩增。PCR 反应体系:ITS1 和 ITS4 各 1 μ L, 2 \times PCR Mix(天根公司) 12.5 μ L, ddH₂O 9.5 μ L, DNA 模板 1 μ L。PCR 反应程序:94 ℃预变性 4 min; 94 ℃变性 40 s, 56 ℃退火 40 s, 72 ℃延伸 1 min, 循环 34 次; 最后 72 ℃延伸 10 min。对 PCR 产物进行凝胶电泳,并将扩增后的 ITS 片段送至上海生工生物股份有限公司测序。

将获得的 ITS 序列在 NCBI 网站上进行 Blast 比对,根据序列覆盖率确定该菌所属种类,然后用 MEGA 软件通过邻接法(Neighbor-joining)构建进化树。

1.4 病原菌对 3 种药剂的敏感性测定

1.4.1 供试药剂 供试 3 种药剂分别为:70% 甲基硫菌灵可湿性粉剂(山东百农思达生物科技有限公司),2.6% 靓果安水剂(主要成分为生物碱和栀子甙,山东潍坊奥丰作物病害防治有限公司),2% 溃腐灵水剂(主要成分为苍术素和厚朴酚,山东潍坊奥丰作物病害防治有限公司)。

1.4.2 含药培养基的制备 配制含药培养基时,3 种药剂均设置 6 个质量浓度,每个质量浓度有 10 个平板,3 次重复。其中 70% 甲基硫菌灵可湿性粉剂先用无菌水溶解后再按比例加入培养基,终质量浓度分别为 0.25, 0.5, 1, 2, 4, 8 μ g/mL; 2.6% 靓果安水剂直接按比例加入 PDA 培养基中倒平板,终质量浓度分别为 10.835, 21.67, 43.3, 86.7, 173.3, 520 μ g/mL; 2% 溃腐灵水剂直接按比例加入 PDA 培养基,终质量浓度分别为 10.45, 20.83, 41.67, 166.67, 666.7, 20 000 μ g/mL。以不加药的 PDA 培养基为对照。

1.4.3 敏感性的测定 将分离得到的病原菌转接到 PDA 培养基中培养 4 d,用直径 4 mm 打孔器在菌落边缘打取菌饼,转接到含药和对照平板中央,25 ℃恒温培养箱中培养至对照平板基本长满菌丝,采用十字交叉法测量各平板上的菌落直径,计算菌丝生长抑制率(抑制率=(对照菌落直径-含药菌落直径)/对照菌落直径×100%)。用 SPSS 23 对不同处

理的菌落直径进行差异显著性分析。将药剂质量浓度转换为以 10 为底的对数值(X),菌丝生长抑制率转换为概率值(Y),通过 Excel 软件对药剂质量浓度对数值和菌丝生长抑制率概率值进行线性回归分析,计算各药剂对病原菌的半最大效应浓度(concentration of 50% of maximal effect, EC₅₀)。

2 结果与分析

2.1 杏树流胶病病原菌分离物 SXZZ 的致病性

用实验室人工接种分离的杏树流胶病病原菌病原菌(编号为 SXZZ)接种健康杏树枝条 3 d 后,接种部位呈黑褐色,有少许白色或灰白色菌丝生长;6 d 后接种处呈黑色并开始出现流胶症状;对照枝条仅伤口处略呈深褐色,无菌丝生长或流胶症状(图 1)。用接种后发病的枝条重新进行病原菌分离纯化,得到的病原菌菌落形态和分生孢子与接种所用的病原菌完全一致,根据柯赫氏法则,可以确定分离得到的病原菌就是引起杏树流胶病的病原菌。



A. 对照枝条;B. 接种 SXZZ 3 d 后的枝条;C. 接种 SXZZ 6 d 后的枝条
A. Control twig; B. Twig inoculated with SXZZ for 3 d;
C. Twig inoculated with SXZZ for 6 d

图 1 杏树流胶病病原菌 SXZZ 的致病性观察

Fig. 1 Evaluation of the pathogenicity of the apricot gummosis pathogen SXZZ

2.2 杏树流胶病病原菌分离物 SXZZ 的形态特性

杏树流胶病病原菌分离物 SXZZ 在 PDA 平板上菌落为圆形,菌丝为绒毛状,培养初期菌落呈白色或灰白色(图 2-A),随后逐渐变为灰色,至培养后期呈黑色(图 2-B)。在显微镜下观察,菌丝无色有隔(图 2-C);分生孢子纺锤形,无色,单孢,基部钝圆,

顶部稍尖(图 2-D)。根据病原菌形态特征,参照葡萄座腔菌属(*Botryosphaeria*)真菌相关文献,初步

鉴定其为葡萄座腔菌(*Botryosphaeria dothidea*)。



A. 培养 4 d 后的菌落形态;B. 培养 2 周后的菌落形态;C. 菌丝形态;D. 分生孢子形态

A. Colony morphology after 4 days culture; B. Colony morphology after 2 weeks culture;
C. Mycelium morphology; D. Conidia morphology

图 2 杏树流胶病病原菌分离物 SXZZ 的形态特征

Fig. 2 Morphology of apricot gummosis pathogen SXZZ

2.3 杏树流胶病病原菌分离物 SXZZ 的 ITS 序列分析

对杏树流胶病病原菌分离物 SXZZ 的通用引物 PCR 产物进行测序,将获得的序列上传至 GenBank (序列登录号为 MW250225), Blast 比对后发现,其

与葡萄座腔菌的同源性高达 99%(图 3)。结合形态学鉴定结果,可以确定陕西周至地区杏树流胶病病原菌为葡萄座腔菌(*Botryosphaeria dothidea*),其分类地位为子囊菌门腔菌纲格孢腔菌目葡萄座腔菌科葡萄座腔菌属。



图 3 杏树流胶病病原菌分离物 SXZZ 的系统发育树

Fig. 3 Phylogenetic tree of apricot gummosis pathogen SXZZ

2.4 杏树流胶病病原菌对 3 种药剂的敏感性

甲基硫菌灵、靓果安和溃腐灵为市面常用的防治果树流胶病的 3 种药剂,为探究其能否用于防治由葡萄座腔菌引起的杏树流胶病,本研究测定了这 3 种药剂对杏树流胶病病原菌的抑制作用,并计算了相应 EC₅₀ 值,结果见表 1。由表 1 可知,与对照组相比,甲基硫菌灵质量浓度为 1 μg/mL 时能显著抑

制杏树流胶病病原菌菌丝生长,其 EC₅₀ 为 2.831 5 μg/mL;而靓果安和溃腐灵分别在质量浓度达到 86.7 和 20.83 μg/mL 时,才能显著抑制杏树流胶病病原菌菌丝的生长,且抑制率较低,二者的 EC₅₀ 非常高。这说明甲基硫菌灵对葡萄座腔菌菌丝生长有较强的抑制作用,但靓果安和溃腐灵的抑制效果较差,即该菌对靓果安和溃腐灵不敏感。

表 1 杏树流胶病病原菌对 3 种药剂的敏感性

Table 1 Susceptibility of apricot gummosis pathogen to three fungicides

| 药剂处理 Treatment | 药剂质量浓度/ ($\mu\text{g} \cdot \text{mL}^{-1}$) Concentration | 菌落直径/mm Colony diameter | 抑制率/% Inhibition rate | 回归方程 Regression equation | 相关系数 Correlation coefficient | EC ₅₀ / ($\mu\text{g} \cdot \text{mL}^{-1}$) |
|-----------------------------|--|----------------------------|--------------------------|-----------------------------|---------------------------------|--|
| 甲基硫菌灵 Thiophanate-Methyl | 0.25 | 78.95±0.42 ab | 1.80 | $Y=2.1707X+4.0188$ | 0.8982 | 2.8315 |
| | 0.5 | 78.90±0.44 ab | 1.87 | | | |
| | 1 | 71.00±1.19 d | 11.69 | | | |
| | 2 | 29.85±0.65 e | 62.87 | | | |
| | 4 | 25.60±0.43 f | 68.16 | | | |
| | 8 | 20.95±0.42 g | 73.94 | | | |
| 靓果安 Liangguoan | 10.835 | 77.60±0.19 ab | 3.48 | $Y=0.2892X+2.8770$ | 0.8572 | 23 944 181.73 |
| | 21.67 | 76.65±0.48 ab | 4.66 | | | |
| | 43.3 | 76.45±0.39 ab | 4.91 | | | |
| | 86.7 | 76.15±2.44 bc | 5.29 | | | |
| | 173.3 | 75.80±0.35 bc | 5.72 | | | |
| | 520 | 71.80±0.97 cd | 10.70 | | | |
| 溃腐灵 Kuifuling | 10.45 | 79.05±0.34 ab | 1.68 | $Y=0.552X+2.7637$ | 0.9184 | 11 252.9917 |
| | 20.83 | 77.70±0.49 bc | 3.36 | | | |
| | 41.67 | 77.50±0.19 bc | 3.61 | | | |
| | 166.67 | 75.60±0.37 bc | 5.97 | | | |
| | 666.7 | 75.30±0.27 bc | 6.34 | | | |
| | 20 000 | 70.90±0.86 d | 11.82 | | | |
| 对照 Control | | 80.40±0.45 a | | | | |

注:菌落直径为“平均值±标准差”。数据后标不同小写字母表示差异显著($P<0.05$)。X 为药剂质量浓度对数值(以 10 为底),Y 为菌丝生长抑制率概率值。

Note: Data is “mean±standard error”. Different letters indicate significant difference($P<0.05$). X is the lg value of chemical mass concentration and Y is the probability of the mycelial growth inhibition rate.

3 讨论与结论

流胶病是杏树最主要的病害之一,可造成巨大经济损失。本研究采集有流胶病症的杏树枝条,分离纯化得到分离物的纯培养,根据分离物的致病性,其菌落、菌丝和分生孢子的形态,以及 ITS 序列分析结果,认为引起陕西周至地区杏树流胶病的病原菌为葡萄座腔菌(*Botryosphaeria dothidea*),其分类地位为子囊菌门腔菌纲格孢腔菌目葡萄座腔菌科葡萄座腔菌属。这与 Li 等^[14]报道的浙江省杏树流胶病病原菌一致。

葡萄座腔菌属真菌分布广泛、寄主多样,是农林业上重要的病原菌或内生菌,尤其是葡萄座腔菌可在桃、杏、樱桃、核桃、柳树、橡树、桉树等 45 个属的木本植物上引起溃疡、枯梢、叶斑病、花果腐烂枯萎等病害,造成巨大经济损失^[15-18]。目前对于葡萄座腔菌引起的植物病害主要依赖化学防治,即喷洒、涂抹或灌根各种杀菌剂,主要有氟硅唑、扑海因、多菌灵、三唑类杀菌剂和有机氯类杀菌剂等^[19-28]。研究表明,甲基硫菌灵在实验室条件下对葡萄座腔菌的菌丝生长和孢子萌发都有很好的抑制效果,在田间试验中也有较好的防治效果,但其对不同菌株的敏

感性有所差异^[24-29]。另外,靓果安和溃腐灵也是市面常见的用于防治流胶病的药剂,也曾被报道可用于防治葡萄座腔菌引起的果树流胶病^[5]。本研究通过测试这 3 种药剂对陕西周至地区杏树流胶病病原菌的抑制效果发现,甲基硫菌灵对该菌株有较好的抑制作用,因此针对陕西周至地区杏树流胶病的防治可采用甲基硫菌灵喷雾或伤口涂抹等处理;靓果安和溃腐灵虽然在高质量浓度时对病原菌菌丝生长有一定抑制作用,但结合 EC₅₀ 值来看,其抑制效果不佳,但这 2 种药剂的有效成分均为植物源生物碱,除了对病原菌生长有直接影响外,此类化合物还可促进植物的生理生长^[30],所以不排除靓果安和溃腐灵可以促进杏树长势,从而增强其对流胶病抵抗性的可能。在后续研究中,将进行活体试验以验证这一推测。

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