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哈茨木霉菌剂防治葡萄病害及土壤盐渍化

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摘　要:以"巨峰""黄玫瑰"葡萄为试材,喷施哈茨木霉菌等5种药剂,通过测定病情指数、田间防效等指标,研究了哈茨木霉菌剂对葡萄霜霉病的抑制效果及对葡萄土壤盐渍化的影响。结果表明:哈茨木霉菌粉剂150、250、350 g,72%霜脲·锰锌80 g、56%噻菌脂百菌清60 g防效分别为87.80%、86.78%、80.68%、82.71%、82.37%;哈茨木霉菌能抑制葡萄灰霉病菌的生长;木霉菌颗粒剂667 m^2用量为10 kg时,能有效降低土壤盐分含量。

关键词:哈茨木霉菌;防治;葡萄病害;盐渍化

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由于设施葡萄长期使用化学肥料及农药,土壤已经"不再健康",土壤性状恶化,土地板结、酸化、盐渍化及病害发生严重,土壤微生物多样性被破坏等问题越来越突出。目前主要采用化学药剂防治病虫害,但是效果不够理想,还存在农药残留、土壤板结、污染环境弊端[1-6]。现以"巨峰""黄玫瑰"葡萄为试材,于2014—2015年喷施哈茨木霉菌剂(*Trichoderma harzianum*),通过测定病情指数、田间防效等指标,调查葡萄病害的种类及对土壤盐渍化的影响,研究了哈茨木霉菌剂对葡萄的安全

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Bioinformatics Analysis and Prediction of Rubisco Large Subunit(RbcL) in Sect. *Ponticum* G. Don

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Abstract:Sect. *Ponticum* G. Don belongs to the representative of the family *Rhododendron* (Sect. *Ponticum* G. Don). Plants of the sect are evergreen shrubs or arbors with important ornamental and medicinal value. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the rate-limiting enzyme of Calvin cycle and also has involved in photosynthesis and photorespiration of plants. The catalytic sites of Rubisco mainly located in the Rubisco large subunit (RbcL),so it has a great significance to study the structure and character of RbcL. The RbcL of Sect. *Ponticum* G. Don and the basic physical and chemical characters of these obtained RbcL were investigated and analyzed in the present study. Four representative RbcL fragments were selected to analyze the hydrophobicity. Structures were predicted by bioinformatics and the phylogenetic relationship of the RbcL of Sect. *Ponticum* G. Don was also been analyzed. The results showed that the catalytic sites of Rubisco and the RbcL were highly conservative in Sect. *Ponticum* G. Don., but they still had differences in evolution. This study may lay a solid foundation for further research into the photosynthesis mechanism of Sect. *Ponticum* G. Don as well as the catalytic mechanism of RbcL.

Keywords:Sect. *Ponticum* G. Don;Rubisco;RbcL;bioinformatics

性,以期为葡萄病害的科学防治提供参考。

1 材料与方法

1.1 试验材料

供试葡萄品种为“巨峰”“黄玫瑰”,供试哈茨木霉菌剂(颗粒剂和粉剂)和灰霉病株由上海交通大学农业环境微生物工程研究所提供。对照菌剂为56%嘧菌脂百菌清 amistaropti(南京博士邦化工科技有限公司生产)和72%霜脲·锰锌 cymoxanilmancozeb(北京中保绿农科技集团有限公司生产)。

1.2 试验方法

1.2.1 哈茨木霉菌剂对葡萄病害的田间防效试验 试验在松江区泖港镇葡萄种植基地进行。每个小区选择25株葡萄,小区面积为20 m^2。2014年5月20、29日每小区分别喷洒哈茨木霉菌粉剂150、250、350 g;以喷施等量的72%霜脲·锰锌80 g;56%嘧菌脂百菌清60 g为对照。分别在2014年6月1、8、15日调查葡萄植株的病情指数及防治效果,采用随机调查等方法调查葡萄病害种类及危害状况,重复3次。病虫害等级分轻度1/3以下(+)、中度1/3～2/3(++)、重度感病2/3以上(+++)3个等级[7]。

1.2.2 对峙试验 采用生长速度法,培养3～5 d的木霉菌种及葡萄灰霉菌落边缘用无菌打孔器打成0.5 cm菌落圆片,分别接入已制备好的PDA平板中央,放入26 ℃恒温培养箱中培养,每处理重复3次,24 h后逐日观察木霉菌与病原菌的生长情况,并分别测量木霉菌菌落指向病原菌的半径、病原菌落指向木霉菌的半径,并计算木霉菌对病原菌抑菌率。当2个菌落接触相交后,观察记载木霉菌对病原菌的抑制、侵入并占领病面菌营养空间的过程。病害等级分1～5级,木霉菌丝分别占病原菌平皿的100%、2/3以上、1/3～2/3、1/3以下、病原菌丝占木霉菌100%。抑菌率(%)=(病原菌对照菌落半径-病原菌落指向木霉菌的半径)/病原菌对照菌落半径×100。

1.2.3 木霉菌对葡萄土壤次生盐渍化的影响 2014年3月5日在葡萄树根部撒木霉菌颗粒剂,共2个处理:667 m^2 用量分别为5、10 kg。采用五点取样法,采集15份葡萄基地土壤样品,每点取土壤剖面5、10 cm的土壤样品,重复3次。土壤样品采回后于室内风干、磨碎后分别过20、60目筛保存备用。土壤全盐含量采用烘干残渣法测定;电导率采用土∶水为1∶5(质量体积比)电导仪法测定[8],土壤盐分与电导率换算 $Y=0.041X-0.113\ 4$,其中 Y 为全盐量($g\cdot kg^{-1}$),X 为电导率($mS\cdot cm^{-1}$)[9]。土壤盐分程度分级标准见表1。

表1 土壤盐分程度分级标准

Table 1 Classification standard of soil salinity

指标 Index	1	2	3	4
盐化程度 Classification standard of salinization	非盐化	轻度盐化	中度盐化	重度盐化
含盐量 C Salt content/($g\cdot kg^{-1}$)	$C<2$	$2<C<5$	$5\leqslant C<7$	$7\leqslant C<10$

1.3 数据分析

试验数据采用DPS法进行多重比较并进行方差统计分析。

2 结果与分析

2.1 哈茨木霉菌剂对葡萄霜霉病的田间防效

由表2可知,药后14 d哈茨木霉菌粉剂150、250、350 g,72%霜脲·锰锌粉剂80 g、56%嘧菌脂百菌清60 g,防效分别为87.80%、86.78%、80.68%、82.71%、82.37%。哈茨木霉菌粉剂150 g的防效与其它处理间达到差异极显著水平($P<0.01$)。

表2 哈茨木霉菌剂对葡萄霜霉病的田间防效

Table 2 Field control effect of *Trichoderma harzianum* on grape downy mildew

药剂处理 Treatment	药前病情指数 Disease index before treatment	药后3 d After treatment for 3 days		药后7 d After treatment for 7 days		药后14 d After treatment for 14 days	
		病情指数 Disease index	防效 Control effect/%	病情指数 Disease index	防效 Control effect/%	病情指数 Disease index	防效 Control effect/%
哈茨木霉菌粉剂150 g *Trichoderma harzianum* 150 g	11.3	10.1	21.71Aa	9.6	57.71Aa	3.4	87.80Aa
哈茨木霉菌粉剂250 g *Trichoderma harzianum* 250 g	12.4	10.5	18.60Cc	11.1	51.10Dd	3.9	86.78Bb
哈茨木霉菌粉剂350 g *Trichoderma harzianum* 350 g	12.2	10.3	20.16Bb	13.5	40.53Ee	5.7	80.68Ee
72%霜脲·锰锌80 g 72% Cymoxanil mancozeb 80 g	12.6	10.6	17.83Dd	10.2	55.07Bb	5.1	82.71Dd
56%嘧菌脂百菌清60 g 56% Azoxystrobin chlorothalonil 60 g	13.4	10.9	15.50Ee	10.3	54.63Cc	5.2	82.37Ee
对照(CK)	12.3	12.9	—	22.7	—	29.5	—

2.2 木霉菌对葡萄灰霉病防治效果

对峙培养试验表明(表3),葡萄灰霉病的生长受到明显抑制,葡萄灰霉菌菌落生长半径明显小于木霉菌,二者接触后,灰霉病原菌菌落不再扩展,与木霉菌对峙,处于僵持状态或逐渐萎缩。在光学显微镜下镜检发现,木霉菌菌丝缠绕在葡萄灰霉菌菌丝体上或穿入葡萄灰霉菌丝体内寄生生长,吸取内部养分,使灰霉病原菌菌丝变细、断裂。另挑取对峙培养中距较远处灰霉病原菌菌丝进行镜检,发现有大量木霉菌菌丝存在。

表3　木霉菌对葡萄灰霉病菌的防治效果

Table 3　Control effect of *Trichoderma harzianum* on grape gray mould blight　cm

处理 Treatment	木霉菌接种天数 Inoculation days of *Trichoderma harzianum*/d				
	1	2	3	4	5
哈茨木霉菌 *T. harzianum*	2.0	6.7	8.0(满)	8.0(满)	8.0(满)
葡萄灰霉菌 *Botrytis cinerea Pers*	0.3	1.9	3.7	4.4	5.7

2.3　葡萄病害发生种类和危害程度

由表4可知,霜霉病、灰霉病危害程度严重(被害率>30%),白腐病、黑痘病、溃疡病中度危害(被害率10%～30%),根癌病、扇叶病、卷叶病等轻度危害(被害率≤10%)。

表4　葡萄病害发生种类和危害程度

Table 4　Species and damage degree of grape diseases

病害名称 Disease name	危害程度 Damage degree
霜霉病 Grape doumy mildew	+++
灰霉病 Grape gray mold	+++
白腐病 Grape white rot	++
黑痘病 Grape bird,s eye yot	++
溃疡病 *Botryosphaeria* spp.	++
根癌病 Grape crown gall	+
扇叶病 Grape fanleaf uirus	+
卷叶病 Grape spotled wilt uirus	+
日灼病 Grape sunscald	+
缺硼 Grape boron deficiency	+
缺锰 Geape manganese deficiency	+

注:+表示轻度为害(被害率≤10%);++表示中度为害(被害率10%～30%);+++表示严重为害(被害率>30%)。

Note:+indicates slightly damaged(Damage rate≤10%);++indicates moderately damaged(10%<Damage rate<30%);+++indicates seriously damaged(Damage rate≥30%).

2.4　设施葡萄地耕层土壤盐渍化程度分析

由表5可知,667 m^2 喷施哈茨木霉菌5、10 kg土壤,5 cm土层盐分含量分别为1.74、1.65 g·kg^{-1},对照土壤的盐分含量为2.10 g·kg^{-1};10 cm土层盐分含量分别为1.81、1.77 g·kg^{-1},对照土壤的盐分含量为2.43 g·kg^{-1}。由此可见,667 m^2 施用木霉菌10 kg可以有效降低土壤盐分含量。

表5　木霉菌对葡萄土壤次生盐渍化的影响

Table 5　Effect of *Trichoderma harzianum* on secondary salinization　g·kg^{-1}

处理 Treatment	5 cm土层盐分含量 Soil salt of 5 cm depth	10 cm土层盐分含量 Soil salt of 10 cm depth
木霉菌5 kg *Trichoderma harzianum* 5 kg	1.74	1.81
木霉菌10 kg *Trichoderma harzianum* 10 kg	1.65	1.77
对照CK	2.10	2.43

3　讨论

“巨峰”“黄玫瑰”葡萄品种抗病性不够、大棚重茬、多种病原真菌侵染引起的土传病害、土壤盐渍化、化肥用增量、未发酵有机肥等因素。有益微生物菌剂(哈茨木霉菌)是上海交通大学农业环境微生物工程研究所研发创制多年比较成熟产品。木霉菌颗粒可以调节土壤微生态平衡、促进植物根系生长、降低次生盐渍化程度。木霉菌对多种病原真菌具有拮抗作用,能解决葡萄霜霉病、灰霉病等实际问题,是现代都市农业发展需要高科技产品。有关木霉菌拮抗微生物在土壤中的定植及根际动态变化还需与各院校农科院土肥生态所进一步合作研究。

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Trichoderma harzianum Control Efficiency on Grape Diseases and Soil Salinization

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Abstract:'Kyoho' 'Yellow Rose' grapes were used as materials,the disease index and field efficiency were researched after treatment of *Trichoderma harzianum*. Control effect on grape downy mildew and soil salinization was studied. The results showed that control efficiency rates of 150 g,250 g,350 g *T. harzianum* powder,80 g cymoxanil mancozeb of 72% ,60 g azoxystrobin chlorothalonil of 56% were 87.80%,86.78%,80.68%,82.71%,82.37%,respectively. *T. harzianum* could control the grape downy mildew. After spying *Trichoderma* granules 10 kg per 667 m^2,soil salt content decreased obviously.

Keywords: *Trichoderma harzianum*;control;grape diseases;salinization