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海南省火龙果溃疡病菌对吡唑醚菌酯敏感性分析

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摘 要：【目的】监测分析海南省火龙果溃疡病菌对吡唑醚菌酯的敏感基线及敏感性水平。**【方法】**采用菌丝生长速率法测定吡唑醚菌酯对海南省8个火龙果种植市（县）采集到的59株火龙果溃疡病菌的EC_{50}值，采用R语言进行数据分析。**【结果】**吡唑醚菌酯对供试菌株的EC_{50}范围为0.254 0~2.223 3 μg·mL^{-1}，EC_{50}均值为0.9841 μg·mL^{-1}；以58株菌株EC_{50}连续性正态分布时的均值0.962 7 μg·mL^{-1}作为海南省火龙果溃疡病菌对吡唑醚菌酯的敏感基线，海南省火龙果溃疡病菌均对吡唑醚菌酯表现为敏感；除乐东和白沙地区间敏感性差异显著外，其他地区间敏感性差异均不显著。**【结论】**海南省火龙果溃疡病菌对吡唑醚菌酯的敏感性差异较小，对吡唑醚菌酯仍较为敏感。

关键词：火龙果；溃疡病；吡唑醚菌酯；敏感性

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Sensitivity analysis of pitaya canker disease *Neoscytalidium dimidiatum* to pyraclostrobin in Hainan province, China

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Abstract:【Objective】Pitaya canker disease caused by *Neoscytalidium dimidiatum* is the main disease of pitaya in Hainan province. With the expansion of pitaya planting area and the increase of planting years, the occurrence of *N. dimidiatum* in the planting area has been increasing. The pathogen mainly damages the stem and the fruit of pitaya and causes great loss in production. According to investigation, pyraclostrobin is used to control the disease in Hainan province all year round, but the sensitivity of *N. dimidiatum* to pyraclostrobin has not been reported yet. Therefore, the purpose of this study was to determine the sensitivity baseline and sensitivity level of *N. dimidiatum* to pyrazolesterim, so as to provide a reference for scientific application of *N. dimidiatum* in Hainan province.【Methods】*N. dimidiatum* collected from eight regions in Hainan province were isolated from the infected tissues. First, some diseased tissue pieces (5 mm×5 mm) were cut off and sterilized with 75% ethanol for 35 s and then with 5% NaClO for 40 s, and washed with sterile water for 3 times. The diseased tissue pieces were then pasted on PDA plates and cultured in an incubator at 28 ℃ for 2-3 days. After mycelia grew on the PDA plate, a single mycelium was transferred to a new PDA plate and cultured for 5 days. The pathogen was preliminarily confirmed by microscopy after sporulating. Next, single spores isolated was used to obtain pure colonies. Finally, a total of 59 purified isolates were obtained and their sensitivities to pyraclostrobin were evaluated through mycelial growth rate method. The medium with final concentrations of 6, 4, 2, 0.5 and 0.2 μg·mL^{-1} were prepared for each isolate. The pyraclostrobin-free PDA medium was added with 1 mL acetone as the solvent control, and the PDA medium with 1 mL sterile water as the blank control. In addition, salicylhydroxamic acid with a final concentration of 50 mg·L^{-1} was

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added to all the tested mediums to reduce the effect of other respiratory pathway on the fungicide. The agar disks containing mycelium of *N. dimidiatum* were inoculated facing up on the pyraclostrobin-containing mediums and the control medium. Each concentration was set up with 3 replicates and cultured in an incubator at 28 ℃. After 48 h, the criss-cross method was used to measure diameters of these colonies. Microsoft Excel 2016 software was used to calculate the regression equation and correlation coefficient for each isolate, and the effective inhibiting medium concentrations (EC_{50}) was calculated according to the regression equation. The EC_{50} values of 59 isolates, were tested for normality and variance homogeneity by R language, and the sampling site was used as single factor for one-way analysis of variance. The resistance level was determined based on the sensitivity baseline of *N. dimidiatum* to pyraclostrobin, and the resistance level (RF) was calculated as EC_{50} (isolates)/sensitivity baseline. When EC_{50} was less than 5 folds of the sensitive baseline, the strain was defined as sensitive strain. When EC_{50} was 5-10 folds of the sensitive baseline, the strain was defined as low resistance. When the EC_{50} value was 10-40 folds of the sensitive baseline, the strain was defined as medium resistant strain. When the EC_{50} value was higher than 40 folds of the sensitivity baseline, the strain was defined as highly resistant strain.【Results】A total of 59 isolates of *N. dimidiatum* were obtained by the tissue isolation method. The EC_{50} values of these isolates to pyraclostrobin were measured and used for the test and analysis. Our results showed that the EC_{50} values of 58 isolates followed the normal distribution pattern, and the mean value of EC_{50} values was 0.962 7 $\mu g \cdot mL^{-1}$, which was ultimately used as the sensitivity baseline of *N. dimidiatum* to pyraclostrobin. The results of regional sensitivity analysis showed that the sensitivity of the 59 isolates to pyraclostrobin were hardly different in Hainan province, with EC_{50} ranging from 0.254 0 to 2.223 3 $\mu g \cdot mL^{-1}$. There was no significant difference in sensitivity among different regions except for Ledong and Baisha. Isolates collected from Ledong county were very sensitive to pyraclostrobin, while isolates from Baisha county were relatively less sensitive. The difference of EC_{50} between Baisha and Ledong county was the most significant. The mean value of EC_{50} in Baisha county was 2.69 times that of Ledong county, and the average resistance was 2.71 times, which was consistent with our field survey results of pyraclostrobin applications.【Conclusion】The sensitivity of *N. dimidiatum* to pyraclostrobin was characterized by the EC_{50} values of the tested isolates. All the EC_{50} values were in the range of 0.254 0-2.223 3 $\mu g \cdot mL^{-1}$, and the average EC_{50} value was 0.984 1 $\mu g \cdot mL^{-1}$. The sensitive baseline of *N. dimidiatum* to pyraclostrobin in Hainan province was 0.9627 $\mu g \cdot mL^{-1}$. In general, the *N. dimidiatum* isolates collected from different regions in Hainan province had minor differences in sensitivity to pyraclostrobin, and the isolates were still sensitive to pyraclostrobin. However, with the increase of planting years and the constant accumulation of the fungicide, the risk of resistance cannot be completely excluded.

Key words: Pitaya; *Neoscytalidium dimidiatum*; Pyraclostrobin; Sensitivity

火龙果（*Hylocereus* spp.）又名红龙果、仙蜜果等，属于仙人掌科（Cactaceae）量天尺属和蛇鞭柱属的多年生攀援性多肉植物。因其拥有较高的经济价值，目前被世界多国引入进行栽培种植[1]。我国主要种植地区有海南、广东、广西、贵州和云南等[2]。火龙果果实不仅外观独特且营养价值丰富，具有促进肠胃消化、降血糖血脂、血压及美容护肤等多种功效，可谓是集食用、药用和观赏于一身的重要热带经济水果[3]。

由新暗色柱节孢菌（*Neoscytalidium dimidiatum*）引起的火龙果溃疡病是一种严重威胁火龙果生产的重要病害，已在多个火龙果种植国家和地区报道过[4-6]。随着火龙果在海南省种植面积的扩大和种植年限的增长，火龙果溃疡病在产区发生情况呈连年递增的趋势，已是影响海南省火龙果产业的第一大病害，不仅影响植株生长和果实外观，而且给生产造

成巨大损失。该病原菌主要危害火龙果的茎部和果实[7-10]，典型发病症状为：病部初期出现褪绿轻微凹陷的圆形小斑点，中期逐渐变为橘黄色，严重时病斑连成一片并逐渐硬化，后期病部中央凹陷，周围木栓化，呈灰白色或灰褐色隆起，似溃疡。

吡唑醚菌酯（pyraclostrobin）是一种广谱甲氧基丙烯酸酯类（QoI类）杀菌剂，其通过在细胞色素b和c1间的电子转移抑制线粒体呼吸，进而抑制真菌孢子萌发和菌丝生长，具有广谱、高效、毒性低、提高对氮的吸收和产量等特点[11-12]。吡唑醚菌酯作为一种广泛使用的杀菌剂，常期使用可能导致植物病原菌对其敏感度下降或产生抗药性：贺瑞等[13]报道海南杧果蒂腐病菌对吡唑醚菌酯产生严重抗药性；贾俊超等[14]报道，田间已发现25种病原菌在14种寄主作物上表现出对QoI类杀菌剂的抗药性。通过前期对海南省火龙果园进行实地田间调查发现，果园常使用有效成分中含有吡唑醚菌酯的农药来防治火龙果病害。然而，海南省内各地的火龙果溃疡病菌对吡唑醚菌酯药剂敏感性水平，尚未见研究报道。本研究中，笔者2019—2020年从海南省火龙果种植产区采集火龙果溃疡病的典型病样标本，经分离、纯化和鉴定，并采用菌丝生长速率法测定了分离菌株对吡唑醚菌酯的敏感性，旨在进一步明确火龙果溃疡病菌对吡唑醚菌酯的敏感性水平，为海南火龙果溃疡病的科学防治提供理论依据。

1 材料和方法

1.1 供试菌株

供试菌株为本实验室于2019—2020年从海南省各火龙果种植市（县）采集、分离、纯化、鉴定和保存的59株火龙果溃疡病菌（*N. dimidiatum*）菌株。

1.2 供试菌株的分离与纯化

对从海南省海口、儋州、琼海、昌江、白沙、澄迈、乐东和东方8个火龙果种植市（县）采回的发病植株样本，采用组织分离法分离病原菌：切取病果和病茎圆形病斑（5 mm×5 mm）若干，75%（φ）乙醇消毒35 s，5%次氯酸钠消毒40 s，无菌水清洗3次后，用无菌纸吸干组织块表面水分至于PDA平板上，培养箱28 ℃培养2~3 d，待长出菌丝，取边缘菌丝转移到新PDA平板上，28 ℃培养5 d产孢，镜检初步确认病原菌。再采用单孢分离法获得病原菌纯菌落：将菌丝放于无菌水中轻轻涮洗，显微镜镜检（孢子悬浮液稀释到在10×下每个视野约观察到1~5个孢子时）进行涂板培养24 h，挑取单条菌丝进行转板，获得病原菌纯菌落。培养7 d后通过形态学观察鉴定、分子鉴定及柯赫氏法则致病性测定后将其转移至PDA斜面试管中保存备用[15]。

1.3 供试药剂

98%吡唑醚菌酯原药（湖北猫尔沃生物医药有限公司），用丙酮为溶剂配制质量浓度为10 mg·mL^{-1}的母液，保存于4 ℃冰箱备用。

1.4 含药培养基制备

采用系列稀释法，经初筛确定有效浓度范围后，将吡唑醚菌酯母液用丙酮稀释为系列梯度浓度，取1 mL稀释药剂加入49 mL融化的PDA培养基中混匀倒板，分别配成终质量浓度为6、4、2、0.5和0.2 μg·mL^{-1}的含药培养基，并以不含药的PDA培养基加入1 mL丙酮作为溶剂对照，加入1 mL无菌水的PDA培养基作为空白对照，所用供试培养基均加入终质量浓度为50 mg·L^{-1}的水杨肟酸，以降低旁呼吸通路对药剂的影响。

1.5 火龙果溃疡病菌对吡唑醚菌酯的敏感性测定

采用菌丝生长速率法[16]：供试菌株在PDA培养基上培养3 d后沿菌落边缘用5 mm直径打孔器取菌饼。将菌饼菌丝面朝上分别接种到5个不同浓度的含药培养基和对照培养基上，每浓度设置3个重复，置于28 ℃培养箱中培养，48 h后采用十字交叉法测量菌落直径，每菌株3次重复试验。

1.6 数据处理

根据公式计算菌丝生长抑制率：菌丝生长抑制率/%= [（对照菌落生长直径-处理菌落生长直径）/对照菌落生长直径-0.5] × 100；采用Microsoft Excel 2016软件计算各菌株的毒力回归方程和相关系数，根据回归方程计算EC_{50}。

针对59株供试菌株EC_{50}值，采用R语言对试验数据进行正态性、方差齐性检验并以采样市（县）为因素进行单因素方差分析[17]，将其中符合连续性正态分布的供试菌株EC_{50}均值作为海南省火龙果溃疡病菌对吡唑醚菌酯的敏感基线[18-20]。抗性水平以火龙果溃疡病菌对吡唑醚菌酯敏感基线为参照，抗性水平（RF）=供试菌株EC_{50}值/敏感基线，当EC_{50}值小于敏感基线5倍时，为敏感菌株；当EC_{50}值为敏感基线的5~10倍时，为低抗菌株；当EC_{50}值为敏感基线的10~40倍时，为中抗菌株；当EC_{50}值大于敏感基线

40倍时，为高抗菌株[21]。

2　结果与分析

2.1　病原菌分离、纯化及保存

于2019—2020年从海南省海口市、儋州市、乐东县和东方市等火龙果种植市（县）采集火龙果溃疡病不同发病情况的果实和茎部样本。挑选具有火龙果溃疡病典型症状的火龙果果实和茎部样本经分离纯化获得59株火龙果溃疡病菌，其中海口8株，儋州14株，琼海3株、昌江3株、白沙3株、澄迈3株、东方15株和乐东10株。

2.2　海南省火龙果溃疡病菌对吡唑醚菌酯的敏感基线

将59株火龙果溃疡病菌对吡唑醚菌酯的EC_{50}值使用R语言的*shapiro.test*函数命令进行正态性检验（*W*检验），$W= 0.964\ 3$，$p= 0.080\ 9$（$p > 0.05$）；结果表明海南省59株火龙果溃疡病菌对吡唑醚菌酯的敏感性呈正态分布，EC_{50}值为0.254 0~2.223 3 μg·mL-1，平均值为0.984 1 μg·mL-1，其中敏感性最弱菌株EC_{50}值是敏感性最强菌株的8.75倍，表明火龙果溃疡病菌对吡唑醚菌酯的敏感性存在差异（表1）。

表1　海南省火龙果溃疡病菌对吡唑醚菌酯的敏感性

Table 1　Sensitivity of *Neoscytalidium dimidiatum* to pyraclostrobin in Hainan

菌株编号 Isolates No.	采样地点 Sampling locations	回归方程 Regression equation	相关系数 Relative coefficient，*R*	EC_{50}值 EC_{50} value/(μg·mL^{-1})	抗性水平 Resistance factor，RF
HNDF20022	东方市Ledong	*Y*=5.509 3+0.886 6*X*	0.998 0	0.254 0	0.263 8
HNHK20041	海口市Haikou	*Y*=5.237 5+0.670 0*X*	0.996 2	0.364 6	0.378 7
HNLD20033	乐东县Ledong	*Y*=5.174 3+0.626 3*X*	0.994 7	0.373 3	0.387 8
HNLD20026	乐东县Ledong	*Y*=5.250 7+0.740 4*X*	0.985 4	0.380 7	0.395 5
HNLD20032	乐东县Ledong	*Y*=5.413 2+0.659 2*X*	0.992 1	0.390 7	0.405 8
HNLD20038	乐东县Ledong	*Y*=5.390 7+0.786 9*X*	0.991 6	0.416 7	0.432 9
HNDF20011	东方市Ledong	*Y*=5.124 2+1.012 7*X*	0.987 9	0.431 8	0.448 5
HNLD20035	乐东县Ledong	*Y*=5.097 8+0.778 7*X*	0.995 8	0.449 3	0.466 7
HNCJ19001	昌江县Changjiang	*Y*=5.310 5+0.817 6*X*	0.980 2	0.477 8	0.496 3
HNDZ19005	儋州市Danzhou	*Y*=5.139 0+0.422 1*X*	0.988 4	0.512 4	0.532 3
HNDF20013	东方市Dongfang	*Y*=5.218 8+0.959 4*X*	0.991 6	0.519 2	0.539 3
HNLD20036	乐东县Ledong	*Y*=5.040 1+0.681 2*X*	0.995 1	0.548 7	0.570 0
HNDZ19003	儋州市Danzhou	*Y*=5.029 6+0.819 8*X*	0.993 0	0.563 4	0.585 2
HNDZ19004	儋州市Danzhou	*Y*=5.436 2+0.840 3*X*	0.991 8	0.619 9	0.643 9
HNHK20001	海口市Haikou	*Y*=4.964 2+0.919 4*X*	0.995 8	0.632 2	0.656 7
HNLD20037	乐东县Ledong	*Y*=5.072 2+0.643 5*X*	0.994 7	0.648 0	0.673 1
HNDF20009	东方市Dongfang	*Y*=5.148 9+0.861 9*X*	0.991 8	0.672 9	0.699 0
HNHK19004	海口市Haikou	*Y*=5.314 7+0.553 6*X*	0.993 6	0.692 2	0.719 0
HNDZ19004	儋州市Danzhou	*Y*=4.984 6+0.517 4*X*	0.977 2	0.748 0	0.777 0
HNLD20031	乐东县Ledong	*Y*=5.295 0+0.738 1*X*	0.993 5	0.781 8	0.812 1
HNDF20012	东方市Dongfang	*Y*=5.436 2+1.044 2*X*	0.985 1	0.804 6	0.835 8
HNDF20025	东方市Dongfang	*Y*=5.048 5+0.670 5*X*	0.999 7	0.817 7	0.849 4
HNDZ19001	儋州市Danzhou	*Y*=5.195 3+0.751 4*X*	0.998 5	0.830 8	0.863 0
HNHK20004	海口市Haikou	*Y*=5.085 8+0.905 2*X*	0.997 9	0.841 9	0.874 5
HNDZ19001	儋州市Danzhou	*Y*=5.170 6+0.830 7*X*	0.986 2	0.842 4	0.875 0
HNQH19002	琼海市Qionghai	*Y*=5.011 6+0.629 9*X*	0.977 0	0.866 5	0.900 1
HNHK19003	海口市Haikou	*Y*=5.239 6+0.739 3*X*	0.993 9	0.874 3	0.908 2
HNCJ20021	昌江县Changjiang	*Y*=4.873 4+0.616 5*X*	0.992 2	0.880 2	0.914 3
HNDF20010	东方市Dongfang	*Y*=5.136 8+0.702 1*X*	0.997 6	0.900 7	0.935 6
HNDF20028	东方市Dongfang	*Y*=4.979 5+0.701 2*X*	0.992 4	0.913 3	0.948 7
HNCM19001	澄迈县Chengmai	*Y*=5.187 2+0.878 3*X*	0.995 3	0.919 7	0.955 3
HNDZ19007	儋州市Danzhou	*Y*=5.050 4+0.571 9*X*	0.988 3	0.966 3	1.003 7
HNDF20016	东方市Dongfang	*Y*=4.971 0+0.803 9*X*	0.994 0	0.970 0	1.007 6
HNLD20030	乐东县Ledong	*Y*=5.054 5+0.434 7*X*	0.992 1	0.984 8	1.023 0
HNLD20034	乐东县Ledong	*Y*=5.064 5+0.462 5*X*	0.981 6	1.031 6	1.071 6
HNHK20005	海口市Haikou	*Y*=5.060 5+0.788 0*X*	0.996 9	1.037 5	1.077 7
HNDZ20019	儋州市Danzhou	*Y*=4.850 4+0.757 6*X*	0.997 8	1.106 0	1.148 9
HNDZ20017	儋州市Danzhou	*Y*=4.6385+1.079 1*X*	0.993 8	1.107 3	1.1502
HNQH19003	琼海市Qionghai	*Y*=4.923 0+0.795 4*X*	0.996 9	1.144 5	1.188 8

续表 Continued Table

菌株编号 Isolates No.	采样地点 Sampling locations	回归方程 Regression equation	相关系数 Relative coefficient, R	EC_{50}值 EC_{50} value/(μg·mL^{-1})	抗性水平 Resistance factor, RF
HNDF20014	东方市Dongfang	Y=4.946 2+0.651 5X	0.996 7	1.162 0	1.207 0
HNDZ19005	儋州市Danzhou	Y=5.051 3+0.559 7X	0.991 8	1.234 7	1.282 5
HNDF20027	东方市Dongfang	Y=4.901 7+0.806 8X	0.996 8	1.239 5	1.287 5
HNHK20003	海口市Haikou	Y=4.946 1+0.652 7X	0.996 4	1.248 6	1.297 0
HNCM19003	澄迈县Chengmai	Y=4.625 0+0.858 8X	0.996 3	1.296 5	1.346 7
HNDZ19002	儋州市Danzhou	Y=4.811 7+0.733 4X	0.996 6	1.299 4	1.349 7
HNCM19002	澄迈县Chengmai	Y=5.114 1+0.643 7X	0.987 8	1.309 0	1.359 7
HNDF20029	东方市Dongfang	Y=5.043 3+0.854 9X	0.999 6	1.309 9	1.360 7
HNDZ19003	儋州市Danzhou	Y=5.300 2+0.797 2X	0.998 6	1.310 3	1.361 1
HNBS20006	白沙县Baisha	Y=4.849 7+1.254 0X	0.998 4	1.312 4	1.363 3
HNCJ20020	昌江县Changjiang	Y=4.824 6+0.657 6X	0.988 9	1.429 5	1.484 9
HNDZ19006	儋州市Danzhou	Y=4.626 8+0.888 8X	0.994 7	1.481 9	1.539 3
HNQH19001	琼海市Qionghai	Y=4.980 2+0.811 0X	0.999 2	1.489 4	1.547 1
HNDF20015	东方市Dongfang	Y=4.882 0+0.604 2X	0.998 5	1.501 5	1.559 7
HNDF20024	东方市Dongfang	Y=4.804 1+0.569 2X	0.998 9	1.530 8	1.590 1
HNBS20007	白沙县Baisha	Y=4.602 3+0.736 8X	0.999 3	1.696 9	1.762 7
HNBS20008	白沙县Baisha	Y=4.811 3+1.238 8X	0.995 8	1.836 4	1.907 6
HNDZ20018	儋州市Danzhou	Y=4.761 4+0.865 2X	0.997 2	1.850 3	1.922 0
HNDF20023	东方市Dongfang	Y=4.762 7+0.499 5X	0.989 1	1.982 7	2.059 5
HNHK20002	海口市Haikou	Y=4.880 3+0.555 4X	0.998 7	2.223 3	2.309 4

去除1株特异性较强的菌株HNHK20002后，将58株火龙果溃疡病菌对吡唑醚菌酯的EC_{50}值使用R语言的*Shapiro test*函数命令进行正态性检验（W检验），W= 0.969 4，p= 0.150 2（$p > 0.05$）；结果表明58株供试菌株对吡唑醚菌酯的EC_{50}分布符合正态分布，以EC_{50}为横坐标，以菌株分布频率为纵坐标，绘制频数分布直方图（图1），其EC_{50}分布符合连续性正态分布，EC_{50}均值0.962 7 μg·mL^{-1}即为海南省火龙果溃疡病菌对吡唑醚菌酯的敏感基线。

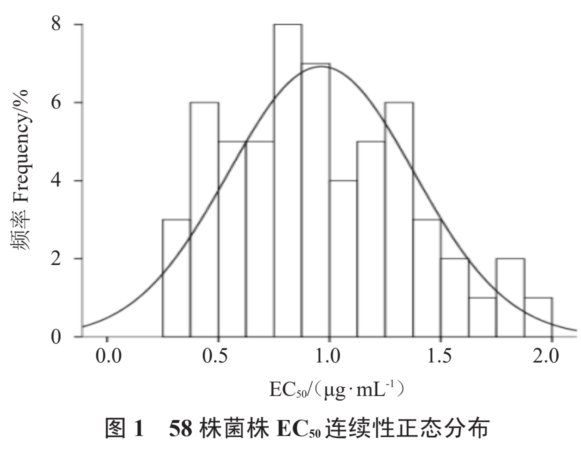

图1 58株菌株EC_{50}连续性正态分布

Fig. 1 Continuous normal distribution for EC_{50} of 58 strains to pyraclostrobin

2.3 不同地区火龙果溃疡病菌对吡唑醚菌酯的敏感性

从海南省8个火龙果种植市（县）采集的59株火龙果溃疡病菌对吡唑醚菌酯的敏感性存在一定的差异。最敏感菌株为东方HNDF20022，EC_{50}值为0.254 0 μg·mL^{-1}，最不敏感菌株为海口HNHK20002，EC_{50}值为2.223 3 μg·mL^{-1}。使用R语言的*Bartlett test*在α=0.05水平下进行方差齐性检验p=0.462 2（$p > 0.05$），结果表明，数据在不同水平下为等方差。对种植市（县）间进一步使用TukeyHSD测试，结果表明，海南8个地区除白沙和乐东的菌株在α=0.05水平上差异显著外，其余地区间对吡唑醚菌酯的敏感性差异均不显著。根据敏感基线0.962 7 μg·mL^{-1}，海南省火龙果溃疡病菌群体目前对吡唑醚菌酯仍均较为敏感，59株病菌的平均抗性为1.02，最高抗性为2.31，均小于敏感基线5倍范围。经各火龙果种植市（县）间EC_{50}均值比较，白沙＞澄迈＞琼海＞儋州＞东方＞海口＞昌江＞乐东（图2），其中乐东县分离的病原菌对吡唑醚菌酯的敏感性最高，EC_{50}平均值为0.600 6 μg·mL^{-1}，平均抗性为0.62，最高抗性为1.07；白沙县分离的病原菌对吡唑醚菌酯相对敏感性较低，EC_{50}平均值为1.615 2 μg·mL^{-1}，平均抗性为1.68，最高抗性为1.91；白沙县的EC_{50}均值是乐东县的2.69倍（表2）。根据敏感基线白沙县产生抗药风险的可能性最高。2019和2020年2 a间火龙果溃疡病菌菌株对吡唑醚菌酯的EC_{50}分别为0.974 0 μg·mL^{-1}和0.989 3 μg·mL^{-1}，下降了0.01 μg·mL^{-1}，说明2019和2020年2 a间火龙果溃疡病菌菌株之间敏感性差异不明显。

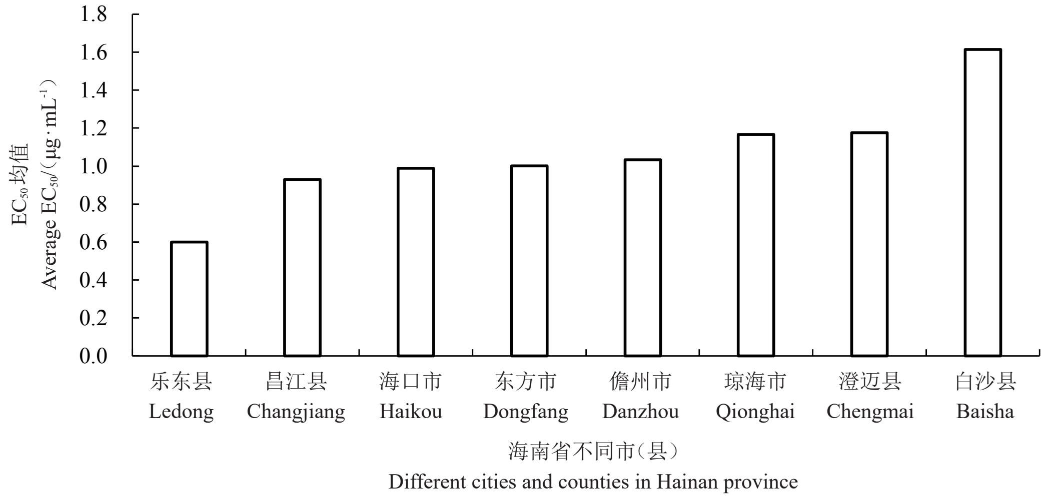

图2　不同市(县)火龙果溃疡病菌对吡唑醚菌酯的敏感性比较

Fig. 2　Sensitivity of *Neoscytalidium dimidiatum* to pyraclostrobin in different cities and counties

表2　海南省各地火龙果溃疡病菌对吡唑醚菌酯的抗性水平

Table 2　Resistance factor for *Neoscytalidium dimidiatum* to pyraclostrobin in different areas in Hainan

采样地点 Sampling locations	菌株数量 Numbers of strains	EC_{50}值分布范围 EC_{50} range	EC_{50}均值 Average EC_{50}	平均抗性水平 Mean resistance factor	最高抗性风险 The highest resistance factor
乐东县 Ledong	10	0.373 3~1.031 6	0.600 6	0.623 9	1.071 6
澄迈县 Chengmai	3	0.919 7~1.309 0	1.175 1	1.220 6	1.359 7
昌江县 Changjiang	3	0.477 8~1.429 5	0.929 2	0.965 2	1.484 9
琼海市 Qionghai	3	0.866 5~1.489 4	1.166 8	1.212 0	1.547 1
白沙县 Baisha	3	1.312 4~1.836 4	1.615 2	1.677 8	1.907 6
儋州市 Danzhou	14	0.512 4~1.850 3	1.033 8	1.073 8	1.922 0
东方市 Dongfang	15	0.254 0~1.982 7	1.000 7	1.039 5	2.059 5
海口市 Haikou	8	0.364 6~2.223 3	0.989 3	1.027 7	2.309 4
合计 Total	59	0.254 0~2.223 3	0.984 1	1.105 1	2.309 4

3　讨　论

海南省地处热带、亚热带地区，具有典型的热带季风气候，是火龙果的优质产区，但由于高温高湿常有台风等自然条件的影响，火龙果种植区易有病虫害发生。火龙果溃疡病是制约火龙果产业发展的重要病害之一，除了危害茎部，影响火龙果植株的生长外，还危害果实，进而影响产量及质量。目前，火龙果溃疡病菌的防治主要依赖于化学药剂。吡唑醚菌酯作为一种广谱性杀菌剂，具有高效、低毒、低残留等诸多优点，在火龙果种植地区被作为推荐药剂，大范围地用于田间病害防治，但由于其本身作用位点单一，病原菌很容易因产生变异，而使吡唑醚菌酯作用效果降低[12,22]，这对病害的防控极为不利。目前已有许多病原菌建立了对吡唑醚菌酯的敏感基线，如海南省杧果蒂腐病菌[13]、水稻稻瘟病菌[23]、苹果腐烂病菌[24]和山东葡萄白腐病菌[25]等。到目前为止，还没有火龙果溃疡病相关药剂敏感性的监测分析报道，因此非常有必要建立火龙果溃疡病菌对吡唑醚菌酯的敏感基线，用于监测海南地区火龙果溃疡病菌对吡唑醚菌酯的敏感性发展情况。由于海南省内几乎不可能采集到未用药前的野生菌株，因此笔者采用火龙果溃疡病菌对吡唑醚菌酯的EC_{50}值进行检测分析，当EC_{50}值符合连续正态分布时，EC_{50}均值作为敏感基线，结果表明供试菌株EC_{50}值间符合连续性正态分布，EC_{50}均值0.962 7 μg·mL^{-1}作为海南火龙果溃疡病菌对吡唑醚菌酯的敏感基线。根据前人的研究，王会会等[9]使用25%吡唑醚菌酯乳油对海南省琼海市火龙果溃疡病菌进行室内药剂筛选，设置质量浓度为4、2、1、0.5、0.25 μg·mL^{-1}，得到EC_{50}结果为1.029 1 μg·mL^{-1}；林珊宇等[26]使用42.4%吡唑醚菌酯•氟唑菌酰胺悬浮剂对广西火龙果溃疡病菌进行室内药剂筛选，得到EC_{50}结果为0.057 5 μg·mL^{-1}；贤小勇等[27]使用96%吡唑醚菌酯原药对广西火龙果溃疡病菌进行室内药剂筛选，设置质量浓度为4、2、1、0.5、0.25 μg·mL^{-1}，得到EC_{50}结果为1.857 2 μg·mL^{-1}，前人研究与本研究获得

的EC_{50}均值均较相近，广西壮族自治区和海南省火龙果溃疡病菌对吡唑醚菌酯的敏感性较相近。

笔者实验室于2019—2020年间对海南8个火龙果种植市（县）进行了实地调查，其中乐东和昌江作为新开发的火龙果产区，种植年限相对较短，用药量相对于其他地区较少，而白沙和琼海为火龙果种植的传统产区，火龙果种植年限相对较长，且用药量也相对较高，其中白沙主要由企业承包种植，所以用药量相对更大。笔者测定了从海南省海口、乐东、东方、儋州、白沙、昌江、澄迈和琼海8个地区分离的59个菌株对吡唑醚菌酯的敏感性，表明海南59株火龙果溃疡病菌对吡唑醚菌酯的敏感性差异性较小，EC_{50}为0.254 0~2.223 3 $\mu g \cdot mL^{-1}$。其中，乐东县菌株对吡唑醚菌酯十分敏感，而白沙县菌株对吡唑醚菌酯敏感性相对较低，地区间以白沙和乐东之间EC_{50}的差异最为显著，白沙县EC_{50}均值为乐东县的2.69倍，平均抗性的2.71倍，这与笔者实验室的田间调查结果相吻合。从研究的结果来看，目前海南地区火龙果溃疡病菌对吡唑醚菌酯仍较敏感，吡唑醚菌酯仍可作为防治火龙果溃疡病的杀菌剂使用。但是随着种植年限的增长，药剂不断累积使用，不排除存在产生抗药性的风险。海南省火龙果溃疡病菌对吡唑醚菌酯药剂敏感性水平目前尚未见报道，笔者在本研究中采用菌丝生长速率法测定了溃疡病菌菌株对吡唑醚菌酯的敏感性，进一步明确了近年内海南省火龙果溃疡病菌对吡唑醚菌酯的敏感性水平。对某种杀菌剂的敏感性监测是一个长期的工作，本研究结果为海南省火龙果溃疡病菌对常用杀菌剂吡唑醚菌酯的敏感性长期监测奠定了基础，为优化杀菌剂使用策略和科学防治火龙果溃疡病等工作提供了一定的科学依据。

4 结 论

海南省火龙果产区火龙果溃疡病菌对吡唑醚菌酯目前未产生抗药性，但不同种植市（县）的火龙果溃疡病菌对吡唑醚菌酯的敏感性存在差异，因此各火龙果种植地区使用吡唑醚菌酯时应根据其敏感性情况对施药比率与频率进行调整。其中白沙地区建议与其他相关药剂交替使用，减缓耐药性的发生，避免产生抗药性菌株。

参考文献 References:

[1] FABRICE L B，FABRICE V，ERIC I. Pitahaya (*Hylocereus* spp.)：A new fruit crop，a market with a future[J]. Fruits，2006，61(4)：237-250.

[2] 易润华，甘罗军，晏冬华，吴泽菁，童依婷，吴凤发. 火龙果溃疡病病原菌鉴定及生物学特性[J]. 植物保护学报，2013，40(2)：102-108.
YI Runhua，GAN Luojun，YAN Donghua，WU Zejing，TONG Yiting，WU Fengfa. Identification and biological characteristics of *Neoscytalidium dimidiatum* causing pitaya canker[J]. Acta Phytophylacica Sinica，2013，40(2)：102-108.

[3] 赵湖冰，姚媛媛，杨乐乐，田野，田孟伦，刘晓柱. 火龙果研究现状与开发前景[J]. 山东化工，2018，47(14)：52-54.
ZHAO Hubing，YAO Yuanyuan，YANG Lele，TIAN Ye，TIAN Menglun，LIU Xiaozhu. Pitaya research status and its development prospect[J]. Shandong Chemical Industry，2018，47(14)：52-54.

[4] MASRATUL H M，BAHARUDDIN S，LATIFFAH Z. Identification and molecular characterizations of *Neoscytalidium dimidiatum* causing stem canker of red-fleshed dragon fruit *(Hylocereus polyrhizus*) in Malaysia[J]. Journal of Phytopathology，2013，161(11/12)：841-849.

[5] EZRA D，LIARZI O，Hershcovich M. First report of internal black rot caused by *Neoscytalidium dimidiatum* on *Hylocereus undatus* (pitahaya) fruit in Israel[J]. Plant Disease，2013，97(11)：1513.

[6] CHUANG M F，NI H F，YANG H R，SHU S L，LAI S Y. First report of stem canker disease of pitaya (*Hylocereus undatus* and *H. polyrhizus*) caused by *Neoscytalidium dimidiatum* in Taiwan[J]. Plant Disease，2012，96(6)：906-907.

[7] 张荣，刘爱媛，白成艳，刘成明，罗笑容，姜峰，梁瑞伟，郑红莉. 火龙果溃疡病的症状观察和病原菌鉴定[J]. 果树学报，2013，30(5)：854-856.
ZHANG Rong，LIU Aiyuan，BAI Chengyan，LIU Chengming，LUO Xiaorong，JIANG Feng，LIANG Ruiwei，ZHENG Hongli. Symptom observation and pathogen identification on canker disease of pitaya[J]. Journal of Fruit Science，2013，30(5)：854-856.

[8] 戴俊，王会会，符碧海，王萌，陶挺燕，谢昌平，朱朝华. 火龙果溃疡病和茎腐病病原菌的生物学特性测定[J]. 中国南方果树，2017，46(1)：78-82.
DAI Jun，WANG Huihui，FU Bihai，WANG Meng，TAO Tingyan，XIE Changping，ZHU Chaohua. Determination of biological characteristics of *Neoscytalidium dimidiatum* and *Fusicoccum* sp. on pitaya[J]. South China Fruits，2017，46(1)：78-82.

[9] 王会会，符碧海，戴俊，徐倩，王萌，陶挺燕，谢昌平，朱朝华. 火龙果溃疡病菌的鉴定及室内药剂筛选[J]. 中国南方果树，2016，45(1)：8-12.
WANG Huihui，FU Bihai，DAI Jun，XU Qian，WANG Meng，TAO Tingyan，XIE Changping，ZHU Chaohua. Identification of dragon fruit canker pathogen and indoor screening of fungicides[J]. South China Fruits，2016，45(1)：8-12.

[10] XU M，PENG Y，QI Z，YAN Z，YANG L，HE M D，LI Q X，LIU C L，RUAN Y Z，WEI S S，XIE J，XIA Y Q，TANG H. Identification of *Neoscytalidium dimidiatum* causing canker disease of pitaya in Hainan，China[J]. Australasian Plant Pathology，

2018，47(5)：547-553.

[11] 杨丽娟，柏亚罗 . 甲氧基丙烯酸酯类杀菌剂：吡唑醚菌酯[J]. 现代农药，2012，11(4)：46-56.
YANG Lijuan，BAI Yaluo. Strobilurin fungicide：Pyraclostrobin [J]. Modern Agrochemicals，2012，11(4)：46-56.

[12] 杜宜新，阮宏椿，石妞妞，甘林，杨秀娟，陈福如 . 福建玉米小斑病菌对戊唑醇、吡唑醚菌酯和硝苯菌酯的敏感性[J]. 西北农林科技大学学报(自然科学版)，2017，45(8)：69-75.
DU Yixin，RUAN Hongchun，SHI Niuniu，GAN Lin，YANG Xiujuan，CHEN Furu. Sensitivity of *Bipolaris* in Fujian to tebuconazole，pyraclostrobin and meptyldinocap[J]. Journal of Northwest A＆F University (Nature Science Edition)，2017，45(8)：69-75.

[13] 贺瑞，赵磊，符瑞，陈芷岑，林晓翠，杨叶 . 海南芒果蒂腐病菌对吡唑醚菌酯的抗药性测定[J]. 植物保护，2018，44(4)：188-193.
HE Rui，ZHAO Lei，FU Rui，CHEN Zhicen，LIN Xiaocui，YANG Ye. Resistance of *Botryodiplodia theobromae* caused mango stem end rot to pyraclostrobin in Hainan[J]. Plant Protection，2018，44(4)：188-193.

[14] 贾俊超，马琳，范志金，夏倩，刘秀峰 . 病原菌对 Strobilurin 类杀菌剂抗药性机理的研究进展[J]. 农药学学报，2008，10(1)：1-9.
JIA Junchao，MA Lin，FAN Zhijin，XIA Qian，LIU Xiufeng. Progress on study of resistance mechanism of strobilurin fungicides[J]. Chinese Journal of Pesticide Science，2008，10(1)：1-9.

[15] 方中达 . 植病研究方法[M]. 3 版 . 北京：中国农业出版社，1998.
FANG Zhongda. Research method of plant pathology[M].3 ed. Beijing：China Agricultural Press，1998.

[16] 杨凤珍，郭建国，杜蕙，王春明，郭成，金社林，王生荣 . 甘肃玉米大斑病菌对吡唑醚菌酯的敏感性监测分析[J]. 西北农业学报，2016，25(10)：1548-1553.
YANG Fengzhen，GUO Jianguo，DU Hui，WANG Chunming，GUO Cheng，JIN Shelin，WANG Shengrong. Monitoring and analyzing sensitivity of *Setosphaeria turcica* to pyraclostrobin in Gansu[J]. Acta Agricultural Boreali-Occidentalis Sinica，2016，25(10)：1548-1553.

[17] ARABIAT S，KHAN M F R，BOLTON M，SECOR G. Stability of tetraconazole-resistant isolates of *Cercospora beticola* after exposure to different temperature and time treatments[J]. Journal of Plant Pathology，2017，99(1)：177-184.

[18] 杨敬辉，许媛，肖婷，褚姝频，芮东明，姚克兵 . 葡萄炭疽病菌（*Colletotrichum* spp.）种群对多菌灵的抗药性监测[J]. 果树学报，2021，38(2)：242-249.
YANG Jinghui，XU Yuan，XIAO Ting，CHU Shupin，RUI Dongming，YAO Kebing. Resistance monitoring of *Colletotrichum* spp. population to carbendazim in grape vineyards[J]. Journal of Fruit Science，2021，38(2)：242-249.

[19] 徐杰，冀志蕊，王娜，张俊祥，林云光，周宗山 . 葡萄炭疽病菌对 4 种杀菌剂的敏感性分析[J]. 果树学报，2020，37(6)：882-890.
XU Jie，JI Zhirui，WANG Na，ZHANG Junxiang，LIN Yunguang，ZHOU Zongshan. Sensitivity analysis of *Colletotrichum gloeosporioides* to four fungicides[J]. Journal of Fruit Science，2020，37(6)：882-890.

[20] 顾乃图，范昆，成世杰，李本杰，付丽，夏晓明 . 苹果轮纹病菌对腐霉利的敏感性检测[J]. 果树学报，2016，33(10)：1277-1285.
GU Naitu，FAN Kun，CHENG Shijie，LI Benjie，FU Li，XIA Xiaoming. Detection of the sensitivity of *Botryosphaeria dothidea* to procymidone[J]. Journal of Fruit Science，2016，33(10)：1277-1285.

[21] 刘世江，丁怡，赵琪君，李明，文小东，宋星陈，李荣玉 . 贵州省水稻纹枯病菌对丙环唑和吡唑醚菌酯的敏感性[J]. 福建农业学报，2019，34(11)：1294-1301.
LIU Shijiang，DING Yi，ZHAO Qijun，LI Ming，WEN Xiaodong，SONG Xingchen，LI Rongyu. Sensitivites of *Rhizoctonia solani* to propiconazole and pyraclostrobin on rice plants in Guizhou province[J]. Fujian Journal of Agricultural Sciences，2019，34(11)：1294-1301.

[22] 王丽，石延霞，李宝聚，刘长令，向文胜 . 甲氧基丙烯酸酯类杀菌剂研究进展[J]. 农药科学与管理，2008，29(9)：24-27.
WANG Li，SHI Yanxia，LI Baoju，LIU Changling，XIANG Wensheng. The progresses of research on strobilurin fungicides[J]. Pesticide Science and Administration，2008，29(9)：24-27.

[23] 梁梦琦 . 长江中下游稻区稻瘟病菌对稻瘟灵和吡唑醚菌酯的抗性监测[D]. 北京：中国农业科学院，2018.
LIANG Mengqi. Monitoring sensitivity of *Magnaporthe oryzae* to isoprothiolane and *Pyraclostrobin* in middle and lower reaches of Yangtze River[D]. Beijing: Chinese Academy of Agricultural Sciences，2018.

[24] 王帅 . 苹果树腐烂病菌对吡唑醚菌酯的抗药性风险评估及生物源杀菌剂的室内活性评价[D]. 杨凌：西北农林科技大学，2018.
WANG Shuai. Resistance risk assessment of *Valsa mali* to pyraclostrobin and antifungal activity of biological fungicides[D]. Yangling: Northwest A＆F University，2018.

[25] 李宝燕，栾炳辉，石洁，汪少丽，田园园，聂乐兴，王英姿 . 胶东地区葡萄白腐病菌对吡唑醚菌酯的敏感性及与其他 4 种药剂的敏感性比较[J]. 农药学学报，2020，22(6)：959-966.
LI Baoyan，LUAN Binghui，SHI Jie，WANG Shaoli，TIAN Yuanyuan，NIE Lexing，WANG Yingzi. Sensitivity of *Coniella diplodiella* to pyraclostrobin in Jiaodong Area and comparison with four other fungicides[J]. Chinese Journal of Pesticide Science，2020，22(6)：959-966.

[26] 林珊宇，贤小勇，朱桂宁，韦小妹，钟有超，黄黎芳 . 防治火龙果溃疡病的药剂筛选及田间应用[J]. 农药，2018，57(12)：921-924.
LIN Shanyu，XIAN Xiaoyong，ZHU Guining，WEI Xiaomei，ZHONG Youchao，HUANG Lifang. Effective fungicides screening and field application effects against pitaya canker[J]. Agrochemicals，2018，57(12)：921-924.

[27] 贤小勇，林珊宇，朱桂宁，韦小妹，覃武，梁桂东，钟有超 . 杀菌剂对火龙果溃疡病的室内毒力和田间防效[J]. 南方农业学报，2018，49(7)：1338-1345.
XIAN Xiaoyong，LIN Shanyu，ZHU Guining，WEI Xiaomei，QIN Wu，LIANG Guidong，ZHONG Youchao. Indoor virulence and field effects of fungicides on pitaya canker[J]. Journal of Southern Agriculture，2018，49(7)：1338-1345.