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Allied Toxicity Measurement of 8 Kinds of Bactericide on Hylocereus Undulatus Britt Scab

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This paper adopts growth rate method to launch indoor measurement on the inhibitory effect of 8 kinds of bactericide on Hylocereus undulatus Britt scab original bacteria. The results show that: these 8 kinds of bactericide have relatively good antibacterial effect in high concentrations. The best effect is found in 40% flusilazole and 20% difenoconazole and the others are 25% prochloraz, 50% propiconazole, 80% carbendazim, 50% iprodione, 12.5% myclobutanil and 20% kresoxim-methy in order. The EC50 is 0.07, 0.07, 0.10, 0.15, 0.28, 0.42, 0.78 and 12.30ppm in order.

1. Introduction

H. undulatus Britt is also called red dragon fruit, H. polyrhizus and love fruit, belonging to Cactaceae, H ylocereus and Selenicereus plant (Huang et.al, 2015; Mizrahi and Nerd, 1997). The origin of H.undulatus Britt is Costa Rica in Central America, Nicaragua, Mexico, Cuba and Vietnam, and it is the main fruit for local people (Huang et.al, 2015; Mizrahi and Nerd, 1997).

H. undulatus Britt is a kind of newly emerging fruit tree in tropical and subtropical region, combing fruit, flower, vegetable and health care and having high economic value. The flower of H.s undulatus Britt is rich in nutrition and has unique function and large amount of medicinal composition. There are thousands of small black sesame-shaped seeds inside the fruit of H. undulatus Britt. H. undulatus Britt has relatively high food therapy value, showing good effect in lowering blood pressure, lowering blood fat, detoxicating, nourishing lung, improving eyesight and beauty. H. undulatus Britt is also effective in terms of the treatment of constipation and diabetes. As new, unique, superior and high-tech agricultural project, H. undulatus Britt is ecological agriculture and high-efficiency agriculture, the production and consumption of which conform to the ecological and environmental requirements.

There are now 3 varieties of H. undulatus Britt of Hylocereus, which is H. undatus, H. polyrhizus and H. costaricensis (Chen, et.al, 2004; Valiente-Banuet, 2007). The H. undulatus Britt of these 3 varieties do not have stone and the flesh has no crude fiber, only some sesame-shaped nutlet, which is sweet and tasty. The H. undatus is subdivided into spinulation white flesh, banana white flesh, blood white, white jade dragon and Vietnam white, of which the white jade dragon is the most superior with high yield, high pollination rate, cold-resistant and disease resistant characteristics. The sugar content is 13-16Brix (MPG et.al, 2005). The H. polyrhizus has as many as 10 varieties, mainly pearl dragon and new red dragon fruit. Compared with white jade dragon, pearl dragon has higher anthocyanin content, aromatic flavor, but the size is relatively small and the sugar content is 9-10Brix. The yield is high, but the fruit is easy to crack. The high-yield period is one month longer than that of H. undatus. The H. costaricensis has relatively few varieties mainly because of its low yield, only5000-6500 kg/667m2 (Nobel, 2006; Hong et.al, 2010; Wang et.al, 2007).

The H.undulatus Britt is rich in nutrition, sugar, organic acid, dietary fibre; especially the content of dietary fiber and the protein are much higher than that of apple, sweet orange and peach. H. undulatus Britt contains 18 kinds of amino acid, including 8 kinds of essential amino acid to human body, which accounts for 34.9% of total amino acid content, approximating to reasonable proportion. Therefore, the quality of protein in the H. undulatus Britt is high. Meanwhile, many kinds of mineral nutrient elements are contained in the H. undulatus Britt, especially rich in potassium, calcium, magnesium and phosphorus (Zainoldin and Baba, 2012). When the

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H. undulatus Britt was introduced and planted in small amount, there are not too many diseases and insect pests. However, in recent years, the diseases and insect pests have aggravated gradually with the expansion of planting area. However, H. undulatus Britt lags behind the development of the other fruits in terms of plant protection, especially the system investigation and correlation studies of diseases and insect pests are still in blank field. We haven't learnt the species distribution and occurrence regularity of diseases and insect pests of H. undulatus Britt. The connected investment is not enough, the control strategies of diseases and insect pests are unreasonable and the measures adopted are not effective. What's worse, production is the only focus whiles the diseases and insect pests management is neglected, which leads to the spread of some of diseases and insect pests, affecting the production of H. undulatus Britt. Scab severely impairs H. undulatus Britt. At the beginning, water soaked chlorotic spots are found on the diseased stems, then extend both upward and downward, showing a long oval shape and there are brick red necrotic spots or rust necrotic spots in the sunken central area; water soaked chlorotic spots appear on the surface of diseased fruit and then extend gradually. Oil immersed light is found at the beginning and tawny and pitchy suberification is found later. Diseased spots grow in group and form large diseased spot with 2 to15cm length and 2 to 5cm width. Some are connected to other diseased spots, forming large spot with irregular shape. When the joint of fruit stalk and fruit is injured, then the abscission of fruits becomes common to see (Nerd, et.al, 2004; Dogan, et.al, 2014; levia, et.al, 2007). This study screens the bactericide which has relatively good antibacterial effect on H. undulatus Britt scab, aiming at providing theoretical basis for the field control of H. sundulatus Britt scab and reference for field control practice.

2. Material and Method

2.1 Bacteria for Experiment

Neoscytalidium dimidiatum(Penz.)CrousSlippers is separated and preserved by our laboratory.

2.2 reagent for Experiment and its Applied Concentration

The reagent for experiment and its applied concentration is shown in Table 1.

2.3 Data Analysis

This paper adopts DPS statistical analysis software to launch duncan test variance analysis.

2.4 Experimental Method

2.4.1 Preparation of PDA Medium

Cut up the peeled potato after washing and add 1000mL of water to boil for 0.5h. After that, use gauze to filter the potato and add more water to regain 1000mL and add sugar and agar. Heat until the agar melts completely and uses gauze to filter while it is still hot. Then, put it in the triangular flask respectively and conduct sterilization after adding cotton plug. The composition is as follow: potato 200g, glucose 10 to 20g, agar 10 to 20g, water 1000mL.

2.4.2 Preparation of Medicated Medium

Prepare different reagents into medical solution of different concentration in the above Table respectively and uniformly mix with the medium used in the experiment proportion of 1:49 and then we get the medicated medium in serial concentration gradient.

2.4.3 Determination Method

We take advantage of growth rate method to conduct allied toxicity measurement on the growth of scab bacteria mycelium to screen chemical reagent with high efficiency and low toxicity. Inoculate the bacterial strain to be measured on the PDA plate and cultivate it for 3 hours. Use puncher with inner diameter of 5 mm to put the bacterial colony into bacteria pie and transplant it onto the PDA plate containing different reagents under aseptic operation, making the side with mycelium get in touch with the medium. Take those without reagent as contrast CK, design 5 processing, 3 times of repetition and cultivate it under constant temperature of 29℃. Measure the diameter of bacterial colony applying crossing method after 2 hours of cultivation and take the average to calculate the inhibitory rate.

Inhibitory rate= [(contrast bacterial colony diameter-5 mm)-(processing bacterial colony diameter-5 mm)]/ (contrast bacterial colony diameter-5) ×100%

Convert the inhibitory rate into inhibitory probability value according to reaction rate-probability value conversion table. Take the logarithm of reagent concentration set as abscissa and inhibitory probability value as ordinate and draw the regression line to work out the toxicity regression equation, median inhibitory concentration (EC50) and correlation coefficient (r) of different reagents on H. undulatus Britt scab original bacteria.

Table 1: Reagent for Experiment and its Applied Concentration for Hylocereus undulatus Britt Scab Reagent Inhibition Experiment

Serial Number	Name of Reagent	Mass Concentration/ppm	Colony Diameter/cm	Inhibitory Rate/%
A	Propiconazole (50%)	0.02 0.05 0.1 0.5 1.0 0	6.28 5.23 4.23 3.37 2.93 8.20	24.9 38.6 51.6 62.7 68.4 0
B	Myclobutanil (12.5%)	0.02 0.05 0.1 0.5 1.0 0	6.675 5.63 6.63 4.60 3.95 8.20	19.8 33.4 20.4 46.8 55.2 0
C	Prochloraz (25%)	0.05 0.1 0.5 1.0 5.0 0	4.45 4.20 3.47 2.45 1.47 7.97	47.1 50.5 60.2 73.9 87.0 0
D	Carbendazim (80%)	0.05 0.1 0.5 1.0 5.0 0	7.05 4.45 3.92 2.63 1.93 8.40	17.1 50.0 56.7 73.0 81.9 0
E	Iprodione (50%)	0.05 0.1 0.5 1.0 5.0 0	6.85 6.10 4.83 2.62 1.40 8.10	16.4 26.3 43.0 72.1 88.2 0
F	Flusilazole (40%)	0.02 0.05 0.1 0.5 1.0 0	4.82 3.54 3.53 2.40 2.14 6.89	32.4 52.4 52.6 70.3 74.3 0
G	Difenoconazole (20%)	0.02 0.05 0.1 0.5 1.0 0	4.175 3.925 4.02 3.42 2.43 7.27	45.9 49.3 47.9 56.7 71.4 0
H	Kresoxim-methyl (20%)	0.05 0.1 0.5 1.0 5.0 0	6.32 6.27 5.53 5.08 4.38 7.07	16.4 26.3 43.0 72.1 88.2 0

3. Result and Analysis

3.1 Symptom of H. undulatus Britt Scab

At the beginning, water soaked chlorotic spots are found on the diseased stems, then extend both upward and downward, showing a long oval shape and there are brick red necrotic spots or rust necrotic spots in the sunken central area; water soaked chlorotic spots appear on the surface of diseased fruit and then extend gradually. Oil immersed light is found at the beginning and tawny and pitchy suberification is found later. Diseased spots grow in group and form large diseased spot with 2 to15cm length and 2 to 5cm width. Some are connected to other diseased spots, forming large spot with irregular shape. When the joint of fruit stalk and fruit is injured, then the abscission of fruits becomes common to see.

3.2 Allied Toxicity Measurement

3.2.1 Antibacterial Effect of Different Reagent Concentration on H. undulatus Britt Scab

The 8 kinds of reagents for experiment all have different degree of inhibitory effect on H. undulatus Britt scab. We can see from the size and definition that the 8 kinds of reagents have relatively good antibacterial effect in high concentrations when the cultivation comes to 2 hours. The best effect is found in 50% iprodione and 20% kresoxim-methy and the others are 80% carbendazim, 50% propiconazole, 40% flusilazole, 12.5% myclobutanil, 25% prochloraz and 20% difenoconazole in order. Figure 2 is a group of experimental photos of 8 kinds of reagents after dilution of 5 gradient multiples showing good inhibitory effect on bacteria.

Figure 2-1 EC antibacterial effect of 50% propiconazole

Figure 2-2 WP antibacterial of 12.5% myclobutanil

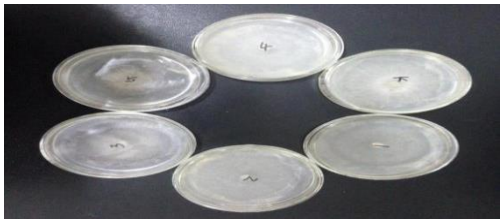

Figure 2-3 WP antibacterial effect of 25% prochloraz

Figure 2-4 WP antibacterial effect of 80% carbendazim

Figure 2-5 WP antibacterial effect of 50% iprodione

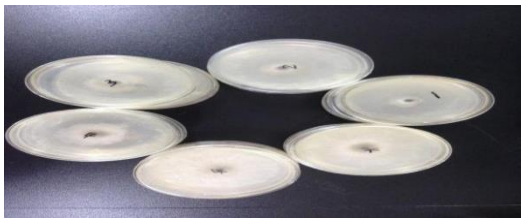

Figure 2-6 WP antibacterial effect of 40% flusilazole

Figure 2-7 WP antibacterial effect of 20% difenoconazole

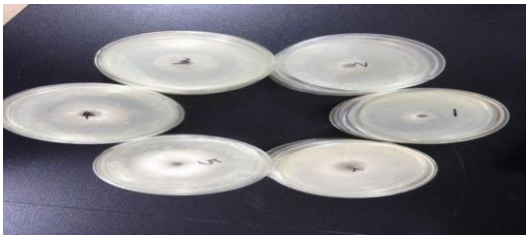

Figure 2-8 EC antibacterial effect of 20% kresoxim-methy

3.2.2 Allied Toxicity Measurement of Bactericide of Different Concentrations on H. undulatus Britt Scab We can see from the determination result (Table 1) applying growth rate method that the 8 kinds of reagents for experiment all have different degree of inhibitory effect on the growth of H. undulatus Britt scab mycelium under experiment concentration. The inhibitory rate grows with the increase of concentration while the

inhibitory rate varies distinctly among reagents. Compare the toxicity of different bactericide based on EC50 value and the toxicity sequence is 40% flusilazole=20% difenoconazole>25% prochloraz>50% propiconazole>80% carbendazim>50% iprodione>12.5% myclobutanil>20% kresoxim-methy. The EC50 is 0.07, 0.07, 0.10, 0.15, 0.28, 0.42, 0.78 and 12.30ppm in order (Table 3).

Table 2: Inhibitory Effect of Different Reagents on the Growth of H. undulatus Britt Scab Mycelium

Serial Number	Name of Reagent	Mass Concentration/ppm	Colony Diameter/cm	Inhibitory Rate/%
A	Propiconazole (50%)	0.02 0.05 0.1 0.5 1.0 0	6.28 5.23 4.23 3.37 2.93 8.20	24.9 38.6 51.6 62.7 68.4 0
B	Myclobutanil (12.5%)	0.02 0.05 0.1 0.5 1.0 0	6.675 5.63 6.63 4.60 3.95 8.20	19.8 33.4 20.4 46.8 55.2 0
C	Prochloraz (25%)	0.05 0.1 0.5 1.0 5.0 0	4.45 4.20 3.47 2.45 1.47 7.97	47.1 50.5 60.2 73.9 87.0 0
D	Carbendazim (80%)	0.05 0.1 0.5 1.0 5.0 0	7.05 4.45 3.92 2.63 1.93 8.40	17.1 50.0 56.7 73.0 81.9 0
E	Iprodione (50%)	0.05 0.1 0.5 1.0 5.0 0	6.85 6.10 4.83 2.62 1.40 8.10	16.4 26.3 43.0 72.1 88.2 0
F	Flusilazole (40%)	0.02 0.05 0.1 0.5 1.0 0	4.82 3.54 3.53 2.40 2.14 6.89	32.4 52.4 52.6 70.3 74.3 0
G	Difenoconazole (20%)	0.02 0.05 0.1 0.5 1.0 0	4.175 3.925 4.02 3.42 2.43 7.27	45.9 49.3 47.9 56.7 71.4 0
H	Kresoxim-methyl (20%)	0.05 0.1 0.5 1.0 5.0 0	6.32 6.27 5.53 5.08 4.38 7.07	16.4 26.3 43.0 72.1 88.2 0

Table 3: Control Effect of Different Bactericide on H.undulatus Britt Scab Bacteria

Serial Number	Name of Reagent	Toxicity Regression Equation	EC_{50}/ppm	Correlation Coefficient/r
A	Propiconazole (50%)	Y=0.6455x+5.5354	0.15	0.9524
B	Myclobutanil (12.5%)	Y=0.5502x+5.0622	0.78	0.7771
C	Prochloraz (25%)	Y=0.6049x+5.6195	0.10	0.9428
D	Carbendazim (80%)	Y=0.8262x+5.4625	0.28	0.8578
E	Iprodione (50%)	Y=1.0916x+5.4112	0.42	0.9677
F	Flusilazole (40%)	Y=0.6124x+5.6975	0.07	0.9478
G	Difenoconazole (20%)	Y=0.3459x+5.4096	0.07	0.7899
H	Kresoxim-methyl (20%)	Y=0.5215X=4.4298	12.30	0.9847

4. Conclusion and Discussion

After toxicity determination of indoor reagent shows that 40% flusilazole and 20% difenoconazole WP has great inhibitory effect in terms of the growth of H. undulatus Britt scab original bacteria mycelium. However, because of the complicated relation existing among the pathogen, host and environment in the field and large dosage, the waxiness of H. undulatus Britt stem is thick, which affects the function and control effect of reagent. Therefore, further experimental study is necessary to see whether there is evident control effect if we directly apply the selected reagents to control corresponding diseases in the field.

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