Pak. J. Bot., 51(6): 2291-2294, 2019. DOI: http://dx.doi.org/10.30848/PJB2019-6(32)

GROWTH-INHIBITION OF 12 FUNGICIDES AGAINST *BOTRYTIS CINEREA* IN TOMATO AND THEIR PREVENTIVE EFFECTS IN FIELD

XIAOMEI WANG[1*], XIAOFENG DU[1], YUAN NONG[1] AND HONGBO SHAO[2,3,4*]

[1]*College of Plant Protection, Jilin Agricultural University, Changchun 130118, China*
[2]*Salt-soil Agricultural Center, Key Laboratory of Agricultural Environment in the Lower Reaches of Yangtze River Plain, Institute of Agricultural Resources and Environment, Jiangsu Academy of Agriculture Sciences(JAAS), Nanjing 210014, China*
[3]*Jiangsu Key Laboratory for Bioresources of Saline Soils, Jiangsu Synthetic Innovation Center for Coastal Bio-agriculture, Yancheng Teachers University, Yancheng 224002, China*
[4] *College of Environment and Safety Engineering, Qingdao University of Science&Technology, Qingdao 266000, China*
**Corresponding author's email: wxm820@126.com; shaohongbochu@126.com*

Abstract

Grey mold disease caused by *Botrytis cinerea* Pers. ex Fr. is a severe threat to tomato (*Lycopersicon esculentum*) yield in field and greenhouse in many countries worldwide. In this study, 12 fungicides agents were selected to determine their inhibiting effects on mycelium growth of *Botrytis cinerea* Pers ex.Fr. and 4 of them with better effects were used to further test the disease control in field. Our results showed that 70% thiophonate-methyl (WP), 50% methyl sulfur bacteria-spirit shine (WP), 50% iprodione (WP) and 99% wichol (TC) exhibited stronger inhibition of the mycelial growth at EC_{50} value<100 mg/L. In addition, 50% cycloamines (WG) and 40% iminoctadine (WP) provided partial inhibition at EC_{50}>10000 mg/L. But 80% carbendazim (WP) and 80% Phenyl ether Jiahuan azole (WG) provided minimal inhibition at EC_{50}>10000 mg/L. Furthermore, the field test showed that 70% thiophonate-methyl (WP), 50% methyl sulfur fungal spirit shine (WP), 50% iprodione (WP) and 99% wichol (TC) provided stronger control effects than those of the boscalid, and these agents could be used in grey mound disease control in practical tomato production.

Key words: *Botrytis cinerea*; Fungicides; Mycelium growth; Tomato; Control efficacy.

Introduction

Recently, tomato has received much more attention due to its inhibition on growth of cancer tumor. When extracts of tomato pigments were added into culture solution containing incubated cancer cells, the cells will quickly lost activity and gradually cause apoptosis over several days (Chen *et al*., 2010; Hussain *et al*., 2019). Overall, tomato can eliminate the risk associated with the tumor of prostate, digestive canal, pancreatic gland, and mamma gland. In addition, tomato is one of the most important economic crop in China with the cropping area of over 1.455×10^6 hm^2 and with a yield of 8.376×10^6 t per year. But with the increasing area for tomato planting, various plant diseases, especially grey mound disease caused by *Botrytis cinerea* Pers. ex Fr., have occurred throughout and caused yield losses of 20-40% in average, at worst reached 60% in China (Ding *et al*., 2009). In details, according to the investigation in 2000, 22.4 % of eggplants are infected by *Botrytis cinerea* at the breeding stage. Furthermore, tomato, cucumber and strawberry are subject to 20%-50% infection and 10-36% yield loss in Zhejiang Province (Zhao *et al*., 2014), over 60% yield loss in Gansu Province (Feng *et al*., 2013), 50% yield loss in Liaoning Province (Ji *et al*., 2012), and 10-30% loss in Shandong Province (Jia *et al*., 2012) caused by this disease. Currently, the disease has occurred throughout China and has presented an increasing trend, and has been the determining factor for the yield losses, and this prevents tomato from high production. Thus this disease has been a major problem in the tomato production in China (Zhao *et al*., 2014).

To control *Botrytis cinerea*, lots of work has been conducted to investigate this disease, including the biological characteristic, resistance development, mechanisms of the infection, and its integrated management. *Botrytis cinerea* has 235 host species, including tomato, eggplant, cucumber, pepper, strawberry, grape, garlic, lettuce, citrus, etc. (Feng *et al*., 2013). The disease causes many kinds of damage on the above vegetable both in field and in the process of storage and transportation. The bacteria mainly damages on the fruit, and sometimes on the leaf and the stem, etc. Regarding fruit damage, residual petal and receptacle are easily subject to damage, and the disease initially causes gray-brown irregular spots, and the spots will gradually develop into the wet rot from sepals to the around (Huang *et al*., 2015; Munir *et al*., 2019). This damage causes fruit rot and a hairy moldy-layer in gray that will occur and cover more than 1/3 area of the fruit surface. Generally, immature fruits are likely to be damaged, and fruit will be damaged also sometime immediately before maturation (Jia *et al*., 2012). Regarding the infection of leave, the damage triggers initially from leaf tip or leaf margin, causing leaf wilting and rotting with brown spot in irregular shape. Damage on the stem will cause the death of leave and stem which live above the spot on stem, and this damage also causes the occurrence of long-oval or irregular-strip spot of the brown (Li *et al*., 2011).

Grey mold disease was developed from the Deuteromycetes fungi and infected tomato. The sclerotium or conidiophore of this fungi can respectively stay over winter in soil or on disease-damaging crop plants (Yu *et al*., 2016). When appropriate environment is available, the mycelium will germinate and produce conidiophores, and these conidiophores will spread through wind, rain, or producing activities of people (Hussain *et al*., 2019). The appropriate temperature for the fungi growing is among 20-25°C, with air humidity not less than 90%. If the high humidity have occurred for a relative long time, the disease will cause more damage (Li *et al*., 2010; Ji *et al*., 2012).

Nowadays there are 4 fungicides generally used for the disease control and management in China. We used 12 fungicides to evaluate the inhibition effects on mycelium growth of *Botrytis cinerea* in laboratory. Then 4 efficient fungicides were applied in tomato field for further determination. The purpose of this study was to evaluate the fungicides efficacy and control grey mound disease, and provide theoretical and experimental basis for the larger-scale application in practice.

Materials and Methods

Fungal strains: The fungal strain (*Botrytis cinerea* Pers. ex Fr.) was provided by Plant Disease Institute of Jilin Agricultural University. The strain was identified and isolated in laboratory in Changchun city.

Fungicides: The12 fungicides agents used in this study were listed in Table 1.

The collection, isolation, purification, and deposition of the strain: The fungal strain (*Botrytis cinerea* Pers. ex Fr.) was obtained at Changchun City. Six *Botrytis cinere*-damaging plants were collected and brought back to the laboratory. Then the damaged plants were entirely rinsed with pure water for 10 minutes. Then, cut off a piece of plant disease tissue (3 mm×3 mm) from the junction of disease and health issue. The surface of the tissue was treated with 75% alcohol for 10 s, and soaked in 0.1% mercury for 1 min, and then clean with dH_2O for 2-3 times. After that the tissues were cultured in potato glucose medium (PDA) at 28°C for 2-3 d. According to the monosporium separation method, the spores were initially collected from growing mycelium, and then cultured in non-antibiotic-based potato glucose medium. The strains were purified several times according to the above procedure, and the purified strains were stored in the refrigerator at 4°C.

Inhibition of mycelium growth by fungicides: Here, the effects of various fungicides were tested against *Botrytis cinerea* Pers. ex Fr. grown on PDA. In details, 12 fungicides were used in following 6 concentrations, i.e. 1×10^4, 1×10^3, 1×10^2, 1×10^1, 1×10^0, and 1×10^{-1} mg/L. Different concentration agents were mixed with PDA by the ratio of 1:9. After the purified *Botrytis cinerea* growing for 3 d, 8 mm-diameter fungus cake was cut to culture in the new PDA medium containing different kinds of fungicides concentrations, and incubated at 26-27°C for 3-7 d. The fungus cake cultured in PDA medium without fungicide were as control. Each treatment had three replicates.

Cross Patch method was used to determine the diameter of a strain, and compared the treated stains with control stains to obtain the inhibition effect of different kinds of concentrations of fungicides. Inhibition % = 100% × (the diameter of stain in CK-the diameter strain in treatment) / (the diameter of stain in CK –the diameter of the whole bacteria medium). The inhibition % of each individual fungicide concentration was transformed to log X, and according to least squares techniques, linear equation between logarithm of concentration and possibility was gained by Excel. According to the linear equation, when y＝5，the value of -x was equal to EC_{50}; And when y=6.28, the value of -x was equal to EC_{90}.

Test in green houses: In Lishu County, Jilin Province, the tests were performed in 12 green houses with typical black soil and normal fertilizing condition. The tested fungicides were sprayed on the disease-damaging tomato plants, on the 20th September (4 groups + 4 controls; each group contains 50 plants). The plants sprayed with 50% boscalid WG and water was used as positive and negative control, respectively.

Investigation method: The investigation was conducted according to guidelines related to pesticide test when the fungicides had been sprayed for 7d. Within each plot, 5 plants growing at diagonal line were randomly selected for the investigation into leaf damage. Those damaging leaves were counted and categorized according to the following equation (Yu *et al*., 2016).

$$\text{Disease parameter} = \frac{\Sigma(\text{Parameter of damaged leaf} \times \text{value of various damaging levels}) \times 100}{\text{Tested leaf number} \times \text{value of the highest damage}}$$

$$\text{Control efficacy} = \frac{(\text{Disease parameter in CK-Disease parameter in treatment}) \times 100\%}{\text{Disease parameter in CK}}$$

Table 1. Fungicides tested in this study.

Number	Brand name	Manufacturer
1.	75% Chlorothalonil WP	Limin Chemical CO., Ltd
2.	99% Hypogeacean TC	Yantai Xinrun Fine Chemical Co., Ltd.
3.	70% Mancozeb WP	Limin Chemical Co., Ltd.
4.	50% Iprodione WP	Jiangxi Wo Yi Chemical Co., Ltd.
5.	50% Cyprodinil wg	Shaanxi Road on the grid Bioscience Co., Ltd.
6.	70% Thiophanate methyl WP	Beijing Mause Technology Co., Ltd.
7.	50% Thiram WP	Hebei Guanlong Agrochemical Co., Ltd.
8.	15% Triadimefon WP	Sichuan Guoguang Chemical Co., Ltd.
9.	10% Difenoconazole WG	Jiangsu Feng Deng Crop Protection Co., Ltd.
10.	80% Carbendazim WP	Jiangsu Taicang Agrochemical Co., Ltd.
11.	40% Iminoctadine WP	Japan soda Co., Ltd.
12.	50% Procymidone WP	Sumitomo Chemical Co., Ltd.

Table 2. Inhibition of 12 fungicides on the fungal strain.

Number	Fungicide	Virulence regression equation	Correlation coefficient (r)	EC_{50} (mg/L)	EC_{90} (mg/L)
1.	70%Thiophanatemethyl WP	y=7.0369x-0.1163	0.9854	0.02	1490.97
2.	50% Procymidone WP	y=8.2138x-0.2870	0.9401	13.72	1186.11
3.	50% Iprodione WP	y=8.4866x-0.3410	0.9720	36.32	1549.11
4.	99% Hypogeacean TC	y=8.0261x-0.3179	0.9246	73.38	4115.53
5.	50% Cyprodinil WG	y=6.4909x-0.1837	0.9619	298.16	317188.27
6.	15% Triadimefon WP	y=8.5948x-0.4549	0.9291	370.41	6174.20
7.	40% Iminoctadine WP	y=6.0518x-0.1412	0.9960	583.14	5029499.76
8.	75% Chlorothalonil WP	y=6.3232x-0.2127	0.9341	1987.95	816375.29
9.	70% Mancozeb WP	y=6.4474x-0.2555	0.9680	3463.67	519306.78
10.	50% Thiram WP	y=5.6268x-0.4060	0.8107	11592.53	104008045.64
11.	10% Difenoconazole WG	y=5.0844x-0.0860	0.9688	374791	1081633370924
12.	80% Carbendazim WP	y=4.1300x-0.0968	0.9175	8017472897	4439038734478

Table 3. The control efficacy of 4 fungicides in field tests in 2017.

Fungicide	Dosage (ga.i/hm-2)	Disease parameter	Control eficacy (%)	Significance	
				5%	1%
70% Thiophanatemethyl WP	521	3.54	83.21	a	A
50% Procymidone WP	521	3.82	82.56	ab	AB
99% Hypogeacean TC	521	4.57	80.53	ab	AB
50% Iprodione WP	521	9.21	69.72	bc	B
50% Boscalid WG	521	8.57	75.54	c	C
CK	Water	18.25			

Results

Inhibition efficacy of fungicides in laboratory: Most of the tested 12 fungicides could inhibit the growth of the fungi, but their inhibition efficacy was significantly different (Table 2). thiophanate methyl (70%, WP), procymidone (50%, WP), iprodione (50%, WP), and hypogeacean (99%, TC) significantly inhibited the growth of the strain at EC_{50}<100 mg/L; cycloamines (50%, WG) and iminoctadine tris (40%, WP) to some degree inhibited the strain growth at EC_{50}<1000 mg/L; carbendazim (80%, WP) and difenoconazole (10%, WG) inhibited the strain growth at EC_{50}>10000 mg/L (Table 2).

The better four fungicides were used for field tests on the basis of their inhibition on the strain growth in laboratory. The commonly used fungicide boscalid (50%, WG) and water were sprayed on damaged crop plants as control. The results showed the control efficacy of thiophanate methyl (70%, WP), procymidone (50%,WP) and hypogeacean (99%, TC) was significantly better than that of boscalid (50%, WG), whereas iprodione (50%,WP) was worse than that (Table 3).

Discussions

Here, the inhibition of 12 fungicides on growth of strain was tested for determine their control efficacy in laboratory. Results indicated that thiophanate methyl (70%, WP), procymidone (50%, WP), iprodione (50%, WP) and hypogeacean (99%, TC) significantly inhibited the growth of the strain, and the EC_{50} was less than 100 mg/L. In addition, thiophanate methyl (70%, WP) worked best in laboratory with EC_{50} at 0.02 mg/L. Cycloamines (50%, WG) and biguanide trioctyl sulfonate (40%, WP) somewhat inhibited the strain growth, and their EC_{50} was less than 1000 mg/L; carbendazim (80%, WP) and difenoconazole (10%, WG) worst inhibited the strain growth, and the EC_{50} was more than 10000 mg/L (Tables 2 and 3).

Therefore, we here recommend using thiophanate methyl (70%, WP), procymidone (50%, WP) and hypogeacean (99%, TC) in field control in practice, which were more effective than boscalid (50%, WG) and iprodione (50%, WP). Identification of practicable fungicides with high efficacy and low toxicity still need to test both in laboratory and in field to provide solid base for future disease control on large scale.

Botrytis cinerea caused disease in tomato production has obtained greater concerns in the world recently (Huang *et al*., 2007; Huang *et al*., 2015).Currently, our effective method by the selected fungicides has provided a better choice in practice with more potential application according to recent reports (Zhao *et al*., 2014; Hussain *et al*., 2019; Munir *et al*., 2019), which has more value for the population in different parts of the globe. Because of complex mechanisms involved in soil types and property, climate, microbial interaction and molecular biology, more work is needed to conduct in terms of unified refine management and tomato-resistant-disease species cultivation by traditional method and biotechnology.

Acknowledgements

This work has been supported by National Key Research and Development Project (N0.2016YFD0201004; 2016YFD0200708; 2018YFD0201200).

References

Chen, F.P., P. Han, Z.Z. Zhang, J.L. Liu and X.P. Liu. 2010. Studies on antifungal activity of fungicide 5-(4-chlorophenyl) 2, 3-dimethyl 3-(pyridine -3) –oxazoline against *Bot rytiscinerea*. *Chin. J. Pest. Sci*., 1: 42-48.

Ding, J.C., H.Y. Hu, X.L. Liu, C.G. Ni, Z.R. Wang, G.Y. Liang and Q.B. Zheng. 2009. Study on control of effect to different medicine to B*otrytis cinerea*. *J. Anhui Agri. Sci.*, 26: 604-605.

Feng, D.L., Q. Wang, J.C. Zheng, C.P. Zhang and H.Y. Chou. 2013. Comparative experimental study of several fungicides to *Botrytis cinerea*. *Acta Agri. Zhejiangensis*, 1: 119-123.

Huang, H., X. Zhang, H. Zou, H.Y. Yang, J.J. Cao and D.R. An. 2015. Toxicity and control efficiency of fungicides against *Botrytis cinerea*. *J. Northwest A&F University*, 2: 184-190.

Huang, Y.Q. 2007. Tomato gray mold disease prevention and treatment screening. *Advances in Plant Protection in Henan Province Ⅱ*, 2: 50-52.

Hussain, T., T. Adesemoye, M. Ishtiaq, M. Maqbool, A. Azam and S. Azam. 2019. Control measures of root rot fungal pathogens by producing resistance against twelve wheat cultivars under greenhouse in Nebraska State of USA. *Pak. J. Bot*., 51(2): 38-43.

Ji, J.J., X.F. Zhang, W.Q. Wang and J.L. Zhang. 2012. Research progress on control of tomato gray mold. *Chin. Agri. Sci. Bull*., 31: 109-113.

Jia, L.Y., W. Tian and X.S. Liu. 2012. Effects of six fungicides against *Botrytis cinera* on tomato under protected facility cultivation. *J. Henan Agri. Sci*., 9: 103-105.

Li, L.K., L.L. Zhai, L.L, H.Y. Wang and Y. Wang. 2010. Sensitivity of *Botrytis cinerea* to Six Fungicides. *Chin. Agri. Sci. Bull*. 2: 230-233.

Li, M.L. and Q. Li. 2011. Occurrence and chemical control of *Botrytis cinerea*. *Chang. Vegetab*., 20: 67-68.

Munir, S., N. Ahmed, M. Abid, S.U. Rehman, M. Ashraf, L. Zhang and M. Anees. 2019. Chitinolytic activity of the indigenous Trichoderma spp. from the north west of Pakistan against the fungal phytopathogens. *Pak. J. Bot*., 51(2): 37-42.

Yu, G.W., S.C. Li and Q. Lu. 2016. Comparative experiment on control effect of different biocides on *Botrytis cinerea*. *Agri. Sci.*, 8: 66-74.

Zhao, Y., Z.Y. Miao, Y. Li and Y.J. Bai. 2014. Research progress on controlling against tomato gray mold. *China Plant Protec.*, 7: 21-29.

(Received for publication 8 May 2018)