Journal of Plant Pathology (2021) 103:915–921
https://doi.org/10.1007/s42161-021-00875-5

ORIGINAL ARTICLE

Activity of eugenol derivatives against *Fusarium graminearum* Q1 strain and screening of isoeugenol mixtures

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Received: 21 October 2020 / Accepted: 31 May 2021 / Published online: 10 June 2021

Abstract

The inhibition effects of ten eugenol derivatives against *Fusarium graminearum* Q1 strain were determined using the mycelia growth rate process. Our results revealed that the toxicities of various derivatives differed substantially as compared to that of eugenol. Isoeugenol had the highest toxicity, about 4.2 times that of eugenol, with the EC_{50} value of 279.7 μM. Based on the toxicity ratio (TR) and synergistic coefficient (SR), the optimum mixing mass ratios of isoeugenol to phenamacril and isoeugenol to tebuconazole against *F. graminearum* were 7:1 and 6:1, respectively. Based on their additive effects, these mixtures could be used as potential alternatives to control *F. graminearum* resistance against phenamacril and tebuconazole due to different mechanism of action.

Keywords Eugenol derivatives · *Fusarium graminearum* · Isoeugenol · Phenamacril · Tebuconazole · Mixture screening

Introduction

Fusarium head blight of wheat (FHB) caused by *Fusarium graminearum* is a devastating disease of cereal crops worldwide (Bai and Shaner 2004; Goswami and Kistler 2004). In addition to reducing grain yield and quality, *F. graminearum* produces harmful mycotoxins in infected grain (Sutton 1982; Bottalico 1998; Varga et al. 2015). The most favourable scenario to avoid deoxynivalenol (DON) contamination (ploughing, moderately resistant variety, triazole application at heading) reduced the DON content by 97% compared to the worst one (direct sowing, susceptible variety, no fungicide application) (Blandino et al. 2012). The most successful single measure was tested triazole fungicides, applied at the time of wheat anthesis, which lowered DON content to 53.4 percent than the grain harvested from untreated control plots (Beyer et al. 2006). Phenamacril (2-cyano-3-amino-3-phenylancryic acetate, JS399-19) and tebuconazole were the two most effective fungicides being used. Phenamacril could reduce both the FHB index and mycotoxin level by 80% (Chen and Zhou 2009; Zhang et al. 2010). Phenamacril is a novel cyanoacrylate fungicide introduced by Jiangsu Branch of China National Pesticide Research & Department South Center. This fungicide was demonstrated to have activity against *Fusarium* spp, especially against *F. graminearum*, and had reasonable control of FHB in the field (Li et al. 2008). So far, registered phenamacril fungicidal products including 15% SC and 25% SC in China to control FHB, rice bakanae disease and strawberry fusarium wilt (http://www.chinapesticide.org.cn/hysj/index.jhtml). FRAC has given phenamacril a separate classification code B6 for the mechanism of action and resistance risk in 2015 (FRAC 2020). Tebuconazole has played an essential role in the management of FHB for more than ten years (Mesterhazy et al. 2003; Muellenborn et al. 2008), and its EC_{50} value against *F. graminearum* was 0.41828 mg/L (Lei et al. 2018). However, long-term application has resulted in poor effectiveness against FHB (Chen et al. 2008), and the mechanism of resistance to phenamacril and tebuconazole in *F. graminearum* revealed the risks of continuing applying them. Mutations in myosin-5 conferred phenamacril resistance in *F. graminearum*, and tebuconazole resistance was linked to the fungal sterol 14α-demethylase (CYP51) gene, and a Tyr137 amino acid mutation in the FgCYP51B gene could contribute to tebuconazole resistance (Zheng et al. 2015; Qian et al. 2018).

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Syzygium aromaticum has the highest antifungal effect among 17 medicine plants against 8 tested phytopathogenic fungi, including *Rhizoctonia cerealis*, *F. graminearum*, *Gaeumannomyces graminis*, *F. oxysporum* f. sp. *vasinfectum*, *Valsa mali*, *Colletotrichum gloeosporioides*, *F. oxysporum* f. sp. *cucumebrium* and *Colletotrichum lagenarium* (Yang et al. 2020). Eugenol (4-allyl-2-methoxyphenol) as an active substance in *S. aromaticum* was traced and isolated using sensitive indicator *F. graminearum*, with EC_{50} value of 190.58 mg/L (Yang et al. 2020). Eugenol contains three functional groups: phenolic hydroxyl, allyl, and methoxy. The antifungal activities of eugenol and its analogues against phytopathogenic and human fungi were reported, and their structure–activity relationships were observed that activities were the influence of C4-allyl, C1-OH, and C2-OCH_3, or one/two NO_2 in various positions of the phenyl ring (Carrasco et al. 2012). The activities of a series of phenylpropanoids derived from eugenol against mycelial growth of a virulent and multi-resistant isolate of *Botrytis cinerea* were strongly related to their chemical structures, and increasing activity has been obtained by isomerization of the double bond or introduction of a nitro group on the aromatic ring (Olea et al. 2019).

The purpose of this study was to identify eugenol derivatives with higher toxicity against *F. graminearum* and mix them with phenamacril or tebuconazole. Currently, phenamacril and tebuconazole are the most effective fungicides for controlling FHB in China. The optimal mixtures screened out could be used as potential alternatives to regulate *F. graminearum* resistance against phenamacril or tebuconazole, as well as improve FHB control efficiency.

Materials and methods

Eugenol and its derivatives

Eugenol and its ten derivatives were listed in Table 1.

Table 1 Eugenol and itsstructural derivatives

Compounds	Purity level	Content (%)	Manufacturer
Eugenol	CP	≥98.5	Sinopharm Group Chemical Reagent Co., LTD
Isoeugenol	AR	98	Sinopharm Group Chemical Reagent Co., LTD
4-ethyl-2-methoxyphenol	AR	99	Aladdin Reagent (Shanghai) Co., LTD
2-methoxyphenol	AR	≥99.0	Aladdin Reagent (Shanghai) Co., LTD
2-methoxy-4-vinylphenol	AR	≥98	Aladdin Reagent (Shanghai) Co., LTD
4-bromo-2-methoxyphenol	AR	98	Aladdin Reagent (Shanghai) Co., LTD
2-methoxyl-5-nitrophenol	AR	98	Aladdin Reagent (Shanghai) Co., LTD
Methyleugenol	AR	98	Aladdin Reagent (Shanghai) Co., LTD
Eugenol acetate	AR	98	Aladdin Reagent (Shanghai) Co., LTD
Phenol	AR	99.5	Sinopharm Group Chemical Reagent Co., LTD
2-allylphenol	AR	>98.0	TCI (Shanghai)

CP Chemically Pure, *AR* Analytical Reagent

Fungicides used for mixing

98.5% phenamacril TC, supplied by the Pesticide Laboratory, College of Plant Health and Medicine, Qingdao Agricultural University, China. 97.2% tebuconazole TC, supplied by Wuhu Duowei Agricultural Chemical Co. LTD.

Origin of *F. graminearum*

The tested *F. graminearum* Q1 strain was isolated from the infected wheat plant in 2016, subcultured and grown at potato dextrose agar (PDA) medium in 4°C at the Pesticide Laboratory, College of Plant Health and Medicine, Qingdao Agricultural University, China.

Toxicity determination of compounds

Different doses of each compound dissolved in 100 µL of dimethyl formamide (DMF) were added to 30 mL of sterilized melted PDA medium to obtain desired concentration. The concentration gradients of each compound were set as follows: eugenol (2436, 1218, 609, 305, and 153 µM), isoeugenol (609, 305, 153, 77, and 39 µM), 4-ethyl-2-methoxyphenol(657, 328, 164, 82, and 41 µM), 2-methoxyphenol (6444, 3222, 1611, 805, and 403 µM), 2-methoxy-4-vinylphenol (3996, 2664, 1332, 666, and 333 µM), 4-bromo-2-methoxyphenol (985, 492, 246, 123, and 62 µM), 2-methoxyl-5-nitrophenol (1182, 591, 296, 148, and 74 µM), methyleugenol (2244, 1122, 560, 280, and 140 µM), eugenol acetate (970, 485, 242, 141, and 70 µM), phenol (4255, 2128, 1064, 832, and 416 µM), 2-allylphenol (745, 372, 186, 93, and 47 µM), phenamacril (15.4, 7.7, 3.8, 1.9, and 1.0 µM) and tebuconazole (5.2, 2.6, 1.3, 0.6, and 0.3 µM). These melted PDA were poured into Petri dishes at 10 mL per dish, with three replicates. Parallel control was maintained to mix 100 µL of DMF with PDA medium. At the center of each Petri plate fungal mycelia block

(0.6 cm in diameter) was inoculated, then incubated at 27 ± 2 °C in the dark. All colony diameters were measured when the control colony had almost covered the Petri dishes for 4 days after inoculation. Colony growth inhibition rate was calculated from the formula: Colony growth inhibition rate (%) = [(DC-DT) / (DC-0.6)] × 100, where DC and DT were average diameters of control and treatment colonies, respectively. The concentrations of all tested compounds inhibiting fungal colony growth by 50% (EC_{50}) on *F. graminearum* were calculated using probit analysis.

Mixed ratio screening of the highly active eugenol derivative with fungicides

The highly active eugenol derivative against *F. graminearum* was screened out based on the EC_{50} value. Then, the mixture of a highly active derivative with phenamacril or tebuconazole was further studied to inhibit *F. graminearum*.

The optimum mixing ratio was screened by cross-determination method (Wu and Si 2006). Based on the EC_{50} values of the selected highly active derivative, phenamacril and tebuconazole, 11 concentration gradients of each mixing combination (the highly active derivative and phenamacril or the highly active derivative and tebuconazole) were set, and the volume ratio of two single ingredient solution in each concentration gradient was 10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 and 0:10 (each approximate EC_{50} was 100%, and the mass ratio of in different volume proportions could be calculated), respectively. Antifungal activities were tested as mentioned above. The actual inhibition rate (AIR), theoretical inhibition rate (TIR) and toxicity ratio (TR) were calculated according to the following formula: TIR = AIR of highly active derivative EC_{50} × volume ratio of highly active derivative + AIR of phenamacril or tebuconazole × volume ratio of phenamacril or tebuconazole. TR = AIR/TIR (TR > 1.25, synergistic effect; TR < 0.75, antagonistic effect; TR≈1.00, additive effect) (Wu and Si 2006).

EC_{50} and synergistic coefficient determination of optimum mixtures

Based on TR, the optimum ratio of the highly active eugenol derivative to phenamacril or to tebuconazole was screened out, and its EC_{50} value was determinated and synergistic coefficient (SR) was calculated by Wadley method (SR < 0.5, antagonistic effect; 0.5 ≤ SR ≤ 1.5, additive effect; SR > 1.5, synergistic effect). $SR = EC_{50}(th)/EC_{50}(ob)$, $EC_{50}(th) = (a+b)/(a/EC(A)_{50} + b/EC(B)_{50})$, Here, A and B are the single compound; a and b are the proportions of the corresponding compound in the mixture; $EC_{50}(th)$ is the theoretical EC_{50} value of mixture; and $EC_{50}(ob)$ is the measured EC_{50} value of mixture (Lei et al. 2018).

Statistical analysis

Data were subjected to statistical analysis of variance (ANOVA) using IBM SPSS Statistics Software, ver. 20. Duncan test was used to analyze the significant differences among treatments at $P < 0.05$.

Results

Antifungal activity of eugenol and its derivatives against *F. graminearum*

According to EC_{50}, the toxicities of derivatives against *F. graminearum* were stronger than that of eugenol except for 2-methoxy-4-vinylphenol, phenol and 2-methoxyphenol (Table 2). The toxicity of isoeugenol was about 4.2 times higher than that of eugenol, with an EC_{50} value of 279.7 μM. The EC_{50} values of 2-methoxy-5-nitrophenol, methyleugenol, 4-ethyl-2-methoxyphenol, 4-bromo-2-methoxyphenol, eugenol acetate and 2-allylphenol were 758.9 μM, 539.6 μM, 508.0 μM, 471.0 μM, 467.0 μM and 395.7 μM, respectively, about 1.5 ~ 3.0 times higher than that of eugenol. The EC_{50} value of eugenol was 1163.9 μM, and the EC_{95} value was 9427.0 μM, indicating that the sensitivity of *F. graminearum* to eugenol was far lower than their derivatives. Based on the antifungal activity, isoeugenol was screened out for mixing with phenamacril or tebuconazole for FHB control.

Toxicity of two fungicides against *F. graminearum*

Phenamacril and tebuconazole are the two most effective fungicides used to regulate FHB in China at present, which had potent toxicity in vitro against *F. graminearum*, with EC_{50} values 3.194 μM and 2.749 μM, respectively (Table 3).

Screened mixing ratios of isoeugenol with two fungicides by TR

According to TR, the EC_{50} volume ratios of 3:7, 2:8 and 1:9 of isoeugenol to phenamacril all had additive effects on *F. graminearum*, but the other ratios performed antagonistic effects with TR < 0.75 (Table 4). When the EC_{50} volume ratio was 2:8 (mass ratio: 16:1), it had the maximum TR of 0.94. But the volume ratio of 1:9 (mass ratio of 7:1) had the highest inhibition rate of 53.76%, and the TR was 0.93. There was no significant difference in TR between EC_{50} volume ratio of 2:8 and 1:9. Therefore, the toxicities of both ratios of isoeugenol to phenamacril would be measured simultaneously to determine the final mixing ratio.

Based on TR, all EC_{50} volume ratios of isoeugenol to tebuconazole had additive effects on *F. graminearum*, with

Table 2 Toxicities of eugenol and its structural derivatives against *F. graminearum*

Compounds	Regression equation	(*r*)	EC_{50} /μM	EC_{95} /μM	EC_{50} 95%Confidence Interval/μM
Eugenol	3.241x-4.937	0.973	1163.9	9427.0	995.2–1390.8
Isoeugenol	2.934x-2.179	0.909	279.7	2818.7	163.2–697.9
4-ethyl-2-methoxyphenol	3.622x-4.908	0.871	508.0	3235.3	285.0–4180.3
2-methoxyphenol	0.976x + 2.008	0.960	1165.3	56,535.1	890.5–1611.6
2-methoxy-4-vinylphenol	3.922x-7.526	0.981	1562.0	8797.8	1372.6–1780.9
4-bromo-2-methoxyphenol	5.175x-8.833	0.945	471.0	1745.7	326.7–742.1
2-methoxyl-5-nitrophenol	4.144x-6.936	0.909	758.9	3896.4	452.4–2358.2
Methyleugenol	4.521x-7.353	0.947	539.6	2416.9	301.8–954.6
Eugenol acetate	4.079x-5.888	0.963	467.0	2461.5	345.1–684.0
Phenol	3.828x-6.895	0.937	1240.0	9633.4	417.1–2290.3
2-allylphenol	4.896x-7.716	0.991	395.7	1580.5	351.4–450.5

TR of 1.08 ~ 1.12 (Table 5). All TR had no significant difference, but the volume ratio of 4:6 (mass ratio of 35:1) had the maximum TR of 1.12, and the volume ratio of 1:9 (mass ratio of 6:1) had the highest actual inhibition rate 67.71%. Similarly, the toxicities of both volume ratios of 4:6 and 1:9 of isoeugenol to tebuconazole would be measured simultaneously to determine the final mixing ratio.

Determined optimal mixing ratios of isoeugenol with fungicides

All mixture ratios screened by TR were further tested for toxicity, and SR was calculated. For mixtures of isoeugenol to phenamacril, volume ratio of 2:8 (mass ratio of 16:1) had the EC_{50} value of 61.96 μM and SR of 0.92, and volume ratio of 1:9 (mass ratio of 7:1) had the EC_{50} value of 26.71 μM and SR of 1.10 (Table 6). Therefore, the optimum ratio of isoeugenol to phenamacril against *F. graminearum* was mass ratio of 7:1.

As well, for mixtures of isoeugenol to tebuconazole, volume ratio of 1:9 (mass ratio of 6:1) had the EC_{50} value of 48.74 μM and SR of 0.62, and volume ratio of 4:6 (mass ratio of 35:1) had the EC_{50} value of 228.63 μM and SR of 0.48 (Table 6). So, the optimum ratio of isoeugenol to tebuconazole against *F. graminearum* was mass ratio of 6:1 (Supplementary material).

Discussion

Eugenol isolated from plants, was environmentally safe and easily biodegradable, and generally assumed to be more acceptable and less hazardous (Romanazzi 2012). Regarding eugenol, its concentration that causes no toxicological effects in rats is 500 mg/kg of body weight which is in contrast with 2.5 mg/kg of body weight as estimation of temporary acceptable daily intake for humans (Campaniello et al. 2010). Therefore, eugenol may be an ideal alternative to synthetic fungicides as an eco-friendly pesticide for plant disease control (Zaker 2016). Eugenol has a broad-spectrum of activities against plant pathogenic fungi, especially to *F. graminearum* with the EC_{50} value of 190.58 mg/L (Yang et al. 2020), and its derivatives had significantly different activities against *F. graminearum*.

The antifungal activity of eugenol was attributed to the destruction of membrane structure, which accumulated to the phospholipid bilayer through its lipotropism, interacted to change the fluidity and penetrability of the fungal membrane, and affected the activity of membrane binding enzymes or proteins (Gill and Holley 2006; Braga et al. 2007; Wang et al. 2010; Campaniello et al. 2010). Isoeugenol, as a double-bond isomer of eugenol, had almost no effect on its lipophilicity. However, this small structural modification increased the toxicity on *F. graminearum* by about 3.2 times by conjugation of the side chain of the double bond with aromatic system, and other possible mechanisms must exist. A possible explanation was the enhanced Michael-type reaction between the side propenyl chain and biological nucleophiles (Olea et al. 2019). The increase of 4-ethyl-2-methoxyphenol toxicity may also be consistent with this mechanism. When allyl was replaced by ethyl, the

Table 3 Toxicity of phenamacril and tebuconazole against *F. graminearum*

Fungicids	Regression equation	(*r*)	EC_{50} /μM	EC_{50} 95% Confidence interval/μM
Phenamacril	3.535x + 4.465	0.963	3.194	2.694–3.760
Tebuconazole	1.332x + 4.415	0.978	2.749	1.962–4.536

Table 4 Screening of mixture ratio of isoeugenol to phenamacril against *F. graminearum*

Mixture	EC_{50} volume ratio	Mass ratio	AIR (%)	TIR (%)	TR
Isoeugenol: Phenamacril	10:0	–	44.80 c	–	–
	9:1	590:1	31.18 e	46.23	0.67 c
	8:2	262:1	25.81 f	47.66	0.54 d
	7:3	153:1	26.52 f	49.10	0.54 d
	6:4	98:1	32.62 e	50.53	0.65 c
	5:5	65:1	32.62 e	51.97	0.63 c
	4:6	43:1	36.56 d	53.40	0.68 c
	3:7	28:1	45.53 c	54.84	0.83b
	2:8	16:1	53.05 b	56.27	0.94 a
	1:9	7:1	53.76 b	57.71	0.93a
	0:10	–	59.14 a	–	–

Lower case letters represent significant differences at 0.05 levels

AIR actual inhibition rate, *TIR* theoretical inhibition rate, *TR* toxicity ratio

lipophilicity of side-chain increased, and the destruction of membrane structure was enhanced.

The compound position and orientation in the membrane were determined by the chemical structure of the adsorbed molecule. Compared with eugenol, 2-allylphenol was not conducive to the insertion of phospholipid bilayer into the membrane, but its toxicity to *F. graminearum* was increased by 1.9 times. This perhaps related to the metabolism of more active antifungal compounds from 2-allylphenol. Four hydroxylation metabolism products of 2-allylphenol were isolated and identified from its metabolites of *Rhzoctonia solani* (Qu et al. 2008). Among them, the activity of 2-(2-hydroxypropyl) phenol was about 2 times of matrix structure. Therefore, the inhibition effect of 2-allylphenol on *R. solani* did not decrease with the increase of drug degradation rate between 0 and 144 h (Qu et al. 2008).

Considering that the polarity of nitro compounds is higher than that of related eugenol derivatives, the antifungal activity of these compounds, associated to lipophilic concentration in the membrane, should be lower than that of eugenol. But the toxicity of 4-bromo-2-methoxyphenol and 2-methoxy-5-nitrophenol against *F. graminearum* was 2.5 and 1.5 times than that of eugenol, respectively. Therefore, it was speculated that the nitro group may act directly on the double bond of the membrane as a strong electron-withdrawing group. Pal and Bandyopadhyay (2012) found that compounds containing nitro aromatic ring could produce reactive oxygen species (ROS) by an enzymatic process. Olea et al. (2019) further confirmed that production of ROS might be the primary mechanism, and production of ROS was consistent with activity.

Based on the existing studies, the antifungal effects of eugenol and its derivatives may be two different or parallel mechanisms: accumulation in the fungal membrane by lipophilic interaction and Michael-type reactions between eugenol derivatives and membrane components or ROS

Table 5 Screening of mixture ratio of isoeugenol to tebuconazole against *F. graminearum*

Mixture	EC_{50} volume ratio	Mass ratio	AIR (%)	TIR (%)	TR
Isoeugenol: Tebuconazole	10:0	–	45.48 f	–	–
	9:1	482:1	51.40 e	47.39	1.08ab
	8:2	214:1	54.17 d	49.30	1.10 ab
	7:3	125:1	56.60 c	51.21	1.11ab
	6:4	80:1	58.69 c	53.12	1.10 ab
	5:5	53:1	58.33 c	55.03	1.06b
	4:6	35:1	63.90 b	56.94	1.12 a
	3:7	23:1	65.27 b	58.85	1.11 a
	2:8	13:1	65.63 ab	60.76	1.08ab
	1:9	6:1	67.71 a	62.67	1.08 ab
	0:10	–	64.58 b	–	–

Lower case letters represent significant differences at 0.05 levels

AIR actual inhibition rate, *TIR* theoretical inhibition rate, *TR* toxicity ratio

Table 6 EC_{50} and SR of screened ratios of isoeugenol to phenamacril or tebuconazole against *F. graminearum*

Mixture	EC_{50} volume ratio	Mass ratio	Actual EC_{50}/μM	Theoretical EC_{50}/μM	SR
Isoeugenol: Phenamacril	2:8	16:1	61.96	56.70	0.92
	1:9	7:1	26.71	29.5	1.10
Isoeugenol: Tebuconazole	4:6	35:1	228.63	110.71	0.48
	1:9	6:1	48.74	30.24	0.62

SR synergistic coefficient

production by enzymatic reduction of nitro compounds (Wang et al. 2010; Marchese et al. 2017; Olea et al. 2019). Among all tested eugenol derivatives, isoeugenol had the hightest toxicity to *F. graminearum*, with the EC_{50} value of 279.7 μM. However, the mixtures of isoeugenol and phenamacril or isoeugenol and tebuconazole did not produce synergistic effects. The optimum mass ratios of isoeugenol to phenamacril or tebuconazole against *F. graminearum* were 7:1 (EC_{50} value of 26.71 μM and SR of 1.10) and 6:1 (EC_{50} value of 48.74 μM and SR of 0.62), respectively, and both them were additive effects. Nevertheless, isoeugenol is a natural product (Maurizio et al. 2020), and its mechanism of action was different with that of phenamacril and tebuconazole. Isoeugenol worked by destructing membrane structure and enhancing reaction between the side chain and the biological nucleophile (Olea et al. 2019). Phenamacril acted on cytoskeleton and motor protein, presumed to be a myosin-5 inhibitor. Myosin-5 is an actin-dependent ATPase motor in *F. graminearum*, and the mutations of myosin-5 might lead to the change in the expression of itself or other corresponding genes in the resistant strain (Zheng et al. 2015). Resistance to phenamacril in *F graminearum* had medium to high resistance risk (FRAC 2020). Tebuconazole inhibited sterol biosynthesis in membranes, with medium resistance risk (FRAC 2020). The Y137H mutation in the cytochrome P450 FgCYP51B protein conferred reduced sensitivity to tebuconazole in *F. graminearum* (Qian et al. 2018).

Globally, FHB was the most severe and hazardous floral disease of wheat (Dweba et al. 2017), and accumulated various mycotoxins in the grain (Varga et al. 2015). Chemical fungicides were still one of the most effective means to control it. Benzimidazole fungicides as carbendazol, and triazole fungicides as tebuconazole and phenamacril were mainly adopted, but *F. graminearum* had appeared serious resistance (Liu et al. 2014; Chen and Zhou 2009). Therefore, although the mixture of isoeugenol and phenamacril or tebuconazole only showed an additive effect, it could effectively control the generation and aggravation of resistance due to different mechanisms.

Conclusions

In vitro, the inhibitory activities of eugenol and its ten derivatives against *F. graminearum* were significantly different, and isoeugenol showed the highest toxicity with the EC_{50} value of 279.7 μM, which was about 4.2 times than that of eugenol. The toxicity differences among eugenol and its derivatives may be due to the dose accumulated in the cell membrane by lipophilic effect, and the ability to react with membrane components by nucleophilic reaction or to induce ROS production. The optimum mixing mass ratios of isoeugenol to phenamacril and isoeugenol to tebuconazole against *F. graminearum* were 7:1 and 6:1, respectively.

Based on different fungistatic mechanisms of isoeugenol, phenamacril and tebuconazole, and their additive effects, these mixtures could be used as the potential alternatives for the FHB control.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s42161-021-00875-5.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no conflict of interest.

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