Establishment of baseline sensitivity of *Rhizoctonia solani* to thifluzamide in corn and its field application

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Abstract

In recent years, banded leaf sheath blight in corn has become an important disease in corn that seriously affects quality and yield. This paper aims to evaluate the sensitivity of *Rhizoctonia solani* to thifluzamide in corn, to clarify the effect of seed coating using a thifluzamide suspension agent on safety and physiological indicators and to determine banded leaf sheath blight in corn control effectiveness in the field, thereby providing a basis for the application of thifluzamide suspension agent as a seed coating. In this study, the thifluzamide sensitivity of 102 strains of *R. solani* in corn in different regions of Shandong was determined using the mycelial growth rate method, and the average half-maximal effective concentration value (EC_{50}) was 0.086±0.004 μg/mL. The sensitivity was consistent with a continuous and skewed normal distribution, and the sensitivity distribution frequency exhibited a continuous, unimodal curve, indicating that thifluzamide had strong inhibitory activity on the mycelial growth of *R. solani* in corn. The impacts of using a thifluzamide suspension agent for seed coating on safety and physiological indicators as well as the control effect in corn were evaluated by combining seed coating, an indoor pot test, and a field trial. The root activities under 24 g a.i. 100 kg^{-1} seed and 12 g a.i. 100 kg^{-1} seed were found to increase by 78.01% and 77.40%, respectively, compared with that under the blank control; the chlorophyll content of corn increased most significantly at a dosage of 24 g a.i. 100 kg^{-1}, which was a 32.32% increase compared to the blank control. Thifluzamide (FS) could significantly increase the hundred-grain weight of corn and the per-plot yield. Among the examined dosages, 24 g a.i. 100 kg^{-1} seed had the most significant treatment effect, with the hundred-grain weight increasing by 12.47% and the yield rate increasing by 15.72% compared to the control in 2016, Simultaneously, the hundred-grain weight increasing by 13.44% and the yield rate increasing by 14.11% compared to the control in 2017. Three dosages of 24% thifluzamide (FS) increased the emergence rate and seedling growth of corn to varying extents. The field control effectiveness against banded leaf sheath blight in corn was best at the dosage of 24 g a.i. 100 kg^{-1} seed for seed dressing with thifluzamide (FS); in 2016 and 2017, the control effects in the small bell stage, large bell stage, tasseling and pollen-shedding stage, silking stage, milk-ripening stage, and wax-ripening stage were 100%, 66.73%, 52.8%, 67.81%, 68.48%, and 62.68% (2016), respectively, and 74.97%, 63.17%, 50.90%, 53.60%, 61.42%, and 55.88% (2017). These results indicated that thifluzamide had enormous potential for controlling banded leaf sheath blight in corn.

Introduction

To promote the integrated control of air pollution to construct an ecological civilization in recent years, straw burning has been fully prohibited, while straw returning has been widely promoted in various places throughout China. However, due to improper treatment methods, straw returning has provided habitats for many soil-borne pathogens. As an important cereal crop in the global agricultural economy [1], corn is critical to increasing grain yield, but the incidence of banded leaf sheath blight in corn has been increasing annually, resulting in a decline in the quality and yield of corn and serious economic losses. Currently, farmers have a weak sense of prevention and control of banded leaf sheath blight in corn, and there is little use of control agents. Therefore, the development of safe, efficient agents for the prevention and treatment of this disease is urgently needed.

Rhizoctonia spp [2]. are destructive soilborne pathogens of many crops around the world that can utilize organic residues in the soil during the saprophytic period to survive as an aseptic mycelium (mycelium or sclerotia) [3,5]. Banded leaf sheath blight in corn is a soil-borne disease caused by infection by fungi in the soil habitat [6] such as *Rhizoctonia cerealis*, *Rhizoctonia solani*, and *Rhizoctonia zeae*. *Rhizoctonia solani* is a dominant pathogen in Shandong Province, China [7]. Its sexual stage is *Thanatephorus cucumeris*, and its main floras include AG-1-IA, AG-1-IB, AG-3, AG-5, AG-A, and AG-K [8,9,10]. The isolated strain of AG-1-IA readily causes banded leaf sheath blight in corn [11]. Disease incidence can span from the seedling period to the late growth period and be severe in the event of crop rotation [4,5]. The infection begins at the base of the leaf sheath, and peak damage occurs during the period from tasselling (VT) to grain filling. Initially, leaf sheaths have dark-green hygrophanous spots that gradually develop into cloud-shaped/wavy or irregular lesions from the bottom upward. The lesions are brown with the colour gradually becoming lighter from the outside to the inside; then, the lesions continue to expand and result in rotting of the leaf sheaths. In severe cases, stems become rotted and lodged/broken [12,13], and ears and grains become infested, causing insufficient grain filling, which seriously affects the quality and yield of corn[14].

At present, the methods for preventing and controlling banded leaf sheath blight in corn mainly include agricultural control, biological control, and chemical control, among which agricultural control has a limited effect and is time and labour consuming. Biological control has become an important area of research in plant protection in recent years. Tagele found that KNU17BI1 has great potential to control banded leaf sheath blight in corn caused by *R. solani* AG-1 (IA) [15], but the control effect is not ideal due to the limits of the growth environment. Hence, chemical control is still the most important prevention and control method in agricultural production. A previous study showed that the control effect of 25% triadimefon wettable powder (WP) could reach 44.17% when a 200-fold solution is applied for soil disinfection [16], and the control effect of 20% Jinggang mycin (AF) in fertilizer can exceed 80.1%. In addition, triazole fungicides, such as tebuconazole, have been used. Traditional control methods involve foliar spraying during the corn tasseling stage, which is limited by the height of the corn plants and is time-consuming and labourious. Thifluzamide is a thiazole amide fungicide that has both protection and treatment effects, and it can be used as a foliar spray or for soil treatment and can be quickly absorbed by plants. Thifluzamide is mainly used to prevent and control diseases caused by *Rhizoctonia* spp. of the phylum Basidiomycota [17,18].

Corn seed coating technology has also been widely used in corn planting. Through seed coating, the active ingredients of fungicides/pesticides are slowly released, which can, to some extent, enhance plant resistance and promote plant growth [19,20,21], thus having beneficial effects for corn [22]. In China, thifluzamide has achieved a good control effect as an agent against rice sheath blight. However, this effect has not been registered for corn, and no study on the control of banded leaf sheath blight in corn by seed dressing with thifluzamide has been reported. As a specific control agent of *Rhizoctonia* spp., investigating thifluzamide (FS) for the prevention and control of banded leaf sheath blight in corn is of great value. In this study, the baseline sensitivity of *R. solani* to thifluzamide was established in corn; the safety of thifluzamide coating was evaluated in corn, and the effects of thifluzamide on physiological and biochemical indicators of corn and its control of banded leaf sheath blight in corn were studied through pot and field fungicide tests to provide a basis for the application of a thifluzamide suspension agent for seed coating.

Materials and methods

Test materials

Test strains: In 2016-2017, diseased leaf sheaths, leaves, and stalks subjected to banded leaf sheath blight in corn were collected in 6 regions of Shandong, China: Tai'an (TA), Linyi (LY), Weifang (WF), Laiwu (LW), Rizhao (RZ), and Qingdao (QD). Upon isolation and purification, 102 strains of *R. solani* in corn were obtained. The sampling fields were never exposed to any thifluzamide or other SDHI. The identities of all isolates in the study were confirmed by morphology, phylogenetic analysis and pathogenicity testing. Isolates were kept for long-term storage in cryogenic tubes with 15% glycerol solution at –80°C. The test corn variety in this study was Zhengdan 958, (Henan Goldoctor Seed Co., Ltd., China). Test agents: The thifluzamide (96% TC; Shandong Kangqiao Bio-technology Co., Ltd.); the tebuconazole (94.7% TC; Shandong Weifang Runfeng Chemical Co., Ltd.); the thifluzamide (24% FS; made in the laboratory; Contains the following materials: FS3000, FS7PG, 2%XG, Deionized water, Magnesium aluminium silicate, White carbon black, LXC, D625, EP60P, Film former); and the 60 g/liter tebuconazole (FS) was provided by Bayer CropScience (China) Co., Ltd.

Establishment of baseline sensitivity of *Rhizoctonia solani* to thifluzamide in corn

The mycelial growth rate method was used to determine the susceptibility of each of the 102 strains to thifluzamide, and a baseline sensitivity was established. Thifluzamide was dissolved with acetone and was prepared as a 500-μg/mL stock solution with 0.1% Tween-80 and sterilized deionized water. Using the stock solution for dilution, drug-containing PDA plates with thifluzamide concentrations of 1, 0.5, 0.25, 0.125, and 0.0625 μg/ml were prepared; a PDA plate with the same volume of sterilized water was used as a control. A puncher (5 mm in diameter) was sterilized; Mycelial plugs (5 × 5 mm) were cut from the periphery of 3-day-old colonies of each isolate a mycelia-carrying disc was taken at the edge of the fungal colonies, and the mycelial disc was transferred to a plate with an inoculation needle, with the mycelia facing downward. Four replicates were included for each treatment. Plates were placed in a 25°C biochemical incubator for 4 days, and the colony diameter (minus the original diameter of the inoculation plug) was determined as the average of two perpendicular measurements. Calculate the mycelial growth inhibition rate and a virulence regression equation was established to obtain the half-maximal effective concentration (EC_{50}) value. The experiment was performed twice.

Safety test

The safety test was conducted by referring to "Crop safety evaluation criteria for farm chemicals" and "Indoor test methods for crop safety evaluation of seed treatment agents" NY/T1965.3-2013(People's Republic of China Agricultural Industry Standard), and the experimental setup was as follows: Before seed sowing, fully developed corn seeds of uniform size were selected for disinfection and placed in sterilized river sand (60 to 70 mesh) in germination boxes(ABS material, transparent, 360mm×29mm×12mm in volume) with the moisture content controlled at 60% to 80%. For each treatment, 1 kg of seed was dressed uniformly and air dried. The thifluzamide (24% FS) dosages were set as 192 g a.i. 100 kg^{-1} seed, 96 g a.i. 100 kg^{-1} seed, 48 g a.i. 100 kg^{-1} seed, 24 g a.i. 100 kg^{-1} seed, 12 g a.i. 100 kg^{-1} seed, 6 g a.i. 100 kg^{-1} seed, and a control (CK). Thus, a total of 7 treatments were included with 4 replicates per treatment and 50 seeds per replicate. A label was pasted on the side of each germination box with the sample number, species name, and time. Germination boxes were maintained in a GXZ light incubator (28°C, 14 h of light). On the 7th day after establishment, the germination rate, seedling height, root length, root number, and fresh plant weight were measured, and the germination index and vigour index were calculated. The experiment was performed three times.

$$\text{Germination index (Gi)} = \sum \frac{Gt}{Dt} \quad (1)$$

$$\text{Vigour index (Vi)} = S\sum \frac{Gt}{Dt} = S \times Gi \quad (2)$$

Note: where Gt is the number of germinated seedlings on the T^{th} day; Dt is the corresponding days needed for germination; and S is the fresh weight per plant on the

7th day.

Greenhouse pot test

The greenhouse pot test included a total of 6 treatments: the 24% thifluzamide (FS) dosages of 48 g a.i. 100 kg^{-1} seed, 24 g a.i. 100 kg^{-1} seed, 12 g a.i. 100 kg^{-1} seed, 6 g a.i. 100 kg^{-1} seed, the control agent tebuconazole at a dosage of 12 g a.i. 100 kg^{-1} seed, and CK. The root activity and chlorophyll content of corn were sampled at the 3-leaf stage. The root activity was determined by the TTC reduction method [23], and the chlorophyll concentration was determined by the extraction method of Ming et al [24,25]. The experiment was performed three times.

Field fungicide test

The test site was established in Ningyang County of Tai'an City in field plots where the incidence of sheath blight was severe. The test plots had a total acreage of 1,000 m^2. The soil was loam with uniform fertility, and the irrigation conditions were good. In the 2016 test, seed sowing occurred on June 21, and harvest occurred on September 24; in the 2017 test, seed sowing occurred on June 19, and harvest occurred on September 21. Seeding with mealie socket seeder(Zhengzhou Minle Agricultural Machinery Co., Ltd.), first adjust the sowing depth to 30 mm, insert the tip of the mealie socket seeder into the soil, the seeds fall into the soil, pull out the mealie socket seeder, and level the soil with the foot. Sowing was implemented using the single-seed dibble seeding method with 2 rows per film and plant spacing of 22 cm and row spacing of 45 cm. The dosages of 24% thifluzamide (FS) included 48 g a.i. 100 kg^{-1} seed, 24 g a.i. 100 kg^{-1} seed, and 12 g a.i. 100 kg^{-1} seed; the control fungicide tebuconazole was applied at a dosage of 12 g a.i. 100 kg^{-1} seed; and seed dressing treatments without thifluzamide were taken as a control. Thus, there was a total of 5 treatments in a randomized block design with 3 replicates per treatment, and each plot was 30 m^2. Corn seedlings were evaluated as follows. One week after planting, 5 sites were sampled in each plot, and 30 plants were surveyed at each site. On the 10^{th} day after sowing, 5 sites were sampled in each plot, and 15 plants were excavated to investigate plant height, stem thickness, root length, and the number of fibrous roots. The fresh plants were weighed, and the root-to-crown ratio was calculated. Before the corn was harvested, 5 sites were sampled for each plot, and samples were brought back to the laboratory for investigation, which included ear length, ear thickness, number of rows per ear, number of grains per ear, and the hundred-grain weight. The yield per 667m^2 and yield increase rate were calculated as well. The condition index of banded leaf sheath blight in corn was investigated at the small bell stage, large bell stage, tasseling and pollen-shedding stage, silking stage, milk-ripening stage, and wax-ripening stage. At each plot, 5 sites were diagonally sampled, and 20 plants were surveyed at each site to determine the number of diseased plants and the disease grades. The disease rate, condition index, and control effect were calculated according to Eqs. (6), (7), and (8), respectively. The disease grading was conducted according to the grading standards of the International Maize and Wheat Improvement Center (CIMMYT) (Table 1).

Table 1. Grading standard for corn sheath blight.

Disease grade	Typical value	Grading standard
0	0	No disease incidence in the whole plant

1	1	Disease incidence at sheaths, at and above the 4^{th} sheath below the ear position
2	3	Disease incidence at sheaths, at and above the 3^{rd} sheath below the ear position
3	5	Disease incidence at sheaths, at and above the 2^{nd} sheath below the ear position
4	7	Disease incidence at sheaths, at and above the 1^{st} sheath below the ear position
5	9	Disease incidence at the ear position and at sheaths above the ear position

Data processing

All data were processed using SAS statistical software package (version 9.2; SAS). The EC_{50} values of each isolate were calculated by plotting the relative inhibition against the log10 of the fungicide concentration used. To detect differences between treatments, the means of control efficacy were arcsine transformed, then compared with Fisher's Least Significant Difference test (LSD, $P<0.05$).

Results

Establishment of baseline sensitivity of *Rhizoctonia solani* to thifluzamide in corn

The sensitivity of 102 strains of *R. solani* in corn to thifluzamide was determined using the mycelial growth rate method. It was shown that *R. solani* was highly sensitive to thifluzamide, with an EC_{50} range of 0.0103-0.1942 and an EC_{50} average value of 0.086±0.004 μg/m. The skewness=0.298, kurt=-0.298, and p=0.0884>0.05, which agrees with continuous skewed normal distribution, and the sensitivity frequency distribution had a continuous unimodal curve (Figure 1) and can be used as the baseline sensitivity of *R. solani* in corn to thifluzamide in the Shandong region.

Fig 1. Frequency distributions of 50% effective concentration (EC_{50}) of 102 *R. solani* in corn isolates treated with thifluzamide based on mycelial growth. EC_{50}

values were calculated by performing a regression of the percentage relative growth against the $\log_{10}$ fungicide concentration.

Safety of thifluzamide in corn

Thifluzamide (24% FS) was generally safe for corn, but excessive use (192 g a.i. 100 kg^{-1} seed) had an adverse effect on indicators, including seedling height, root length, and germination rate. When the dosage was 6-96 g a.i. 100 kg^{-1} seed, corn was safe, and the dosage of 12 g a.i. 100 kg^{-1} seed promoted plant height, root length, root number, the root-to-crown ratio, and the germination index. The dosage of 6 g a.i. 100 kg^{-1} seed had the most favourable effect on the seedling emergence rate, plant fresh weight, and vigour index (Table 2).

Table 2. Safety of thifluzamide in corn [a].

Dosage [b] (g a.i. 100 kg^{-1} seed)	Plant height (cm)	Root length (cm)	Root number (piece)	Fresh mass (g)	Germination rate (%)	Shoot ratio (%)	Germination index (%)	Vigour index (%)
192	7.99±1.05d [c]	7.15±0.46c	5.04±0.21a	1.15±0.05d	66.67±1.26f	1.54±0.04d	9.73±0.08f	11.19±0.14g
96	14.76±0.76bc	11.73±0.01a	5.14±0.05a	1.67±0.17b	90.00±1.65d	1.67±0.06c	13.63±0.09d	22.71±0.09e
48	13.49±0.45bc	13.03±0.93a	5.22±0.71a	1.69±0.20b	93.33±1.44c	1.95±0.15ab	14.30±0.07c	24.13±0.20d
24	14.49±0.01bc	13.54±0.65a	5.25±0.32a	1.71±0.17b	95.00±2.10b	1.94±0.04b	14.88±0.02b	25.41±0.08c
12	17.98±0.02a	13.81±0.25a	5.33±0.23a	1.76±0.07ab	98.33±1.04a	2.06±0.05a	15.58±0.01a	27.37±0.07b
6	15.52±0.26b	13.42±0.64a	5.23±0.15a	1.83±0.04a	98.33±1.28a	1.88±0.10b	15.50±0.01a	28.39±0.07a
CK	13.07±0.16c	9.1±0.24b	4.41±0.28b	1.45±0.07c	76.67±1.37e	1.73±0.12c	12.33±0.02e	17.88±0.21f
p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a] The experiments performed in the laboratory in 2016.

[b] "Dosage" means the effective concentration.

[c] Mean values followed by the same letter in the columns were not significantly different according to Fisher's LSD test at P=0.05.

Effects of thifluzamide on root activity and chlorophyll content

Seed dressing with thifluzamide could improve the root activity and increase the chlorophyll content of corn seedlings, among which the dosages of 24 g a.i. 100 kg^{-1} seed and 12 g a.i. 100 kg^{-1} seed had the most significant promotional effect and outperformed the tebuconazole treatment (Figures 2 and 3).

Fig 2. Effect of seed dressing with thifluzamide on the root activity of corn seedlings

Fig 3. Effect of seed dressing with thifluzamide on the chlorophyll content of corn seedlings

Effect of thifluzamide on field emergence of corn

Three dosages of 24% thifluzamide (FS) increased the emergence rate and seedling growth of corn to varying extents. Among them, in 2016 and 2017, the 24 g a.i. 100 kg^{-1} seed dosage had the most favourable effect on the seedling emergence rate, plant height, main root length, fibrous root number, and plant fresh weight. In 2016, The seedling emergence rate was 15.91% higher than the control, and the plant height, main root length, fibrous root number, and plant fresh weight were increased by 4.16 cm, 2.94 cm, 0.87, and 0.64 g, respectively. The dosage of 12 g a.i. 100 kg^{-1} seed had a better promotional effect on stem thickness, which was 0.75 mm higher than that of the control (Table 3). Three doses of thifluzamide (FS) significantly increased the corn root-to-crown ratio, which was obviously better than that under the tebuconazole treatment. Similarly, the 2017 study further validated the 2016 conclusion. 3 dosages of 24% thifluzamide (FS) increased the emergence rate and seedling growth of corn to varying extents (Table 4).

Table 3. Effect of thifluzamide on field emergence of corn (2016) [a].

Fungicide	Dosage (g a.i. 100 kg^{-1} seed)	Emergence rate (%)	Plant height (cm)	Stem thickness (mm)	Main root length (cm)	Fibrous root number (piece)	Fresh weight (g)	Shoot ratio
Thifluzamide (FS) 24%	48	92.67±1.16a	17.01±0.05c	2.93±0.11b	14.45±0.15b	3.47±0.21c	2.11±0.15bc	0.57±0.02ab
	24	95.56±0.84a	18.61±0.08a	3.10±0.12ab	16.20±0.07a	3.89±0.09a	2.53±0.20a	0.61±0.02a
	12	93.78±1.86a	18.06±0.05b	3.16±0.11a	15.58±0.16a	3.76±0.04ab	2.31±0.23ab	0.55±0.02b
Tebuconazole (FS) 60 g/liter	12	95.11±1.65a	17.01±0.13c	2.95±0.03ab	15.39±0.08a	3.56±0.15bc	2.32±0.12ab	0.54±0.02b
CK	-	82.44±1.92b	14.45±0.12d	2.41±0.09c	13.26±0.35c	3.02±0.13d	1.89±0.13c	0.39±0.02c
p-value	-	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0293

Table 4. Effect of thifluzamide on field emergence of corn (2017) [b].

Fungicide	Dosage [c] (g a.i. 100 kg^{-1} seed)	Emergence rate (%)	Plant height (cm)	Stem thickness (mm)	Main root length (cm)	Fibrous root number (piece)	Fresh weight (g)	Shoot ratio
Thifluzamide (FS) 24%	48	99.33±0.33a [d]	16.70±0.21c	2.92±0.04c	14.24±0.00d	3.44±0.17b	2.29±0.08ab	0.58±0.03a
	24	97.33±0.67a	19.08±0.24a	3.17±0.03a	16.13±0.04a	4.38±0.25a	2.69±0.08a	0.62±0.02a
	12	92.67±0.88bc	18.09±0.34ab	3.13±0.03ab	14.79±0.14c	3.78±0.20ab	2.39±0.09ab	0.55±0.01a
Tebuconazole (FS) 60 g/liter	12	98.67±0.33a	17.36±0.54bc	2.99±0.01bc	15.57±0.14b	3.84±0.25ab	2.66±0.09a	0.60±0.01a
CK	-	90.00±0.58c	14.80±0.13d	2.49±0.00d	13.85±0.02d	3.16±0.10b	2.19±0.07b	0.42±0.02b
p-value	-	<0.01	<0.01	<0.01	<0.01	<0.01	0.0123	<0.01

[a] The experiments performed in the field in 2016. [b] The experiments performed in the field in 2017.

[c] "Dosage" means the effective concentration. [d] Mean values followed by the same letter in the columns were not significantly different according to Fisher's LSD test at *P*=0.05.

Effects of thifluzamide on corn yield

Three doses of thifluzamide could increase the ear length, ear thickness, number of rows per ear, and number of grains per ear in the field test of this study. The laboratory seed investigation showed that thifluzamide (FS) could significantly increase the 100-grain weight of corn and the yield per plot. The 24 g a.i. 100 kg^{-1} seed treatment increased the 100-grain weight by 12.47% (2016) and 13.44% (2017) compared with the control, leading to a yield increase of 15.72% (2016) and 14.11% (2017) (Table 5 and 6).

Table 5. Effects of thifluzamide on corn yield(2016) [a].

Fungicide	Dosage (g a.i. 100 kg^{-1} seed)	Ear length (cm)	Ear width (cm)	Row (number/ear)	Ear grain number (grains/ear)	Hundred-grain weight (g)	Plot yield (kg)	Yield increase (%)
Thifluzamide (FS) 24%	48	21.19±0.52b [c]	16.74±0.08ab	15.33±0.05a	506.80±3.95b	31.50±0.01b	24.30±0.29ab	9.18b
	24	22.88±0.30a	17.12±0.06a	15.47±0.38a	516.63±0.68a	32.73±0.25a	25.76±0.64a	15.72a
	12	20.15±0.22b	16.39±0.31bc	15.13±0.31a	508.50±0.56b	31.15±0.46b	24.04±0.14ab	8.00bc
Tebuconazole (FS) 60g/liter	12	20.20±0.21b	17.06±0.21b	15.00±0.34a	507.90±1.70b	31.34±0.10b	23.41±0.27b	5.18c
CK	-	17.77±0.20c	15.78±0.20c	14.73±0.38a	471.73±2.98c	29.10±0.07c	22.26±0.63b	-
p-value	-	<0.01	<0.01	0.7812	<0.01	<0.01	<0.01	<0.01

Table 6. Effects of thifluzamide on corn yield(2017) [b].

Fungicide	Dosage (g a.i. 100 kg^{-1} seed)	Ear length (cm)	Ear width (cm)	Row (number/ear)	Ear grain number (grains/ear)	Hundred-grain weight (g)	Plot yield (kg)	Yield increase (%)
Thifluzamide (FS) 24%	48	20.41±0.60ab	16.58±0.16bc	14.50±0.25a	508.2±0.001b	32.38±0.16b	24.79±0.29abc	7.50c
	24	22.73±0.11a	17.90±0.23a	14.57±0.34a	516.5±0.000a	33.26±0.23a	26.31±0.08a	14.11a
	12	19.87±0.24bc	16.34±0.19c	14.73±0.37a	504.3±0.003b	30.47±0.09c	24.26±0.63bc	5.20d
Tebuconazole (FS) 60g/liter	12	21.17±0.23ab	17.35±0.14ab	14.47±0.07a	509.7±0.002b	32.10±0.13b	25.12±0.37ab	8.93b
CK	-	17.67±1.02c	15.85±0.10c	14.70±0.56a	482.4±0.003c	29.32±0.25d	23.06±0.16c	-
p-value	-	<0.01	<0.01	0.9827	<0.01	<0.01	<0.01	<0.01

a The experiments performed in the field in 2016.
b The experiments performed in the field in 2017.
c Mean values followed by the same letter in the columns were not significantly different according to Fisher's LSD test at P=0.05.

Effects of thifluzamide on the prevention of banded leaf sheath blight in corn in the field

In the field test of this study we found that there were fewer incidences of banded leaf sheath blight in corn from the seedling stage to the large bell stage, during which the control effect was high. The tasseling and pollen-shedding stage was the disease-spreading period, with high temperature and humidity conditions being conducive to the spread of sheath blight, and the maturity stage was the abrupt surge period of the disease. The 2-year field trial showed that 3 doses of thifluzamide (FS) had good control effects on banded leaf sheath blight in corn throughout the entire growth period and significantly reduced the incidence of banded leaf sheath blight in corn during the high-incidence period. Among these, the dosage of 24 g a.i. 100 kg^{-1} seed had the optimal field control effect, and the control effects during the small bell stage, large bell stage, tasseling and pollen-shedding stage, silking stage, milk-ripening stage, and wax-ripening stage were 100%, 66.73%, 52.8%, 67.81%, 68.48%, and 62.68% (2016), respectively, and 74.97%, 63.17%, 50.90%, 53.60%, 61.42%, and 55.88% (2017). Through field observation and data analysis, the disease rate in the plots under the seed dressing with thifluzamide treatment was significantly higher during the period from the late wax-ripening stage to corn harvest than during other stages (Table 7).

Table 7. Effects of thifluzamide on the prevention of corn sheath blight in the field in 2016 and 2017 [a].

Growth period	Fungicide	Dosage (g a.i. 100 kg^{-1} seed)	2016			2017		
			Disease rate (%)	Condition index (%)	Control effect (%)	Disease rate (%)	Condition index (%)	Control effect (%)
Small bell stage	Thifluzamide (FS) 24%	48	1.67±0.43a	0.19±0.14a	66.93±0.33b	8.33±0.18b	0.93±0.06b	37.44±0.56d
		24	0±0.00a	0±0.00a	100±0.00a	3.33±0.43d	0.37±0.14d	74.97±0.32a
		12	0±0.00a	0±0.00a	100±0.00a	5±0.01bc	0.56±0.01bc	62.46±0.23b
	Tebuconazole (FS) 60 g/liter	12	1.67±0.43a	0.19±0.14a	66.93±0.44b	6.67±0.61bc	0.74±0.20bc	49.95±0.34c
	CK	-	5±0.55a	0.56±0.18a	-	13.33±0.27a	1.48±0.08a	-
p-value			0.3640	0.3647	<0.01	0.1640	0.1660	<0.01
Large bell stage	Thifluzamide (FS) 24%	48	6.67±0.18b	0.74±0.59b	55.64±0.20b	11.67±0.15b	1.67±0.12bc	52.65±0.80b
		24	5±0.55b	0.56±1.80b	66.73±0.45a	11.67±0.40b	1.3±0.12c	63.17±0.26a
		12	6.67±0.67b	0.74±2.15b	55.64±0.60b	16.67±0.34ab	1.85±0.10abc	47.39±0.42c
	Tebuconazole (FS) 60 g/ liter	12	8.33±0.33b	0.93±1.04b	44.56±0.36c	26.67±0.11a	2.96±0.03ab	15.82±0.62d
	CK	-	15±0.45a	1.67±0.73a	-	28.33±0.21a	3.52±0.06a	-
p-value			0.4488	0.2450	<0.01	0.0285	0.0298	<0.01
Tasseling and pollen-shedding stage	Thifluzamide (FS) 24%	48	20±0.63b	4.07±0.22b	38.92±0.28b	33.33±0.30bc	6.67±0.21bc	36.87±c
		24	18.33±0.24bc	3.15±0.16bc	52.8±0.33a	26.67±0.20c	5.19±0.93c	50.9±a
		12	25±0.32ab	5±0.28ab	25.04±0.53d	35.00±0.45ab	6.48±0.21bc	38.62±b

	Tebuconazole (FS) 60 g/ liter	12	21.67±0.63b	4.63±0.21b	30.59±0.64c	36.67±0.19ab	7.78±0.69ab	26.35±d
	CK	-	30±0.18a	6.67±0.25a	-	45.00±0.29a	10.56±1.62a	-
p-value			0.4435	0.4279	<0.01	<0.01	<0.01	<0.01
Silking stage	Thifluzamide (FS) 24%	48	36.67±0.26b	9.26±1.28bc	42.52±0.62b	38.33±0.27b	10.93±1.19ab	39.17±0.94c
		24	26.67±0.20c	5.19±0.41c	67.81±0.42a	31.67±0.35c	8.33±0.33c	53.6±0.53a
		12	41.67±0.27ab	9.07±0.18bc	43.67±0.79b	41.67±0.33bc	10.56±0.60bc	41.23±0.64b
	Tebuconazole (FS) 60 g/ liter	12	41.67±0.27ab	12.04±1.29ab	25.28±1.31c	46.67±0.27abc	14.81±2.37ab	17.51±0.57d
	CK	-	55±0.17a	17.04±1.92a	-	51.67±0.11a	17.96±0.76a	-
p-value			0.0121	0.0209	<0.01	0.0654	0.0106	<0.01
Milk-ripeness stage	Thifluzamide (FS) 24%	48	40±0.34bc	9.63±1.27b	43.49±0.60b	60.00±0.2a	13.70±1.06b	41.74±0.35b
		24	31.67±0.65c	5.37±2.59b	68.48±0.23a	48.33±0.26b	9.07±0.09b	61.42±0.43a
		12	41.67±0.67bc	9.44±2.25b	44.57±0.25b	53.33±0.32ab	16.3±0.38b	30.71±0.18c
	Tebuconazole (FS) 60 g/ liter	12	48.33±0.25ab	12.04±1.52ab	29.36±0.30c	56.67±0.10ab	17.78±0.72b	24.41±0.12d
	CK	-	60±0.17a	17.04±0.79a	-	61.67±0.24a	23.52±2.34a	-
p-value			0.0197	0.0377	<0.01	0.4185	0.0151	<0.01
Wax-ripeness stage	Thifluzamide (FS) 24%	48	53.33±2.53ab	13.33±2.19bc	49.3±0.37b	66.00±0.28a	21.48±2.88ab	31.76±0.18d
		24	38.33±1.67d	9.81±1.59c	62.68±0.36a	51.67±0.17b	13.89±1.83c	55.88±0.20a
		12	41.67±1.95c	13.52±0.61bc	48.6±0.14b	53.33±0.18b	21.85±0.65bc	30.58±0.42b
	Tebuconazole (FS) 60 g/ liter	12	50±1.66abc	17.78±1.04b	32.4±0.28c	61.67±0.21ab	24.63±2.15abc	21.76±0.42c
	CK	-	60±0.01a	26.3±0.48a	-	66.67±0.34a	31.48±1.08a	-
p-value			<0.01	<0.01	<0.01	0.0579	0.0542	<0.01

[a] The experiments performed in the field in 2016 and 2017.

[b] "Dosage" means the effective concentration.

[c] Mean values followed by the same letter in the columns were not significantly different according to Fisher's LSD test at *P*=0.05.

Discussion

Being a fungicide of the succinate dehydrogenase inhibitor (SDHI) type, thifluzamide inhibits the synthesis of succinate dehydrogenase [26], thereby preventing pathogens from transmitting electrons in the mitochondria [27], thus inhibiting their growth [28]. Studies have shown that thifluzamide has high inhibitory activity against *R. solani* and can be used as a more effective substitute for boscalid and Jinggang mycin to control sheath blight [29]. Hence, we established the baseline sensitivity of *R. solani* in corn to thifluzamide and found that it was highly sensitive. Of the 55 fungicides listed by the Fungicide Resistance Action Committee (FRAC), the SDHI class is growing at the fastest rate among the new compounds that have been produced and put on the market [26]. As an SDHI fungicide, thifluzamide has high biological activity, but it only has a single action site, so it runs a high risk of drug resistance [30]. A previous study found that the risk of resistance to thifluzamide is moderate in *R. solani*, which can develop resistance to QoI fungicides, and the Fungicide Resistance Action Committee (FRAC) states that the use of this fungicide should be in accordance with the manufacturer's recommended effective dose, with particular attention to adhering to safety intervals [31]. In this study, we did not spray and reduced the number of fungicide applications, and the optimal dosage was determined in the indoor safety test and the greenhouse pot experiment using the seed dressing method. When the thifluzamide dosage (24% FS) was 6-96 g a.i. 100 kg^{-1}

seed, seed coating with this fungicide was safe for corn. The field study found that the seed coating treatment at the dosage of 24 g a.i. 100 kg^{-1} had the highest field control effect on banded leaf sheath blight in corn and could provide a theoretical basis for control using thifluzamide. Thifluzamide has strong adsorption capacity in the soil, but its adsorption intensity is weak, with 19.5%-54.0% digestion in 90 days [32]. In the field test of this study, the disease rate of banded leaf sheath blight in corn at each plot treated with thifluzamide (FS) was found to significantly increase after the late milk-ripening stage, but the control effect was still higher than that of the blank control and the control fungicide. It can be basically guaranteed that thifluzamide would not be applied to corn during the whole growth period.

Currently, the main prevention and control measures for banded leaf sheath blight in corn are chemical. Jiang stated that the control of banded leaf sheath blight in corn should be based on agricultural methods, with seed treatment with chemical agents being the main approach. The study by Xue et al. showed that the control effect of banded leaf sheath blight in corn was significantly different when fungicide application occurred during different growth stages, and the jointing stage was the best period for application [33]. Taking the traditional fungicide Jinggang mycin as an example, although 2 consecutive applications by leaf sheath spraying in the early tasseling stage has a good control effect, the application method is time consuming, labourious, and causes severe air pollution at large dosages that is unsafe for natural enemies, humans, and livestock, which has caused the chemical to be banned in many countries. In addition, spraying is ineffective for controlling soil-borne diseases and has a short duration of effectiveness. Furthermore, multiple applications are required, and the awareness of disease control is weak among farmers. Therefore, it is necessary to develop efficient, safe and time-saving fungicides. In this study, the control effect of thifluzamide suspension (FS) on banded leaf sheath blight in corn in the field was significantly higher than that under seed dressing with the control fungicide tebuconazole. Compared with traditional fungicide agents and fungicide application methods, thifluzamide has the advantages of an increased utilization rate, guaranteeing precise application, reduced application frequency, which saves seeds and fungicide, and reduced production costs, and it has broad prospects for development. In conjunction with the call of the public for environmental protection, biological control has also made great breakthroughs in recent years. Chaurasia et al. isolated antagonized *Bacillus subtilis*, which produces diffusive and volatile compounds that can induce the separation of the tested mycelia and conidia [34], and Stein found that the peptide and non-peptide metabolites produced by *B. subtilis* have antibacterial activities [35]. However, the effectiveness of biological control is greatly affected by environmental conditions, and it is difficult to meet expectations. In a greenhouse test, the effect of biocontrol with *B. subtilis* was lower than that of Jinggang mycin [36]; meanwhile, the control effect of *Trichoderma* spp. against banded leaf sheath blight in corn can reach up to 68.52% [37]. Considering various aspects such as economic benefits and natural environmental conditions, biological control still needs to be developed. Many studies have shown that SDHI fungicides have good health protection effects on plants and can promote crop growth and enhance the ability of crops to tolerate adverse environments. A previous study by Lde and Dubois showed that Benodanil can prevent and control diseases caused by *Rhizoctonia* in a variety of crops and can increase yield [38], and field trials have found that Carboxin can stimulate wheat growth and increase yield [32]. When thifluzamide is applied at 240 g/L, rice leaves become broader, thicker, and greener, and rice stalks exhibit enhanced toughness, which promotes robust growth. Worthing CR et al. found that compound products such as penflufen, Emesto, and EverGol can improve the crop viability, improve resistance in plants, and increase crop quality [39]. Through a greenhouse pot test in this study, the effects of seed coating using a thifluzamide suspension agent on the root activity and chlorophyll content of corn were preliminarily determined, which showed that the fungicide had a significant promotional effect and has further research value.

Supporting Information

S1 Table. Meteorological data sheet during the test (2016)

S2 Table. Meteorological data sheet during the test (2017)

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Author Contributions

Conceived and designed the experiments: DLS XYJ. Performed the experiments: DLS CTY FLH XS SSS. Analysed the data: DLS HLT. Contributed reagents/materials/analysis tools: DLS XDL JWZ. Wrote the paper: DLS.

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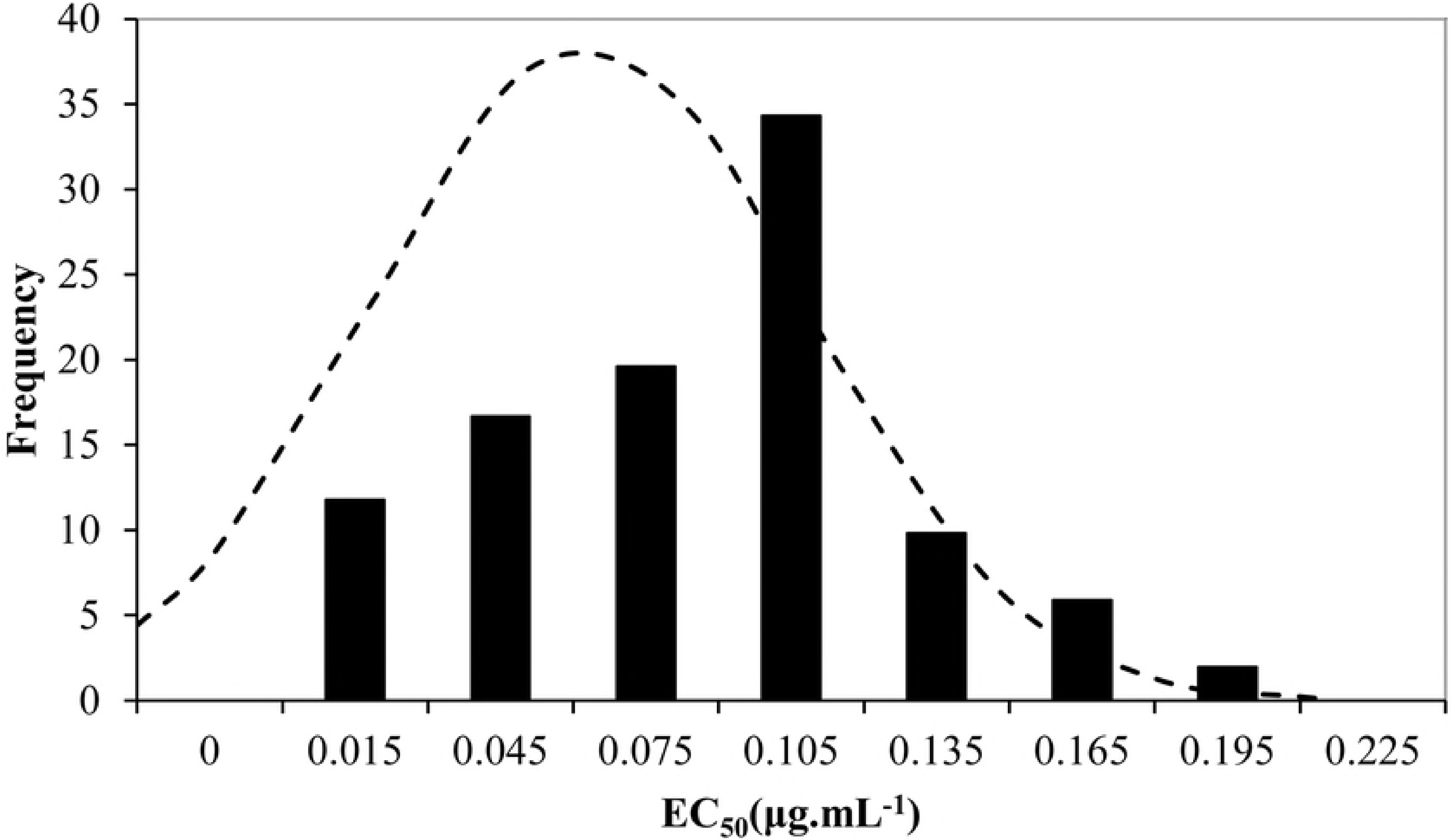

40
35
30
25
20
15
10
5
0
Frequency
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0.075
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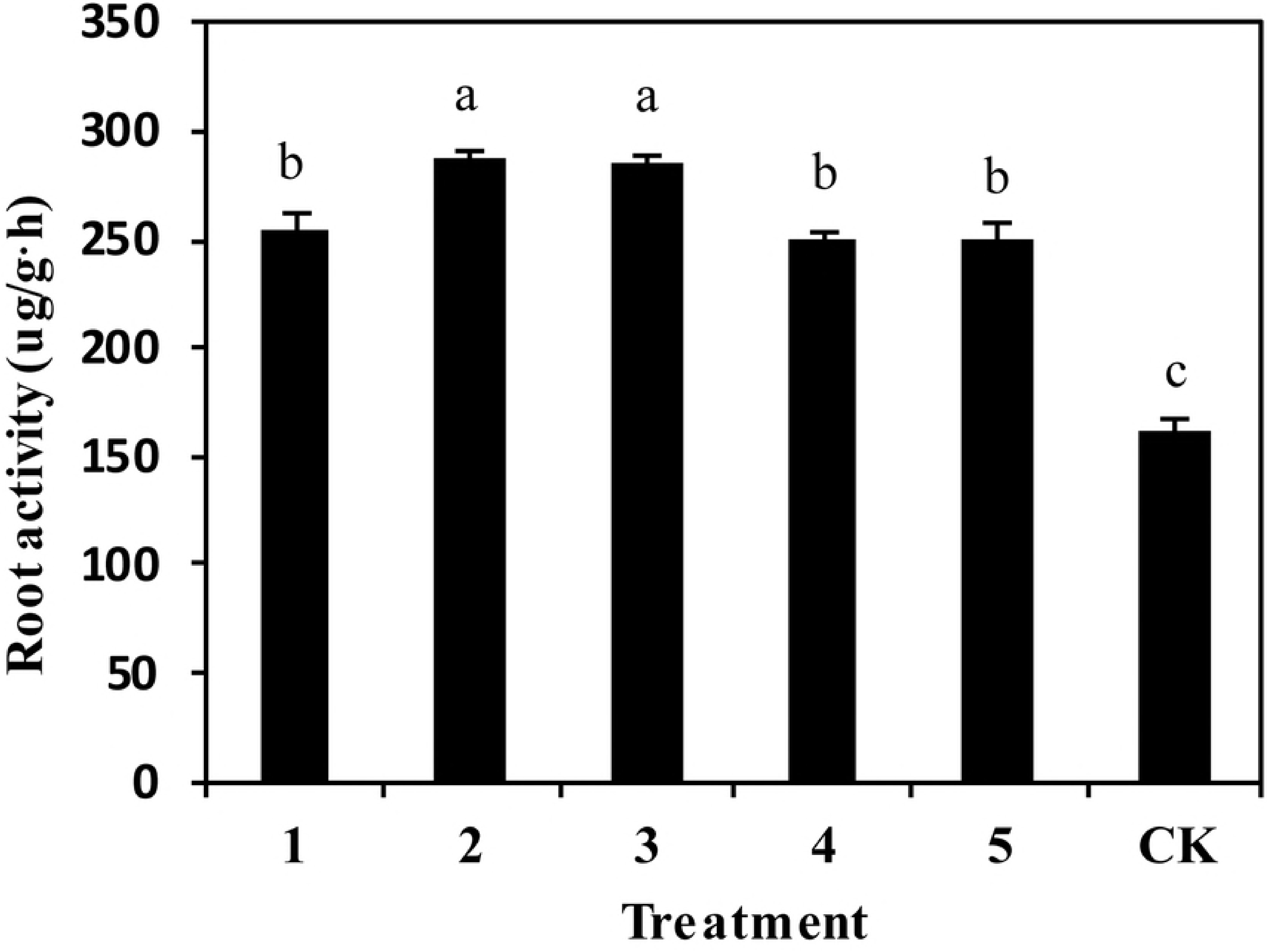
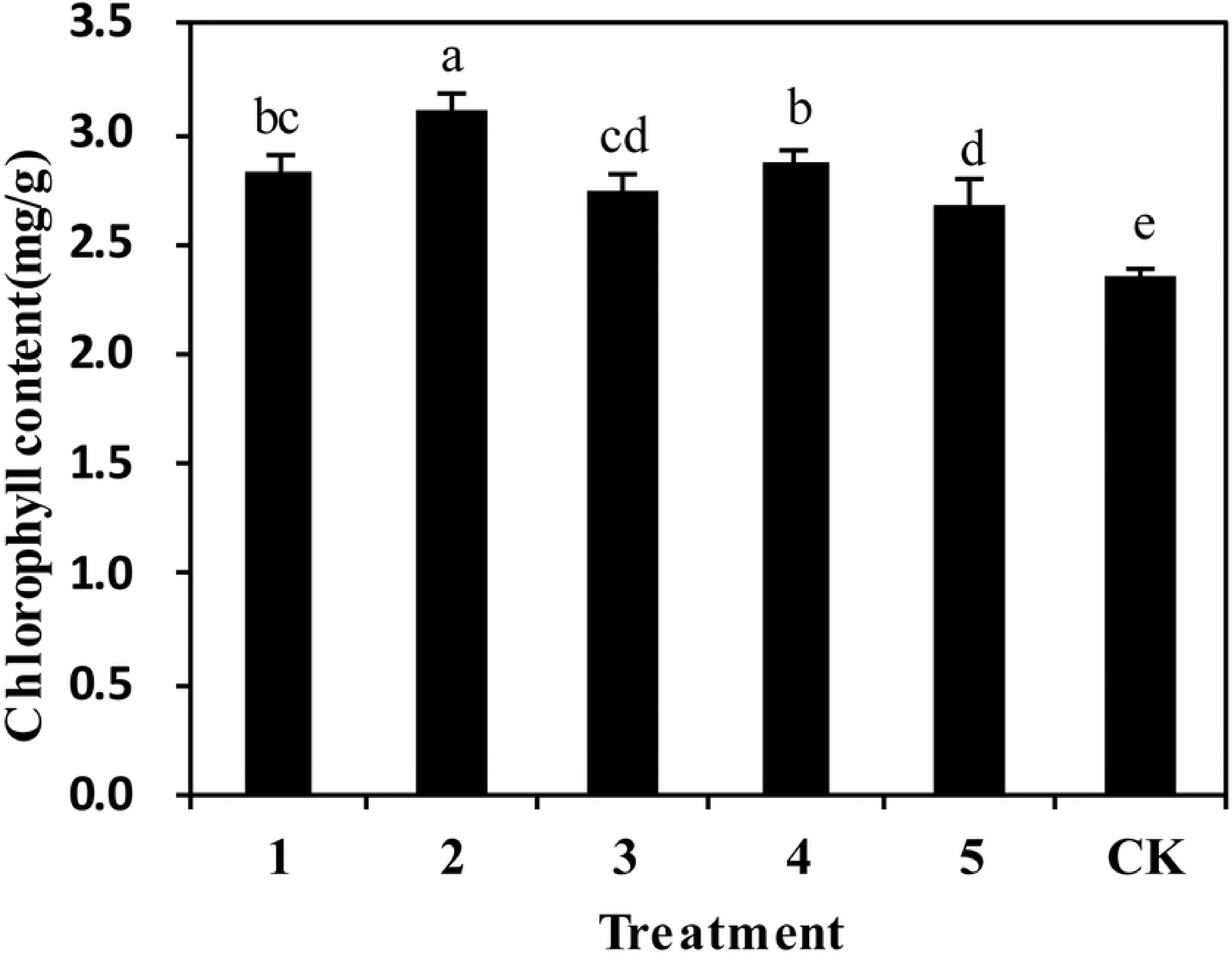
0.135
0.165
0.195
0.225
EC50(µg.mL-1)



Root activity (ug/g·h)
350
300
250
200
150
100
50
0
a
a
b
b
b
c
1
2
3
4
5
CK
Treatment


Chlorophyll content(mg/g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
bc
a
cd
b
d
e
1
2
3
4
5
CK
Treatment