

Fungicidal activity of 12-propoxyimino-1, 15-pentadecanlactam on selected crops[☆]

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ABSTRACT

Fungicide candidate **7B3**, featuring a structure of macrolactam with an oxime side chain, was evaluated through pot culture test and field efficacy trials. The results showed that **7B3** has excellent fungicidal activity against *Rhizoctonia solani* and *Botrytis cinerea*. In the pot culture test **7B3** showed good control against *R. solani* on cucumber and *B. cinerea* on tomato. In two-year four-place field efficacy trials the control of *R. solani* on cotton by **7B3** reached 64–92% at a rate of 140 g ai ha⁻¹ and was better than or comparable to the commercial fungicide, carbendazim at the same dose. In a one-year one-place field efficacy trial the control effect of **7B3** against *B. cinerea* on cucumber reached 95% at a rate of 450 g ai ha⁻¹ and was comparable to the commercial fungicide, huimeike at a rate of 504 g ai ha⁻¹. Study on the dynamic distribution in cotton plants showed that **7B3** has almost no acropetal translocation or systemic activity in cotton plants. The toxicological profile of **7B3** indicated that it is a low toxicological compound and safe to humans, i.e. teratogenesis, mutagenesis and carcinogenesis tests were negative. All of the results suggested that **7B3** may be expected to be further developed as a practical fungicide.

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1. Introduction

In the search for novel pesticides with high activity, high selectivity, are non-polluting, and rapidly degrade, a series of macrolactone and macrolactam derivatives have been designed and synthesized in our laboratory by mimicking the chemistry of a biologically active natural product. Among them, 12-propoxyimino-1,15-pentadecanlactam (**7B3**) (Fig. 1) exhibited a broad spectrum of fungicidal activity and especially excellent fungicidal activity against *Alternaria kikuchiana*, *Phyllosticpa physaleos* Sacc, *Rhizoctonia solani* Kuhn and *Botrytis cinerea* Pers in vitro. The EC₅₀ values were 1.2, 1.9, 4.6 and 8.6 µg mL⁻¹ respectively (Huang et al., 2007).

The diseases caused by *A. kikuchiana* and *P. physaleos* are not common in China. However, *R. solani* is an important agricultural fungus which causes serious decrease in yield especially in major cotton-growing regions in China (Chen et al., 1998; Gong et al.,

2004; Deng et al., 2006), and grey mould caused by *B. cinerea* has become one of the most widely distributed diseases in vegetable (cucumber, tomato, calabash, eggplant, onion etc) and fruit (apple, grape, strawberry etc) growing regions and seriously affects crop production in China (Zhang et al., 2005; Xu et al., 2005; Chen et al., 2006; Wang et al., 2006).

In this paper we report the evaluation results of **7B3** as a fungicide controlling cotton sheath blight (*R. solani*) and vegetable (cucumber and tomato) grey mould (*B. cinerea*) through pot culture and field efficacy trials, its dynamic distribution in cotton plants, and its toxicology.

2. Materials and methods

2.1. Materials

7B3 (12-propoxyimino-1,15-pentadecanlactam, a mixture of *Z* and *E* isomers) was prepared according to the method of Huang et al. (2007), and formulated as either a 28% (w/w) or 25% (w/w) WP (wetttable power) from **7B3** (28% or 25%), NNO (condensed polymer of naphthalenesulfonate and formaldehyde, 10%) and white carbon black (62% or 65%) respectively. Carbendazim 50% WP was purchased from Jiangsu Xinxi Pesticide Factory, Jiangsu Province,

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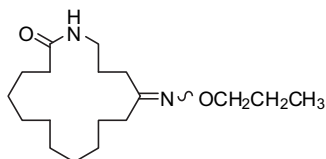


Fig. 1. Chemical structure of 7B3.

China. Thiram 50% WP was purchased from Shijiazhuang Lufeng Chemical industry Co., Ltd., Hebei Province, China. Iprodione 50% WP was purchased from Bayer CropScience (China) Co., Ltd., and huimeike 28% WP [a mixed preparation of diethofencarb and chlorothalonil (1:1.8, w/w)] was provided by the pesticide factory of the Institute of Plant Protection, Shandong Academy of Agricultural Sciences, Shandong Province, China.

grade 3: Brown disease spots on caudex were depressed. Disease spots on 11–25% of the root, 25% of the leaves were yellow and wilting.

grade 5: Dark brown disease spots on caudex now sunken and thinner. Disease spots on 26–50% of the root, 50% of the leaves were faded and dead.

grade 7: Dark brown disease spots around the caudex were softened and lodged. Disease spots on 51–75% of the root, 75% of the leaves were faded and dead.

grade 9: The whole plant was faded or dead.

The disease index was calculated according to the formula as follows:

The control effect was calculated according the following formula based on the disease index and DMRT (Duncan's multiple range tests) was calculated.

$$\text{disease index} = \frac{\sum (\text{diseased plant number of every grade} \times \text{corresponding grade number})}{\text{total plant number investigated} \times \text{the highest grade number}} \times 100$$

$$\text{control effect} = \frac{\text{disease index of untreated control} - \text{disease index of control tested}}{\text{disease index of untreated control}} \times 100$$

R. solani Kuhn and *B. cinerea* Pers for pot culture tests previously collected from cucumber field in Jinan, China, were provided by the Institute of Plant Protection, Shandong Academy of Agricultural Sciences, Shandong Province, China.

2.2. Pot culture test against *R. solani* on cucumber

The experiments were conducted in the green house of the Institute of Plant Protection, Shandong Academy of Agricultural Sciences.

R. solani was cultivated on PDA (potato dextrose agar) plates for 6–7 d at room temperature. Cucumber seeds (cv Shengfeng No. 3) were soaked in a suspension of carbendazim (1 g of carbendazim 50% WP + 500 mL of water) for half an hour, then washed with water and sown in pottery pots (300 mm in diameter) containing 0.6 kg of soil sterilized with formaldehyde (4–5 seeds for each pot). When plants were grown to the five-leaf stage, an aqueous solution of 7B3 28% WP at doses of 168, 315, and 504 g ai ha⁻¹ were used to irrigate the soil for protection control. Thiram was used as the standard of comparison. The soil was inoculated with the *R. solani* along with PDA at 1% weight of the soil 48 h after pesticide irrigation. There were three replicates for each concentration. The state of disease was investigated according to a nine-grade classification, 6 d after inoculation by which time the disease in the untreated control has fully developed and the disease index was calculated.

According to Pesticide-guidelines for field efficacy trials (II) (People's Republic of China, 2004) the classification standard is as follows:

grade 0: No disease spots on the caudex and root.

grade 1: A few brown disease spots on caudex, disease spots on less than 10% of the root, or less than 10% of the leaves on the lower part were yellow.

The disease index and the control effect are shown in Table 1.

2.3. Field efficacy trials against *R. solani* on cotton

Experiments were conducted on Jinan, Shandong Province, and Yuncheng, Shanxi Province, China, based on the Pesticide-guidelines for field trials (II) (People's Republic of China, 2004). Fengkang No. 6 (first year, Shandong Province), Jinmian No. 31 (first year, Shanxi Province), Handan No. 103 (second year, Shandong Province) and Luyan No. 21 (second year, Shanxi Province) were the experimental cultivars.

2.3.1. Cotton seed dressing

7B3 25% WP was used at 0.4% and 0.6% the weight of the seed for seed dressing. Carbendazim 50% WP was used as the standard of comparison. The test fungicides were diluted with water (15–30 mL for 4–6 g of WP), to form a slurry which was mixed with cotton seed. After air drying the seeds were sown.

2.3.2. Experimental

The treatments were arranged randomly in the experimental field. In each piece of land (0.003 ha) 60 holes were dug with five dressed seeds sown in each hole. A total 1200 seeds (280 g)

Table 1
Control effect of 7B3 against *R. solani* on cucumber in pot culture test.^a

Treatment	Dosage, g ai ha ⁻¹	Disease index	Control effect (%)
7B3 28% WP	168	59.88bB	38.99bB
7B3 28% WP	315	45.68bBC	53.46abAB
7B3 28% WP	504	25.92bC	73.59aA
Thiram 50% WP	450	56.79bB	42.14bB
Untreated control		98.15aA	

^a Values followed by the same letter(s) are not significantly different.

were used for each treatment. There were four replicates per treatment.

2.3.3. Control effect investigation

When the disease in the untreated control had fully developed according to the nine-grade classification as in Section 2.2 disease on all treatments was assessed. The disease index and control effect were calculated according to the formulas in Section 2.2, and DMRT (Duncan's multiple range tests) was calculated (Table 2).

$$\text{disease index} = \frac{\sum (\text{diseased leaf number of every grade} \times \text{corresponding grade number})}{\text{total leaf number investigated} \times \text{the highest grade number}} \times 100$$

$$\text{control effect} = \frac{\text{disease index of untreated control} - \text{disease index of control tested}}{\text{disease index of untreated control}} \times 100$$

2.4. Pot culture test against *B. cinerea* on tomato

Experiments were conducted in a green house of the Institute of Plant Protection, Shandong Academy of Agricultural Sciences.

B. cinerea was cultured on PDA plates for 6–7 d at room temperature in the dark to reduce the production of spores. Tomato seeds (cv Shanghai 908) were soaked in a suspension of carbendazim (1 g carbendazim + 500 mL of water) for 30 min, then washed with water. When plants were grown to the seven to eight-leaf stage, an aqueous solution of **7B3** 28% WP at a rate of 168, 315, and 504 g ai ha⁻¹ was used to spray the plants. Huimeike and thiram were used as the standards. The plants were inoculated with a water suspension of spores (2.9 × 10⁶ spores mL⁻¹, 20–30 mL for each pot) 24 h after the application of pesticide. There were three replicates of each. The state of disease was investigated according to a nine-grade classification 5 d after inoculation by which time the disease in the untreated control has fully developed and disease index was calculated.

According to Pesticide-guidelines for the field efficacy trials (I) (People's Republic of China, 2000) the classification standard is as follows:

grade 0: No disease spot on the leaves.

grade 1: Disease spots appeared on less than 5% of the leaves.

grade 3: Disease spots appeared on 6–15% of the leaves.

grade 5: Disease spots appeared on 16–25% of the leaves.

grade 7: Disease spots appeared on 26–50% of the leaves.

grade 9: Disease spots appeared on more than 50% of the leaves.

The disease index was calculated according to the following formula:

The control effect was calculated according the following formula based on the disease index and DMRT was calculated.

The disease index and the control effect are shown in Table 3.

2.5. Efficacy trials against *B. cinerea* on cucumber

Experiments (cv Shandong Caoba cucumber) were conducted in the green house of the Institute of Plant Protection, Shandong Academy of Agricultural Sciences based on the Pesticide-guidelines for the field efficacy trials (I) (People's Republic of China, 2000).

2.5.1. Experiment

The treatments were arranged in 0.0009 ha of land with 60 plants growing on it. There were four replicates per treatment arranged randomly in one green house.

2.5.2. Pesticides' application method

When sporadic disease spots appeared on the lower leaves at the beginning of blossom and bearing, an aqueous solution of **7B3** 25% WP at a rate of 281 and 450 g ai ha⁻¹ was sprayed onto the plants. Huimeike 28% WP was used as the standard. Two applications were made at an interval of 6 d. Disease was assessed 7 d after the last application.

2.5.3. Control effect investigation

Five observation plots were chosen randomly from each piece of land, in each plot three plants were checked and three young cucumbers at the upper, middle and lower parts were checked for

Table 2
Control effect of **7B3** against *R. solani* on cotton in field efficacy trials.^a

Treatment	Dosage		First year		Second year	
	g, WP/ 1 kg seed	g, ai ha ⁻¹	Disease index	Control effect (%)	Disease index	Control effect (%)
Site: Jinan, Shandong Province, China						
7B3 25% WP	4	93.3	3.25bB	88.89abA	18.45bB	65.33bAB
7B3 25% WP	6	140	2.49bB	91.50aA	13.45bB	74.71aA
Carbendazim 50% WP	3	140	5.23bB	82.11bA	22.20bB	58.27bB
Untreated control			29.24aA		53.19aA	
Site: Yuncheng, Shanxi Province, China						
7B3 25% WP	4	93.3	6.10bB	69.21b	5.47aA	61.89A
7B3 25% WP	6	140	5.44bB	72.73bc	4.75aA	64.37A
Carbendazim 50% WP	3	140	5.32bB	73.13c	4.89aA	63.31A
Untreated control			19.8a		13.33bB	

^a Values followed by the same letter(s) are not significantly different.

Table 3
Control effect of **7B3** against *B. cinerea* on tomato.^a

Treatment	Dosage, g ai ha ⁻¹	Disease index	Control effect (%)
7B3 28% WP	168	18.63cC	80.67cC
7B3 28% WP	315	13.50cdCD	85.82bcABC
7B3 28% WP	504	12.04cdeCD	87.51abcABC
Huimeike 28% WP	315	3.24eD	96.64aAB
Iprodione 50% WP	563	9.26cdeCD	90.39abcABC
Untreated control		96.37aA	

^a Values followed by the same letter(s) are not significantly different.

each plant. Disease was assessed following the nine-grade classification (People's Republic of China, 2000):

- grade 0: No disease spot detected.
- grade 1: Disease developed on fruit umbilicus.
- grade 3: The disease spot length was less than 5% length of the fruit.
- grade 5: The disease spot length was 6–10% length of the fruit.
- grade 7: The disease spot length was 11–25% length of the fruit.
- grade 9: The disease spot length was more than 26% length of the fruit.

The disease index was calculated according to the following formula:

$$\text{disease index} = \frac{\sum (\text{diseased fruit number of every grade} \times \text{corresponding grade number})}{\text{total fruit number investigated} \times \text{the highest grade number}} \times 100$$

The control effect was calculated according to the formula in Section 2.4, and DMRT was calculated. The disease index and the control effect are shown in Table 4.

2.6. Dynamic distribution in cotton plants

Plants were selected at the trifoliate stage for study. Each culture bottle contained 20 cotton plants with roots immersed in 100 mL of 500 mg L⁻¹ **7B3** treatment solution. The treated plants were maintained in the growth cabinet and collected after 4, 8, 16, 24, 36, 48 h. The extraction of **7B3** from plants and determination of **7B3** residue in plants was conducted according to the method described in Ref. (Yan et al., 2009).

2.7. Toxicological test

The toxicological test of **7B3** was based on the standard procedure (People's Republic of China, 1995).

3. Results and discussion

3.1. Control effect of **7B3** against *R. solani*

The test fungicide, **7B3** gave good control against *R. solani* on cucumber in the pot culture test (Table 1). The control effect of **7B3** reached 53.46% at a rate of 315 g ai ha⁻¹, and was better than thiram at a rate of 450 g ai ha⁻¹ (42.14%). The control effect of **7B3** reached 73.59% at a rate of 504 g ai ha⁻¹ and is much better than thiram at a rate of 450 g ai ha⁻¹.

In two-year four-place field efficacy trials, the control effect of **7B3** against *R. solani* on cotton reached 64–92% at a rate of 140 g ai ha⁻¹ and was better than or comparable to carbendazim at the same rate (Table 2).

Table 4
Field efficacy trials of **7B3** against *B. cinerea* on cucumber.^a

Treatment	Dosage, g, ai ha ⁻¹	Disease index	Control effect (%)
7B3 25% WP	281	2.59bB	89.51aAb
7B3 25% WP	450	1.20bB	95.14aA
Huimeike 28% WP	504	0.98bB	96.06aA
Untreated control		24.72aA	

^a Values followed by the same letter(s) are not significantly different.

Table 5

Distribution of **7B3** in cotton plants when the chemical applied as root treatment.*

Treatment time (h)	Content of 7B3 in the different parts of cotton plants (μg g ⁻¹)		
	Root	Stem	Leaf
4	54.44 ± 1.64 ^{eE}	0.19 ± 12.30 ^{fF}	0.04 ± 5.52 ^{eD}
8**	66.69 ± 2.81 ^{dD}	0.87 ± 1.91 ^{eE}	0.11 ± 2.52 ^{eD}
16**	96.99 ± 1.29 ^{cC}	2.71 ± 2.08 ^{dD}	0.33 ± 4.87 ^{dD}
24**	98.02 ± 2.08 ^{cC}	4.27 ± 2.93 ^{cC}	0.89 ± 2.48 ^{cC}
36	116.25 ± 2.13 ^{bB}	8.95 ± 6.84 ^{bB}	2.93 ± 6.50 ^{bB}
48	137.05 ± 4.39 ^{aA}	5.12 ± 3.84 ^{aA}	2.53 ± 5.80 ^{aA}

*a–f and A–F show a significant difference at 0.05 and 0.01 level, respectively.

They were used as examples for residue analysis of **7B3 in Ref. (Yan et al., 2009).

3.2. Control effect of **7B3** against *B. cinerea*

7B3 controlled *B. cinerea* on tomato in the pot culture test (Table 3). The control effect of **7B3** reached 85.82% at a rate of 315 g ai ha⁻¹ and 87.51% at a rate of 504 g ai ha⁻¹ respectively, which is not as good as huimeike, but is comparable to iprodione. However, in a one-year one-place field efficacy trial (Table 4) **7B3** displayed excellent fungicidal activity against *B. cinerea* on cucumber. The control effect reached 95.14% at a rate of 450 g ai ha⁻¹, and was comparable to huimeike (control effect was 96.06% at a rate of 504 g ai ha⁻¹).

3.3. Dynamic distribution in cotton plant

The concentration of **7B3** reached 54.44 μg g⁻¹ in roots of cotton plants 4 h after treatment with 500 μg mL⁻¹ of **7B3** (Table 5), which showed that **7B3** can penetrate through the epidermis of the root and be absorbed by cotton plants. The content of **7B3** in roots, stems and leaves all increased with extension of treatment time and the concentration of **7B3** reached 137.05, 5.12 and 2.53 μg g⁻¹ respectively, 48 h after treatment. However, the concentration of **7B3** in roots was almost 27 and 54 times of the concentration in stems and leaves respectively, which revealed that **7B3** has almost no acropetal translocation and systemic activity in cotton plant. The results provide a theoretical basis for an application method of **7B3** for soil treatment or seed coating treatment.

3.4. Toxicological test of **7B3**

The results of toxicological tests indicated that **7B3** was a low toxicological compound (LD₅₀ > 5000 mg kg⁻¹ for acute oral and LD₅₀ > 2000 mg kg⁻¹ for acute dermal) based on classification standard procedure of People's Republic of China. The teratogenesis, mutagenesis and carcinogenesis tests were negative; therefore **7B3** is safe for humans (Table 6).

Table 6
Toxicological profile of **7B3**.

Tested item	Value
Acute oral (rats)	LD ₅₀ > 5000 mg kg ⁻¹
Acute dermal (rats)	LD ₅₀ > 2000 mg kg ⁻¹
Eye irritation (rabbits)	Slight irritation
Skin irritation (rabbits)	Not irritation
Teratogenesis	Negative
Mutagenesis	Negative
Carcinogenesis	Negative

4. Conclusion

7B3 has very weak systemic activity and is suitable for controlling cotton sheath blight with soil treatment or seed coating treatment. The field efficacy trials showed that the control effect of **7B3** against *R. solani* on cotton is better or comparable to carben-dazin and against *B. cinerea* on cucumber is comparable to hui-meike. In addition, **7B3** with low toxicological properties and novel structure may be expected to be further developed as a practical fungicide.

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