DEVELOPMENT OF A NOVEL FUNGICIDE, PENTHIOPYRAD

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ABSTRACT

Penthiopyrad, (*RS*)-*N*-[2-(1,3-dimethylbutyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide is a novel fungicide belonged to the carboxamide group. This compound was discovered by Mitsui Chemicals Agro, Inc., and launched in Japan in 2010 under the trade name of Affet® SC in fruit and vegetable market and in 2009 under the name of Gaia® WDG in turf market. It has been well known that carboxamide type fungicides such as carboxin have an activity against Basidiomycetes pathogen such as wheat bunt and *Rhizoctonia* diseases. On the other hand, penthiopyrad shows a remarkable activity against not only these diseases but also gray mold, powdery mildew and apple scab classified into Ascomycetes or Deuteromycetes. Here, we describe the discovery of penthiopyrad, chemical and biological properties and recent development status of penthiopyrad.

INTRODUCTION

As the first generation compound of carboxamide, carboxin was developed over 40 years ago and has been used as an important seed treatment fungicide. Mepronil and flutolanil, developed in 1980s, have been also used to control some diseases caused by Basidiomycetes such as *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*). Then furametpyr and thifluzamide were developed in late 1990s and launched to control rice sheath blight caused by *R. solani*, but their spectrum were relatively restricted.

On the other hand, there were some precedents suggesting that ortho-substituted carboxanilides could show broader spectrum activity. Edigington reported that some oxathiin compounds showed broader spectrum of fungicidal activity. For example, *N*-(biphenyl-2-yl)-2-methyl-1,4-thioxolane-3-carboxamide (F-427) has fungicidal activity not only on Basidiomycetes but also on Ascomycetes. In 1989, Mitsubishi Kasei Corporation (now Nihon Nohyaku Co., Ltd.) discovered a 2-chloropyridine-3-carboxamide derivative, BC-723 that exhibited fungicidal activity against gray mold caused by Ascomycetes such as *Botrytis cinerea*. And ortho-substituted carboxanilides was of interest as basic structure of broad spectrum fungicide.

OBJECTIVES

The objectives of our research were to discover a novel carboxamide compound that has high and broad fungicidal activity against Basidiomycetes, Ascomycetes and Deuteromycetes. In the course of our research, we paid special attention to the fact that the fungicidal activities of some *N*-biphenyl carboxamide compounds were moderate but their spectra were broad against various kinds of pathogenic fungi. After an extensive research, we found a highly active novel carboxanilide derivative that contained two heteroaromatic rings and also found that branched alkyl substitution on the heteroaromatic ring in the amino part of the carboxanilide expanded its antifungal spectrum. With further research, we finally discovered penthiopyrad that is a unique carboxanilide fungicide candidate containing both pyrazole and thiophene ring.

MATERIALS AND METHODS

1. Preparation of test chemicals and fungal materials
1.1. Chemicals
Tested compounds were synthesized by Mitsui Chemicals, Inc. A stock solution of penthiopyrad (5,000 mg/L) was prepared in acetone and diluted appropriately for each assay. A 40% suspension concentrate formulation of penthiopyrad was also used for in field trial.
1.2. Fungal materials
B. cinerea and *Pyricularia oryzae* for pot test were grown and maintained on a potato dextrose agar (PDA) plate. The plates of *B. cinerea* were illuminated with black light blue lamps (Panasonic FL20S-BL-B) for 3 days to induce spore formation and then spore was collected from plates with a small painting brush. *P. oryzae* was grown on PDA plates to produce conidiospores. *Sphaerotheca cucurbitae* (subcultured in greenhouse) and *Puccinia recondita* (subcultured in growth chamber at 18ºC) for pot test were grown and maintained on leaves of cucumber and wheat respectively and conidiospores and urediniospores were collected from the diseased leaves just before tests.
Alternaria mali, *B. cinerea*, *Botrytis squamosa*, *Cercospora beticola*, *Colletotrichum acutatum*, *Corticium rolfsii*, *Diplocarpon rosae*, *Elsinoe ampelina*, *Fulvia fulva*, *Glomerella cingulata*, *Microdochium nivale*, *Monilinia mali*, *Monilinia fructicola*, *Mycosphaerella melonis*, *Mycovellosiella nattrassii*, *Phomopsis* sp., *P. oryzae*, *R. solani*, *Sclerotinia sclerotiorum*, *Ustilago maydis*, *Venturia inaequalis* for mycelial growth inhibition test were grown and maintained on PDA plates.
Some AG group of *R. solani*, AG1 IA, AG1 IB, AG2-1, AG2-2 IIIB, AG2-2 IV, AG2-3, AG3 and AG5, for mycelial growth inhibition test were grown and maintained on PDA plates.

2. Pot tests
2.1. Preparation of test plants
Soaked in water for two days at room temperature, about 30 rice seeds were sown in 7.5 cm diameter plastic pot filled with soil and grown for 10 to 14 days before the inoculation test. About 20 wheat seeds were sown in 6 cm diameter pot and grown for 10 to 14 days and a cucumber seed and two kidney bean seeds were sown in 7.5 cm diameter pot respectively and grown for 10 to 14 days before the inoculation test. 2.2. Application methods
Fifty mg of penthiopyrad was dissolved in 10 mL of acetone and then diluted with water to adjust the concentration. The compound solution was sprayed to 2-3 leaf stage (LS) rice seedlings at 250, 100, 50 and 25 ppm, 1.5 LS wheat seedlings at 250, 100, 50 and 25 ppm, 1.5 LS cucumber seedlings at 250, 100, 50 and 25 ppm, seed-leaf stage of kidney beans seedlings at 500, 200, 100 and 62.5 ppm, respectively. Sample water volumes were 30 mL/3pots for rice, cucumber, kidney bean and tomato, and 15 mL/3pots for wheat. After air drying, plants were inoculated with spores of pathogens by the following method.
2.3. Inoculation methods
1) Rice blast (*P. oryzae*)

The treated plants were sprayed with spore suspension of *P. oryzae* adjusted at 1 x 10^5 spores/mL and then kept in a chamber that was controlled by 12-hour dark and 12-hour light cycle condition under high humidity at 25 ºC for 7 days.

2) Wheat brown rust (*P. recondita*)

The treated plants were dusted with spores of *P. recondita* and then kept in a plastic bag to maintain dark and high humidity at 4 ºC for two days followed by incubation in greenhouse for 8 days.

3) Cucumber powdery mildew (*S. cucurbitae*)

The treated plants were dusted with spores of *S. cucurbitae* with a painting brush and incubated in greenhouse for 7 days.

4) Kidney bean gray mold (*B. cinerea*)

The treated kidney bean leaves were cut and put in plastic cups in which humidity was maintained with wet paper filter. A paper disk (8 mm diameter, thick type) dipped in the spore suspension of *B. cinerea* ($1x10^5$ spores/mL, containing 20% potato broth and 2% glucose) was placed on the center of each seed-leaf. The plastic cups were kept in dark condition at 20 ºC for four days.

2.4. Assessment methods

The number of lesions and the uredinial colonies on the inoculated leaves were counted in case of rice blast and wheat brown rust, respectively, and the disease index was adapted by following criteria; 0: no symptom, 1: 1-3 lesions, 2: 4-7 lesions, 3: 8-14 lesions 4: more than 14 lesions. In case of cucumber powdery mildew and kidney bean gray mold, disease index was calculated by following criteria; 0: no symptom, 1: less than 12.5%, 2: 12.5-25.0%, 3:25-50% and 4: more than 50% of the leaf area was covered with lesions.

Fungicidal activities of tested compounds were scored by the following index, 0, 1, 2 and 3.

0: A ppm < 95%

1: A ppm ≥ 95% and B ppm < 50%

2: B ppm ≥ 50-95%

3: B ppm ≥ 95%

Kidney bean gray mold A: 500ppm, B: 62.5ppm, Cucumber powdery mildew and wheat brown rust A: 250ppm, B: 25ppm, Rice blast A: 250ppm, B: 50ppm

3. Mycelial growth inhibition tests on agar media

3.1. Application methods

PDA plates containing penthiopyrad at 250, 50, 10, 2 and 0.4 ppm were prepared. Replication was three for each concentration.

3.2. Inoculation methods

A. mali, *B. cinerea*, *B. squamosa*, *C. beticola*, *C. acutatum*, *C. rolfsii*, *D. rosae*, *E. ampelina*, *F. fulva*, *G. cingulata*, *M. nivale*, *M. mali*, *M. fructicola*, *M. melonis*, *M. nattrassii*, *Phomopsis* sp., *P. oryzae*, *R. solani*, *S. sclerotiorum*, *U. maydis*, *V. inaequalis* were previously cultured on PDA plates for 7 days and mycelial discs were cut off from fresh part of the colonies by a 6 mm cork borer. The mycelial discs were put on the PDA plates containing each tested chemical. Controls were incubated on the PDA without any chemicals tested.

3.3. Assessment methods

Incubated 4 days, the colony diameter on agar medium was measured and inhibition rate was calculated by the following formula.

Inhibition rate (%) = ((colony diameter on the control – colony diameter on the treated medium)/colony diameter on the control) x 100
Minimum inhibitory concentration (MIC) values were calculated by the approximation formula of concentration-effect curves against each AG group strain of *R. solani*. The approximation formulas were obtained from the least-square method.

4. Sugar beet field trials in Netherland
Trial protocol was as follows,
Dosage: penthiopyrad 7, 14 and 28 g a.i./SU(seed unit)
Sowing and Inoculation date: June 7, 2011
Inoculation: 5 kg/ha of infected millet was put beside seeds
Assessment: Every week after sowing

RESULTS & DISCUSSION

1. Pot tests
The fungicidal activity of various 1-methyl-3-(trifluoromethyl) pyrazole-4-carboxamides against various diseases was tested by pot tests. A compound that has an 1,1,3-trimethylindane-4-yl group similar to that of the *N*-substituent at the amide group in BC-723, exhibited higher activity against gray mold and wheat brown rust than BC-723. A compound having a *sec*-butyl group on the *N*-phenyl ring at the amide group also exhibited high activity against gray mold and wheat brown rust and higher activity against powdery mildew than a previous compound that has 1,1,3-trimethylindane.

2. Mycelial growth inhibition tests on agar media
Penthiopyrad showed strong inhibition effects on spore germination of various plant pathogens tested compared with the results on mycelial growth inhibition.
Especially, penthiopyrad has higher antifungal activity against spore germination than mycelial growth of *B. cinerea*. In case of the field application, penthiopyrad showed highly preventive control effects rather than curative effects against gray mold. The strong inhibition effect of penthiopyrad on spore germination seems one reason why preventive application brings better performance of gray mold control than by curative application.

3. Sugar beet field trials
Sugar beet field trial was conducted by the following protocol.
Dosage: 7, 14 and 28 g a.i./SU
Sowing and inoculation date: June 7, 2011
Inoculation: 5 kg/ha of infected millet was put beside seeds
We observed the field after 51 days from sowing and could watch the big difference between treated plots with penthiopyrad and untreated plots. There was big void where plants were disappeared by the damage of *Rhizoctonia solani* in untreated plots. There was no difference between the dosages of penthiopyrad.

The results of pot tests on various kinds of diseases showed that penthiopyrad had broad spectrum of fungicidal activity. Especially against wheat brown rust, cucumber powdery mildew and beans gray mold, penthiopyrad was significantly effective even at the low dosage. No phytotoxicity could be observed in all the tested plant at any application dosage. We have evaluated the activity of penthiopyrad against crown and root rot in the sugar beet field trials in USA. Penthiopyrad showed very good efficacy of control crown and root rot caused by *R. solani* at low dosage.

MCAG submitted Kabina® ST as a seed treatment use of penthiopyrad to EPA in September, 2012. We expect that penthiopyrad can contribute to sugar beet production by controlling soil born and seed born diseases in the future.