

Ethaboxam – its Fungicidal Activity as an Oomycetes Fungicide

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Abstract

Ethaboxam is a novel thiazole carboxamide fungicide that controls various diseases caused by Oomycetes, registered in Korea in 1999, and currently in the registration track in European countries. The main target diseases are potato late blight caused by *Phytophthora infestans*, tomato late blight caused by *P. infestans*, Phytophthora blight of red-pepper caused by *P. capsici*, grape downy mildew caused by *Plasmopara viticola*, cucumber downy mildew caused by *Pseudoperonospora cubensis* and lettuce downy mildew caused by *Bremia lactucae*. This fungicide has reasonable preventative, persistent, curative, translaminar and systemic activity against potato late blight. Ethaboxam completely stopped mycelial growth of *P. infestans* and *P. capsici* at the concentrations of 0.5 and 10 mg/liter, respectively, but weakly inhibited germination of zoospores or cysts when observed with microscope. The Giemsa and DAPI staining showed that Ethaboxam inhibited migration of nuclei from cyst to the growing germ tube and mycelia. Ethaboxam appeared to inhibit oxygen consumption by mitochondria prepared from *P. capsici*. These results indicate that Ethaboxam may have multiple biochemical action sites. In the resistance studies, Ethaboxam did not induce any mutants resistant at the concentrations ranging from 2.0 ~ 250.0 mg/liter when tested with eight isolates of *P. infestans* and seven isolates of *P. capsici*. Furthermore, Ethaboxam was effective against several strains of *P. infestans* and *P. capsici* that were resistant to Metalaxyl. These results indicate that the probability to develop mutants resistant to Ethaboxam is low in field conditions, and that strains resistant to the phenylamide can be also controlled by Ethaboxam. Ethaboxam was formulated mainly into WP and SC formulations for foliar application. For potato late blight, it was applied with 7~10 days of the spray interval and 200 g ai/ha of the application rate. Ethaboxam expressed synergistic fungicidal activity when mixed with Mancozeb applied at 800~1400 g ai/ha. All of these results imply that Ethaboxam is a new competitive active ingredient compared to the leading commercial products in the world.

Introduction

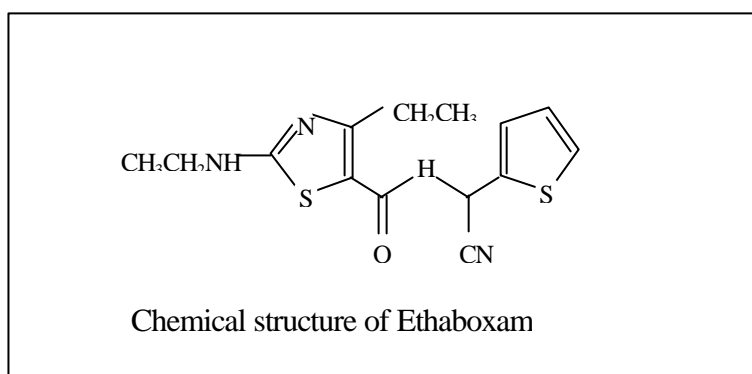
The global total market size of fungicides in 2000 was approximately 6.5 billion US dollars and that of fungicides to control diseases on fruits and vegetables was 2.7 billion US dollars (46%). Approximately one billion US dollars was used to control the diseases caused by

Oomycetes. Two major diseases in this area are potato late blight caused by *P. infestans* and grape downy mildew caused by *P. viticola* throughout the world, especially in Europe.

Ethaboxam is a new Oomycetes fungicide discovered by LGCI (separated from LG Chemical Ltd.), Korea, in 1993 and controls various diseases caused by Oomycetes including potato late blight and grape downy mildew as well as other vegetable diseases. Thus far, Ethaboxam has been tested on grape downy mildew, potato late blight, tomato late blight, cucumber downy mildew, red-pepper blight and lettuce downy mildew in fields. In this workshop, Ethaboxam will be introduced with emphasis on its biological activity against potato late blight including its physicochemical properties, *in vitro* and *in vivo* fungicidal activity, mode of action, resistance and cross resistance.

Physicochemical properties and formulation

Ethaboxam is a novel thiazole carboxamide derivative which is odorless white powder at room temperature. Its water solubility is 12 mg/liter, n-octanol/water partition coefficient (logP) is 2.86 and vapor pressure is 1.5×10^{-7} Pa which is not volatile. Ethaboxam is hydrolytically stable at pH 7 at room temperature; its half-life is 89 days at pH 4.0 and 46 days at pH 9.0 at room temperature. Both Ethaboxam and the formulated product were also stable 2 months after storage at 50 °C when tested with the 25% WP formulation.



Fungicidal activity in the growth chamber tests

Ethaboxam completely inhibited mycelial growth of *Phytophthora infestans* at 0.1~0.5 mg/liter and *P. capsici* at 1.0 mg/liter, respectively. The biological activity of Ethaboxam was evaluated as preventative, persistent, curative, translaminar and systemic fungicide in the growth chamber. In preventative activity, Ethaboxam almost completely suppressed development of grape downy mildew, potato late blight, tomato late blight and red-pepper blight at 50 mg/liter, and its control efficacy for each disease at 10 mg/liter was 100, 60, 94

and 95%, respectively. In persistent activity, its preventative activity 6 days after treatment on tomato late blight was 98% at 250 mg/liter, 94% at 125 mg/liter and 93% at 63 mg/liter. In curative activity, zoospores of *P. infestans* were inoculated 24 h prior to the application of Ethaboxam at 250 mg/liter. The control efficacy of Ethaboxam was 38%, and that of Cymoxanil was 43% under the same condition. Translaminar activity was measured by applying 250 mg/liter of Ethaboxam on either side of leaf surface and inoculating the pathogen on the other side. Its control efficacy was 94% when applied on lower surface and inoculated on upper surface, and 98% when applied on upper surface and inoculated on lower surface. Systemic activity was measured by estimating disease severity on stem and leaves after soil drenching of Ethaboxam at 250 mg/liter, and the control efficacy was 78%. Its systemic activity was further studied using ¹⁴C-labeled Ethaboxam; the label was evenly distributed throughout the seedlings of tomato and red-pepper when treated by soil drenching, however small amount of the label was recovered from the root when treated on leaves or stem. These results indicate that Ethaboxam is preventative, persistent, curative, translaminar and systemic, and each activity may vary depending on the treatments.

Field performance

Ethaboxam formulated has been tested mainly to determine its *in situ* performance at several doses in the fields in Korea and Europe since 1995; 10 trials in 1995, 64 trials in 1996 and 53 trials in 1997. Most field trials were focused on the six diseases; they are grape downy mildew, potato late blight, tomato late blight, cucumber downy mildew, Phytophthora blight of red-pepper and lettuce downy mildew. In the field trials of potato late blight, application of Ethaboxam with 7~10 days interval performed effectively and better in mixture with Mancozeb than as solo. In other crop and disease combinations, application of Ethaboxam with 7~10 days interval performed excellent as solo or in mixture with other fungicides including Mancozeb.

Mode of action

Ethaboxam stopped mycelial growth of *P. infestans* and *P. capsici* when treated zoospores or cysts at 10 mg/liter; however, it did not inhibit their germination at this concentration. When treated at the concentration on growing mycelia, this fungicide caused branched hyphae and then malformed swollen hyphal tips, and the mycelia finally stopped their growth. No leakage of cell contents was observed from mycelial walls. However, migration of nuclei from cyst to hyphal cell was inhibited by Ethaboxam when observed with microscope after the Giemsa staining. In addition to these microscopic observations, effect of Ethaboxam on polymerization of tubulin prepared from calf brain and on succinate dehydrogenase complex

was studied, and Ethaboxam was turned out to have no effect on these target sites in our study system. However, Ethaboxam inhibited oxygen consumption by mitochondria prepared from *P. capsici* in the dose-dependent manner. These observations suggest that Ethaboxam may have novel and more than one action mechanisms. Further studies are required to determine the specific biochemical action sites.

Resistance and cross resistance

New fungicides with novel and multiple mechanisms are preferred in these days for more efficient management of fungicide resistance and cross resistance of the pathogens.

In laboratory, zoospores of *P. infestans* and *P. capsici* were treated with a chemical mutagen MNNG at 30 mg/liter or with UV light at 254 nm to induce mutant strains, and attempted to select any mutants that are resistant to Ethaboxam at concentrations ranging 2.0 ~ 250.0 mg/liter. No resistant mutants have been obtained using this method up to now; however this method efficiently induced mutants resistant to other fungicides including the phenylamide fungicide. This result indicates that Ethaboxam has low probability to develop resistant mutants in field conditions. Using seven strains of *P. capsici* resistant to phenylamide at 100 mg/liter, cross resistance of these strains to Ethaboxam were tested in the laboratory. All of these strains were completely inhibited at 5.0 mg/liter, and this result indicates that there is little probability for cross resistance between Ethaboxam and the phenylamide.

Closing remarks

Ethaboxam is a new excellent fungicide to control the diseases caused by Oomycetes. Phytotoxicity was not observed as solo or in mixtures with other fungicides. Ethaboxam is proved to be competitive to the commercialized leading fungicides in its biological performance throughout the field trials. Furthermore, this fungicide has the preventative, persistent, curative, translaminar and systemic activity. Our studies indicate that Ethaboxam may have novel and multiple action mechanisms. No resistant strains have been obtained by now, and strains resistant to the phenylamide were sensitive to Ethaboxam as the susceptible strains were. However, further studies are required to determine the specific biochemical action sites and to monitor resistance problem in field conditions. In addition, Ethaboxam is relatively safe when tested for mammalian and ecotoxicity. The long-term chronic toxicology studies are scheduled to be completed by the end of year 2001.