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Improvement in Fungicidal Activity of Ethaboxam by a Non-ionic Surfactant, Polyoxyethylene Cetyl Ether

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Ethaboxam is a fungicide controlling plant diseases caused by Oomycetes. Efforts were made to improve its fungicidal activity applying formulation technology. Fungicidal activity of ethaboxam against cucumber downy mildew caused by *Pseudoperonospora cubensis* was improved by incorporating polyoxyethylene cetyl ether (PCE) in a wettable powder formulation. It was found that the optimum combination ratio of PCE and ethaboxam was 3:1, and a tank-mix of 150 μg/ml of ethaboxam and 450 μg/ml of PCE would be as good as the standard 25% WP formulation diluted to 250 μg/ml ethaboxam without PCE in controlling cucumber downy mildew. Based on this results, a wettable powder (WP) co-formulation containing 15% of ethaboxam and 45% of PCE was developed in this study, and tested for its performance in the fields. This co-formulation showed significant improvement in persistence of fungicidal activity and curative efficacy of ethaboxam against cucumber downy mildew. The improved control efficacy was also confirmed for control of grape downy mildew caused by *Plasmopara viticola* and potato late blight caused by *Phytophthora infestans* in the field tests.

Keywords : ethaboxam, surfactant, oomycetes, polyoxyethylene cetyl ether

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Ethaboxam (CAS name: *N*-(cyano-2-thienylmethyl)-4-ethyl-2-(ethylamino)-5-thiazolecarboxamide; CAS registration number: 162650-77-3) is a thiazole carboxamide fungicide effective against plant diseases caused by Oomycetes (Kim et al., 1999). Currently, ethaboxam of 25% WP formulation is registered for cucumber downy mildew, potato late blight and red pepper Phytophthora blight under the trade name 'Guardian®'(Bayer CropScience Ltd., Korea) in Korea (Korea Crop Protection Association, 2005). In the baseline resistance monitoring tests conducted in Korea, the minimum inhibition concentration (MIC) of ethaboxam was lower than 5.0 μg/ml for *P. infestans* (Kim et al., 2002, Zhang et al., 2005). In the growth chamber tests, ethaboxam showed effective control at 32 μg/ml when tested against late blight of tomato and phytophthora blight of red pepper caused by *P. infestans* and *P. capsici*, respectively (Kim et al., 2004). However, the application rate of ethaboxam was registered at 250 μg/ml that is much higher than the MIC value of 5.0 μg/ml or effective dose of 32 μg/ml in the growth chamber tests. Not only for ethaboxam but also for many other pesticides, it is common to apply pesticides at higher rates than their MIC levels or effective doses in the laboratory and growth chamber experiments (Steurbaut, 1993). In the field conditions, significant amount of active ingredients of pesticides can be lost in the process of delivery from application sites to biochemical target sites of target organisms due to precipitation, run-off, wash-off, evaporation, light and metabolic degradation. Hall et al. (1993) reported that 99% of the active ingredient may be released into environment before they provide expected effects.

Efforts have been made to improve delivery of pesticides to target sites and to accelerate their penetration by using various surfactants together with active ingredients (Foy, 1993). However, a specific surfactant is usually not expected to improve biological efficacy of diverse active ingredients and it is necessary to select one for any specific active ingredient (Kirkwood, 1993). Among surfactants, polyoxyalkylene alkyl ethers with alkyl groups having 10-12 carbons have been reported to improve fungicidal activity of metconazole (Grayson et al., 1995; Sampson et al., 1995), and dimethomorph (Grayson et al., 1996a; Grayson et al., 1996b; Yu et al., 2001). For ethaboxam, polyoxyethylene cetyl ether (PCE) having 12 carbons of the alkyl group was found to enhance control efficacy of ethaboxam by LG Life Sciences Ltd., Korea (not published). This study was conducted to determine the optimum combination ratio of ethaboxam and PCE to develop a co-formulation, and confirm the improved fungicidal activity of ethaboxam in the field conditions. We assumed that if ethaboxam was applied with PCE in a form of co-formulation in the field, the amount of ethaboxam could be

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reduced without loss of disease control efficacy and/or the number of sprays could be reduced by extending the spray intervals.

Materials and Methods

Determination of the optimal ratio between ethaboxam and PCE. An optimal ratio between ethaboxam and PCE (Green Soft Chem, Korea) was determined based on control efficacy against cucumber downy mildew. Cucumber cultivar 'Eunkwang-baekdadagi', which is susceptible to cucumber downy mildew caused by *Pseudoperonospora cubensis,* was grown in plastic pots (9 cm diameter, 10 cm deep) using artificial soil (Potground H, Germany) up to the 3-4 leaf stage. Healthy seedlings were transplanted 20 cm apart in 80 cm-spaced rows in a plastic house (3 m wide × 160 m long × 2.5 m high) located at Osan, Gyeonggi-do. Plot size varied among experimental units but always larger than 15 m^2 (=3 m × 5 m), and each plot was spaced with 2 m-wide border of untreated plants. Experimental plots were arranged by randomized complete block design with three replicates. Cucumber plants were grown following the general farmer's practice and exposed to natural infection by *P. cubensis*. Ethaboxam (Guardian®) was diluted with water to reach 125, 150, 175 and 200 μg/ml. For each concentration of ethaboxam, PCE was added at 1:1, 2:1, 3:1 and 4:1 ratios to ethaboxam by weight. No spray check and application of Guardian® which was diluted with water to reach 250 μg/ml ethaboxam without addition of PCE were also included in the experiment. Fungicide applications were made when the cucumber plants reached up to the 10-12 leaf stage and sprayed four times at 7 day intervals onto foliage at water volume of 1,500 l/ha by a electric knapsack sprayer (Apollo Industries Ltd., Korea). At 7 days after the final application, disease severity assessment was made for 2-3 leaves positioned between the 6th and 10th leaves from the top of individual plants. A total of 100 leaves per plot were assessed for disease severity according to the Evaluation Guideline of Registration Trial in Korea (NIAST, 2000). Statistical analysis was performed using the ANOVA procedure of the SAS System for Windows Ver. 8 (SAS Institute Inc., USA). Mean separations were determined using the least significant difference ($P = 0.05$).

Persistent activity of ethaboxam against cucumber downy mildew. Effects of PCE on persistence of ethaboxam efficacy against cucumber downy mildew were determined in a field trial at Osan, Gyeonggi-do. Experimental plots were prepared using cv. Eunkwang-baekdadagi cucumber plants in a farmer's plastic house following the same practice as described above. Four treatments including 150 μg/ml ethaboxam with 450 μg/ml PCE, 250 μg/ml ethaboxam with 750 μg/ml PCE, 250 μg/ml ethaboxam with no PCE, and no spray check were arranged by randomized complete block design with three replicates. For this experiment, a WP co-formulation containing 15% ethaboxam and 45% PCE was made and it was diluted to 150 μg/ml ethaboxam with 450 μg/ml PCE and 250 μg/ml ethaboxam with 750 μg/ml PCE by adding 1.0 g and 1.67 g of the co-formulation to 1 l of water, respectively. The standard 25% WP formulation, Guardian®, was diluted with water to obtain 250 μg/ml ethaboxam with no PCE. All treatments were applied at 5, 7-8 and 10 day intervals starting from the 10-12 leaf stage of cucumber plants. The different spray intervals resulted in different spray numbers of four, five and seven applications, respectively. Fungicide application, disease severity assessment and statistical analysis were conducted using the same methods as described above.

Curative activity of ethaboxam against cucumber downy mildew. This experiment was conducted to examine fungicidal activity of the co-formulation when the fungicide was applied after symptom development on cucumber plants (cv. Eunkwang-baekdadagi) at the 15-18 leaf stage. Four treatments including ethaboxam 150 μg/ml with PCE 450 μg/ml, ethaboxam 250 μg/ml with PCE 750 μg/ml, ethaboxam 250 μg/ml without PCE and no spray control were arranged by randomized complete block design with three replicates. Experimental plots were prepared by the same methods as described above. A total of 100 leaves per plot were assessed for disease severity before the fungicide application and marked with yellow ribbons. Disease severity of the marked leaves was assessed again 5 days after the fungicide application to determine curative activity of each treatment. Treatment means were compared with each other using the least significant differences ($P = 0.05$).

Control efficacy against grape downy mildew and potato late blight. After confirming improvement in persistent and curative activity of the ethaboxam co-formulation with PCE against cucumber downy mildew, the co-formulation was further evaluated for its control efficacy against grape downy mildew and potato late blight. Four treatments including 250 μg/ml ethaboxam with 750 μg/ml PCE, 150 μg/ml ethaboxam with 450 μg/ml PCE and 250 μg/ml ethaboxam with no PCE were tested with no spray control. The experiment for grape downy mildew was conducted at a vineyard located in Cheonan, Gyeonggi-do using eight year old Geobong, a susceptible cultivar to grape downy mildew caused by *Plasmopara viticola.* The experiment plots were arranged by randomized complete block design with three replicates. Each experimental unit consisted of a row of 30 healthy vines and was spaced with one row of untreated vines. Fungicide applications were made four times at 7 day intervals at a water volume of 3,000 l/ha by a motorized knapsack sprayer (MS057, Maruyama, Japan). For potato late blight, cultivar Sumi, which is susceptible to potato late blight caused by *P. infestans*, was planted in an open field located at Hoenggye, Gangwon-do. Experiment plots were arranged by randomized complete block design with three replicates. Each plot consisted of approximately 150 healthy potato plants and was bordered by 1 m-wide of untreated plants. Fungicide applications were made four times at 7 day intervals at a water volume of 1,200 l/ha by an electric knapsack sprayer (Apollo Industries Ltd., Korea). Disease severity of both diseases was assessed based on the Evaluation Guideline of Registration Trial in Korea (NIAST, 2000). Treatment means were compared by the least significant difference ($P = 0.05$) using the SAS System for Windows Ver. 8 (SAS Institute Inc., USA).

Results

Optimal combination ratio between ethaboxam and PCE. Addition of PCE significantly improved fungicidal activities of ethaboxam against cucumber downy mildew regardless of ethaboxam concentrations (Fig. 1). Quadratic regression models were appropriate to describe effects of PCE on ethaboxam efficacy against cucumber downy mildew. For all concentrations of ethaboxam, the models indicated that disease severity decreased as the proportion of PCE increased, and disease severity approached the minimum levels when PCE to ethaboxam ratio was 3:1. No more increase in control efficacy was apparent when the ratio was 4:1 in the tank mix. Depending on ethaboxam concentrations, different combination ratios of PCE in the tank mix were required to reach the control efficacy equivalent to that of the standard WP formulation which was Guardian® diluted to 250 μg/ml ethaboxam without PCE. Application of Guardian® resulted in 5.1% disease severity. The 125 μg/ml ethaboxam mixed with PCE showed no better control efficacy than the standard WP formulation regardless of the PCE concentrations. At 200 μg/ml ethaboxam, however, even 1:1 combination ratio with PCE provided a control efficacy similar to that of the standard WP formulation, and when PCE concentrations increased, 200 μg/ml ethaboxam was better in reducing disease severity than the standard WP formulation. At least 150 μg/ml of ethaboxam with 450 μg/ml of PCE was

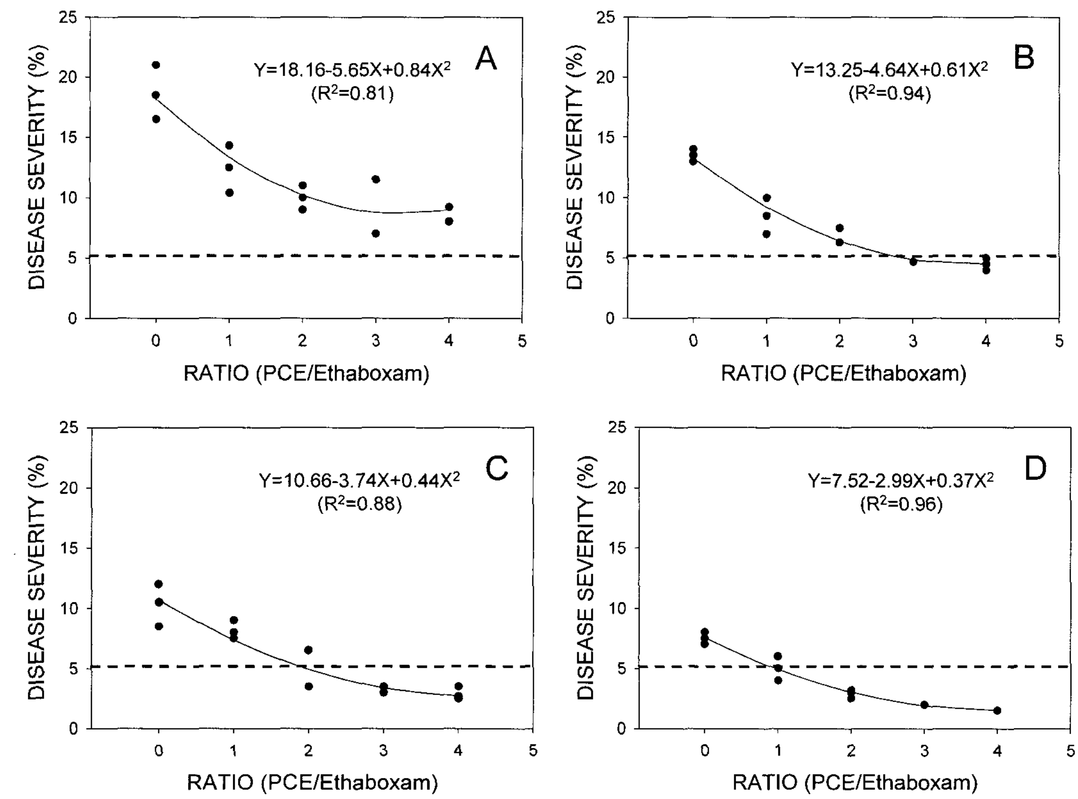

Fig. 1. Effects of PCE:ethaboxam ratios in the WP co-formulation on disease development of cucumber downy mildew. The dotted line indicates disease severity (5.1%) when treated with Guardian®, the standard WP formulation of 250 μg/ml ethaboxam without PCE. (A), 125 μg/ml ethaboxam; (B), 150 μg/ml ethaboxam; (C), 175 μg/ml ethaboxam; and (D), 200 μg/ml ethaboxam. The no spray check resulted in 38.0% disease severity.

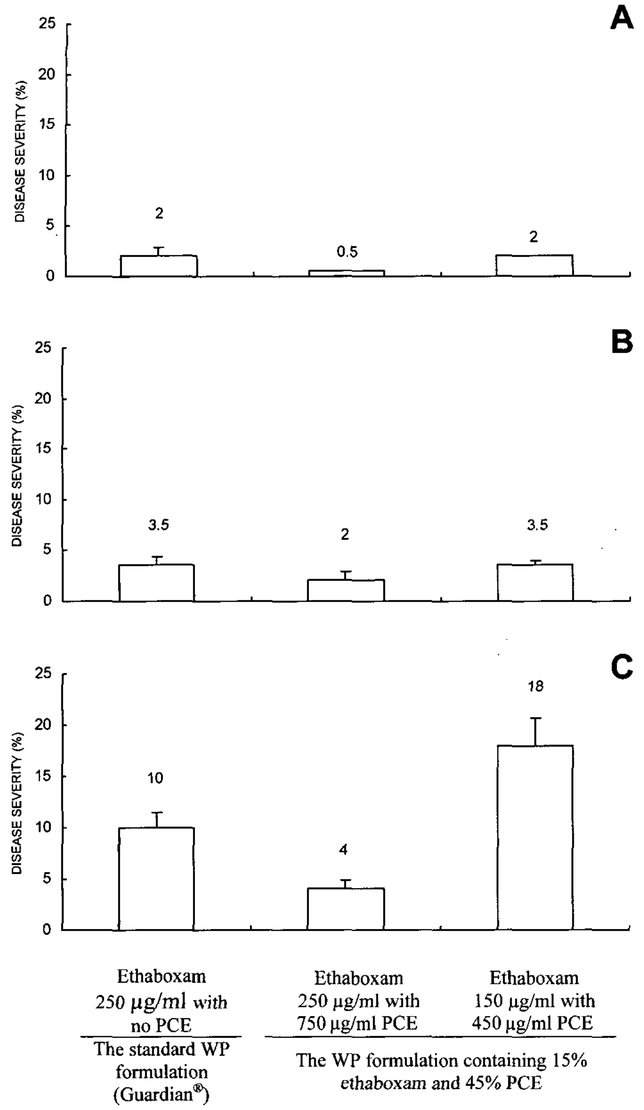

Fig. 2. Effects of ethaboxam concentrations with and without incorporation with PCE on disease development of cucumber downy mildew when sprayed at intervals of 5 days (A), 7-8 days (B), and 10 days (C). The no spray check resulted in 33.3% disease severity.

required to obtain the control efficacy equivalent to that of the standard WP formulation. Based on these results, the optimal ratio of PCE to ethaboxam appeared to be 3:1 by weight for practical applications. A WP co-formulation containing 15% of ethaboxam and 45% of PCE was used for further studies.

Persistent activity of ethaboxam in co-formulation. The co-formulation of PCE and ethaboxam at 3:1 ratio appeared to improve persistence of ethaboxam activity against cucumber downy mildew (Fig. 2). At 250 µg/ml ethaboxam, the co-formulation and the standard WP formulation without PCE showed similar control efficacy when applied at 5 or 7-8 day intervals. However, a substantial difference in control efficacy between the co-formulation and the standard WP formulation was observed when applied at 10 day intervals. This result suggested that control efficacy of ethaboxam would become more persistent by applying the co-formulation instead of the standard WP formulation. At 150 µg/ml ethaboxam, the co-formulation showed the control efficacy equivalent to that of the standard WP formulation with 250 µg/ml ethaboxam when sprayed at 5 or 7-8 day intervals. However, the co-formulation of 150 µg/ml ethaboxam with PCE did not suppress disease development as much as the standard WP formulation when applied at 10 day intervals.

Curative efficacy of ethaboxam in co-formulation. The co-formulation also significantly improved curative efficacy of ethaboxam against cucumber downy mildew (Table 1). No disease progress was observed at 5 days after the application of the co-formulation treatment of 250 µg/ml ethaboxam. Even at 150 µg/ml of ethaboxam, the co-formulation suppressed disease development significantly, and resulted in lower disease development compared to the standard WP formulation. Furthermore, the symptoms of cucumber downy mildew which developed on the back side of leaves were turned into a brown color indicating

Table 1. Curative efficacy of ethaboxam against cucumber downy mildew

Treatments	Disease severity (%) at 0 DAA[c]	Disease severity (%) at 5 DAA	Disease increase[d] (%)
[a]Ethaboxam 250 µg/ml + no PCE	7.0 ± 1.0 ab[e]	28.3 ± 2.5 b	304.3 b
[b]Ethaboxam 250 µg/ml + PCE 750 µg/ml	7.0 ± 1.7 ab	7.0 ± 0.6 c	0 c
[b]Ethaboxam 150 µg/ml + PCE 450 µg/ml	6.0 ± 1.7 b	6.7 ± 1.5 c	11.7 c
Untreated	9.0 ± 1.7 a	56.7 ± 1.0 a	530.0 a

[a]Commercial standard of ethaboxam formulated into 25% WP (Guardian®).
[b]Co-formulation containing 15% of ethaboxam and 45% of PCE.
[c]Days after application.
[d]Increase of disease severity was obtained by a formula $I = (A_5 - A_0)/A_0 \times 100$, where I = % disease increase, A_0 = disease severity at 0 DAA, and A_5 = disease severity at 5 DAA.
[e]Means in each column with the same letter are not significantly different by LSD at $P = 0.05$.

Table 2. Fungicidal activity of ethaboxam against grape downy mildew and potato late blight

Treatments	Disease severity[c](%)	
	Grape downy mildew	Potato late blight
[a]Ethaboxam 250 μg/ml + no PCE	14.3 ± 1.2 b[d]	1.7 ± 0.3 b
[b]Ethaboxam 250 μg/ml + PCE 750 μg/ml	5.5 ± 1.3 d	0.5 ± 0.0 c
[b]Ethaboxam 150 μg/ml + PCE 450 μg/ml	11.0 ± 2.0 c	1.3 ± 0.3 b
Untreated	58.8 ± 5.8 a	18.3 ± 2.9 a

[a]Commercial reference of ethaboxam formulated into 25% WP (Guardian®).
[b]Co-formulation containing 15% of ethaboxam and 45% of PCE.
[c]Disease severity was assessed 7 days after the final application.
[d]Means for disease severity in each column with the same letter are not significantly different by LSD at $P = 0.05$.

inactivation of the downy mildew pathogen by the co-formulation treatment.

Fungicidal efficacy of the co-formulation against grape downy mildew and potato late blight. Improvement in fungicidal activity of ethaboxam by applying the co-formulation with PCE was confirmed in the field experiments against grape downy mildew and potato late blight (Table 2). Disease severity of both grape downy mildew and potato late blight was significantly lower when 250 μg/ml ethaboxam was applied in co-formulation with PCE than when applied without PCE. Especially, application of ethaboxam 150 μg/ml with PCE 450 μg/ml resulted in significant increase in control efficacy against grape downy mildew as compared with application of ethaboxam 250 μg/ml without PCE. In the case of potato late blight, 150 μg/ml ethaboxam with 450 μg/ml PCE showed almost the same control efficacy as 250 μg/ml ethaboxam with no PCE.

Discussion

It was found in this study that ethaboxam can be improved for its fungicidal activity by incorporating a surfactant in the WP co-formulation. PCE itself did not have any control efficacy against cucumber downy mildew in a separate experiment (not published). However, control efficacy of ethaboxam was improved by incorporating PCE against cucumber downy mildew, potato late blight and grape downy mildew, suggesting that PCE probably helped ethaboxam exhibit its intrinsic fungicidal activity more effectively. Improvement of fungicidal activity of fungicides by incorporating polyoxylalkylene alkyl ethers as tank-mix has been reported. For example, when mixed with polyoxylakylene alkyl ethers, only two third of metconazole of the original application rate was enough to control cereal diseases effectively (Grayson et al., 1995, Sampson et al., 1995). Similar results were achieved in the case of dimethomorph when applied with polyoxylalkylene alkyl ethers to control cucumber downy mildew (Yu et al., 2001) and potato late blight (Grayson et al., 1996a). Foliar uptake of dimethomorph by cucumber plants was found to be increased when dimethomorph was applied with polyoxylalkylene alkyl ethers (Yu et al., 2001). The results in the present study confirmed that fungicidal activity of ethaboxam increased effectively when PCE was incorporated as a co-formulation, and the amount of ethaboxam in the co-formulation could be reduced to 60% of the standard WP formulation without loss of control efficacy against cucumber downy mildew, grape downy mildew, and potato late blight.

Grayson et al. (1996b) reported that the optimal combination ratios of a non-ionic surfactant to dimethomorph were 9:1 and 6:1 to control grape downy mildew effectively in the glasshouse and in the field, respectively. In the case of the co-formulation of ethaboxam and PCE, we found that the optimum combination ratio of PCE to ethaboxam was 3:1 by weight. The co-formulation of PCE and ethaboxam would be more flexible in the development process than the case of co-formulation of dimethomorph with a non-ionic surfactant because less amount of surfactant is required in the co-formulation of PCE and ethaboxam than the case of dimethomorph. Kirkwood (1999) reported that the combination ratio of surfactant to pesticide was more important than the total content of surfactant in the solution to maximize pesticide efficiency.

The increase in persistence of disease control efficacy when applied the co-formulation of ethaboxam and PCE could provide advantages not only economically for farmers but also ecologically for environmental safety. Farmers can reduce the number of sprays without loosing control efficacy by applying the co-formulation of ethaboxam and PCE at a longer time intervals. Also, it would be possible to achieve a certain level of control efficacy with a less amount of the active ingredient than that in the standard WP formulation of ethaboxam. For example, the co-formulation of ethaboxam 150 μg/ml and 450 μg/ml PCE at 1:3 ratio provided a same level of disease control efficacy as the standard WP formulation of 250 μg/ml ethaboxam when sprayed at 5 or 7-8 day intervals.

Curative efficacy of ethaboxam against cucumber downy mildew was improved by applying the co-formulation of ethaboxam and PCE. Incorporation of PCE in the co-formulation may have facilitated spread of ethaboxam on the surface of cucumber plants and infiltration of ethaboxam into plant tissues, resulting in improvement of both preventive and curative efficacy to control cucumber downy mildew. Enhancement of control efficacy of ethaboxam by applying the co-formulation of PCE and ethaboxam at 3:1 ratio was also proved in the case of grape downy mildew and potato late blight.

In conclusion, we found that PCE was a suitable surfactant to improve control efficacy of ethaboxam against cucumber downy mildew, grape downy mildew, and potato late blight. The optimal combination ratio of PCE to ethaboxam was 3:1 and a 15% WP co-formulation diluted to 250 μg/ml ethaboxam and 750 μg/ml PCE provided significantly and consistently better disease control efficacy than the standard 25% WP formulation diluted to 250 μg/ml ethaboxam without PCE. It may be possible to reduce the amount of ethaboxam from 250 μg/ml to 150 μg/ml without losing significant control efficacy if the co-formulation of PCE and ethaboxam at the 3:1 combination ratio is applied at the intervals of shorter than 7-8 days. The results indicated that other ethaboxam formulations such as water dispersible granule or suspension concentrate may also show improved control efficacy for the Oomycetes diseases of other crops not included in this study. Further studies are needed with respect to different formulations of ethaboxam.

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