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Efficacy of Fluazinam and Iprodione+Propineb in the Suppression of *Diaporthe phaseolorum, Colletotrichum truncatum* and *Cercospora kikuchii*, the Causal Agents of Seed Decay in Soybean

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Seed decay of soybean caused by *Diaporthe phaseolorum, Colletotrichum truncatum,* and *Cercospora kikuchii* is a serious disease when soybean is harvested under warm and wet weather conditions. Benomyl has been used for controlling the disease, however, benomyl application may be limited due to common occurrence of resistance. The efficacy of 21 fungicides against the pathogens was evaluated *in vitro*. Among the fungicides tested, benomyl, carbendazim, fluazinam, iprodione+propineb, thiophanate-methyl, and triflumizole were found effective and were evaluated for their ability to control the seed pathogens. Fluazinam completely inhibited mycelial growth at a concentration of 100 μg/ml for *D. phaseolorum*; and at a concentration of 500 μg/ml for *C. truncatum* and *C. kikuchii*. EC_{90} values of fluazinam were similar to that of benomyl. Because fluazinam, iprodione+propineb, and triflumizole were found effective against the seed pathogens, these were subjected for field-testing. Suppression of pod and seed infection by fluazinam and iprodione+propineb was as high as that of benomyl without any reduction in agronomic characters of soybean. This study shows that fluazinam and iprodione+propineb may be used in combination with benomyl to control seed pathogens, manage resistance, and ensure production of high quality soybean seeds.

Keywords **:** benomyl, fluazinam, iprodione+propineb, seed pathogens, soybean.

Soybean has been grown for food and animal feeds for thousands of years in Korea. As soybean acreage expands throughout the world, soybean diseases have increased in number and severity. More than a hundred pathogens are known to affect soybeans (Sinclair and Backman, 1989), some of which are associated with soybean seed infection. Of major economic importance are *Diaporthe phaseolorum, Colletotrichum truncatum,* and *Cercospora kikuchii*. These pathogens generally occur as mixed infection and produce poor seed quality, which lead to lower seed viability and yield reduction. Seed decay associated with the pathogens is more serious when soybean matures in warm, wet weather and when harvesting is delayed. Soybean plants are susceptible to the pathogens at all stages of development, particularly from bloom to pod fill during warm, moist weather. Yield reductions by *D. phaseolorum* and *C. truncatum* sometimes reach as much as 89.9% (Lee and Park, 1994) and 57.6% (Oh and Kim, 2001) in Korea, respectively. Purple blotch caused by *C. kikuchii* does not reduce yield directly, but heavily infected seeds produce diseased seedlings and reduced stands. Sometimes stained seeds reach more than 50% in the country (Oh and Kwon, 1981).

To manage soybean seed pathogens, fungicides are important. Primarily benomyl has been used to reduce losses attributed to seed pathogens (Ellis and Sinclair, 1975; Jeffers et al., 1982; TeKrony and Egli, 1985). Unfortunately, because of benomyl's specific mode of action, there is a tendency for the plants to develop resistance to this fungicide. Resistance to benomyl was already widespread based on the reduced efficacy and control failures that occurred in several fungicide efficacy experiments (Lapeyre and Duvoic, 1997; McGrath, 2001; Romero and Sutton, 1998; Yourman and Jeffers, 1999). Hence, it is necessary to search for new chemicals, which can substitute for or can be used alternately with benomyl without the risk of developing resistance. Some fungicides may be equally effective against soybean seed pathogens as benomyl, but such efficacy has yet to be identified.

The objective of this study was to evaluate the impact of carbendazim, fluazinam, iprodione+propineb, thiophanate-methyl, and triflumizole on the fungal growth *in vitro* and on the disease development in the field of soybean seed pathogens, *D. phaseolorum, C. truncatum,* and *C. kikuchii*.

Materials and Methods

Fungi and fungicides. Isolates of *D. phaseolorum, C. truncatum,*

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and *C. kikuchii* were used in this investigation. The isolates of *D. phaseolorum* and *C. kikuchii* were recovered from infected soybean seed, whereas, that of *C. truncatum* was isolated from infected soybean stem in a commercial field. Twenty-one fungicides registered for different crops in Korea were used for the fungicide screening test. For this study, the following fungicides were used: benomyl (benomyl 50% WP), carbendazim (garbenda 60% WP), fluazinam (fluazinam 50% WP), iprodione +propineb (iprodione/propineb 50+20% WP), thiophanate-methyl (thiophan 70% WP), and triflumizole (rifzol 30% WP).

Inhibition of pathogens in culture. The controlling activity of 21 different fungicides was determined against the mycelial growth of *D. phaseolorum, C. truncatum,* and *C. kikuchii in vitro.* Fungicides were added to PDA after autoclaving when the agar had cooled to about 55°C. Solutions of each fungicide were mixed with melted PDA media to give recommended concentrations. A mycelial disk (ϕ5 mm) from the hyphal tips of the pathogen incubated for 5 days at 25°C was inoculated in the center of the fungicide-amended PDA. After incubation for 5 days at 25°C, the colony diameters of each pathogen were measured in two perpendicular directions. There were three replicates for each compound and the test was performed twice. Five fungicides, those highly effective on three pathogens, were selected for further tests.

For the inhibitory effects of five fungicides on mycelial growth, mycelial agar disks (5 mm in diameter) were inoculated on PDA amended each with fungicide at 0.1, 1.0, 10, 100, 500, and 1,000 μg/ml using the same method described above. After incubation for 7 days at 25°C in the dark, the colony diameter of each plate was measured. The inhibition ratio (%) of mycelial growth was determined as follows: inhibition ratio (%)=(1-colony diameter on treated media/colony diameter on untreated control media) × 100. Five replicate plates of each fungicide concentration as well as the control were prepared for each pathogen. The effective dose causing 90% inhibition of mycelial growth (EC_{90}) was calculated from the regression equation of the logit transformed of dose-response curves.

Management of the pathogens in field tests. The efficacy of the fungicides fluazinam, iprodione+propineb, and triflumizole in suppressing three seed pathogens was compared with that of benomyl in two field experiments conducted in the Cheonan district. The first experiment was conducted in summer 1999 and the second in 2000 using the soybean cultivar Seokryang. Experiment plot was arranged in randomized block design with four replicates, 60 cm × 20 cm. Fertilizers N:P:K=1.74:2.8:2.0 kg/10a were applied as basal fertilization and common cultural management practices were carried out.

Fungicides were sprayed to run-off from flowering stage (R1), four times at 10-day interval and subsequently inoculation of each pathogen was made with a mixed suspension of 10^6 conidia/ml.

Disease severity of pods was assessed at R6 stage by means of visual observation of symptoms on the pods of 10 plants per plot and seed infection was assessed at harvest by the method of McGee (1986). Agronomic characteristics and yield components

Table 1. Mycelial growth of *Diaporthe phaseolorum, Colletotrichum truncatum,* and *Cercospora kikuchii* on PDA amended with indicated concentrations of different fungicides at 25°C for 5 days

Fungicide	Concentration (mg/ml)	Colony diameter (cm)		
		D. phaseolorum	*C. truncatum*	*C. kikuchii*
Azoxystrobin	1	6.4	5.4	2.8
Benomyl	1	0.0	0.0	0.0
Carbendazim	1	0.0	0.2	0.0
Carbendazim+kasugamycin	1	5.4	8.4	6.0
Chlorothalonil	1	2.8	4.8	4.0
Cymoxanil+chlorothalonil	2	2.9	6.5	3.5
Dichlorofluanid	1	3.5	2.7	1.2
Difenoconazole	0.5	1.7	4.8	1.0
Ethaboxam-A	1	3.4	4.9	1.5
Ethaboxam-B	1	2.8	6.3	3.9
Fluazinam	0.5	0.0	0.6	0.6
Folfet	2	4.1	1.6	4.3
Iprodione	1	1.4	5.3	2.9
Iprodione+propineb	2	0.0	1.4	0.6
Imidachloprid	0.5	9.0	8.2	3.8
Iminoctadintris+dimethomorph	1	0.0	4.2	1.9
Dxadixyl+propineb	2	1.8	6.4	1.0
Propineb	2	1.5	5.5	0.6
Thiophanate-methyl	1	3.1	1.6	0.0
Triflumizole	1	0.0	4.6	1.0
Control	0	9.0	9.0	8.0

were measured at R6 stage.

Results

Inhibition of seed pathogens in culture. Of the 21 fungicides tested, benomyl, carbendazim, fluazinam, iprodione+propineb, thiophanate-methyl, and triflumizole showed good activity against the mycelial growth of pathogens on PDA. As shown in Table 1, the mycelial growth of all the pathogens was completely inhibited at indicated concentration. With carbendazim, only *C. truncatum* showed mild growth of mycelia on PDA amended with 1.0 μg/ml.

Fig. 1 shows that benomyl and fluazinam did not inhibit mycelial growth of *D. phaseolorum* on PDA amended with a concentration of 10 μg/ml, but completely inhibited the pathogen at a concentration of 100 μg/ml. Carbendazim, iprodione+propineb, and thiophanate-methyl inhibited mycelial growth only by 45% and 75% each at a concentration of 100 μg/ml, but completely inhibited growth at a concentration of 500 μg/ml. Meanwhile, iprodione+propineb and triflumizole inhibited mycelial growth by 78% and 66%, respectively, at a concentration of 500 μg/ml, and completely inhibited at 1,000 μg/ml. As for *C. truncatum*, benomyl and fluazinam inhibited mycelial growth by 86% and 79% at 100 μg/ml, respectively, and completely inhibited at 500 μg/ml. However, carbendazim, iprodione+ propineb and triflumizole inhibited not more than 62%, 77% and 32%, respectively, even at a concentration of 1,000 μg/ml. As for *C. kikuchii*, all except for iprodione+propineb and triflumizole inhibited mycelial growth completely at 500 μg/ml. Meanwhile, iprodione+propineb and triflumizole inhibited mycelial growth only by 29% and 41%, respectively; even at 1,000 μg/ml, inhibition rate was not more than 68% and 74%.

Benomyl and fluazinam were shown to have excellent inhibitory effects on mycelial growth among the fungicides tested. These results were corroborated by the EC_{90} values of the fungicides. EC_{90} values of the fungicides differ depending on the pathogens (Table 2). Against *D. phaseolorum*, EC_{90} values of six fungicides were from 6.45 to 14.20 μg/ml. However, iprodione+propineb and triflumizole showed high EC_{90} values of 144.94 and 119.95 μg/ml against *C. kikuchii*, respectively.

Management of seed pathogens in the field. Carbendazim and thiophanate-methyl were excluded in the field test because they have the same modes of action as benomyl. The efficacy of the fungicides fluazinam, iprodione+propineb, and triflumizole was compared with benomyl in suppressing seed pathogens in the soybean field. In fluazinam-treated plants, plant height and number of branches were slightly less, but the number of pods and the weight of seed were higher compared to that of benomyl-treated plants. However, the results were not statistically significant (Table 3).

Suppression of disease infection by fluazinam and iprodione+propineb was as high as that of benomyl, but there was no significant difference in pod and seed infections. However, triflumizole was less effective than

Table 2. EC_{90} values of six fungicides against *Diaporthe phaseolorum*, *Colletotrichum truncatum*, and *Cercospora kikuchii*, the causal agents of seed decay in soybean

Fungicide	EC_{90} (μg/ml)		
	D. phaseolorum	*C. truncatum*	*C. kikuchii*
Benomyl	6.85	6.45	6.92
Carbendazim	10.50	11.49	7.04
Fluazinam	6.79	6.94	6.99
Iprodione+propineb	7.13	9.54	144.94
Thiophanate-methyl	6.91	7.70	15.76
Triflumizole	8.51	14.20	119.95

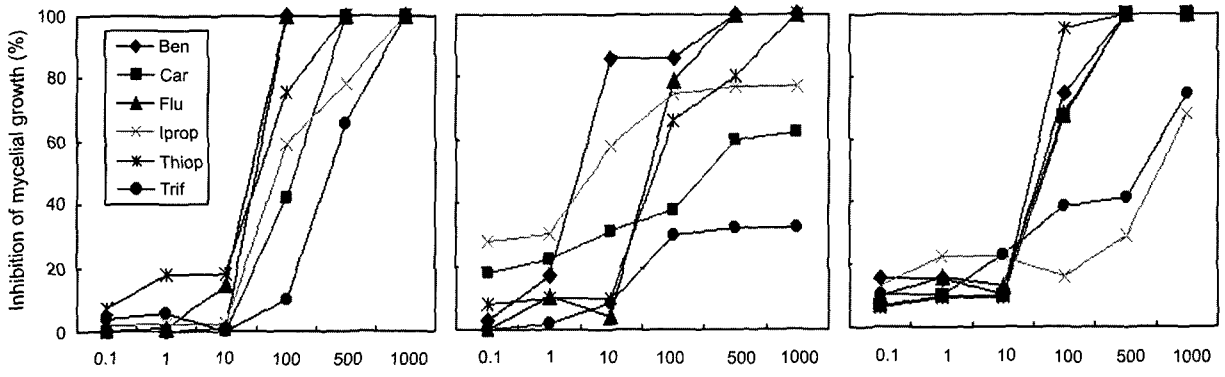

Fig. 1. Inhitory ratio (%) of mycelial growth of *Diaporthe phaseolorum, Colletotrichum truncatum, and Cercospora kikuchii* on the fungicide mediated PDA. Benomyl (Ben), Carbendazim (Car), Fluazinam (Flu), Iprodione+Propineb (Ipr-p), Thiophanate-methyl (Thio-m) and Triflumizole (Trif).

Table 3. Effect of fungicide application on the agronomic characteristics of vegetable soybean cv. Seokryang in 1999 and 2000

Fungicide	Plant height (cm)		No. of branches/plant		No. of pods/plant		Fresh pod[x] wt. (g)		100-seed wt. (g)		Seed weight/plot (g)		
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	Ave.
Benomyl	18.0	18.9	4.6	3.7	343	236	840	590	39.0	38.5	165	182	174
Fluazinam	19.5	19.4	4.9	4.2	275	278	671	729	35.5	37.9	155	188	172
Iprodione+propineb	8.7	18.6	4.3	3.5	292	290	712	643	37.0	37.8	159	176	168
Triflumizole	18.6	19.6	3.3	3.4	264	256	556	701	36.8	37.0	163	161	162
Control	18.5	18.7	4.5	3.8	331	254	576	580	38.0	37.0	159	170	164
F-value	0.56ns[y]		1.72ns		0.30ns		0.27ns		0.11ns		1.38ns		

[x]Fresh pod weight was measured at full pod stage (R6), and 100-seed and total seed weight were measured at harvest.
[y]ns = Not significantly different with the yearly average.

Table 4. Effect of fungicide application on the disease infection and total seed weight of vegetable soybean cv. Seokryang in 1999 and 2000

Fungicide	Pod infection (%)			Seed infection (%)		
	1999	2000	Ave.	1999	2000	Ave.
Benomyl	41.4	30.8	36.1c[x]	19.8	21.0	20.4c
Fluazinam	41.6	39.6	40.6c	29.4	28.6	29.0bc
Iprodione+propineb	43.7	38.7	41.2c	28.3	32.4	30.4b
Triflumizole	48.0	49.1	48.6b	36.2	33.9	35.1b
Control	83.5	79.9	81.7a	62.5	64.0	63.3a
F-value	1.20*[y]			5.31*		

[x]Mean separation within columns by DMRT at 5% level.
[y]Significant differences at $p<0.05$ level.

benomyl (Table 4).

Discussion

The adverse effect of seed pathogen infection on seed production is well documented (Oh and Kim, 2001; Oh and Kwon, 1981; Sinclair, 1993; Wrather et al., 1996). Fungicide application is a good strategy for the control of seed pathogens. Benomyl, a systemic fungicide, is very effective against a broad spectrum of seed pathogens (Ellis and Sinclair, 1975; Lee and Park, 1994; Jeffers et al., 1982; TeKrony and Egli, 1985). However, plants develop resistance to benomyl because of its specific mode of action. Resistance by different pathogens to benomyl has been reported in the past (Lapeyre and Duvoic, 1997; McGrath, 1985; Romero and Sutton, 1998; Yourman and Jeffers, 1999) and there were some reports that the fungicide's control effects have been reduced to a certain extent (Oh and Kim, 2001). Even though benomyl is still effective in many locations and is still recommended for the disease, it is possible that its use will be banned within a few years. Hence, alternative chemicals for disease suppression are urgently needed.

Agar amended with each fungicide reduced mycelial growth of the seed pathogens (Fig. 1). Among them, benomyl completely inhibited mycelial growth at the lowest concentration. This finding was similar to previous reports where benomyl sufficiently controlled *D. phaseolorum* (Lee and Park, 1994; Wrather et al., 1996), *C. truncaum* (Ellis and Sinclair, 1975), and *C. kikuchii* (TeKrony and Egli, 1985). Fluazinam also inhibited completely mycelial growth of *D. phaseolorum* at 100 μg/ml of PDA. It also completely inhibited mycelial growth of both *C. truncatum* and *C. kikuchii* at 500 μg/ml of PDA. The EC_{90} values in this study were 6.85 and 6.79 μg/ml for benomyl and fluazinam, respectively (Table 2). Accordingly, fluazinam was the most effective against all the seed pathogens tested, comparable to that of benomyl. Carbendazim and thiophanate-methyl completely inhibited mycelial growth of all the pathogens at 500 μg/ml. However, these chemicals have the same modes of action as benomyl, in which the butylcarbamoyl group is split off to give the relatively stable carbendazim, followed by slow degradation to nontoxic 2-aminobenzimidazole in plants (Clive, 1994). Therefore, these must not be used with benomyl because they might cause problems of developing cross-resistance.

Because the control efficacy of fluazinam and iprodione +propineb was prominent against pod and seed infection of soybean in the field test (Tables 3 and 4), these fungicides were considered to be effective against soybean seed pathogens as much as benomyl, without obstructing the agronomic characters and yield components of soybean culture.

Even though benomyl was effective in controlling soybean seed pathogens under all tested conditions, caution should be taken regarding resistance or tolerance of seed pathogens to this fungicide. Therefore, fungicides such as benomyl must be used alternately with other fungicides with different modes of action, or combined with multi-site fungicides with low resistance risk. In this regard, fluazi-

nam and a mixture of iprodione and propineb may be used in combination or alternately with benomyl to ensure high quality seeds, manage resistance, and control seed decay caused by *D. phaseolorum, C. truncatum,* and *C. kikuchii.*

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