Communications to the Editor

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IMPROVED ANTIFUNGAL EFFECT OF THIABENDAZOLE AGAINST *PENICILLIUM DIGITATUM* BY ITS APPLICATION TO POST-HARVEST CITRUS FRUIT IN COMBINATION WITH CARBOHYDRATE ESTERS OF FATTY ACIDS[1,2)]

Yoshihiro Nishikawa *,[a] and Masanori Ohkawa[b]

Faculty of Pharmaceutical Sciences, Kanazawa University,[a] 13-1 Takara-machi, Kanazawa 920, Japan and Faculty of Education, Kanazawa University,[b] 1-1 Marunouchi, Kanazawa 920, Japan

The antifungal activity of thiabendazole against *Penicillium digitatum* inoculated artificially on the exocarps of post-harvest citrus fruit was enhanced by adding various carbohydrate esters of fatty acids. Especially strong adjuvant effects occurred with sucrose monoesters of lauric, palmitic and stearic acids, commercial sucrose-tallowates, and 1-*O*-lauroyl-D-glucopyranose. Some related laurates derived from α,α-trehalose, sorbitol, sorbitan, isosorbide, and glycerol were also moderately effective.

KEYWORDS——thiabendazole; sucrose ester; 1-*O*-lauroyl-D-glucopyranose; antifungal activity; *Penicillium digitatum*; *Citrus unshiu*

The preservatives for citrus fruits, *O*-phenylphenol, its sodium salt, and diphenyl were first permitted officially in Japan. But they were not satisfactorily effective in preventing citrus green mold, a representative storage disease caused by *Penicillium digitatum*. So later in 1978 thiabendazole (TBZ) was allowed to be used in addition.

In the present study, we have found that the antifungal activity of TBZ against *P. digitatum* artificially inoculated on the exocarps of post-harvest citrus fruit can be enhanced by adding various carbohydrate esters of fatty acids such as sucrose esters (Table I), 1-*O*-lauroyl-D-glucopyranose (GL),[3)] and miscellaneous related laurates (Table II).

Bioassay was carried out in the following manner. Freshly picked individual Satsuma mandarins (*Citrus unshiu* Marc.) were each given, with a surgical knife, four X-shaped scratches (ca. 1 mm in depth, ca. 3 mm in line-length) on the peel at equal intervals around the shoulder. The injured fruit (eleven in one experiment) were dipped into an aqueous suspension containing TBZ (Merck Co., Inc.) (100 or 200 ppm) and a carbohydrate ester preparation (100 ppm) for a few minute, then dried at room temperature. The inoculums of *P. digitatum*, which had been pre-cultured on agar medium for seven days, were transferred to the scratches with a cork borer (ID, 3 mm). Subsequently, the fruit were incubated in a chamber at 25°C with a relative humidity of 100 %. On the third day, the antifungal activity was evaluated based on the infection ratio and the average lesion size: infection ratio (%) = [number of fruit with infection in at least one scratch / number of treated fruit] X 100, and average lesion size (mm) = sum of the maximum diameters of individual lesion / total number of scratches given.

Figure 1 shows the antifungal effects of TBZ (100 or 200 ppm) against *P. digitatum*

Table I. Sucrose Ester Preparations Used

Sucrose ester preparation	Abbreviation	Ester composition (%)	
		Mono-	≧ Di-
A. Laboratory-prepared monoesters[a]			
Sucrose-mono-laurate	SL	100	0
Sucrose-mono-palmitate	SP	100	0
Sucrose-mono-stearate	SS	100	0
B. Commercial products[b]			
Sucrose-tallowate, S-1570[c]	ST_1	70	30
Sucrose-tallowate, S-770[c]	ST_2	40	60
Sucrose-tallowate, DKF-10[d]	ST_3	0	100

a) Y. Nishikawa and K. Yoshimoto, Chem. Pharm. Bull., 25, 624 (1977). b) Sucrose-tallowate: sucrose ester of hydrogenated beef tallowic acid consisting mainly of stearic and palmitic acids (ca. 7:3). c) Mitsubishi Kasei Syokuhin Co., Ltd. (Previously, Ryoto Co., Ltd.). d) Dai-ichi Seiyaku Kogyo Co., Ltd.

in the presence or absence of the sucrose ester preparations (100 ppm each) listed in Table I. As expected, treating the fruit with TBZ alone at concentrations of 100 and 200 ppm were both effective, giving infection ratios of 62.8 and 43.2 % and lesion sizes of 6.2±3.9 mm and 5.2±3.0 mm, respectively. These values were significantly lower than those (93.2 % and 13.3±3.4 mm) of untreated controls (Fig. 1a). None of the sucrose esters tested exerted the antifungal activity by themselves (Fig. 1b-g). But these were able to enhance the antifungal activity of TBZ significantly, when they (100 ppm each) were applied in combination with TBZ (100 or 200 ppm) (Fig. 1b-g). For example sucrose-mono-laurate (SL) + TBZ at 100 + 100 ppm and 100 + 200 ppm resulted in infection ratios of 11.4 and 4.5 % and lesion sizes of 0.8±0.7 and 0.3±0.8 mm, respectively (Fig. 1b). The sucrose esters mono-substituted with shorter acyl moieties appeared to be more advantageous for the adjuvant effect. But precise studies of the structure-activity relationship remain to be done by comparing the activities at the doses proportional to the individual molecular weights.

For 1-*O*-lauroyl-D-glucose (GL), not only *P. digitatum* but also *P. fructigenum* (blue-green mold) and *P. italicum* (blue mold) were used as test fungi. It is surprising that, unlike sucrose esters, GL (100 ppm) exhibited antifungal activity against *P. digitatum* by itself, the effect being comparable, or even somewhat superior to that of TBZ alone (Fig. 2a). Single use of GL also had a considerable effect against *P. fructigenum*, while TBZ alone was only slightly effective (Fig. 2b). A combined application of GL and TBZ at 100 + 100 ppm almost completely prevented the occurrence of both green and blue-green molds, demonstrating here again the marked enhancement of the antifungal action (Fig. 2a,b). Blue mold could not be controlled by either GL or TBZ singly and also by their mixed application, though TBZ and TBZ + GL tended to be only weakly inhibitory (Fig. 2c). Since SL and GL had especially promising adjuvant effects, various related laurates derived from α,α-trehalose, sorbitol, sorbitan, isosorbide and glycerol were also subjected to the antifungal test against *P. digitatum* in the presence of TBZ (100 ppm of laurate + 200 ppm of TBZ). As shown in Table II, all of the laurates had adjuvant effects, but their activities were generally moderate and not so strong as with SL and GL.

At this time, the mechanisms involved in the enhancement caused by the carbohydrate esters are yet unclear, but it may be significant that such an effect could not be ob-

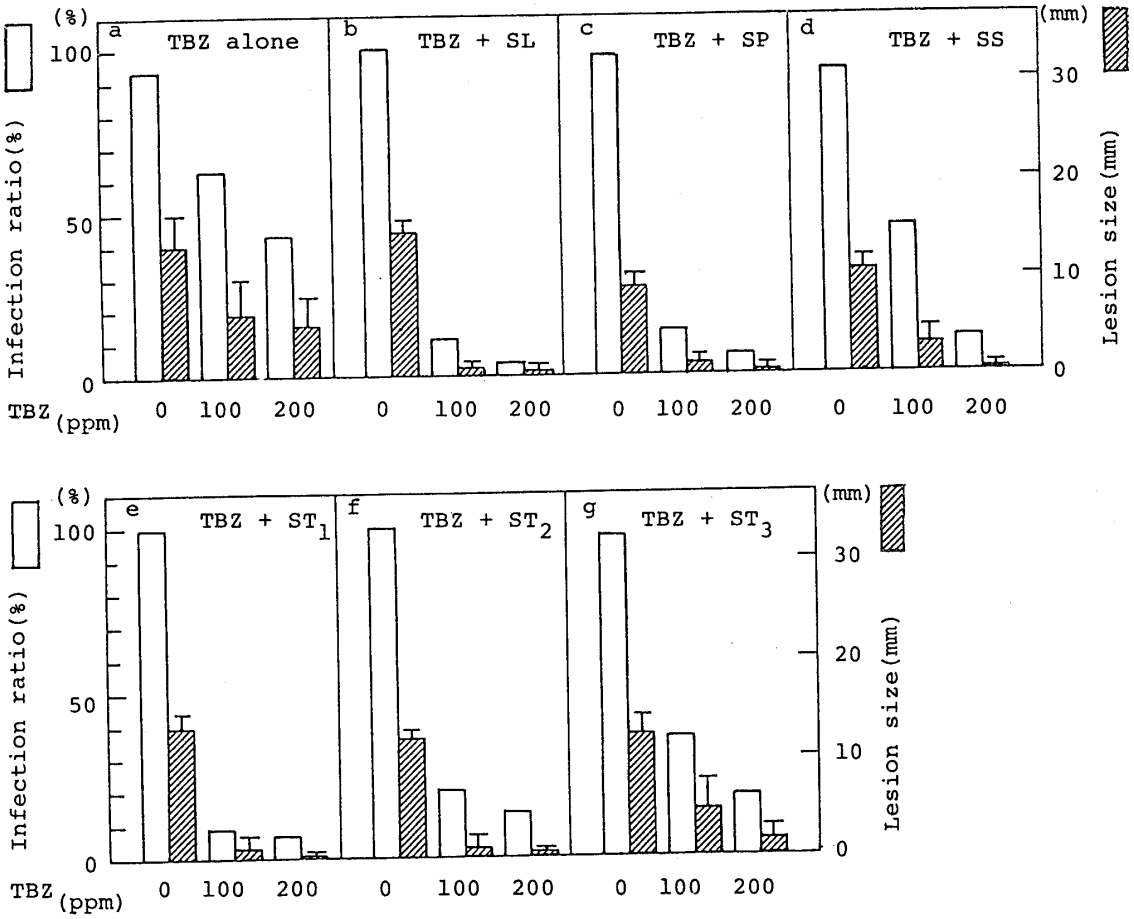

Fig.1. Antifungal Effect of TBZ against *P. digitatum* in Combination with 100 ppm of Sucrose Ester Preparation

For abbreviations used for the sucrose esters, see Table I.
Each vertical bar of lesion size represents the mean ± standard error.

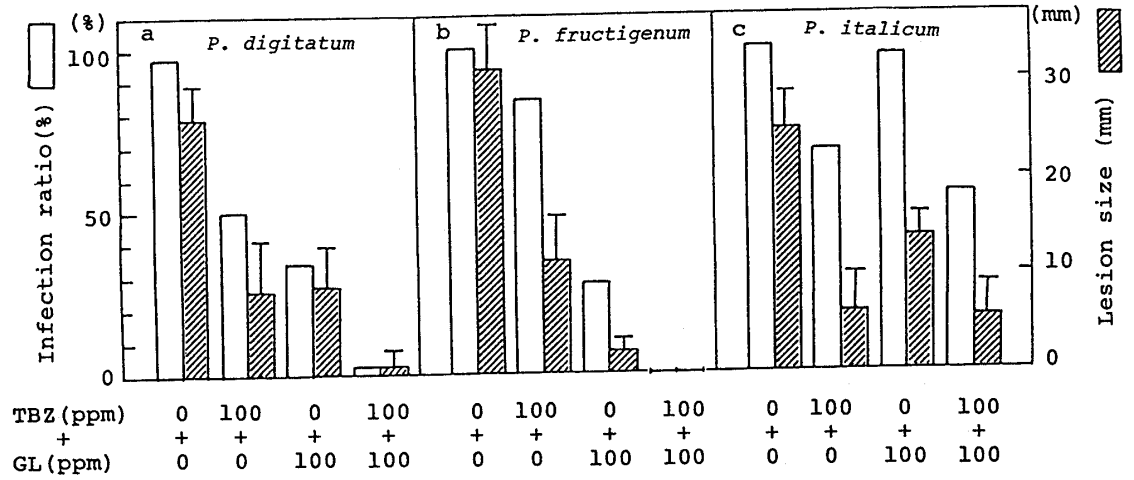

Fig.2. Antifungal Activity of TBZ against *P. digitatum*, *P. fructigenum* and *P. italicum* in Combination with 1-*O*-Lauroyl-D-glucopyranose (GL)

Each verticl bar of lesion size represents the mean ± standard error.

Table II. Antifungal Effects of Miscellaneous Laurates (100 ppm) against *P. digitatum* in Combination with TBZ (200 ppm)

Laurate added to TBZ	Infection ratio(%)	Lesion size(mm)
Untreated control	93.2	13.2 ± 3.4
TBZ alone	43.2	5.2 ± 3.0
6-O-Lauroyl-α,α-trehalose[a)]	25.0	4.5 ± 3.6
6,6'-Di-O-lauroyl-α,α-trehalose[a)]	27.3	2.3 ± 1.6
Sorbitol-mono-laurate[b)]	27.3	3.7 ± 2.9
Sorbitan-mono-laurate[b)]	22.7	2.7 ± 1.7
Isosorbide-mono-laurate[b)]	27.3	2.7 ± 2.1
Glycerol-mono-laurate[b)]	29.5	2.7 ± 1.9

a) Synthesized according to the procedures reported previously: K. Yoshimoto, T. Wakamiya and Y. Nishikawa, Chem. Pharm. Bull., 30, 1169 (1982). b) Gifts from Nihon Surfactant Ind., Co., Ltd.

tained *in vitro* as preliminarily evidenced by the dilution method. Therefore, it is almost certain that the mechanism cannot be explained merely from the physical point of view: participation of some physiological factors should be taken into account also. In spite of the obscurity of the mechanism, our present findings are important practically in the citrus industries, considering the improved prevention of the storage diseases, cost-reduction, and safety for human health.[4)]

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REFERENCES AND NOTES

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3) Y. Nishikawa, M. Okabe, K. Yoshimoto, G. Kurono and F. Fukuoka, Chem. Pharm. Bull., 24, 387 (1976).
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