A report on the use of thiabendazole for the control of fungal pathogens of cultivated mushrooms

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Introduction
Benomyl (methyl 1 (butylcarbanoyl)-2 benzimidazolcarbamate) was released in 1969 for the control of mushroom fungal pathogens. It proved to be very effective against the main fungal pathogens: Verticillium fungicola (Preuss) Hassebr. var. fungicola (Syn. V. malthousei Ware), the cause of dry bubble; Mycogone pennisiosa Magn., the cause of 'wet bubble'; Dactylium (Cladobotryum) dendroides (Bull. ex. Merat) W. Games & Hoozemans, the cause of 'cobweb disease' (Snel & Fletcher 1971; Holmes et al. 1971; Van Zaayen & Van Adrichem 1982).

Soon after the extensive use of benomyl on European mushroom farms, strains of V. fungicola var. fungicola resistant to this fungicide were noticed (Bollen & Van Zaayen 1975). Resistance was also reported from England (Gandy & Spencer 1974). The sudden and widespread occurrence of benomyl-resistant strains of this serious pathogen led to a search for other fungicides to control the disease. Chlorothalonil gave satisfactory results (Gandy & Spencer 1976) but did not, however, control 'dry bubble' completely and hence the search for better fungicides continues.

Fletcher (1981) reported that one of the benzimidazole fungicides, thiabendazole (TBZ) gave good results in the commercial control of 'dry bubble' and Rucklidge (1978) suggested that TBZ might be effective in the control of the latter pathogen because of its lower toxicity to the mushroom, which would make it safer for use at a higher application rate than most other fungicides.

No fungicide has, up to the present, been registered for use against mushroom fungal pathogens in South Africa. The aim of this investigation was to evaluate thiabendazole (commercially available as Tecto) with the aim of petitioning for its registration in this country.

Materials and Methods
1. The fungicide

Two forms of the fungicide were used in these trials. For in vitro sensitivity determinations, a pure, acetone-soluble powder (Tecto Antimycotic A, 991 g/kg pure thiabendazole) was used. For all other experiments Tecto Flowable Fungicide, a water-dispersible suspension containing 450 g a.i. (active ingredient)/dm³ TBZ, was used. Both these products were kindly supplied by the firm MSD Agvet, Private Bag 3, Halfway House, 1685.

A flowable fungicide in a water dispersable suspension containing 450 g/dm³ thiabendazole satisfactorily controlled the four major fungal pathogens of the mushroom cultivated commercially in South Africa, Agaricus brunnescens. These pathogens are Verticillium fungicola var. fungicola, Mycogone pennisiosa, Dactylium dendroides and Papulospora byssina. These results were obtained in commercial mushroom production rooms as well as in artificially inoculated plots in pilot plant research. In vitro activity of this fungicide on the former two fungal pathogens was also determined. The fungicide was found to be non-toxic to the mushroom and low residue levels of the product were recorded on harvested mushroom basidiocarps. The recommended dosages are: 1,838 g a.i. (active ingredient)/m² between each casing and 1,44 g a.i./m² between each break, applied as a drench.

Sukcesvolle beheer van die vier vernaamste funguspatogene van Agaricus brunnescens, die sampioen wat kommersiel in Suid-Afrika verbou word, is verkry deur behandeling met 'n vloeibare swamdoder in die vorm van 'n waterdispersierbaar suspensie wat as aktiewe bestanddeel 450 g/dm³ tiabendasool bevat. Hierdie patogene is Verticillium fungicola var. fungicola, Mycogone pennisiosa, Dactylium dendroides en Papulospora byssina. Hierdie resultate is in kommersiële sampioen­produuserende kamers sowel as met kunsmatig besmette sampioenbeddings in 'n loodsanleg verkry. Die in vitro aktiwiteit van tiabendasool teen die eersgenoemde twee funguspatogene is ook bepaal. Dit is gevind dat die swamdoder nie toksies vir die sampioenmiselium is nie en dat lae residu­vlakke daarvan op geplukte sampioene gemee is. Die aanbevolle toediening is: 1,838 g aktiewe bestanddeel/m² na die oorplaas van die deklaag en 1,44 g aktiewe bestanddeel/m² tussen elke oes. Die swamdoder word tydens watertoediening in die deklaag ingewek.

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2. The fungal pathogens

In vitro sensitivity of mycelial growth was determined for two virulent isolates V₁ and V₂ of V. fungicola var. fungicola and one isolate, M₁ of Mycogone perniciosa. V₁ was isolated from a diseased basidiocarp of the commercial mushroom, Agarius brunescens Peck., grown at the Bothasfontein production unit, Kyalami in room BS 4 on 1982-07-22. V₂ was isolated on 1982-08-22 from a diseased basidiocarp grown in room 6 of the Waterford production unit, Bryanston. M₁ was isolated from a serious outbreak of 'wet bubble' in room 40 of the Dennehof production unit, Lyttelton on 1982-07-21. These three isolates were maintained on potato dextrose agar (Difco).

3. In vitro studies

In vitro sensitivity of mycelial growth of the above isolates was tested using potato dextrose agar to which thiabendazole was added in a series of concentrations from previously prepared stock solutions in acetone. In experiment 1 plates contained 0; 2.5; 5; 10; 15; 25 and 50 mg/dm³ (p.p.m.) TBZ to establish the range of sensitivity of one of the strains (V₁). The results are presented in Figure 1. In subsequent experiments concentrations of 0.25; 0.5; 0.75; 1.0; 2.5; 5.0; 7.5 and 10 mg/dm³ were used. Controls included plates with 10 cm³ acetone/plate and TBZ and acetone free plates. Figures 2, 3 and 4 represent the results of three experiments with ten replications of each TBZ concentration performed on the isolates V₁, V₂ and M₁ respectively.

The plates were inoculated with an inverted disc (diameter 5 mm) of agar with mycelium cut aseptically from the edge of one-week-old, actively growing colonies of the pathogens on PDA. The inoculated test plates were incubated at 24°C for two weeks after which radial growth of the colonies was measured along two axes crossing each other at 90° in the centre of each colony.

To determine the toxicity of TBZ to A. brunescens, the same method as above was employed using plates containing 5; 10; 20; 30; 50 and 100 mg/dm³ TBZ. Inoculation discs were cut from two-week-old cultures of A. brunescens strain A6.5 (Hauser) grown on malt agar (Oxoid).

4. Determination of the persistence of thiabendazole residues

Mushrooms were grown in the phytotron at the University of Pretoria under conditions comparable to commercial production (Eicker 1981). A. brunescens strain A6.5 (Hauser) was grown and a peat/calcite mixture was used as casing material. Asbestos trays (Everite 582-280) of 390 x 390 mm were filled with through-grown compost. Each tray received 7 kg of compost (moist mass) and was cased with a 45 mm layer of peat/calcite. Just after the casing material was placed on the trays, Tecto flowable fungicide was 'watered' onto the casing in a 'normal' application (1.838 g.a.i. TBZ/m²) and a 'double' application (3.676 g.a.i. TBZ/m²). Control trays received water only. After the second break (flush) of basidiocarps was harvested, a further application of the fungicide was 'watered' onto the mushroom beds. In these instances the 'normal' treatment contained 1.44 g.a.i./m² TBZ and the 'double' treatment 2.88 g.a.i./m².

TBZ residue levels were determined spectrofluorometrically in mushroom caps harvested from the first break and
from the third break. Because of the high clay content of the South African 'peat' and the possibility of the absorption of TBZ on to the clay particles, TBZ residues in the casing were also determined.

A detailed description of the method is available, on request, from the firm MSD. The results obtained with two experiments, i.e. two separate mushroom production cycles, are given in Table 1.

Table 1 Persistence of thiabendazole residues on mushrooms and in the casing medium after different fungicidal treatments. Residues determined spectrofluorometrically and expressed as mg/dm² (p.p.m.)

| Material tested               | Control | Normal TBZ application | Double TBZ application |
|------------------------------|---------|------------------------|-----------------------|
| Mushrooms from first break    | <0,01   | 0,14                   | 0,18                  |
| Mushrooms from third break    | <0,01   | 0,36                   | 0,52                  |
| Casing medium after first break| <0,01   | 0,60                   | >1,0                  |
| Casing medium after third break| <0,01  | 0,81                   | >1,0                  |

5. Pilot mushroom production experiments
Mushrooms were grown in asbestos trays in the phytotron as described above. Tecto flowable fungicide was applied to two-thirds of the growing trays in the phytotron room as follows. Immediately after casing, the fungicide was applied to one-third of the trays as a drench treatment to give a TBZ concentration of 1,838 g a.i./m² ('normal') and the other third of the trays received a 'double' drench treatment to give a TBZ concentration of 3,676 g a.i./m². Similar applications were made after harvesting the first and second breaks to give a 'normal' TBZ concentration of 1,44 g a.i./m² and a 'double' TBZ concentration of 2,88 g a.i./m² respectively. Each asbestos tray comprised one experimental plot using a randomized block experimental design, with ten replicates of each treatment, i.e. no TBZ, 'normal' TBZ and 'double' TBZ.

Ten days after casing all the plots were inoculated with Verticillium fungicola var. fungicola (isolate V1). The conidial inoculum was obtained from sporulating two-week-old cultures on PDA. The plates were flooded with sterile distilled water and the conidia removed by rubbing the surface of the culture lightly with a bent glass rod (Gandy & Spencer 1978). The conidial suspension was shaken vigorously for thirty minutes to break up the slimy conidial heads. Each plot, representing 0,152 m², was inoculated with the fungal pathogen by atomizing a 20 cm² conidial suspension containing ca. 10⁶ conidia/cm². The percentage occurrence of each tray was assessed during each break by counting the number of diseased basidiocarps from each treatment and expressing it as a percentage of the total number of basidiocarps of all the plots of the particular treatment. The production of mushrooms in kg/m² for each treatment over three breaks was also recorded. The results of two trials, comprising the full growing cycle of a normal mushroom crop, are presented graphically in Figure 5.

6. Commercial mushroom production experiments
Mushroom production can not be discontinued in a commercial production unit. Furthermore mushroom growers would not allow the artificial spreading of pathogenic fungi on their farms. For this reason experiments using TBZ were performed in mushroom production rooms without inoculating the plots with pathogens. To compensate for this, growing rooms were chosen for their consistently high disease incidence on the commercial crop. Two experiments were conducted in such rooms at the Dennhof production unit of Tongaat Mushrooms (Pty) Ltd, Lyttleton. Each experiment was done on a mushroom crop grown in 96 standard wooden trays, each with a surface area of 1,95 m². The standard method of production (Vedder 1978) was followed but normal treatments, such as the spraying of the casing with a formaldehyde solution were withheld. One-half of the growing room, containing 48 trays (93,6 m² in total) was treated with Tecto (TBZ) while the other half of the room (48 trays) received no treatment and served as a control. The TBZ was applied as a drench during normal watering operations. Three applications were made as follows. Immediately after casing Tecto was applied at the rate of 4,08 cm³/m² to give a concentration of 1,838 g a.i./TBZ/m². Applications of Tecto at 3,20 cm³/m² were also made after removal of the first and second breaks of mushrooms to give TBZ concentrations of 1,44 g a.i./m² respectively. A method was devised (see below) to assess the percentage occurrence of four common fungal pathogens on the treated and control plots. The results of the experiments conducted during November 1982 and January 1983 are presented in Table 2.

7. Determination of the percentage occurrence of fungal pathogens
It has always been difficult to make a correct and objective
From the results of the residue analyses (Table 1) it is evident that very low residue levels are found on mushrooms treated with TBZ in the 'normal' concentrations of application. Even after two 'double' applications, the residue level of the third break mushrooms is less than 1 mg/dm³.

It is important to note that significant quantities of TBZ remain in the casing medium. Some of the peat used in South Africa is quite rich in clay minerals. It is suggested that some of the TBZ is adsorbed onto the clay colloids. This might influence the efficacy of TBZ. Consequently, higher application concentrations might be required in areas using this type of peat casing.

3. Pilot mushroom production experiments
The application of TBZ after casing and between the first and second and the second and third breaks evidently increased the production of mushrooms during both trials in the University's phytotron.

TBZ at a rate of 1,44 g a.i./m² or higher drastically reduced the incidence of V. fungicola var. fungicola symptoms when compared with the untreated control trays. On the untreated control trays 42.4% of all the basidiocarps showed signs of dry bubble infection while only 5.8% of the fruit bodies of the 'normal' TBZ treatment were affected by this pathogen. Double 'TBZ' treatment cut this infection to only 2.4%.

4. Commercial mushroom production experiments
The method using metal rings inserted at random in growing trays was found to be an effective way of assessing the incidence of different fungal pathogens in mushroom growing rooms.

From the results of the two trials conducted at the Dennehof production unit (Table 2) it is clear that TBZ controls Verticillium fungicola var. fungicola reasonably well and the three other most prevalent mushroom pathogens very well. It must, however, be taken into consideration that the trials were done in uninoculated plots. Nevertheless, comparing the treated side with the untreated side of the growing room, it can be seen quite clearly that the application of TBZ at the rate of 1,838 g a.i./m² at casing and 1,44 g a.i./m² between breaks satisfactorily controls the four major pathogens of A. brunnascens without reducing the production of basidiocarps.

If the number of basidiocarps produced during the first trial (Dennehof Room 5) is compared with the basidiocarp assessment of the incidence of fungal pathogens on mushroom beds. A method was devised which proved to be quite helpful. Metal rings with a diameter of 20 cm, representing a surface area of 0.128 m², were constructed from 3 mm diameter steel rods. Each ring was supplied with three evenly spaced metal spikes 10 cm in length. The rings were painted bright red. Twenty such rings were randomly placed on the trays treated with TBZ and twenty on the control trays. The metal spikes were pressed into the casing and compost so that the ring was situated about 1 cm above the casing layer. Each ring was also numbered by means of an aluminum tag. Data sheets were made and at each break the number of mushroom caps of 15 mm and more in diameter were counted in each ring and recorded. The number of diseased caps were also recorded. Four disease groups were recognized, viz: Verticillium fungicola var. fungicola, Mycogone perniciosa, Dactylium dendroides and Papulospora byssina.

The percentage occurrence of each of these pathogens was determined by expressing the number of infected basidiocarps as a percentage of the total number of basidiocarps in the 20 rings of each treatment. Productivity of mushrooms was determined by counting the total number of mushrooms produced in the rings during the entire period of the experiment. (Table 2).

**Results**

1. *In vitro* studies
It was found that thiabendazole had very little influence on the vegetative growth of *Agaricus brunnascens* at the concentrations tested. Even at a concentration of 100 mg/dm³ the radial growth of the mycelium of strain A6.5 (Hauser) was only very slightly inhibited. It can be concluded that TBZ has a low toxicity to the mushroom.

Mycelial growth of both *V. fungicola* var. *fungicola* isolates was severely inhibited by relatively low concentrations of TBZ (Figures 1–3). In all the experiments the ED50 values of TBZ on agar growth of the pathogens was found to be between 2 and 3 mg/dm³. *Mycogone perniciosa* was even more sensitive to the presence of TBZ with an ED50 value of less than 1 mg/dm³ (Figure 4).

2. Persistence of thiabendazole residues
From the results of the residue analyses (Table 1) it is evident that very low residue levels are found on mushrooms treated with TBZ in the ‘normal’ concentrations of application. Even after two ‘double’ applications, the residue level of the third break mushrooms is less than 1 mg/dm³.

**Table 2** The effect of thiabendazole (Tecto) on the % occurrence of fungal pathogens and number of basidiocarps in two commercial mushroom growing trials

| Production unit and treatment | Verticillium fungicola var. fungicola | Mycogone perniciosa | Dactylium dendroides | Papulospora byssina | All fungal pathogens | Production of basidiocarps/m² |
|-----------------------------|--------------------------------------|---------------------|---------------------|---------------------|---------------------|-------------------------------|
| Dennehof Room 5 'Normal' TBZ treatment | 1,11                                | 0,92                | 0,00                | 0,00                | 2,03                | 211,33                        |
| Dennehof Room 5 Control—No treatment | 4,48                                | 11,81              | 0,00                | 0,41                | 16,70               | 191,80                        |
| Dennehof Room 2 'Normal' TBZ treatment | 1,25                                | 0,00               | 0,00                | 1,25                | 602,73              |                               |
| Dennehof Room 2 Control—No treatment | 10,28                               | 2,46               | 0,23                | 0,00                | 12,97               | 508,98                        |
yield of the second trial (Dennehof Room 2), it can be seen that the production of the first trial was very poor. This was, unfortunately, due to a problem experienced in the production unit owing to a temporary shortage of casing substrate. Consequently, the casing of the trays in the experimental growing room had a layer of casing that was too thin and over-pinning ('mass pinning') and 'overlay' occurred. This reduced mushroom production considerably. This overall low mushroom production did not, however, mask the favourable influence of the TBZ applications.

Discussion

This investigation demonstrated that Tecto flowable fungicide (thiabendazole) is effective in controlling the four major pathogens of Agaricus brunnescens under South African growing conditions. This was proved by the control of Verticillium fungicola var. fungicola in trays inoculated with high inoculum densities of this pathogen, as well as the control of fungal pathogens in commercial growing rooms where there is a naturally high occurrence of especially, dry bubble and wet bubble. Although TBZ is not as effective as was the other benzimidazole fungicide, benomyl, (before its effectiveness declined owing to the relatively rapid and widespread appearance of tolerant V. fungicola var. fungicola strains) it is, nevertheless, a useful compound now. The good control of the two important pathogens, wet bubble and dry bubble, is also borne out by the results obtained with the in vitro sensitivity tests.

From the in vitro data and the in vivo experiments it seems that thiabendazole acts rather as a fungistatic chemical and not so much as a fungicide. This observation was also made by Sokolski (1981).

The low toxicity of TBZ to the mushroom owing to its specific mode of action (Rucklidge 1978), counts in its favour. Application rates can thus be increased with safety until good pathogen control is reached. This is not the case with broad-spectrum protectant fungicides, such as Chlorothalonil (Gandy & Spencer 1978).

Thiabendazole has a low mammalian toxicity, about 3 850 mg/kg LD50 for rabbits. The very low residues found on mushrooms treated with even very high Tecto applications during this investigation, show that there is no danger to the consumer in using this fungicide.

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