Bot. Bull. Academia Sinica (1990) **31:** 51-57

An evaluation of azaconazole for the treatment of wood used for *Agaricus bisporus* mushroom growing trays

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(*Received October 30, 1989; Accepted November 24, 1989*)

Abstract. *In vitro* and *in vivo* laboratory tests showed that azaconazole is highly inhibitive to most important strains of *Agaricus bisporus* grown in South Africa. A concentration of 10 ppm azaconazole in agar cultures was sufficient to suppress mycelial growth. Wood impregnated with 100 ppm azaconazole formed inhibition fronts against the mushroom mycelium on agar and wood impregnated with 2500 ppm azaconazole formed inhibition fronts against mushroom mycelium in compost and protected the wood against fungal attack. Azaconazole was found to be highly efficient as wood preservative under commercial growing conditions, preventing growth and penetration of mycelium in the wood used for tray construction. Treated trays were devoid of compost after tipping the trays at the completion of the production cycle. Residue of azaconazole in mushrooms grown in treated trays was less than 10 μg/kg, which is vastly less than the acceptable daily adult intake of 1500 μg. The recommende dosage is 2500 ppm azaconazole at a rate of 600 ml/m^2 of mushroom tray area or until run-off, applied by spraying. Trays may also be dipped in an azaconazole solution of the same concentration.

Key words: *Agaricus bisporus*; Azaconazole; Mushroom growing; Wood preservative.

Introduction

Wood is used extensively in South Africa for the construction of mushroom growing trays. There are several advantages in using wooden trays. According to Van Leemput *et al.* (1987) wood has outstanding mechanical properties, is stable and easy to handle. It is relatively cheap and damaged trays can be repaired easily. Wood also has natural binding forces making it durable and compact (Lelley and Krafack, 1987).

However, wood has certain disadvantages that manifest themselves in the mushroom industry. According to Lelley and Krafack (1987) wood undergoes structural changes as a result of water loss and water absorption. Steaming of growing rooms at the completion of a production cycle also has a detrimental effect on the wood of growing trays leading to deformation and warping. However, the most important disadvantage is the fact that mushroom mycelium uses wood as a food source and can thus penetrate the wood. The mushroom mycelium attacks and reduces the lignocellulose complex and this causes the wooden tray to loose its stability and manageability. Survival of mushroom mycelium in wood between successive crops can lead to the transmission of virus diseases. Virus disease is a very serious condition which can reduce crop production significantly. It can even result in total crop loss (Eicker and Botha, 1989; Van Leemput *et al.*, 1987). To prevent transmission of virus and bacterial diseases colonized compost and wooden trays must be treated at the termination of the crop and preferably a disinfectant applied to the empty trays prior to reuse (Fletcher *et al.*, 1986). The application of live steam definitely safeguards against the transmission of diseases by superficial compost and mycelial fragments. However, steam does not penetrate the wood deeply enough to destroy ingrained,

virus-infected mycelium (Lelley and Krafack, 1987). The only efficient method of wood preservation and protection against virus transmission is by the application of chemicals that protect wood against mycelial penetration. It is important to ensure that such chemical treatment does not transmit harmful substances to the basidiocarp. The application of such a wood preserving chemical has to result in an inhibition front between the tray sides and the mushroom mycelium, which should last throughout the growing period.

Prevention and control of disease is of the utmost importance to the mushroom growers. In the mushroom industry hygiene is the most relevant precautionary measure and this includes the disinfection of growing trays (Lelley and Straetmans, 1987). Pentachlorophenate (NaPCP) was traditionally used world wide for this purpose. The product was applied either by spraying or dipping. Evidence is mounting of human pentachlorophenate poisoning and its detrimental effect on the environment (Sanster *et al.*, 1982; Gray *et al.*, 1985; Jones *et al.*, 1986). The principal exposure of workers to NaPCP occurred during the treatment of empty mushroom trays (Onyon, 1987). The most common effects from occupational exposure to NaPCP is local skin, nose and eye irritation and acute systemic toxicity. A fatal case of pentachlorophenol intoxication was reported by Gray *et al.* (1985). The use of NaPCP in mushroom growing has been forbidden in many countries. It is therefore vital that an effective but safe alternative for NaPCP be found. Azaconazole was shown to have very promising efficacy against wood-destroying fungi (Van Leemput *et al.*, 1987). We report on the results of an evaluation of Rodewod 50 SL (a.i. 52.5 g/l azaconazole) as wood preservative in the South African mushroom industry.

Materials and Methods

Materials

Treatment solutions for the tests were prepared from the Rodewod 50 SL concentrate of azaconazole, 1-[[2-(2,4-dichlorophenyl)-1,3-dioxolan-2-yl] methyl-] H-1,2,4-triazole. The two most commonly grown strains of *Agaricus bisporus* in South Africa, the white button Hauser AX60 and the brown open Hauser C3.8, were used in the evaluation tests. Rodewod 50 SL was kindly supplied by Janssen Pharmaceutica (Pty) Ltd, South Africa and the mushroom strains were provided by the Waterford Spawn Laboratory of Tongaat Mushrooms (Pty) Ltd, Bryanston.

Methods

1. *In vitro* determination of the activity of different concentrations of azaconazole against *A. bisporus*

In vitro sensitivity of mycelial growth of the two strains of *A. bisporus* was tested using Difco malt agar (MA) to which azaconazole was added in a series of concentrations. Active ingredient concentrations of 0.01; 0.05; 0.1; 0.5; 1.0; 5.0 and 10.0 ppm (parts per million or mg.l) were used. Dilutions were made in distilled water. Control plates contained only MA.

Each plate was inoculated with an inverted disc of agar with mycelium cut from the edge of a 2-week-old, actively growing colony of *Agaricus bisporus* on MA. Twenty replicates were used for each treatment. The inoculated test plates were incubated at 25°C for 2 weeks after which the activity of azaconazole was determined by measuring the radial growth of the colonies along two perpendicular axes through the centre of each colony (Eicker, 1984; Eicker, 1987).

2. *In vitro* determination of the effect of *A. bisporus* on wooden blocks treated with different concentrations of azaconazole

Wooden blocks (dimensions 5 x 2 x 0.6 cm), end-sealed with paraplast embedding wax, were immersed for two hours in azaconazole treatment solutions at concentrations of 50; 100; 500; 1000; 2500 and 5000 ppm. Blocks were then exposed for three consecutive days to 100°C for 40 minutes per day. This sterilization process is known as tyndallization. MA plates were inoculated with the particular strain of *A. bisporus* by placing an inoculum near the edge of each plate. The plates were incubated for 9 days. An azaconazole treated, sterile block was then aseptically placed 5 mm from the edge of an actively growing mushroom colony. The plates were incubated for a further two weeks at 25°C. The activity of the wood preservative was evaluated by noting whether an inhibition front formed between the treated block and the fungal mycelium. Untreated, sterile wooden blocks were used as controls. The growth of mycelium on the wood was also evaluated according to the method of Van Leemput *et al.* (1987).

During preliminary trials blocks of pine wood (*Pinus* sp.) as well as gum wood (*Eucalyptus* sp.) were

used. The results were similar for both types of wood and successive tests were there-fore carried out using *Pinus* sp. blocks only.

3. *In vivo* determination of the effect of variously treated wooden disc on the growth of mushroom mycelium in compost

One hundred grams of fresh mushroom compost was placed in every cotton plugged, sterilized glass tube (4.5 cm x 26 cm) and inoculated from one side with 12 g of mushroom spawn. The compost cultures were incubated at 25°C. Round wooden discs (diameter 4.2 cm; thickness 2.0 cm), end-sealed with paraplast embedding wax, were immersed for two hours in four different concentrations of azaconazole, viz 1000; 2500; 5000 and 7500 ppm. On the seventh day of incubation the wooden discs were placed on the compost on the opposite side of the inoculation point (Fig. 1) and incubated at 25°C for a further three weeks.

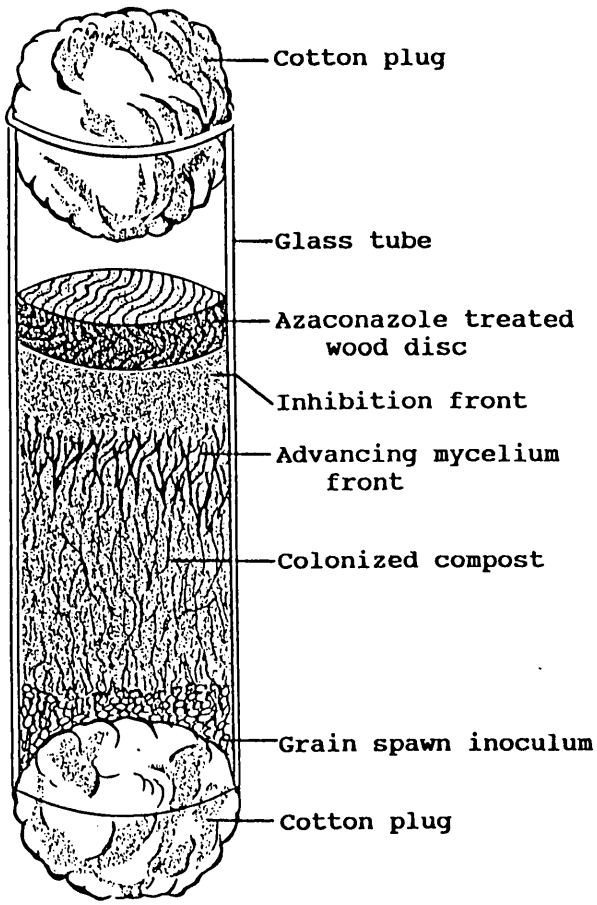

Fig. 1. Apparatus used for the *in vitro* determination of the effect of azaconazole treated wooden discs on the growth of mushroom mycelium in compost.

The presence of an inhibition front between the wooden disc and the advancing mushroom mycelium in the compost was noted. If there was no inhibition front the degree of mycelium growth on the wood was assessed.

4. Commercial mushroom production experiments

Two experiments were conducted under commercial conditions, one at the Dennehof production unit and one an experimental growing room at the Waterford production unit of Tongaat Mushrooms (Pty) Ltd. New trays assembled from pine wood with an internal area of 0.6 m^2 (dimensions 114 x 56 x 17.5 cm) were used. Treatment solutions containing 1500, 2500, 5000 ppm azaconazole were used. The treatment solutions were applied at a rate of 600 ml/m^2 (until run -off) using a sprayer delivering coarse droplets on the internal surfaces of the trays. There were three replicates of each treatment and trays were allowed to dry in a sheltered, well-ventilated place for four days. The usual cultivation practices of the mushroom farm were employed in the experiment. Mushroom yields were recorded on treated and untreated growing trays.

At the end of the production cycle the compost was tipped out and trays were examined visually to determine the relative amounts of compost adhering to the tray.

5. Determination of persistence of azaconazole residues

Samples of mushrooms were harvested randomly during the Dennehof experiments from the first and second break of trays treated with 2500 ppm a.i. azaconazole. Samples of the compost and casing were also taken for residue analysis. Mushrooms picked from untreated trays were submitted as contols. The samples were submitted to the South African Bureau of Standards to test for azaconazole residue content. The analyses were carried out in duplicate using gas chromatography. Recovery determinations were done by adding known amounts of azaconazole to portions of the untreated control samples and analyzing these concurrently with the samples.

Results

1. *In vitro* determination of the activity of different concentrations of azaconazole against *Agaricus bisporus*

Fig. 2 illustrates the drastic inhibition of vegetative growth of two mushroom strains by increasing azaconazole concentrations in the agar medium. Fig. 3 represents the results of the *in vitro* sensitivity of two strains of the cultivated mushroom to various concentrations of azaconazole. Significant inhibition could already be detected at the very low concentration of 1 ppm while 5 ppm a.i. azaconazole severely inhibited growth. A concentration of 10 ppm virtually arrested all growth. The brown C3.8 strain seems to be slightly more sensitive to azaconazole than the AX60 strain.

2. *In vitro* determination of the effect of *Agaricus bisporus* on wooden blocks treated with different concentrations of azaconazole

Inhibition fronts were observed with azaconazole concentrations of 100 ppm and above (Fig. 4).

With the exception of the 50 ppm concentration all the concentrations prevented mycelium growth on the blocks (Table 1).

3. *In vivo* determination of the effect of variously treated wooden discs on the growth of mushroom mycelium in compost

Table 2 shows the influence of wooden discs treated with different concentrations of azaconazole on the vegetative growth of mushroom mycelium in compost. It also indicates whether the wood was attacked by the mushroom mycelium or not.

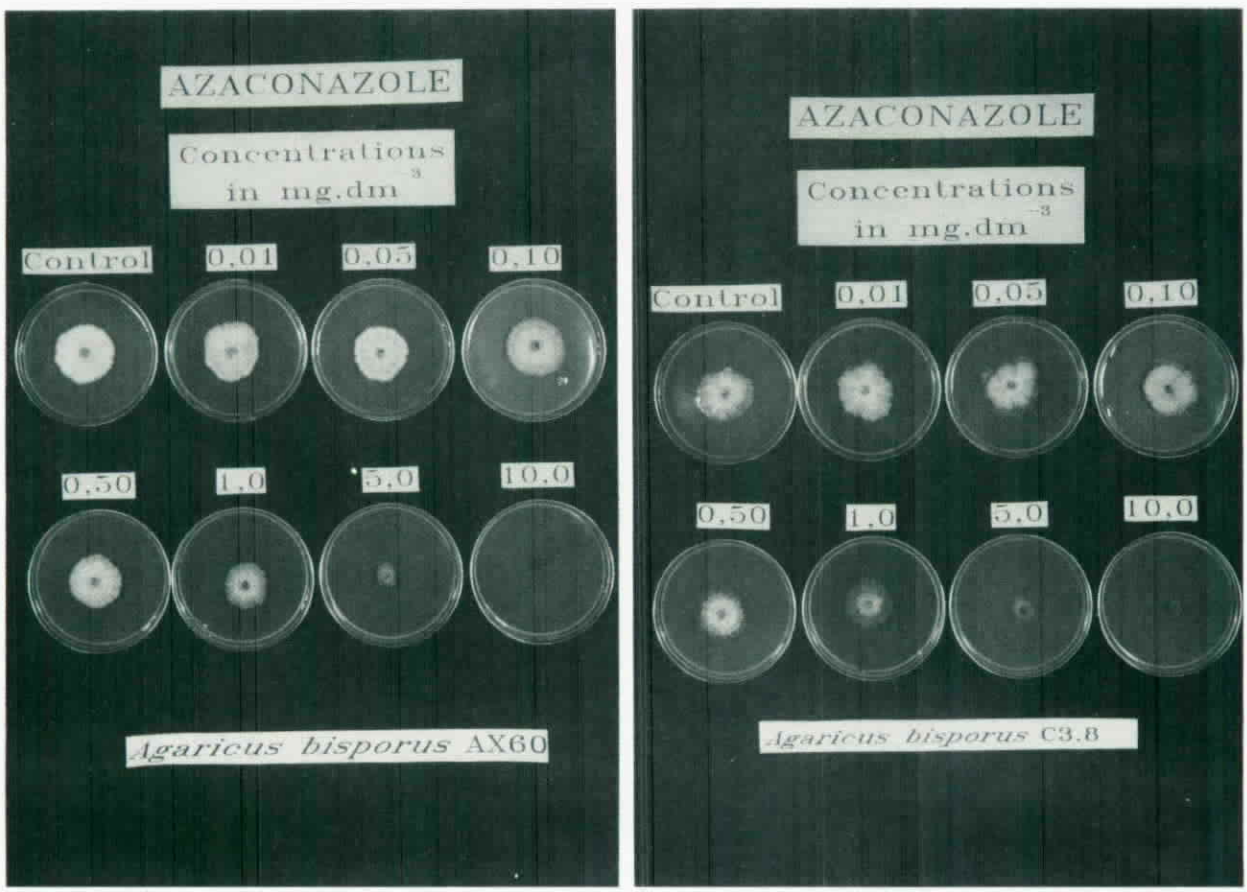

Fig. 2. *In vitro* inhibition of colonies of two strains of *Agaricus bisporus* on agar media with different concentrations of azaconazole.

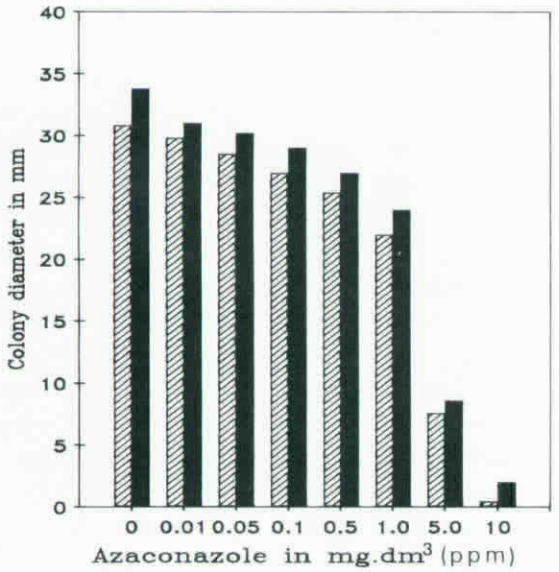

Fig. 3. *In vitro* effect of azaconazole on the vegetative growth of two strains of the cultivated mushroom, *Agaricus bisporus*, ▨, *Agaricus bisporus* C 3.8; ■, *Agaricus bisporus* AX 60.

At a concentration of 1000 ppm no inhibition front was present and mycelial growth was present on the test disc. Inhibition fronts were observed with azaconazole concentrations of 2500 ppm and higher.

4. Commercial mushroom production experiments

No significant differences in the yields were recorded. Average yield on untreated trays was 13.02 kg/m² and on treated trays 13.67 kg/m². A very slight adhesion of mycelia was observed on the trays treated with 1500 ppm azaconazole. The trays treated with 2500 and 5000 ppm showed no adhesion of mycelium and on emptying at the end of the crop the compost block fell cleanly from the tray. No indication of any other wood destroying fungi were found on the treated trays.

5. Persistence of azaconazole residues

An addition of 0.1 mg/kg azaconazole to control mushrooms gave a 106% recovery while a 1.0 mg/kg

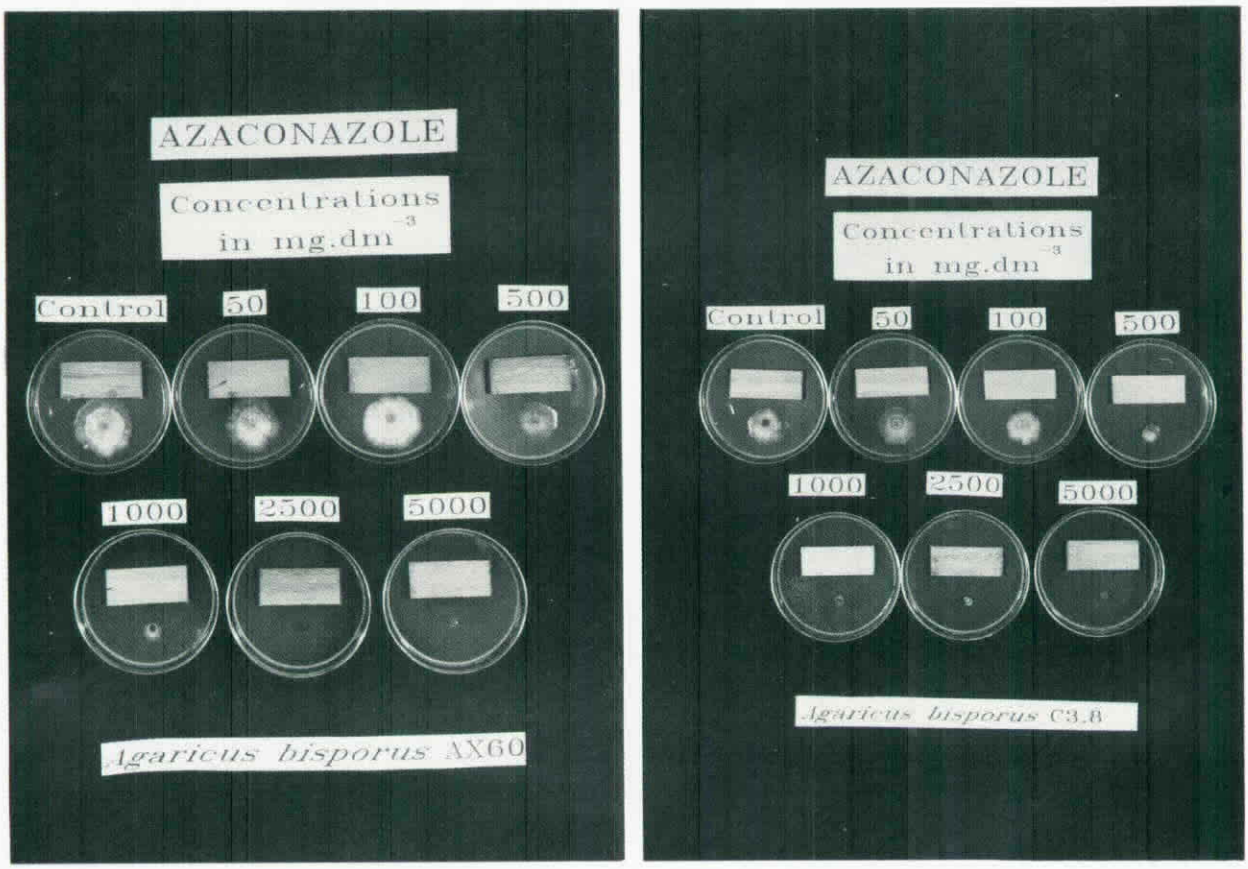

Fig. 4. *In vitro* effect of azaconazole treated wooden blocks on linear vegetative growth of two strains of *Agaricus bisporus*.

Table 1. *In vitro determination of the effect of* ***Agaricus bisporus*** *AX60 and C3.8 on treated wooden blocks*

Concentration (ppm)	Inhibition front[a]	Mycelial growth on test block[b]
0	0	+
50	0	+
100	0	-
500	+	-
1000	+	-
2500	+	-
5000	+	-

[a]0 = not present; + = present
[b]+ = growth; - = no growth

Table 2. *In vivo effect of variously treated wooden discs on the growth of* ***Agaricus bisporus*** *AX60 in compost*

Concentration (ppm)	Inhibition front[a]	Mycelial growth on test disc[b]
0	0	+
1000	0	+
2500	+	-
5000	+	-
7500	+	-

[a]0 = not present; + = present
[b]+ = growth; - = no growth

azaconazole addition yielded a recovery of 114%. In all the substrates submitted for analysis, including peat casing and mushroom compost sampled from azaconazole treated growing trays, azaconazole residues were so low that none could be detected even at the lowest limit of detection which was 10 μg/kg.

Discussion

In vitro and *in vivo* tests showed beyond a doubt that azaconazole is highly inhibitive to different strains of the cultivated mushroom. A very low concentration of 10 ppm is sufficient to suppress growth of the mycelium in agar cultures. Azaconazole impregnated wood forms inhibition fronts against the mushroom mycelium on agar and in mushroom compost. The treated wood is also protected against colonization by the wood destroying mushroom mycelium and other wood attacking fungi. This investigation furthermore demonstrated that azaconazole is highly efficient under commercial mushroom cultivation conditions as wood preservative in the prevention of growth and penetration of the mycelium of *A. bisporus* in the growing trays. To gain the full benefit of preservation of the wood, the prevention of virus disease transmission and clean tray tipping treatment of growing trays with a concentration of 2500 ppm azaconazole is recommended. Our experiments clearly indicate that application of the wood preservative by means of a hand operated spray delivering coarse droplets is very effective. To eliminate a heavy dirt load it is recommended that trays should, where necessary, be precleaned.

Extensive toxicological and environmental impact studies were previously carried out (Valcke and Goodwine, 1985; Van Leemput *et al.*, 1987). Azaconazole proved to be perfectly safe. It has a low oral and dermal acute toxicity with an acceptable daily intake of 1500 μg per day for an average sized adult. The results of residue analyses of mushrooms harvested randomly from treated trays as well as casing and compost samples from these trays showed that the azaconazole content were less than 0.01 mg/kg.

We can unconditionally recommend that RODEWOD 50 SL be registered as a wood preservative in the mushroom industry.

Recommended application

We recommend an application of azaconazole at a concentration of 2500 ppm (equivalent to a 5% solution of the product RODEWOD 50 SL) at a rate of 600 ml/m^2 of mushroom tray area or until run-off is obtained. The solution can be applied by means of a spray delivering relatively large droplets. Trays may also be dipped in an azaconazole solution of the same concentration.

Acknowledgments. We sincerely thank the management and staff of Tongaat Mushrooms (Pty) Ltd for research facilities on their Dennehof and Waterford production units. Their encouragement and help is appreciated. Dr B van der Westhuizen of Janssen Pharmaceutica (Pty) Ltd is thanked for his kind help. Financial support from University of Pretoria, the FRD of the CSIR and Janssen Pharmaceutica (Pty) Ltd is gratefully acknowledged.

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以 azaconazole 處理洋菇栽培用箱床木材之評估

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經活體內外篩選結果，證明 azaconazole 對南非產洋菇之多數主要菌系具有強力抑制效用。培養基中含此藥10 ppm濃度即足以壓制菌絲生長。注入此藥100 ppm之木片置於洋菜培養基，或注入2500 ppm之木片置於堆肥中拮抗洋菇菌絲，均能形成阻止帶且可保護木片受腐朽菌之侵害。在一般商業栽培條件下，此藥仍爲很有效的防腐劑，能阻止菌絲之生長及其侵入箱床用木材。當生產期結束而清理箱床時，木箱則不黏留堆肥。防腐處理木箱栽培之洋菇菌絲之殘留藥量低於10 μg/Kg，遠較成人可攝限量1500 μg之下。故此藥之有效使用量推介如下：使用濃度爲2500 ppm，用量爲600 ml/木箱表面積每平方公尺，使用方法：撒布或浸漬皆可。