

## *In vitro* Inhibition of Teliospore Germination and Its Subsequent Growth of *Tilletia caries* with Fungicides

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### Summary

Four fungicides namely Baytan, Ferrax, Murbenine and Murganic RPB have been evaluated for the *in vitro* inhibition of teliospore germination and its subsequent growth of *Tilletia caries*. To select the appropriate doses of the tested fungicides, six doses ranging from 1 to 10<sup>5</sup> ppm have been evaluated in a preliminary trial. Based on the observation of the preliminary laboratory trial, three doses for each fungicide have been selected in the final laboratory trial. The results of the experiment showed that Murganic RPB appeared to be best with the lower doses followed by Murbenine with the considerably higher doses both in inhibiting spore germination and its subsequent growth of *T. caries*. Baytan and Ferrax also had inhibitory effect on spore germination and growth but with relatively higher doses.

**Key words:** *Tilletia caries*, inhibition, fungicides, spore germination and its subsequent growth.

### Introduction

The losses due to bunt or stinking smut of wheat caused by several *Tilletia* spp. have comprised the major part of the total losses, probably the second only to rusts in importance as diseases of wheat<sup>2)</sup>. *Tilletia caries* (Dc.) Tul. is most predominant among the various species of *Tilletia*. Considerable works have been done for the control of the bunt fungi through seed treatment with different seed-dressing chemicals by the various investigators in the different wheat growing countries of the world<sup>1, 6, 9, 21)</sup>. Because of intrinsic damages of interfering with the subtle host: pathogen relationship by *in vivo* bioassays, *in vitro* spore germination tests have been developed to estimate separately the real toxicity of the antifungal chemicals to a wide range of plant pathogens. The key to the control of bunt fungi is inhibition of spore germination. Spore germination process is the basic to and precedes all other developmental phases in the bunt fungi.

In the present investigation, four fungicide formulations have been used for *in vitro* inhibition of spore germination and its subsequent growth of *T. caries*. Although the selected fungicides have been tested in the field as seed-dressing chemicals<sup>10, 13, 14, 17, 20)</sup>, but there is little recent information on critical *in vitro* spore germination tests on any of these

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modern fungicides. There was nothing published on the *in vitro* inhibition of spore germination and its subsequent growth of *T. caries* with the selected fungicides, but some components of the selected fungicides have been proved most effective as *in vitro* antifungal agents against many other fungi<sup>3, 15, 18, 22</sup>.

The current study has therefore been undertaken to evaluate the selected fungicides based on the inhibition of teliospore germination and its subsequent growth of *T. caries*.

## Materials and Methods

### Inoculum

The teliospores of *T. caries* obtained from the bunt infected wheat grains supplied by ICI (Imperial Chemical Industries, Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY, England) were used as the inoculum in the present study.

### Fungicide formulations

The four selected fungicide formulations used in this experiment were Baytan (25 % triadimenol and 3 % fuberidazole) supplied by Bayer U.K. Ltd.; Ferrax (40 % ethirimal, 3 % flutriafol and 1 % thiabendazole) supplied by Imperial Chemical Industries, England; Murbenine (30 % guazatine) and Murganic RPB (36 % carboxin and 1 % phenyl mercury acetate) supplied by Murphy Chemicals Ltd. England.

### Preparation of spore suspensions

A number of bunt infected grains were soaked with 70 % ethanol for 10 minutes to disinfest the surface. After that the soaked grains were broken aseptically with sterile forceps and suspended in sterile demineralized water and adjusted at  $5.5 \times 10^5$  spores ml<sup>-1</sup> of suspensions. The spores were counted with the help of haemocytometer counting method.

### Inoculation and incubation

Three drops of spore suspensions were placed on sterile plastic petri dishes containing 1 % water agar medium and then one drop of the prepared fungicide solution of different doses was added on each drop of spore suspension and maintained properly three replicated plates as untreated control. After that fungicide treated agar plates including untreated ones were incubated at 20° C for 15 days.

### Preliminary evaluation

To select the optimum doses of the selected fungicides a preliminary trial has been made with the six doses for each of the fungicide. Undiluted fungicides were considered as 10<sup>6</sup> ppm and then diluted the concentration as 1 ml of fungicide mixed with 9 ml of distilled water. Thus prepared six different concentrations were 10<sup>5</sup>, 10<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup>, 10 and 1 ppm. In the preliminary trial observations were taken periodically only for teliospore germination until 15th day of incubation. The percentage of germination was based upon the counts of 900 spores taken randomly 100 spores from each drop of the three replicated plates (each plate contained three drops).

### Final evaluation

Based on the preliminary screening three doses for each of the fungicides were selected

for the final laboratory evaluation. The doses selected for Baytan and Ferrax were 15000, 20000 and 30000 ppm; Murbenine were 750, 1000 and 1200 ppm and for Murganic RPB were 100, 150 and 200 ppm. In the final trial teliospore germination and its subsequent growth (promycelium, formation and fusion of primary sporidia) were observed periodically under a compound light microscope up to 15 days of incubation. The count of the percentage of germination followed the same procedure as in the preliminary trial. Pearson's Chi-Squared Statistics ( $2 \times 2$  contingency table) have been followed to show the significance of differences among the doses of each fungicide.

### Results and Discussions

All the tested fungicides reduced the germination of teliospores of *T. caries* at all the doses compared to that of the control (Fig. 1). The percentage of inhibition of germination increased with increasing doses of all the fungicides tested. Complete inhibition of germination of teliospores were achieved with Murganic RPB at  $10^3$  ppm product, Murbenine at  $10^4$  but only at the highest dose ( $10^5$  ppm) of Baytan and Ferrax. Among the four fungicides tested Murganic RPB appeared to be most effective in inhibiting spore germination of *T. caries* followed by Murbenine, Baytan and Ferrax, respectively. Preliminary trial was performed to select the appropriate doses for selected fungicides for further detail study of the spore germination and its subsequent growth of *T. caries*.

The results of the final laboratory evaluation also showed that all the tested fungicides at all the doses were significantly effective in inhibiting of spore germination of *T. caries* compared to the untreated control (Table 1). Baytan at  $1.5 \times 10^4$ ,  $2 \times 10^4$  and  $3 \times 10^4$  ppm inhibited spore germination about 80, 95 and 98 %, respectively. All the three doses were significantly different with one another in inhibiting spore germination. In case of Ferrax, the highest dose reduced germination by about 93 % which was significantly higher in inhibition than the lower and middle doses of which inhibition were about 83 and 85 %, respectively but there was no significant differences among these two doses. The two higher doses of Murbenine were statistically identical but both were significantly higher in inhibition than that of the lower dose. Although all the doses of Murganic RPB gave above 96 % inhibition, the

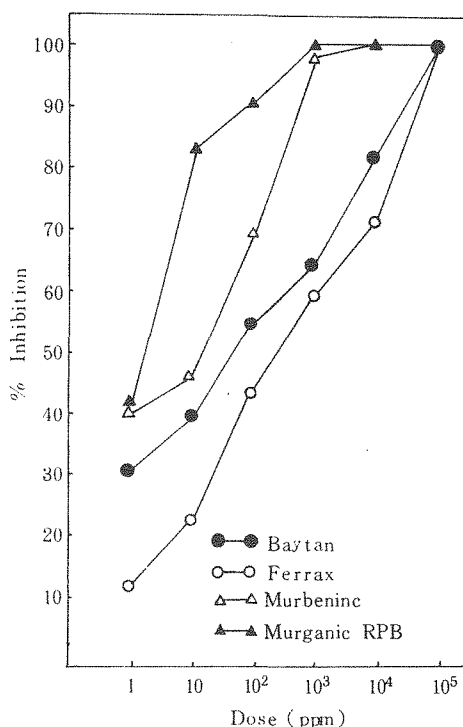


Fig. 1 Efficacy of fungicides in inhibition of teliospore germination of *Tilletia caries* in the preliminary laboratory trial.

Table. 1 Efficacy of fungicides in inhibition of spore germination of *Tilletia caries* in the final laboratory trial.

Treatment	Dose (ppm)	Days after incubation						
		5	6	7	8	9	10	10
		% Germination*					% Inhibition**	
Control	—	10.8	21.0	39.4	53.7	55.4	56.2	—
Baytan	15000	0.6	3.6	10.3	10.4	11.2	11.4	79.7 a
	20000	0.2	1.3	2.1	2.3	2.4	2.2	95.3 b
	30000	0.0	0.8	1.0	1.0	1.1	1.1	98.0 c
Ferrax	15000	1.9	6.3	8.1	9.2	9.3	9.4	83.2 a
	20000	1.1	4.4	7.4	8.3	8.5	8.5	84.8 a
	30000	0.8	3.6	3.6	3.6	4.1	4.1	92.7 b
Murbenine	750	0.0	4.3	9.0	9.2	9.3	9.8	82.6 a
	1000	0.0	0.6	0.6	0.7	0.7	0.7	98.8 b
	1200	0.0	0.3	0.3	0.4	0.4	0.4	99.2 b
	100	0.0	0.8	1.9	2.0	2.1	2.1	96.3 a
Murganic RPB	150	0.0	0.3	0.4	0.4	0.4	0.4	99.2 b
	200	0.0	0.0	0.0	0.0	0.0	0.0	100.0 c

\* The percentage of germination was based on the count of 900 spores from each treatment.

\*\* The percentage of inhibition with different doses of each fungicide followed by the same letter do not differ significantly at 0.05 level.

selected three doses were still significantly different from each other in inhibiting spore germination of *T. caries*. Complete inhibition was observed only with Murganic RPB at 200 ppm.

The results presented in Table 1 show that germination initiated at the fifth day of incubation except for Murbenine and Murganic RPB where germination was initiated at the sixth day of incubation. For all the treatments including control germination became constant at the 10th day of incubation. The results also suggest that 6th to 8th day of incubation were the peak period of germination in case of control whereas in case of fungicidal treatment germination became more or less constant after seven days of incubation. So far, there was no published report on the selected modern fungicides against *in vitro* inhibition of teliospore germination of *T. caries*. The results of the present studies suggest that Baytan at higher doses inhibited spore germination of *T. caries* which differ with Frohberger<sup>5)</sup> who reported that Baytan had no significant effect *in vitro* on spore germination and mycelial growth of most of the important phytopathological fungi. But are in agreement with Vanova<sup>22)</sup> who stated that Baytan had some antifungal action and also with Srikant *et. al.*,<sup>18)</sup> who found *in vitro* inhibition of *Sclerotium rolfii* of wheat with Baytan. There was a very little information on Ferrax, a comparatively new fungicide. The present investigation suggests that it is not effective at the lower doses which is partial agreement with Heaney *et. al.*, who observed no significant response against powdery mildew fungi of barley at the lower doses<sup>8)</sup>. But El-Tobshy reported that thiabendazole, one of the components of Ferrax inhibited the growth of *Gliomastix novae* only at 50 ppm which completely differ with the current results<sup>3)</sup>. It may be due to the fact that thiabendazole content in Ferrax was only 1 % and the tested fungus was also different. The present work demon-

strates that Murbenine is effective in inhibiting spore germination of *T. caries* at comparatively lower doses than Baytan and Ferrax. Although there was no report of Murbenine against spore germination of *T. caries* but inhibition of spore germination of many other fungi have been achieved with Guazatine, the only component of Murbenine at lower doses by many investigators<sup>15, 16, 23, 24</sup>. Among the tested fungicides Murganic RPB appeared to be most effective in inhibiting spore germination of *T. caries* at very lower doses which resembles with the observations of many other investigators who found carboxin, one of the major component of Murganic RPB was most effective *in vitro* inhibition of many other fungi<sup>2, 12, 18</sup>. The present investigation provides a valuable information that the tested fungicides not only inhibited the germination of teliospores but also have a great influence on its subsequent growth (Fig. 2). Among the germinated spores about 72 % have the fused primary sporidia in case of the control whereas only about 28, 39, 17 and 6 % have the fused primary sporidia in the case of Baytan, Ferrax, Murbenine and Murganic RPB, respectively. This suggests that the tested fungicides also inhibited the further growth of the germinated spores which ultimately retard the possibilities of infection. Because upon germination, if a primary sporidium does not fuse with another compatible sporidium, it may produce hyphae which are mononucleate, non pathogenic and can not infect the young seedlings<sup>19</sup>. The basic pattern of the life cycle of *T. caries* investigated by several workers<sup>4, 7, 11, 19</sup> revealed that upon germination teliospore produces a promycelium and a whorl of primary sporidia developed from its tip and compatible haploid sporidium (+ and -) usually fuse in pairs with a conjugation peg. Fused primary sporidia germinate to produce

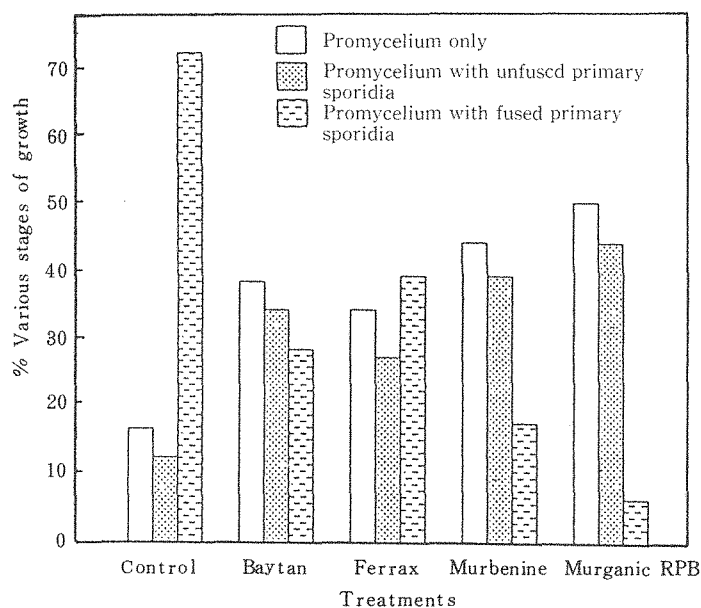


Fig. 2 Effect of fungicides on different stage of growth of the germinated teliospores of *Tilletia caries*.\*

\* Observation was based on the counts of 18 germinated teliospores from each treatment taken randomly six from each of the three replicated plates (only with the lower doses of all the fungicides as in the higher doses have not sufficient germination).

binucleate hyphae or secondary sporidia which can infect the wheat seedlings. Unless fusion occurs, there will be no infection.

However, from the present study it could be concluded that Baytan and Ferrax are not suitable for the *in vitro* inhibition of spore germination and its subsequent growth of *T. caries* as these fungicides required very higher doses. Murbenine is effective but the required doses were also considerably higher and Murganic RPB is the most effective even at the lower doses and obviously out performs all other three tested fungicides in inhibiting both the spore germination and its subsequent growth of *T. caries*. The results of the *in vitro* study may could have fundamental implications for the control of the bunt fungi *in vivo*.

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## 殺菌剤によるコムギなまぐさ黒穂病菌の 冬孢子発芽と発芽管長の阻害

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### 摘 要

ベイタン、ヘラックス、マルベニンおよびマルバニック RPB の4つの殺菌剤を用いて、コムギなまぐさ黒穂病菌の冬孢子発芽と発芽管長の阻害について調べた。

供試した殺菌剤の有効最適濃度を知るため、1 ppm から $10^5$  ppm を含む培地上で冬孢子を培養した。予備的な室内試験の結果に基づいて、3種類の濃度からなる殺菌剤を含む培地上で冬孢子を培養した。その結果、コムギなまぐさ黒穂病菌の冬孢子発芽と発芽管伸長に対して、低濃度のマルバニック RPB は高濃度のマルベニンより強く阻害した。高濃度ではあるが、ベイタンとヘラックスは冬孢子発芽と発芽管長を阻害した。