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IN VITRO STUDIES ON FUNGICIDES AGAINST *COLLETOTRICHUM GLOEOSPOROIDES* (PENZ.) SAC –THE DIE BACK OF TEA.

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To ascertain the inhibiting effect of revised doses of fungicides – Tilt 250 E. C., Calixin E. C. and Macuprax 16 w/w along with newly received chemicals–Folicur 250 EC and Bavistin 50 W.P. in comparison with higher rate of conventionally used copper based Cupravit Ob21 on mycelial growth of *Colletotrichum gloeosporoides*, two more *in vitro* studies were initiated during 1993. Observations recorded on the comparative growth of fungus indicated distrinct difference in the activity with different concentrations of fungicides. Amongst them, even 100 ppm of Bavistin and 200 ppm of Tilt were found most effective and completely inhibited the growth followed by 400 ppm Folicur and 1500 ppm Calixin. On the contrary, higher concentrations of Macuprax and Cupravit were ineffective and failed to arrest the mycelial growth.

INTRODUCTION

Die back of tea is one of the major problems in Bangladesh tea both in mature as well as nursery plants. Die back recorded during the late sixties on the Indian clone TV9 is reported to be widespread in tea, in permanent and temporary shade trees, green crops as well as in rubber plantations in Bangladesh. The initial symptoms shown by the causal organism are small brownish spots on the aerial soft stem which gradually extend in both the upper and lower directions. In advanced stages, the apical portion of the shoot becomes black, with a gradual shedding of leaves with cessation of further sprouting (Fig. 1).

The isolated organism on Potato Dextrose Agar (PDA) sent to the Commonwealth Mycological Institute (CMI), UK was identified as *Colletotrichum gloeosporoides* (Penz.) Sac. and reported to be seen for the first time in tea. The cells of acervulus develop as setae which are brown, septate, slightly swollen at the base and then taper to the tip. Conidia are cylindrical with obtuse ends, hyaline, aseptate 12.0–13.6 (12.95) μ x 4.0–4.8 (4.33) μ formed from unicellular hyaline cylindrical phialidic conidiophores. The conidiophores are sometimes septate and branched. The spore masses are normally small and colourless (Fig. 2). Colonies on PDA show sparse whitish aerial mycelium.

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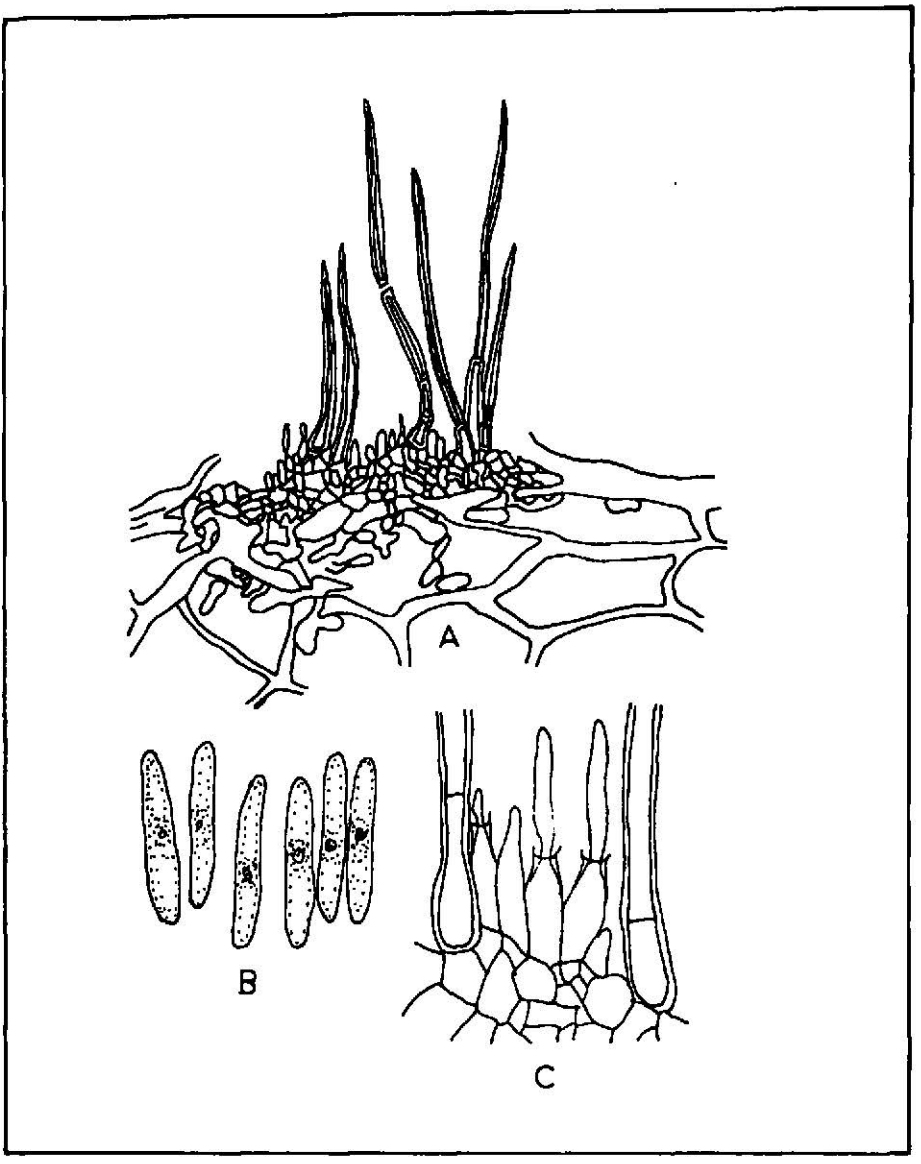

Fig. 2–*Spore masses showing (A) Acervulus, (B) Conidia and (C) Conidiophore of C. gloeosporoides*

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Fig. 1–*Infected shoots of die back*

The air borne pathogen *in vivo* is active under hot and humid conditions with relative humidities of over 92% and with mean daily sunshine of 4–5 hours.

Several new systemic and contact fungicides were screened in 1991 under laboratory conditions to evaluate their inhibiting effect on the causal organism. In the light of these preliminary findings, two more laboratory studies were undertaken in 1993 using modified doses of the fungicides tested earlier along with two new fungicides received thereafter.

MATERIALS AND METHODS

Two *in vitro* trials were initiated in January and April, 1993. The treatments for the second trial conducted in April were modified on the basis of the findings of the first trial conducted in January. The different systemic and contact fungicides evaluated in comparison with the commonly used copper–based Cupravit Ob21 were:

a) Systemic fungicides:
1. Calixin E. C. (Tridimorph)
2. Tilt 250 E. C. (Propiconazol)
3. Folicur 250 E. C. (Tuberconazole)
4. Bavistin 50 WP (Carbendazim)

b) Contact fungicides:
1. Macuprax 16 w/w (Bordeaux mixture and Cupraneb)
2. Cupravit Ob21 (Copper oxychloride)

As before, pure cultures of *Colletotrichum gloeosporoides* grown on PDA were used adopting the Poisoned Food Technique of Schmitz (1930).

An 8–day–old inoculum was used. Different concentrations of fungicides were prepared and 6 ml from each of the concentrations was poured into a conical flask containing 45 ml sterilized nutrient agar medium (PDA). Thereafter, 15 ml of the incorporated medium was poured into a Petri dish (9 cm diam.). After about two hours a fungal disc of the inoculum drawn by a cork borer was inoculated in the centre of the Petri dish. The experiment was done under Laminar Flow and the Petri dishes were kept inside to avoid contamination. The room temperature and relative humidity recorded during the period of both studies were between 23–27°C and 70–94% respectively.

Both trials were conducted in a randomised block design with the treatments replicated thrice.

Treatments in first trial:

T_1	=	Control (untreated)	
T_2	=	Macuprax	1200 ppm
T_3	=	Macuprax	1000 ppm
T_4	=	Macuprax	800 ppm
T_5	=	Cupravit	2000 ppm
T_6	=	Calixin	1200 ppm
T_7	=	Calixin	1100 ppm
T_8	=	Calixin	1000 ppm
T_9	=	Folicur	400 ppm
T_{10}	=	Folicur	200 ppm
T_{11}	=	Folicur	100 ppm
T_{12}	=	Folicur	50 ppm
T_{13}	=	Folicur	30 ppm
T_{14}	=	Tilt	200 ppm
T_{15}	=	Tilt	100 ppm
T_{16}	=	Tilt	50 ppm
T_{17}	=	Tilt	30 ppm
T_{18}	=	Bavistin	400 ppm
T_{19}	=	Bavistin	300 ppm
T_{20}	=	Bavistin	200 ppm

Treatments in second trial:

T_1	=	Control (Untreated)	
T_2	=	Calixin	1500 ppm
T_3	=	Folicur	200 ppm
T_4	=	Folicur	100 ppm
T_5	=	Folicur	50 ppm
T_6	=	Tilt	200 ppm
T_7	=	Tilt	100 ppm
T_8	=	Tilt	50 ppm
T_9	=	Bavistin	200 ppm
T_{10}	=	Bavistin	100 ppm
T_{11}	=	Bavistin	50 ppm

Observations on the growth of the fungal colony were taken at regular intervals. Two measurements at right angles to each other were made. Observations were recorded 3, 6 and 8 days after inoculation in the first and 3, 6 and 10 days after in the second trial.

RESULTS AND DISCUSSION

The measurements of the growth of the fungus in the first and second trials are presented in Tables 1 and 2 respectively.

TABLE 1 – *Growth of the fungus Colletotrichum gloeosporoides on PDA on different treatments*

Date of treatment : 3.1.93

Treatments (ppm)			Growth of fungus (cm) (Mean of 3 replications) 6.1.93	9.1.93	11.1.93	Over all mean
T_1	= Control		3.98	8.00	9.00	6.99 a
T_2	= Macuprax	1200	3.47	7.53	9.00	6.67 a
T_3	= Macuprax	1000	3.53	7.80	9.00	6.78 a
T_4	= Macuprax	800	3.72	8.00	9.00	6.91 a
T_5	= Cupravit	2000	3.58	7.10	9.00	6.56 a
T_6	= Calixin	1200	0.00	1.67	2.58	1.42 bcd
T_7	= Calixin	1100	0.00	1.90	3.40	1.77 bc
T_8	= Calixin	1000	0.50	1.98	3.78	2.09 b
T_9	= Folicur	400	0.00	0.00	0.00	0.00 g
T_{10}	= Folicur	200	0.00	0.00	1.12	0.37 efg
T_{11}	= Folicur	100	0.00	1.35	2.03	1.13 cdefg
T_{12}	= Folicur	50	0.50	2.17	2.90	1.86 bcd
T_{13}	= Folicur	30	0.60	2.80	3.68	2.36 b
T_{14}	= Tilt	200	0.00	0.00	1.36	0.45 efg
T_{15}	= Tilt	100	0.00	1.00	1.58	0.86 defg
T_{16}	= Tilt	50	0.00	1.58	1.97	1.18 cdef
T_{17}	= Tilt	30	0.50	1.93	2.05	1.49 bcde
T_{18}	= Bavistin	400	0.00	0.00	0.00	0.00 g
T_{19}	= Bavistin	300	0.00	0.00	0.00	0.00 g
T_{20}	= Bavistin	200	0.00	0.00	0.00	0.00 g

Note: Means followed by the same letter are not significantly different from each other.

TABLE 2 – *Growth of the fungus Colletotrichum gloeosporoides on PDA on different treatments*

Date of treatment : 6.4.93

Treatments (ppm)			Growth of fungus (cm) (Mean of 3 replications) 9.4.93	12.4.93	16.4.93	Overall Mean
T_1	= Control		3.00	7.00	9.00	6.33 a
T_2	= Calixin	1500	0.00	1.55	2.87	1.47 b
T_3	= Folicur	200	0.50	2.12	2.68	1.77 b
T_4	= Folicur	100	1.38	2.60	3.43	2.47 ab
T_5	= Folicur	50	1.55	3.05	4.07	2.89 ab
T_6	= Tilt	200	0.00	0.00	0.00	0.00 c
T_7	= Tilt	100	0.00	0.00	0.40	0.10 c
T_8	= Tilt	50	0.50	2.12	2.72	1.78 b
T_9	= Bavistin	200	0.00	0.00	0.00	0.00 c
T_{10}	= Bavistin	100	0.00	0.00	0.00	0.00 c
T_{11}	= Bavistin	50	0.00	0.00	0.40	0.10 c

Note: Means followed by the same letter are not significantly different from each other.

From the results of the first trial (Fig. 3), it is seen that the copper-based fungicides, Macuprax and Cupravit failed to check the mycelial growth of the fungus even at high concentrations; slight to moderate growth of mycelium was noticed in the concentrations of Calixin (T_6–T_8) and Folicur (T_{12} and T_{13}). Folicur at 200 and 100 ppm and all the concentrations of Tilt (200, 100, 50 and 30 ppm) showed very slight to negligible growth. Amongst them Tilt (T_{14}) at 200 ppm was comparatively better. All concentrations of Bavistin (T_{18}–T_{20}) and Folicur (T_9) completely inhibited the mycelial growth during the period of study.

The analysis of variance also showed that all the treatments of Bavistin, Folicur (T_9 and T_{10}) and Tilt (T_{14}) were significant to the rest of the treatments at 1% level.

Since none of the concentrations of Macuprax and Cupravit were effective, these were excluded from the second trial. Calixin at a higher dose (1500 ppm) together with Folicur, Tilt and Bavistin each at 200, 100 and 50 ppm were included for comparison.

Observations recorded thrice at regular intervals up to 8 to 10 days (Table 2) indicate superiority against mycelial growth with all the treatments of Tilt and Bavistin and at 200 ppm Folicur (T_3) (Fig. 4). Calixin at 1500 ppm was comparatively better over 1200 ppm used in the first trial. Analysis of variance also showed that all the treatments of Bavistin (T_9, T_{10} and T_{11}), Tilt (T_6 and T_7), Folicur (T_3) and Calixin (T_2) were superior to the other treatments (T_4, T_5 and T_8) at 1% level.

CONCLUSIONS

Die back of young tea shoots is a frequent occurrence. Conventional use of copper fungicides has been ineffective and hence the need to test other suitable systemic fungicides.

It would appear from the studies that all concentrations of Bavistin and Folicur at 400 ppm completely inhibited the mycelial growth of *Colletotrichum gloeosporoides*. Conversely Tilt at 200, 100 and 50 ppm and Folicur at 200 ppm were also excellent where very slight growth was observed. Calixin at 1500 ppm was comparatively better than doses tried up to 1200 ppm and was comparable with Folicur at 200 ppm and Tilt at 50 ppm. The mycelial growth was however, moderate with Folicur at 100 and 50 ppm. Macuprax and Cupravit were ineffective.

It will thus be seen that Bavistan, Tilt and Folicur each at 200 and 100 ppm were comparable to the performance of Calixin and appear promising for field studies.

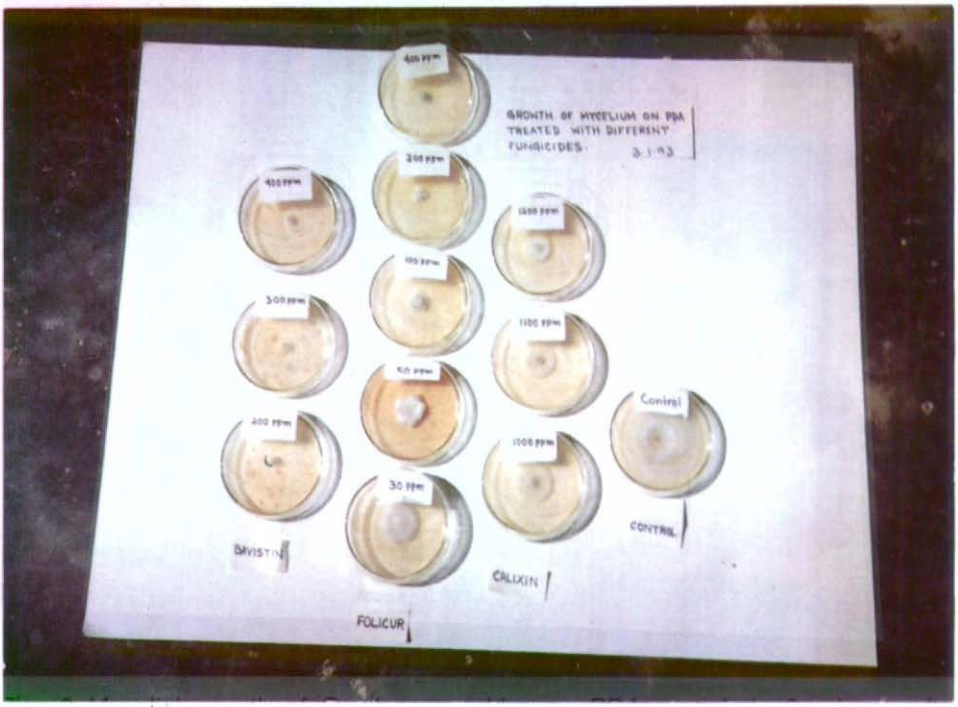

Fig. 3–*Mycelial growth of C. gloeosporoides on PDA recorded 8 days after inoculation on 11.1.'93*

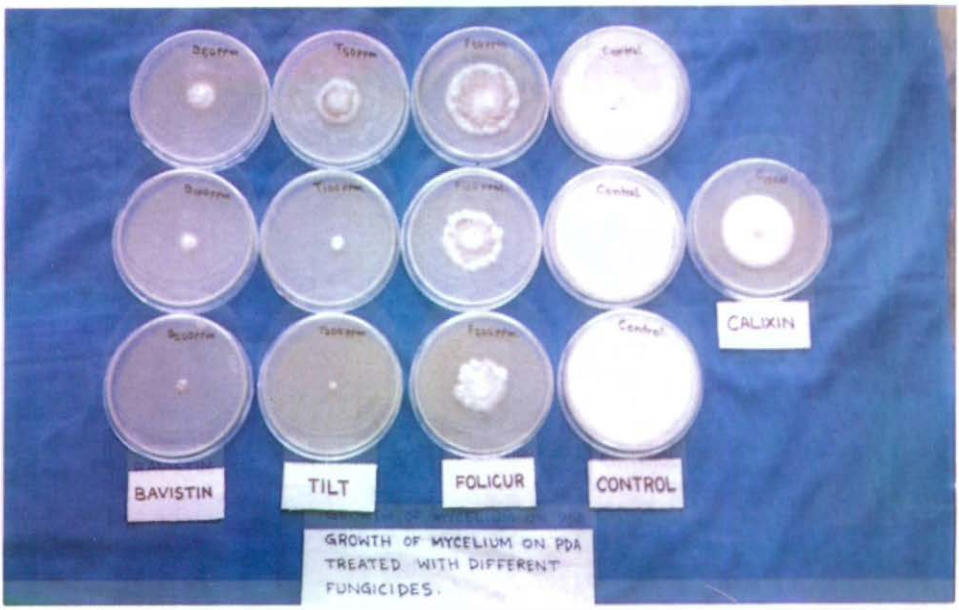

Fig. 4–*Mycelial growth of C. gloeosporoides on PDA recorded 10 days after inoculation on 16.4.93*

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