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Control of *Phytophthora palmivora* on Orchids with Some New Systemic and Standard Fungicides

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RINGKASAN

Tiga fungisida serap yang baru iaitu Aliette (efosite-Al), Ridomil (metalaxyl) serta Ridomil MZ (metalaxyl dengan mancozeb) dan dua fungisida biasa Terrazole (etridiazole) serta Difolatan (captafol) telah diuji kesan profilaktik dan terapeutik ke atas Phytophthora palmivora *pada orkid. Pucuk-pucuk punca haibrid* Aranda *Christine dapat dikawal dengan berkesan daripada jangkitan* Phytophthora *apabila disembur dengan metalaxyl pada 0.05% b.a., campuran metalalaxyl-mancozeb pada 0.15% b.a. dan captafol pada 0.2% b.a. tiga hari sebelum suntekan tiruan dengan* Phytophthora. *Etridiazole dan efosite-Al, keduanya pada kadar 0.1% b.a. tidak memberikan kawalan yang memadai. Metalaxyl (0.05% b.a.) serta campurannya dengan mancozeb (0.15% b.a.) menghalangkan dengan baiknya perkembangan penyakit oleh* Phytophthora *apabila digunakan sebagai semboran terapeutic. Etridiazole (0.1% b.a.) dan efosite-Al (0.1% b.a.) memberikan kawalan terapeutic yang sederhana manakala captafol tidak memberikan sebarang kawalan terapeutic. Ridomil MZ, Terrazole dan Aliette tidak membawakan fitotoksisiti sehingga kadar 0.5% b.a., 0.4% b.a. dan 0.8% b.a. berturut-turut. Fungisida-fungisida Ridomil, Aliette dan Terrazole menunjukkan kadar rintangan yang serupa ke atas perkembangan linear* P. palmivora *tetapi berbeza dari darjah invitrofungitoksisiti. Ridomil, Ridomil MZ serta Terrazole menunjukkan nilai* ED_{50} *yang kurang dari 1 µg/ml manakala Difolatan secara relatif adalah kurang toksik (*ED_{50} *19.9 µl/l).*

SUMMARY

Three new systemic fungicides viz. Aliette (efosite-Al), Ridomil (metalaxyl) and Ridomil MZ (metalaxyl plus mancozeb) were screened against two standard fungicides viz. Terrazole (etridiazole) and Difolatan (captafol) for prophylactic and therapeutic efficacy against Phytophthora palmivora *on orchids. Terminal shoots of* Aranda *Christine hybrids were effectively protected from* Phytophthora *infection when sprayed with metalaxyl at 0.05% a.i., metalaxyl-mancozeb mixture at 0.15% a.i. and captafol at 0.2% a.i. three days before artificial inoculation. Etridiazole and efosite-Al, both at 0.1% a.i. did not give adequate protection. Metalaxyl (0.05% a.i.) and its mixture with mancozeb (0.15% a.i.) retarded significantly disease development by* Phytophthora *when used as a curative spray. Etridiazole (0.1% a.i.) and efosite-Al (0.1% a.i.) gave moderate therapeutic control while captafol gave no therapeutic control. No phytoxicity was observed with Ridomil MZ, Terrazole and Aliette up to concentrations of 0.5% a.i., 0.4% a.i. and 0.8% a.i. respectively. The Ridomil products, Aliette and Terrazole exhibited similar rates of inhibition of linear extension of* P. palmivora *but differed in the degree of* in vitro *fungitoxicity. The Ridomil products and Terrazole had* ED_{50} *values of less than 1 µg/ml while Difolatan was relatively less toxic (*ED_{50} *19.9 µl/l).*

INTRODUCTION

The small scale commercial cultivation of orchids in Malaysia does not do justice to the favourable climate, the adequate land and labour and the abundance of a wide diversity of potential commercial hybrids. The recent estimate of Malaysia's export of orchids amounts to a meagre M$2.57 million a year compared to the total of M$43.5 million exported, by ASEAN countries (Alvensleben, 1980) or to Singapore's M$13.7 million, although about 70 percent of Singapore's

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exports are actually supplied by Johore growers (ANON, 1980). The phlegmatic growth of our orchid export industry could be attributed to many factors such as the lack of well coordinated market facilities, lack of a strong rapport with foreign importers, the high risk involved and the expensive nature of orchid cultivation. In addition, the popularity of orchids does not spare them from devastation by insect pests and diseases.

In Malaysia, viruses and fungi constitute the most important and widespread pathogens of orchid diseases (Lim, 1980). Of the latter group, *Phytophthora palmivora* Butler, appears to be the most important, causing root rot and crown rot (synonymous to heart rot, orchid blight). *P. palmivora* was first reported on *Vanda* orchids by Thompson in 1958. Recently, the pathogen was also isolated from *Aranda* Christine hybrids, *Aranda* Deborah, *Aranda* Wendy Scott var Greenfield, *Aranthera* James Storei, *Arachnis* Maggie Oei and several *Vanda* hybrids (Lim 1980). Since the work by Thompson (1958, 1959) very little has been done on *Phytophthora* diseases on orchids and its control. Fungicides such as Perenox (Thompson, 1958) or Difolatan recommended for controlling *Phytophthora* on orchids are preventive rather than curative, besides yielding inconsistent results. In this paper, some aspects of the efficacy and phytotoxicity of some new systemic fungicides on orchids are presented.

MATERIALS AND METHODS

Phytophthora palmivora Butler isolated from *Aranda* Christine hybrid, designated P-90-2 was used as it was found to be the most virulent isolate on the *Aranda* hybrids tested (Lim, 1981 unpublished data). The fungus was maintained in the dark at 25±1.2°C on vegetable juice agar (VJA) composed of 10% Campbell's V-8 juice, 0.2% $CaCO_3$ and 2% agar (Miller, 1955).

The chemicals selected for the series of tests include Ridomil(R) 25% W.P. (metalaxyl), Ridomil MZ(R) 58% WP (metalaxyl plus mancozeb), Aliette(R) 80% W.P. (efosite-Al or phosetyl aluminium), Terrazole(R) 35% W.P. (etridiazole) and Difolatan 4F(R) 39% flowable (captafol) unless otherwise stated.

In vitro-efficacy test

Linear extension of *P. palmivora* Butler from a 9mm-diameter agar plug on VJA incorporated with various concentrations (0 to 50 μg/ml) of the above fungicides except Aliette was measured after five days at 25±1.2°C. Aliette was excluded as it exhibited negligible *in vitro*-fungitoxicity against local *Phytophthora palmivora* strains from various hosts (Lim and Varghese, 1979). The results were subjected to probit analysis (Finney, 1971) whereby the percent inhibition of linear growth was converted to probit inhibition which was then plotted against the log concentration of the test chemicals to compare the rates of inhibition (slope) and the ED_{50} values of the fungicides. The ED_{50} value represents the amount of fungicide required for 50% inhibition of growth.

In vivo-prophylactic efficacy test

The systemic fungicides viz. Ridomil(R) at 0.05% a.i., Ridomil MZ(R) at 0.15% a.i., Aliette(R) at 0.2% a.i. and Terrazole(R) at 0.1% a.i. were screened against the standard protectant fungicide Difolatan 4F(R) at 0.2% a.i. for both prophylactic and therapeutic efficacy. Two month-old *Aranda* Wendy Scott and *Aranda* Christine hybrids, about 50 cm in height grown in pots in the open, were used for the prophylactic and therapeutic tests respectively. Each chemical was screened in three replicates of ten plants per replicate and the design used in both tests was a randomized complete block design. The plants were sprayed to run-off with 20 ml of the test chemical using a 2 liter volume pressurized handsprayer. The plants in the blank check treatment were sprayed with water minus the chemical.

For the prophylactic test, the plants were sprayed with the test chemical three days before inoculation. Inoculation was done by spraying the paired-crown leaves with *P. palmivora* sporangial suspensions and by placing VJA agar discs of the fungus from the periphery of five-day old cultures in the axils of the paired crown leaves. Inoculated plants were covered with polyethylene bags for 48 hours. Disease development was recorded at weekly intervals and the percent infected crowns at two weeks after inoculation were transformed ($\sqrt{\chi + 0.5}$) and analysed using an analysis of variance and the New Duncan Multiple Range Test for signifcance between means (P=0.05) (Thomas and Hill, 1975). The mean percentage disease control (MPDC) was also determined from the following formula (Horsfall and Baratt, 1945):

$$MPDC = \frac{\text{Mean Percent Disease Incidence in Control} - \text{Mean Percent Disease Incidence in Treatment}}{\text{Mean Percent Disease Incidence in Control}} \times 100$$

In vivo-therapeutic efficacy

For the therapeutic studies, the plants were sprayed twice with the test chemicals at five-days and 19 days after inoculation which was done as described previously. The progress of *Phytophthora*

infection down the stem from the point of inoculation at the uppermost crown leaves was measured at weekly intervals after the second spray. As the disease advanced downwards, the strap leaves in the infected region wilted and discoloured, turning necrotic. Five weeks after inoculation the stem was sliced longitudinally and the final length of the disease lesion was measured from the uppermost apical leaves *(Plate 1)*. Data were analysed using analysis of variance and significant differences between treatment means were tested at P=0.05 using New Duncan Multiple Range Test. In addition, the therapeutic value of the chemical was determined from the formula:

$$\text{Therapeutic value} = \frac{\text{Mean length of stem lesion in check} - \text{Mean length of stem lesion in treatment}}{\text{Mean length of stem lesion in check}} \times 100$$

Phytoxicity studies

Aranda Christine hybrid plants were separately sprayed with Ridomil MZ, Terrazole and Aliette used at rates up to 0.5% ai, 0.4% a.i and 0.8% a.i. respectively. Plants were sprayed to run-off twice at weekly intervals and observed for phytotoxicity symptoms weekly till three weeks after the last spray.

RESULTS

In vitro-efficacy

The fungicides tested were found to have varying degrees of fungitoxicity (Table 1) but similar rates of inhibition of linear extension of *Phytophthora palmivora* strain from *Aranda* orchid *(Fig. 1)*. The rates for inhibition (slope values) of *P. palmivora* for the various chemicals were 1.25 for Ridomil and Ridomil MZ and about 1.16 for both Difolatan and Terrazole. Ridomil

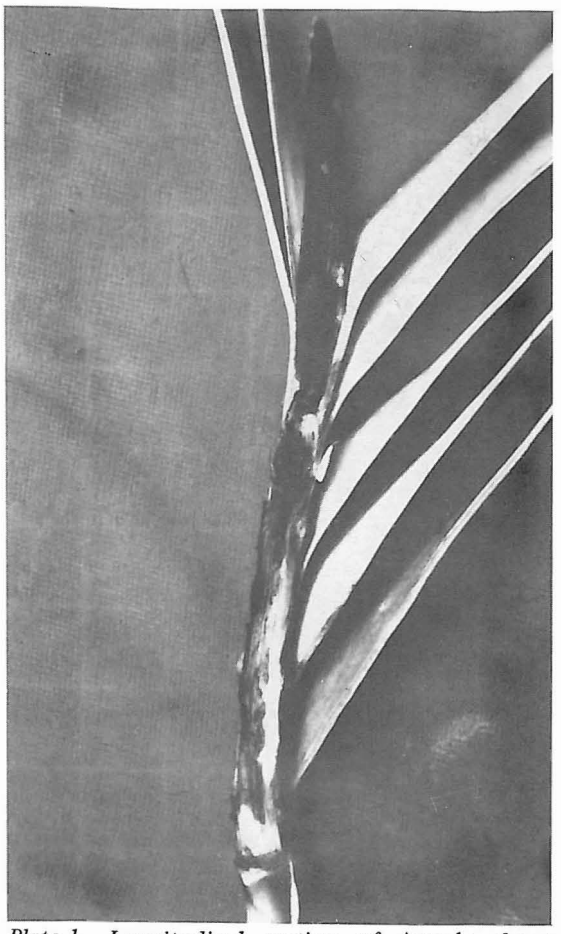

Plate 1. Longitudinal section of Aranda *shoot showing the advance of* Phytophthora *infection from the apex five weeks after inoculation.*

25% W.P. was the most toxic with an ED_{50} value of 0.045 μg/ml. The mixture product of metalaxyl plus mancozeb (Ridomil MZ 58% W.P) and etridia-

Table 1
In vitro toxicity of chemicals to *Phytophthora palmivora* based on the ED_{50} value and rate of inhibition of mycelial growth.

Fungicide	ED_{50} value (μg/ml or μl/l)	Rate of inhibition (slope value)
Ridomil 25% W.P.	0.045	1.25
Terrazole 35% W.P.	0.266	1.16
Ridomil MZ 50% W.P.	0.334	1.25
Difolatan 4F	19.95	1.15

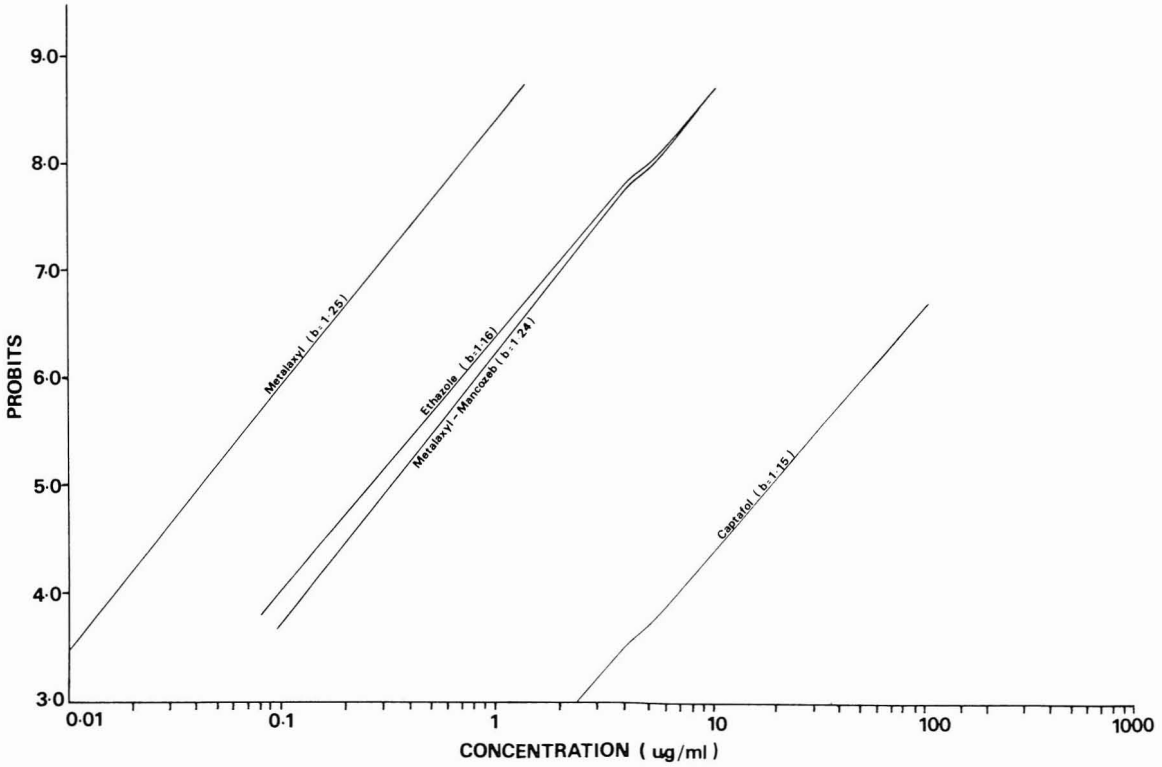

Fig. 1. Log-probit curves of chemicals showing the rate of inhibition(b) on Phytophthora palmivora.

zole (Terrazole 35% W.P.) were also highly potent with an ED_{50} of 0.33 μg/ml and 0.27 μg/ml respectively. Captafol (Difolatan 4F) had an ED_{50} of 19.95 μl/l.

Complete inhibition of growth of *P. palmivora* occurred at concentrations of <2 μg/ml for Ridomil 25 WP and ≏ 10 μg/ml for both Ridomil MZ 58% W.P. and Terrazole 35 WP. Mycelial distortion was observed at concentration as low as 0.1 μg/ml for Ridomil 25 W.P. and 0.5 μg/ml for Terrazole and Ridomil MZ.

In vivo-prophylactic efficacy

The results (Table 2) indicated that the fungicides varied greatly in their efficacy in

Table 2
Prophylactic efficacy of fungicides on *Phytophthora palmivora* infection on *Aranda* orchid*

Chemical	Rates used % a.i.	Mean percent infected Crown*	Mean percent Disease Control (MPDC)
Ridomil 25 W.P.	0.05	0 d	100
Ridomil MZ 58% W.P.	0.15	0 d	100
Difolation 4F (39%)	0.2	13.33 cd	81
Aliette 80% W.P.	0.2	33.33 bc	52
Terrazole 35% W.P.	0.1	60.00 ab	14
Water check	–	70.00 d	0

* Means of 3 replicates consisting of 10 plants per replicate taken 2 weeks after inoculation. Means followed by similar alphabet are not significantly different at P = 0.05.

protecting the *Aranda* Christine hybrids. Disease lesions developed on the unprotected plants three to four days after inoculation. Crown infection in the sprayed plants ranged from 0% to 60%. Ridomil at 0.05% a.i. and Ridomil MZ at 0.15% a.i. gave the best protection (MPDC = 100%) when sprayed three days before inoculation. Difolatan at 0.2% a.i. also exhibited good protection (MPDC 81%) which was not significantly different to the protection provided by the metalayxl products. Aliette at 0.2% a.i. gave only moderate protection while Terrazole at 0.1% a.i. gave significantly the lowest protection of only 14% MPDC.

In vivo-therapeutic efficacy

From Table 3, it is evident that Ridomil MZ at 0.15% a.i. and Ridomil at 0.05% a.i. provided the best mitigation of disease severity after two sprays at five days and 19 days after inoculation. Approximately five weeks after inoculation, the disease lesion advanced only 8.68 cm and 9.98 cm from the site of inoculation for Ridomil MZ and Ridomil respectively. The weekly rates of disease development in the inoculated plants sprayed with water were more than two times faster than those observed for the metalaxyl products. Aliette at 0.1% a.i. provided moderate therapeutic activity while Terrazole at 0.1% a.i. and Difolatan at 0.2% a.i. provided negligible therapeutic control. Disease development in the inoculated plants sprayed subsequently with Terrazole or Difolatan were the most severe and were not significantly different from the inoculated check plants.

Phytoxicity

No phytoxicity symptoms in the form of chlorosis, spotting or scorching were observed on the *Aranda* Christine hybrids after spraying with Ridomil MZ, Terrazol and Aliette at the high concentrations of 0.5% a.i., 0.4% a.i. and 0.8% a.i. respectively. However, heavy residues were observed on the plants sprayed with Ridomil MZ and Aliette.

DISCUSSION

The results indicated that both Ridomil 25% W.P. and Ridomil MZ 58% W.P. provided a similar magnitude of protectant and therapeutic efficacy which was more superior than the other chemicals tested on *Phytophthora* crown rot in *Aranda* orchids. This is not surprising as Ridomil 25% WP when used at 0.05% a.i. contains the same proportion of the active moiety metalaxyl as Ridomil MZ 58% W.P at 0.15% a.i. Metalaxyl at 0.05% a.i. also provided good preventive and therapeutic control of cocoa seedling dieback caused by *Phytophthora palmivora* (Lim and Ang, 1980). The excellent protectant and therapeutic activity of metalaxyl could be attributed to its high inherent fungipotency against *Phytophthora* spp and its apoplastic and symplastic translocation in plant tissues after penetration (Staub, Damen and Schwinn, 1978).

Despite its negligible *in vitro* fungitoxicity against local *Phytophthora* spp. (Lim and Varghese, 1979), one pre-inoculation spray and two post-inoculation sprays of Aliette at 0.1% a.i. gave moderate preventive and therapeutic control of *P. palmivora* on orchids respectively. This implies that one or two applications of Aliette is not adequate for effective control. This observation is further supported by studies in Thailand where Aliette at 1.0g a.i./litre (i.e. 0.1% a.i.) applied in high volume at weekly intervals effectively con-

Table 3
Therapeutic efficacy of fungicides on *Phytophthora palmivora* infection on *Aranda* orchid.

Chemical	Rates used (% a.i.)	Mean Length of infected Stem* (cm)	Therapeutic value
Ridomil MZ 58% W.P.	0.15	8.68c	40.6
Ridomil 25% W.P.	0.05	9.98 bc	31.1
Aliette 80% W.P.	0.1	11.32 b	22.7
Terrazole 35% W.P.	0.1	13.29 a	8.9
Difolatan 4F	0.2	14.29 a	2.3
Water Check	0.0	14.63 a	–

* Mean of 3 replicates consisting of 10 plants per replicate taken 33 days after inoculation. Means followed by similar alphabet are not significantly different at P = 0.05.

trolled established *Phytophthora* spp in *Vanda* TMA (Beach, 1979).

Difolatan had no therapeutic value against *Phytophthora* infection in orchids but provided excellent protection for 17 days which was as good as that provided by the metalaxyl treatments when used at 0.2% a.i. despite the fact that the plants were heavily watered every day. The high prophylactic value of Difolatan could be attributed to many factors such as its good tenacity and resistance to ambiental degradation.

Contrary to the manufacturer's claim that Terrazole at 15 gm per 4.5 litre (ie. 0.1% a.i) can control *Phytopthora* crown rot in orchids up to three to four weeks with one single application, our results showed that under daily watering, one application of Terrazole at 0.1% a.i. did not give satisfactory preventive or therapeutic control of crown rot. Cultural practices that can alter disease pressure can influence fungicide efficacy and hence account for variation in results among studies.

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