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# Effect of Fungicides on the Viability of *Phytophthora cactorum* Propagules in the Soil

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## Abstract

Out of eleven fungicides, Ridomil, Aliette (systemic) and Euparen M were most effective in inactivating the fungus mycelium in the soil within 2 days. They also completely inhibited the production of sporangia and oospores. These fungicides and Antracol also reduced the viability (50—100 %) of sporangia in the soil. They also reduced the viability of oospores along with Mancozeb, Zineb and liquid Ziram. Euparen M, Antracol, Mancozeb, Zineb and liquid Ziram completely killed zoospores; Ridomil, Previcur and Aliette were also effective. These fungicides can thus reduce the inoculum potential of the pathogen in the soil and reduce the chances of new infections.

## Zusammenfassung

### Wirkung von Fungiziden auf die Lebensfähigkeit von *Phytophthora cactorum* im Boden

Drei von elf Fungiziden (Ridomil, Aliette und Euparen M) inaktivierten das Myzel des Pilzes innerhalb zwei Tagen und hemmten die Bildung von Sporangien und Oosporen. Diese Fungizide und Antracol verminderten die Lebensfähigkeit der Sporangien im Boden (50—100 %) und diejenige der Oosporen (neben Mancozeb, Zineb und Ziram). Euparen M, Antracol, Mancozeb, Zineb und Ziram töteten Zoosporen vollständig; Ridomil, Previcur und Aliette wirkten teilweise. Diese Fungizide können so das Inokulumpotential des Pilzes im Boden und damit die Gefahr neuer Infektionen vermindern.

*Phytophthora cactorum* is recognized as the major pathogen involved in the collar and crown rot of apple trees in the world (BAINES 1939, MCINTOSH 1975). Since the pathogen survives in the soil in the form of resistant propagules including dormant mycelium, oospores and sporangia (SNEH and

McIntosh 1974), a reduction in their numbers can help in reducing its inoculum potential and thus control the disease outbreaks. Zoospores of the pathogen also play a major role in its short-term survival and are incitants of new infections (McIntosh 1972). The present studies were undertaken to see the efficacy of some selective fungicides including systemic, against the various pathogen propagules in the soil. Similar attempts have earlier been made only against zoospores (McIntosh 1971).

## Materials and Methods

Suspensions of eleven test fungicides (Table 1) were thoroughly mixed in separate soil lots to give the desired concentrations of the toxicant (w/w). Sandy loam soil at pH 7.5 with 45 % water holding capacity (WHC) was used. The soil moisture content was adjusted to 40 % and the treated soil lots were kept in separate polythene bags to reduce the loss of moisture. In control treatment only tap water was added.

For the production of fungus mycelial mats, sunhemp seed extract broth (McIntosh 1966) was taken in 150 ml flasks and inoculated by pipetting mycelial fragments from a homogenized culture. These flasks were incubated in the dark at room temperature (16—25 °C) for five days. No sporangia or oospores were apparent at this interval. These mycelial mats were washed with distilled water and used in further studies.

For the production of sporangia, the mycelial mats were transferred to 0.1 M $KNO_3$ solution in 250 ml glass beakers. Within two days, large numbers of sporangia were produced which were separated by shaking and passing the suspension through two layers of cheese cloth. Subsequently the filtrate was centrifuged (1000 g for one minute), washed with distilled water and then used.

Oospores were produced in clarified oat meal extract in petri dishes. The inoculated dishes were incubated in the dark at 27 ± 1 °C. Within one week, large numbers of oospores were produced which were separated from the mycelium by homogenisation after three months.

### Mycelium lysis and sporulation

Mycelial mats were placed in two folds of polyester cloth which were kept in the fungicide treated soil in polythene bags. Each treatment was replicated thrice. The observations on the mycelial lysis and sporulation were taken at 40 × magnification after 2 and 5 days incubation at room temperature (15—25 °C). A part of the mycelium was placed on the selective nutrient agar medium (Sneh and McIntosh 1974) to know its viability. The degree of lysis of the mycelium was classified into eleven grades from 0 (no lysis) to 10 (complete lysis) according to the percentage of hyphae lysed based on observations in 10 microscopic fields. The number of sporangia and oospores produced in each case was also recorded.

### Viability of sporangia

Sporangia were placed in two folds of polyester cloth discs and kept in the fungicide treated soil by the same method as in case of mycelium. After 24 h incubation at room temperature (15—25 °C), the sporangia were recovered from the soil and incubated on a selective nutrient agar medium at 27 ± 1 °C for germination. After 48 h, these were washed on the glass slides and examined for their germination at 40 × magnification. The observations were based on 10 microscopic fields and each treatment was replicated thrice.

### Viability of oospores

Three months old oospores of *P. cactorum* were placed in the fungicide treated soil in [illegible] folds of polyester cloth discs, as described earlier. After 24 h storage at room temperature (16—25 °C), these were recovered and incubated on the selective nutrient medium at

27±1 °C for 72 h. The oospores were then washed on the glass slides and examined for germination under the microscope at 40 × magnification. Observations were based on counts from 10 microscopic fields and each treatment was replicated thrice.

### Viability of zoospores

For production of zoospores, the fungus was grown on white bean agar medium in culture dishes. After five days of incubation at 23±1 °C, the colonies were flooded with distilled water. The water was changed twice daily and after 2—3 days, sporangia were produced in large numbers. The plates were then kept at 4 °C for 30 minutes to enhance the release of zoospores. Suspensions were poured from the plates and passed through four layers of cheese cloth to remove mycelial fragments and dehisced non-germinated sporangia. The zoospores so obtained were mixed in the fungicide treated soil. Fungicides were mixed in water at such concentrations that 20 ml added to 200 g non-sterile air dry soil would provide the desired concentrations of the toxicant (w/w). Control soil received water only. Fifteen ml of zoospore suspension were added to each lot of the treated as well as control soil and the moisture was adjusted to 40 %. After thorough mixing, the soil lots were stored in polythene bags for 24 h at room temperature (16—25 °C). Each treatment was replicated thrice. The viability of the zoospores was determined by the dilution plate method (McIntosh 1972). Ten g soil (air dry equivalent) were taken and sufficient water was added to make 100 ml, thus making 1 : 10 dilution. One ml aliquots from each were pipetted into 70 mm culture dishes containing selective nutrient agar medium. After 24 h incubation at 26±1 °C, the soil was washed from the plates and 1 ml of Rose bengal (50 ppm) solution was added. After additional 48 h the colonies of *P. cactorum* were counted. The composition of the selective nutrient agar medium was as follows: $KH_2PO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $CaSO_4 \cdot 2H_2O$, 0.1 g; Thiamine HCl, 0.02 g; DL-threonine, 1 g; sucrose, 20 g; Agar agar, 20 g; and distilled water, 1000 ml. Benomyl dissolved in dimethyl sulfoxide (DMSO) was added into the molten medium before pouring to give a concentration of 20 ppm benomyl and 0.5 % DMSO. In addition to this, chloroamphenicol, Penicillin G (60 ppm each) and PCNB (100 ppm) were also added into the medium.

## Results

### 1. Effect of fungicides on the lysis of *P. cactorum* mycelium in the soil

Out of the eleven fungicides mixed in the soil, three namely, Aliette (0.20 %), Ridomil (WP) (0.02 %) and Ridomil 5 g (0.20 %) made the mycelium unviable within 2 days (Table 1). In other fungicide treatments the mycelium remained viable till this time.

After five days, the mycelium remained viable only in case of Emisan (0.02 %) and untreated control soil. In the control and in soil treated with Aliette, Manocozeb (0.2 %) and liquid Ziram (0.02 %) mycelium lysis was not apparent after two days whereas 40 % of the mycelium was lysed in previcur (0.2 %) and 30 % in Euparen M (0.1 %) treated soils at the same interval. After 5 days, maximum lysis was observed in case of Panoctin (90 %) and in other fungicides, it was 80 % except Aliette (0.20 %) and Antracol (0.05 %) where only 40 % and 70 % mycelium was lysed respectively. In the control treatment there was 80 % lysis.

### 2. Effect on sporulation

Sporangia were not produced in the mycelium kept in fungicide treated soil after 2 days. After five days sporangia were not produced in the soil

*Table 1*
Effect of fungicides on the lysis of *P. cactorum* mycelium in the soil

| Fungicide | Concentration* (%) | Degree of lysis after days | | Viability of mycelium | |
|---|---|---|---|---|---|
| | | 2 | 5 | 2 | 5 |
| Aliette** [Aluminium ethylphosphide] | 0.20 | 0 | 4 | — | — |
| Ridomil** [N-(2,6-dimethyl-phenyl)-N-(Methoxy-acetyl)alaninemethylester] | 0.02 | 1 | 8 | — | — |
| Ridomil** [5 % granules] | 0.20 | 2 | 8 | — | — |
| Euparen M [N-dichlofluoro-methane sunfenyl-N' N'-dimethyl-N-p-tolylsulphamide] | 0.10 | 3 | 8 | + | — |
| Emisan-6 [Mercurychloride (6 % Hg)] | 0.02 | 1 | 8 | + | + |
| Panoctin [Di-(guanidino-octyl)amine] | 0.05 | 2 | 9 | + | — |
| Antracol [Zinc propylenene bisdithio-carbamate] | 0.05 | 2 | 7 | + | — |
| Previcur** [N-(3-dimethyl-aminopropyl)-thio-carbamic acid-S-ethylester hydrochloride] | 0.20 | 4 | 8 | + | — |
| Mancozeb [Manganous-ethylene bisdithio-carbamate (78 %) + Zinc ions (2 %)] | 0.02 | 0 | 8 | + | — |
| Zineb [Zinc ethylenebisdithio-carbamate] | 0.02 | 1 | 8 | + | — |
| Liquid Ziram [Zinc dimethyl-dithiocarbamate] | 0.02 | 0 | 8 | + | — |
| Control | | 0 | 8 | + | + |

* = Concentration of commercial formulation. (+) = Fungus viable.
** = Systemic in nature. (—) = Fungus dead.

treated with Aliette, Ridomil and Ridomil 5 G. The number of sporangia produced after five days was maximum in case of Zineb treated soil (27.4) followed by liquid Ziram (24.7) and these were higher than untreated soil (23.3) whereas in Panoctin treated soil the number was equal to that in control (23.3). In Euparen M treated soil, the sporangia number was minimum (9.2). It was followed by Antracol (10.2) and Previcur (17.0). In Mancozeb and Emisan treated soils, the number was slightly less than the control. Oospore production was scanty both in the fungicide treated as well as untreated soil.

### 3. Effect on the viability of sporangia in the soil

Euparen M completely reduced the viability of sporangia kept in the soil. Antracol gave 95.1 % inhibition of sporangial germination followed by Ridomil (87.8), Ridomil 5 G (86.6) and Aliette (78.5). Zineb (74.9), Mancozeb and liquid Ziram (64.6) were also quite effective. In the control soil 82 % sporangia were viable. Previcur was least effective and Emisan was slightly better.

### 4. Effect on viability of oospores

None of the eleven fungicides could inhibit the oospore germination completely after 24 hours of their addition to the treated soil. The reduction in the viability of oospores was however maximum (74.7) in Mancozeb treated soil followed by liquid Ziram, Euparen M and Anthracol (67.2). In case of Ridomil and Zineb treated soils, the per cent reduction was 63.8 while in Aliette and Ridomil 5 G treated soil, it was 55.8 and 62.9 respectively. Minimum reduction of germination was in case of Panoctin (32.8). In the untreated soil, 58 % oospores germinated after 24 hours of incubation.

### 5. Effect on the viability of zoospores

No zoospores could be recovered in the dilution plates from the soil treated with Euparen M, Antracol, Mancozeb, Zineb and Ziram. This was followed by Ridomil and Previcur which gave 67.8 and 69.1% reduction in the recovery of zoospores respectively. Next in efficacy were Emisan (60.2), Aliette (59.7) and Ridomil 5 G (50.0). Pancotin proved almost ineffective in killing the zoospores in the soil within 24 hours. In the control, 787 zoospores per g soil were recovered after 24 hours.

## Discussion

Aliette and Ridomil seem to be the most effective fungicides in reducing the viability of *P. cactorum* in the soil. These two fungicides completely inactivated the fungus mycelium in the soil within two days; other fungicides were less effective. The lysis of the mycelium was not affected much by the addition of fungicides in the soil. In Aliette treated soil, though the mycelium was killed faster, the rate of lysis was slow thereby indicating that the fungicide may be effective against other soil microorganisms which play some role in the lysis (SNEH and MCINTOSH 1974). Effect of fungicide on the autolytic enzymes of the fungus seems inevitable since the mycelium was inactivated within 2 days (SNEH and MCINTOSH 1974).

Sporulation of the fungus which normally starts within 24 hours of the addition of mycelium into the soil was completely inhibited by three fungicides (Ridomil, Ridomil 5 G and Aliette). In addition to these, Euparen M and Antracol were also quite effective in reducing the production of sporangia and oospores. In addition to these effects fungicides also affected the survival of sporangia, oospores and zoospores in the soil. Sporangia lost viability com-

pletely with Euparen M within 24 hours. Antracol, Ridomil, Ridomil 5 G and Aliette were also very effective in reducing their viability within same duration. Euparen M, Antracol, Ridomil and Mancozeb were also quite effective in reducing the viability of oospores in the soil. Other fungicides (liquid Ziram, Zineb and Aliette) also gave sufficient reduction in viability of oospores within 24 hours of their addition to the treated soil. Euparen M, Antracol, Mancozeb, Zineb and Ziram completely killed the zoospores in the soil. Other fungicides were also quite effective giving more than 50 % reduction in the recovery of zoospores. Previcur, though little effective against the other propagules, gave sufficient reduction in the recovery of zoospores.

These fungicides seem to reduce the propagule density of the pathogen in the soil by reducing their production as well as their viability. These fungicides can also check new infections if they are applied in the spring when the activity of the pathogen and the susceptibility of the host are higher (SEWELL *et al.* 1979) and thus effectively reduce the disease incidence in the field.

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