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Investigation note

Efficacy of benomyl and folpet on *Fusarium oxysporum* a strawberry pathogen

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Abstract

In Costa Rica, the strawberry crop is important due to the generation of jobs in rural areas; nevertheless, it has been affected by fungal pathogens that have caused reductions in the area planted. The fungus *Fusarium oxysporum* is associated with wilt and collapse of plants, which is why the *in vitro* biological efficacy of the fungicides benomyl and folpet was tested, in three concentrations, to determine if it is possible to use them as a combat alternative. The research was carried out in the Phytopathology Laboratory of the Fabio Baudrit Moreno Agricultural Experimental Station, Alajuela, Costa Rica in 2021. By means of the poisoned food technique, the growth diameters of the mycelium of three strains of the fungus, exposed in quintuplicate to the commercial dose, the tenth and hundredth part of this, as well as to a treatment without fungicide, were compared. The percentage of growth inhibition caused by each dose was then calculated. Benomyl showed 100% inhibition at all doses for the three strains, while folpet reached a maximum of 60% in the commercial dose. According to the results, *F. oxysporum* strains isolated from strawberry plants with wilt symptoms respond differentially to benomyl and folpet.

Keywords: *Fragaria* × *ananassa* Duchesne ex Rozier, Costa Rica, wilt.

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Strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier) is a small-scale crop in Costa Rica, there are about 300 production systems between 1-5 ha (INEC, 2015). According to Zumbado *et al*. (1994), it is an activity that requires 12 day's wages per each hectare planted to cover the work of nursery, production, harvest and packaging; in addition, per each day's wage there is a generation of five indirect jobs, which is why it is an important activity for the communities of Llano Grande of Cartago (main growing area), Poás and Fraijanes in Alajuela, Los Santos in San José and Vara Blanca in Heredia (SNITTA, 2017).

Since 2014, the incidence of a phytosanitary problem in the strawberry crop has been increasing, which has caused many small producers to leave this activity, which translates into economic and social problems for these families and their employees, since the existing sources of employment in the producing areas have been reduced by at least 1 800 (Avendaño, 2017; Zuñiga, 2017). One of the pathogens associated with this symptomatology in Costa Rica is *Fusarium oxysporum* Schltdl. sensu lato (Granados-Montero *et al*., 2022). In the strawberry crop, this fungus can produce 60% plant loss (Golzar *et al*., 2007).

Among the symptoms are reduction in growth, ascending wilt, necrosis in the roots and crown, reduction in the number of fruits and eventual death of the plant, which Williamson *et al*. (2012); Koike *et al*. (2013) associate with *Fusarium oxysporum* f. sp. *fragariae* (Fof). According to Koike and Gordon (2015), there are no effective management strategies against this disease; however, in Costa Rica, strawberry growers frequently use at least seven molecules for the chemical management of wilt (Zúñiga, 2020. Per. Commun.), among them benomyl and folpet. Therefore, the objective of this research was to know the biological efficacy of these two active ingredients in three concentrations, on the mycelial growth of *Fusarium oxysporum* sl. isolated from strawberry plants with symptoms of wilt.

The test was carried out in the Phytopathology Laboratory of the Fabio Baudrit Moreno Agricultural Experimental Station (EEAFBM, for its acronym in Spanish), of the University of Costa Rica. Located in La Garita of Alajuela, Costa Rica. Three strains of *F. oxysporum* s.l. (F2, F239 and F352) were selected, which were identified by means of the nuclear ribosomal DNA internal transcribed spacer nrDNA (ITS) with the primers ITS 5 and ITS 4 (White *et al*., 1990). These were isolated from necrotic roots of strawberry plants with wilt symptoms, collected in Llano Grande and Vara Blanca, Costa Rica, in 2017. Granados-Montero *et al*. (2022) verified the pathogenicity of the F2 strain in strawberry plants of the Festival variety. The strains were stored in cellulose at 4 °C.

To produce the actively growing mycelium needed for the test, a section of the cellulose was taken and a Petri dish with 25% potato dextrose agar (PDA) was placed for 10 days in the dark at 24 °C.

The efficacy of the molecules benomyl (AFUNGIL 50 WP® commercial dose 400 g 200 L^{-1} water) was determined in three concentrations: the commercial dose (CD= 500 ppm), one tenth of the commercial dose (CD/10= 50 ppm) and one hundredth of the commercial dose (CD/100= 5 ppm) and folpet (Folpan 80 WG® commercial dose 125 g 200 L^{-1} water) in three concentrations: the commercial dose (CD= 1.01 ppm), one tenth of the commercial dose (CD/10= 0.1 ppm) and one hundredth of the commercial dose (CD/100= 0.01 ppm). In addition to a control treatment without fungicide.

The poisoned food technique described by Dhingra and Sinclair (1985) was used, which consists of placing 5 mm discs of actively growing mycelium in the center of a Petri dish with PDA medium plus the fungicide. Each dose per product was repeated five times. The measurement of mycelial growth was carried out after 10 days, time necessary for the control to occupy 100% of the Petri dish. Orthogonal diameters were quantified and an average by dose was obtained. With the data obtained, the percentage of mycelium growth inhibition (PMGI) was calculated according to Masiello *et al*. (2019), namely the difference between control growth and treatment growth divided by control growth and multiplied by 100.

The data were analyzed by means of an Anova, after corroboration of the assumptions, and a comparison of means through the Tukey test ($p \leq 0.05$). The two fungicides in the three concentrations were able to inhibit the mycelial growth of the three strains of *Fusarium oxysporum* sl., compared to the control (Figure 1). Benomyl achieved 100% inhibition of all strains at all doses, unlike folpet, which reached only 60% at the highest dose (CD). According to Castellanos *et al*. (2015), benomyl is considered very toxic for the strains evaluated, since it obtained more than 90% inhibition of mycelium, in contrast, folpet is slightly toxic, being in the range of 30-75% inhibition.

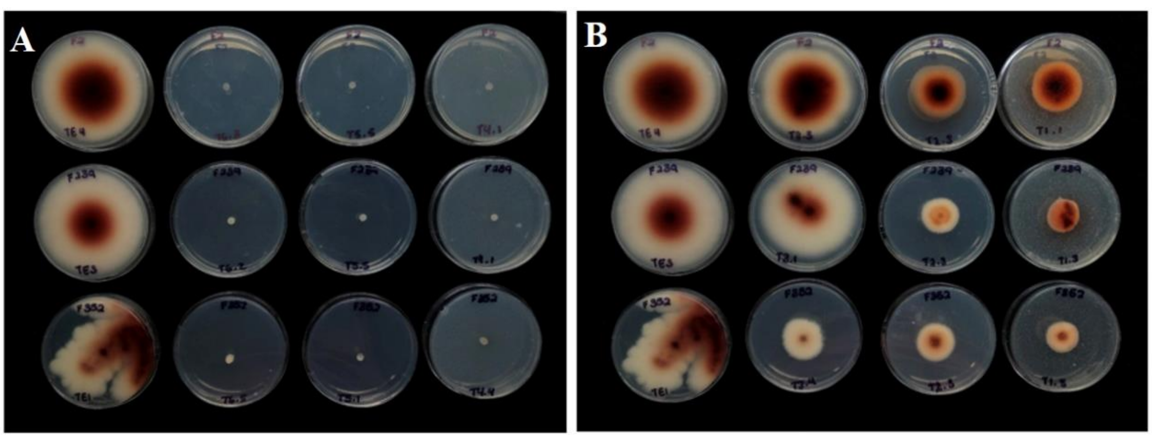

Figure 1. Mycelial growth of *Fusarium oxysporum* Schltdl. sensu lato (strains F2, F239 and F352) isolated from the roots of strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier) with wilt symptoms exposed to the active ingredient. A) benomyl at one hundredth of the commercial dose (DC/100= 5 ppm), one tenth of the commercial dose (DC/10= 50 ppm) and the commercial dose (DC= 500 ppm); and B) folpet at one hundredth of the commercial dose (DC/100= 0.01 ppm), one tenth of the commercial dose (DC/10= 0.10 ppm) and the commercial dose (DC= 1.01 ppm).

The efficacy of benomyl on *F. oxysporum* isolated from strawberry was reported by Cho and Moon since 1984, these authors indicated that doses of 100, 300 and 600 ppm inhibit 100% of mycelial growth, and that at the highest dose there is no sporulation. Then, in 1985, they reported that it prevents the germination of chlamydospores. This fungicide has been shown to be effective in inhibiting the development of this pathogen, also in other crops; for example, Yadav *et al*. (2017), for *F. oxysporum* recovered from soybean, found 100% inhibition in mycelial growth at 250, 500 and 1 000 ppm.

Other researchers have reported lower percentages of inhibition, as is the case of Bashir *et al*. (2017), who found for *F. oxysporum* f. sp. *capsici*, efficacies of 79, 8, 90, 2 and 93, 4 at doses of 300, 500 and 700 ppm of benomyl, respectively. Additionally, Rafique *et al*. (2016) reported 90% inhibition of mycelial growth of *F. oxysporum* f. sp. *lentis* at 100 ppm. There is little literature regarding the chemical management of Fusarium wilt of strawberries caused by *Fof*; probably due to the fact that, as mentioned by Koike and Gordon (2015), the strategies are oriented to preventive management, with an integrated management approach, fundamentally based on the use of resistant materials, since there are no effective curative treatments.

Recently, Coronel *et al*. (2022) found that *Fusarium solani* is also linked to wilt and death of strawberry plants and report 100% inhibition of mycelial growth *in vitro* at concentrations of 250, 500 and 750 ppm of benomyl. On the other hand, folpet showed a 60% efficacy of inhibition of mycelium growth at the recommended dose (Table 1). No references were found to the use of this active ingredient to combat wilt in strawberry, but Masiello *et al*. (2019) reported low efficacy of this active ingredient on the mycelial growth of three strains of *F. graminearum*, *Fusarium proliferatum* and *F. verticillioides*, thus, at 120 ppm they obtained 42%, 50% and 44% on average, respectively; while, at 1 200 ppm, the average efficacies were 64%, 61% and 72%, respectively, close to those obtained in this study.

Table 1. Percentage of average inhibition of mycelium growth of the three strains of *Fusarium oxysporum* Schltdl. *sensu lato.**

	CD/100	CD/10	CD
Benomyl	100 a	100 a	100 a
Folpet	23.9 b	56.5 b	60.4 c

*= exposed to one hundredth of the commercial dose (CD/100), one tenth of the commercial dose (CD/10) and the commercial dose (CD) of the active ingredients folpet and benomyl. The letters represent significant differences (Tukey, $p \leq 0.05$).

Folpet has been used for more than 55 years, while benomyl has been on the market for 40 years (Gupta, 2018). According to FRAC (2020), folpet is a multisite fungicide with low risk of developing resistance; for its part, benomyl is considered high risk and resistance has been achieved in many species of fungi. However, according to the results of this research, the *F. oxysporum* strains evaluated are sensitive to benomyl and resistant to folpet; which could be indicating that the latter molecule has been used more frequently for the management of the disease in Costa Rica. In fact, in accordance with the list of products registered for the crop, the State Phytosanitary Service (SFE, 2022) indicates that three formulations with active ingredient folpet are registered, recommended for the management of *Fusarium oxysporum* in strawberry, but no benomyl-based products.

This could explain, in part, the resistance of the strains studied to folpet. According to the above, an alternative for the chemical combat of wilt of strawberry plants could be the use of benomyl; nevertheless, it is important to perform germination tests of conidia and corroborate the efficacy with more doses and strains of the fungus to have greater statistical and biological support.

Conclusions

The fungus *Fusarium oxysporum* s.l. associated with the wilt of strawberry plants in Costa Rica showed differential sensitivity to fungicides with active ingredient benomyl and folpet.

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