Testing of germ-inhibiting effects of different essential oils on conidia of *Monilia fructigena* and *Blumeriella jaapii*

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Abstract

In March 2017 a new research project was started in the organic stone fruit sector. The project is split up into three parts: regulation of insects in sweet cherry orchards under roof (DLR Rheinpfalz), regulation of plum moth (DLR Rheinpfalz, ÖON Jork, LVWO Weinsberg). The third part deals with fungal diseases affecting sour cherries. From August to December conidia germinating tests were carried out in the laboratory of the pomicultural experimental station Heuchlingen (LVWO Weinsberg). Essential oils of Curcuma xanthorrhiza*,* Curcuma longa *(both extracted from rhizomes),* Zanthoxylum rhetsa *and* Zanthoxylum alatum *(both produced from fruits), Palmarosa (distilled from gras) and* Cinnamomi ceylanici *(extracted from the bark) were tested for their ability to inhibit conidial germination of* Monilia fructigena *and* Blumeriella jaapii*. Additionally, a powder form of Curcuma longa and a plant extract of Primula veris were tested.* Curcuma xanthorrhiza*,* Zanthoxylum alatum*,* Palmarosa*,* Cinnamomi ceylanici*, the powder form of* Curcuma *and* Primula veris *showed high efficacy under laboratory conditions.*

Keywords: Stone fruit, essential oils, *Monilia fructigena*, *Blumeriella jaapii*, germinating tests

Introduction

Cherry leaf spot is a fungal disease that infects mostly sour cherries. Premature fall of leaves weakens the trees and can result in low blossom setting in the next spring or in their death (Stegmeier *et al.* 2014). *Monilia fructigena* is the most important stone fruit disease worldwide (Fritsch 2009) and can cause great fruit losses. Only a few plant protection products are registered for use in organic stone fruit orchards (Rank, 2003). The fungicidal effect of essential oils has been demonstrated in several studies (detailed literature see bachelor thesis of Stoll, 2017). In this research project several essential oils were tested for potential to inhibit of conidia germination.

Material and Methods

The conidial germination tests were based on results of a bachelor thesis (Stoll 2017) and results of BÖLN-projects 02OE109 (Kollar et al. 2003) and 2809OE103 (Kollar et al. 2013). Conidia germination tests with *Blumeriella jaapii* were performed with the method of KOLLAR (2003), similar to tests with apple scab. Each conidia germination test included a control variant and two variants with the fungicide *Funguran progress* in two different concentrations (400 g copper/ha and 250 g copper/ha) for comparison with the other variants.

In August 2017 infected leaves of *Blumeriella jaapii* were collected in an organic orchard, dried and frozen. In order to get conidia of cherry leaf spot, frozen leaves were given into a bottle with distilled water and shaken on a horizontal shaker (300 mot./min) for 10 minutes. The number of conidia in the produced suspension varied from 40.000 to 60.000 conidia/ml. The conidia suspension was mixed with the test products, the essential oils were weighed

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(see tables 1+2). For the formulation of the essential oils Trifolio S-forte was added until a stable emulsion was found. 1 ml from this mixture was filled in sterile Cellstar-Multiplates, which were closed with a special tape. The plates were incubated 24 hours at room temperature.

Fruit mummies of organic grown apples and plums with symptoms of *Monilia fructigena* were collected. Fruiting bodies of infected mummies were scraped away and shaken with a horizontal shaker (300 mot./min) for 10 minutes in distilled water. A conidia suspension with 40.000-60.000 conidia/ml was produced. Test products and conidia suspension were mixed in decreasing concentrations. For conidia germination tests with conidia of *Monilia fructigena* another method (Joseph, 2011) had to be applied. 0.5 ml from the mixture was given on microscope slides and put into the plastic box, which was totally water-tight and air-tight. The plastic box had been stored for 48 hours at room temperature in the laboratory. One plant extract from the roots of *Primula veris* was produced. 50 g of the roots were heated in tap water, rested for 10 minutes and filtered. Six essential oils, *Curcuma* longa in powder form and a plant extract of *Primula veris* were tested in varying concentrations under laboratory conditions.

For each variant and replicate 100 conidia were classified as “not germinated”, “with a short germinating tube” or “with a long germination tube”. Each variant included 4 replicates (leaf spot) or 4 replicates (Monilia). Based on the percentage of the third class the efficacy according to ABBOTT had been calculated for each variant. In total 8 conidia germination tests with *Blumeriella jaapii* and 7 conidia germination tests with *Monilia* were performed up to now. The following tables are including results of 2 tests with *Monilia fructigena* and 2 tests with *Blumeriella jaapii* from the bachelor thesis of Stoll (2017).

In the first tests (both fungi) with powder of *Curcuma longa* the powder was filled into a tea-filter, stayed for 15 minutes in the suspension of conidia and was removed until the suspension was filled into the cellplates. In the following tests the powder remained during the whole time of germinating.

Results

Table 1 summarizes the main results of the conidia germination tests with *Blumeriella jaapii*.

Table 1: Efficacy (ABBOTT) of test products from conidia germination tests with *Blumeriella jaapii*.

test product (type of substance)	**Amount (g) per 50 ml conidia suspension**	**efficacy (%)**
Funguran progress (400 g cu/ha)	0,115	100 in nearly all tests
Funguran progress (250 g cu/ha)	0,072	100 in nearly all tests
Curcuma xanthorrhiza (oil)	0,250 / 0,200 / 0,210 / 0,150	76-100 / 74-97 / 74-100 / 71-74
Curcuma longa (oil)	0,400 / 0,350 / 0,300	88 / -2.4 / 16-100
Curcuma longa (powd).	0,500 / 0,450 / 0,400	87-100 / 67-100 / 73-100
Curcuma longa (powd).	0,350 / 0,300 / 0,250 / 0,200	80-100 / 100 / 100 / 100
Zanthoxylum rhetsa (oil)	0,500 / 0,250	100 / 71
Zanthoxylum alatum (oil)	0,500 / 0,300	100 / 100
Palmarosa (oil)	0,250 / 0,200 / 0,180	100 / 100 / 100
Cinnamomi ceylanici (oil)	0,250 / 0,200	100 / 100
Primula veris (pl.ext)	3 ml (= 6 %)	98 / 100

The best results were obtained with the essential oils (oil) *Curcuma xanthorrhiza*, *Zanthoxylum rhetsa*, *Zanthoxylum alatum*, Palmarosa, *Cinnamomi ceylanici, Curcuma*

longa in powder form (powd) and the plant extract (pl.ext) *Primula veris*. The efficacy of *Curcuma longa* used as essential oil varied.

Table 2 shows the main results of 7 conidia germination tests with *Monilia fructigena*. The best results were achieved with the essential oils *Curcuma xanthorrhiza*, Palmarosa, *Zanthoxylum alatum*, *Cinnamomi ceylanici*, *Curcuma longa* in powder form and a plant extract of *Primula veris*.

Table 2: Efficacy (ABBOTT) of test products from conidia germination tests with fungus *Monilia fructigena*.

test product (type of substance)	**Amount (g) per 50 ml conidia suspension**	**efficacy (%)**
Funguran progress (400 g cu/ha)	0,115	100 in nearly all tests
Funguran progress (250 g cu/ha)	0,072	100 in nearly all tests
Curcuma xanthorrhiza (oil)	0,275 / 0,250 / 0,225	16 / 21-100 / 76-83
Curcuma longa (oil)	0,400 / 0,300 / 0,290	100 / -24 / 34
Curcuma longa (powd).	0,500 / 0,450 / 0,400	61-100 / 72-100 / 70-100
Curcuma longa (powd).	0,350 / 0,300 / 0,250 / 0,200	100 / 100 / 100 / 100
Zanthoxylum rhetsa (oil)	0,500 / 0,350 / 0,300	98-100 / 100 / 59-79
Zanthoxylum alatum (oil)	0,500 / 0,450 / 0,400 / 0,350	92 / 93-100 / 78 / 17
Palmarosa (oil)	0,300 / 0,280/ 0,250	100 / 100 / 100
Palmarosa (oil)	0,200 / 0,180	100 / 88-95
Cinnamomi ceylanici (oil)	0,250 / 0,210 / 0,200 / 0,150	60-100 / 100 / 94-100 / 100
Primula veris (pl.ext)	3 ml	100

Between the germination tests with the same products there were some variations in efficacy. *Curcuma longa* used as essential oil showed too much variation in efficacy. When the powder of *Curcuma longa* was given directly in solution until evaluation of germinating, better results were achieved. With decreasing concentrations of *Zanthoxylum rhetsa* and *Zanthoxylum alatum* the inhibition was reduced.

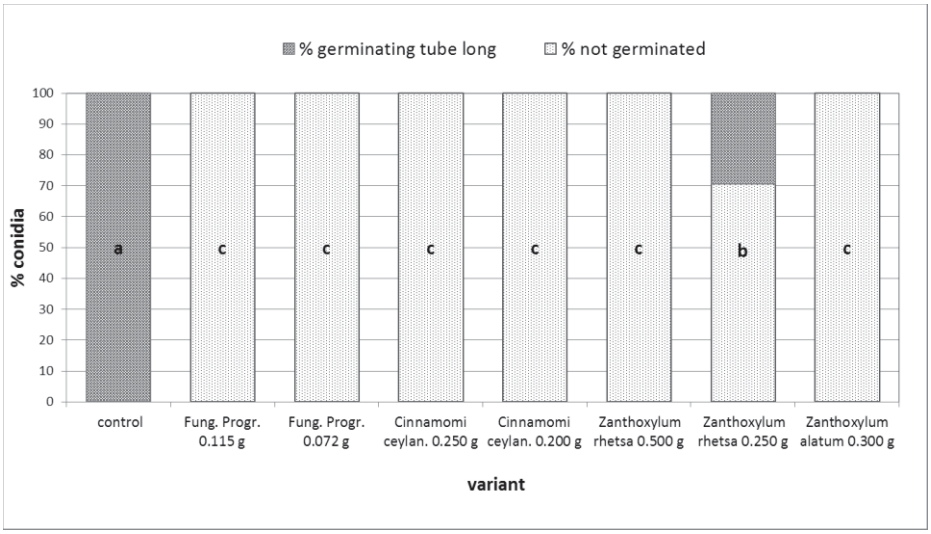

Figure 1: Percentage of conidia in three germinating classes, laboratory tests at *Blumeriella jaapii*, description of variants according to table 1.

In figures 1 and 2 the percentage of “not germinated”, conidia “with a short germinating tube” and conidia “with a long germination tube” were presented as example for two tests.

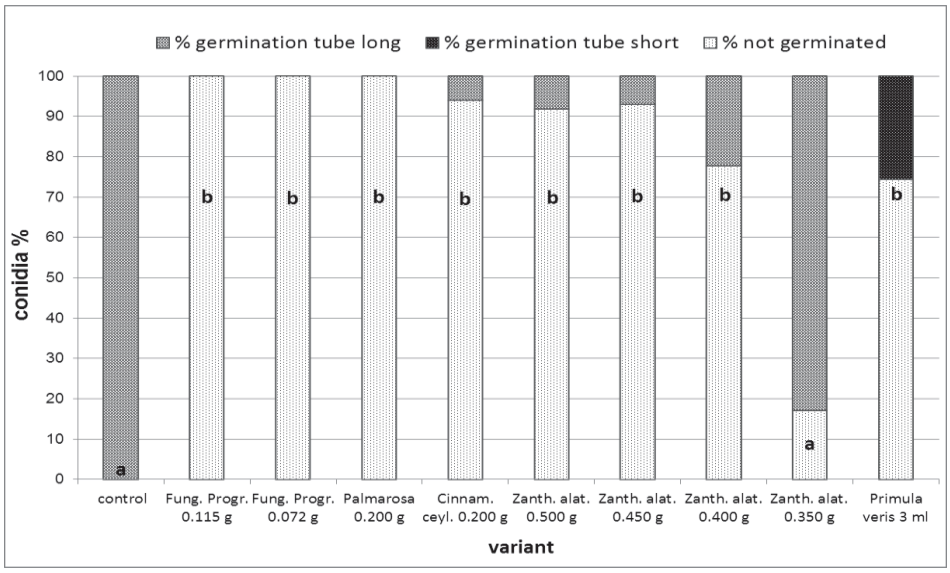

Figure 2: Percentage of conidia in three germinating classes, laboratory tests at *Monilia fructigena*, description of variants according to table 2.

Discussion

For both fungal diseases essential oils or extracts could be identified, which showed a good inhibiting effect on the germination of conidia in laboratory. Powder of Curcuma longa e.g. had a good efficacy in a wide range of concentration. For some extracts a concentration was found, under which the efficacy was decreasing. Further laboratory tests are planned for the next months with focus on possible variants for the trials in the orchard in spring and summer 2018. Side effects like phytotoxicity, stability and adhesion of the test products to the leaves will be evaluated.

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