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AN *IN VITRO* STUDY OF COMMERCIAL FUNGICIDE EFFECTS ON POLLEN GERMINATION IN APPLE

Sultan Filiz GUCLU[1], Fatma KOYUNCU[2]

[1]Süleyman Demirel University, Atabey Vocational School, Isparta, Turkey
[2]Süleyman Demirel University, Agricultural Faculty, Isparta, Turkey

Corresponding author email: sultanguclu@sdu.edu.tr

Abstract

In this study some fungicides effects (Captan, azoxystrobin, mycolobutanil, thiophanate - methyl, maneb) on pollen viability tests and in vitro pollen germination investigated in Red Chief's pollens. Pollen viability test was made by TTC (2, 3, 5-triphenyl tetrazolium chloride) 15% +0.5% agar-agar+5 ppm boric acid at 25^{0}C medium as control medium. Pollen germination was conducted at three concentrations: the recommended fields rate (100% RFR), 10% RFR and 1% RFR of each fungicide. 'Agar in plate' method was used for pollen germination tests. Statistical analyses performed with GLM models Using SPSS. Pollen germination rate was inhibited by increasing doses of fungicides when compared with control medium. Captan and azoxystrobin were most inhibitory. Germination was not significantly affected by mycolobutanil. Also Thiophanate –methyl was found inhibitory.

Key words*: pollen germination, pesticide, 'Red Chief'.*

INTRODUCTION

Rapidly increasing world population is regarded as one of the most important problems for mankind in the decades to come. A decline in the agricultural areas plays an important role in this direction and lead to certain restrictions in feeding the growing population. Therefore, an evaluation of the available land for obtaining the maximum yield has become a major goal. One of the applications in this connection has been the use of pesticides in plants against harmful organism for improving agricultural productivity. However, in addition to the benefits they provide, an excessive use of these chemicals, wrong applications as well as their side-effects have started creating serious environmental problems as well as posing toxicity threat to living organisms.

Inhibitory effects of pesticidal sprays during pollination would be of particular concern in areas where pollination and fertilization are limiting factors in fruit production. Recently, there has been a lot of press related to pollinator health, and some troubling information indicates that certain fungicides, when used during bloom, can negatively affect the health of honey bees. This is a complicated problem with the solutions relying on understanding the detailed relationships among chemicals, pollinators and pest management needs. The objective of this study was to evaluate the effects of selected fungicides from different class.

MATERIALS AND METHODS

Plant material

Red Chief's pollens were used for pollen tests. Pollens were obtained from flowers of the above mentioned at balloon stage. The flowers were transferred to the laboratory immediately. Anthers were removed and placed into the dark-colored bottle to promote dehiscence at room temperature. The fungicides, which were commonly used for apple in Isparta selected for tests.

Table 1. Used fungicides

Active ingredient	Class	Trade name	Formulation*
Captan	Dicarboximide	Captan	WP
Azoxystrobin	Strobilurin	Quatris	WG
Mycolobutanil	Azole	Rally	WSP
Thio phanate-methyl	Benzimidazole	Topsin M	WSP
Maneb	Dithiocarbomate	Manex	Flowable

*WP=wettable powder, WG=water-dispersable granule, WSP=water-soluble pouches

The class, trade name, formulation and recommended field rate for each of compounds are shown in Table 1. Applies were made

according to Yi et al., 2003. Pollen germination and tube growth were conducted at three concentrations: the recommended fiels rate (100% RFR), 10% RFR and 1% RFR of each 'fungicide'. The pollen morphological homogeneity percentages of pollens were assessed with thehaemocytometer (Marienfeld, Germany) slide (Eti, 1990). Imperfectly shaped pollen grains were considered as aborted pollen. The final percentage of morphological homogeneity was defined as:

(number of normal shaped pollen) – (number of aborded pollen) per field

MH = total number of pollen field × 100

Pollen viability was determined by TTC (2, 3, 5-triphenyl tetrazolium chloride) stain test. Pollens were scattered onto TTC and stained pollens were counted after 2 hours and 15 minutes, respectively.

To determine the pollen viability, pollens of Red Chief apple (of four different areas) were observed onto two slides under a light microscope (×100 magnification). The stained pollen was considered as viable in these tests.

Germination medium without fungicide served as control.

For the *in vitro* test, pollen grains were sowed in the medium containing 0.5 agar + 15% sucrose + 5 ppm H_3BO_3 (boric acid) and incubated at the constant temperature of 25°C. The 'agar in plate' method was used to assisgn pollen germination and pollen tube growth (Koyuncu and Güçlü, 2009).

An ocular micrometer was used to measure pollen tube lenght, under a light microscope, at a magnification.

Four Petri dishes were used for germination and pollen tube growth experiments. For each assay, 2mL of medium with or without 'fungicide' was placed into Petri dishes.

Counts were made from 4 different microscope fields (100-150 pollen grains per field for each Petri dishes) (Hedly et al., 2004; Koyuncu, 2006). A factorial design was used in this study. Each treatment (5 'fungicide's x 3 concentrations plus control) was replicated 4 times.

Statistical analysis was conducted using Duncan's multiple range test within the general linear model procedure of SPSS 16.0.

For germination percentage, data were transformed with arcsine to meet the equal variance assumption.

RESULTS AND DISCUSSIONS

Pollens were evaluated from morphological homogeneity and viability there is no significant effect comperad to the control group. (Table 2).

Table 2. Morphological homegeneity and viability ratios (%)

	Morphological homogeneity (%)	Staining Test TTC (%)
Control (No fungicide)	98	97
Captan	96	96
Maneb	94	92
Mycolobutanil	93	90
Thiophanate-methyl	94	92
Azoxystrobin	92	90

Morphological homogeneity and viability rates were obtained upper 90%. However the lightest colour pollens were observed from Azoxystrobin but they were adopted 'viable'. In this case, the azoxystrobin fungicide may impair the structure of the enzyme when exposed to long-term or ex suggesting potential damage layer.

Different staining tests were carried out different fruit cultivars by different researches. Tosun and Koyuncu 2007, studied on cherry pollens, Koyuncu (2006), studied strawberry pollens using TTC and reported that pollen viability ratios reached 82% (Allstar and Elvira) and 86,5% (Chandler).

There are a lot of studies different fruit species about pollen viability tests. Junqueira, 2016 pointed that the viability of pollen grains was affected by the application of fungicide P + E, regardless of the application time. On the other hand, pollen grain germination was not affected by the fungicide or the stage.

Azoxystrobin and captan remained extremely inhibitory, with germination less than 1% of control. Maneb also inhibitory effect of pollen germination with 13.7%.

The highest pollen germination obtained from medium which was contain mycolobutanil with 87.7%. Also thiophanate-methyl have 68.8% germination. The inhibitory effects of some fungicides have been reported different study. Especially in apple, Yi et al 2003 reported the pollen germination in apples treated with Captan decreases by 20 % as compared to the control. Also captan was reported the inhibit pollen germination in pear (Butt et al., 1985).

The new azole product, mycolobutanil, had little or no inhibition, while the other new product, azoxystrobin, had very toxic effects (Yi et al., 2003b). Parallel these findings from our study captan and azoxystrobin were found severely inhibitor for pollen germination. After then maneb has found inhibitory for pollen germination. Mycolobutanil had little inhibition.

A number of compounds tested included fungicides different chemical class, i.e., dicarbomixide, strobilurin, azole, benzimidazole, dithiocarbomates were evalueted. The benzimidazo (thiophanate-methyl) had no to-intermediate effects. The dithiocarbamate compound (maneb) more severely suppressed pollen germination. Azoxystrobin, a strobilurin, was highly inhibitory. shown Table 3.

Table 3 Germination of apple pollen in presence of selected fungicides

Fungicides	Fungicide conc. (%of RFR)		
	100	10	1
Control (No 'fungicide')	100a[y]	100a	100a
Captan	0.0b	0d	0.9e
Maneb	0.0b	0.0d	13.7d
Mycolobutanil	0.0b	46.9b	87.7b
Thiophanate-methyl	0.0b	10.2c	68.8c
Azoxystrobin	0.0b	0d	0.3e

[z]Germination percentages shown are relative to the control, which is expressed at 100%. Actual pollen germination in the no-'fungicide' control was 77.4%. RFR=recommenden field rate

[y] Mean values within a column followed by the same letter are not significantly different at p=0.05, Duncan's multiple range test.

With 88.6% pollen germination rate obtained from the control medium (0.5 agar + 15% sucrose + 5 ppm H_3BO_3).

There is no pollen germination was observed in assays incorporatting any of the fungicides at 100% RFR (Recommended field rate).

Pollen grains typically exhibit high sensitivity to chemicals with *in vitro* germination assays where contact with chemicals is intense. Although germination was inhibited severely at 10% RFR, the fungicides showed differential effects as pollen germination was observed in the presence of maneb, captan and azoxytrobin. Germination in thiophanate- methyl was only 10.2%.

The highest relative germination occured in the presence of mycolobutanil which was 46.9% of control. Assays conducted at 1% were effective in delineating differences in polen sensitivity to different fungicides. Found that fungicide sprays caused detrimental effects on stigma morphology and enhanced exudates production in almond flowers.

Percent fruitset was not measured in the study, however increased exudates production was raised as possibly causing inhibition of pollen tube growth and germination. It was also suggested that the increased exudates production may be a stress response which could decrease the period of stigma receptivity (Yi et al., 2003). Cyprodinil promoted a copious increase in exudates secretion and caused the most severe collapse of stigmatic cells of all the fungicides evaluated in the almond study.

Fungicides incorporated into the media, or sprayed on the surface of the medium, reduced pollen germination and pollen tube growth at concentrations lower than those commercially recommended for successful disease control (Heazlewood, 2004).

The mode of action of the fungicide, systemic or contact, is thought to alter the level of damage caused to pollination.

It should be noted that effects of 'fungicide's on pollen under in vio conditions will be affected by additional considerations, such as the persistence of the chemical, whether orr not it is systemic, and how it may interact with the constituents of the stigmatic papilae (Yi et al., 2003).

CONCLUSIONS

Commercial 'fungicide's haven't affected so much to morphological homogeneity and pollen viability. Pollen viability rates changed between 90% and 97%. Pollen germination rates decreased by incresing doses of all 'fungicide's.

All 'fungicide's must have used recommended dose. Azoxystrobin was found the most dangerous.

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