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Evaluation of microbial-based products and an inorganic chemical for control of *Monilinia* spp. in plum organic production

Оценка на микробиални продукти и неорганичен химикал за контрол на *Monilinia* spp. при биологично производство на сливи

Aneliya BORISOVA (✉), Vilina PETROVA

Department of agrotechnology, plant protection, and economics on crops, Institute of Agriculture, Agricultural Academy, Sofiysko shosse str. 1, 2500 Kyustendil, Bulgaria

✉ Corresponding author: anelija@gmail.com

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ABSTRACT

The plum (*Prunus domestica* L.) is one of the most important fruit crops in Bulgaria and ranks first place among all fruit species in terms of production. Organic fruit cultivation has increased rapidly worldwide in the last two decades, including in Bulgaria. Brown rot caused by fungi *Monilinia laxa*, *M. fructigena*, and *M. fructicola* is one of the economically most important and challenging diseases to manage in organic stone fruit production. In a 3-year study, the effectiveness of the organic liquid fertilizer Ecosist-Arbanasi (*Bacillus subtilis TS 01*), commercially available biofungicide Serenade ® ASO (*Bacillus amyloliquefaciens* QST 713), and inorganic chemical (calcium polysulphide) to control brown rot in organic plum production was investigated. The study was conducted in an organic orchard of the Institute of Agriculture – Kyustendil at Kyustendil region of Bulgaria with plum cultivar Stanley during 2020-2022. The agro-climatic characteristics of the region have all the objective factors for the development of *Monilinia* spp. Calcium polysulphide applied two times alone insufficiently reduced the incidence of brown rot (45.5% efficacy). Two soil applications of Ekosist-Arbanasi (77.5% efficacy), three foliar spraying with it (71.6% efficacy), and seven spraying applications of Serenade ® ASO (73.2% efficacy) may be sufficient against brown rot in plums. However, none of the treatments completely controlled *Monilinia* spp. These results show that improved integrated control management is needed for brown rot control in organic plum production.

Keywords: *Prunus domestica*, Brown rot, Biological control, *Bacillus subtillis TS 01*, calcium polysulphide

РЕЗЮМЕ

Сливата (*Prunus domestica* L.) е една от основните овощни култури в България и заема първо място по производство от всички овощни видове. През последните две десетилетия биологичното производство на плодове нараства бързо в световен мащаб, включително и в България. Кафявото гниене, причинено от *Monilinia laxa*, *M. fructigena* и *M. fructicola* е една от икономически най-важните и предизвикателни болести за управление в органичното производство на костилкови плодове. В 3-годишно проучване е изследвана ефективността на третирането с органичен течен тор Екосист-Арбанаси (*Bacillus subtilis TS 01*), биофунгицид Serenade ® ASO (*Bacillus amyloliquefaciens* QST 713) и неорганичен химикал (калциев полисулфид) за контрол на кафявото гниене при биологично производство на сливи. Проучването е проведено в биологична овощна градина на Института по земеделие – Кюстендил, област Кюстендил, България със сливов сорт Стенлей през 2020-2022 г. Агроклиматичната характеристика на района притежава всички обективни фактори за развитието на *Monilinia* spp. Калциевият полисулфид, приложен два пъти самостоятелно, има незадоволителен ефект срещу причинителите на кафявото гниене (45.5% ефикасност). Две почвени приложения на Екосист-Арбанаси (77.5%

ефикасност), три листни пръскания с него (71.6% ефикасност) и седем приложения на Serenade ® ASO (73.2% ефикасност) са достатъчно ефикасни срещу кафявото гниене при сливата. Въпреки това нито едно от леченията не контролира напълно *Monilinia* spp. Този резултат показва, че е необходим усъвършенстван комплексен подход за борба срещу причинителите на кафяво гниене при биологичното производство на сливи.

Ключови думи: *Prunus domestica*, кафяво гниене, биологичен контрол, *Bacillus subtillis TS 01*, calcium polysulphide

INTRODUCTION

Organic production of fruits has experienced rapid growth worldwide during the last two decades. Organic fruit production has also increased in the past ten years in Bulgaria. The total areas in which organic production methods were applied as permanent crops in 2009 were 2 688 ha and in 2020- 24 829 ha (MAF 2011; MAFF 2021a). The rapid development of organic agriculture requires new approaches to control pests and diseases without synthetic pesticides.

Brown rot caused by three main fungal species *Monilinia laxa*, *M. fructigena*, and *M. fructicola* is especially destructive to stone fruits, but it also attacks other fruits (Batra, 1991; Ogawa et al., 1995) and governs an important economic impact. The greatest loss from brown rot occurs directly as a result of rotting in the orchard, in transit, and/or on the market. Yield reduction also occurs due to blossom blight and twig cankers (Martini and Mari, 2014). Losses depend on weather conditions and are especially severe if high humidity, warm temperatures, and abundant rainfall prevail before harvest (Bonaterra et al., 2003).

Two species of the genus *Monilinia*, *M. laxa*, and *M. fructigena* are widely distributed in Bulgaria. They regularly appear on pome and stone fruits every year, causing particularly high yield losses when the rainy period coincides with blooming and fruit ripening (Borovinova, 2015). *M. fructicola* has been detected recently in Bulgaria on plum, peach, nectarine, and cherry fruits (Bobev et al., 2020). The plum (*Prunus domestica* L.) is one of the main fruit crops in Bulgaria and takes first place from all fruit species in terms of production 60 024 t in 2020 (MAFF 2021_b).

Brown rot is one of the most challenging diseases to manage in organic stone fruit production (Tamm et al., 2004; Holb and Schnabel, 2005). In a wet season, for commercial stone organic orchards, it is not unusual to suffer 75% or more crop losses due to brown rot, even though copper-based fungicides are used (Larena et al., 2005; Xu et al., 2007). All these facts point to the importance of finding sufficient methods to control this disease. If the proposed approach would be safe for humans, such as biocontrol agents, would be even better.

The aim of this three-year study was to compare the effectiveness of the treatments with microbial-based products (Ecosist-Arbanasi and Serenade ® ASO) and an inorganic chemical (calcium polysulphide) for the control of brown rot in organic plum production.

MATERIALS AND METHODS

Plant material and orchard

The study was conducted in an organic plum orchard (located at 42°13'52''N and 22°46'39''E) of the Institute of Agriculture – Kyustendil at Kyustendil region (Bulgaria) in 2020, 2021, and 2022. The 7-ha orchard was planted in 2013 with the self-fertile plum cultivar Stanley. The cultivar was grafted on *Prunus cerasifera*. The distance between trees was 5 m x 5 m. The orchard was not irrigated. Cultivar Stanley has moderately early blooming and its fruit maturity dates around early September (Vitanova et al., 1998). The cultivar is susceptible to *Monilinia* spp. (Holb et al., 2011).

Environmental monitoring

Precipitation (mm) and mean daily temperature (°C) were recorded during the test periods in 2020, 2021, and 2022 using a meteorological station located at Lat 42.650, Long 23.383, and Altitude 531 m.

Treatments

Treatments were performed from the middle of April to the beginning of August of 2020, 2021, and 2022. Each treatment was replicated at least five times (single tree replications). The treatments were:

Ecosist-Arbanasi (soil application) 2.3 l/ha + Carbo active - 2.0 l/ha;
Ecosist-Arbanasi (foliar spraying) – 1.2%;
Serenade ® ASO (foliar spraying) – 8.0 l/ha;
Calcium polysulphide (foliar spraying) – 4.0 l/ha;
Untreated control

The organic liquid fertilizer Ecosist-Arbanasi of company Biochum ®, Bulgaria is a universal bacterial based natural product, which includes several strains of *Bacillus subtilis* as well as the bacteria *Bacillus licheniformis*, *Azotobacter chroococum* and *Azotobacter vinelandii*. The strain *Bacillus subtilis TS 01* is registered with number NBIMCC No. 8718 in the Bulgarian national bank of industrial microorganisms for patent proceedings and is known to has a stronger effect against plant pathogens than other strains of the same bacterium (Todorova, 2009). The microbial product also contains other complementary bacteria and organic substances useful for plants and animals (Yakimov et al., 2015). This product is combined with the liquid organic fertilizer Carbo-Active, which is a product based on molasses, enriched with macro- and microelements, and serves to activate the microorganisms in the Ekosist-Arbanasi. Two treatments were carried out with Ekosist-Arbanasi + Carbo-Active (soil application) in BBCH 56-60 (beginning of raceme elongation - first flowers open) and BBCH 73-77 (30%-60% of fruits formed). Three sprayings were carried out with Ekosist-Arbanasi in BBCH 56-60, BBCH 67-69 (flowers fading- end of flowering), and BBCH 73-77, following the manufacturer's recommendations.

Serenade ® ASO a suspension concentrate (SC) containing 1 x 10^{12} CFU/g *Bacillus amyloliquefaciens* QST 713 (formerly *Bacillus subtillis*) is a biofungicide of company Bayer Crop Science, Leverkusen, Germany. The biofungicide was applied 7 times during the growing season from the first flowers open to the beginning of fruit coloring (BBCH 56-81) with an interval of about 15 days between sprayings.

The inorganic chemical calcium polysulphide of company Biofa, GmbH, Germany was applied 2 times in BBCH 56-60 and BBCH 69-72 (end of flowering-ovary growing; fruit fall after flowering). The producer recommends up to 3 applications per season.

All treatments were applied with backpack sprayers STIHL SR 200 depending on weather conditions.

Brown rot assessment

For each treatment and year, disease assessment was done based on the percentage of *Monilinia* spp. infected fruits in the growth stage - fruit ripe for picking (BBCH 87). Fifty randomly selected fruits from each quadrant of five trees of each treatment were examined for disease symptoms. The fruit was considered to be diseased if at least one visible brown rot lesion was present on a fruit (Figure 1). Brown rot incidence was calculated as the percentage of diseased fruits.

Figure 1. Symptoms on plum fruits caused by *Monilinia* spp.

Statistical analysis and effectiveness

The data were statistically processed by analysis of variance using the F-test to test significance and the LSD-test to test the significance of the differences between variant means and control, at levels $P < 0.05$, 0.01, and 0.001, depending on data dispersion. The data analysis was performed by computer programs developed by

Maneva, 2007 based on standard statistical algorithms suitable for small sets of data with biological origin (Sokal and Rohle, 1981). The different treatments were compared to the untreated control and standard deviation (Sd) was given.

The effectiveness of the products was calculated according to Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

Environmental monitoring

From the climatic characteristics, it was seen that in the area of the village Zgurovo, Kyustendil region, Bulgaria, where the orchard is located, all the objective factors for the development of brown rot (*M. laxa* and *M. fructigena*) during the investigated period (Figure 2) were noticed. Infection is highly dependent on wetness duration and temperature. Infection and development of disease symptoms occur over a relatively wide temperature range between 4 and 30 °C, the optimum being about 24 °C (Holb, 2008). Low-moisture conditions limit infection, little or no infection occurs in rainless weather, even if humidity is high (Holb, 2008). In April 2020 the rainfall was 56.4 mm (a total of 8 days with rain) with a mean daily temperature of 9.9 °C, while in August 2020 the rainfall was 83.7 mm by a large amount during the first ten days of August, with a mean daily temperature 23.2 °C (Figure 2). Next year, in April 2021 the rainfall was 78.1 mm (a total of 14 days with rain) with a mean daily temperature of 8.5 °C and in August the rainfall was 109.1 mm mostly during the last three days of August with a mean daily temperature of 23.2 °C.

Disease assessment was done immediately after these rainfalls, which suggests that there was no opportunity for the development of fruit symptoms. Last year, in April 2022 the rainfall was 69.2 mm (14 days with rain) with a mean daily temperature of 10.2 °C, and in August the rainfall was 42.0 mm, which is significantly less than the previous two years with a mean daily temperature 21.8 °C (Figure 2). During the season of all studied years, it is evident that several successive cycles of secondary infection occurred.

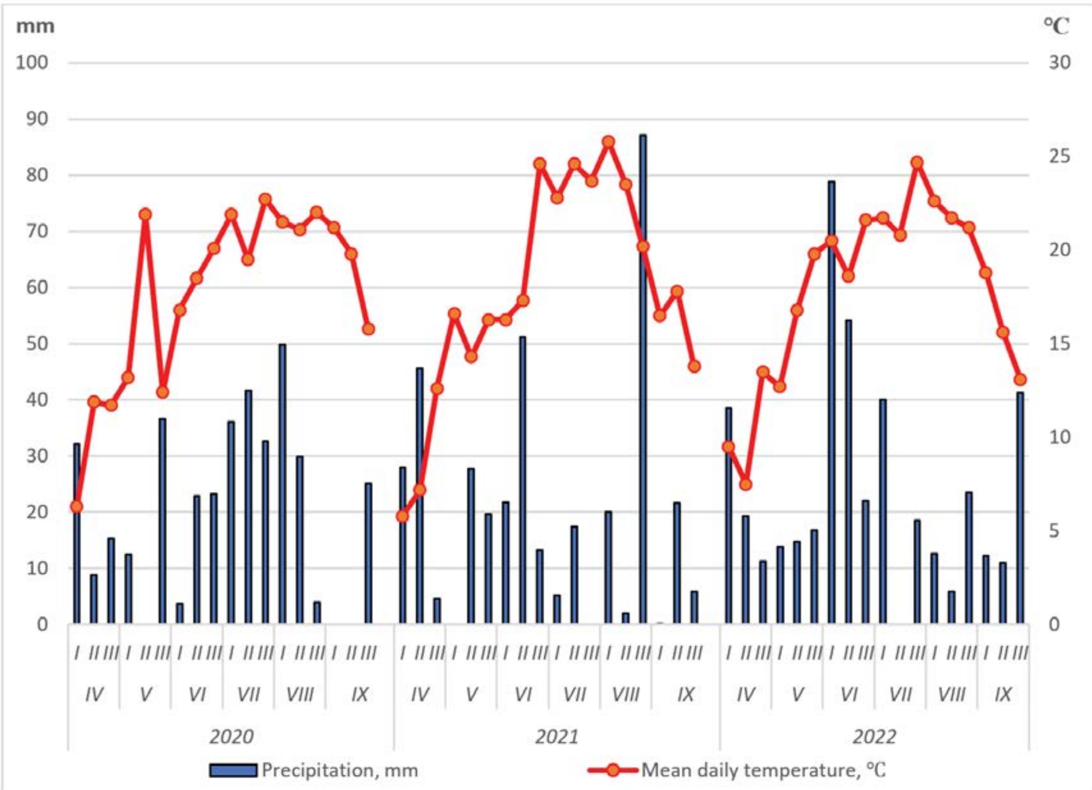

Figure 2. Amount of precipitation (mm) and mean daily temperatures (°C) every ten days in a month for the period April – September (2020-2022)

Brown rot incidence

Analyses of variance on brown rot incidence values indicated differences among years and treatments (Table 1). All treated variants differed significantly from the untreated control during all the years of the study. The highest percentage of brown rot fruits in all treatments, including untreated control, was recorded in 2020, while the lowest was in 2021. In 2020 the foliar spraying with Ecosist-Arbanasi provided much better control than soil application, but in 2022 the opposite was observed. However, the use of Ecosist-Arbansi as a foliar spray or watering into the soil significantly reduces the occurrence of brown rot in all years. Results that the organic fertilizer Ecosist-Arbanasi is appropriate for suppression of *Monilinia* spp. are confirming the results obtained by Pashev et al. (2020). Their study was carried out with the same plum cultivar Stanley in Dryanovo, Bulgaria. The highest rate of damage on the fruits was reported at the untreated control, respectively 8.9% in 2016 and 12.6% in 2017, while in the variant treated with Ecosist-Arbanasi (soil application) was 4.8% (2016) and 5.3% (2017) and in treatment Ecosist-Arbanasi (foliar spraying) was 4.3% (2016) and 4.9% (2017).

The disease incidence was significantly reduced also with the Serenade®ASO applied seven times from BBCH 56 to BBCH 81. The percentage of brown rot fruits recorded in years 2020, 2021 and 2022 for this treatment was 25.6%, 4.3%, and 6.5%, compared to the untreated control 75%, 14.7%, and 39.2%, respectively (Table 1).

In recent years, the commercial bioformulate Serenade®Max has been included in several studies comparing biological control agents (BCAs) and different strategies to manage brown rot disease on stone fruits in Europe (De Curtis et al., 2019; Palmieri et al., 2022). In organic farming it is appropriate to use a control strategy based on the application of BCAs, including Serenade®, paying attention to the critical periods for the onset of latent infections (the phenological phase 77–81 BBCH for plums is a vulnerable period for the entry of the pathogen), and carrying out a sufficient number of treatments (Palmieri et al., 2022). Research and evidence on the efficacy of other strains of *Bacillus amyloliquefaciens* as an alternative for controlling brown rot in stone fruit has been conducted by many authors (Bellamy et al., 2021; Calvo et al., 2017; Casals et al., 2021; Gotor-Vila et al., 2017; Lahlali et al., 2020).

Calcium polysulphide treatments, applied two times before and after bloom, resulted in significantly higher brown rot incidence compared to microbial-based products during the three years of the research. However, all calcium polysulphide treatments had a significantly lower incidence of brown rot compared to untreated control. Calcium polysulphide as a lime sulphur was widely used for controlling *Monilinia* spp. in organic stone fruit production (Holb, 2006; Holb and Kunz, 2013; Louise et al., 2014).

Table 1. Percentage of infected fruits caused by *Monilinia* spp. in cultivar Stanley in an organic plum plantation (2020–2022)

Treatments	Brown rot fruits (%)			
	2020	2021	2022	Average
Ecosist- Arbanasi (soil application)	25.03 ***	2.10 ***	7.82 ***	11.65 ***
Ecosist -Arbanasi (foliar spraying)	18.90 ***	2.50 **	16.87 ***	12.75 ***
Serenade ® ASO	25.66 ***	4.32**	6.54 ***	12.17 ***
Calcium polysulphide	37.00 ***	5.05 **	25.40 **	21.72 ***
Untreated control	75.00	14.73	39.2	42.97
Sd	4.80	3.14	4.00	2.31
F	44.29	5.42	21.25	66.47
LSD 0.05	10.17	6.66	8.48	4.89

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 2. The efficiency determined in the individual treatments in organic plum plantation (cultivar Stanley)

Treatments	Efficacy according to Abbott (%)			
	2020	2021	2022	Average
Ecosist-Arbanasi (soil application)	66.63	85.75	80.05	77.48 ± 5.66
Ecosist-Arbanasi (foliar spraying)	74.8	83.04	56.96	71.60 ± 7.69
Serenade ® ASO (foliar spraying)	65.77	70.62	83.32	73.24 ± 5.23
Calcium polysulphide (foliar spraying)	50.67	50.47	35.20	45.45 ± 5.12

Protection Efficacy

The efficacies determined in the individual treatments are given in Table 2. The highest efficiency was achieved with the Ecosist- Arbanasi (soil application) – 77.5%, followed by Serenade ® ASO – 73.2% and Ecosist-Arbanasi (foliar spraying) – 71.6% averaged over the study period. The lowest efficiency had calcium polysulphide – 45.5%, which was probably caused by insufficient applications.

Biological control can be very effective, but its effectiveness is highly dependent on environmental conditions. Just like the pathogen, the biocontrol organism requires optimal temperature and moisture conditions to reproduce and spread. This may explain the different efficacy of the same product in the different years of the study, and even between studies.

Most of the biocontrol research focuses on the application of BCAs after harvest. However, more could be done to investigate the potential for BCAs for use in the field to protect blossoms and fruits to reduce post-harvest losses. The application of the organic liquid fertilizer Ecosist-Arbanasi against brown rot in the field in this study and the previous one (Pashev et al., 2020) clearly shows its high potential for use against *Monilinia* species. Future research needs to understand the ecology of this product and its population dynamics in relation to external conditions in order to optimize its use in combination with other management strategies.

CONCLUSIONS

In conclusion, the agro-climatic characteristics of the Kyustendil region of Bulgaria have all the objective factors for the development of *Monilinia* spp.

Two applications of calcium polysulphide insufficiently reduced the incidence of brown rot. Three foliar and two soil applications of microbial-based products Ekosist-Arbanasi (*Bacillus subtilis TS 01*) and seven applications of commercially available biofungicide Serenade ® ASO (*Bacillus amyloliquefaciens* QST 713) could be sufficient against the brown rot in organic plum production.

However, none of the treatments completely control *Monilinia* spp. This result shows that an improved integrated control strategy is needed for brown rot control in organic plum production.

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