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RESEARCH ON EFFECTIVENESS OF SULPHUR BASED – ON FUNGICIDES TREATMENTS AGAINST *PODOSPHAERA LEUCOTRICHA* IN APPLE IN VOINESTI AREA, DAMBOVITA COUNTY

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Abstract

Apple powdery mildew caused by Podosphaera leucotricha is one of the most common and severe diseases of this plant in all production areas. In years under favorable conditions for the disease, especially for susceptible varieties, it causes significant damage to apple orchards of up to 80% of the harvest. The research was carried out between 2019 and 2021 within the Research and Development Station for Fuit Growing Voinești and aimed at the effect of Polisulf product type MIF and Sulfomat 80 PU on the attack of the monitored pathogen on Jonathan and Golden Delicious varieties. The highest degree of attack was calculated for the Jonathan variety of 60.8% in 2021. The effectiveness of the treatments was 80.5% - 84.8% for the Jonathan variety and 88.2% - 96.4% for the Golden Delicious variety. Sulfur-based products have provided good protection against powdery mildew and are recommended in integrated apple protection systems.

***Key words**: apple orchard, degree of attack, effectiveness, powdery mildew, sulphur based - on product.*

INTRODUCTION

Caring for the trees, making them produce more, better, with tastier fruits, has preoccupied man since ancient times and will continue to be an everlasting activity. Fruits are essential in human nutrition because they are an indispensable source of vitamins and energy.

Apple growing has occurred since oldest times and apple has become one of the most important fruits that are fit for human consumption. Anatolia, Caucasia, Turkistan, and Europe were centres of origin for the domestic apple (*Malus domestica* Borkh.), now grown in continental climates in the Northern and Southern Hemispheres (Pirlak et al., 2003). Apples are among the world`s fruits crops, figuring in both the Bible and the tales of Homer (Jackson, 2003). They are considered to be one of the oldest fruit species and ancient Roman and Greek historians have offered extensive descriptions (Jaloba et al., 2019).

The apple is very well adapted to the temperate climate whose extremes endures much better than other fruit tree species (Hoza, 2000). Our country has favorable climatic and pedological conditions for a large number of fruit species such as stone fruits, pome fruits, fruit bushes, which are found from the sea to the mountains. Within these, apple has a great ecological resilience in Romania where it could find very favourable pedo - climatic conditions to obtain good quality yields (Ghena & Branişte, 2003).

By the other hand, in the apple growing areas, a significant number of pathogens may cause large qualitative and quantitative losses in apple orchards and warehouses. Under the pedoclimatic conditions of the Voinești orchard traditionally consacrated to apples cultivation since ancient times, there is a high biological reserve of diseases and pests, a high risk of infection, which allows in favorable years the successive or simultaneous attack of specific or non-specific pathogens and pests, which can cause very serious damage.

In the Voinești area, the most harmful are scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*), which makes it mandatory to apply phytosanitary treatments.

A strong infection of powdery mildew, especially on susceptible varieties, can defoliate the tree and affect the growing shoots with serious consequences in the following year by weakening the trees. Briefly, the apple requires a large number of phytosanitary treatments that make fruit growers to allocate about 40-50% of total production costs (Petre et al., 2006).

Its importance emerges from the fact that it can start very early, from the bud burst and can manifest throughout the growing season (Gheorghieș & Geamăn, 2003; Dulugeac, 2011). The attack can be manifested on leaves, shoots, flowers and less often on fruits. The young leaves are most susceptible to attack.

A fine mycelium is noted on the shoots grown from infected buds (Gheorghieş & Geamăn, 2003), which shortly can reach the entire shoot, but also the leaves and flowers.

Severely infected shoots have shortened internodes and are covered with a silver-gray mycelium. White-gray mycelial felt turns brown, numerous dark brown fruits appear - chasmothecia (Hickey & Yoder, 1990; Gheorghieș & Geamăn 2003). From shoots with primary infection, the fungus spreads to other organs, causing secondary infections.

On the leaf surface, symptoms of powdery mildew appear as irregular chlorosis of gray-white with white powder and also may include whitish lesions on curled or longitudinally folded leaves, stunted whitish gray twig growth and fruit russeting. Due to attack, the leaves do not fully open, remain small and thicken, curled and distorted and twist to the top, taking on the appearance of a “little boat” (Paraschivu, 2010).

Most economic damages occur in the form of aborted blossoms, reduced fruit finish quality, reduced vigor and yield of the bearing trees and stunting and poor form of young, nonbearing trees. Powdery mildew can cause considerable loss due to nutrient removal, reduced photosynthesis, increased respiration and transpiration, and impaired growth (Retrieved from:https://www.saillog.co/PowderyMildewOfApples.html).

P. leucotricha may overwinter as chasmothecia (cleistothecia) but is tipically observed as mycelium developing from within buds produced by the apple host during the previous growing season (Strickland et al, 2021). Perennating in buds, it makes it difficult to control and, although it can cause fruit russet, it primarily causes losses due to its chronic effect on tree vigor and yield (Biggs et al., 2009). Infection and the evolution of the attack are favored by temperatures of 18-22°C and 80% humidity. Powdery mildew can spread to lower humidity (70%), for infection being sufficient moisture formed by perspiration, on the surface of green organs (Gheorghieș & Geamăn, 2003).

Elemental sulphur is known, undeniably, the oldest pesticide ever to control powdery mildew. Ancient Greeks were aware of its pesticide properties as early as 1000 B.C. In the scientific published literature, Forsyth (1802) cited by Tweedy (1969) was the first to suggest the application of sulphur for disease control. Unfortunately, the fungicidal properties of sulphur were apparently forgotten during the middle age and were not rediscovered until the beginning of the nineteenth century. Ever after, sulphur has been used in various forms for disease and pest control, especially against powdery mildew and mites on plant (Jalobă & Grădilă, 2019). On the other hand, sulphur is an element essential for plant growth and development. It is the building block for the synthesis of amino acids (cysteine, methionine), proteins, coenzymes, sulpholipids and polysaccharides (Bělíková et al., 2019). It is generally accepted that the efficacy of sulphur for powdery mildew control is related to contact and vapour activity. Contact activity appears to have a minor role in the efficacy of sulphur products. Vapour activity has a major role but is temperature dependent. Below 15ºC, sulphur activity is confined to contact activity because vapour activity is negligible (Retrieved from https://www.saillog.co/PowderyMildewOfApples.html). The aim of this paper was to monitor the behaviour towards the powdery mildew of two apple varieties and the effectiveness of the sulphur based – on fungicides treatments applied against the monitored pathogen.

MATERIALS AND METHODS

A three years trial (2019-2021) was established on April 2019 at the Research and Development Station for Fruit Growing Voineşti (Middle North Muntenia, Romania).

The climatic conditions of the area are favorable for apple growing, with an average annual temperature of 8.8°C and an average annual rainfall exceeding 750 mm. The typical soil in the area is brown-eumezobasic, weakly pseudogleized, loamy texture, weakly acidic pH (5.6-6). The humus content is medium at the surface: 2.15-3.25. Jonathan and Golden Delicious were the two classic varieties tested, within a 18-year-old orchard. The planting distance for Jonathan and Golden Delicious varieties is 4 meters between rows and 3 meters between trees within a row. (833 trees/ha) The shape of the crown is palmette with oblique arms. Trees in the orchard were on M9 rootstock grafted. During the period of vegetative rest, autumn tillage was carried out by ploughing on the intervals between rows. Pruning works for growth and bearing control were carried out early spring, before the growing season began. The trial was conducted using the randomized complete block method in four repetitions which included four untreated plots. The application equipment was a mist blower Grùnman 3 WF-3 knapsack mist blower, suitable for fungicide treatments in orchards. 100 leaves per tree were assessed before each spray and after ten days until the latest treatment by visually rating. Assessments were made on the frequency (F, %, or Pest Incidence) and the intensity (I, % or Pest Severity) of pathogen attack and the degree of attack (DA, %) and efficacy (E, %) were calculated. The degree of attack was calculated using the formula: F% x I%/100. The efficacy of fungicides was calculated according to Abbott`s formula: (degree of attack in untreated control - degree of attack in treated plot)/ degree of attack in untreated control x 100. All data were subjected to statistical analysis provided by ARM-8 sofware (Jalobă et al., 2021). At harvest time, the yield/tree was also recorded by weighing all the fruits per tree.

Three treatments with Polisulf and Kumulus DF and five treatments with Sulfomat 80 PU and Kumulus DF were proceeded in the experimental orchard. The tested products are shown in the Table 1. Treatments were scheduled at the following stages:

Polisulf and Kumulus DF:

- 1st treatment (A): mouse ear (BBCH 10);
- 2nd treatment (B): shoots about 40% of final length (BBCH 34);
- 3rd treatment (C): at the end of flowering (BBCH 69).

Table 1. Fungicides used to control powdery mildew in the trials

Product	Active ingredient	Rate (kg, l/ha)	Water volume (l)
Polisulf	thiosulphuric sulphur 3% + polisulfhydric sulphur 12%	30	1500
Sulfomat 80 PU	sulphur 800 g/kg	4.5	1500
Kumulus DF	sulphur 800 g/kg	4.5	1500

Sulfomat 80 PU and Kumulus DF:

- 1st treatment (A): mouse ear (BBCH 10);
- 2nd treatment (B): shoots about 40% of final length (BBCH 34);
- 3rd treatment (C): red bud stage (BBCH 57);
- 4th treatment (D): end of flowering (BBCH 69);
- 5th treatment (E): second fruit fall (BBCH 73).

Polisulf is a fungicide that acts directly by the decomposition of elemental sulphur as a protective fungicide. Thus, sulphur interrupts the transfer of electrons, causing sulphur reduction in hydrogen sulphide (H_2S). Sulphur also forms a protective layer, which inhibits germination of spores.

Sulfomat 80 PU is a dust free, flowable micronised sulphur granules, containing 80% sulphur as active ingredient and balance adjuvants, easy of measuring and handling. It has a lot of advantages as follow: it has instant dispersion and high suspensability in water, therefore it don't cause scorching; it has triple action as fungicide, micronutrient (Sulphur) and miticide and it has sustained action for longer effect; there are no stains on fruits and leaves after spraying, nor do leaves get burnt.

RESULTS AND DISCUSSIONS

While the Jonathan variety are known to be very sensitive to powdery mildew and Golden Delicious is supposed to be middle sensitive, the applied treatments considerably reduced the attack of *Podosphaera leucotricha* in the treated plots (Table 2). Under the weather conditions of Voinești area, the pathogen attack was noted every year, being detecteted even with the begining of the spring. Assessments made on the Pest Incidence (Pesinc %) and Pest Severity (Pessev %) of the pathogen attack were recorded in the database Tables 2, 3, 6 and 7. As a result, the degree of attack (DA%) was calculated for each of the four repetitions in untreated checks, non-polluting products and standard product applied, for both Jonathan variety and Golden Delicious variety. Also, photographs and samples were taken to be studied.

Table 2. Development of *P. leucotricha* in Polisulf trial (Jonathan variety)

Assessment time	Assessment	2019			2020			2021		
		Untreated	Polisulf	Kumulus	Untreated	Polisulf	Kumulus	Untreated	Polisulf	Kumulus
0 DA-A (0 day after first treatment)	Pesinc % Pessev %	34 25	34 25	34 25	7.5 15	7.5 15	7.5 15	22 20	22 20	22 20
10 DA-A (10 days after treatment A)	Pesinc % Pessev %	41 30	32 25	31.4 25	17.5 20	12.5 15	12 15	36 25	18.7 15	22.5 14
10 DA-B (10 days after treatment B)	Pesinc % Pessev %	65.5 40	40 25	35 25	35 25	18 15	16.5 15	45 35	25.5 15	27 15
10 DA-C (10 days after treatment C)	Pesinc % Pessev %	76 50	37 20	32.5 20	55 35	24 15	19.7 15	64 40	26 15	28 16

Polisulf trial

Infections on leaves appeared first on the lower surface. These lesions may spread to the upper surface and cover the entire leaf with a white spores and mycelium. Infections noted along the leaf margin often resulted in leaf curling and crinkling.

The degree of attack (DA%) evolution for each study is shown in Figure 1 and Figure 2. As it can be seen, the degree of attack at untreated check has had an ascending development and has increased with each assessment of untreated controls due to weather conditions and lack of phytosanitary treatments.

Table 3. Development of *P. leucotricha* in Polisulf trial (Golden Delicious variety)

Assessment time	Assessment	2019			2020			2021		
		Untreated	Polisulf	Kumulus	Untreated	Polisulf	Kumulus	Untreated	Polisulf	Kumulus
0 DA-A (0 day after first treatment)	Pesinc % Pessev %	16 10	16 10	16 10	12 10	12 10	12 10	11 15	11 15	11 15
10 DA-A (10 days after treatment A)	Pesinc % Pessev %	26 20	25 15	24 15	18 15	13 10	12 10	30 25	25 15	25.5 12.25
10 DA-B (10 days after treatment B)	Pesinc % Pessev %	33 30	25 12.25	23 12.5	24 30	18 10.25	20 10	32 30	22 10	27 10
10 DA-C (10 days after treatment C)	Pesinc % Pessev %	36 40	11 12.5	8 10	32 35	8.5 7.5	14 7.25	40 35	5 10	9 10

As it can be seen in Figures 1 and 2, at the experiment with Polisulf, the degree of attack ranged from 8.5% to 38% (in 2019), from 1.13% to 19.25% (in 2020) and from 4.4% to 25.6% (in 2021) for the Jonathan variety. In the Golden delicious variety, DA% evolved from 1.6% to 14.4% (in 2019), from 1.2% to 11.2% (in 2020) and from 1.65% to 14% (in 2021).

Regarding the samples treated with fungicides against powdery mildew, in Figures 1 and 2 are shown the values between which the degrees of attack ranged in this trial where the non-polluting product Polisulf and the standard product Kumulus DF were applied. Thus, for the Jonathan variety, the samples where the product Polisulf type MIF was applied registered a degree of attack registered between 1.88% (in 2020, 10 days after treatment A) and 9.85% (in 2019, at 10 days after treatment B). In the Golden Delicious variety, Polisuf type MIF proved to be even more effective, registering a lower degree of attack, risen from between 1.3% (in 2020, 10 days after treatment A) and 3.75% (in 2021, 10 days after treatment A). These results were almost similar to those of the standard product Kumulus DF in efficacy and there were no statistically significant differences between the two fungicides.

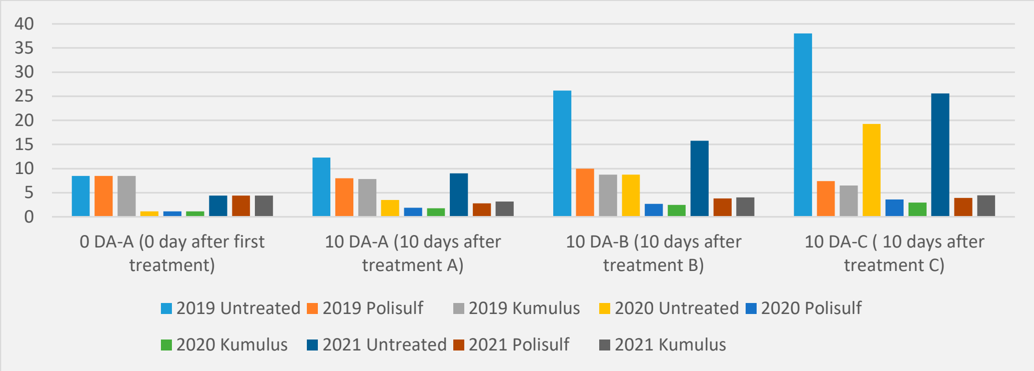

Figure 1. Degree of attack in Polisulf trial (Jonathan variety)

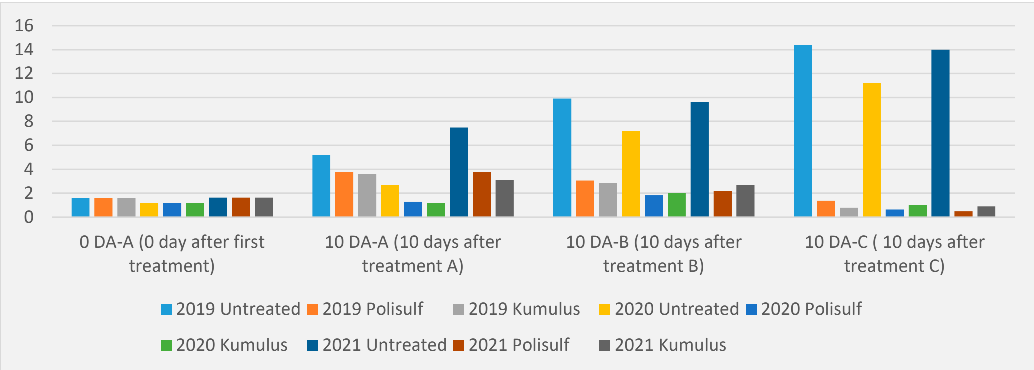

Figure 2. Degree of attack in Polisulf trial (Golden Delicious variety)

Under such conditions, the non-polluting product applied performed very well in both apple varieties compared to that of the standard product, their effectiveness being shown in Tables 4 and 5. As one can be seen, the product was effective in those three years of experimentation, the efficacy after the last treatment ranging between 80.6% and 84.8% for the Jonathan variety and between 90.5% and 96.4% for the Golden delicious variety.

Table 4. Efficacy% of products applied to control powdery mildew in the Jonathan variety

Year	2019		2020		2021	
Assessment time	Polisulf	Kumulus DF	Polisulf	Kumulus DF	Polisulf	Kumulus DF
10 days after A	35.4	36.3	46.4	49.1	68.8	65.3
10 days after B	62.4	66.8	69.2	71.8	75.8	74.2
10 days after C	80.6	82.8	81.4	84.7	84.8	82.9

Table 5. Efficacy% of products applied to control powdery mildew in the Golden Delicious variety

Year	2019		2020		2021	
Assessment time	Polisulf	Kumulus DF	Polisulf	Kumulus DF	Polisulf	Kumulus DF
10 days after A	28.4	31.6	52.4	55.5	50.00	58.5
10 days after B	68.6	70.8	74.5	72.2	77.4	72.2
10 days after C	90.5	94.5	94.3	90.9	96.4	93.5

Sulfomat 80 PU trial

Simultaneously with the testing of the Polisulf, the effectiveness of the Sulfomat 80 PU product in controlling powdery mildew in both varieties was also monitored.

Table 6. Development of *P. leucotricha* in Sulfomat 80 PU trial (Jonathan variety)

Assessment time	Assessment	2019			2020			2021		
		Untreated	Sulfomat	Kumulus	Untreated	Sulfomat	Kumulus	Untreated	Sulfomat	Kumulus
0 DA-A (0 day after first treatment)	Pesinc % Pessev %	35.0 20.0	35 20	35 20	10 15	10 15	10 15	20 25	20 25	20 25
10 DA-A (10 days after treatment A)	Pesinc % Pessev %	40.0 25.0	24.5 20.25	28 17.5	20 25	16.75 15	16 15	32 25	25.25 15	17.75 20
10 DA-B (10 days after treatment B)	Pesinc % Pessev %	62.5 40.0	49 20	48.5 20	38.0 25.0	19.25 17.5	24.25 13	48 30	29.5 20	29.5 20
10 DA-C (10 days after treatment C)	Pesinc % Pessev %	65.0 45.0	42 20	43 20	44.0 30.0	24.25 15	22.5 15	50 40	31 20	29.25 20
10 DA-D (10 days after treatment D)	Pesinc % Pessev %	72.0 60.0	47.5 20	42.5 20	65.0 50.0	33.75 20	32 20	74 60	43.5 25	43 20
10 DA-E (10 days after treatment E)	Pesinc % Pessev %	84 70	48 22.5	44 22	72.0 60.0	38 20	36.5 17.5	76.0 80.0	47.5 25	48.5 20

In the experiment where Sulfomat 80 PU and Kumulus DF were applied, the symptoms on the leaves were identical to those of Polisulf trial. First, in the primary infection, the leaves were colonized as they emerged from the buds, while in the secondary infections windborne spores landed on young leaves as they unfurled and expanded. Fungal colonies composed of mycelium and spores appeared as white, felt-like patches. As it was expected, secondary infections appeared first on the lower leaves surface, and was detectable on the upper leaf surface as chlorotic spots.

Table 7. Development of *P. leucotricha* in Sulfomat 80 PU trial (Golden Delicious variety)

Assessment time	Assessment	2019			2020			2021		
		Untreated	Sulfomat	Kumulus	Untreated	Sulfomat	Kumulus	Untreated	Sulfomat	Kumulus
0 DA-A (0 day after first treatment)	Pesinc % Pessev %	12 10	12 10	12 10	8 10	8 10	8 10	14 15	14 15	15 15
10 DA-A (10 days after treatment A)	Pesinc % Pessev %	28 20	8 5	7 5	16 15	10.6 7.5	7.2 10	30 20	15.3 15	14.5 15
10 DA-B (10 days after treatment B)	Pesinc % Pessev %	34 25	18 7.5	16 7.5	22 20	10.8 10	9.9 10	28 30	15.4 17.5	19.25 15
10 DA-C (10 days after treatment C)	Pesinc % Pessev %	38 40	30.5 10	28.1 10	30 35	12.6 10	10 10	36 40	19 18	18.5 17.5
10 DA-D (10 days after treatment D)	Pesinc % Pessev %	45 50	27 12.5	30.4 10	34 40	14.6 7	12.1 7.5	46 45	20.6 20	18.1 20
10 DA-E (10 days after treatment E)	Pesinc % Pessev %	48 50	22.6 10	20.4 10	38 40	13 8.5	10.5 7.5	52 45	18.4 15	13 15

In the Sulfomat experiment, the assessments were staggered over a longer period of time due to the higher number of treatments (up to the second fall of the fruit), so that the degree of attack after the last treatment on the untreated control was higher than that recorded in the experiment with Polisulf, implicitly the incidence and severity recorded in Tables 6 and 7. Thus, the highest degree of attack reached 60.88% in 2021. As we can see, the degree of attack evolved from 7.0% to 58.8% (in 2019), from 1.5% to 43.2% (in 2020) and from 5.0% to 60.8% (in 2021) for the Ionathan variety (Figure 3). In the Golden delicious variety trial, the degree of attack increased from 1.20% to 24.0% (in 2019), from 0.8% to 15.2% (in 2020) and in 2021 from 2.1% to 23.40% (Figure 4). Under this infection pressure, the product Sulfomat 80 PU proved to be very effective, comparable to the standard product Kumulus DF. Analyzing the results obtained after the last treatment, efficacy ranged from 80.5% to 82.4% for the Ionathan variety and from 88.2% to 92.7% for the Golden delicious variety, quite similar to standard product Kumulus DF (Tables 8 and 9). Such good results were also obtained by Mitre et al., 2018, using wettable sulphur to control powdery mildew in Cluj Napoca, Romania.

Other researches (Cristea et al., 2017; Buzatu et al., 2018; Alexandru et al., 2019; Chitulescu et al., 2019; Chiriac et al., 2021; Mandru et al., 2021) also obtained favourable results testing unpolluted products which are suitable to be used in ecological production.

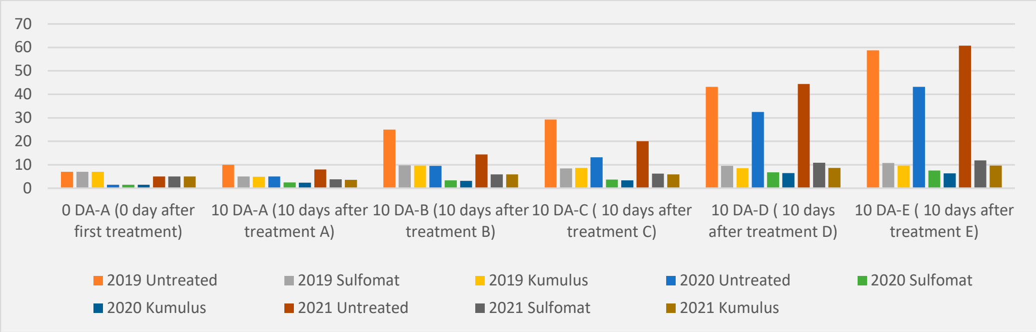

Figure 3. Degree of attack in Sulfomat trial (Jonathan variety)

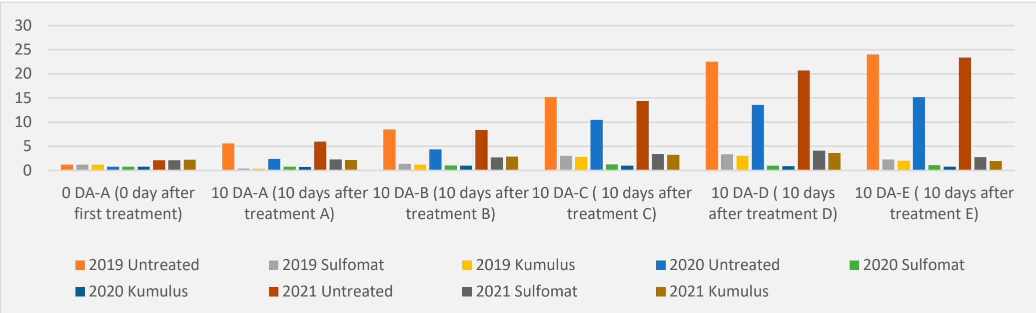

Figure 4. Degree of attack in Sulfomat trial (Golden Delicious variety)

Table 8. Efficacy% of products applied to control powdery mildew in the Jonathan variety

Year	2019		2020		2021	
Assessment time	Sulfomat 80 PU	Kumulus DF	Sulfomat 80 PU	Kumulus DF	Sulfomat 80 PU	Kumulus DF
10 days after A	50.4	51.2	49.8	52.3	52.7	55.6
10 days after B	60.8	61.3	64.6	66.8	59.4	59.1
10 days after C	71.2	70.6	72.4	74.5	69.1	70.7
10 days after D	78.1	80.3	79.1	80.3	75.5	80.6
10 days after E	81.6	83.5	82.4	85.2	80.5	84.1

Table 9. Efficacy% of products applied to control powdery mildew in the Golden Delicious variety

Year	2019		2020		2021	
Assessment time	Sulfomat 80 PU	Kumulus DF	Sulfomat 80 PU	Kumulus DF	Sulfomat 80 PU	Kumulus DF
10 days after A	66.4	69.8	68.2	70.1	61.9	63.9
10 days after B	75.7	78.2	75.4	77.6	67.8	65.6
10 days after C	79.9	81.5	88.0	90.5	76.3	77.4
10 days after D	84.9	86.5	92.5	93.3	80.1	82.5
10 days after E	90.6	91.5	92.7	94.8	88.2	91.7

CONCLUSIONS

Sulphur is a low cost, multi-site fungicide that is widely used to control powdery mildew all over the world. Good coverage of sulphur sprays on apple trees foliage is required for effective powdery mildew as it was proved in this paper.

Spray programs in which sulphur - based on products are included continously are also important to prevent development of resistance to newer fungicides and guarantee an optimum control of powdery mildew. They are mandatory to be included in each management control program of apple diseases.

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