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EVALUATION OF TWO METHODS OF TREATING *CROCUS SATIVUS* PATHOGENS

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ABSTRACT

Crocus (*Crocus sativus* L.) is a very important plant species for Western Macedonia, but also for Greece. In recent years, the flowering of *Crocus sativus* L. has shown a declining trend due to various causes including fungal infections. So it is important to find methods to deal with *Crocus sativus* pathogens. The research has been conducted in the area of Kozani, a city where crocus has been cultivated for the last 300 years. The aim of this research was the evaluation of two methods concerning the respective response of the crop. For this purpose, a physical method (sundisinfection), a chemical one (with systemic fungicide) and the control were installed. In addition, two different sowing depths (15, 20 cm) were tested to find the most suitable one. The experimental design was RCB with four replications. Specifically, the flowers of the crocus were measured and the number of infected bulbs from each experimental plot as well. The results showed that the number of flowers obtained by the two treatments (sundisinfection, chemical method) and the control were very close. Regarding the infected bulbs the most effective method was the chemical one and the most suitable sowing depth was that of 20 cm, except the control. Higher stigma yield was obtained from the control and the sun disinfection.

Key words: *crocus*, *sun disinfection*, *yield*, *sowing depth*.

INTRODUCTION

Crocus (*Crocus sativus* L.) is a very important plant species for Western Macedonia, and for Greece as well because it is of great economic interest due to its use as a spice (saffron) and for medicinal purposes (Vogdopoulos and Lazaridou 2022). Saffron is derived from the stigma of *Crocus sativus*, a sterile autotriploid herb belonging to the family *Iridaceae* (Shah and Tripathi 2009, Ambardar and Vakhlu 2013).

Globally, the area under saffron cultivation has shown significant decline due to several biotic and abiotic factors. Among the various biotic constraints, bulb rot has been recognized as a major limiting factor for successful cultivation of crocus in both traditional and non-traditional areas. The disease is also known as dry rot, brown rot, violet rot, death blight and yellows. Infected plants die early, resulting in the

reduction in size and number of daughter bulbs and flowers. This leads to the reduction in flowering period and subsequently to low yield and poor quality of saffron. Pathogenic fungi infect the bulbs by penetrating the protective sheaths, subsequently converting the white-colored bulb surface to yellow and ultimately to black, resulting in the rotting and death of the bulbs. Under moist conditions, the infection progresses very gradually and ultimately causes drying of the infected parts. In newly infested fields, the disease first occurs in small patches that gradually enlarge with each passing year until the whole field is infested. Unfortunately, the origin of the disease and the conditions that contribute to its development remain unknown. Several species of *Penicillium* have been isolated from infected crocus bulbs. *Rhizopus nigricans* has been isolated from injured bulbs of *Crocus sativus* L. (White et al. 2002). The genus *Fusarium* includes several species of fungi and spreads rapidly in soils and organic substrates. One of the pathogens of this genus is *Fusarium oxysporum* which is the most destructive disease of crocus and more than 100 plant species (Berrocal Lobo and Molina 2008) and has caused severe losses in Italy (Cappelli 1994). Of the pathogens isolated from saffron, *Fusarium* has been detected in many different saffron growing regions causing the highest bulb losses. Infected plants die early, resulting in reduced yield, quality, and flower and stigma production (Palmero et al. 2014). *Rhizoctonia* and *Sclerotium fungi* are soil basidiomycetes, which cause serious diseases in saffron. *Phytium* species are soil pathogens, which under conditions of increased moisture cause disease in many plants, including saffron plants (de Souza et al. 2003). Therefore it is important to find methods to deal with *Crocus sativus* pathogens.

Chemical soil disinfection, when it is applicable, it is often the only way out of dealing with a large number of soil pathogens. As a pre-emergence application, it drastically affects their survival soil pathogens, while disinfection with sublethal doses of disinfectants in combination with non-chemical methods such as sun disinfection are considered as biologically milder methods (Katan, 1981). Sun soil disinfection is a recent agricultural phytopathological soil disinfection technique. The method is based on the utilization of solar radiation by using transparent polyethylene plastics during the warm period of the year (Katan & DeVay 1991). Sun disinfection effectively reduces pathogen populations to a fairly large extent (30-50 cm). The presence of moisture is also considered an important factor for the successful application of sun disinfection. The actual sensitivity of the various microorganisms increases with the level of humidity.

This research was undertaken to study the effectiveness of two different methods treating the pathogens of crocus and how two different sowing depths influence their effectiveness.

MATERIALS AND METHODS

The experiment was established in the area of Kozani in Greece, in a field that has proven to be contaminated with pathogenic organisms. The treatments used included a physical method (sun disinfection), a chemical one (with systemic fungicide) and the control (no application). In addition, two different sowing depths (15, 20 cm)

were tested to find the most suitable one. The experimental design was RCB with four replications. Regarding the sun disinfection method, a special 3-layer impermeable sheet with a thickness of 30-35µm, Orgasum, was used, which significantly reduces chemical losses during soil solar disinfection. Its permeability to chemicals is 100-200 times less than that of a simple polyethylene sheet of the same thickness. The sun disinfection method was installed on the 21st of July, 2022 and completed on the 10th of September, 2022. Regarding the chemical method, the systemic fungicide Neotopsin 70 WG was used, which was sprayed at the depth where the crocus was sowed, with a special machine that has the ability to penetrate the soil and spray at the desired depth. No treatment was applied on the control. The sowing of saffron took place on the 17th of September, 2022, one week after the application of sun disinfection. The traditional way of planting (saffron planter) was used. In order to evaluate the effectiveness of the plant protection methods the flowers of the crocus were measured. Additionally a sample of 25 bulbs was taken from each experimental plot separately, which was examined in the Laboratory, both for the isolation and identification of pathogens and for the effectiveness of the plant protection methods.

RESULTS AND DISCUSSION

A phytosanitary check was carried out on all the samples studied for the presence of both external and internal symptoms on the saffron bulbs. A small percentage of infestation was observed in all the samples tested, while in the majority of them the affected bulbs presented mainly external changes. In particular, in the treatment of sun disinfection at the depth of 15 cm and 20 cm, the presence of both external and internal necrotic spots was 12%, and 8% respectively. Regarding the chemical method, the percentage of infestation at the depth of 15 cm and 20 cm, the presence of both external and internal necrotic spots was found to be 9% and 6% respectively. Concerning the control the external lesions as necrotic spots at the depth of 15 cm and 20 cm was 12% and 8% respectively, while there was no lesions inside the bulb (Table 1).

Table 1. Infection frequency in collected bulbs of *Crocus sativus* L. in three different treatments (plant protection methods)

Treatments	No of samples	Internal symptoms %	External symptoms %
Control 15cm depth	93	0	12
Control 20 cm depth	93	0	8
Sun disinfection 15 cm depth	104	12	12
Sun disinfection 20 cm depth	92	8	8
Fungicide 15cm depth	93	9	9
Fungicide 20 cm depth	94	6	6

As can be seen in the table above, in the control at both sowing depths (15, 20 cm), there are only external infestations and no internal lesions were observed. On the other hand, in the two other treatments used (sun disinfection, and chemical application), external and internal lesions were observed. This is because these treatments (sun disinfection, and fungicide) require irrigation for their proper effectiveness, during their installation in the field. The moisture that was created may have activated the pathogenic organisms, resulting in the acceleration of the start of their biological cycle, while in the experimental plots of the control, there was no moisture at all and the pathogenic organisms were deactivated. Tjamos and Faridis (1980) and Tjamos (1984) reported that sun disinfection, alone or in combination even with reduced doses of methyl bromide, successfully treated the fungus *Pyrenochaeta lycopersici* in a greenhouse experiment and the fungus *Verticillium dahliae* (Tjamos and Paplomatas 1987). Tjamos and Paplomatas (1988) also reported the long-term action of sun disinfection. Therefore, in our research the effectiveness of sun disinfection can be proven in the coming years.

Regarding the sowing depth in all treatments including the control, reduced infestation was observed at the 20 cm sowing depth compared to the 15 cm. Comparing the two methods (sun disinfection, and fungicide), less damage was observed in the chemical method (9 and 6% internal and external symptoms instead of 12 and 8%). Regarding the infected bulbs the most effective method was the chemical one and the most suitable sowing depth was that of 20 cm.

The results showed that the number of flowers obtained by the two treatments (sun disinfection and control) were very close both in total and at each of the two sowing depths separately. Slightly fewer flowers were obtained by the chemical method in total (Table 2). It should be emphasized that in both sun disinfection and control, more flowers were obtained from the 20cm sowing depth, while in the chemical method more flowers were obtained from the 15 cm sowing depth. So the appropriate sowing depth is dependent on the method applied. Higher stigma yield was obtained from the control and the sun disinfection.

Table 2. Number of flowers in three different protection methods and two different sowing depth

Treatments	Number of flowers Sowing depth 15 cm	Number of flowers Sowing depth 20 cm	Total Number of flowers
Control	1705	1948	3653
Chemical method	1876	1561	3437
Sun disinfection	1729	1918	3647

CONCLUSION

It was concluded that concerning the infected bulbs the most effective method was the chemical one, except the control, and the most suitable sowing depth was that of 20 cm. Regarding the yield it was concluded that the total number of flowers obtained by the two treatments and the control were very close.

However the results concerned one year experiments. So further research, for more than one year, is needed to confirm the results of the present study.

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