The Incidence Rate of White Rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) Disease in Sunflower Cultivation Areas in Konya and Aksaray Provinces and its Pathogenic Potential

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ABSTRACT

This study was conducted between June-September 2017-2018 in sunflower cultivation areas of Konya and Aksaray to determine the incidence rate of white rot disease and its pathogenic potential and to identify the disease agent *Sclerotinia* spp., Surveys were carried out 11,750 decares in total from Karatay, Altınekin, Cihanbeyli, Karapınar, Kadınhanı and Çumra districts of Konya province and 3000 decares in total from Centre of Aksaray (Hırkatol and Topakkaya village) and Eskil districts of Aksaray province. In the study, it was determined that the isolates obtained from diseased plants belong to *Sclerotinia sclerotiorum* (Lib.) de Bary. Although the disease was not seen in the first year in Konya, the disease rate in Altınekin district was determined as 9.38% in the second year. As a result of the survey studies, the disease rate in the Center of Aksaray in 2017 and 2018, respectively, was 2.83% and 3.97%; in Eskil, while no disease was occurred in the first year, it was determined at a rate of 4.16% in 2018. In the pathogenicity tests carried out with sunflower seedlings using approximately 20% of the isolates, it was found that the disease severity of the isolates varied between 56% and 66% and the difference between the disease severity and scale values of the isolates was statistically significant (P <0.05). Aksaray / Hırkatol was found to be the most virulent isolate in the pathogenicity test.

1. Introduction

Sunflower (*Helianthus annuus L.*), which ranks fourth in the world and is one of the most important oil plants, is essential in terms of vegetable crude oil production due to the high oil content (22-50%) in its seed (Tekçe 2015).

It also ranks first in terms of cultivation area and production among the oilseed plants cultivated in our country. The highest amount of sunflower for oil is produced in the Thrace-Marmara Region (47.2%), followed by the Central Anatolia Region (29.2%). As a snack, it is mostly produced in the Central and Eastern Anatolia Regions (Anonymous 2016).

Many investigations are being carried out in order to expand the production areas of oilseed plants, which will be the raw material for vegetable oil production in Turkey. Accordingly, sunflower cultivation for oil is also encouraged in the irrigable areas of provinces in the inner parts of the Black Sea Region and provinces such as Konya and Aksaray in the Central Anatolia Region. Crop alternation of sunflower with sugar beet or wheat, especially in the Central Anatolia Region or its replacement in areas where sugar beet cultivation is restricted have contributed significantly to the increase in sunflower production for oil in our country (Onan et al. 1992; Öztürk et al. 2008).

According to 2017 statistics, in our country, 1 million 800 thousand tons of sunflower for oil was produced in 681 thousand hectares of cultivation area and approximately 10% of the cultivation area and the highest yield taken from the unit area is in Konya (Anonymous 2017). Sunflower consumes less water than sugar beet, corn and carrot plants, which have an important place in Konya's plant pattern. This is one of the reasons for the farmer to prefer sunflower and it is also a suitable plant for alternation with wheat (Yavuz 2016).

Sclerotinia species, one of the crown rot diseases in sunflower cultivation, cause significant losses (Benlioğlu et al. 2004; Kırbağ and Turan 2006; Yıldız et al. 2010; Koike et al. 2013). *Sclerotinia* diseases are defi-

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ned by different names such as cottony rot, white mold, watery soft rot, stem rot and head rot. The most important pathogen of our study, sunflower crown rot *Sclerotinia sclerotiorum* (Lib.) de Bary is a disease that is common in almost every region of the world where sunflower is produced, including our country. *S. sclerotiorum* is distributed in all regions with temperate, tropical or subtropical climates (Gulya et al. 1997). *Sclerotinia minor* Jagger is also another species that has been reported to cause root rot and wilting on sunflower, but it is much less common than *S. sclerotiorum*. The pathogen is a polyphagous and facultative parasite and is a host in more than 400 plant species belonging to 75 families (Lazar et al. 2011). *Sclerotinia sclerotiorum* can remain dormant with its sclerots in dry soil without hosts and causes early infections at the seedling stage (Khan 2007).

For the determination of *S. sclerotiorum*, which causes root and crown rot disease in sunflower in Turkey, Yucer (1980) conducted a study in the Thrace region, Çınar and Biçici (1982) in Çukurova, Cetinkaya and Yildiz (1988) in the Marmara region, Onan et al. (1992) in the Aegean Region, Tozlu and Demirci (2008) in the Pasinler Plain of Erzurum province and reported that it is an important disease of sunflower due to economic losses. Yield loss varies depending on the developmental period of the sunflower. Yield losses can reach 100% if the infection occurs at the early stage of sunflower development (Lamey et al. 2000; Saharan and Mehta 2008). Sunflower plants infected at the beginning of the flowering stage may lose 98% of their potential yield, while plants infected 8 weeks after flowering may lose only 12% of their potential yield (Maširević et al. 2006).

Since cultural measures and the use of resistance varieties are limited in the control against *S. sclerotiorum* in sunflower and the chemicals used have negative effects and are not economical, early diagnosis of the pathogen is very important in order to develop and increase disease control strategies (Kotan et al. 2009).

In Turkey, there are no studies conducted so far on the presence of fungal diseases that are among the agents that cause yield and quality losses in sunflower production areas in Konya and Aksaray provinces of the Central Anatolia region, which have a significant share in sunflower production. Therefore, this study aims to investigate *S. sclerotiorum*, which causes root and crown rot disease in sunflower production areas of Konya and Aksaray provinces of Central Anatolia Region. Although studies related to sunflower diseases in Turkey are limited in number, at least researching and analyzing the situation in the region will be of great importance in terms of providing important contributions to the literature as well as guiding and informing the producers of the region and the relevant organizations for the struggle.

2. Materials and Methods

2.1. Mateial

2.1.1. Test pathogen fungus

In our study, *Sclerotinia sclerotiorum* isolates obtained from diseased sunflower plants cultivated in Konya and Aksaray provinces were used as test pathogens. The isolates were grown on Potato Dextrose Agar (PDA) medium with streptomycin sulfate 0.9 g/L. Potato Dextrose Agar (PDA) is a general purpose nutrient medium used for the cultivation of a large number of fungi and yeasts. It was supplemented with acid or antibiotic (40 mg streptomycin sulfate / 100 ml) to inhibit bacterial growth. The nutrient medium was prepared as 4.0 g / lt Potato Extract, 20.0 g / lt Dextrose, 20.0 g / lt Agar and adjusted to pH 6.8 (Dhingra and Sinclair 1995).

2.1.2. Test plant

In the study, the sunflower plant was used as the host. When selecting the sunflower plant variety, the most cultivated varieties in the villages of Aksaray province, where *S. sclerotiorum* is common, were taken into consideration. Considering this, "İnegöl Alası", which is a sunflower variety for snack, was selected to be used in our study. The variety that has a wide cultivation area in the Aksaray region was obtained from the producers.

2.1.3. Cultivation environments used in pot experiments

In the pathogenicity test, sterile peat and perlite mixture at a ratio of 3: 1 in pots and 15-15-15 (N-P-K) commercial compound fertilizers were used for cultivation environments. The experiments were carried out with 2.5 lt (130 x180 mm) volume plastic pots.

2.2. Method

2.2.1. Disease survey

Sunflower cultivation areas in districts of Konya (Karatay, Altınekin, Cihanbeyli, Karapınar, Kadınhanı and Çumra) and Aksaray provinces (Center (Hırkatol and Topakkaya villages) and Eskil) were selected as survey areas. In the surveys conducted between June and September of 2017 and 2018, quite a lot of fields were examined and diseased and healthy plants were counted. The samples taken from the fields were examined as 20 plants per 1-5 da, 40 plants per 6-10 da, and 60 plants in fields larger than 10 da.

In order to determine the disease rates (%), at least 60 plants were selected from the areas selected from each province, in randomly determined rows, with approximately 10 plants each. Diseased and healthy sunflowers taken from different points of the field were counted, the results were converted into percentages and disease rates were calculated according to Bora and Karaca method (1970).

2.2.2. Isolation from diseased plants

Diseased plant samples collected from the study areas constituted the main material of the study. Depending on the number of diseased plants, 5-10 plants

were sampled per field and used for isolation. After the plant samples brought to the laboratory to isolate the agent were washed in tap water, the pieces or sclerotiums taken were disinfected superficially in 1% sodium hypochlorite (NaOCl) for 1-2 minutes, then rinsed 3 times in sterile distilled water, and left to dry for 5 minutes on sterile blotting paper in a laminar flow cabinet. Then, each sample was planted in 2 petri dishes on Potato Dextrose Agar (PDA + Streptomycin sulphate) medium. 3-4 diseased tissue pieces were planted in each petri dish. These petri dishes were left to incubation at 20-25°C and started to be monitored from the 2nd day. Pure cultures were obtained by transferring micellar discs of 5 mm diameter taken from the tip of colonies that developed from tissue pieces or sclerotiums within 2-3 days to petri dishes containing PDA. These *S. sclerotiorum* isolates were kept in a refrigerator at 10°C and used in the next sta-ges of the study.

2.2.3.Identification of the isolates

It has been stated that it is possible to separate sclerotiums by size in order to define *S. sclerotiorum* and determine its differences from other species (Ekins et al 2005). The width and length of 5 sclerotium obtained from each isolate developed in PDA for 3 weeks were measured with a caliper and the arithmetic averages of the obtained values were taken (Lucas 1998).

2.2.4.Pathogenicity test

Pathogenicity was established with 4 repetitions according to the Random Parcel Trial Pattern and 2 (two) *S. sclerotinia* isolates obtained from sunflower fields in Aksaray province were applied to sunflower seedlings as a limited time stem inoculation method.

When the plants are in the R2 stage, which is the beginning of flowering (Nelson et al 1988), the mycelium discs of 4 mm diameter taken from the actively growing parts of the cultures of *S. sclerotiorum* developed in PDA for 7 days, were inoculated by placing a mycelium disc on each plant and into the wounds opened to the stem of the plant with a diameter of 5 mm, 4 cm above the soil surface, and wrapped with parafilm. The sterile PDA discs were placed in control plants. At the end of the incubation period (7 days), the length of light or dark brown lesions on the stem was measured from the point of inoculation with a digital caliper. 1-6 Root rot scale modified according to James (1971) (1: No symptoms in the plant, 2: Area with 1-10% necrosis, 3: Area with 10-25% necrosis, 4: Area with 25-50% necrosis. 5: Area with 50-75% necrosis, 6: Area with 75 -100% necrosis) was used and the disease severity (%) was calculated according to the Townsend-Heuberger (1943) formula.

The obtained data were analyzed with one-way ANOVA using the SPSS statistical program (SPSS Inc., version 17.0) and the significant differences between the averages were determined by Tukey multiple com-parison test ($P \leq 0.05$).

3. Results and Discussion

3.1.The results of disease survey

Sunflower production areas in Konya and Aksaray districts were continuously checked with an average interval of 20-30 days (30, 45, 60 and 90 days) starting from June (emergence) in 2017 and 2018. The results of the years surveyed in Konya are given in Table 1.

Table 1
Ratio (%) of White Rot Disease (*Sclerotinia sclerotiorum*) in Sunflower cultivation areas of Konya province

Years	Location	Production Area (da)	Number of fields surveyed	Survey area (da)	Number of diseased fields	Infestation % of field	Disease rate (%)
2017	Karatay	183710	25	1900	0	0	0
	Karapınar	96000	20	1000	0	0	0
	Cihanbeyli	83765	12	900	0	0	0
	Altınekin	79904	24	850	0	0	0
	Kadınhanı	78500	13	800	0	0	0
	Çumra	36670	14	400	0	0	0
2018	Karatay	204743	32	2100	0	0	0
	Karapınar	71523	13	700	0	0	0
	Altınekin	109002	28	1300	8	28	9,38
	Kadınhanı	76255	15	800	0	0	0
	Çumra	88722	16	900	0	0	0

While no plants showing symptoms such as root and crown rot, wilting, growth retardation, weak plant growth were occurred in 5,850 da area surveyed in 2017, abiotic related problems were observed more (Table 1). In 2018, *S. sclerotiorum* was detected in 4.74% of 5,900 da area, and abiotic related problems seen in the previous year were occurred again. Monthly

(6th and 7th months) average relative humidity is 40.4% in Konya province while the average temperature is 24 ° C, and these climatic values are thought to prevent the emergence of the disease (*S. sclerotiorum*). Because the importance of humidity and precipitation in the development of the disease has been emphasized in many studies (Çetinkaya and Yıldız 1998; Huang et

al. 1998; Matheron and Porchas 2000). Considering the average humidity, temperature and monthly precipitation data in Konya / Altınekin district, it is predicted that there are increasing values between 2017-2018 and that disease agents causing sunflower root rot may appear in this region in 2018. Considering these evaluations, a disease agent was encountered in the Oguzeli village of Altınekin during the 2018 surveys. As a result of the examination, 45 of 480 plants examined were found to be diseased and the disease rate was determined as 9.38%. In addition to climate data, faulty agricultural processes have triggered the emergence of disease. In particular, entering alternation for only 1 year, not leaving the field fallow and excessive irrigation are examples of these and also show that this disease may increase in the coming years. Among them, long-term crop rotation is very important in combating the pathogen (*S. sclerotiorum*) before it becomes a serious problem in a region (Rashid 2003).

The results of the surveys carried out in the sunflower fields of Aksaray province in 2017-2018 are given in Table 2.

Table 2

Ratio (%) of White Rot Disease (*Sclerotinia sclerotiorum*) in Sunflower cultivation areas of Aksaray province

Years	Location	Production Area (da)	Number of fields surveyed	Survey area (da)	Number of diseased fields	Infestation % of field	Disease rate (%)
2017	Merkez	49950	13	600	3	23	2,83
	Eskil	86500	20	900	0	0	0
2018	Merkez	64825	17	800	5	29	3,97
	Eskil	65066	18	700	4	22	4,16

The disease rate in the center was 2.83% in 2017, it was 3.97% in 2018. While no disease was occurred in Eskil in 2017, the disease rate was 4.16% in 2018 (Table 2). 23% of the survey area in the Aksaray region was infected with *S. sclerotiorum* in 2017. In 2018 surveys, it was observed that the disease occurred in districts where there was no disease in the previous year, and the rate of infection was between 22% and 29%. There may be different reasons for the emergence of the disease to increase compared to previous years or for the emergence of the disease in districts where it has never appeared. The disease started to appear in increasing density due to the fact that sunflower producers do not fully comply with the alternation in the production areas, successive sunflower cultivation is widespread, the time of cultivation is not appropriate, the cultivation is performed frequently, the diseased plant residues are not destroyed and the surface irrigation (wild irrigation) is applied. Crop rotation should be initiated before the pathogen becomes a serious problem in an area, and cultivating non-host crops for 3-5 years will reduce the number of sclerotia in the soil and minimize the impact of sunflower root infection (Gulya et al. 1997; Rashid 2003).

The fact that monthly (6th and 7th months) average relative humidity of Aksaray is quite high at 60% and the average temperature is around 21,6°C and lower than other provinces can be considered among the reasons why the disease is seen in this region. Sclerots from the previous year are the most important means of penetration and their germination is best performed at 24% soil moisture. However, destructive infections occur when sensitive plants are close (Irany et al. 2001; Lazar et al. 2011).

3.2.Isolation results and symptoms of the disease

When the sunflower fields were visited during the vegetation period, it was found that *Sclerotinia sclerotiorum* was the white rot agent of the disease which was obtained as a result of both macroscopic observations and isolations performed from diseased plants.

In the observations about the pathogen, it was seen that irregularly shaped light and dark brown lesions starting from the base of the sunflower stem and the expansion of these lesions in time led to the symptoms of cottony mycelium. The disease spreads completely by covering the stem. The most obvious and typical symptom of the pathogen is the formation of amorphous sclerotium on the infected sunflower stem, which are initially white but then turn black on the outside and begin to harden. Over time, the stem of the infected plant can be broken easily by taking a thready appearance, and sclerotiums are seen to form when these parts are opened. Similar symptoms were observed in a survey carried out on sunflower in Pasinler plain of Erzurum and 169 *S. sclerotiorum* isolates were obtained in isolation from roots (Tozlu 2003).

S. sclerotiorum was found in only 1 locality in Konya and 3 different localities in Aksaray. As a result of a 2-year survey study, 2 isolates from Aksaray Province Center (Topakkaya village) in 2017, 4 isolates from Aksaray Province Center (Hırkatol village), 3 isolates from Eskil (Kökez village), 1 isolate from Konya province Altınekin (Oguzeli village) was obtained. Thus, a total of 10 isolates were determined.

3.3.Identification of isolates

The agents of white rot disease are *S. sclerotiorum* and *S. minor*, and the distinction of these two species is based on the size of the sclerotium. S. *minor* forms more, small, irregular sclerots. It was determined that

all isolates belong to *S. sclerotiorum* by the measurements of sclerot sizes of the isolates obtained as a result of the isolations. When all localities are considered in general, the length of the sclerots varies between 4.4-8.19 mm (5.8 mm) and the width is between 2.13 and 5.9 mm (4.0 mm). The results in this study are in accordance with the sclerots of *S. sclerotiorum* in the literature with sizes ranging from 5-20 mm (Singleton 1992) and 3-10 mm (Ekins et al. 2005).

3.4.Pathogenicity test of isolates

In pathogenicity tests, Aksaray (Eskil and Hırkatol) isolates obtained from the regions where the disease occurred for two consecutive years were used and it was found that the scale values of the isolates varied between 5-6. As a result of the pathogenicity tests, a difference was observed between the lesion lengths of the symptoms (Table 3). When the lesion lengths of the repetitions were evaluated in general, Hırkatol isolate was measured as the highest 12 cm and the lowest 10 cm, and the Eskil (Kökez) isolate as the highest 9.5 cm and the lowest 5.5 cm. It was observed that this difference is related to the humidity and precipitation during the vegetation period, which was supported by the studies (Minkevich and Kosorukova 1987; Tozlu 2003; Onaran 2009). Both isolates were observed to cause complete death in plants and are highly virulent. Differences in virulence among the isolates of *S. sclerotiorum* have been revealed by many researchers (Marciano et al. 1983; Kohn et al. 1990).

Table 3
Disease severity rate (%) of *Sclerotinia sclerotiorum* isolates

Isolates	Average lesion lengths (cm)	Disease severity (%)
Aksaray/Hırkatol	11 a	66 a
Aksaray/Eskil (Kökez)	7.5 b	56 b

* There is no statistical difference between groups bearing the same letters (P <0.05)

One of the most important reasons why the disease severity occurs most in the isolate taken from Hırkatol is thought to be caused by the continuous cultivation of the same product due to the intensive sunflower cultivation in this region. In many studies, the reason for the high inoculum density has been reported to be due to the continuous cultivation of the same crop in the same fields and continuous precipitation (Morton and Hall 1989; Dorrance and Lipps 2002).

4. Conclusion

In the study, it was determined that the agent that causes white rot disease in sunflower cultivation areas in Konya and Aksaray provinces is a fungal agent named *S. sclerotiorum*. Determining the disease in sunflower is important since no detailed studies have been carried out in these regions before.

In the study, it was aimed to determine the prevalence rates of *S. sclerotiorum* and it was observed that the disease appeared at increasing rates in the districts of Aksaray every year and the average disease rate for two years was found to be 3.5%. As a matter of fact, in studies conducted on sunflower in our country, it has been stated that the prevalence of *S. sclerotiorum* varies between 2.5-100% (Onan et al. 1992; Demirci and Kordalı 1998; Tozlu 2003).

In Konya province, the course of the disease, which was observed only in the second year, was quite high with a rate of 9.38%. Altınekin District Directorate of Agriculture and the producers stated that the disease was seen several times in previous years. Considering the temperature and humidity values of 2017, it is thought that the emergence of the disease may increase in the following years. In addition, it can be said that the amount of inoculum will increase over the years depending on sunflower production and even this disease will cause an epidemic by posing a potential danger. It is thought that detecting the areas where *S. sclerotiorum* may occur and diagnosing the disease will contribute greatly to combat this pathogen and reduce its inoculum potential.

5. Acknowledgements

6. References

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