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# The possibility to control diseases caused by *colletotrichum* species in strawberries

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**Abstract**

Colletotrichum species are important fungal pathogen that cause significant crop losses before and after harvest worldwide. It is known that three species of Colletotrichum are caused by Anthracnose Disease in Strawberry. These are Colletotrichum fragariae A.N.Brooks, Colletotrichum acutatum J.H.Simmonds, and Colletotrichum gloeosporioides (Penz.)Penz.&Sacc.in Penz.(telemorph Glomerella cingulata (Stoneman) Spauld.&H.Scherenk). Strawberry anthracnose symptoms caused by C. *acutatum*, C. *fragariae* and C. *gloeosporioides* are similar. This pathogen causes losses in strawberry production, seedling, and crops in all stages of the plant from seedling production from harvest period. The pathogen has become widespread on seedling material throughout the world for the last two decades. This review covers detailed information on the anthracnose diseases caused by Colletotrichum species in strawberries as well as disease cycle and control methods. The review was conducted to assist researchers studying on strawberry diseases, extension services, strawberry farmers, and seedling producers.

**Key Words:** *Strawberry, Fragaria x ananassa, Anthracnose Disease, Colletotrichum spp.*

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## Introduction

Strawberry (*Fragaria x ananassa* Duch.), which has an important place among berry fruits, is one of the species under Fragaria genus belonging to Rosaceae family of Rosales order (Hancock, 1990). Being a perennial and evergreen plant, strawberry is native to North and South America. While traditional strawberry cultivation started in AD 1300s in Europe, modern strawberry cultivation started in the 1970s in Turkey (Anonymous, 2009). Strawberry has become popular as it is rich in vitamins and minerals, is consumed as fresh, and is used in producing jam, marmalade, ice-cream, cake, liqueur, and fruit juice. Also, ellagic acid in this fruit species, which is rich in vitamin C, has anti-carcinogenic properties. Strawberry fruits contain significant amounts of mineral matters such as salicylic acid, vitamins A and B, calcium, iron, and phosphorus and small amounts of bromine, silicon, iodine, and sulphur (Paydaş, 1998; Aybak, 2005; Anonymous, 2009).

Based on data of FAO, Turkey was ranked as the fifth with a strawberry cultivation area of 154.310 da in 2016. The worldwide strawberry production was 9.118.336 t in 2016 and the USA was followed by Turkey (415.150 t) and Spain (366.161 t), respectively (Anonymous, 2016). Upon the increased strawberry production and export around the world, the economic importance of strawberry has increased in domestic and foreign markets. Based on the export figures of 2016, Spain has the highest rate of strawberry export (314.256 t), which is followed by the USA (134.406 t) and Mexico (102.631 t). Turkey had a strawberry export of 9557 tons in 2016 and the export market of Turkey includes Romania, Iraq, and Georgia, respectively (Anonymous, 2016).

Many fungal disease agents are seen in strawberry plants. One of those agents is Colletotrichum. It is known that 3 species of *Colletotrichum* cause Anthracnose Disease in strawberry. These species are *Colletotrichum fragariae* A.N. Brooks, *Colletotrichum acutatum* J.H. Simmonds, and *Colletotrichum gloeosporioides* (Penz.) Penz.&Sacc.in Penz. (teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Scherenk).

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These agents cause similar symptoms in strawberry, spread rapidly in strawberry plantations in temperate weather (optimum growth temperature 25 ºC for *C. acutatum* and 30 ºC for *C. gloeosporioides*) in rainy and harvest times and lead to decay. As a result, the diseases Anthracnose Fruit Rot and Anthracnose Crown Rot occur. The other symptoms of Anthracnose are stolon and petiole infections and bud rot, black spot on leaves, irregular leaf spot, and blossom blight. Sometimes, *Colletotrichum dematium* (Pers.) Grove also causes fruit rot in strawberries. Blossom and maturing fruits are very susceptible to anthracnose. Anthracnose fruit rot is seen commonly in hot areas in the world and more severely in the areas where plastic mulch is used and ridge planting is performed. In general, *C. acutatum* causes fruit rot (Maas,1998).

The symptoms of the disease occur in different periods. Upon *Colletotrichum* infection of mother plants, all plants collapse due to crown rot. Paleness is seen in seedbeds and unrooted companion plants and lesions are seen in stolons. Infected plants spread in fields through sick seedlings taken from seedbeds. In strawberry cultivation areas, yield loss is seen in all of the products due to Anthracnose disease under suitable environmental and cultural conditions. Leaves, flowers, and fresh and mature fruits can be too susceptible to Anthracnose disease that causes losses in all the fruits under some conditions (Howard et al., 1992).

Even if cultivation activities in the production area is managed optimally, there may be a loss of > 50% in the product due to fruit rot when suitable conditions occur for development of the disease. As Anthracnose disease causes epidemic in strawberry fields in Brazil, losses of 30-68% occur. This disease appeared in the hot regions of North America first and it is considered that the disease might have occurred in the south, as well (Legard, 2000). Anthracnose disease that causes serious losses in strawberry cultivation areas and fruit production has been reported in Florida, Brazil, and Argentina and it has been stated that this disease outbreak destroyed strawberry cultivation areas there. For more than twenty years, losses have increased in North America, California, New York, Massachusetts, Pennsylvania, Ohio, Canada, Ontario and China due to Fruit Anthracnose (Turechek et al.,2006; Dai et al., 2006 ).

*C. acutatum* is a necrotrophic pathogen in strawberry tissues and causes necrosis in flowers, fruits, leaves, leafstalks and roots. In seedbeds, seedling roots are infected with the agent during harvesting, cleaning, and packaging. Normally, roots are not easily infected in perennial strawberry plants. However, as strawberry is usually produced annually or every two years, the agent infects in seedbeds which are very different and far away from the cultivation fields and it is transferred to the fields through active or dormant infections. The biotrophic phase of the agent is quite short. In this phase, the infected leaves, leaf stalks, and branches continue growing without showing any symptoms. The agent, which is dormant on the surface of these parts, produces secondary conidia and thus flowers and fruits are infected. The primary inoculum source in strawberry field is the conidia in acervulus on leaves and fruits (Figure 1).

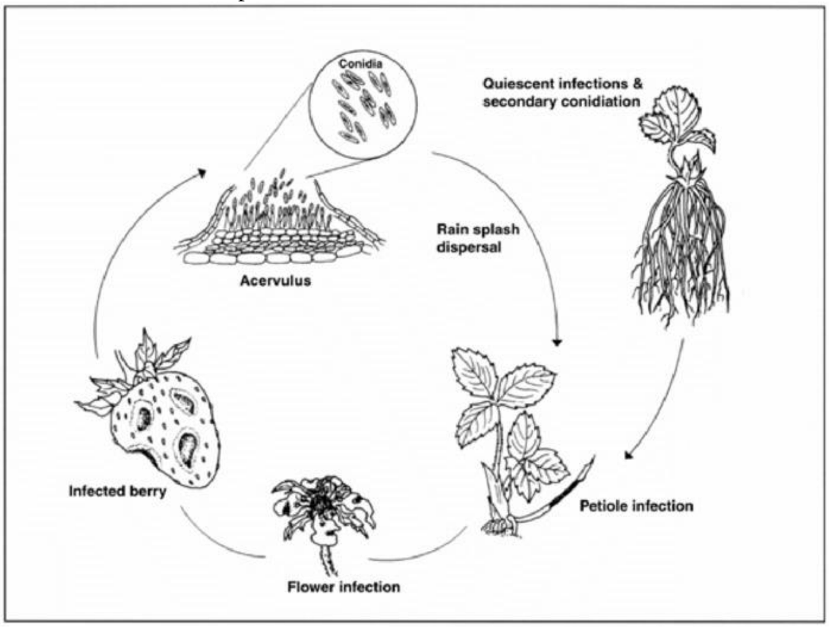


**Figure 1.** Fruit anthracnose lifecycle caused by *Colletotrichum acutatum* in strawberries. Biotrophic phase on seedlings and long necrotrophic phase in leaf stalks, fruits and flowers (Peres et al., 2005)

The conidia of the agent spreads through animals, tools-equipment, farmers, insects, splashing water drops, and rain dragged by wind. The development and spread of the disease are quite low under cool and dry conditions. Crown infections develop very often in seedbeds but infections are not seen after plants are transplanted. Sudden deaths occur in temperate weather conditions in autumn or in the spring of the following year. Therefore, infected plants continue fungus growth in new seedbeds and are the inoculum source of the following year.

**The Studies on Controlling the Anthracnose Disease in Strawberries**

In controlling the diseases caused by *Colletotrichum*, generally, one of the following methods or a combination of several of them can be used (Wharton and Diéguez-Uribeondo, 2004).

Resistant varieties
Cultural measures
Chemical control
Biological control

**Resistant varieties**

It is known that the host plant resistance is a suitable and effective way in controlling Anthracnose disease (Wharton and Diéguez-Uribeondo, 2004). Among the strawberry varieties, Sweet Charlie, Carmine, Florida Radiance and Florida Elyana are known as the varieties tolerant against the disease (Chandler et al., 2006; Seijo et al.,2008). Field trials were performed between 2001-2004 in order to determine the susceptibility of some strawberry varieties (Treasure, Sweet Charlie, Earlibrite, FL 99-164, Carmine, Camino Real, Aromas, Strawberry Festival, Camarosa and Gaviota) against *C. gloeosporioides* isolates obtained from strawberry; on the other hand, the field trials were performed for their reactions against *C. fragariae* during production season of 2003-2004. Every year, 4-6 isolates have been tested against 4-7 different strawberry varieties. Camarosa was the most susceptible variety in strawberry cultivation season of 2001-2002, which was followed by Aromas. Earlibrite and Sweet Charlie were determined to be the most resistant varieties. These varieties had similar results also in production season of 2002-2003. Treasure, Strawberry Festival and Gaviota were also assessed in strawberry production season of 2002-2003. It was found that Treasure was more resistant and Gaviota was more susceptible compared to the other varieties. Strawberry Festival had a moderate level of resistance similar to Aromas. In strawberry production season of 2003-2004, Treasure was the most resistant variety and Camarosa was the most susceptible variety. The reaction of strawberry varieties against *C. fragariae* was very similar to *C. gloeosporioides*, Treasure was quite resistant in *C. fragariae*, FL 99-164 and Carmine had a moderate level of resistance, and Strawberry Festival and Camarosa were quite susceptible (MacKenzie et al; 2006).

**Cultural measures**

In general, they include methods of sanitation, changing product designs, increasing resistance and avoiding predisposition. Additionally, these methods include the use of suitable sanitation techniques in order to prevent fruits from being exposed to pathogen during carrying, packaging and storing. Also, abiotic factors such as mechanical injury, excessive temperature and oxygen shortage that predispose fruit against pathogenic infections during packaging should be avoided. Disposal of sick leaves and fruits in the field increases the effect of chemical control and is generally crucial in terms of garden health (Wharton and Diéguez-Uribeondo, 2004).

*C. acutatum*, that causes Fruit rot in strawberries, can survive over winter in strawberry plant residues or soil or mummified fruits. As *C. acutatum* can survive and reproduce on leaves without showing any symptom (dormant infection) (Figure 1), it has been stated that infected plants overlooked before planting may cause severe infections (Leandro et al.,2001). Conidia of *C. acutatum* may spread locally with splashing water and rain drops and to further places through air and wind (Yang et al., 1990). In their studies, some researchers have revealed that plastic cover makes the spreading of disease easier compared to bare soil or hay mulch. It has been stated that precipitation lasting for a short period such as 15 minutes in places where plastic mulch is used is enough for the 100% infection of fruits 60 cm far away from a sick fruit (Madden et al., 1993). However, it is known that the plastic cover is essential for weed control in strawberries and it is not a realistic option to eliminate this cover (Yang et al., 1990). The use of plastic mulch in strawberry farming in recent years has decreased spreading of inoculum and infection in both nursery gardens and cultivation fields and thus it can control significantly the incidence of anthracnose disease (Freeman, 2008). Also spores of the agent spread from sick fruits toward healthy fruits during harvesting (Legard, 2000). Wilson et al (1990) stated that the optimum temperature for infection in immature and mature fruits was between 25-30 °C for Midway variety and the prevalence of disease was higher than 80% if leaf wetness was seen for more than 13 hours. They also determined that while there was no infection on mature or immature fruits below 4°C, there was no infection on immature fruits above 35°C (Madden et al.,1993).

*C. acutatum* was isolated from all of the strawberry fruits kept at constant temperatures (-12 and -30 °C) for 18 weeks in the laboratory or kept at 5 °C for 1 week and then at -12 and 30 °C. In two-year field studies (1988-1989, 1989-1990), *C. acutatum* was isolated from almost all of the fruits located on the surface of soil or 5-8 cm under soil after being kept under winter conditions for 3 months (from November to January) but *C. acutatum* decreased 3 months later. The recovery rate of *C. acutatum* from the mummified fruits kept above or below soil for 6 months (from November to May) was found to be 80% and 67% in 1988-89, 60% and 0% in 1989-90 and 7% and 7% in 1990-91, respectively. And the recovery rates of *C. acutatum* from the mummified fruits above or below soil were determined to be 53% and 7% in 1989-90 and 47 and 40% in 1990-91 (Wilson et al., 1992).

An experiment was set up under field conditions in Florida in 1998 and 1999 in order to determine whether *C. gloeosporioides* continued its vitality in crown parts of strawberry plants during summer months. Strawberry crowns naturally infected by *C. gloeosporioides* were put into cloth pouches containing field soil, they were buried at soil depths of 5 or 13 cm in the field and they were taken from soil in order to make assessment in both of these years. It was determined that 96% of 428 *Colletotrichum* spp. isolates obtained from the strawberry crowns buried in the soil were *C. gloeosporioides* and 4% were *C. acutatum*. *C. gloeosporioides* had more vitality in beginning, its vitality was stable during the following 2-3 weeks and then decreased. *Colletotrichum* spp. was not determined in the inoculum in the pouch buried for 56 days in 1998 and 98 days in 1999. As the period between strawberry seasons is usually longer than 170 days, it was determined that *C. gloeosporioides* could not maintain its vitality in the sick plant residuals buried in soil in Florida and, therefore, the plant residuals in soil did not contribute to the epidemic of *C. gloeosporioides* during summer months in Florida (Ureña-Padilla et al., 2001).

It is known that the main species causing anthracnose in strawberries in Israel is *C. acutatum*. In the studies, *C. acutatum* obtained from strawberry was examined in pepper, eggplant, tomato, bean and strawberry plants under greenhouse conditions. It was reported that the agent was isolated from all of the tested plant species 3 months later but it caused disease symptoms only in strawberries. Epiphytic and endophytic fungal growth in several plant species was validated by re-isolation performed on leaf tissues and using PCR tests. *C. acutatum* was isolated from healthy *Vicia* spp. (broad bean) and *Conyza* spp. (Erigeron) showing no symptoms. The isolates obtained from weeds caused disease symptoms in strawberry plants and the agent was validated to be *C. acutatum* by using PCR. Although *C. acutatum* causes disease only in tulips and strawberries in Israel, it is present in many plant species. For this reason, plants that are invisible hosts of *C. acutatum* may act as a potential inoculum source for strawberry infections and in order for the pathogen to maintain its vitality (Freeman et al., 2001).

The cross-inoculation trials in strawberry and tulip revealed that all tulips died within 14 days after the inoculation of all tulips (tulip or strawberry) regardless of the source of the isolate, typical anthracnose symptoms were observed in strawberry plants inoculated with the isolates obtained from both hosts and then the plants died (Freeman, 2000).

**Chemical control**

In general, the diseases caused by *Colletotrichum* species can be controlled with copper compounds, Dithiocarbamates, Benzimidazoles, Triazoles and other fungicides (such as chlorothalonil, imazalil and prochloraz) (Wharton and Diéguez-Uribeondo 2004). It has been proved that the newest fungicide group such as strobilurin (i.e.; azoxystrobin and pyraclostrobin) are considerably effective in infected fruits. Fungicides may be applied against the infections that may develop during humid periods in order to protect the young tissues of leaves, flowers or fruits (Wharton and Diéguez-Uribeondo 2004).

In America, treatments of captan and thiram, which are the protective fungicides, are performed safely in disease controls. On the other hand, benzimidazole fungicides are not effective against Colletotrichum species as they form resistance (Freeman et al., 1997; Smith and Black, 1993).

The effect of some fungicides and hot treatment on the strawberry anthracnose caused by *C. acutatum* was examined under laboratory, greenhouse and field conditions in a study. In laboratory trials, it was determined that the dose values (ED50) preventing mycelial growth by 50% were 30.5, 12.2, 0.2, 0.15, 0.05, 0.07, and 0.05 μg/ml respectively for folpet, captan, propiconazole, difenoconazole, prochloraz-Zn/folpet, prochloraz-Zn, and prochloraz-Mn. When Prochloraz-Zn treatment was compared with prochloraz-Zn/folpet (90%), captan, folpet and water (100%) treatments, it was found that the infection in strawberry stolons decreased significantly and was 60%. In greenhouse trials, the naturally infected seedlings were exposed to hot treatment for 5 minutes at 49°C in all fungicides and the treatment decreased the number of dying seedlings significantly compared to control. Prochloraz-Zn was the most effective fungicide but it was not different from hot treatment. In the field trials performed between 1995 and 1996, the decrease percentage in seedling death was found to be 93.3, 93.1, 66.7, 37.7, and 29.1%, respectively for prochloraz-Mn, prochloraz-Zn, prochloraz-Zn/folpet, propiconazole, and difenoconazole (Freeman et al., 1997).

Nowadays, anthracnose control in blueberry and almond is performed mainly with chemical methods and it has been reported that fosetyl-Al, captan, benomyl, chlorothalonil, ziram, fenbuconazole, myclobutanil, thiophanate-methyl, azoxystrobin and pyraclostrobin are effective against *C. acutatum*. However, benomyl, one of these fungicides, has been withdrawn by the producing companies. Among other fungicides, fosetyl-Al is expensive, chlorothalonil has phytotoxicity in fruits, the use of captan by fruit processing companies is limited since it has B-2 carcinogenic effect today, the harvest range of ziram is minimum 14 days and azoxystrobin and pyraclostrobin treatments are permitted only once in a season due to fungicide resistance. All of these reasons restrict the control

(Adaskaveg and Förster,2000). In a previous study, the effect of pyraclostrobin treatments on anthracnose fruit rot during the pre-and post-infection periods in New York and Florida was assessed in Tristar strawberry variety under controlled climate conditions and by field studies. *C. acutatum* was inoculated to Tristar strawberry varieties and the plants were placed into humid rooms at 14, 22, and 30 °C. They were removed from the room 3, 6, 12,14,24 hours later and taken to greenhouse in order to allow disease development. Pyraclostrobin was treated to strawberry fruits at the dose of 168gr/ha, 3, 8, 24, and 48 hours before the inoculation of fruits and before humid period or 3, 8, 24 and 48 hours after inoculation and during the humid periods. All pyraclostrobin treatments suppressed the disease compared to control. The prevalence of disease was mostly observed when plants were exposed to humid conditions for a long time (12 and 14 h) and high temperatures (22 and 30°C). Pyraclostrobin treatments provided significant control under humid conditions in 3 and 8 h after infection; however, this treatment was found to be less effective than protective disinfection. However, pyraclostrobin provided the control by 50% 24 hours after infection under long humid conditions and by 75% with protective treatment. For this reason, it was stated that it was possible to obtain a result from pyraclostrobin corresponding to protective disinfections only with the fungicide treatments following the short-wet periods (Turechek et al., 2006).

In another study, protective (captan) and systemic (fludioxonil + cyprodinil) fungicides were assessed as pre- and post-disease treatments for the control of anthracnose fruit rot caused by *Colletotrichum acutatum* under short (6 and 8 h) and long (18-24h) humid conditions. The assessments were conducted in Maryland and Florida, 2 seasons for each. In the control of anthracnose fruit rot, the combined use of captan and cyprodinil + fludioxonil before inoculation was effective but the most effective result was obtained under the shortest humid conditions. Cyprodinil + fludioxonil was found to be effective when applied 4, 8 and 24 h later than inoculation and before inoculation. However, the treatment controlled the disease better when applied under short humid conditions. Captan was effective when applied under short humid conditions 4 and 8 h after inoculation but it was not effective 24 h after inoculation. Captan treatment became ineffective in any time after inoculation under long humid conditions. The effect of cyprodinil + fludioxonil after infection brought a great flexibility in controlling anthracnose fruit rot (Peres et al., 2010).

The *in-vitro* effect of 28 fungicides against *C. fragariae* causing root rot in strawberry was tested. Diniconazole, flusilazole, benomyl, propiconazole, nuarimol, fenarimol and bitertanol agents decreased the growth of the agent at 0.5 ppm and greater concentrations. PCNB, mancozeb, maneb, myclobutanil, thiram, DCNA, iprodione, triforine, chlorothalonil and triadimefon decreased the colony diameter by 20% or higher at 5 ppm agent. Commonly used systemic fungicides (especially Ergosterol Biosynthesis Inhibitors) inhibited the growth of *C. fragariae* more compared to classic fungicides. However, it has been stated that most of these systemic fungicides are not licensed in strawberries in America (Smith and Black, 1993).

Whether *C. gloeosporioides* and *C. acutatum* obtained from strawberry continue their vitality in crown and soil in various inoculum levels were examined under laboratory and field conditions. In this study using 2 isolates for each species, the species were identified based on their morphological characteristics and molecular techniques. The conidia of 4 isolates survived in sterilized soil for more than 1 years and its vitality decreased rapidly at soil moisture of 22% (field capacity) in the untreated soil and the population decreased by 95% within 4-9.8 days. When methyl bromide (MB) was applied to soil in field capacity, it was determined that the vitality of the conidia of two species decreased by 95% within 8.9 to 12.9 days. In the soil with humidity of 11%, the period required to decrease the populations of *C. gloeosporioides* and *C. acutatum* isolates at the rate of 95% was found to be 124.5 and 114.4 days in two isolates for *C. gloeosporioides* and 72.8 and 74.2 days in two isolates for *C. acutatum*. *C. acutatum* could not be obtained from the naturally infected crowns buried for 5 months in a depth of 10-20 cm in MB-treated and untreated soils under field conditions. Also, after the artificially inoculated mummified fruits were buried under the soil for 5 months, a decrease of 15-39% was observed in the isolation of the pathogen. Soil solirization and MB treatments for four weeks destroyed the pathogen in the artificially inoculated fruits and soil. Based on this study, it has been stated that the mummified fruits should also be taken into consideration as much as conidia in disease epidemic (Freeman et al., 2002). In a study evaluating the effect of some fungicides (bitertanol, carbendazim, hexaconazole, imazalil, propiconazole and thiabendazole) against *Colletotrichum acutatum* in some strawberry varieties (cv. Pajaro, Chandler and Oso Grande) under laboratory, greenhouse and field conditions, 0.5, 1, 1 and 2 ppm doses of propiconazole, bitertanol, imazalil and hexaconazole, respectively, inhibited the mycelial growth of fungus by 50%. The disease incidence was assessed in 3 strawberry varieties under greenhouse conditions and propiconazole treatment decreased death rate (32-54%). But a slight phytotoxicity was observed in the treated plants. In the field trials of three years, when the plants immersed in carbendazim, bitertanol and thiabendazole suspensions were transplanted in the field, the disease incidence decreased significantly (De los Santos and Romero, 2002).

In the studies conducted on the control of fruit rot in strawberries in Louisiana and Mississippi between 2002-2005, 60 fungicides were assessed. They revealed that the most frequent fruit rot in the harvest period was *Botrytis* Fruit Rot caused by *Botrytis cinereal,* Stem Rot caused by *Gnomonia comari*, and Fruit Rot Anthracnose caused by *Colletotrichum acutatum*. In this study, it was observed that the fungicides included in 7 groups were effective in controlling fruit rot in strawberries. The commercial combinations of fungicides were usually very effective. When at least 2 treatments of Pyraclostrobin+boscalid, cyprodinil +fludioxonil, azoxystrobin, fenhexamid+captan and captan were compared with untreated control, the total fruit rot was observed less. Fruit rot was observed less in cyprodinil +fludioxonil and azoxystrobin treatments in fruits. Stem end Rot was also less in cyprodinil+fludioxonil, pyraclostrobin +boscalid, captan, azoxystrobin and pyraclostrobin treatments (Wedge et al., 2007).

In Florida, captan and thiram protective fungicides are usually used weekly in the control of fruit rot anthracnose during the production season (Mertely et al., 2009). In addition to some treatments, the protective fungicides other than Strobilurin and Quinone as well as pyraclostrobin, azoxystrobin and other systemic fungicides may replace previously formulated cyprodinil+fludioxonil mixture especially when the conditions are suitable for the development of disease (Mertely et al., 2005; Mertely et al., 2006; Mertely et al., 2008a; Mertely et al., 2008b). In Mid-Atlantic and Midwestern countries, the protective fungicides other than Strobilurin and Quinone as well as cyprodinil +fludioxonil, boscalid + pyraclostrobin systemic fungicides and the tank mixture of captan are recommended in the control of anthracnose fruit rot (Ellis et al., 2004; Ernest et al., 2010).

In recent years, new generation succinate dehydrogenase inhibitors (SDHI) have been developed. In this study, the inhibitive effect of five SDHI fungicides against *Colletotrichum* species was determined. It was found that C. *gloeosporioides*, *C. acutatum*, *C. cereale* and *C. orbiculare* isolates were naturally resistant to boscalid, fluxapyroxad and fluopyram in YBA agar medium under *in vitro* conditions. On the other hand, these isolates were relatively susceptible to penthiopyrad except for *C. orbiculare*. Benzovindiflupyr had the most inhibitive effect against all of these four species. Benzovindiflupyr had effective results against *C. globosporioides* and *C. acutatum* in apple and peach fruits and also in cucumber plant inoculated with *C. orbiculare*. This study is the first report on determining the effect of benzovindiflupyr against *Colletotrichum* species. Due to the broad-spectrum effectiveness of Benzovindiflupyr against the species in *Colletotrichum* genus, it has been reported that it can be used in disease control strategies against various pathogens in a broad range of products (Ishii et al., 2015).

**Biological control**

Although antagonists living on surfaces of plants have a potential effect in biological control, there is a limited number of studies on the biological control of *Colletotrichum* species (Lenne and Parbery, 1976). A high number of studies on biological control with *T. harzianum* have been conducted under commercial conditions and significant results have been obtained in greenhouse areas and vineyards (Elad and Shtienberg, 1995).

However, the recent studies have reported that *Trichoderma* isolates take the plant pathogens under control (Elad and Freeman, 2002). In a previous study, it was observed that various *Trichoderma* isolates obtained from 76 BCA (biocontrol agent) isolate, including T-39 isolate, were effective in controlling botrytis cinerea and anthracnose disease in strawberry under laboratory and greenhouse conditions (Freeman et al., 2001; Elad et al., 2001). It was reported that TRICHODEX which is T-39 commercial preparation of *T. harzianum* and various *Trichoderma* isolates were effective in the control of anthracnose (*Colletotrichum acutatum*) and *Botrytis cinerea* diseases in strawberry under controlled and greenhouse conditions. Three *Trichoderma* species (T-39(*T. harzianum*), T-161 (*T. atroviride*), and T-166(*T. longibrachiatum*)) were applied in different treatment times and at different doses and they were assessed against strawberry anthracnose and Botrytis cinerea. It has been recorded that the use of all the possible single, double, or triple combinations of *Trichoderma* species decreased anthracnose severity in the concentrations of 0.4% and 0.8% and in high concentration (0.8%) applied with 7 or 10-day intervals and single and double combinations showed a decrease in the concentration of 0.4% compared to control (Freeman et al., 2004).

Although the studies on biological control against the pathogens in green parts have been still in research stage, it is known that many commercial products such as Aspire TM, BioSave TM, Trihode TM, AQ10 TM and Avogreen TM have been put on the market in recent years. But none of the mentioned preparations has been developed specifically to be used against anthracnose. However, these preparations have been assessed for the control of anthracnose. Today, there is no commercially licensed product to be used against *C. acutatum* in almond and blueberry in America. However, *Candida oleophila* and *Bacillus subtilis* biofungicides containing antagonistic bacteria have been in test stage in order to determine their efficiency against *C. acutatum* (Wharton and Diéguez-Uribeondo, 2004).

Chemical fungicides are an important component used in disease control in many products. Various analysis systems have been developed in order to use some natural product-based fungicide for disease control so that their antifungal effects are assessed. Also in this study, The antifungal effect of Angelica sinensis (Dong Quai) new natural product based fungicide in control of strawberry anthracnosis caused by Colletotrichum species was assessed. For this purpose, the antifungal effect of A. sinensis was determined using direct bioautography test of *A. sinensis* oil against three Colletotrichum species (*C. acutatum*, *C. fragariae* and *C. gloeosporoides*) and using the leaves of the plant against *C. fragariae*. It was found that the antifungal effect of *A. sinesis* oil was not different in *Colletotrichum* species. However, it was found that it had an effective inhibitive effect against *C. acutatum* almost as much as Captan (fungicide) and Benomyl (benzimidazole class fungicide) was ineffective. This study revealed that it would shed light on the following studies (Tabanca et.al., 2008).

Strawberry is one of the fresh products that spoils faster due to its sensitivity to the factors such as mechanical injury, physiological defects, water loss and rot. Anthracnose is one of the factors causing post-harvest rot and product loss in strawberry production. Although several *Colletotrichum* species cause strawberry anthracnose, the most frequent and destructive one is *C. acutatum*. The antifungal effects of thyme, cortex cinnamomi and clove bud essential oils (EO) against mycelial growth, conidial germination, and appressorium formation of *Colletotrichum acutatum* under in vitro conditions and against the disease incidence under in vivo conditions were determined. It was found that all the essential oils added into the potato dextrose agar medium inhibited mycelial growth of *C. acutatum* and had a fungistatic effect in 667 µl/l concentration. It was determined that the volatile effect of cortex cinnamomi, thyme, and clove bud essential oils inhibited conidial germination completely at the lowest concentrations (1.53, 15.3, and 76.5 µl/l, respectively) and inhibited appressorium formation at 1.53 µl/l concentration. The volatile effect of thyme and cortex cinnamomi essential oils decreased anthracnose incidence at 15.3 and 76.5 µl/l concentrations, respectively, in disease inoculated strawberry fruits. In the study, it was found that thyme and cortex cinnamomi essential oils had volatile effect against *C. acutatum* under both in vitro and in vivo conditions (Duduk et al., 2014).

The antifungal effects of the chitosan coating (ChEC), the effect of which was enhanced (made functional) by cinnamon essential oil, and *Roselle calyces* water extract on the mycelial growth of *Colletotrichum fragariae* at two temperatures (5 and 20 °C) and under different storage conditions (0, 3, 5, 10, 13, and 17 days) as well as their physical, chemical, physiological and nutraceutical (preventative and therapeutic) characteristics were assessed. ChEC, water vapor permeability (WVP) and mechanical properties were determined. 5 treatments were applied for fruits in the trial. T1) control (strawberries without ChEC and non-inoculated) (FC); T2) fruit with ChEC and non-inoculated (FEC); T3) fruit coated and after 24 h inoculated with *C. fragariae* ($10^5$ $mL^{-1}$) (FECI); T4) fruit inoculated ($10^5$ $mL^{-1}$) and coated after 2 h (FIEC), and T5) fruit inoculated and non-coated (FI). The antifungal effect of fruit coating on disease incidence and severity was measured. According to the results, it was determined that growth of *C. fragariae* was affected by all the treatments and storage temperatures, except for FEC treatment at 5°C. It was observed that *C. fragariae* developed on the third day in the strawberries stored at 20°C but disease symptoms were observed 10 days after in the fruits (FECI) stored at 5°C due to ChEC treatment performed after 24 hours by inoculating disease. The disease incidence in the fruits stored at 5°C decreased 4-5 times compared to the fruits stored at 20°C regardless of the treatment. Additionally, fungus growth was observed even in ChEC treatment at 20°C, as it is the most suitable condition for *C. fragariae* growth, but the disease incidence was found 3 times less compared to the control and FI (fruit inoculated and non-coated) treatments. It was determined that only FECI and FIEC treatments delayed growth of *C. fragariae* by 50% and these treatments were protective and therapeutic. The antioxidant capacity of strawberries increased only in control group at the end of storing. As a result, it may be an effective method to store strawberries for 17 days at 5°C through ChEC treatment (Ventura-Aguilar et al., 2018).

## Conclusion

Strawberries (*Fragaria × ananassa*), which are grown worldwide and have a high economic value, are exposed to numerous plant pathogens. Colletotrichum species cause significant economic losses in crops in tropical and subtropical temperate regions. Economic importance of disease agent, host specialization, changes in cell biology of infection function, fungi-host relationship, genetic diversity and epidemiology have led to conduct intensive studies on the biology of the pathogen. In Turkey, further studies should be conducted on the existence, prevalence and control of *Colletotrichum* species in strawberries. Many international studies have been conducted on chemical control of the diseases caused by Colletotrichum species in strawberry but it is considered that there is a need for the studies conducted with the biological agents and alternative natural products in disease control.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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