DOI: https://doi.org/10.55302/JAFES18721037y

THE FUNGAL DISEASES IN KIWIFRUIT STORAGE, AND NON-CHEMICAL METHODS USING TO PREVENT THESE DISEASES

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Abstract
The kiwifruit is originated Southeast Asia whose production and consumption is increased each day. The production quantity has reached 3.447.605 tonnes around the world. It is also important to protect the quality of kiwifruit which has a high vitamin C, in the postharvest period. One of the most important problems of kiwifruit is infections originated by fungus during storage. The most common fungal disease in kiwifruit storage is *Botrytis cinerea*. The first symptom is seen after a few week storage. Because the pathogen can develope in the cold storage conditions, it causes decay and serious economical damage. Although the chemical methods is used to prevent this disease, with the increasing consumer awareness and due to caused the risk both environmental and human health, the tendency towards non-chemical methods of combat has been increased. The aim of this study is to compile the studies on non-chemical struggle methods used in *Botrytis cinerea* struggle. It was found that the most common non-chemical treatments are curing, heat applications, ultraviolet light applications, gamma radiation applications, ozone application and essential oil applications. Because these physical methods are not risk to the human health, it will guide for future research.

Keywords: Kiwifruit, storage, *Botrytis cinerea.*

Introductıon
Kiwi was not a well-known fruit until the end of the 20th century. But nowadays, it is a specie that is recognized all over the world and its production and consumption increases every year. The origin is Southeast Asia. The kiwi is a plant belonging to the genus *Actinidia* in *Actinidiaceae* family. The genus of *Actinidia* contains more than 50 species, all of which are of Asian origin (Koday 2000). Some of these are grown as ornamental plants in various parts of the world. In this genus, only five species can be eaten as fruits. These species are *Actinidia deliciosa*, *A. chinensis*, together with the species that fruits are small and their shells are hairless such as *A. arguta*, *A. colomikta* or *A. eriantha*. The cultivated species is *A. deliciosa* (Koday, 2000). Species of *A.deliciosa* and *A.chinensis* have economic importance. The Hayward variety occupies almost 95% of commercially cultivated areas. Over the world, the fruit species that have increased the most in the last 100 years in terms of production area and consumption are the kiwifruit. The Hayward variety with green fruit is the most preferred variety by producers and consumers for its delicious taste, its high nutritional value and yield, and its long shelf life. For this reason, a large part of the world's kiwi production is composed by the Hayward variety (Yılmaz, 2016). In 2014, China is in the 1st place in world kiwi production. Turkey is in the 8th place in world production with 31,795 tons (Table 1).

Damages in kiwi storage
a) Fungal Diseases

1.Botrytis cinerea; It has a very broad host directory. The pathogen acts as saprophyte in aged and injured plant tissues. *Botrytis cinerea* can cause serious losses in more than 200 plant species worldwide. The disease occurs during kiwi storage and it causes infections between 0-35˚C temperatures. The diseased fruit flesh is wet and glassy, from the outside the affected area seems that is darker than the firm part of the fruit. The first sign of the infection appears a few weeks after the fruit is placed in a cold storage. Pathogen causes serious economic damage in the last stages of production, but it also developes during storage and lead to decay.

Table 1: The kiwi production in the world (FAO 2014)

	Countries	Production(ton)		Countries	Production (ton)
1	China	1.840.000	13	Korea	9.158
2	Italy	506.958	14	Australia	4.239
3	New Zealand	410.746	15	Israel	3.500
4	Chili	266.017	16	Switzerland	501
5	Greece	171.510	17	Montenegro	500
6	France	620.00	18	Kirghizistan	400
7	Iran	43.165	19	Slovenia	260
8	Turkey	31.795	20	Bulgaria	208
9	Japan	31.600	21	Cyprus	100
10	USA	25.855	22	Tunusia	32
11	Spain	20.881	23	Kanada	30
12	Portugal	18.150			
	Total	3.447.605			

2. Alternaria Rot; Alternaria alternata is a fungal pathogen which occurs mainly in ripe fruits as saprophytes. The pathogen causes decay by entering fruits by wounds on the fruit after harvest.
3. *Penicillium spp.*; *Penicillium* species (especially *Penicillium expansum*) are common in apples and pears, but also in kiwifruits. It is a fungal pathogen that causes the blue mold disease. This disease occurs in apple, pear and kiwi after harvest and is also called soft or wet decay.
4. *Phoma destructiva*; The pathogen can affect all parts of the plant above the ground. The pathogen forms stains on the leaves ranging in color from dark brown to gray. As these stains develop, they are seen as rings. The pathogen is primarily effective in old leaves whereas all leaves are susceptible to disease. Leaves can fall when the disease is severe. Stains on the leaf are similar to early blight, but there are numerous picnidiums in Phoma lesions. Dark brown lesions in the form of intervening rings form on the stems and both green and mature fruit may be infected. Disease symptoms often occur as small submerged lesions on the sepal end of the fruit, and then a large number of picnidium occurs in the middle of them.
b) Mechanical Disorders
1. Vibration damage necrosis; During the transportation of the product, when the inner flesh is exposed to fibrillation and the fruit flesh is watered and softened.
2. Freeze damage; This status is occured when the temperature of the warehouse is below the freezing point. Cells break apart and become jelly-like.
3. Water Loss; It is occures due to the excessive decrease of relative humidity of storage rooms. On the surface of the fruit, firstly shrinkage shown and is followed by desiccation.
4. Ammonia damage; Discoloration caused by ammonia leaks on the surface of the fruit, it can be seen in cold room cooled by ammonia.
5.Granulation; It is also called sac drying and crystallization. The granulation occurs due to the fruit juice sac in the stem part of fruit appears to be enlarged and hardened and discolored.

The methods of kiwi storage
1. Ventilated Cold Storages (Traditional, Simple, Ordinary); The cooling air of the night is taken in by convection or ventilation and used for cooling the product.
2. Conventional cooled storages In cooling the storage room, the change of situation of the refrigerant is utilized. This provides the desired degree of cooling.
3. Controlled Atmposphere (CA) Storages; Storage in the CA prevents the binding of auto-catalytic ethylene which is required for maturation to the receptors, thereby prolonging the shelf life of the

product and ensuring that the fruit does not lose quality after storage. Storage in CA Kiwi is carried out in commercially developed countries. In the CA storage, with the increase of CO_2 and the reduction of O_2 is slow down the operation of the enzymes, the ethylene synthesis and respiration rate is decreased and therefore storage life and fruit quality of kiwifruit are increased (Öz and Eriş 2009). Hayward kiwi variety was stored in controlled (CA) and normal atmospheric (NA) storage for 180 days at 0±0.5°C temperature and 90-95% relative humidity. As a result of the study, kiwi was found to be successfully stored for 6 months in storage at CA in combination of 5:5 and 5:2 (%CO_2:%O_2) when compared to NA warehouse (Eris et al. 1996). 1-methylcyclopropene (1-MCP) was applied to Hayward kiwifruit fruit and stored in controlled atmosphere (KA) and normal (NA)+ethylene controlled (EC) conditions. As a result of the study, especially CA (2% O_2, 5% CO_2) + EC application had positive effects on the preservation and shelf life of 'Hayward' kiwi variety fruits. 1-MCP treatment caused deterioration of taste and foreign smell formation. The 'Hayward' kiwifruit fruits were stored successfully at 0°C and 95% relative humidity at CA (2% O_2; 5% CO_2) and NA + ethylene controlled conditions for 6 months without loss of quality (Yıldırım, 2010).

4. Modified Atmosphere Packaging; Modified Atmosphere Packing (MAP) is the process whereby products are packaged with a passive or active gas composition in a package that limits carbon dioxide, oxygen and water vapor permeability. MAP application in kiwis; not only reduces the rate of fruit softening but also prolongs the storage period because of the increase of CO_2 and the decrease of O_2 in the environment. In one study, the Hayward kiwi variety was packaged in three different packages: consumer packaging, classical packaging, modified packaging, and maintained at 0°C temperature and 90-95% relative humidity conditions. According to the result of this research, the modified packaging reduced the weight loss during storage. In classical packaged fruit, weight loss has increased. The level of total soluble solids (TSS) increased while the shell thickness of the fruit, the fruit stiffness, the vitamin C content and the titratable (TE) acidity level decreased during the cold storage in general. It was found that the effect on the skin thickness of packaging types is negligible. Fungal rotting factors were increased in the modified packaged fruit, but decreased in the consumer packaged fruit. Color lightening was occured in fruit flesh and skin during the storage. Flavor characteristics of the fruit decreased during storage. As a result, it was determined that Hayward kiwi fruit has been successfully preserved for 6 months with the modified packaging and 5 months with the consumer and classic packaging (Namdar, 2005). The effects of different post harvest practices and different packaging types on kiwi storage time and fruit quality were investigated. In this study, 3-dose 1-MCP such as 312.5 ppb, 625 ppb and 1250 ppb were applied to some of the kiwifruits. Some of them are packed with LDPE and PVC packaging material. The kiwi were then stored for 2, 4 and 6 months in cold air storage at 1°C and 95% relative humidity. The results obtained from the research showed that the applications of PVC and LDPE were successful in terms of the criteria examined. Among the 1-MCP applications, the application of 1250 ppb 1-MCP showed more positive results than the other doses. This application was followed by application of 625 ppb 1-MCP and 312.5 ppb insufficient in terms of preservation of the desired fruit quality (Duman, 2011).

Prevention methods of diseases

Heat Treatments

It is required that before all harvested crops are stored, the disease agents must be free from bugs and dirt and dust. The sensitivity of the harvested product to postharvest diseases and the physiological changes that cause the pathogens to develop in the fruit are increased during long-term storage. This shortens the life of the stored product. Nowadays, disinfecting methods are preferred so as not to leave residue on the products. Temperature treatments are also the most preferred method among them economically (Fallik, 2004). These applications kill pathogens causing surface degradation, while preserving fruit quality during long-term storage and marketing. Three different methods are used in temperature applications. These are hot water applications, hot steam applications and hot air applications. In these three applications, the first objective is to reduce the microorganism load, while at the same time stimulating the skin structure and increasing the resistance.

Curing-hot air applications

The curing application is done just before the storage of many vegetables and fruits just after the harvest. This practice prevents the germination of spores on the products. Products are kept in relatively high temperature conditions for a while. Thus, the fruit and vegetables are stored in a way that gives them resistance. In a study conducted, the effects of temperature and humidity on curing *Botrytis cinerea* infection levels were investigated. After harvesting, each fruit was inoculated with 17 ml of Tween 20 containing 25 000 spores in the drop. The best curing effect was obtained at 10°C; The disease intensity was highest at 0°C, whereas the curing effect was reduced at temperatures above 10°C (Bautista-Banos et al. 1997). In another study, flower bud decay in kiwi fruit inoculated with *Botrytis cinerea* showed a decrease with increase in ambient temperature holding time between harvest and packaging / classification. The holding period at 0°C prior to packing / classification was effective in reducing decay. When the effect of the holding time was examined, the degree of decay was reduced by 20% with 7 days of waiting at 0°C, while not decreasing at 4°C for 4 days. In non-inoculated kiwi fruit subjected to the same treatments, *Botrytis* decay intensity was similarly reduced. The crack rate in packed and chilled fruits immediately after harvest was higher than in other applications. This is due to the pre-cooling and the interaction between the polyethylene coated plates.(Pennycook and Manning, 1992).

Hot water and steam applications

Hot water application is usually used to control fungal disease and it is necessary to dry the product after application. Hot steam applications are mostly used for insect control whereas hot air applications are applied against both fungal agents and insects. The last two applications can be carried out in different forms, hot air is stationary or circulated, and humidity control can also be performed during application (Bal, 2009). When the effect of hot water treatments on decaying of Galia melon in long term storage is investigated, it is stated that decay caused by *Alternaria alternata* and *Fusarium* spp. is less in hot water applied fruits compared to fruits that untreated or dipped to water. Gray moldy inoculated strawberries were stored in water at temperatures of 40º, 42º, 44º, 46º, 48º and 50ºC for 15 minutes after inoculation. This application delayed the development of *Botrytis cinerea* in strawberry fruit. Waiting in water at temperatures of 44º and 46ºC is the best result in terms of preservation of fruit flesh stiffness and internal quality. At this temperature range, there was no negative effect on the external color and taste. The kiwi were kept in the water at 46, 48 and 50˚C temperature for 4, 8 and 15 minutes and the effectivity against Botrytis cinerea of this application was examined. After these applications, the kiwi were stored at 0°C for 13 weeks. According to the results of the study, 15 min at 46˚C and 8 min at 48˚C were found to be more effective in *Botrytis cinerea* control than other applications. These applications did not have a positive effect on the flesh severity and the application of 15 minutes at 48˚C caused damage to the kiwifruits (Cheah et al. 1992). In another study, the Kiwi inoculated with *Botrytis cinerea* was dipped in hot water (45, 50 and 55°C) for 2, 4 and 8 minutes after 3 weeks. All fruits were stored at 0.5˚C and 85-90% relative humidity for 18 weeks. Hot water treatments increased weight loss and weight loss was two fold higher than control samples in $55^{o}C$ hot water samples. In contrast, the infection rate was significantly inhibited at 6 and 12 weeks of storage in hot-water treated kiwi. In addition, hot water applications slow down the softening of the fruit. At the end of storage, however, there were no significant differences between applications and control. Hot water applications did not have any positive or negative effects in terms of other quality parameters (Moghadam and Ebadi 2012). The hot water immersion treatment at 50°C and 1.5°C for 1.5 minutes after storage to kiwifruit was being positively effect on preservation of fruit quality after prolongation of shelf life and storage (8 days at 20°C temperature and> 90% relative humidity). With the hot water immersion treatment for 1.5 minutes at 55°C temperature, it is provided that preservation from *Botrytis cinerea* of Hayward kiwi fruits, and quality was maintained during marketing. Therefore, this application suggests that it provides an effective, non-chemical method for protecting the kivin from infection after harvest (Koukounaras et al. 2008).

Gamma Rays and UV-C Applications

Fruits and vegetables are deteriorated due to physical, chemical and biological factors. Several methods of conservation have been developed to reduce this deterioration effect. One of these preservation methods is to store fruits or vegetables by irradiating them. The radioactive materials emit alpha, beta, gamma, X-rays into the environment during the continuous disintegration of their atoms. These rays cause electric charged ions to form in the material they hit. These rays are called ionizing rays. Ionized beam has more energy than non-ionized visible light, television and radio waves and microwave (Acar, 1999). UV irradiation has advantages such as no residue, no legal limitations, and no need for a large safety area to be used, compared to other methods. It is necessary to act in accordance with some rules in food irradiation applications. Firstly, the irradiation method is not applied to the degraded products, but it does not provide an alternative to the Good Manufacturing Practices (Atasever and Atasever 2007). Ultraviolet irradiation is performed using UV-C lamps with the most lethal effect on microorganisms and with a wavelength of 254 nm. At the same time, this wavelength creates a slight stress response, increasing the post-harvesting resistance of the product (Kasım and Kasım 2007). It is stated that UV-C heat has two positive effects on the control of the storage decay of fruits and vegetables. These;

1. The deadly effect of pathogen damage to DNA structure,
2. It encourages the accumulation of antimicrobial compounds that will provide resistance to pathogens in fruit shells. (Bal, 2009).

The labels of irradiated foods must contain the symbol known as radura.

Figure 1: Symbol indicating that the food is irradiated

In a study of the effect of gamma irradiation on kiwi quality has shown that the practitioner can inactivate three pathogens of *Botrytis cinerea*, *Diaporthe actinidiae* and *Botryosphaeria dothidea*. Irradiation application reduced the L* and b* values while increasing the a* values of kiwifruit during storage. In contrast, the irradiated kiwi were softer than the unirradiated kiwi. Irradiation application reduces the amount of total soluble solids during storage while the kiwifruit has no effect on the organic matter content. The amount of vitamins C of irradiated kiwifruit was found to be lower than that of non-irradiated control fruits. The antioxidant activity of kiwi irradiated with 2 and 3 kGy radiation was lower than in control fruits and in treated with 1 kGy radiation. In addition, 3 kGy gamma radiation application also increased sensory quality. In general, gamma irradiation positively affects sensory and hygienic quality by controlling the microbiological population, while cause adverse effects on the vitamin C content, antioxidant activity and structural properties of kiwi (Yook, 2009). UV-C (between 0.01 and 1.50 J/cm^2) and temperature applications (temperatures between 35 and 48°C for 3, 5, 10, 15 and 20 minutes) against *Botrytis cinerea* and *Monilinia fructicola* fungi that causing postharvest decay on kiwifruit were investigated. *Botrytis cinerea* spores were inactivated by UV-C application at 45˚C for 15 minutes (1.00 J/cm^2) and *M. fructigena* spores at 45˚C for 3 minutes (0.50 J/cm^2) (Marquenie et al. 2002).

4. Ozone Application: The shelf life of products that are subjected to ozone treatment and stored become long. Because ozone has antimicrobial effect. Ozone treatment slows the softening of many fruit tissues and reduces weight loss. It has been reported that high doses of ozone adversely affect the sensory properties of foods such as color and flavor (Tan et al. 2005) The effect of ozone application varies according to the product variety. The effect of ozone gas on blossom end rot of Kiwifruit that caused by *Botrytis cinerea* (*Actinidia deliciosa*, cv Hayward) investigated. Artificially inoculated kiwis were kept at 0°C and 95% relative humidity in conventional cold storage for 4 months. The ethylene in the medium was removed by catalytic oxidation and the ozone gas was applied to the cold storage chamber by the continuous flow method (0.3 µL L-1). Ozone treatment reduced the severity of the disease by 56% and the development of the disease on the infected fruit was stable. Pathogen spores did not occur in the presence of ozone while the infected fruits formed sclerotia. In a study that to determine the cause of ozone suppressive effect, kiwi fruits were exposed to ozone (0.3 µL L-1) before and after inoculation on a traditional cold storage for 0, 2, 8, 24, 72 and 144 hours, and the effectivity of ozone treatment on severity of diseases has been monitored. As the duration of exposure to inoculation increased for the fruits exposed to ozone before inoculation, the disease was markedly suppressed and applications after inoculation remained ineffective. Pre-inoculation ozone application is highly recommended because it increases the resistance of kiwifruit to pathogen and prevents the formation of the disease. Measures of antioxidant and antioxidant activity in fruits that were exposed to ozone for a certain period showed a strong negative correlation between disease intensity or severity and phenol content (Minas et al. 2010).

5. Essential Oil Applications: Essential oils; which are obtained by different ways from plants, are terpene-like oil-like natural substances which can be transported by burning intense smelling, water vapor. Essential oils obtained from some medicinal aromatic plants have antioxidant and antimicrobial effects. The most common and reliable methods used in food preservation include heat treatment, freezing, drying, irradiation. In cases where these methods can not be applied or are inadequate, the addition of antimicrobial agent to food is a problem. Antimicrobial agents are used to destroy microorganisms that are undesirable in food but for any reason, and prevent them from multiplying (Cerit, 2008). Extracts from 345 plants and 49 essential oils were evaluated for antifungal activity against *Botrytis cinerea*. At the end of this study, lemongrass (*Cymbopogon martini*), thyme (*Thymus zygis*), cinnamon (*Cinnamomum zeylanicum*) and carnation buds (*Eugenia caryophyllata*) from the 49 essential oils showed the highest antifungal activity against *Botrytis cinerea* (Wilson et al. 1997). The effects of moth, thyme, mother-of-pearl, marjoram, lavender, rosemary, sage and ylang essential oils on *Botrytis cinerea* were investigated. As a result, it was determined that essential oils such as mint, thyme, sedum, marjoram applied at relatively low concentrations (85-300 µg/mL) prevent *Botrytis cinerea* development. In contrast, lavender, rosemary, sage, and yarn essential oils have been found to have less inhibitory effect (Daferera et al. 2002). The antifungal effect of essential oils of *Carnation carnium* (*Carum carvi*) and anise (*Pimpinella anisum*) on kiwifruit was investigated. For this purpose *Botrytis cinerea* spores, which are propagated and pre-disinfected in tissue culture, are inoculated with kiwi fruit. Four different doses of essential oils (200, 400, 600 and 800 µL/L) were then applied. As a result it was determined that blackcurrant caraway and aniseed essential oils increased kiwi shelf life and completely inhibited gray mold formation when compared to the control group. It has been determined that the antifungal effect increases as the dose treated increases (Fatemi et al. 2013).

Conclusıons

In many fresh fruits and vegetables, chemical substances are used against fungal pathogens. These substances are known to affect human health negatively. In this study, it has been shown that non-chemical physical and natural substances can be used as antifungal agents.

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