REPUBLIC OF TURKEY
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

CONTROL OF GRAY MOLD DISEASE AGENT *Botrytis cinerea* ON CUCUMBER *Cucumis sativus* BY PEPPERMINT *Mentha piperita L.* ESSENTIAL OIL MIXED NANOPARTICLES

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Supervisor
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M. Sc. Thesis

August 2018
KAYSERI

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SCIENTIFIC ETHICS SUITABILITY

I, Layth Hussein ASHOUR, hereby declare that this piece of work is the result of my research and that this work has never been submitted anywhere for any degree. All the sources of information used were obtained in accordance with academic ethics and have been duly acknowledged.

Layth Hussein ASHOUR

COMPLIANCE WITH GUIDE

This master's thesis write up on the topic "Control of gray mold disease agent *Botrytis cinerea* on cucumber (*Cucumis sativus* L.) By peppermint (*Mentha piperita* L.) Essential oil mixed nanoparticles" was prepared with accordance Erciyes University's graduate Thesis Writing Directive

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ACCEPTANCE AND APPROVAL

Under the supervision of Prof. Dr. Mehmet ARSLAN, this write up on the topic **"Control of Gray Mold Disease Agent *Botrytis Cinerea* on Cucumber by Peppermint (*Mentha Piperita L.*) Essential Oil Mixed Nanoparticles"** was prepared by Layth Hussein ASHOUR was accepted as master degree thesis at the Department of Agricultural Sciences Technologies; Graduate School of Natural and Applied Sciences, Erciyes University.

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ABSTRACT

Nanotechnology have great potential to improve existing crop control protocols in the near future. The antifungal activity of ZnO, CuO and $AgNO_3$ nanoparticles and their mixtures with mint (*Mentha piperita* L.) essential oil were tested *in vitro* and *in vivo* to control gray mold disease (*Botrytis cinerea* Pers.) on cucumber (*Cucumis sativus* L.). The 0, 5, 50, 100, and 200 mg/l concentrations of Nano ZnO CuO and $AgNO_3$ were used to determine their antifungal effect *in vitro* growth of *Botrytis cinerea*. 50 and 200 mg/l of $AgNO_3$ nanomaterials alone and its essential oil mix with 10, 20, 40, 80, 160, 320 and 640µl/l concentrations were tested on *B. cinerea* growth on cucumber leaf disc *in vitro*. Also 0, 50 and 200 mg/l of Nano ZnO, CuO and $AgNO_3$ were used alone and mixed with 80 and 160 µl/l mint essential oil in an *in vivo* whole plant experiment to test their antifungal effect on *B. cinerea*. *In vitro* petri dish assays showed that nanoparticles had significant inhibitory effect on the colony formation of *B. cinerea*. *In vivo* inoculation assays further confirmed that $AgNO_3$ nanoparticles significantly decreased colony formation of spores and disease growth on cucumber. Antifungal activity of Nano ZnO, CuO and AgNO3 significantly increased when mixed with mint essential oil. Antifungal effects of nanoparticles and essential oil increased as their concentrations increased.

Key words: $AgNO_3$, *Botrytis cinerea, Cucumis sativus*, CuO, essential oil, *Mentha piperita,* cucumber, nanoparticles, ZnO.

HIYARDA(*Cucumis sativus* L.) KURŞUNİ KÜF (*Botrytis cinerea*) HASTALIĞININ KONTROLÜ ÜZERİNE NANE (*Mentha piperita*) UÇUCU YAĞI VE NANOPARTİKÜL KARIŞIMININ ETKİSİ

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ÖZET

Nanoteknoloji, yakın gelecekte mevcut bitki koruma kontrol protokollerini iyileştirebilecek bir potansiyele sahiptir. Hıyarda (*Cucumis sativus* L.) kurşuni küf hastalığı (*Botrytis cinerea* Pers.) üzerinde ZnO, CuO ve $AgNO_3$ nano partikülü ve nane (*Mentha piperita* L.) uçucu yağı nanopartikül karışımlarının antifungal etkisi *in vitro* ve *in vivo* olarak test edilmiştir. Kurşuni küf gelişimi üzerine 5, 50, 100, and 200 mg/l konsantrasyonlardaki nano ZnO, CuO ve $AgNO_3$'ın antifungal etkisi *in vitro* olarak tespit edilmiştir. Ayrıca *in vitro* koşullarda 50 ve 200 mg/l konsantrasyonda ZnO, CuO ve $AgNO_3$ 0, 10, 20, 40, 80, 160, 320 ve 640 µl/l nane uçucu yağı ile karıştırılarak yaprak diskleri üzerinde kurşuni küf gelişimi test edilmiştir. Sera koşullarında nanomateryal 0, 50 ve 200 mg/l konsantrasyonlarda 80 ve 160 µl/l uçucu yağla karıştırılıp tüm bitki üzerine uygulanarak *in vivo* antifungal etkisi belirlenmiştir. *In vitro* denemeleri nanomateryalirin koloni oluşumu üzerinde baskılayıcı etkiye sahip olduğunu göstermiştir. *In vivo* inokülasyon denemeleri ise ZnO, CuO ve $AgNO_3$ nano partikülerinin hıyar üzerinde sporların koloni oluşumunu azalttığını ve hastalığın ilerlemesini engellediğini teyit etmiştir. Nano ZnO, CuO ve $AgNO_3$ partiküllerinin antifungal etkisi uçucu yağ ile birlikte karışım olarak kullanıldığında önemli derecede artmıştır. Nano partiküllerin ve uçucu yağın antifungal etkisi nano partikül konsantrasyonunun artışıyla birlikte artmıştır.

Anahtar kelimeler: $AgNO_3$, *Botrytis cinerea, Cucumis sativus,* CuO, hıyar, nanopartikül, *Mentha piperita*, uçucu yağ, ZnO

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LIST OF ABBREVIATIONS

Abbreviate	Details
NPs	Nano particles
AgNO3	Silver nitrate
ZnO	Zinc Oxide
CuO	Copper Oxide
mg/l	milligram /liter
µl	Microliter
µl/l	Microliter/ liter
µg/mL	Micrograms /milliliter
EO	essential oil
MIC	Minimum Inhibitory Concentration
ISR	Induced Systemic Resistance
EPA	United States Environmental Protection Agency
OPPs	Organophosphorus pesticides
DDT	Dichlorodiphenyltrichloroethane
PDA	potato dextrose agar
SAS	Statistical Analysis System
LSD	Least Significant Difference

CHAPTER 1

INTRODUCTION

As one of the most widely used members of the Cucurbitaceae family, cucumber (*Cucumis sativus* L.) is a crucial vegetable to human dietary habits around the world (Lower and Edwards, 1986). The plant is a vine that creeps with deep roots and sprouts through supportive frames that wrap around the ribbing through spiraled and thin tendrils.

A minimum of 200 plant species are affected by the *Botrytis cinerea* pathogen (gray mold), both before and after harvest (Jarvis, 1977). Some of the world's major crops are impacted by gray mold, including strawberries, cucumbers, and flowers of all varieties. Gray mold is necrotrophic, which means it kills the host cells and seriously damages the plant tissues, causing rotting in the crops or flowers (Staples and Mayer, 1995).

Fruits and vegetables are most affected by this pathogen, including lettuce, beans, cabbage, grapes, and all berry types Droby and Lichter (2004). Gray mold rot after the harvest can occur on all areas of the plant, including leaves, flowers, fruits, shoots, and organs stored beneath the soil.

This fungus has gained great attention of researchers due to the growing global trade of products. *Botrytis cinerea* grows at temperatures just over freezing over extended time periods to greatly affect apples and kiwis though "dry eye rot" and also impact the cut flower trade of roses. Herbs, flowers, fruit, and vegetables grown in greenhouses with or without heating are particularly prone to infection, especially cucumbers (the basis of this research) and peppers (Pande et al., 2002).

The gray mold pathogen has several enzyme and toxins that degrade the walls of a cell, including oxalic acid. It has been recently discovered that it causes its host to kill off its own cells as part of its plan of attack. Symptoms are noticeable throughout the plant parts, both old and new. The onset of these symptoms is a mark soaked with water, with the infected part eventually withering away or collapsing. Fruit rot, damping of seedlings, and blossom blight are all common symptoms. For greenhouse cucumbers, excessive gray rot of the fruit is most observed (Ten Have et al., 2002). The fungus spreads best during the spring season when conidia (asexual spores) are produced in excess and are spread to any available hosts. During other seasons, the sclerotia is small and hardened. New spores are continuously grown during growth seasons for the infection of new plants. Optimal temperatures for infection are warm, with a range between 18 and 25 ℃, with a moist environment. The most common defense against gray mold in the past were from fungicides based in either copper or sulfur that were sprayed weekly. However, if the climate conditions were optimal for growth, these fungicides had little effectiveness of stopping the spread or infection. Beyond this, fungicides are facing criticism for their negative effects on the environment and on human health. As such, innovative and safer techniques for combating this pathogen are needed. A technique that has been growing in popularity is control through biological agents (Elad et al., 1992). Beyond the negative effects of fungicides and pesticides, various fungi, including gray mold, are prone to develop resistance to chemical control.

The purpose of this study is to evaluate environmentally safe control methods as an alternative for combating gray mold for cucumber plants. Biological techniques have proven effective at the complete or partial destruction of the pathogens *Trichoderma* spp. and *Gliocladium* ssp (Agrios, 1997). They also have shown the potential of improving the self-defense of plants through bio elicitors. This is called Induced Systemic Resistance (ISR) (Sticher et al., 1997). Applying some types of oligo glucosides (chemical elicitors) and essential oils has been shown to trigger ISR (Benhamou et al., 1994) and have been recognized as strong antifungals (Siripornvisal et al. 2009). Agriculturists have begun to adapt essential oils, plant extracts, and their various components because of their eco-friendliness, safety, biodegradability, and consumer approval (Tzortzakis and Economakis, 2007). Several essential oils used for medicinal purposes have also been effective against bacterial and fungal pathogens (Amini et al., 2012). With a particular interest of those from thymus, eucalyptus, and citrus. Nanomaterials are also growing in popularity in the agricultural sector for their antimicrobial effects for both plant and human pathogens such as various geometric shapes of nanomaterials, like Nano-spheres, Nano-rods, nanowires, nanotubes and Nanoprisms of inorganic materials are developed and synthesized Some of the examples include a silver nanoparticle, nanoscale, silicate, platelets, carbon nanotubes, and the nanoparticles of TiO_2, MgO, ZnO, copper, and gold. (Taniguchi, 1974; Murphy, 2002; Huang et al., 2016).

Postharvest outbreaks of *Bipolaris sorokiniana*, Raffaele sp., *Colletotrichum* ssp., and *Botrytis cinerea* are reported (Kim et al., 2009; He et al., 2011). This study evaluates the possibilities of using nanoparticles mixed with peppermint essential oils to control *Botrytis cinerea* on cucumbers.

CHAPTER 2

LITERATURE REVIEW

2.1. Cucumber

Cucumber (*Cucumis* *sativus*) is a member of the gourd family Cucurbitaceae that is cultivated across the globe. *C. sativus* is an annual climber with morphological variations. It has prostate, angular stems that are covered in white pubescence. There aren’t any stipules. The plant has axillary tendrils that are not branched of up to 30 cm in length. Its leaves are organized in an alternating pattern across petioles between 10 and 16 cm in length. Its palm leaves almost orbit around the plant and are between 7 and 20 cm in length (Schaefer and Renner 2009).

This creeping vine has cucumiform fruit which are used as vegetables. The cucumber comes in three main varieties: sliced, pickled, and seedless. Many cultivars are developed from these three types. The “wild cucumber” plants in North America are members of the genera *Marah* and *Echinocystis* but are not close relatives. Although a native of Southern Asia, cucumbers now grow across the world, with many varieties traded as crops (Kubitzki, 2004). Cucumbers are often regarded as vegetables, but are actually fruit grown in warmer seasons. Consisting of 95 percent water, with minimal calories, and skins rich in Vitamins A and C and folic acid, cucumbers are vital for many healthy diets. They also contain iron, calcium, potassium, and phosphorus.

Cucumber plants have large leaves that canopy over their long, cylindrical fruit. This fruit has tapered ends and can grow as big as 60 cm in length with a 10 cm diameter. This fruit can be eaten either pickled or fresh. Green cucumbers, which are most common, are unripe. Ripe cucumbers are yellow, and are sour and bitter in taste (Zomlefer, 1994; Ghebretinsae et al., 2007).

Originally grown in India, a great number of cucumber varieties are observed in that country. The plant has been cultivated for at least 3,000 years and was most likely introduced to Europe by the Greeks or Romans. Cucumber cultivation is recorded in France by the 9th Century, England by the 14th, and North America by the middle of the 16th Century.

It is known as one of the common foods of the ancient Ur people. Within the Legend of Gilgamesh, people are known to be eating cucumbers. Some historical records show production in ancient Thrace. This can be seen through the connection to the modern dietary habits of the Bulgarian and Turkish people, with both countries formerly part of this ancient state. The cucumber is also named in the Bible as among the foods of the Egyptian Israelites. The plant spread from India to Greece and Italy, before making its way into Chinese cuisines. In fact, the researcher Robert Daniel recently connected a Greek word with the Arabic *al-qitta*, the name of cucumber in the standard Arabic language. Pliny the Elder named cucumbers are crops grown by the Ancient Greeks and listed different types in Africa, Italy, and Middle Asia (Pliny at el, 1938).

Although cucumbers are among the world's healthiest fruit, its overall health benefits remain unknown to many civilizations. Cucumbers aren't as tasty as other squash varieties, but they exceed them in the ability to quench thirst and cool one's hot body. Cucumbers are great at maintaining healthy and toned skin and also for the prevention of acidity.

2.2. *Botrytis cinerea* (Gray mold)

Gary mold (*Botrytis cinerea* Pers.) effects more than 200 plants. *Botrytis cinerea* is noticed by fluffy, gray mycelium. The severity of this pathogen causes global losses between $10 and $100 billion. Because of these losses and the fungus' ability to resist common chemical treatments, gray mold is among the world's most researched pathogens. Gray mold develops in a number of vegetables, fruit, and other crops and has a negative impact on all the plant's components including the flowers, fruit, stems, and leaves (Sharma et al., 2009; Soulie et al., 2003). A recent study ranked *B. cinerea* as the second most economically devastating plant pathogens in the world (Milena and Evelyn, 2005). This pathogen is of particular importance because the transportation of crops and cut flowers are prone to its development and spreading, including the high humidity, injury to fruit, and high sugar content of much of the transported crops. It devastates the potential sale of the strawberry, cucumber, cucumber, cherry, pear, eggplant, pepper, lettuce, and carrot trade. This ubiquitous pathogen can easily spread through the air to find its next viable host (Droby and Lichter, 2004). Although the pathogen was most usually controlled using chemical treatments, these fungicides have faced criticisms in recent years due to their negative effects on the environment and human health, as well as the pathogen's resistance to the chemicals (Tripathi and Dubey, 2004).

2.2.1. Taxonomic Position and Classification

Botrytis was first listed as a genra in 1729 by Micheli for his famous book "*Nova Plantarum Genera".* It was often confused with *Sclerotinia* spp. until the two were further detailed in 1900 by Smith and the conflict between the two was solved in 1945 by Whetzel. It was further redefined in 1973 by Hennebert into 22 species (Hennebert, 1973) with most varieties limited to a single host, such as tulips or onions. However, *B. cinerea* can impact many different plants both before and after the harvesting. The name of *Botrytis cinerea* was given in 1801 by Persoon and labeled as a vine pathogen. The connection between the fungus' two forms was observed in 1939 by Drayton and Groves (1939).

2.2.2. Disease Cycle

Figure 2.1 below shows the different stages in the gray mold cycle. The first step is the conidium landing on a host. Because it is an airborne pathogen, it can travel a great distance before finding a new host. Once a host is found, the conidium starts the germination process when moisture is present. This produces a germ tube which turns into an appressorium which penetrates into the surface of the host. After this, the host's underlying cells are killed to establish a primary lesion. Here, necrosis or host self-defense is possible. It is possible for quiescence to occur indefinitely, where the growth of the fungus is limited (Prusky, 1996). In some situations, the entire cycle can complete within three or four days, depending on the host type and external conditions.

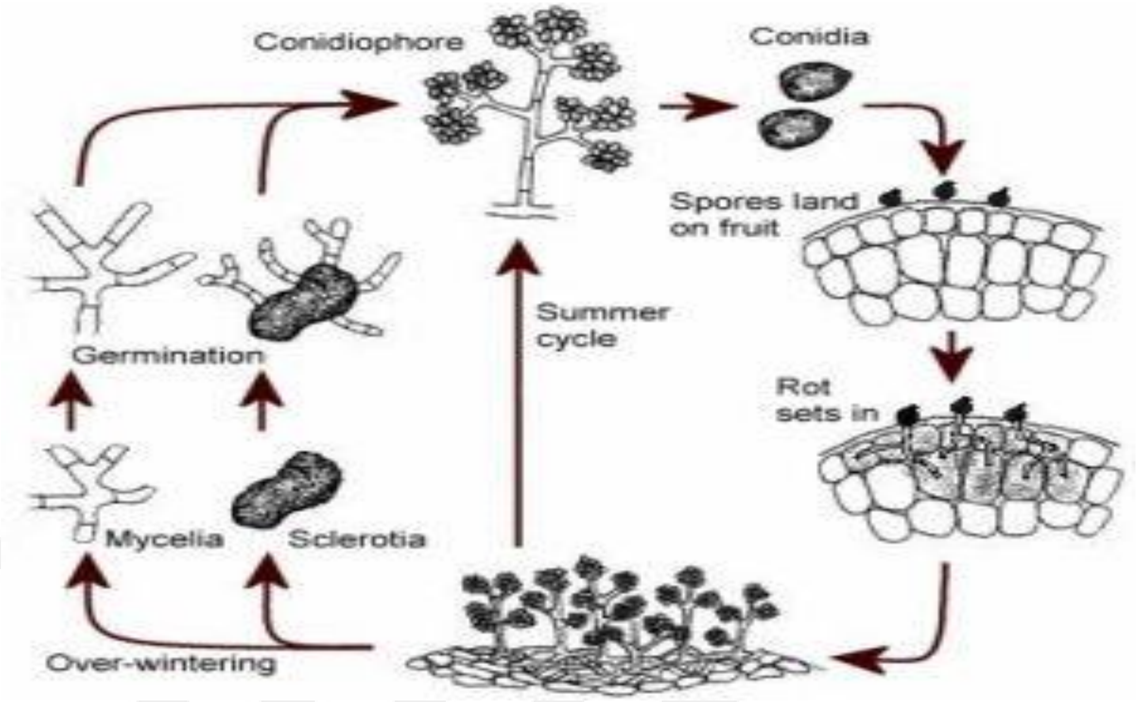


Figure 2.1. Development of Botrytis gray mold diseases.

2.2.2.1 Attachment of Conidia

Attachment is speculated as being accelerated by surface interactions on the plant's cuticle. There are two phases of attachment. The first one occurs before the conidia is hydrated and uses adhesion caused by hydrophobic interactions of the host and conidia (Doss et al., 1993). The second stage involves a stronger binding that takes place after germination, several hours after infection. The germ tube tips have an extracellular matrix similar to fibrillary made up of proteins and carbohydrates (Doss, 1999). This works as the adhesive to the host surface and also protects the hyphae from dehydrating as well as host defense (Doss et al. 1995).

2.2.2.2. Germination

There are many contributing elements to conidium germination. Either free surface water or a relative humidity more than 93 percent is crucial for germination and penetration into the host. The germ tubes will remain short before surface penetration in the absence of free surface water (Salinas and Verhoeff, 1995). Conidia inoculation in aqueous suspension needs various nutrients that could emulate a rupture in the epidermis of the plant (Benito et al., 1998). A connection was established between ethylene levels and gray mold (Elad and Volpin, 1988).

2.2.2.3. Differentiation of Infection Structures on the Host Surface

During penetration, *B. cinerea* produces appressoria, but not the typical type for normal fungal pathogens (organized 79). The germ tubes' hyphal tips have been known to swell (Cole et al., 1996). Through histochemical and microscopic inspection of the gene function, it was recorded that this serves as the functional appressoria. The mentioned swelling could result from osmotic rises that lead to water being absorbed (Tenberge et al., 2002).

2.2.2.4. Penetration of the Host Surface

Host tissue invasion is carried out by either active penetration or passive ingression. This fungus finds any opportunity, including infection where the plant is wounded or where other pathogens have been present. It is also possible for entrance to occur at open stomas. This penetration of dead or injured tissues is labelled expansion instead of penetration (Williamson et al., 1995). Cutin is present in the cuticle, where is a polyester of the C16 and C18 fatty acids and is covered by wax (Salinas J. S. and K. Verhoeff and Verhoeff, 1995).

2.2.2.5. Killing the Host

The gray mold pathogen kills the hosts' cells before hyphae invasion. When *B. cinerea* invades plant tissue, a nuclear condensation and damage to the membrane occur. These occurrences point to a programmed cell death and indicate the phytotoxic activity of diffusible factors (either directly or indirectly). They can include proteins or compounds with a low molecular weight that are secreted. All of this indicate that a programmed cell death may be necessary for an effective infection (Govrin and Levine, 2000).

2.2.2.6. Formation of Primary Lesions, Defense Responses in the Host

Numerous processes within both the host and fungus are caused by the penetration and rupturing of cell walls. Several are listed below.

Necrotic lesions are caused by the defense system of the host triggered by a cell death. It has been shown that an oxidative burst happens in the plant tissue a few layers removed from the hyphae. Cytological staining showcased proof of a cell death process, including membrane damage and rapid nuclear condensation (Govrin and Levine, 2000). Permanent infection is found in several tissues where symptoms are undetectable. They were noticed in some soft fruit varieties including grapes and berries. Here, the infection occurs in the flower and is dormant within the developing fruit tissue for many weeks. The fungus continues is growth once the fruit begins to ripen (Prusky, 1996).

2.2.2.7. Evasion of Chemical Defense Pathogenic

Fungal pathogens have designed methods to overpower the defense system of plants. One of these is a secretion by the ABC-transporters that converses a level of tolerance to the toxicity of the fungus (De Waard, 1997). The role of these transporters within the *B. cinerea* pathogen are not yet understood (Schoonbeek et al., 2001).

2.2.2.8. Disease Expansion and Tissue Maceration

It is necessary for *B. cinerea* to macerate plant tissue and change it into a fungal biomass. This is first carried out by the execution of nearby cells. The biggest obstacle in the path of this pathogen is the cell wall of the host, and as such, the plant cell degradation is crucial to its development (Ten Have et al., 2002).

Through studies, it has been observed that once cuticle penetration occurs, the pathogen will regularly invade the anticlinal wall that is between two epidermal cells. The cell wall swelling displays pectin degradation with the epidermal wall matrices, which causes water to be absorbed (Mansfield and Richardson, 1981). As such, there is evidence to support the role of pectinases in the initial infection.

2.2.3. Symptoms

Different types of plants display a variation of symptoms of gray mold infection. Most commonly, as the name indicates, a gray-colored coating that is fuzzy is seen on the ripened fruit as well as the flower blossoms. The infected leaves showcase a grayish cloud of spores. The initial stages are characterized by brown or gray spots that are circular in fashion and develop fuzz as the fungus develops. If strawberries are kept in cold storage for extended periods, it is common for gray mold to develop. Flowers, fruit, and leaves display spots and decaying. Roses display stem cankers (Donald, 2012).

On flowering plants, the stems, flowers, and/or leaves will first display spots soaked with water that soon grow into fuzz. Flower blights are often first seen because the oldest petals face the first attack. As the infection spreads, flowers will dry out and become brown. On fruiting plants, such as cucumber, a fluffy mold that is either white, tan, or gray will first develop. If the fruit is disturbed, gray spores can spread everywhere. The fruit will also display spots soaked with water that are first brown and then start cracking through fungus development. Berries will become totally water-soaked before rotting.

2.2.4. Management

The control of gray mold disease is difficult due to the pathogen's variety of infection strategies, wide host range, genetic diversity, and survival as conidia, mycelia, and sclerotia (Anjani et al., 2004). The best way to control gray mold is thus by the integration of a variety of cultural management practices with the application of fungicides or biological agents (Batista et al., 1996).

Strict sanitation is of utmost importance and cannot be overemphasized. Remove dead or dying tissue from the plants and from the soil surface as the fungus readily attacks them and produces tremendous quantities of airborne spores. All old blossoms and dead leaves should be removed, and all fallen leaves and plant debris in the garden and greenhouse should be carefully collected and burned or hauled away with the trash. Ideally, all diseased plants and plant parts should be removed and destroyed. Botrytis spores are always present, but they don’t germinate until exposed to cool and moist conditions, especially high humidity. Try and prevent these conditions from being prevalent for long (Thind, 2012).

Water on foliage and flowers from overhead watering, especially on cool, cloudy days, promotes the disease. Try keeping the plant parts above the surface dry. Soil surface irrigation and watering in the morning is recommended (Anjani et al., 2004).

The fungus thrives in areas that are cool and moist and in densely packed areas. Prune or stake the interior of dense plants to increase air movement. In greenhouses, ensure maximum air circulation by either increasing heat in the greenhouse or inculcating forced circulation and ventilation. Even lowering the humidity slightly can have a significant effect on Botrytis (Anjani et al., 2004). Do not over-feed your plants, especially with nitrogen as tender growth is susceptible to fungus (Thind, T.S. 2012).

Wounds are possible entry sites for pathogens of all types including Botrytis, so avoid injuring plants in any way. Do not leave large stubs of tissue on stock plants when taking cuttings (Batista et al., 1996).

Only blemish-free, flowers, fruits, vegetables and plant material should be stored in a clean, cool and dry environment without moisture on the walls, ceilings or floor. The temperature should be as close to freezing as possible (Thind, 2012).

Biological control are growing in popularity because of the growing concerns with fungicides for environmental and human health. Beyond these concerns, biological agents can also help combat the other major issue with chemical treatment: fungal resistance to the medicine (Agrios, 1997; Sticher et al., 1997; Benhamou et al., 1994). Whenever possible grow plant varieties that are known to be less susceptible to Botrytis (Batista et al., 1996). Spray susceptible plants with an organic fungicide like Neem oil or Soap-Shield to keep fungal diseases and other pests at bay (Leroux, et. al., 1999).

Chemical control remains the most effective method for controlling gray mold**.** Various fungicides are used for control. Multi-site inhibitors such as captain and thiram are chemicals affecting multiple chemical pathways in the pathogen. They suppress conidial germination by inhibiting several thiol-containing enzymes involved in respiration, but show little activity against mycelial growth. Multi-site fungicides act as a protective layer only on the plant surface and therefore need frequent applications at high doses (Leroux, et. al., 1999). The risk of resistance to these fungicides is generally low, and control failure based on resistance development has rarely been reported (Williamson, et. al., 2007). In fact, resistance emerged soon after the introduction of these chemical groups**.** In the last decade, researchers have developed a number of site-specific fungicides that have stronger activities against *B. cinerea* and are applied at substantially lower doses per acre**.** Many of these chemicals were categorized as "reduced risk" fungicides by the United States Environmental Protection Agency (EPA) due to their "low-impact on human health, low toxicity to non-target organisms (birds, fish, and plants), low potential for groundwater contamination, lower use rates, low pest resistance potential, and compatibility with Integrated Pest Management" (EPA,2011). "Reduced risk" fungicides include aniline pyrimidines (APs), hydroxyl annelids (HAs), methyl Benzimidazole carbamates (MBCs), phenylpyrroles (PPs), Quinone outside inhibitors (Qois), and succinate dehydrogenase inhibitors (SDHIs) (Schnabel, et, al., 2012).

2.2.5. Plant Essential Oils

Because of the growing concerns associated with fungicides (the primary pathogen treatment method), that include harm to both the environment and human health, as well as fungal resistance to the chemical treatment, essential oils have been sought after as an alternative (Dellavalle et al. 2011). These plant extracts have recently been studied for their effective antifungal characteristics (Siripornvisal et.al. 2009).

Even if not for the concerns with chemical treatments, essential oils and plant extracts have attracted attention because of the biodegradability, environmentally safe qualities, safety, and volatility (Amini et al. 2012). Even the extracts of medicinal plants have been discovered as effective treatment against some plant pathogens, with a particular interest in the oils from thymus, eucalyptus, and citrus. (Katooli et al. 2011).

The essential oils from spearmint have been proposed to combat cucumber crown rot caused by *Fusarium oxysporum* f. sp. *Radical-cucumerinum* (Nosrati et al. 2011**)** Beyond this, the oils of cinnamon, cassia, fennel, close, lemongrass, and basil have been studied for use on cucumbers in combating pathogen infection.

Essential oils were first coined in the 16th Century by Paracelsus. He named the active element for each herb or drug as the “quintessential” (Edris, A. 2007). Nearly 500 active compounds are in essential oils, including terpenes, terpenoids, aromatic, and aliphatic compounds. They are made from plants using airing, fermentation, and crushing, extraction, or hydrolysis methods, with the most common method being distillation.

2.2.5.1. Mint Essential Oil

Both peppermint and spearmint are from the Lamiaceae family of plants. The official name for peppermint is *Mentha piperita* and of spearmint is *Mentha spicata*. Peppermint was made through combining spearmint and water mint. Both of these mint varieties contain menthol, so they share many common benefits. When mint is named alone, the reference is usually spearmint. However, the essential oils from both are used for a variety of purposes, including healing and chewing gum flavor (Soković and van Griensven., 2006).

The important essential oil of menthol is produced from peppermint plants, which has showcased a serious ability to combat plant pathogens (Soković and van Griensven., 2006). Many of the species in the Mentha family have displayed strong pharmacological properties, including herbal medicine and antimicrobial benefits (Soković and van Griensven, 2006). It was discovered that the main components of *M. Piperita* oil are: menthol at 37.4 percent, methyl acetate at 17.4 percent, and menthone at 12.7 percent. For *M. spicata*, the main components are carvone at 69.5 percent and menthone at 21.9 percent. This revealed that the strongest antifungal activities were found in menthol and carvone, with limonene having the weakest activities. Through a 2006 study, the essential oils from spearmint and peppermint were used to reduce *T. virde* growth by 0.25 µg/mL and three mushroom pathogens: *P. totaasii, T. harzianum*, and *L. fungi cola* (Soković et al., 2009; Bouchra et al., 2003).

It was found in 2003 (Durović-Pejčev et al., 2014) that *M. pulegium* was mildly successful against gray mold with 58.5 percent mycelial growth at 250 µg/mL. A study showed peppermint oil had great effectiveness against the *T. aggressive f. europarum*, with the antifungal and prohibitive properties at 0.64 µg/mL (Soković and van Griensven, 2006; Soković et al., 2009). Through chemical analysis, Serbian peppermint oil had active compounds of menthone at 37.02 percent, menthol at 29.57 percent, and isomenthone at 9.06 percent. The antimicrobial elements of peppermint oil were also proven to be quite effective. In 2010, it was found that it reduced growth of *P. fluorescens* by 1.12 µg/mL (Tyagi and Malik, 2010). Despite this effectiveness, a similar 2014 study into mint oil showed the English horsemint oil was very ineffective against *P. aeruginosa* at 25 µg/mL (Biljana Todorović et al. 2016). Also, Biljana

Todorović et al. (2016) showed no antimicrobial effects of mint oil against *P. tolaasii* using the tested amount of 0.32 µg/mL.

2.2.6. Nanotechnology

2.2.6.1 Nature and Types of Nanomaterials

Nanoparticles refer to objects with sizes between 1 and 100 nm, in one of three possible dimensional arrangements. Scientists have shown that the nanoparticles of any given item have chemical, physical, and biological differences between the larger particles or elements of the same item. There are many diverse nanoparticle arrangements, either of metals, polymers, carbon, organics, metal oxides, silicates, and non-oxide ceramics. Their morphologies include shapes such as spherical, tubular, cylindrical, and more. These nanoparticles are designed with a surface arrangement suitable for the given application, as seen in Figure 2.2.

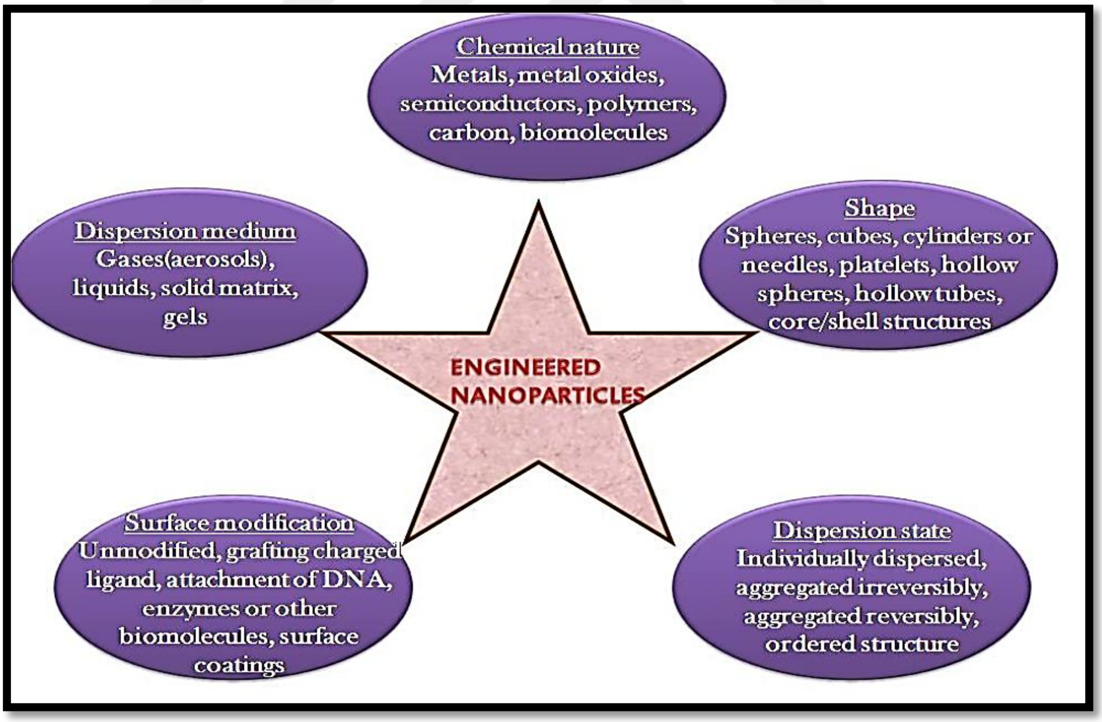


Figure 2.2. Various features contributing to the diversity of engineered nanoparticles. The same chemical can generate a wide variety of nanoparticles.

2.2.6.2 The Ranges of Nanotechnology

Nanotechnology is a growing field of utmost importance to modern research and development and its arrival opened many never-before-imagined pathways and uses within a broad array of fields. They are currently used in the healthcare, optical, mechanical, chemical, electrical, medicinal, agricultural fields, among many others. They are currently being used to combat many challenges across these fields for their technological and environmental adaptability. This technology has revolutionized the way materials and devices are developed because of their “bottom-up” approach.

The topic of interest to this research is the possibility to improve the agricultural standards. Because the topic of agriculture is ever-growing with the needs for healthy and fresh food for the global population, new developments in the way this is achieved is necessary. The importance becomes ever clearer with the knowledge that nearly one-third of the world’s crops are destroyed on an annual basis due to pests, pathogens, natural disasters, and weed infestations. As such, researchers are always looking for ways to improve the system and reduce the rate of loss. Nanoparticles are a topic of recent discussion for such an endeavor. This technology can be used in place of chemical fungicides and pesticides that cause damage to the ecosystem. The development of Nano-herbicide and pesticides that can treat crops after harvest at the site are in constant consideration (Liu et al., 2009; Jo et al., 2009; Chun et al., 2010; Solgi et al., 2009; Jung et al., 2010, and Park et al., 2006; Kim et al. 2012).

Because of the above-mentioned concerns and problems facing the global crop trade, nanoparticles in the agricultural sector have been sought after for effective fungicide, pesticide, and plant growth solutions (Jung et al., 2010, and Park et al., 2006; Jo et al., 2009; Kim et al. 2012). Their usage in the field is summarized by Figure 2.3 below. A major point of worry for the production of facile nanoparticles is the use of toxic elements during their synthesis. There are numerous problematic elements in their current development, including generation high heat levels that need toxic elements for proper synthesis. Because of this toxicity, the agricultural community is active in seeking out environmentally safe synthesizing methods that have the desired characteristics. A possible solution is the use of biogenic sources for their synthesizing, including plants and microbes that can stabilize the nanoparticles with little concern.

Figure 2.3. Application of nanoparticles in agriculture

2.2.6.3. Applications of Nanotechnology in the Agricultural Sector

2.2.6.3.1. Physicochemical Properties of Nanoparticles

There are increased benefits of the physiochemical properties of nanoparticles when compared to their larger counterparts (Donaldson and Stone, 2003). Particle size plays an important role in their activity, with a direct ratio of reduction in size and increase in performance (Baker and Satish, 2012). This also means that the fewer number of nanoparticles to perform the desired task is optimal. Their surface design is also important in how effective they will be for the intended task, with adjustments made for specific activity enhancement. A crystalline nature is reported to have the most benefit for agricultural nanoparticles (Kuchibhatla et al. 2012).

Zinc is of particular importance for the enzymes that assist with crop metabolism for increased growth (Ali et al., 2008; Mousavi, 2011). Also, the use of copper has been used as far back as the 17th Century to combat pathogens (Stampoulis et al., 2009; Mondal and Mani, 2012). Magnesium is also noted for having an assisting role in photosynthesis, as reports have shown that crops lacking in magnesium have a drastically reduced yield (Guo et al., 2016). As such, researchers are studying and developing new agricultural techniques that involve these, and other similar, elements for their benefits.

2.2.6.3.2. Parameters Influencing the Activity of Nanoparticles

There are various influencing parameters that indicate nanoparticle effectiveness. When seeking an effective treatment against a particular pest or fungus, the factors of importance are origin, synthesis, solubility, and protocols. It has been found that most agrochemicals are poorly soluble with water and require a solvent. As mentioned above, most of these solvents have toxicity and negatively affect the ecosystem (Ishaaya et al., 2007). For nanoparticles, they are water soluble and this solubility can be adjusted through the increase of decrease of hydrophilic groups when synthesizing (Baker and Satish, 2012). The protocols used during synthesis can impact the nanoparticles' toxicity which can cause health and environmental concerns (Kavitha et al., 2013). [Because of this, the aforementioned biogenic sources are being implemented to synthesize safe and healthy nanoparticles. During synthesis, the various bioactive elements can reduce and stabilize and can either prevent or protect against the toxic elements (Syed et al., 2016). Several of the used compounds (as shown in Table 2) are saponins, flavonoids, tannins, phenols, and terpenoids (Baker et al., 2013b). They have crop compatibility, but more importantly, are minimally toxic. As it currently stands, many toxic chemicals are used in agricultural production for the increase of crop harvest.

Agricultural products from chemicals have several serious implications. They are usually unable to reach their target location because of leaching, microbe degradation, or hydrolysis. This means that the same chemicals are overused, which destroys both the soil and water quality (Mishra et al., 2014). Nanoparticles, on the other hand, have proven more effective at reaching the target, performing their intended task, and the need to use them less often. This means they are much cheaper, more effective, and better suited at all environmental conditions (Sekhon, 2014).

2.2.6.3.3. Widely Used Nanoparticles in the Agricultural System

Nanoparticles developed for agriculture have ushered in a new era of agricultural production with problems of the past being largely neutralized (Fraceto et al., 2016). These nanoparticles can be developed based on the required action and expected properties of the location. Some of the most commonly used elements include, zinc, copper, gold, silver, silica, aluminum, chitin, titanium, and graphene (Sabir et al., 2014). Nanoparticles are, for the most part, tagged of conjugated with molecules from the carrier to produce emulsion, hydrogel, immobilized association, and liposome products.

2.2.6.3.4. Nanoparticles as Potent Antimicrobial Agents against Phytopathogens

Microbial management is crucial to effective agricultural techniques because microorganisms can destroy an entire crop (Bhardwaj et al., 2014). Fungicides and pesticides being overused because of fungal resistance to the chemicals have given way to new health risks in the modern world (Baker and Satish, 2015). Because of the size of nanoparticles, they can easily enter the pathogen's cell wall and perform a different, more effective, duty than the chemicals. These actions can include damaging the cell membrane which destroys the contents of the cell and disrupts metabolism metabolisms (Baker and Satish, 2012, 2015; Baker et al., 2015).

2.2.6.3.5. Nanoparticles as Potential Fungicides

Fungal diseases make up more than 70 percent of crop issues and cause a massive economic problem for farmers and produce traders (Agrios, 2005). Many of the most commonly traded crops, including rice, cotton, wheat, groundnut, grapevine, and barley are prone to fungal infection (Dhekney et al., 2007). Traditional fungicides are used to help combat this loss, but they are being less supported because of their health and environmental complications (Patel et al., 2014). Current research has proven that nanoparticles are extremely effective against a wide variety of pathogens (Mishra et al., 2014). An example is the silver nanoparticles used to great results against rice blast disease from *Magnaporthe grisea* that removed the need for the common fungicides of azoxystrobin and isoprothiolane (Rabab and El-Shafey., 2013). Another example is copper being used to combat both the *Alternaria alternate* and *Botrytis cinerea* pathogens cinerea (Ouda, 2014). Zinc and magnesium oxide have also displayed great effectiveness against the spores from the fungal pathogens *Rhizopus stolonifer, Fusarium oxysporum,* and *A. alternata* (Wani and Shah. 2012). Sulfur nanoparticles were used to treat wilt and apple scab disease from the *Fusarium solani* and *Venturia inaequalis* (Rao and Paria, 2013). Further, silver was used to treat fungal attacks in asparagus (Al- Othman et al., 2014). Magnesium oxide against cucumber wilt (Ramy and Ahmed 2013). and zinc oxide defeated the fungal pathogens *F. oxysporum* and *Penicillium expansum*. Through extensive laboratory experimentation, these nanoparticles reduced the hyphal growth, prevented germination, and prohibited the budding of the mentioned fungal pathogens (Medda et al., 2014).

2.2.6.3.6. Nanoparticles as Potential Nano Bactericides

Nanoparticles have proven quite effective antibacterial agents through a high volume of scientific research (Baker et al., 2015). One such study showed the power of silver nanoparticles with strong normal and multidrug resistance (Chowdhury et al. 2014). Silver nanoparticles also displayed powerful antibacterial activity against *Citrobacter freundii* and *Erwinia cacticida* (Paulkumar et al., 2014). And phytopathogens (Aravinthan et al. 2015), as well as limiting the growth of *Ralstonia solanacearum* and *Xanthomonas axonopodis* (Bhor et al. 2014).

2.2.6.3.7. Nanoparticles as Herbicides

Beyond bacterial and fungal pathogens, farmers also have serious problems with weeds destroying their crops. Chemical herbicides are generally used, but they face similar concerns as the other chemical agricultural treatments for their toxicity. On the other hand, nanoherbicides have proven strong chemical stability, soil sorption, bioavailability, solubility, and photodecomposition. Research has shown that nanoparticles working with atrazine with poly has improved herbicidal power (Pereira et al., 2014). In addition, herbicides which encapsulated chitosan and sodium triphosphate reduced the sorption and toxicity (Grillo et al., 2014). For a timed herbicide release, a recent study cross-linked chitosan and diuron to high success against weed growth, lessened toxicity, and promoted plant growth (Yu et al., 2015).

2.2.6.3.8. Nanoparticles as an Alternative to Pesticides

Chemical pesticides, as mentioned throughout this research, are receiving negative attention due to their harmful effects. The commonly used pesticides, thiamethoxam and clothianidin, are toxic to bees and water (Krupke et al., 2012). Organophosphorus pesticides (OPPs) are also highly damaging to the ecosystem and food chain because they accumulate with tropical level organisms (Gill and Garg, 2014). It is also possible for these chemicals to infiltrate and contaminate drinking water reserves (Baker et al., 2013c). More recently, common OPPs have shown toxicity to the human body (Guyton et al., 2015). And trace amounts of them have been detected within breast milk. These pesticide agents have been linked to the growing rates of such diseases including: cancer, Parkinson’s disease, diabetes, genetic disorders, fetal diseases, respiratory issues, and neurological problems (Hu et al., 2015). Several methods for combating the harmful effects of pesticides have been implemented, including transporting the harmful toxins to dumps, adding reactants, incineration, excavation, and vitrification (Singh et al., 2016). However, these procedures are expensive and cost between $25 and $50 billion each year (Kang, 2014). In order to alleviate both the economic and health concerns, biopesticides and green agrochemicals are being used. However, these techniques have proven ineffective against the pest infestations and their availability does not meet the pesticide needs. As such, nanoparticles connected to a biological origin has been studied to solve these problems.

A study published in 2009 (Teodoro et al. 2010). Showed nanostructured aluminum as an effective insecticidal agent within food in storage against the common pests of Sitophilus oryzae and Rhyzopertha Dominica. Silica nanoparticles were also proven effective at reducing the toxicity and activity of the validamycin pesticide through controlled delivery. Nanoparticles as pesticidal agents are dependent upon the temperature where they were dissolved and the pH (Liu et al., 2006).

Silver and silver-zinc nanoparticles showed a mortality of LC50 at 424.67 and 539.46 mg/mL against Aphis nerii Boyer de Fonscolombe (Rouhani et al., 2012).

2.2.6.3.9. Nanoparticles in Soil Improvement

The fertility of soil is a major point of agricultural interest. Because of numerous natural events, soil can become less fertile (Gallagher and Mitchel, 2002). However, the mechanical strength present in nanoparticles has been used as a deterrent to such naturally degrading events. Silica nanoparticles have shown effectiveness at recovering damaged or weakened soil because they can cause natural water and gradients to have a uniform distribution which enhances the soil quality (Diaz-Rodrigues et al., 2008). More than silica, nanoparticles from bentonite and laponite are great for increasing natural water flow (Gratchev et al., 2007).

2.2.6.3.10. Nano Agro-Sensors for Detection and Onsite Diagnosis

Beyond treating diseases and pests, early detection is crucial to preventing a massive crop loss. Biosensors are commonly used for the monitoring of pesticides, pathogens, toxins, and other pollutants. These sensitive and intelligent sensors can work without highly skilled labor. Scientists are now developing Nano agro-sensors that can monitor disease and contaminant activity, which can reduce economic losses through proper control (Liu et al., 2008). Through current research, sensors from gold nanoparticles have been able to detect DDT Baker et al. 2013c). And carbon nanotube sensors have shown strong detection of methyl parathion, paraoxon, and their metabolites (Zhang et al., 2014).

2.2.6.3.11. Nanoparticles in Plant Growth Promotion and Seed Germination

Plant cells have a sieving property that prevent foreign particles from entry. Most of the agrochemicals are macro-sized and cannot enter. However, nanoparticles have no issues penetrating the cell to perform their task. Carbon nanoparticles have proven effective at improving plant growth. A 2014 study revealed that multiwall carbon nanotubes successfully penetrated the seed coating and improved water uptake through the production of new pores (Srivastava and Rao, 2014). The results of this study displayed an increase in root and shoot growth, number of leaves, and biomass. These experiments proved fruitful for peanuts, wheat, garlic, and corn crops. In addition, nanoparticles of zinc-oxide at concentrations of 20 and 30 μg/mL improved seed production, flowering, and growth in onions (Laware and Raskar, 2014). The growth of soya beans using monocrystalline powders of iron, copper, and cobalt were also improved (Ngo et al. 2014). An overall crop increase of 16 percent was recorded, with germination using iron was at 65 percent improvement, and cobalt and copper showed an 80 percent improved germination rate.

2.2.6.3.12. Nanomaterial Coated Water Filters

The irrigation system is one of agriculture's biggest impactors. It is common that contaminated water is used for irrigation, particularly in third-world countries or places with prolonged drought. Beyond negatively impacting crop yield, contaminated water is also disastrous to soil fertility (Chong et al., 2010). Among the common water contaminants are metals, as well as organic and inorganic pollutants (Fatta et al., 2011). Beyond destroying crop production rates, contamination also negatively impacts the humans who consume these crops. While the reduction of contamination is desirable, it is often not feasible due to the difficult and expensive process of chemical treatments, radiation, filtration, and desalination (Zhang and Fang, 2010; Oller et al., 2011). For this purpose, using nanoparticles to treat wastewater has provided a viable alternative solution. Technologies using nanomaterials are comprised of numerous filters and membranes that include magnetic elements, carbon nanotubes, and nanoporous ceramics. This system is effective at water treatment and doesn't require expensive or complex equipment. Another benefit of these water filtration systems is the potential to purify potable water, which could eliminate waterborne pathogens that produce microbial infections. Nanoparticles of iron-oxide are drawing attention because of the strong absorption, easy separation, and minimal cost (Girginova et al., 2010; Fan et al., 2012). Magnetic nanoparticles are ideal for this solution because of their Nano size, surface changeability, and paramagnetic qualities (Qu et al., 2013). Other suggested nanoparticles for water treatment include titanium, aluminum, zinc, and silver.

CHAPTER 3

MATERIALS AND METHODS

3. 1. Plant Materials and Growth Conditions

The cucumber Beith Alpha cultivar (Paşa Tohumculuk) was used as plant material for the present study. The seeds were planted in 3 L plastic pots filled with soil, sand and vermiculite mixture in the ratio of 2:1:1. Four grams of ammonium sulfate, superphosphate, and potassium sulfate was applied in each pot. After emergence, the seedlings in each pot were thinned to one. The plants were irrigated with tap water when irrigation was needed.

3.2. Sample Preparation and Characterization of Nanomaterials

The ZnO, CuO and $AgNO_3$ were purchased as dry powders. Nanomaterials will be suspended in deionized water at concentrations of 50 and 200 mg/L for the stock suspensions. Sonication for 30 minutes follows for the production of the necessary concentrations for further experimentation.

3.3. Fungal Strain and Growth Conditions

The *B. cinerea* standard strain used for this experiment was provided by Prof. Dr. Soner Soylu (Mustafa Kemal University). The isolate was routinely cultured on potato dextrose agar (PDA) that induced prolific conidia production for seven days at 22 degrees. *B. cinerea* conidia were gathered from seven to 14-day-old fungal plates in 20 mL of water. The suspended conidia were washed with sterile, deionized water. The resuspended conidia were then diluted with sterile, deionized water to a final concentration of 10^5 conidia/mL in potato dextrose broth medium, with varying nanomaterials.

3.4. Essential Oil Source and Essential Oil Analysis

Aerial parts of peppermint (*Mentha piperita* L.) plants were collected at the flowering stage from Erciyes University Experimental Farm. The used essential oils were obtained through water distillation of leaves dried at room temperature for three hours using a Clevenger.

The used stems, flowers, and leaves of the plant were dried in shade. After the essential oil was obtained using the above-mentioned method, it was dried over anhydrous sodium sulfate and stored in a refrigerator set to four degrees until the experiment was performed. The GC analyses were done with a Hewlett-Packard 6890 GC with FID. An HP-5 MS capillary column (30 m x 0.25 mm i.d. 0.25 µm film thickness) was used. The used carrier gas was helium at 1.4 ml per minute. The column’s temperature was programmed at: 45 degrees for five minutes, then an increase of three degrees per minute until reaching 220 degrees where the samples were held for ten minutes. The injector had a temperature of 220 degrees and the detector was 250 degrees. Injection was done using the automatic setting. Samples (0.5 µl of the oil solution in hexane (1:100) were injected using the split-less technique into the Helium. Electronic integration was used to measure the peak area and retention time. The essential oil’s GC/MS analysis was done using the Hewlett Packard 5970A mass selective detector (MSD), directly coupled to an HP 6890 GC. The column, temperature program, and injection were carried out using the above technique. The library search was done with "Wiley Library, WILEY275, NBS75K, NIST98, and FLAVOR". EI mass spectra were measured at 70 eV ionizations voltage over the mass range 10 - 400 u. The compounds were identified through a comparison of the retention times and mass spectra of the experimental items and library standards (Stenhagen et al., 1974; Adams, 1995).

3.5. Antifungal Effect of Different Concentrations of Nanoparticles on Mycelial Growth *In Vitro* Conditions

The ZnO, CuO and $AgNO_3$ were suspended in 20 % potato dextrose agar medium to the final concentrations of 5, 50, 100, and 200 mg/L poured into Petri dishes of 90 mm in diameter. Three potato dextrose agar plates of each nanomaterial concentration were tested. After cooling and solidification, well-cultivated 5-mm diameter *B. cinerea* colony was then inoculated in the center of the medium, all the colonies were moved to a room with a controlled climate of 22 degrees. The mycelia diameter was measured after 72 hours of incubation.

Figure 3.1. Measurement of fungal growth

3.6. Antifungal Effect of Different Concentrations of Nanoparticles and Essential Oil on Leaf Disc *In Vitro* Conditions

The ZnO, CuO and $AgNO_3$ were suspended in 4% water-agar to the final concentrations of 50 and 200 mg/L. Each NPs was mixed alone and with different concentrations of essential oil (0, 10, 20, 40, 80, 160, 320 and 640 µl). Tween-20 was incorporated into the agar medium to enhance essential oil solubility and allowed to solidify. For inoculation, cucumber leaf was collected from a greenhouse and brought to the laboratory. One-centimeter diameter discs were excised from the leaf’s center with a hole punch. The leaf discs were rinsed with deionized water, placed onto 4 percent water-agar plates amended with or without nanomaterials. The leaf discs were carefully placed in potato dextrose agar media containing different concentrations of NPs, and then the suspension of conidia (2 µL, 10^5 conidia/mL in potato dextrose broth medium).

Figure 3.2. Leaf discs taken from the greenhouse grown cucumber plants

Different nanomaterials were placed onto the petals to find the nanoparticle impact on fungal infection. Using the method, the microenvironment of the leaf can be emulated when the disc is soaked with nanoparticles on both sides. Even distribution can be ensured through suspending the conidia and nanoparticles onto the disc. Deionized water and conventional fungicide procymidone containing the same concentration of conidia were used in the leaf disc as a negative control and positive control for the second and third groups. After inoculation, the Petri dishes were instantly moved to a room with a controlled climate of 22 degrees. A photographic record and diameter measurement of the location of infection were measured after 72 hours, with the data averaged and means calculated.

3.7. Fungi Infection in the Whole Leaf *In Vivo* Conditions

Cucumber leaves were selected on the greenhouse growing plants, and the leaves were washed with deionized water. When the water on the leaves evaporated 50 and 200 mg/L concentration of NPs were sprayed on each selected leaves. The fungal suspension of conidia (2 μL, 10^5 conidia/mL in potato dextrose broth medium) was sprayed in the upper and lower surfaces of the leaves. The treated plants were then placed in a growth chamber, in which the temperature was set at 22°C and the moisture was adjusted to 90%. After 96 h of incubation, the leaf was photographed and the total fungal plaque areas were measured.

For each nanoparticle application, three replicates were used. PDA medium without nanoparticles and essential oil were used as a control. The zones of growth were measured at the fourth and sixth days of incubation. The following formula was used to calculate the fungal toxicity of the oils based on the growth inhibition of the mycelia.

Growth inhibition (%) = dc-dt/dc x 100

Where dc = Average mycelial growth increase in the control,

dt = Average mycelial growth increase in the treatment (Singh and Tripathi, 1999).

3.8. Statistical Analyses

The experiments were performed three times. Statistical analysis, a one-way analysis of variance followed by LSD test ($P \leq 0.05$), was carried out using SAS for Windows, version 9.3; SAS Institute.

CHAPTER 4

RESULTS

4.1. Essential Oil Components of *Mentha piperita*

Essential oil components of *Mentha piperita* are given in Table 4.1. Thirty five essential oil components were detected from the analysis of *Mentha piperita* essential oil (Table 4.1). Thirteen major essential oil components greater than 1% was detected from mint essential oil (Table 4.1). The major essential oil components were β-Pinene (1.25%), 1, 8-Cineole (2.15%), β-phellandrene (5.60%), menthone (29.60%), isomenthone (4.70%), menthofuran (7.40%), neomenthol (3.20%), menthol (28.16%), menthol acetate (4.41%), β-caryophyllene (3.15%), germacrene D (1.89%) and Pulegone (4.39%).

Table 4.1. Essential oil components of *Mentha piperita*

Compound Name	**Retention Time**	**Retention Index**	**Area %**
α-Thujene	3.69	922	0.05
α-Pinene	4.33	930	0.82
Sabinene	4.61	965	0.62
β-Pinene	5.12	970	1.25
3 Octanol	5.42	978	0.05
Myrcene	5.42	981	0.17
α-Terpinene	6.06	1007	0.09
p-Cymene	6.25	1010	0.14
1,8-Cineole	6.47	1019	2.15
β-Phellandrene	6.86	1020	5.6
β-ocimene	7.39	1024	0.02
γ-Terpinene	7.65	1048	0.17

Compound Name	**Retention Time**	**Retention Index**	**Area %**
trans-Sabinene hydrate	8.05	1053	1.53
Linalool	8.53	1082	0.14
Menthone	8.53	1142	29.6
Isomenthone	8.8	1149	4.7
Menthofuran	9.05	1153	7.4
Neomenthol	9.63	1155	3.2
Menthol	12.38	1168	28.16
Is menthol	13.94	1174	0.24
α-Terpineol	16.11	1177	0.36
Pulegone	16.53	1219	4.39
Piperitone	19.16	1229	0.48
Neomenthol acetate	19.67	1261	0.28
Menthol acetate	19.85	1280	4.41
Is menthol acetate	20.18	1295	0.14
β-Bourbonene	21.33	1388	0.23
β-Elemene	21.72	1390	0.12
β-Caryophyllene	21.89	1425	3.15
(Z)-β-farnesene	22.56	1446	0.35
α-Humulene	24.01	1457	0.11
Germacrene D	25.3	1483	1.89
Germacrene A	25.61	1496	0.41
Caryophyllene oxide	26.32	1578	0.13
Viridiflorol	31.44	1590	0.42

4.2. Antifungal Effect of Nanoparticles Dose on Mycelial Growth of *Botrytis cinerea* under *In Vitro* Conditions

Table 4.2. Variance analysis of nanomaterials on growth inhibition of *Botrytis cinerea*.

Variation Resource	S.D.	Sum of Square	Mean Square	F
Dose	3	16180.14	5393.38	47.62**
Treatment	2	3886.28	1943.14	17.16**
Dose x Treatment	6	10543.29	1757.21	15.51**
Error	132	14951.14	113.26	
Total	143	45560.80		

Variance analysis table showed that dose, treatment, and dose x treatment interaction were significant in terms of growth inhibition on *Botrytis cinerea* (Table 4. 2).

Table 4.3. Growth inhibition of nanomaterials on grey mold

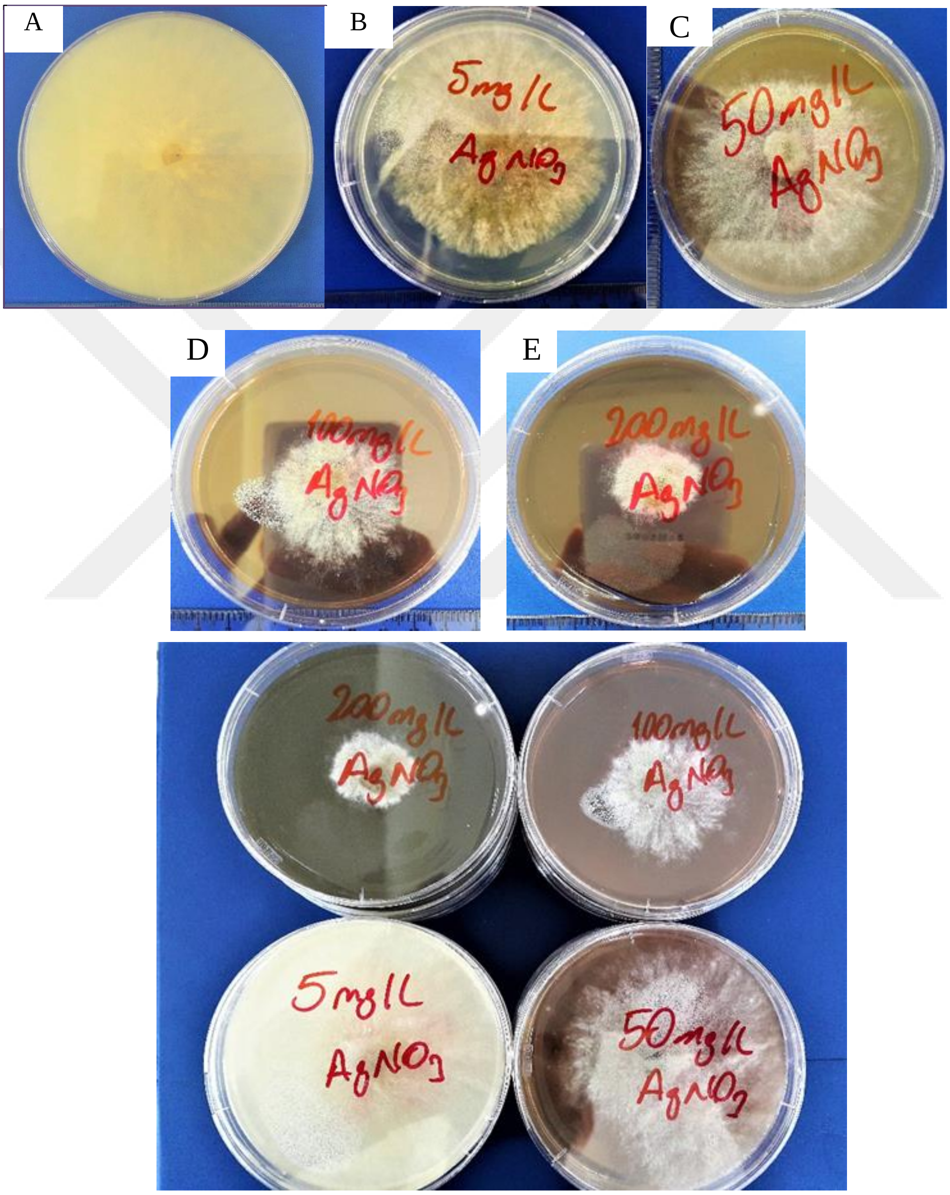
Nanomaterial	Growth inhibition (% of the control)
$AgNO_3$	38.32 A
ZnO	31.48 B
CuO	25.61 C
LSD (% 0.05)	4.30

Compared with the control, growth inhibition difference among AgO_3, ZnO and CuO was significant (Table 4. 3). The growth inhibitions varied between 25.61 and 38.32 %. The highest and the lowest inhibition rates were obtained from $AgNO_3$ and CuO respectively.

Table 4.4. Growth inhibition of nanomaterial doses on *Botrytis cinerea*.

Dose (mg/l)	Growth inhibition (% of the control)
5	22.08 C
50	22.39 C
100	34.95 B
200	47.82 A
LSD (% 0.05)	4.96

Growth inhibition of nanomaterials on *Botrytis cinerea* varied among application doses (Table 4.4). Growth inhibition rates varied between 22.39 and 47.82 %. The highest growth inhibition was obtained from 200 mg/l with 47.82 % and the lowest one was obtained 5 mg/l with 22.08. There was no significant difference between 5 and 50 mg/l application doses.



A: The control zero conc., B: 5mg/l, C: 50 mg /l, D: 100mg/l, E: 200 mg/l, (AgNo3)

Figure 4.1. Mycelial growth of *Botrytis cinerea* on PDA.

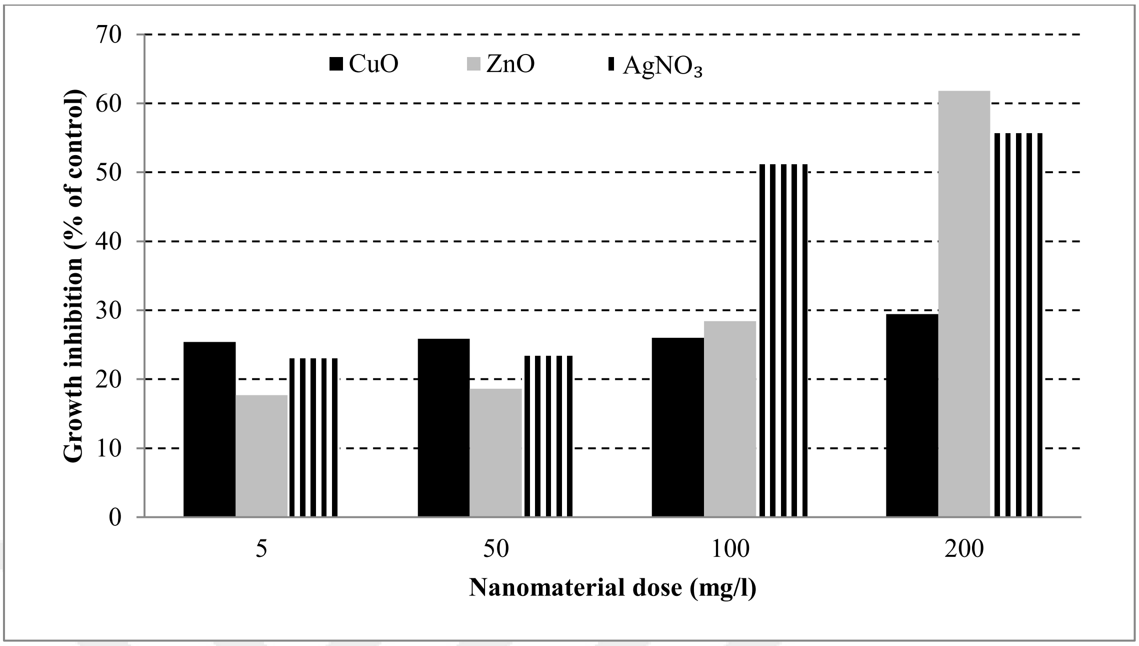


Figure 4.2. Effects of nanomaterial x dose interaction on growth inhibition of *Botrytis cinerea*.

In terms of growth inhibition, dose x nanomaterial interaction was significant (Figure 4.1). The significant interaction was resulted from growth inhibition of $AgNO_3$, since growth inhibition of $AgNO_3$ was lower than ZnO in the 200 mg/l dose while growth inhibition of $AgNO_3$ was higher than ZnO in 100 mg/l application. The growth inhibitions of $AgNO_3$ in 5 and 50 mg/l concentrations were not significant. The growth inhibitions of CuO in 5, 50 and mg/l concentrations were not significant. Growth inhibition of $AgNO_3$ significantly greater at 100 mg/l then CuO and ZnO. However, at 200 mg/l growth inhibition of ZnO was higher than $AgNO_3$. It can be seen from the Figure 4.1 that different concentrations of CuO did not cause serious growth inhibition but at 200 mg/l growth inhibition of ZnO and AgNO3 greatly increased. The maximum growth inhibition was obtained from 200 mg/l. The Nano ZnO had great inhibitory effect on *B. cinerea* growth at 200 mg/l $AgNO_3$ hat the highest inhibition rate at 100 mg/l concentration.

4.3. Antifungal Effect of Different Nanoparticles and Essential Oil on Leaf Disc *In Vitro* Conditions

Table 4.5. Variance analysis of nanomaterial mint essential oil mix on growth inhibition of *Botrytis cinerea*.

Variation source	**D.F.**	**Sum of Square**	**Mean Square**	**F**
Nanomaterial (A)	6	170582.46	28430.41	593.86**
Nanomaterial dose (B)	2	3882.96	1941.48	40.55**
Mint dose (C)	12	53272.31	4439.35	92.73**
A x B	1	29317.17	29317.17	612.38**
A x C	2	34131.72	17065.86	356.47**
B x C	6	10744.64	1790.77	37.41**
A x B x C	12	12040.30	1003.35	20.96**
Error	336	16085.71	47.87	
Corrected Total	377	330057.30		
C.V.	9.25			

Based on the ANOVA analysis for *B. cinerea*, when it was subjected to the nanomaterial and essential oil treatments, significant differences in the growth inhibition of the fungus on leaf disc were found (Table 4.5). It was clearly seed from the Table 4.5 that nanomaterial, nanomaterial dose, mint dose, nanomaterial x nanomaterial dose, nanomaterial x mint dose, nanomaterial dose x mint dose and nanomaterial x nanomaterial dose x mint dose interactions were significant.

Table 4.6. Growth inhibition of mint essential oil doses on *B. cinerea* infected leaf disc.

Mint essential oil doses (µl)	Growth inhibition (% of the control)
10	42.95 E
20	49.85 D
40	62.32 C
80	80.87 B
160	95.68 A
320	95.90 A
640	95.98 A
LSD 0.05	2.61

Growth inhibition of grey mold on leaf disc was significant among mint essential oil doses (Table 4. 6). Compared with the control, growth inhibition of mint essential oil doses varied between 42.95 and 95.98%. The lowest grey mold inhibition on leaf disc was obtained from 10 µl essential oil application fallowed by 20 µl essential oil applications, respectively. Growth inhibition on leaf disc rate increased with the increased rate of mint essential oil. Great inhibition on leaf disc was obtained at 160 µl and above essential oil applications.

Table 4.7. Growth inhibition of nanomaterial type on *Botrytis cinerea* infected leaf disc.

Nanomaterial	Growth inhibition (% of the control)
$AgNO_3$	77.78 A
ZnO	76.25 A
CuO	70.35 B
LSD 0.05	1.71

Compared with the control, growth inhibition rate of nanomaterial on *B. cinerea* was significantly different (Table 4. 7). The inhibition rate varied between 70.35 and 77.78 %. The highest inhibition was obtained from $AgNO_3$ with 77.78% and the lowest was obtained from CuO with 70.35%.

Table 4.8. Growth inhibition of nanomaterial dose on *Botrytis cinerea* infected leaf disc.

Nanomaterial doses (mg/l)	Growth inhibition (% of the control)
50	65.99 B
200	83.60 A
LSD 0.05	1.40

Table 4.8 shows that growth inhibition rate increased with the increasing nanomaterial dose. The growth inhibition rate was significantly higher in 200 mg/l nanomaterial dose than 50 mg/l nanomaterial dose.

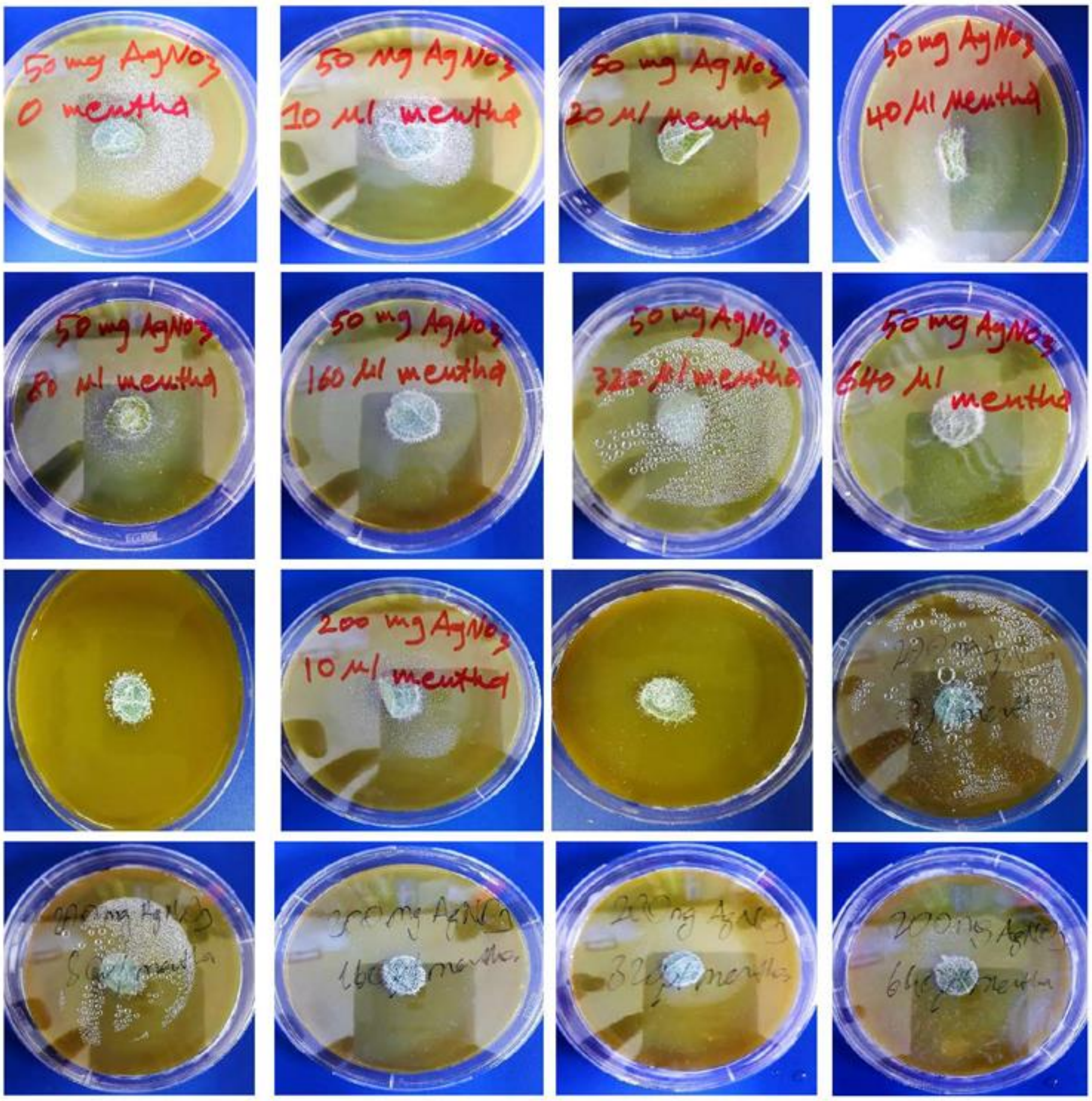


Figure 4.3. Fungal growth on inserted leaf disc of Potato DA.

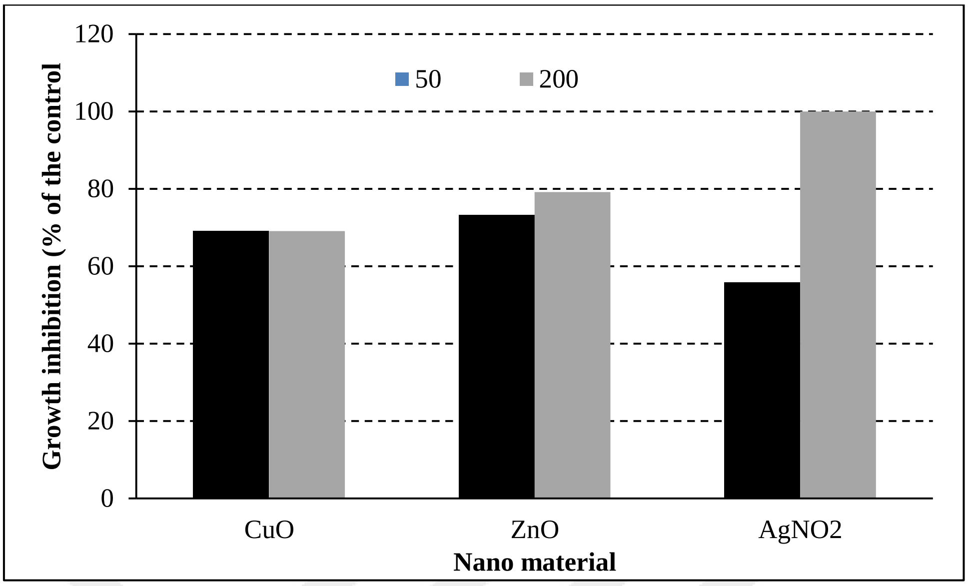


Figure 4.4. Effects of nanomaterial x nanomaterial dose interaction on *Botrytis cinerea* infected leaf disc.

When growth inhibition of nanomaterial and nanomaterial dose interaction was in consideration, the nanomaterials had highly significant effect on grey mold growth at 200 mg/l concentration (Figure 4.4), except for ZnO and CuO. The inhibitory effect difference of CuO between 50 and 200 mg/l concentrations was not significantly different. However, the inhibitory effects of ZnO was slightly different between 50 and 200 mg/l concentrations.

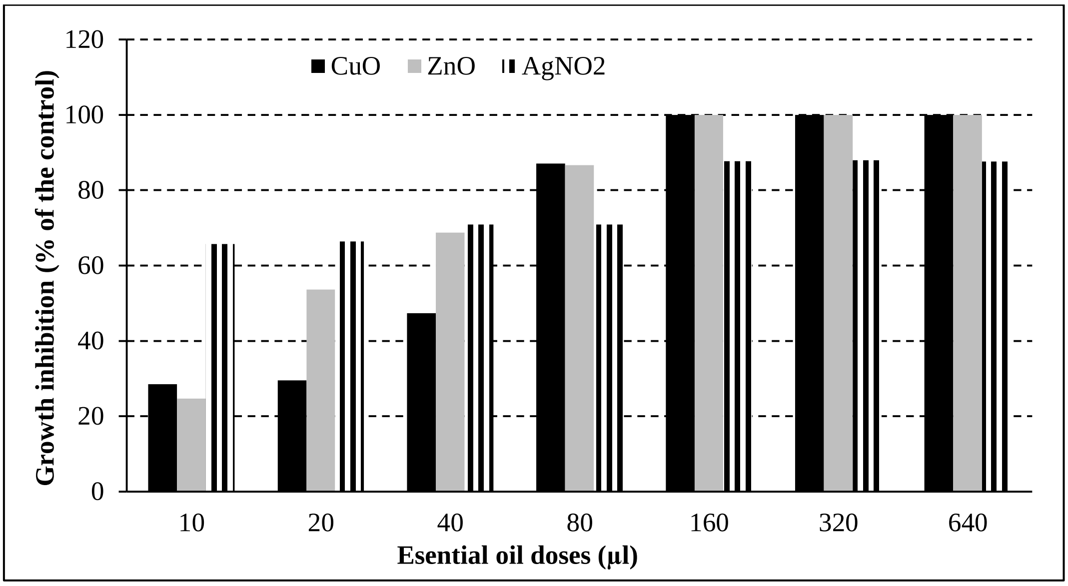


Figure 4.5. Effects of nanomaterial x essential oil dose on growth inhibition of *Botrytis cinerea* infected leaf disc.

Figure 4.5 showed that Nano CuO and ZnO at 160, 320 and 640 µl/l essential oil concentrations had complete inhibition on grey mold growth while, $ZnNO_3$ had no complete inhibition on grey mold disease at the highest concentrations (Figure 4. 3). However at 10, 20, 40 and 80 µL essential oil concentration $AgNO_3$ had higher inhibitory effect on gray mold growth then ZnO and CuO. Inhibitory effect of CuO greatly increased at 80 µl essential oil concentration and had complete inhibition at 160 ZnO µl and above doses. Inhibitory effects of ZnO linearly increased until 160 µl essential oil dose.

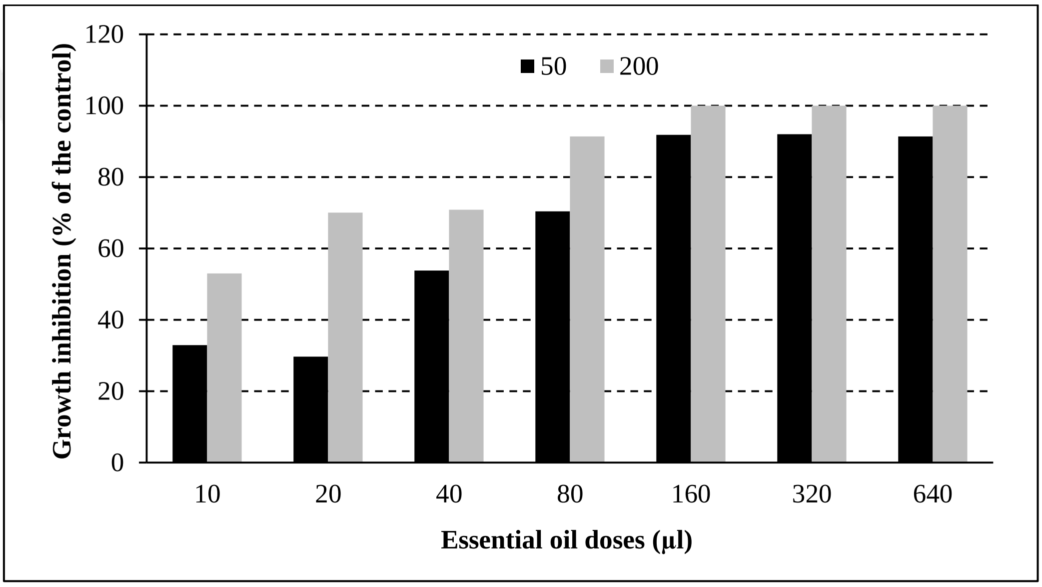


Figure 4.6. Effects of nanomaterial dose x essential oil dose interaction on growth inhibition of *Botrytis cinerea* infected leaf disc.

The highest differences in the growth inhibition were found in the Nano material treatments at concentrations of 200 mg/l with 160 µl essential oil treatments (Figure 4.6.). Complete growth inhibition was achieved at 160 µl essential oil treatments with 200 mg/l nanomaterial doses. 160, 320 and 640 µl essential oil treatments had similar inhibitory effects on grey mold growth at 200 mg/l nanomaterial concentration.

Table 4.9. Nanomaterial x Nanomaterial dose x mint dose interaction on growth inhibition of *B. cinerea* infected leaf disc

Nanomaterial	**Nanomaterial dose (mg/l)**	**Mint dose (µl)**	**Growth inhibition (% percent of the control)**
AgO	50	10	51.31
AgO	200	10	100.00
CuO	50	10	25.14
CuO	200	10	31.87
ZnO	50	10	22.36
ZnO	200	10	27.04
AgO	50	20	32.74
AgO	200	20	100.00
CuO	50	20	10.96
CuO	200	20	48.09
ZnO	50	20	45.32
ZnO	200	20	61.98
AgO	50	40	41.81
AgO	200	40	100.00
CuO	50	40	47.36
CuO	200	40	47.36
ZnO	50	40	72.22
ZnO	200	40	65.20
AgO	50	80	37.71
AgO	200	80	100.00
CuO	50	80	100.00
CuO	200	80	74.12
ZnO	50	80	73.39
ZnO	200	80	100.00
AgO	50	160	75.43
AgO	200	160	100.00
CuO	50	160	100.00
CuO	200	160	100.00
ZnO	50	160	100.00
ZnO	200	160	100.00
AgO	50	320	75.87
AgO	200	320	100.00
CuO	50	320	100.00
CuO	200	320	100.00
ZnO	50	320	100.00
ZnO	200	320	100.00
AgO	50	640	74.12
AgO	200	640	100.00
CuO	50	640	100.00
CuO	200	640	100.00
ZnO	50	640	100.00
ZnO	200	640	100.00

When nanomaterial x nanomaterial dose, nanomaterial x mint dose, nanomaterial dose x mint dose and nanomaterial x nanomaterial dose x mint dose interactions were considered, it was clearly seen that the significant interaction was resulted from $AgNO_3$, since complete growth inhibition was obtained at 10 µ essential oil dose with 200 mg/l concentration. CuO and ZnO did had no complete inhibition on grey mold at the same nanomaterial and essential oil concentrations (Table 4.9). Mixing essential oil with nanomaterials enhanced antifungal effect of Nano ZnO, $AgNO_3$ and CuO.

4.4. Antifungal Effect of Different Nanoparticles on Gray Mold Infection in the Whole Leaf *In Vivo* Conditions

Variance analysis of nanomaterial + mint essential oil mix on disease incidence of gray mold was given in Table 4.10.

Table 4.10. Variance analysis of nanomaterial + mint essential oil mix on disease incidence of *Botrytis cinerea*.

Variation source	D.F.	Sum of Square	Mean Square	F
Treatment	7	6673.72	953.38	4.45**
Error	16	3426.00	214.12	
Corrected total o	23	10099.72		
C.V.	19.42			

Variance analysis table showed that nanomaterial + mint essential treatments were significant in terms of disease incidence (Table 4. 10).

Table 4.11. Disease incidence of gray mold on nanoparticle + essential oil treatment on cucumber leaf

Treatment	**Incidence (%)**
CuO 50 mg/l + 80 µl/l essential oil	22.03 B
CuO 200 mg/l + 160 µl/l essential oil	7 .53 B
ZnO 50 mg/l + 80 µl/l essential oil	18.78 B
ZnO 200 mg/l + 160 µl/l essential oil	29.85 B
AgNO3 mg/l + 80 µl/l essential oil	17.91 B
AgNO3 mg/l + 160 µl/l essential oil	8.56 B
Untreated control	62.12 A
Fungicide	10.32 B
LSD 0.05	25.32

Disease incidence varied between 7.53 and 62.12 % among the treatments (Table 4.11). The highest disease incidence was obtained from untreated control and the lowest was obtained from 200 mg/l CuO with 160 160 µl/l essential oil treatments. Both essential oil and nanoparticle dose were increase the disease incidence greatly reduced.

Variance analysis of nanomaterial + mint essential oil mix on disease severity of gray mold was given in Table 4.12.

Table 4.12. Variance analysis of nanomaterial + mint essential oil mix on disease severity of *Botrytis cinerea*.

Variation source	D.F.	Sum of Square	Mean Square	F
Treatment	7	395.45	56.49	35.34**
Error	16	25.57	1.99	
Corrected total	17	297.03		
C.V.	19.42			

Variance analysis table showed that nanomaterial + mint essential treatments were significant in terms of disease severity (Table 4. 12).

Table 4.13. Disease severity of gray mold *Botrytis cinerea* on nanoparticle + essential oil treatment on cucumber leaf

Treatment	**Severity (%)**
CuO 50 mg/l + 80 µl/l essential oil	10.77 B
CuO 200 mg/l + 160 µl/l essential oil	2.44 D
ZnO 50 mg/l + 80 µl/l essential oil	9.27 BC
ZnO 200 mg/l + 160 µl/l essential oil	1.77 D
AgNO3 mg/l + 80 µl/l essential oil	7.77 C
AgNO3 mg/l + 160 µl/l essential oil	3.17 D
Untreated	13.28 A
Fungicide	3.63 D
LSD 0.05	2.18

Various concentration of nanoparticles and essential oil were used *in vivo*. Nanoparticles, CuO, ZnO and $AgNO_3$ (50, 200 mg/l) mix with 80 and 160µl/l essential oil had great antifungal effect on cucumber (Table 4.11 and 4.13). When disease severity was considered, it varied between 1.77 and 13.28 %. The lowest disease severity was obtained from ZnO 200 mg/l + 160 µl/l essential oil and the highest was obtained from untreated control treatment with 13.28 %.

CHAPTER 5

DISCUSSION

Nano biotechnology comprises Nano sized organic and inorganic particles with biological functions. Various kind of organic and inorganic nanomaterials such as titanium, silver, copper, magnesium, zinc, gold and alginate have been available, yet Nano silver has proved to be most effective antimicrobial agents on fungi, bacteria and viruses (Guo et al., 2003). Nanoparticle application can open a research door of managing fungal pathogens. Since nanomaterials have different morphological, structural, and physiological properties that enhance antimicrobial efficiency of essential oils. Based on enhanced effectiveness, polymers, metals or ceramics have several biological applications.

More than 70% of known major crop diseases are Fungal disease (Agrios, 2005) and great amount of yield loss is occur in major world crops such as wheat, rice, corn, barley, cotton, grapevine, groundnut, cucumber and tomato (Dhekney et al., 2007). Great amount of crops are affected by mycotoxins such as ergot toxins, aflatoxins, Fusarium toxins, ten azonic acid and patulin (Schneider and Ullrich, 1994). Several studies have conducted on the fungicidal effects of nanoparticles against phytopathogenic fungi (Kim et al., 2012), but the fungicidal effects of essential oil mixed nanoparticles has not been elucidated yet.

Botrytis cinerea (grey mold), first described over 200 years ago, is one of the economically important pathogens of greenhouse and open-field grown vegetable crops due to saprophytic nature (Bessey, 1950). Whenever environmental conditions are favorable, *B. cinerea* can be pathogenic and destructive. Fruit rots, neck and bulb rots, stem cankers and rots, leafspots, blossom blight and tuber and root decay are the well-known injuries caused by *B. cinerea.*

Fungi are responsible for more than 70% of known major crop diseases (Agrios, 2005) and considerable crop losses are occurring in major World crop species such as rice, wheat, barley, cotton, peanut and soybean (Dhekney et al., 2007) Grey mold, *Botrytis cinerea*, is one of the economically important pathogens of greenhouse and open-field grew cucumber. Known chemical controls are not always effective to control grey mold, therefore, alternative methods to protect the crop have become more attractive. Different nanoparticles such as copper, zinc, titanium (Retchkiman-Schabes et al., 2006) magnesium, gold (Gu et al., 2003) alginate (Ahmad et al., 2005) and silver have been developed. Nanoparticles offer a successful alternative solution for protecting agricultural crops from bacterial and fungal infection.

Spraying with effective chemical fungicides to control *B. cinerea* is not fully effective, consequently, alternative methods are needed to protect the crops have gained great attention. Different nanoparticles such as silver, aluminum, gold, zinc, carbon, titanium, palladium, magnesium, iron, fullerenes, alginate and copper have been developed (Schabes-Retchkiman et al., 2006; Gu et al., 2003; Ahmad et al., 2005; Pinna and Niederberger, 2008). Nanoparticles have great antimicrobial activity on bacteria and fungi (Baker et al., 2005; Melaiye et al., 2005; Lok et al., 2006; Krishnaraj et al., 2012). Diffusion of nanoparticles into fungal or bacterial cells disrupts DNA and RNA synthesis that causes direct cell death (Ing et al., 2012). Also nanoparticles damage the fungal and bacterial cell walls showing formation of pits and cause accumulation of cell membranes that increase permeability and cell death (Egorova, 2010; Sintubin et al., 2012; Sharma et al., 2009; Li, et al., 2010; Chauhan, 2013).

Essential oils, the secondary plants’ metabolism, are mostly made up of complex mixtures of odoriferous, and liquid substances comprising monoterpenoids, sesquiterpenes and phenols (Simões and Spitzer, 2004). Essential oils accumulate in the cytoplasmic membrane and damage the membranes consequently change the selective function of the membranes (Sikkema, Bont and Poolman 1994). It was reported that essential oil has strong antifungal effect on fungi (Soliman and Badeaa, 2002; Hammer et al. 2004; Pinto et al., 2006; Tabanca et al., 2007; Tullio et al., 2007; Dutta, et al., 2007).

The compositions of essential oils vary with the plant species, environmental conditions, harvesting stage, time and processing (Mishra 1994). As displayed by Table 4.1, the essential oils of *M. piperita*, contained β-Pinene, 1, 8-Cineole, β-phellandrene, menthone, isomenthone, menthofuran, neomenthol, menthol, menthol acetate, β-caryophyllene, germacrene-D and Pulegone.

Similar results were reported by Lawrence (1987), Vorin et al. (1990), Topalov and Zheljazkov (1991), Court et al. (1993), Chalchat et al. (1997), Chalchat and Michet (1997), Khanna et al. (2014) and Balakrishnan (2015) that β-Pinene, 1, 8-Cineole, β-phellandrene, menthone, isomenthone, menthofuran, neomenthol, menthol, menthol acetate, β-caryophyllene, germacrene-D and Pulegone were main compounds found in *M. piperita* essential oils.

The major essential oil components were it is problematic to connect the activity of a complex mixture to particular components (Pauli and Schilcher 2010). Still, it is reasonable to speculate that the activity of *M. piperita*, essential oils can be related to the presence of α-Pinene, 1,8-cineole, β-phellandrene, menthone, isomenthone, menthofuran, neomenthol, menthol, menthol acetate, β-caryophyllene, germacrene-D and Pulegone. These major essential oil compounds were found to be the most active components (Table 4.1). For most scenarios, the essential oils' prohibitory effects are linked to the dominant elements and not to their other elements (Farag, 1989). Still, some scientists discovered a bigger effect when using the entire essential oil rather than only the dominant compound. This highlights a potential synergistic effect of the minor elements (Bullerman, 1977). (Tampieri et al., 2005) reported that mint essential oil had great inhibition activity on the growth of fungal pathogens due to major components, such as menthone, menthol.

This work revealed the effects of different concentrations of CuO, ZnO and $AgNO_3$ on the growth inhibition of *B. cinerea*. The results showed that different concentration of Nano CuO ZnO and $AgNO_3$ had significant inhibition on growth inhibition of *B. cinerea* as compared with the control treatment. The highest nanoparticle doses had great inhibition of *B. cinerea*. The highest growth suppression of *B. cinerea* was observed at the highest Nano $AgNO_3$ ZnO and CuO concentrations. The antifungal effects of Nano $AgNO_3$ ZnO and CuO may be due to its morphological, structural, and physiological properties. Antifungal effect of Nano ZnO on some plant pathogenic fungi has also been reported by some workers (Sawai and Yoshikawa, 2004; Violeta et al., 2011; Lipovsky et al., 2011; Hassan et al., 2013).

The applied nanoparticles had growth inhibition on *B. cinerea*. However, inhibitory effect of $AgNO_3$ and ZnO found most effective on *B. cinerea* growth under *in-vitro* condition. Inhibitory effect was found to be dependent on the concentration of Nano $AgNO_3$, CuO and ZnO as indicated in the current work. (Pérez -de-Luque and Diego, 2009) indicated that inhibitory effect of nanomaterials could be attributed to the release of metabolites and extracellular enzymes. It was reported that antifungal activity of nanoparticles was due to suppression of enzymes (Bhainsa, and D'Souza, 2006; Vahabi et al., 2011). Broad range of biological activities of silver on microorganisms including the alteration of cell membrane structure and functions were reported (McDonnell and Russell, 1999; Sondi and Salopek-Sondi, 2004).

Nanoparticles have great potential to protect crops from bacterial and fungal attacks. They allow better scientific management of plant diseases as compared to synthetic chemicals (Park et al., 2006) Silver nitrate, copper oxide and zinc oxide nanoparticle are effective antifungal agents (Nadia et al., 2010; Kim et al., 2007). The nanoparticles of Nano silver nitrate, copper oxide and zinc oxide at highest concentration were found most effective in growth inhibition of grey mold. But growth inhibition increased with the increased concentration of nanoparticles.

CONCLUSION

The present study revealed the efficacy of different concentrations of silver nitrate ($AgNO_3$), copper oxide (CuO) and zinc oxide (ZnO) on the growth inhibition of *B. cinerea*. It was clearly seen from the results, that different concentration of the applied nanomaterials had significant inhibition on *B. cinerea* as compared with the control. The highest inhibitions were observed at the highest concentrations of nanomaterials. Considering the reduction in mycelial growth and germination of *B. cinerea in vitro*, it can be concluded that mint essential oil could be used as possible bio fungicides, as an alternative to synthetic fungicides, against *B. cinerea* on cucumber plant. ZnO, CuO and $AgNO_3$ nanoparticles possess significant antifungal properties against *B. cinerea* and the inhibitory effects increase as the concentrations of nanoparticles increase. The inhibitory effects of nanoparticles increased when they combined with mint essential oil. ZnO, CuO and $AgNO_3$ are heavy metals that treat human health and environment. When developing a commercial nanoparticle and essential oil combinations, environmentally safe nanomaterials must be chosen. Since nanoparticles have greater toxicity than micro ones.

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