Salahaddin University Kurdistan– Iraq

College of Agriculture Engineering Sciences

Department Plant Protection stage 4

Control of *Botrytis cinerea* using different plant extracts

A Report submitted to the Department of Plant Protection at Salahaddin University- Erbil in Partial Fulfillment of the Requirement for the Degree of BC of Science in College of Agriculture Engineering Sciences

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1. Introduction

Botrytis cinerea is one of the most extensively studied necrotrophic fungal pathogens and causes gray mold rot in more than 500 plant species (Williamson et al., 2007). This pathogen has a disastrous economic impact on various economically important crops including grape, strawberry, and tomato (Dean et al., 2012) and is able to be present inside stems, leaves, flowers, fruits, and seeds. It may trigger obvious disease symptoms in the pre-harvest period or remain quiescent until post-harvest period (Fillinger and Elad, 2016). *Botrytis cinerea* has been reckoned as one of the most important post-harvest pathogens in fresh fruits and vegetables (Zhang et al., 2014). The annual economic losses of *B. cinerea* easily exceed $10 billion worldwide (Weiberg et al., 2013). Due to its scientific and economic importance, *B. cinerea* has been classified as the second important plant pathogen (Dean et al., 2012). It is difficult to control *B. cinerea* because it has broad host range, various attack modes, and both asexual and sexual stages to survive in favorable or unfavorable conditions (Fillinger and Elad, 2016). The asexual spores of *B. cinere*a are conidia, which are easily to be dispersed by wind or water, and the sexual spores of *B. cinerea* are sclerotia, which are essential for survival under adverse environment (Brandhoff et al., 2017).

Biological agents and chemical compounds from plants are increasingly used for plant protection (Shuping, D.; Eloff, J 2017). Many types of plant essential oils have the potential to preserve food products (Zheng,J et al.,2019) . The application of plant extracts as well as plant metabolites for plant disease management has become an important viable, sustainable and environmentally friendly component of integrated disease management (Harish et al., 2008; Stavi et al., 2016; Zhang et al., 2016).

Therefore, the number of studies on the application of natural extracts in the food safety field is increasing, including the investigation of plant oils for inhibition activity in the context of postharvest gray mold caused by *B. cinerea*(Safaei-Ghomi, J.; Ahd, A.A. 2010; Olea, A.F. et al., 2019). Studies have shown extracts from pimento demonstrated the presence of bioactive agents that can inhibit gray mold on strawberry leaves (Sernaite,L. et al., 2019). Finally some studies showed that there are more than 280 phytochemicals are reported to have fungicidal properties, among these phytochemicals, phenolic compounds, terpenoids, alkaloids and ketones are among the top mentioned (Boulogne et al., 2012).

1.1 The **aim** of project

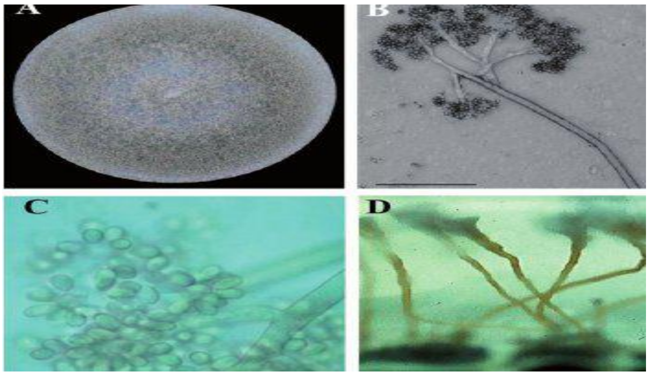
1. Increase awareness about using plant extract and its benefit on environment in our region.
2. Finding the most affective plant extract agents gray mold.

1. Literature Review

1.1 Gray Mold (*Botrytis cinerea*)

The genus name refers to the structure of the macroconidia, which rise and form clusters with the shape of grape bunches: 'botryose'. *Botrytis* is the asexual stage of *Botryotinia* (Kirk et al. 2013). *B. cinerea* is one of the most common fungal plant pathogens and infects well over 200 different plant hosts. In addition to being an aggressive primary pathogen, *B. cinerea* is a many-sided organism, able to grow and reproduce on damaged, aging, and dead tissues of strawberry, vegetables, and many other plants. *B. cinerea* reproduces primarily by making asexual spores, or conidia (Grant-Downton et al. 2014). These gray masses of conidia are readily spread by wind, splashing water, and physical/mechanical activity. Like many fungi, *B. cinerea* also has a second, sexual form named *Botryotinia fuckeliana* that consists of a tiny mushroom-like structure (apothecium) that contains a different spore type (ascospore). However, this phase apparently has not been found in strawberry fields in California or elsewhere (Petrasch et al. 2019).

Under some conditions, *B. cinerea* can produce an overwintering structure, the sclerotium, which is a hard, black, and oblong to spherical structure. Sclerotia can withstand dry, warm, and cold conditions. Under more suitable conditions, sclerotia will germinate to form mycelium that can colonize a host and produce spores. And sometimes can important source of primary inoculum (Whetzel 1945).



Fig(1): *B. cinerea* A. colony morphology on PDA B. Conidiophore C. Conidia D. Apothecia.

2.2 Taxonomy

This fungus belong to phylum Ascomycota, subphylum Pezizomycotina, class Leotiomycetes, order Helotiales, family Sclerotiniaceae. Phylogenetic analysis of 22 species of the genus *Botrytis* revealed that *B. cinerea* forms a small clade with three other species, which all of them are specialized pathogens of dicots (Staats et al., 2005). The current status of *B. cinerea* and its teleomorph *Botryotinia fuckeliana* have two type of conidia macroconidia are multinucleate and the microconidia (male spermatia) are uninucleate (Beever and Weeds, 2007). (Angelini et al., 2016) reported that there are 16 chromosomes in developing Asci. Apothecia of *B. cinerea* are rare in the field, but are more common in other *Botrytis* spp. Most strains are heterothallic, carrying one or other allele of the mating type (Faretra et al., 1988); however, there are also field isolates with dual mating phenotypes (Faretra et al., 1996; van der Vlugt-Bergmans et al., 1993). Sexual crossing in vitro involves incubating sclerotia of the female (sclerotial) parent for long periods at zero degrees (preconditioning) before fertilizing them with a suspension of microconidia from the spermatial parent obtained by irrigation of an ageing culture (Williamson et al., 2007)

2.3 Disease Cycle

Botrytis cinerea can affect leaves, stems, crowns, flowers, flower buds, seeds, seedlings, bulbs, and just about any other part of a plant with the exception of the roots. On most susceptible plants, new infections may begin in the spring as soon as weather conditions are favorable for disease development. Wet or very humid weather may be highly favorable for the spread of the disease. For some *Botrytis* spp., sclerotia develop in dead plant tissue and form the overwintering stage of the fungus. Fungal mycelium may also overwinter in woody stem debris. Sclerotia then germinate in the spring, or mycelium grows out of infected debris and conidia (infectious spores) develop. Conidia may be windborne or rain-splashed to cause new infections on susceptible host tissue (Williamson et al., 2007).

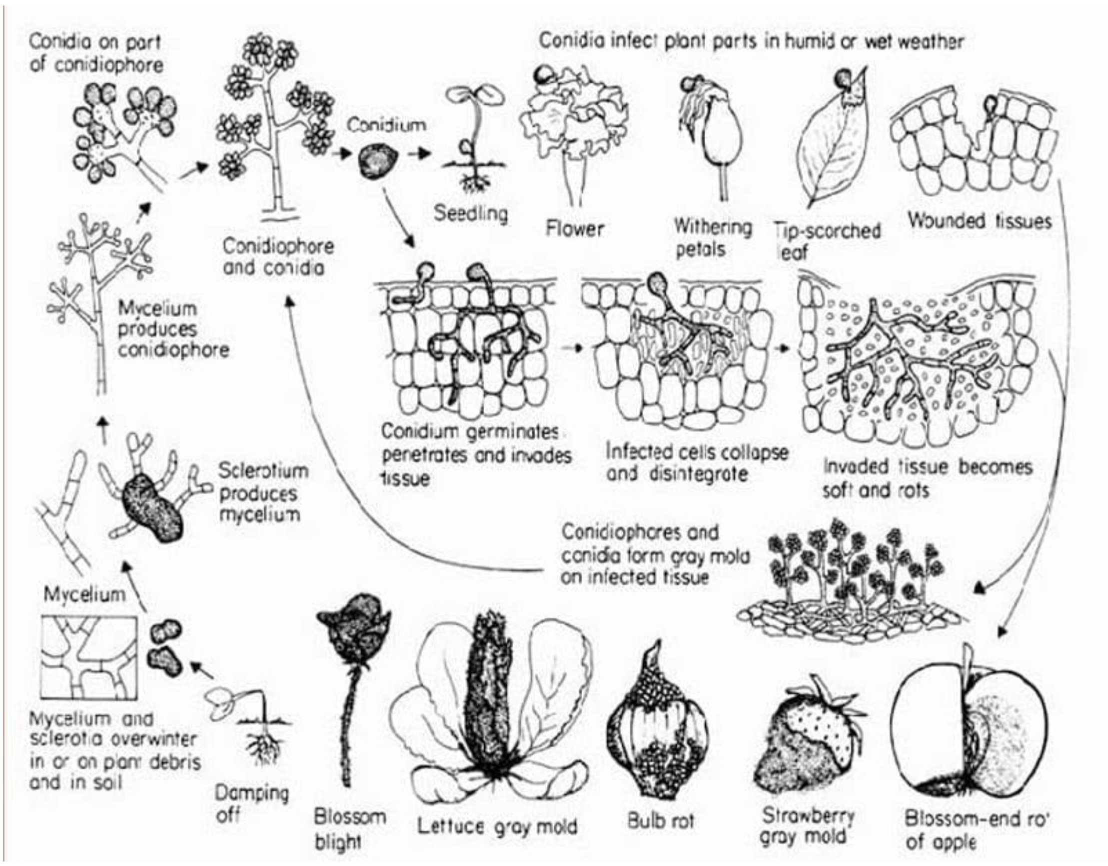


Fig2. Life cycle *B. cinerea.*

2.4 Symptoms

B. cinerea is responsible for a very wide range of symptoms (Fig. 3) and these cannot easily be generalized across plant organs and tissues. Soft rots, accompanied by collapse and water soaking of parenchyma tissues, followed by a rapid appearance of grey masses of conidia are perhaps the most typical symptoms on leaves and soft fruits (Fig.3 a, b). In thick-skinned fruits, such as kiwifruits, the dark water-soaking symptom is evident only after cutting. On many fruits and vegetables the infection commonly begins on attached senescent flowers and then as a soft rot it spreads to affect the adjacent developing fruit (blossom-end rot), as in courgettes (zucchini), cucumbers, French beans, strawberries and apples. On flower petals, symptoms range from minute ‘pock’ marks to full-scale soft rotting (Fig. 3c) depending on the environmental conditions. In greenhouse-grown tomato, the greatest damage occurs on stems at pruning wounds where the fungus can rot through the entire stem. Soft rotting of mature tomato fruits occurs mainly post-harvest; an unusual ‘ghost spot’ symptom in unripe tomato is associated with a successful host defense, but the symptom renders fruits unmarketable.

Fig. 3 Symptoms of infection by Botrytis cinerea. (a) Grey mould of strawberry fruit. (b) Grey mould of raspberry fruit. (c) Rose petals blemished by lesions (right).

2.5 Management

2.5.1 Chemical control

In 35 years since the first commercial use of methyl benzimidazole carbamate (MBC)-generating fungicides, acceptance has grown that for each new chemical the risk of resistance arising in *B. cinerea* is strong if the product is applied repeatedly. Consequently, mixed spray programs have been devised, ideally with each spray chosen from a different fungicide group, to reduce the risk of substantial field resistance arising and to keep below the permitted maximum residue level for each active ingredient. The problem arises, however, when some horticultural crops need protection over extended periods because of sequential flowering and fruiting. The molecular target sites of modern fungicides and the mechanisms of resistance are gradually becoming clear and such studies will be greatly facilitated when the complete *B. cinerea* genome is analysed and annotated. The chemicals used for control of *B. cinerea* have recently been reviewed (Leroux, 2004). Five categories of fungicides are recognized, namely those affecting respiration, microtubule assembly, osmoregulation, sterol biosynthesis inhibitors and those whose toxicity is reversed by amino acids (Rosslenbroich and Stuebler, 2000). Several multisite toxicants affecting fungal respiration have been used against *B. cinerea* over a long period without substantial resistance developing in field populations (e.g. thiram, mancozeb, captan, dichlofluanid, tolylfluanid).

2.5.2 Biological control

Early studies on microbial ecology of the phyllosphere showed that there was considerable potential for use of microbial antagonists for control of Botrytis on crops. At least seven products have now been approved for use on food and non-food plants in greenhouses, under plastic tunnels or in the field in different countries (Elad and Stewart, 2007). Leaf extracts of different plants have significant antimicrobial potential (Shabana et al., 2017) and can be exploited in agriculture for biological diseasemanagement (Galeane et al., 2017). Antifungal activity of volatile components extracted from leaves, stems and flowers of *Lantana camara, Malvaviscus arboreus* and *Hibiscus rosa-sinensis* were tested against*,* *Botrytis cinerea* and some other pathogenic fungi (Boughalleb et al., 2005). They have achieved niche markets in situations where heavy use of conventional fungicides has been restricted because of residues accumulating, or because of the restrictions imposed by importing countries. The original aspirations to deliver a single biological control agent (BCA) infrequently and then rely on its ability to self-disperse in a crop canopy to the required target zones has in most cases turned out to be unrealistically optimistic, but great advances have been made in the understanding of their biological modes of action. BCA formulations may include filamentous fungi such as *Trichoderma harzianum, Clonostachys rosea (Gliocladium roseum) and Ulocladium oudemansii,* the yeast *Candida oleophila*, or bacteria including *Streptomyces griseoviridis, Bacillus subtilis and Pseudomonas syringae. (*Gurjar et al., 2012)

2.5.3Cultural practices

Grey mould is exacerbated by high humidity, reduced light and moderate temperature. Hence it is helpful in crop management to create an open canopy to provide adequate air movement and good light interception so that water droplets from rain or irrigation dry as soon as possible. High RH promotes conidial generation and allows germination and penetration of the host. Cultural practices that alleviate the effects of grey mould are diverse and often specific to particular species and cropping systems. In perennial woody plants, such as grapevines, pruning to reduce excessive vegetative growth of the plant has been shown to be beneficial (Gubler et al., 1987). Excessive use of nitrogen fertilizer encourages rapid vegetative growth and increases the risk of grey mould and other diseases. Some of the problems in soft fruit production caused by rainfall during the blossom period have been overcome by plastic rain shelters and tunnels, and facilitated a massive expansion in crop area for strawberries and raspberries. For example, 90% disease reductions in strawberries grown under plastic have been reported, compared with field-grown plants (Xiao et al., 2001). However, it is still important to encourage ventilation to reduce high RH inside these structures and minimize wetting of foliage. When the plastic covers are removed in late summer there is still infection of leaves and stems, leading to over-wintering mycelium and sclerotia. Spectral modification of daylight by near-UV filters incorporated into plastic covers has been useful to reduce conidiation and infection in a number of crops (Reuveni and Raviv, 1992; Reuveni et al., 1989; West et al., 2000). In unheated greenhouses, the night temperature of plants can be lower than the air temperature due to irradiative cooling; heating briefly before sunrise to raise plant temperature above the ambient air temperature reduces dew formation on leaves and can control grey mould (Dik and Wubben, 2004).

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