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Diseases of Kiwi fruit and Control of Dominant Patogen among Them in Adjara, Georgia

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Abstract

During 2021 and 2022, research was carried out at the Plant Protection Laboratory of Batumi State University and in the Khelvachauri district (Sameba village) of Adjara, Georgia. The purpose of this study was to identify and determine the dominants pathogens of Kiwifruit and determine the efficacies of different concentration i.e. 1000ppm, 2000ppm, 3000ppm, 4000ppm, 5000ppm of 12 different methanolic extracts from different medicinal plant species against main pathogen. Our results show that The bacterium *Erwinia amylovora* affects the fruits during ripening. The fungus *Sclerotinia sclerotiorum* affects immature fruit on the tree. *Botrytis cinerea* affects fruits during storage. *Botryosphaeria dothidea* affects the fruit during ripening. It was clarified that among them the most common and dangerous is the fungus *Botrytis cinerea*. It was found out that revealed that all the concentrations of plant extracts except *Althaea officinalis*, *Betonica officinalis* and *Jasminum officinale* brought about inhibition in the growth of fungi *Botrytis cinerea*. Highly significant inhibition percent of mycelial growth against *Botrytis cinerea* was observed in PDA media amended with the 5000ppm concentration of *Inula racemosa* extract. Among the 12 extracts evaluated, 4 extract showed mycelia inhibition above 82.1-97.4%, 3 showed moderate effect of 68.7 - 79.2 % and 2 showed least 30.6% - 41.3%. The study ascertains the value of plants used in traditional medicine thereby concluding that *Stevia rebaudiana*, *Inula racemosa*, *Rubia coradifolia* and *Panax ginseng* extracts can be emerged as safe alternatives to replace chemical fungicides against pathogens. Based on a general analysis, it is very clearly shown that the investigation is an important step towards developing plant bioprotection strategies for antifungal activity against the important phytopathogen. That can be beneficial for farmers and researchers who involve in agriculture.

Keywords: kiwifruit, fire blight, field rot, ripe rot, storage rot, antifungal activity, inhibition effect

INTRODUCTION

Among subtropical cultures, Kiwi, *Actinidia deliciosa* (Chevalier) Liang et Ferguson (syn.: *A. chinensis* Planchon var *hispida* Liang), is considered one of the main cultures of the subtropical zone of Georgia. From fruits of actinidia prepare compotes, jam, marmalade, they can be eaten fresh. The kiwifruit is healthy and contains many vitamins and minerals. Kiwis are rich in vitamin C, vitamin K, potassium, and fiber. Kiwis have more vitamin C than an equivalent amount of orange (Boeing et al., 2012; Boland, 2013; Beever et al., 1990; Sivakumaran et al., 2017).

The orography of the region and the edaphic - climatic conditions (frequent rain, warm humid temperature, excess of soil moisture, etc.) can forward the wide spread and development of disease-causing agents.

In the first years the harvest kiwi was considered practically painless. In recent years, various diseases have prevented the mass production of kiwi in Georgia. Information on diseases of kiwi is found in various works (Fletcher, 1971; Ford, 1971; Sale, 1980; Pennycook, 1985; Shainidze, 2004; et al.). The main pathogens (*Erwinia amylovora*, *Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Botryosphaeria dothidea*) of kiwi fruits causes considerable damage to the Actinidia crop especially in subtropical plains. It is increasingly becoming a limiting factor for successful cultivation of kiwi in these regions. Several fungicides recommended to control this pathogens not only add up to human and environmental hazards, but also have been found to be inconsistent and insufficient to deal with this disease. However, the application of this agrochemical is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Harris et al., 2001).

In many countries the use of synthetic fungicides has been banned due to polluting nature. In order to have safe methods for plant disease control in sustainable agriculture there is a need for reducing the use of synthetics chemicals fungicides there by replacing it with biocides with plant origin. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. The plants are rich sources of numerous bioactive secondary metabolites such as alkaloid, flavonoids, terpenoids, saponins, tannins and phenolic compounds which are the important sources of microbiocides, antifungal activity and many pharmaceutical compound (Mahesh and Satish, 2008; Arif et al., 2009). Plants also are rich sources important organic compounds, pharmaceuticals and pesticidal compounds. Many reports on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants have reviewed. Thus plant extracts which is a source of natural pesticides can be developed into new biocidal pesticides (Samy and Ignacimuthu, 2000; Palombo and Semple, 2001; Behera and Misra, 2005; Govindarajan et al., 2006).

Therefore, this study was intended to determine the composition of pathogenic microbionts on kiwi in all agroecosystems in Georgia; study of the symptoms, etiology and harmfulness of one bacterial and three fungal kiwi pathogens and application of the biological method (various extracts of high medicinal plants) against the most common fungi *Botrytis cinerea* in Adjara, Georgia.

Materials and Methods

Collection of samples: Study was conducted from March 2021 to December 2022 in Laboratory Plant Protection Laboratory of Batumi State University, and field studies in the Khelvachauri district (village Sameba) of Adjara, Georgia.

Sampling location: The objects of research were various varieties of Kiwi plants (Actinidia), pathogenic bacteria and fungi inhabited on them in agroecosystems of the coastal zone of Georgia. The materials were collected and analyzed (dissemination and development of diseases) by using the well-known methods (Foster et al., 2004). The symptoms such as rot, mummification, wilting, spotting, necrosis, mold, galls, canker, deformation, chlorosis, mosaic, etc., were recorded.

Isolation of fungi: For isolation of fungi from Kiwi plants, the agar method was applied. Fungal pathogens responsible for disease were isolated from fruit. From the samples obtained, serial dilutions (1:10) were prepared in test tubes with 9 ml sterile water, adding 1 g of fruit samples until dilutions 10^{-5} , 10^{-6} and 10^{-7} , of which 50 μ L and shaken at 200 rpm for 3 hours. Samples collected were seeded by duplicate through diffusion technique on Petri dishes of 90 mm diameter containing one of the following artificial Potato Dextrose Agar (PDA) and Czapeck's agar medium. After incubation (23°C for 6 days) the number for each fungi was calculated. All fungi were cultured using a single spore technique on PDA and Czapeck's medium. After incubation, fungi were identified by their macroscopic and microscopic characteristics

Identification of fungi: Fungal cultures identification was based on macroscopic characteristics like colony morphology, color, texture, shape and appearance were observed on potato dextrose agar (PDA) and microscopic characteristics like conidia shape, hyphae color, concentric zone, and pigmentation. Species identification was based on the morphological characteristics of single-spored isolates as described

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(Domsch, 1993; Navi et al., 1999; Watanabe, 2000). Collections of the fungi have been examined by standard light microscopy (Pereval, Carl Zeiss, Jena and Olympus, BX50, Hamburg, Germany). The SEM micrographs have been prepared by means of a JSM-35 (Japan) SEM microscope. The specimens examined are deposited at HAL, KW and TGM (Holmgren et al., 1990).

Plant Material: The present investigation deals with the screening of extracts of selected 12 different plant species (*Althaea officinalis*, *Betonica officinalis*, *Corylopsis sinensis*, *Eucalyptus cinerea*, *Galium aparine*, *Hippophae rhamnoides*, *Inula racemosa*, *Jasminum officinale*, *Panax ginseng*, *Rubia coradifolia*, *Salvia officinalis* and *Stevia rebaudiana*). The leaves were washed thoroughly 2-3 times with running tap water and dried in the shaded area. After then, dried plant material was ground into powder using a blender and sealed in polythene bags for further use.

Preparation of Leaf Extracts: Leaf extract was prepared by Soxhlet extraction method (López-Bascón, 2020). About 20 g of dried powdered leaves and roots material was uniformly packed into a thimble and run in Soxhlet extractor with ethanol or DMSO (dimethyl sulfoxide) for 48 hours. The extract was then filtered with the help of filter paper, and solvent was evaporated from the extract. For aqueous extraction, 20 g of powdered plant material was macerated by a blender with 200 ml of distilled water and the solvent powder mixture was kept at room temperature for 48 hours; the extract was filtered through filter paper. The extracts were kept in the refrigerator at 40°C for further experiments.

Preliminary Phytochemical Screening: The preliminary phytochemical testing of leaf extracts to detect the presence of different secondary metabolites was done. Air-dried and powdered leaf materials were screened for the presence of alkaloids, flavonoids, phenols, terpenoids and steroids, proteins, amino acids, saponin, reducing sugar, and glycoside compounds as described in literature. The ethanolic leaf extracts of selected medicinal plants were tested for preliminary phytochemical screening using basic standard procedures (Harborne, 1998).

Antimycotic Activity: The antimycotic study of leaf extracts of selected medicinal plants was determined by using the disc diffusion method (Kirby, 1956). Filter paper discs of 6 mm diameter were soaked with 1 ml of extracts. Sabouraud's Dextrose Agar (SDA) plates were inoculated with culture by point inoculation. The plates were done in triplicates and were incubated at 28°C. The antimycotic activity was taken on the basis of diameter of zone of inhibition, which was measured after 7 days of incubation, and the mean of three readings is presented.

Control Experiment: The presence of the inhibition zone of the selected fungus was calculated using griseofulvin as standard.

Data Analysis: Data were analyzed following a procedure appropriate to the design of the experiment as described by Gomez and Gomez (1984) and logistic model and, general linear model (GLM) of SAS version 9.2 (SAS, 2009). The treatment means were separated using the least significant difference (LSD) test at 5% probability level. Correlation and regression analysis was used to determine the relationships between growth, yield and yield related traits, and disease severity and AUDPC across the treatments. It was performed to determine the association of disease parameters with yield obtained from the different fungicide schedules.

RESULTS

On the fruits of *Actinidia deliciosa*, 15 species of microbionts, including 3 view bacteria (*Erwinia amylovora*, *Pseudomonas syringae* pv. *Syringae*, *Pseudomonas syringae* pv. *actinidiae*) and 11 fungi (*Phytophthora cactorum*, *Diaporthe actinidiae*, *Botryosphaeria dothidea*, *Alternaria alternate*, *Aspergillus niger*, *Fusarium moniliforme*, *Mucor* sp., *Penicillium citrinum*, *Sclerotium rolfsi*, *Rhizoctonia solani*, *Rhizopus psapzzh*) were identified as a result of phytopathological and mycological studies conducted in different places of agroecosystem in Georgia.

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Sometimes, during storage, the infected fetuses can be covered with a multi-colored mycelium, which includes 8 species of fungi (*Alternaria* sp., *Aspergillus niger*, *Fusarium moniliforme*, *Mucor* sp., *Penicillium citrinum*, *Sclerotium rolfsi*, *Rhizoctonia solani*, *Rhizopus psapzzh*).

The results of studies have shown that among the detected mycobionts on kiwi the most dangerous and harmful species are: *Erwinia amylovora*, *Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Botryosphaeria dothidea*. These pathogenic microbionts species associated with kiwifruits spoilage are of economical and public health significance.

As shown in Fig. 1, the distribution of *Erwinia amylovora* was 19%, *Sclerotinia sclerotiorum* - 22%, *Botryosphaeria dothidea* - 27% and *Botrytis cinerea* - 39%.

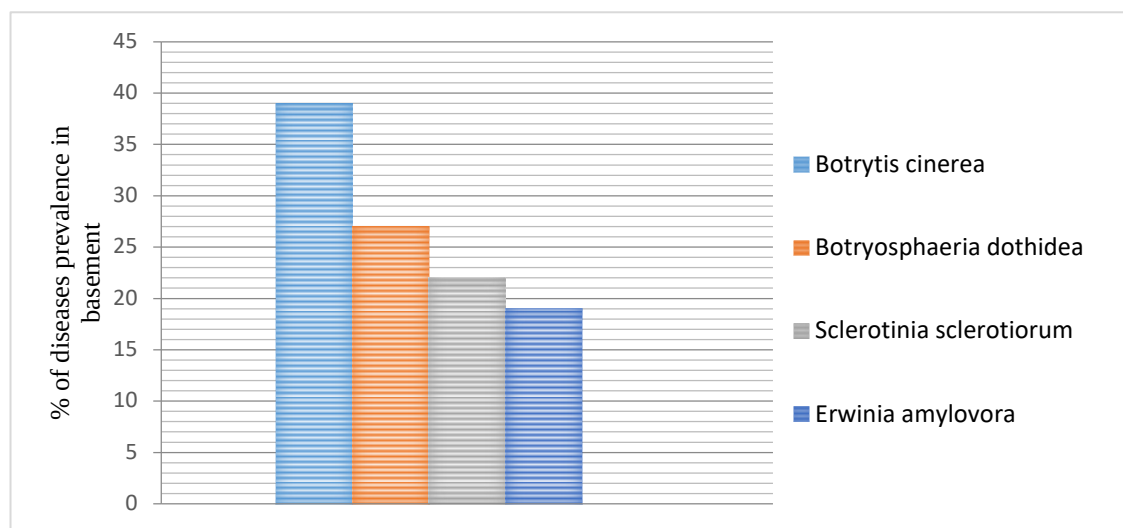


Figure 1. Spreads of the main pathogens of Kivi Fruits in Adjara, Georgia, %

As shown in Fig. 2, *E. amylovora* causes a characteristic watery exudate from infected parts of plants. In fruits, the symptoms may vary depending on when and how pathogen affect. Infected fruits very small, wrinkled and black, remaining attached to the cluster base. When the pathogen progresses from the branches to the fruit, the fruits tend to appear less wrinkled and discolored. Damage to hail or insects often leads to red, brown or black damage after infection. Infected fruits can also exhibit clear or milky silt during high humidity. This sludge changes from red to brown in later stages and can dry out to look shiny or glassy on the surface of the fruit. Our results show that The bacterium *E. amylovora* especially affects the fruits during ripening and disease incidence was approximately 39%. Pathogen can survive on the outside of the plant for weeks at a time prior to infection. At this time, the bacteria population size will increase its population until weather conditions or pollinators allow for widespread dispersal. Cankers can also be a source of primary inoculum during a new growing season. The bacterium is washed into the flower by rainfall and the movement of pollinating insects (most notably bees), gaining entry where it begins to infect the host. This is the beginning of blossom blight, which then travels down the new growth and kills younger stems. Secondary infections can occur through wounds created by mechanical damage, insect feeding, or cultivation. The pathogen grows optimally at 26°C, but can grow anywhere from 4 to 32°C.

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Figure 2. Symptoms of bacterial scorch of kiwi fruit caused by *Erwinia amylovora*

On fig. 3 clearly shows that the damage on the fruit is deeply penetrating, watery, sunken, more or less whitish (depending on the degree of superficial mycelial growth); black sclerotia often develop amongst the mycelium on the surface of the lesions. Lesions rarely occur on clean, exposed fruit surfaces, but are usually centred on sites at which either infected buds or blossom debris (particularly senescing stamens and petals) are in contact with the fruit surface, or adjacent fruits are in contact with each other so that they retain a water droplet between them.

Studies have shown *S. sclerotiorum* overwinters in kiwifruit orchards as Sclerotia in the soil. Some of the Sclerotia will have developed on diseased kiwifruit blossoms and fruits during the previous summer, but probably their major source is saprophytic growth of the fungus on grass and weed debris and other ground litter. After a period of winter dormancy, Sclerotia 'germinate' during spring and summer in response to moisture and rising soil temperatures. In the Kobuleti district, the earliest apothecia have been found in kiwifruit orchards in late September; they become plentiful during late October, November and December. Apothecial production usually terminates in mid summer as the soil dries out or as the supply of overwintered Sclerotia is depleted. However, apothecial production may continue throughout the summer.



Figure 3. Symptoms of field rot of kiwi fruits caused by *Sclerotinia sclerotiorum*

As shown in Fig. 4, Symptoms of ripe rot is the development of shallow, brown dimples, c. 1,8-5,5 mm in diameter, on the surface of the fruits; the skin within each dimple is unbroken, with a thin layer of dry, yellowish flesh beneath. Some, but not all, of these dimples develop into expanding rot lesions as the fruits ripen, whereas, in other instances, identical rot lesions develop without a preliminary dimple having been observed.

The presence of ripe rots reduces the crop's shelf-life directly, by the deterioration of flesh Quality and the development of unpleasant odours and flavours in affected fruits, and indirectly, by the accelerated ripening of adjacent fruits because of ethylene evolution.

In samples of kiwifruits from the Kobuleti district, up to 15% of fruits have been affected with the distinctive, early symptoms of *B. dothidea* ripe rot, while the total incidence of this fungus has been up to 27%.

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Figure 4. Symptoms of dothidea ripe rot of kiwi fruits caused by *Botryosphaeria dothidea*

On fig. 5 shows symptoms caused by *B. cinerea* on kiwi fruits. Subsequent experimental data have indicated that *B. cinerea* often becomes conspicuous in kiwifruit orchards during late blossom and petal fall. On unsprayed orchards, profuse sporulation may be visible on 60 - 70% of blossoms. During wet weather, leaf lesions may develop from infected blossoms. Fruit infection occurs during harvesting, grading, and packing operations, by direct Botrytis contamination of the picking wound that is formed where the fruit is snapped from its pedicel. Harvested fruits that have been graded and packed as healthy and unblemished will often develop rot symptoms while being held in storage at 00C. Symptoms to appear after of 4 weeks of cold storage. The affected area retains the normal shape of the fruit, and feels soft but resilient. The unaffected area remains firm and does not differ from healthy fruits. The rot advances more or less evenly through all the internal tissues of the fruit; after several weeks it may have spread throughout the fruit, but often the distal end remains unaffected.

Initially, there is little or no visible fungal growth on the outside of the rotting fruit. Later, however, an uneven, fluffy, dull white layer of Botrytis mycelium may develop on the skin of the affected portion of the fruit. This white mycelium usually resembles the typical 'white mould'. After prolonged cold storage, the mycelium may assume a grey, fuzzy appearance, because of the growth of tufts of dark conidiophores bearing numerous, powdery, grey conidia; in other instances, the mycelium may aggregate to form small, flat, irregularly shaped, black sclerotia, closely appressed to the fruit surface. External mycelium frequently spreads to adjacent fruits within the tray, ultimately causing secondary infections ('nesting').

When the storage life of the fruits is coming to an end, more variable symptoms develop, associated with a number of fungal pathogens.

Botrytis stem-end rot first became a problem in Georgia 1978, when the overall loss to the industry was estimated as c. 25-100%. Incidence is unpredictable and highly variable, not only from year to year and from orchard to orchard, but even between fruits picked from the same vines on different days.



Figure 5. Symptoms of gray rot of kiwi fruits caused by *Botrytis cinerea*

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As has been clarified, among the discovered mushrooms the most common and dangerous is the mushroom *Botrytis cinerea*. Therefore, the purpose of this study was to evaluate and apply a biological method (various extracts of tall medicinal plants) against the most common and dangerous fungal pathogen *Botrytis cinerea*.

In fig. 6 illustrated the efficacies of different concentration i.e. 1000ppm, 2000ppm, 3000ppm, 4000ppm, 5000ppm of 12 different methanolic extracts from different medicinal plant species against main pathogen. It was found out that revealed that all the concentrations of plant extracts except *Althaea officinalis*, *Betonica officinalis* and *Jasminum officinale* brought about inhibition in the growth of fungi *Botrytis cinerea*. Highly significant inhibition percent of mycelial growth against *Botrytis cinerea* was observed in PDA media amended with the 5000ppm concentration of *Inula racemosa* extract. Among the extracts evaluated, 4 extract showed mycelia inhibition above 82.1-97.4%, 3 showed moderate effect of 68.7 - 79.2 % and 2 showed least 30.6% - 41.3%. The study ascertains the value of plants used in traditional medicine thereby concluding that *Stevia rebaudiana*, *Inula racemosa*, *Rubia coradifolia* and *Panax ginseng* extracts can be emerged as safe alternatives to replace chemical fungicides against pathogens.

Based on a general analysis, it is very clearly shown that the investigation is an important step towards developing plant bioprotection strategies for antifungal activity against the important phytopathogen.

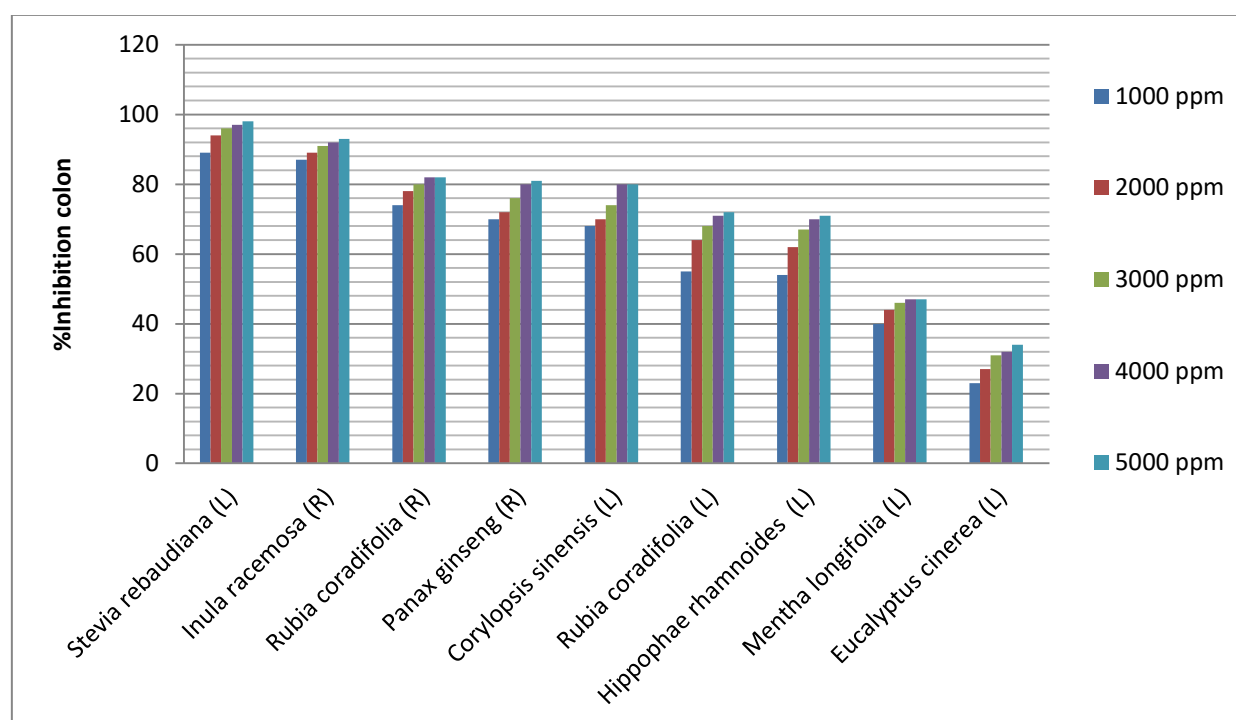


Figure 6. Efficiency of different concentration of herbal extracts of medicinal plants against *Botrytis cinerea*

Note: The value means of three replicates $\pm$ standard error. The values followed by different alphabets differ significantly when subjected to Tukey HSD at 0.5 subset; R: Root; L: Leaf.

DISCUSSION

Long-term observations have shown that various pathogens are not only able to cause the infection but also responsible for the severe deterioration of trees and harvested fruit quality during the cold- or post-storage ripening. Of more significance is a distinctive type of lesion, caused by bacteria *Erwinia amylovora*. Fire blight attacks blossoms, leaves, shoots, branches, roots and especially fruits. Generally, symptoms of fire blight are easy to recognize and distinguishable from other diseases. This sludge changes from red to brown in later stages and can dry out to look shiny or glassy on the surface of the fruit. *B. dothidea* caused ripe rot affects harvested fruits during post-storage ripening. While during cold storage, *Botrytis cinerea* based *Botrytis* storage rot affects harvested fruits. Sclerotinia rot, caused by *Sclerotinia sclerotiorum*, mainly affects immature fruits on the trees.

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On fungal diseases of kiwi fruits mentioned above almost similar data have been obtained by different researchers (Gyoung et al., 2018; Michailides et al., 2000; Pennycook, 1982, 1985; Li et al., 2019). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Samy, 2000; et al).

The purpose of this study was to evaluate and apply a biological method (various extracts of tall medicinal plants) against the most common and dangerous fungal pathogen *B. cinerea*.

Used various extracts of tall medicinal plants against *B. cinerea*. It was found out that revealed that all the concentrations of plant extracts except *Althaea officinalis*, *Betonica officinalis* and *Jasminum officinale* brought about inhibition in the growth of fungi *Botrytis cinerea*. Among the 12 extracts evaluated, 4 extract showed mycelia inhibition above 82.1-97.4%, 3 showed moderate effect of 68.7 - 79.2 % and 2 showed least 30.6% - 41.3%. The study ascertains the value of plants used in traditional medicine thereby concluding that *Stevia rebaudiana*, *Inula racemosa*, *Rubia coradifolia* and *Panax ginseng* extracts can be emerged as safe alternatives to replace chemical fungicides against pathogens.

Conclusion

The study ascertains the value of plants used in traditional medicine thereby concluding that *Stevia rebaudiana*, *Inula racemosa*, *Rubia coradifolia* and *Panax ginseng* extracts can be emerged as safe alternatives to replace chemical fungicides against pathogens.

Based on a general analysis, it is very clearly shown that the investigation is an important step towards developing plant bioprotection strategies for antifungal activity against the important phytopathogen. That can be beneficial for farmers and researchers who involve in agriculture.

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