Essential Oils Effect on Cucumber Powdery Mildew

Subjects: Agronomy

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Essential oils of lemongrass, lemon, thyme, peppermint, abundance blend, purification blend, and thieves blend were tested in vitro and under greenhouse conditions in two separate experiments. The effects of essential oils were tested against powdery mildew disease at concentrations of 1.0–2.5 mL/L, and the consequent impact of the oils on plant growth was evaluated. Powdery mildew fungus, Podosphaera xanthii, was identified using sequencing of the ITS region. The essential oils significantly reduced disease incidence up to 77.3% compared with the positive control ($p < 0.5$).

Keywords: cucumber ; essential oils ; crop management ; disease control ; Podosphaera xanthii ; powdery mildew

1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important and widely distributed vegetable crops due to its rapid growth and early maturity. Cucumber is produced either in protected houses or in open fields [1][2]. The global cultivation of this crop covers 2,231,402 hectares, with production of about 87.805 tons [3]. However, cultivation in the Arab countries is about 67,000 hectares. Among these countries, Saudi Arabia cultivates 11,764 hectares and produces 149,074 tons, with a production rate of 1.27 tones/ha [4]. Cucumber fruit is a good source of minerals and vitamins and delivers Na, Mg, K, Ca, S, Si, F, and Fe. Cucumber contains carbohydrates, protein, thiamin, riboflavin, vitamin C, and niacin. Moreover, the presence of minerals is important to maintain human blood alkalinity. In addition, the vitamins in cucumber fruit facilitate strength and the growth of connecting tissue, as well as relieve joint pain. Cucumber also contains secoisolariciresinol, lignans, lariciresinol, and pinoresinol, which help to reduce the risks of different cancers [5].

In many countries of the Middle East, such as Egypt, open fields are the common cultivation areas for cucumbers [6]. However, in other countries suffering from water shortages, such as Gulf countries, greenhouses are the main production sources of cucumber [7]. In Saudi Arabia, the total greenhouse area for cucumber production in 2013 was 2605 hectares, which produced 236,087 tons [8].

This crop is affected by several destructive diseases that reduce its productivity and increase production costs. Among these diseases, powdery mildew, caused by the fungus *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (Syn. *Sphaerotheca fuliginea*), represents a challenge for both growers and researchers [9][10]. This pathogen infects the shoot system and causes a severe reduction in crop productivity [11][12]. The conidia of *P. xanthii* are typically airborne, take approximately 24 h to germinate, and invade plant tissue [13]. Disease development usually takes 7 days when relative humidity exceeds 75% [14].

This disease is controlled by synthetic fungicides, but these compounds can lead to pathogen resistance towards the relevant chemical agents [15]. Moreover, global public awareness has increased regarding the contamination of the environment and food sources with chemicals and pesticides [16], which has motivated researchers and producers to seek a safe alternative to manage this vital crop.

The management of diseases using resistant cultivars has been very successful in many cases; however, due to pathogen adaptation, the degree of protection achieved with resistant cultivars varies and is not always sufficient as a single-management practice. Some reports noted that resistant cultivars can produce less than susceptible cultivars [17]. Thus, the use of resistant cultivars alone may not be a suitable method to manage powdery mildew and achieve good crop yields. The successful control of powdery mildew would require an integrated pest management (IPM) that includes the application of good cultural practices, as well as the selection of appropriate resistant varieties and fungicides. Good cultural practices should focus on shortening the period of leaf wetness through, e.g., early planting, good circulation of air, expanding planting space, and avoiding overhead irrigation [18][19]. Currently, there is a global preference for vegetable production using organic farming, which relies on safe biopesticides and biofertilizers. However, the application of natural biopesticides instead of chemicals in the control of diseases still represents a major challenge for researchers [20].

Biopesticides play a significant role as alternatives for chemical pesticides and as a part of the IPM in controlling the powdery mildews of cucurbits [17], but some of these biopesticides may have negative effects on saprophytic microorganisms [21]. Biofungicides are natural products derived from microorganisms, plants, animals, or minerals that can suppress fungal organisms directly or by stimulating the defense responses of plants. Biofungicides generally have a narrow spectrum, with low toxicity to the non-target organisms, quick decomposition, and a low negative impact on the environment. Biofungicides based on bioagents, such *Bacillus subtilis*, potassium bicarbonate, phosphorous acid, Tricon, and oils, were approved to control powdery mildew in some crops [17][22]. Currently, several biofungicides have been registered and approved for controlling the powdery mildew of cucumber in many countries [17][23]. In the Middle East, however, there is a lack of information on the status of biopesticide availability and application. While organic farmers and growers do their best to avoid chemical pesticides through the application of good cultivation practices and organic fertilizers, the normal cultivation of cucumber mainly relies on chemical fungicides to control powdery mildew.

As low-risk substances, natural products of plant origin are an important group of candidates for inclusion in IPM [24]. Despite the publication of reports attesting to the antimicrobial activities of plant extracts and essential oils, relatively few natural products are used in crop protection [25]. Natural compounds have lower perseverance and toxicity than synthetic pesticides, which reduces their potential negative impacts on the environment [18]. Essential oils (EOs) are natural compounds used for many purposes due to their antimicrobial properties [26][27][28]. Essential oils present a wide spectrum of activities against diverse plant diseases [29] and have been successfully applied in the suppression of many plant pathogens causing foliar diseases, including powdery mildews [18][29][30] and leaf spots [31]. The application of essential oils as an alternative to chemical pesticides in the control of plant diseases is associated with potential advantages because these oils are relatively non-toxic to mammals and fish. Essential oils are usually rich in various compounds, including 20 to 60 active substances [32]. Furthermore, microbial resistance to essential oils develops more slowly than resistance to other compounds due to the complex mixtures of constituents that characterize certain oils [33]. Increasingly more EOs have shown interesting activities from an agricultural perspective against a broad spectrum of microorganisms in vitro and in situ [32]. Although many studies have reported the efficacy of EOs at a lab scale, plant–pathogen–EO interactions remain understudied, leading to a lack of field knowledge [32][34]. Based on the scarcity of works from field trials on the use of EOs for controlling plant diseases, the present study offers valuable information about the efficiency of EOs in suppressing powdery mildew on a large scale.

2. Essential Oils Effect on Cucumber Powdery Mildew

The results of the morphological and molecular characterization of cucumber powdery mildew fungus (strain KKUY-6001) confirmed the identity of this fungus as *Podosphaera xanthii*. This fungus was previously described by other investigators as an obligate biotrophic pathogen that colonizes the shoot systems of many cucurbits, negatively affects host growth and physiology, and is associated with severe harvest losses [18][25].

The in vitro experiments undertaken in this study demonstrated the effectiveness of essential oils, individually or in a mixture, in suppressing *P. xanthii*. An oil concentration of 2.0 mL/L caused clear deformation in the mycelia, conidiophore, and conidia, and a concentration of 2.5 mL/L resulted in plasmolysis and distortion of the hyphae and conidia of the pathogen, resulting in complete death of the pathogen. Tripathi et al. [35] suggested that the fungitoxicity of the essential oils may be due to their effective ingredients, including carvacrol and thymol, which have been shown to exert a suppressive effect against fungal species. While the specific mechanisms of action of these compounds against fungi have not been fully explored, the potential capabilities of these compounds to soften or disrupt the reliability of cell membranes and cell walls were previously suggested [36]. Our findings are supported by those of Ahmed [1], who found that clove, nigella, olive, and rocket oils significantly reduced the conidial germination of *P. xanthii.* Similarly, Raj and Shukia [37] found that clocimum and lemongrass oils caused 100% inhibition of the conidial germination of powdery mildew affecting opium poppy. Nada [38] reported that applying volatile oils as a film on slides completely prevented the spore germination of *P. xanthii*.

Research study's results showed that the application of essential oils, individually or in a mixture, significantly reduced the disease incidence and disease severity of powdery mildew in cucumber. The most effective treatment was the oil mixture, followed by lemongrass oil and lemon oil. The results of our study are consistent with those of Abd El-Sayed [39], who found that the foliar application of some plant extracts (thyme, henna, eucalyptus, and garlic), individually or in a mixture, decreased the powdery mildew intensity on cucumber compared to the control. Further, Ko, Wang, Hsieh, and Ann [40] found that canola oil, corn oil, grape seed oil, peanut oil, safflower oil, soya bean oil, and sunflower oil (0.1%) greatly reduced the severity of tomato powdery mildew caused by *Oidium neolycopersici*. Sturchio, Donnarumma, Annesi, Milano, Casorri, Masciarelli, Zanellato, Meconi, and Boccia [18] reported that treating zucchini plants with combined clove and rosemary oil could reduce the incidence of powdery mildew under controlled conditions. Additionally, there are several reports on the efficiency of applying plant extracts and essential oils to control plant diseases, including powdery mildew, throughout the world [9][17][41][42]. Considering these previous reports alongside the present study, essential oils represent an important group of candidates for inclusion in IPM because essential oils are considered low-risk substances [43]. Essential oils are composite mixtures of compounds and include terpenes, such as terpinene, terpenoids, monocyclic alcohols, bicyclic alcohol, acyclic monoterpene alcohols, aromatic phenols, aliphatic aldehydes, acids, monocyclic ketones, cinnamic acid, esters, and bicyclic monoterpenic ketones [29]. These compounds may work together to suppress plant pathogenic fungi in vitro [44].

The results of the study showed a significant increase in the biomass of the essential-oil-treated plants, including plant length, leaf area, and fresh and dry weight, compared to the positive control. The increase in the plant biomass after oil treatment could confirm the efficacy of the essential oils in suppressing powdery mildew pathogens and decreasing disease severity. Consequently, the cucumber plants became healthy and free from infection, enabling the plants to undergo their normal physiological processes and achieve their normal growth parameters. In addition, the normal growth of the oil-treated plants is a good indicator of the very low phytotoxicity of essential oils when used in appropriate doses (in this case, 2.5 mL/L). Moreover, the observed increase in the number of flowers/plant after essential oil application is a positive indicator of an increase in the expected yield. It is reasonable to attribute this increase in the overall growth of the cucumber plants to: (1) the suppression of fungal pathogens; (2) the direct exposure of plants to growth promoters, such as phenols, which are present as fractions in essential oils; and (3) the induction of plant resistance against pathogens. Our hypothesis agrees with that of Ahmed [1], who reported that, under protected houses, the application of plant oils (e.g., clove, olive, and nigella) was followed by an increase in the number and weight of fruits per plant, as well as a noticeable increase in plant vigor

Research observed that the plants infected with powdery mildew (positive control) showed low chlorophyll, carbohydrate, and protein contents compared to plants treated with essential oils. The obtained results are similar to those reported by Cheah et al. [45], who found that infection with *P. xanthii* reduced the photosynthetic area of cucurbit leaves. In the present study, there was a significant increase in the content of Chl. a and Chl. b due to the application of the essential oils, compared to the positive control. This result could indicate that the application of essential oils kept the cucumber plants free from infection while the leaves remained healthy and contained normal photosynthetic machines that induced normal metabolic processes. Consequently, the yields of metabolic materials, such as carbohydrates and proteins, in the oil-treated plants were higher than those in the positive control. The present findings are in partial agreement with those of Herger et al. [46], who noted that the application of an aqueous extract solution of *Reynoutria sachalinensis* (Milsana) leaf suppressed *Erysiphe cichoracearum* and *P. xanthii* infection and increased photosynthetic pigments in cucumber leaves. These authors suggested that the observed reduction in disease intensity may have been due to an increase in the chlorophyll content, which was reduced as a result of powdery mildew infection. Additionally, the reduction in disease incidence in cucumber plants treated with essential oils could be due to alterations in the physiology and biochemistry of plants, increases in enzyme activity, and increases in barrier defense through greater cell-wall lignification.

The present results revealed that essential oils protect cell membranes against the destruction that can be caused by fungal infection. To the best of our knowledge, there are currently no reports on the effects of either powdery mildew infection or treatment with essential oils on cell membrane stability. However, the obtained results could be supported by the findings of Hashem and Hamada [47], who noted that some biologically active materials reduced the infection of wheat roots and induced the membrane stability of plant leaves compared to the control.

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