



Scientific Journal of Faculty of Science, Menoufia University

<https://sjsfsmu.journals.ekb.eg/>



Effect of some essential oils, some plant extracts and white vinegar against some plant pathogenic fungi

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ARTICLE INFO.

ABSTRACT

Received: 04/04/2024

Accepted: 13/05/2024

Phytopathogenic fungi have caused significant economic losses, which is why it is important to develop and implement preventative treatments that are environmentally friendly. This study aimed to evaluate the antifungal activity of white vinegar, essential oils (clove, garlic and rosemary) and some plant extracts of garlic, onion, clove, chili and rosemary against three fungal species (*Aspergillus* spp., *Alternaria* spp. and *Fusarium* spp.), which were isolated from different parts of plants. Furthermore, the chemical components of vinegar, clove and garlic oils were identified using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) technique. Plants infected with fungi were biologically treated with a mixture of plant extracts of garlic, clove and rosemary by the spraying on the leaves. The essential oils and white vinegar showed greater efficacy than the plant extracts, where white vinegar and clove oil were the most potent. Plants infected that were treated with a mixture of plant extracts showed the disappearance of the fungal infection and improvement in the growth of these plants. Through this research, white vinegar, essential oils and plant extracts used have antifungal activity and can be used as an alternative to synthetic fungicide which are harmful to humans, plants and animals. Traditional cleansing compounds such as vinegar performed better against fungi used. It is also emphasized to use White vinegar to get rid of fungi and microbes found in vegetables and fruits by soaking and washing them. All treatments used against fungi are considered economical in price and environmentally friendly.

Keywords: *Essential oils, vinegar, plant extracts, ATR-FTIR, clove oil, garlic oil, antifungal activity.*

1. Introduction

Under the threat of climate change and pathogen spread, improving crop productivity and avoiding the use of chemical pesticides is a major concern for the agricultural industry (Ramakrishna *et al.*, 2019). Fungal diseases are among the most hazardous biological stresses, causing severe damage to agricultural crops in many countries (Tsitsigiannis *et*

al., 2012) by producing toxic metabolites known as mycotoxins, which are secondary metabolites of fungi (Set and Erkmen, 2010).

Mycotoxins are a diverse group of substances that include aflatoxins, ochratoxin A, patulin, and *Alternaria* toxins, all of which are produced by different mycotoxigenic species (Fernández-Cruz *et al.*, 2010). *Aspergillus flavus* and *Aspergillus parasiticus* produce aflatoxins, secondary toxic fungal metabolites. There are four naturally occurring

aflatoxins, the most toxic of which is aflatoxin B1 (AFB1). The most important mycotoxins are produced by moulds of the *Aspergillus*, *Penicillium*, and *Fusarium* genus, which have a negative impact on crops, particularly vegetables.

Vinegar is a natural product of a two-staged fermentation process (Solieri and Giudici, 2009). Firstly, fermentable sugars are converted to ethanol by the yeasts in anaerobic conditions. Subsequently, ethanol is transformed into acetic acid by bacteria of the *Acetobacter* genus during the oxidation process, also known as acetic acid bacteria (AAB) in aerobic conditions (Li et al., 2015). The most common substrates used for vinegar production are products with a high sugar content (Hutchinson et al., 2019), but they can also be produced from alcohols, including wine (Tesfaye et al., 2002). Acetic acid is the main product of the fermentation process, but small amounts of tartaric acid and citric acid are also present (Hailu et al., 2012).

Plant extracts have led to interest in the fungicide industry as potential agents for controlling plant pathogens in the field. This is primarily due to their antimicrobial properties, which are derived from a diverse range of secondary metabolites such as phenols, polyphenols, alkaloids, flavonoids, glycosides, tannins, and other compounds. Numerous studies have shown that plant extracts are effective against a wide range of pathogenic fungi, including *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Alternaria* spp.

Essential oils (EOs) are important because they protect plants from bacteria, viruses, fungi, insecticides and herbivores by decreasing their appetite for these plants (Arasu et al., 2019). Eos are secondary metabolites that are produced by vascular plants, primarily by various species of the labiate families *Lamiaceae*, *Apiaceae*, and *Asteraceae*, as well as by other families like *Myrtaceae*, *Rutaceae*, and *Lauraceae* (Campolo et al., 2018). EOs, also known as volatile oils, are complex mixtures of volatile constituents biosynthesized by plants, primarily terpenes, terpenoids, aromatic and aliphatic constituents with low molecular weights (Nikmaram et al., 2018; Wińska et al., 2019). EOs can have more than 60 components. The antimicrobial activity of essential oils is due to phenolic compounds (Plavsic et al., 2017). The effect of EOs on moulds can be observed in both the macromorphological and cellular levels. Some macromorphological changes include a shortage of sporulation or pigmentation, a change in the number of conidia, increased branching of hyphae, or a change in their size. It has been proposed that some of the aforementioned changes are caused by oil

activities on enzymatic reactions of cell wall synthesis, which affect mould growth and morphogenesis and also cause the pulling back of the cytoplasm in hyphae, resulting in mycelium death (Carmo et al., 2008). EOs can inhibit the synthesis of DNA, RNA, polysaccharides and proteins in fungi and bacterial cells causing changes similar to the mechanism of antibiotic activity (Leja et al., 2019a; Leja et al., 2019b).

Fourier Transform Infrared (FTIR) is a widely used method for identifying chemical constituents and elucidating compound structures (Topalá and Tătaru, 2016), and it is required to identify medicines in many countries' pharmacopoeia. In recent years, FTIR has played an important role in pharmaceutical analysis due to its fingerprint characteristics and wide range of applications (Baseri and Baker, 2011; Cozzolino, 2015). These IR vibrational spectroscopic techniques provide molecular-level information, allowing for the investigation of functional groups, molecular conformations, quantitative analysis, and structural studies with information on intra and intermolecular bonding types. Transmission mode, attenuated total reflectance (ATR), diffusive reflectance, and microspectroscopy are the most commonly used infrared spectroscopy techniques.

The advantages of the Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) technique include a small amount of material required to experiment and can be repeated many times very quickly to verify results, a short time to obtain spectra, high sensitivity, and in the case of tissue it is not necessary to do any preparatory operation of samples (Topalá, 2013; Topalá and Ducu, 2014). The use of an attenuated total reflectance (ATR) device enabled rapid FTIR measurements of liquids such as oils and plant extracts, allowing for the identification and quantification of valuable plant biomarkers (Schultz and Baranska, 2007).

The aim of this study is to find out some environmentally friendly ways to combat some fungal diseases that affect some plants, vegetables and fruits. Essential oils, vinegar and plant extracts were used for this purpose.

2. Material and methods

Plant samples

The plant samples used were collected from different parts of plants such as orange, mango, guava and rose leaves, onion, tomato, cucumber, and fruits of orange and banana from the village of Al-Hamoul and Shibin Al-Kom, Menoufia Governorate. These

plants are infected with fungal diseases as shown in the figure 1.



Figure 1: Some isolated plants that infected with fungal

Extract preparation:

In this experiment, vinegar, essential oils, and plant extracts were used. We used white vinegar available at home at a concentration of 5%. Aromatic oils were used as raw materials and were obtained from a herbal shop in Shebin Al-Koum, Menoufia Governorate. These oils are garlic, clove and rosemary oil. Some plant aqueous extracts were used from some effective plants found in every home, such as garlic, onion, clove, rosemary, and chili extract.

Isolation and purification

The fungal strains used as test organisms were isolated from different samples of plants as explained above. Potato dextrose agar (PDA) medium was used to isolate fungi using the streak plate technique. Plates were incubated for 5-7 days at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ (Almalki *et al.*, 2020). After isolation, the isolates were purified on PDA media.

Antifungal activity

The antifungal activity was evaluated by using agar well diffusion method (Perez, 1990; Ahmad and Beg, 2001). This technique avoids volatilization of active plant extract compounds. Potato dextrose agar (PDA) was used for fungal cultures. Wells or cups of 8 mm size were made with sterile borer into agar plates containing the fungal inoculum. Each well was inoculated with 100 μl of each compound and then, left at room temperature for 10 minutes to allow the diffusion of these substances into the agar wells (Rios *et al.*, 1988). After incubation for 24-72 hr at 37°C , the plates were observed. If the fungal activity was

present on the plates, it was indicated by an inhibition zone surrounding the well containing the extract. The zone of inhibition was measured and expressed in millimeters. The antifungal activity results were expressed in term of the diameter of the zone of inhibition (Alves *et al.*, 2000). The mean and standard deviation of the diameter of inhibition zones were calculated.

Chemical study using ATR-FTIR technique

The most effective substances with antifungal activity were chemically studied using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) technique for garlic and clove oil and vinegar and the shape of the device (BRUKER ALPHA II model) shown in fig 2. This analysis was performed in the central laboratory of the Faculty of Science, Menoufia University.



Figure 2: Shape ATR-FTIR device used

Biological application

Leaves of Plants infected with fungi which isolated from them were biologically treated with a mixture of plant extracts of garlic, clove and rosemary as shown above by the spraying on the leaves.

Statistical analysis

For calculating the average values and standard deviation of the obtained data, Microsoft Excel 365 was used. The reported values are the mean \pm SD values from the tests repeated three times. All of the statistical methods which were used in this study were according to Bishop (1983), while the

analysis was carried out by Info Stat statistical package.

3. Results and discussion

In this study, fifteen fungal isolates were obtained from the leaves of various trees, vegetables and fruits from the village of Al-Hamoul and Shibin Al-Kom, Menoufia Governorate, they were identified through culture and distinctive morphological features. Based on morphological characteristics, the fungi have been primarily identified as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium*, and *Alternaria*. Petri dishes that show isolation of these fungi in the fig 3.

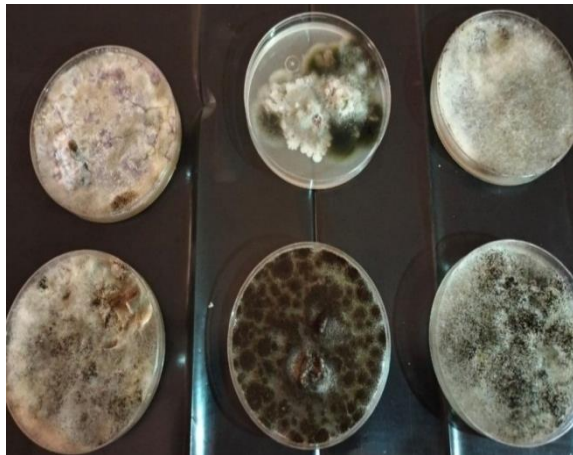


Figure 3: Petri dishes that show the isolation of some fungi such as *Aspergillus*, *Fusarium*, and *Alternaria*

Antifungal activity results by natural substances (essential oils, plant extracts and white vinegar) are represented in the table 1 and fig 4. The activity of fungi is determined by the diameter of inhibition zone and are represented in fig 5. Results show the best essential oils used are clove oil then garlic oil and rosemary oil while the best plant extract is garlic extract. White vinegar affects all isolated used fungi so it has a broad spectrum of pathogenic fungi such as *Aspergillus*, *Fusarium*, and *Alternaria*.

These essential oils are thought to play a role in plant defense mechanisms against phytopathogenic microorganisms (Mihaliak *et al.*, 1991). Clove oil showed complete inhibition against all tested fungi except *Alternaria spp.* so it has antifungal activity. The efficacy of clove oil as an antifungal agent was reported by Soliman and Badea (2002); Velluti *et al.* (2003); Matan *et al.* (2006). Eugenol is the main component of clove oil. The antimicrobial activity of this oil can be attributed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with –SH groups in the active sites of target enzymes, resulting

in the deactivation of enzymes in fungi (Cowan, 1999; Velluti *et al.*, 2003).

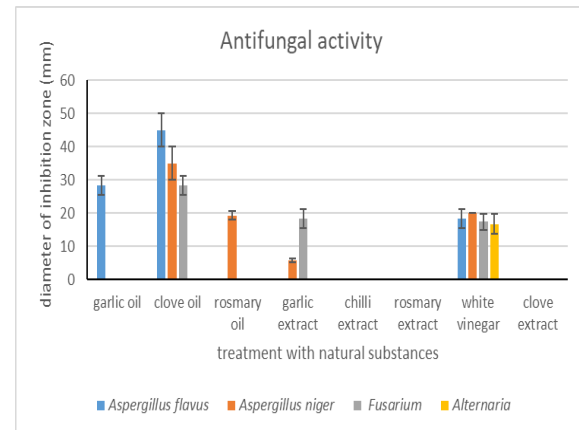


Figure 4: Antifungal activity of natural substances on some pathogenic fungi isolated from different sources of plants

The results also show essential oils such as clove, garlic and rosemary oils and plant extracts such as garlic extract have antifungal activity. Several studies have shown that essential oils have antimicrobial, antiparasitic, antioxidant, and insecticidal activities (Yu *et al.*, 2017). Furthermore, essential oils have been studied as inhibitors of mould growth and aflatoxins production (Zabka *et al.*, 2009), which can be used to control crop diseases caused by soil-borne fungi. Essential oils such as clove (*Syzygium aromaticum*) have been shown to inhibit the growth of phytopathogenic fungi such as *Fusarium*, *Aspergillus*, and *Alternaria* (Ibáñez *et al.*, 2019; Sempere-Ferre *et al.*, 2021).

Plant extracts and essential oils were found to inhibit pathogenic fungi through three modes of action: increasing fungal mortality (fungicidal effect), inhibiting fungal growth and development (fungistatic effect), and/or improving plant growth by inducing defense responses of infected plants (Draz *et al.*, 2019). Although the mechanisms that made essential oils effective as an antimicrobial agent are not fully known, there are several proposed possible mechanisms. Researches revealed that accumulation of the essential oils in the cell, effect of cell permeability, disruption of major organelle membranes, alteration of the general morphology (Bajpai *et al.*, 2013; Hua *et al.*, 2017), which causes leakage and death of the cell of the organism are the mechanism of action by the EOs.

Table 1: Antifungal activity of natural substances on some pathogenic fungi isolated from different sources of plants

treatment		diameter of inhibition zone (mm)			
		<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Fusarium</i>	<i>Alternaria</i>
1	Garlic oil	28.0±2.9 ^c	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
2	Clove oil	45.0±5.0 ^d	35.0±5.0 ^d	28.3±2.9 ^c	0.0±0.0 ^a
3	Rosemary oil	0.0±0.0 ^a	19.3±1.2 ^c	0.0±0.0 ^a	0.0±0.0 ^a
4	Garlic extract	0.0±0.0 ^a	5.7±0.6 ^b	18.3±2.9 ^b	0.0±0.0 ^a
5	Chili extract	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
6	Rosemary extract	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
7	White vinegar	18.3±2.9 ^b	20.0±0.0 ^c	17.3±2.5 ^b	16.7±2.9 ^b
8	Clove extract	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
9	Positive control	84.7±0.6 ^c	84.7±0.6 ^c	84.7±0.6 ^d	84.7±0.6 ^c

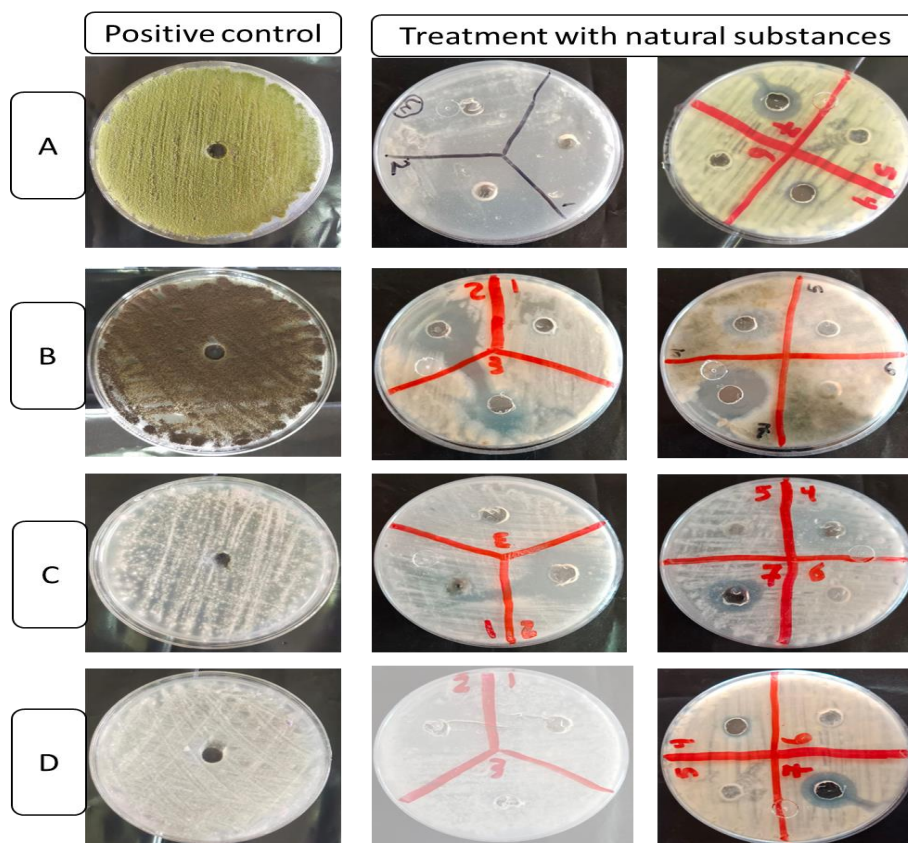


Figure 5: Antifungal activity of natural substances against (A) *Aspergillus flavus* (B) *Aspergillus niger* (C) *Fusarium spp.* (D) *Alternaria spp.*

According to Lagrouh *et al.* (2017), plant extracts and essential oils can affect pathogenic fungi through six mechanisms of action: inhibition of electron transport in mitochondria, inhibition of cell division, interference with nucleic acids synthesis and/or inhibition of protein synthesis, and inhibition of efflux pumps. The host plant activates defense processes involving the production of enzymatic and nonenzymatic antioxidants, including the production of soluble sugars, phenols, flavonoids, and hormones (Tarkowski *et al.*, 2019). Phenols and flavonoids protect diverse components of the cell from damage and play an important role in plant growth and development by altering cellular processes (De Pinto and De Gara, 2004). Another mechanism to prevent pathogen infection is the production of enzymatic antioxidants and scavenging of reactive oxygen species (ROS) (Walter *et al.*, 2009). The accumulation of cell-damaging ROS is abrogated by activation of enzymatic antioxidants as well as non-enzymatic antioxidants (Barna *et al.*, 2012).

The antifungal efficacy of Eos also depends on the presence of different active constituents such as monoterpenes, sesquiterpenes, phenols, aldehyde, and ketones, which are interacted to show synergistic, additive, and complementary effects (Soković *et al.*, 2010). Terpenoids, alcohols, and phenolic terpenes in an oxygenated form precisely increase the antifungal activity of Eos (Bassolé and Juliani, 2012).

Results also show all isolated fungi such as *Aspergillus*, *Fusarium*, and *Alternaria* inhibited with vinegar so it has antifungal activity. These results agree with Zhang *et al.* (2020) that improve Vinegars have strong antimicrobial properties.

ATR-FTIR spectrum of garlic oil is shown in fig 6. The peaks at 3879.91 and 3717.47 cm^{-1} were assigned to -OH groups of amide. Water -OH stretch, alcohol -OH stretch or carboxylic acid -OH stretch were detected at 3549.82 cm^{-1} and that for -C-H stretch bending was observed at 2921.91. The absorption peaks at 2856.55 cm^{-1} was associated with the presence of -C-H aldehydic or -C-H stretch. The absorption peaks at 1743.69 cm^{-1} was associated with the presence of ester or ketone. The peak at 1457.60 cm^{-1} belongs to CH_2 bend or C=C aromatic while C-F appears at 1159.17 cm^{-1} and C-Br at 719.57 cm^{-1} .

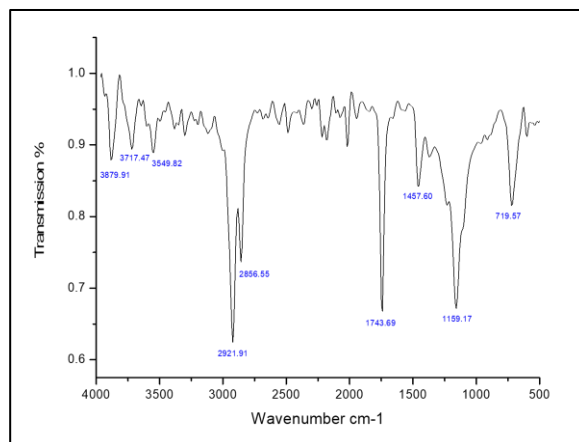


Figure 6: ATR-FTIR spectrum of garlic oil.

ATR-FTIR spectrum of clove oil is shown in fig 7. The absorption peaks at 3286.02 cm^{-1} was associated with the presence of carboxylic acid -OH stretch. The peak at 2933.12 cm^{-1} were assigned to carboxylic acid -OH stretch or -C-H stretch while 2871.63 cm^{-1} vibration corresponded to -C-H aldehydic or -C-H stretch. C=C aromatic was detected at 1409.14 cm^{-1} . The peak at 1035.93 cm^{-1} belongs to C-F while C-Cl appears at 874.40 cm^{-1} and C-Br at 570.58 cm^{-1} .

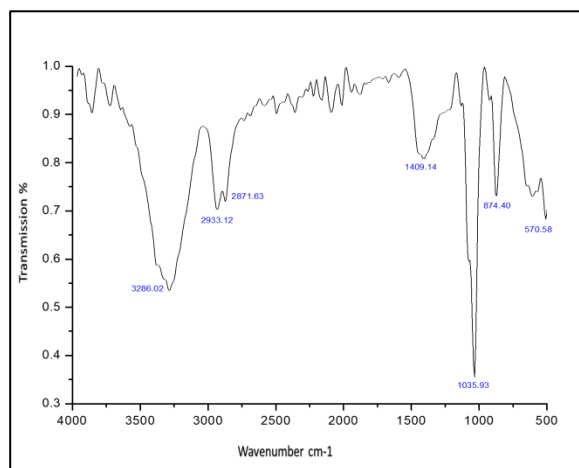


Figure 7: ATR-FTIR spectrum of clove oil

ATR-FTIR spectrum of vinegar is shown in fig 8. Water -OH stretch, alcohol -OH stretch or carboxylic acid -OH stretch were detected at 3301.38, 3259.92 and 3211.75 cm^{-1} . The absorption peaks at 1639.60 cm^{-1} was associated with the presence of C=O amide or C=C alkene. The peaks at 606.50 and 520.27 cm^{-1} were assigned to a halogen compound (C-Br).

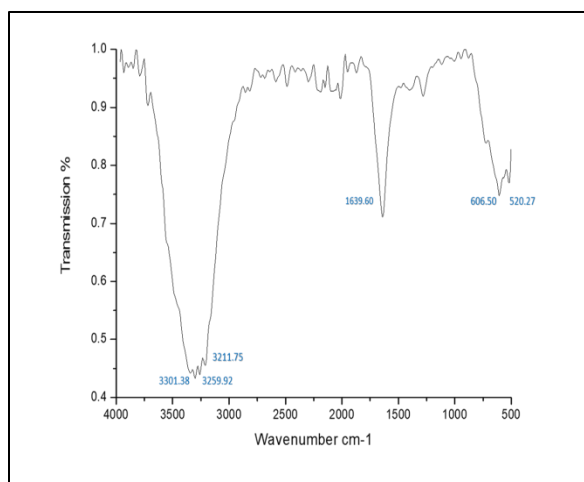


Figure 8: ATR-FTIR spectrum of vinegar

Results that indicate the presence of the O-H bend of carboxylic acids or any OH group (Rastogi and Arunachalam, 2011) revealed the presence of phenols, flavonoids, tannins, saponins, and glycosides. These compounds act as antioxidants to protect the plants from harmful free radical accumulation, so clove oil, vinegar, and garlic acid have an important role in antifungal activity.

Biological application through the spray of leaves infected with fungi with a mix of garlic, clove and rosemary plant extracts. For results, these plant extracts have a strong antifungal effect and lead to the growth and improvement of these plants and is represented in fig 9. This mixture reduces the chance of plants being infected with insects that may cause fungal infection. The reason may be because this mixture contains many active substances that act as antifungal and prevent accumulation of ROS that are harmful to plants. Also, the pungent garlic odor is caused by the release of sulfur, which causes the escape of some insects that cause fungal infections.

4. Conclusion

Essential oils, plant extracts and vinegar are natural substances that have a long history of use. They have a promising action of antimicrobial and insecticidal effect. So, they are used to test their activity by many researchers to see their potential for controlling fungal plant diseases. All the mentioned experiments in this review showed the high capability of these natural substances used to act as antifungal agents. Their environmentally friendly characteristics make them interested by the researchers those exploring products that have desirable effects on the target organisms with no or less negative impact on the environment.



Figure 9: Biological application of a combination of garlic, clove and rosemary plant extracts against infected fungi in plants

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