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Original Research Article

Herbicidal and Anti Pathogenic Potential of Thymol

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Abstract: In recent years, the indiscriminate use of synthetic herbicides involves high resistance of the weeds to these chemicals in addition to the harmful environmental effects leading to the emergence of more resistant varieties as one of the their most important consequences. Therefore, the current global effort is in progress to find natural compounds to control weeds or plant pathogenes. Many secondary metabolites have antifungal, anti-parasitic, anti-bacterial and anti-viral properties. Thymol is a natural volatile compound from monoterpenes group which can be found in the essential oils of Lamiaceae family. In this study, the allelopathic effect of thymol on the germination and seedling growth rate of some weed species (*Amaranthus retrflexus*, *Portulaca oleracea* L., *Rumex acetosa*) as well as its impact on the control of some fungi species (*Asperjillus flavus, Asperjillus fumigats, fusarium graminearum, Sclerotinia sclerotiorum*) and its inhibitory effect on two plant pathogen bacterial species (*Erwinia carotovora and Xanthomonas compestris*) were studied. Results showed that the thymol could effectively reduce weed germination rate and early growth. Also, increasing thymol concentration leads to fungal growth reduction and increased inhibitory effect of this substance on the plant pathogen bacteria.
Keywords: thymol, allelopathic, anti-fungal, anti-bacterial, weed.

INTRODUCTION

Improvement of the crop yield greatly depends on the effective management of competing weeds. Weeds compete crops to access to water, light and nutrients; and cause qualitative and quantitative reduction in the crops [1]. Today, the use of chemical compounds as the cheapest and most common method of controlling plant diseases is received a great deal of attention. However, due to the slow decomposition process of these compounds and their toxicity to humans and other non-target organisms, the research to find new compounds that are renewable, environmental friendly and easy to produce is necessary. The use of secondary plant compounds, extracts and essential oils as antimicrobial agents in food industry, plant protection and pharmaceutical is increasing [2, 3].

There is a few studies on antifungal activity of secondary compounds against soil-borne pathogen, *Sclerotinia* [4, 5]. The fungi are distributed worldwide and have pathogenic traits on more than 480 plant species including oil products [6]. Stem rot caused by this fungus is one of the important diseases of soybean and canola [7].

One of the most important diseases of maize in the world is ear rot. The disease is induced by Fusarium species [8]. Fusarium ear rot caused by *Fusarium graminearum* usually occurs in warm and dry weather during the graining stage [9].

Aspergillus is one of the most common fungi in the environment and the air and causes human and animals' disease [10]. Among the numerous species of this genus, *Aspergillus fumigatus* has a greater role in the pathogenesis. Diversity and strength of the antigens, toxins and enzymes and the smaller size of the conidia in this fungus are the major reason for that [11].

The *Erwinia* bacteria species are plant pathogens and cause necrosis, galls, wilting or soft rot in plants. Those erwinia species which cause soft rot in the plants belong to the Carotovora group. The bacteria of Xantomonas genus is cause of bacterial Xantomonas leaf spot disease [2].

MATERIALS & METHODS

Herbicidal Test

Thymol solution at concentrations of 1, 0.1, 0.01 and 0.001 mg/ml was prepared. The control seeds were irrigated using distilled water. Then, the seeds were disinfected by 1% sodium hypochlorite and were placed in the plates in order to germinate at 25°C. The plumule and radicle length were measured with a millimeter ruler after 8 days.

Antifungal Test

The antifungal effect of thymol was investigated using the mixing material and PDA medium method. For this purpose, different concentrations of thymol were prepared and added to the sterilized media to reach 0.01, 0.1, 0.2 mg/ml. The resulting media were divided immediately in petri dishes with a diameter of 6cm; and it was allowed to obtain a solid medium. Then, fungal discs with a diameter of 7mm were prepared from young cultures using cork borers. A disc was inserted in the center of each petri dish containing medium, and they were placed at 25°C. After 24 hours, the halo growth of each fungus were measured on a daily basis up to 4 days.

The inhibitory percent of thymol concentration using the Abbott (1925) formula is as follows: IP = C-T/C×100,

where:

IP: percentage of inhibition

C: average diameter of fungus halo in control treatment

T: average diameter of the fungus halo in the compound treatment

Antibacterial Test

The disk diffusion method according to Kirby Bauer was applied to determine the quality sensitivity in which the standardized bacterial suspension with McFarland 0.5 was cultured using grass method on Muller Hinton agar medium. Then, in order to evaluate the antibacterial properties, the blank disks were placed on agar with a certain distance from each other and about 20 micro liters of thymol concentrations of 5, 10, 15, 20 and 30 mg/ml were added to the disks. The disks containing antibiotics, gentamicin, chloramphenicol and amoxicillin were used as a positive control. Then, the medium containing the bacteria were kept at 37°C for 24 hours. The results were evaluated by measuring the diameter of formed haloes [12].

RESULT AND DISCUSSION

Our results showed that increased concentrations of thymol significantly decreased seed germination, radicle and plumule growth of tested weeds, *Amaranthus retroflexus*, *Rumex cetosa and Portulaca oleraceae*. So that, it completely inhibited the above indices at the concentration of 1 mg/ml (Fig 1).

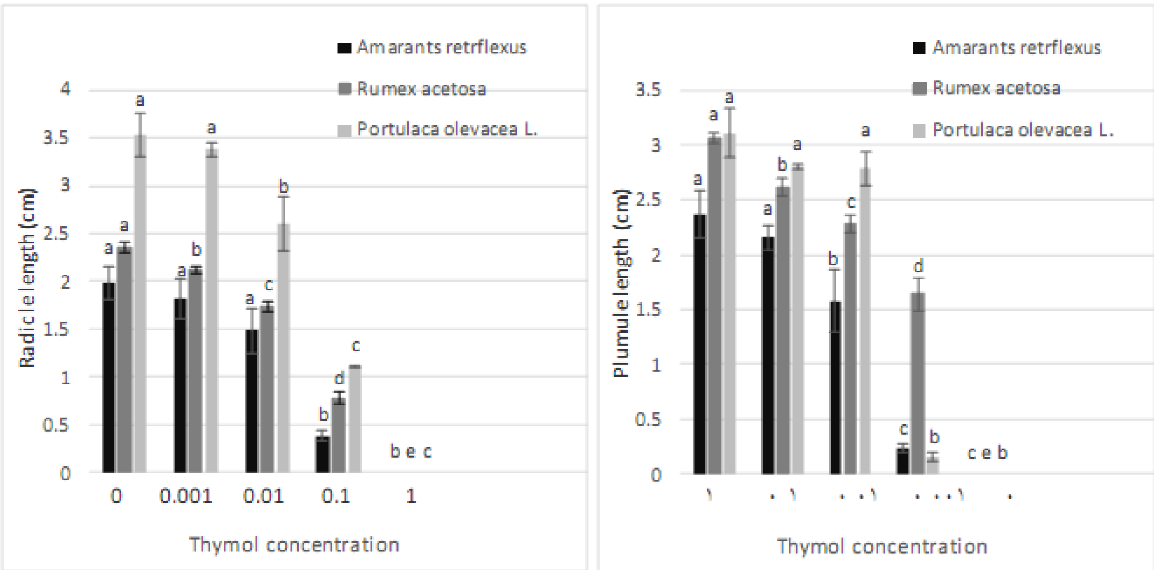


Fig-1: Herbicidal effects of thymol on tested weeds

The results of this study also showed that thymol has strong antifungal effects. The antifungal activity of thymol is generally increased with the concentration in a dose dependent manner. So that, the fungal mycelium growth has been almost inhibited in all tested fungi except *Asperjillus flavus* at concentrations of 0.2 mg/ml (Fig 2).

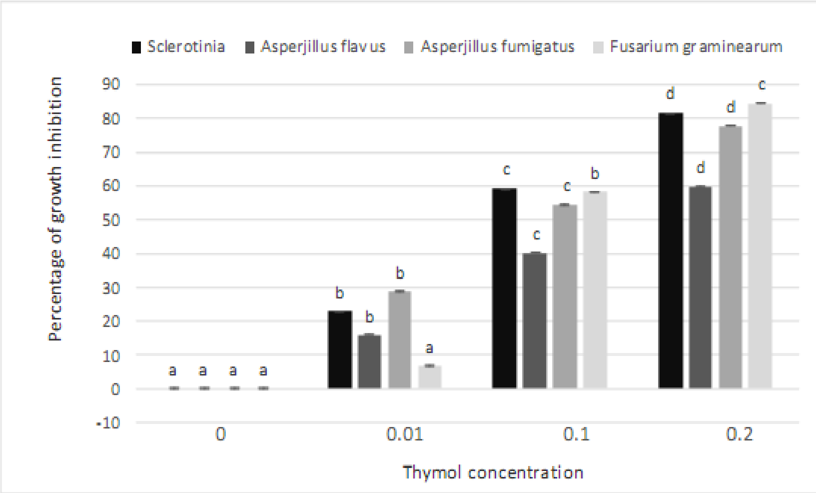
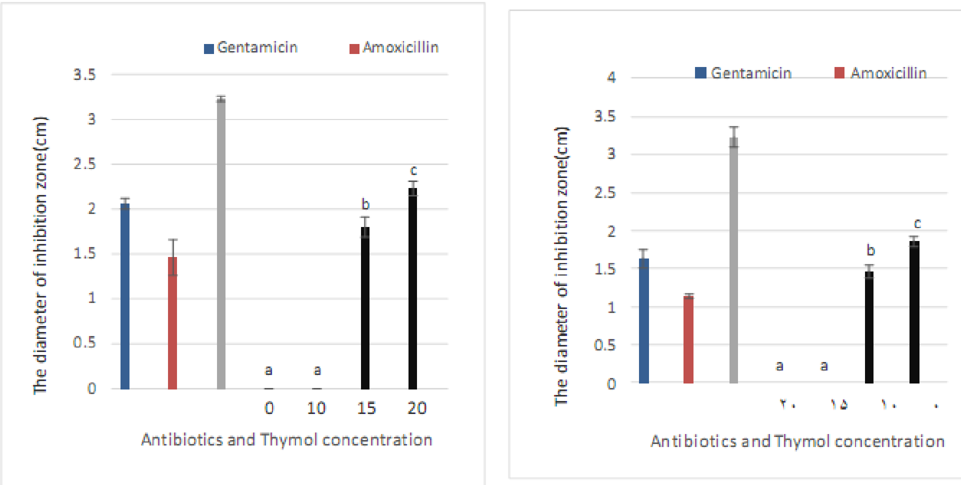


Fig-2: Thymol inhibitory effect on the fungus growth

The results also showed that thymol has antibacterial traits as it has a complete inhibitory effect on both tested bacteria at the concentration of 30mg/ml. This trait varies depending on the concentration and the bacteria. The effect of this substance at the concentration of 20mg/ml on the bacterium, Xantomonas is more than Erwinia (Fig 3).



(A) (B)

Fig-3: Antibacterial effects of thymol on A: *Xanthomonas compestris*. B: *Erwinia carotovora*

Since the 1940s, crops producers are increasingly dependent on chemical herbicides to control weeds. The problems associated with indiscriminate use of herbicides include ground water and soil pollution, development of herbicide resistant weeds [13]. The aims of recent research on the allelopathic effects of the secondary metabolites is to use natural allelopathic substances in order to reduce reliance on chemical herbicides. As the results showed, the allelopathic effects are different in different treatments and different plants. These results dictate we must generalize the allelopathic effects to plant community and all the conditions, carefully.

The fungi causing post-harvest rot or mold grow by producing extra-cellular hydrolases on a vast number of agricultural products and food and infect them by producing Mycotoxins [14]. The use of plant secondary metabolites to control plant diseases as a biological method has been proposed in recent years and received a great deal of researchers' attention as a safe and effective way. These compounds not only have no side effect, but also increase the quality and storage period because of their antioxidant properties [15, 16].

Bacterial resistance against antibiotics is increasing. Secondary metabolites with anti-microbial effects on a wide range of organisms, as well as their potential food uses in some cases and less side effects compared to commonly used antibiotics can eventually be replaced antibiotics[17].

In this study, it is possible that only a mechanism doesn't cause a reduction in the plants' growth. But the allelopathic thymol with its impact on the multiple processes disrupts absorption of water and mineral ions in addition to reduce the synthesis or degrade thylakoids, and ultimately reduces the growth of weeds. Also, thymol, has the ability to inhibit the growth of fungal mycelia and spore production by plants and animals pathogenic fungus.

CONCLUSION

It was concluded that thymol as a monoterpenic volatile compound from lamiaceae family essential oil indicated considerable herbicidal and plant antipathogenic properties. It was regarded as a potent allelochemical and may be candidate as a bio herbicides or biopesticide.

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