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STUDY OF THE INFLUENCE OF PLANT EXTRACTS ON THE MYCELIUM GROWTH OF *ALTERNARIA SOLANI*

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Abstract

The response of fungus *Alternaria solani* to plant extracts originating from the *Fabaceae* family and orange oil was investigated by a modified *in vitro* method of Thornberry. The sensitivity of the pathogen to five different extracts at a concentration of 1 ml/l, 2 ml/l, and 3 ml/l was accessed. The results showed that an orange oil extract (number 7) has the strongest inhibitory effect on the mycelium growth when compared to the other extracts. The effect of this extract has also been observed with all used concentrations. The insignificant effect on mycelium growth was obtained for extract 4; at dose of 1 ml/l since the inhibitory effect reached 25% and at 2 ml/l - 25.45%. Over 50% of inhibitory effect was found for extract number 3. The percentage distribution of the tested products at the lowest dose (1 ml/l) showed that the orange oil extract has the strongest inhibitory effect on mycelium growth when compared to the other plants extracts – 36%. The effect of extract number 4 and number 9 was relatively weak, only 11% and 12%, respectively. Compared to the highest administered dose, the best limiting effect was observed with orange oil – 31%, and with treatment with plant extract number 3 – 26%. It has been shown that the increased concentration of plant extracts increased the sensitivity of the pathogen.

Keywords: *Alternaria solani*, *in vitro*, inhibitory effect, extracts

INTRODUCTION

Alternaria solani is the causal agent of early blight of potato that leads to major damages on potato crops. It is a major foliar disease of potato crops and losses due to early blight typically are around 20-25%; however, there have been cases of 70-80% losses (Pasche et al., 2005). It produces irregular to circular dark brown spots on the lower (older) leaves. The excessive defoliation may lead to the death of plant and consequent yield loss (Wharton & Kirk, 2007). A. *solani* overwinters as mycelium or conidia in plant debris, soil, infected tubers or on other host plants from the same family. The disease is controlled primarily by practices such as use of resistant cultivars and foliar fungicides, crop rotation, removal and burning of infected plant debris, and eradication of weed hosts. These measures reduce the inoculum level to which subsequent plantings could be exposed (Raju, 2001).

The most common and effective method for control of early blight is through application of foliar fungicides. The protective fungicides which have been recommended for the early blight control are maneb (ethylene(bis)dithiocarbamate), mancozeb (dithiocarbamate), chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), and triphenyltin hydroxide (Cuthbertson & Murchie, 2003). The negative aspect of synthetic fungicides is their toxicity to humans, soil, and wildlife. When the fungicides enter the food chain they can cause several deleterious effects on biosphere, they contribute to significant declines in populations

of beneficial soil organisms, soil acidification, and diminished resistance to diseases (Shiva et al., 2004).

In recent years, the research on medicinal plants and plant extracts with biological activity has attracted a lot of attention. The potential of medicinal plants which have been implied by experimental results justified their use not only in the traditional but also in the complementary and alternative approaches towards prevention and treatment of human diseases. The application of medicinal plants is based on variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, and glycosides which have shown antimicrobial properties *in vitro* studies (Dahanukar et al., 2000). Additionally, the different extracts from higher plants are source of antimicrobial agents and they can offer a variety of mechanisms of action (Runyoro et al., 2006). Since the herbs are widely exploited in traditional medicine, their curative potential has been well-documented and, in many countries, the traditional medicine is the primary health care systems. Such use of medicinal plants makes them important for traditional medicine and health but also an option for introduction of new treatments (Houghton, 1995).

To the *Fabaceae* family belong more than 700 genera and about 20 000 species trees, shrubs, vines, and herbs which the makes the *Fabaceae* the third largest angiosperm family after *Orchidaceous* and *Asteraceae* (Stevens, 2001). With its 490 species, which have application in the traditional medicine, the *Fabaceae* is also the second largest family of medicinal plants (Gao et al., 2010).

The aim of this research was to study the *in vitro* effect of four plant extracts with origin *Fabaceae* family and an orange oil extract to control the growth of plant pathogen *Alternaria solani.*

MATERIALS AND METHODS

According to Thornberry's method, the effect of four plant extracts (number 2, 3, 4, 9) originating from the *Fabaceae* family, as well as orange oil (labeled as number 7) at different doses (1 ml/l; 2 ml/l; 3 ml/l) was tested. Nine ml of potato dextrose agar (PDA) was poured for each Petri dish. The tested products with the specified concentration were added immediately. The medium and extracts were mixed, and after solidification a mycelium log of 8 mm of 12–14-day old culture of the pathogen *Alternaria solani* was introduced. The used in this study *Alternaria solani* isolate belongs to the phytopathological collection of the Phytopathology laboratory of the Maritsa Vegetable Crops Research Institute - Plovdiv. Each variant had three repetitions. The colony's diameter was measured on the 3rd, 5th, 7th and 9th day. The data were presented in percentage according to Abbott (1925) and the modified formula: (A-B)/A*100, where:

A – diameter of the fungus colony in the control, in mm.

B – diameter of the fungus colony in the petri dish with fungicides, in mm.

The software products used during the research was "MS Excel Analysis ToolPak Add-Ins" (https://support.office.com) and "R-3.1.3" in combination with "RStudio-0.98" and installed package "agricolae 1.2 -2".

RESULTS AND DISCUSSION

The large-scale use of conventional pesticides and fertilizers puts enormous pressure on agriculture and environment. Using *in vitro* method, the effect of four plant extracts originating from *Fabaceae* family and an orange oil, was tested against the plant pathogen *Alternaria solani* (Figure 1).

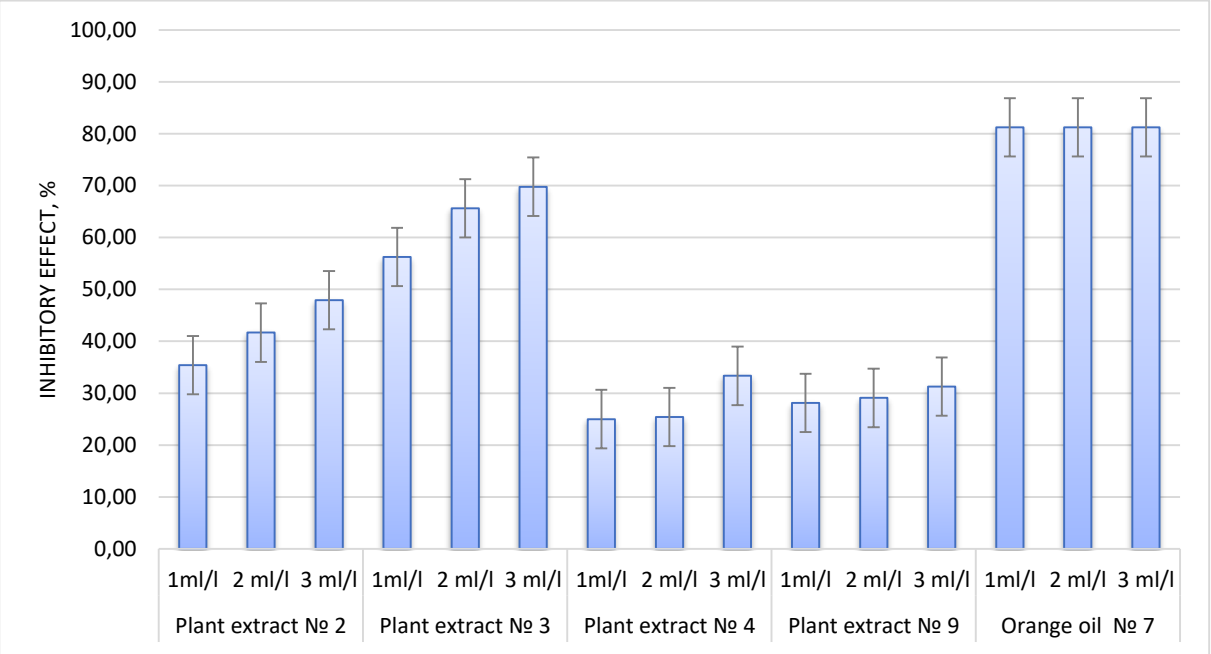

Figure. 1. Effect of plant extract on mycelium growth of *Alternaria solani*

The plant extract 2 in all tested doses has an efficiency in the range of 35.42 % to 47.92 %. The percentage range limit the mycelium growth of *Alternaria solani* with extract 3 was very good and was within the limits of 56.25 % (in a dose of 1 ml/l) to 69.79 % (in a dose of 3 ml/l). Not satisfactory were the data obtained from the plant extract 4, where in all applied doses the limit of the mycelium was below 35 %. A similar trend was found with extract 9, which limited the mycelium growth at a dose of 3 ml/l by 31.25 %. The orange oil affected mycelium growth of the pathogen up to 81.25 % in all administered doses (Figure 1).

The plant extracts have a very important role in defense against insects, herbivores, and microorganisms (Cowan, 1999, Ghosh et al., 2002**)**. The antifungal activity of different organic solvent extracts of *Cassia fistula* was reported by Hajra et al. (2003).

The effective management strategy of the disease could be through cultural practices, chemical, biological control, and use of resistant variety. Wide use of synthetic fungicides can cause environmental hazards and have ill effects on human beings and animals. The chemical fungicides not only develop fungicidal resistance but also can accumulate in food and ground water as residues. To overcome these problems, the development of alternative methods which are safe to the environment, non-toxic to humans and animals and are rapidly biodegradable, one such strategy is use of botanicals to control fungal plant diseases. Plants are the richest source of organic chemicals and produce a wide variety of eco-friendly secondary metabolites with antifungal activities (Riaz et al., 2010).

The effect of different doses of plant extracts and orange oil on the pathogen *Alternaria solani* was investigated (Figure 2). The applied dose of 1 ml/l for either extract 3 or orange oil was the most effective against the pathogen with 25 and 36%, respectively. The dose of 2 ml/l was also the most effective against mycelium growth for extract 3 – 27% and for orange oil 33%. A similar trend was maintained also at the highest administered dose.

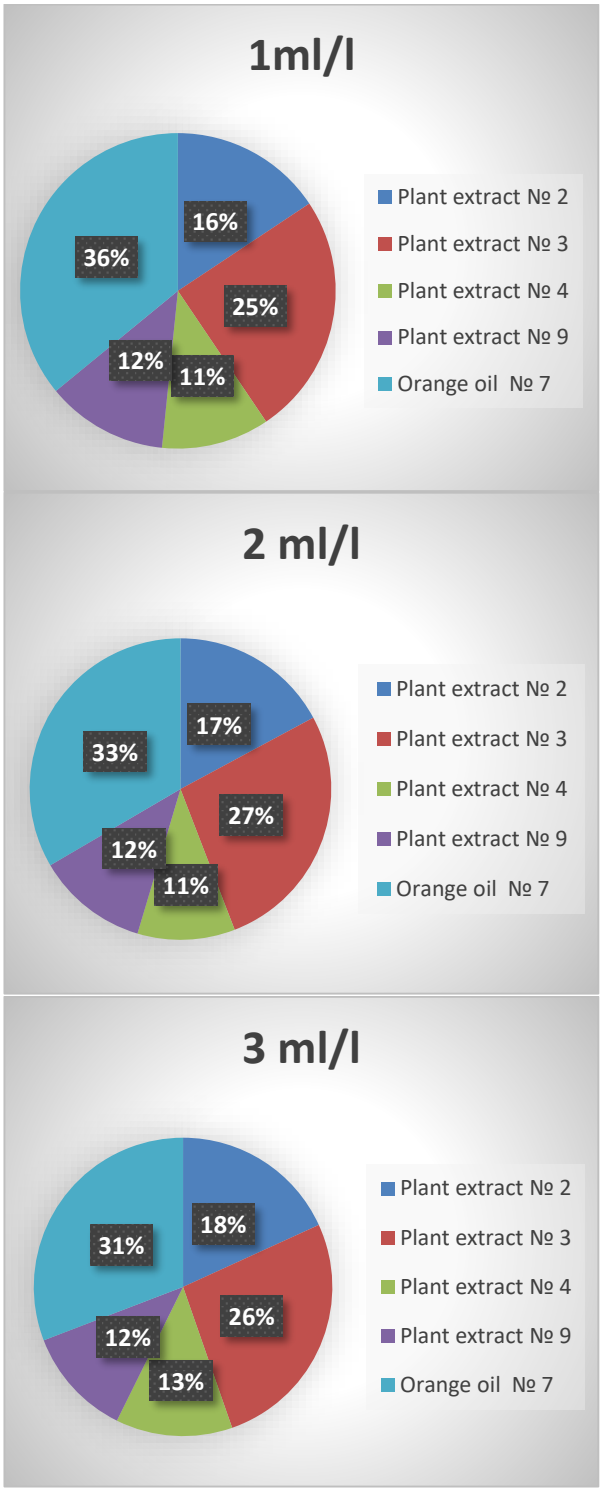

Figure. 2. Comparison of the inhibition effect (%) of plant extracts and orange oil at particular dose on the mycelium growth of *Alternaria solani*

It is known that the free radicals have a negative effect on cells. This effect could be restrained by the scavenger activity of natural antioxidants in plants. Part of the biological activities of the natural compounds extracted from plants is their activity towards free radicals (Baratta et al., 1998). The discovery of new antimicrobial agents that successfully combine a scavenger activity but show non or low toxicity could find application in the phytotherapy and there is a thorough screening of therapeutic agents from higher plants (Kuete & Efferth, 2010).

One of the key challenges in the agricultural industry is the need to address the problems associated with pesticide use (environmental pollution, bioaccumulation and increasing pest resistance), which requires a reduction in the number of pesticides applied in order to protect crops and stored products (Hayles et al., 2017).

CONCLUSION

As a result of the conducted research, it was found that plant extract 3 and orange oil in all studied doses show very good results and to a high extent limit the mycelium growth of *Alternaria solani*. The obtained data indicates that the studied plant extracts could be used as an alternative to the widely used chemical pesticides.

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