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**The Response of Tea Tree Oil as a Biofungicide Against Early Blight Disease in Tomato  
Crop (*Solanum Lycopersicum*) in Sudan**

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**Abstract:** The tomato crop (*Lycopersicon esculentum* Mill.) originated in Tropical Central and South America. In Sudan, tomato is gaining importance and its consumption has increased. It ranks as the second vegetable crop in Sudan. Tomatoes are subject to a large number of pests and diseases from the time of emergence to harvest. Among these; Early Blight is the most important fungal disease of tomato, induced by *Alternaria* spp. Current research is designed to investigate the potential of Early Blight biocontrol strategy through the use natural alternatives to pesticides with the aim of promoting sustainable agricultural development and economic growth. The antifungal effect of tea tree (*Melaleuca alternifolia*) essential oil against *Alternaria* sp. was studied *in vitro* and *in vivo*. The inhibition effect of four different concentrations (0.5%, 1%, 2% & 3%) of tea tree oil or melaleuca oil on the growth rate (mm/hr.) of the pathogen was evaluated in potato dextrose agar. The inhibitory effect of the tea tree oil was examined in the nursery during 2007/2008 winter season using a susceptible open pollinated tomato cultivar Peto 86 and recommended fungicide for early blight disease of tomato Ridomil® Gold 72 W.P. for comparison. The results performed that the antifungal effect of tea tree oil against *Alternaria* spp. was enhanced significantly *in vitro* and *in vivo* with the least disease intensity of 12.50%. When tomato plants were treated with the concentration of 3% of the tea tree oil. Disease incidence of 17.88% and 25.08% was recorded on plants treated with the fungicide and the control treatment, respectively. Biocontrol methods based on inhibition of the spore germination of causal agents are achieving significant results. Some of the advantages of these methods over chemical methods include absence of residual toxicity, the harmlessness to the nature and costless.

**Key words:** *Melaleuca alternifolia*, *Alternaria* spp., Early blight disease, Tomato, Sudan.

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## Introduction

Tomato (*Solanum Lycopersicum*) which belongs to the family Solanaceae is believed to have originated in the coastal strip of western South America (Papadopoulos, 1991). It was first domesticated in Mexico (Anon., 1983). Tomato is the second most important vegetable crop next to potato. Present world production is about 126 million tons (FAOSTAT Database, 2009). Tomato fruit is rich in vitamins A and C and contains an antioxidant, lycopene (Jones, 1999).

In Sudan as reported by Mirghani and El Tahir (1995) tomato is gaining importance and its consumption has increased. It ranks as the second vegetable crop and is usually produced by small farmers in rain-fed areas, irrigated private farms and in the big government schemes. The Central State is the most important production area in Sudan, followed by the Northern State. The production of tomato in Sudan has been increasing steadily up to 145909 hectors (\*FAOSTAT Database, 2009).

Tomatoes plants are subject to a large number of pests and diseases from the time of emergence to harvest. Among these; Early Blight disease induced by *Alternaria* sp is one of the most important limiting factors in tomato production (Rich, 1983). It is widespread in the tropics, subtropics and temperate zones and can attack the plants at any stage of development causing a significant risk to crop productivity in the field and to fruit quality in the market (Anon., 1983). Early blight inflicts a wide range of symptoms at all stages of plant growth. It can cause damping-off, collar rot, stem cankers, leaf blight, and fruit rot. The classic symptoms occur on the leaves where circular lesions up to 1/2" in diameter are produced. Within these lesions, dark concentric circles can be seen. The leaf blight phase usually begins on the lower, older leaves and progresses up wards. Infected leaves eventually wither, die, and fall from the plant (Sherf & Macnab, 1986 and Jones *et al.*, 1991). All *Alternaria* species are highly resistant to adverse weather. They develop in a wide range of temperatures and use the locally available source of moisture. They sporulate best on necrotic and dead tissues and produce a relatively small number of spores, mainly at the end of the season, and they are decisively affected by the age-conditioned susceptibility of host plants (Rotem, 1998).

The best way to manage the disease is to use preventive measures by combining many control measures together. Once early blight is established in the crop, it is very difficult to be controlled (Smith & Kotcon, 2002 and Ben-Noon *et al.*, 2003). Management of early blight disease in tomato is currently relies on fungicides such as Ridomil Gold, Sulphur and Copper compounds (Markham 1999). The widespread use of these fungicides have significant drawbacks including increased cost, handling hazards, pesticide residues in agricultural commodities, and threat to human health and environment (Paranagama *et al.*, 2003). Recently, bio control methods based on inhibition of the spore germination of causal agents are apparently the most acceptable approach. Some of the advantages of these methods over chemical methods include absence of residual toxicity, the harmlessness to the nature and costless (Agrios, 1997). Paranagama *et al.*

(2003) stated that of the bio control methods is the use of natural plant protectants that have pesticidal activity. Essential oils are plant volatiles containing monoterpenes, sesquiterpenes and phenyl propionoids.

The essential oil of *Melaleuca alternifolia* has a long history of use as a topical antiseptic and has been used in Australia as an antiseptic since the 1920s (Carson *et al.*, 2006). The leaves of *M. alternifolia* (tea tree) have long been used in aboriginal traditional medicine of New South Wales (Australia) as remedies for wounds and cutaneous infections (Penfold and Grant 1925). This oil has a wide spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, both aerobic and anaerobic, against yeasts and fungi (Gustafson *et al.*, 1998).<sup>1</sup>

It is also active against clinically isolated fluconazole-resistant *Candida* strains (Carson *et al.*, 2006). Tea tree oil has broad antimicrobial activity and is incorporated into a diverse range of pharmaceutical and cosmetic products. Therefore, the oil and products containing it have been evaluated *in vivo* for the treatment of superficial fungal infections such as Onychomycosis and oral candidiasis, with some favorable clinical outcomes (Fritz *et al.*, 2001). Papadopoulos *et al.* (2006) explained that the antimicrobial activity of tea tree oil has been principally attributed to terpinen -4-ol.

Essential oil from *M. alternifolia* has shown potential to reduce disease incidence and severity induced by *A. solani* in potato and *Cercospora beticola* in sugar beets (Caolotanski *et al.* 2002). Tea tree oil is an essential oil steam distilled from the Australian plant *M. alternifolia*. This natural oil is an effective antiseptic, fungicide and bactericide, and has many safe and effective uses in the health and cosmetics industry (Markham, 1999).

## Materials and Methods

The essential oil of *Melaleuca alternifolia* was purchased from Thursday Plantation Ltd., Ballina, Australia. The fungicide Ridomil Gold W.P. 72% was purchased from Syngenta Co. Tomato open pollinated cultivar (Peto 86) was purchased from local market.

The fungicidal effect of the essential oil in four concentrations against *Alternaria* sp. was assessed *in vitro* under laboratory conditions. The fungal pathogen used in this experiment was isolated on PDA medium from infected tomato leaves showing typical early blight symptoms.

The pure 100% essential oil was mixed with pre-autoclaved PDA medium in liquid condition to give final concentrations of 0.5, 1.0, 2.0 and 3.0%. A 5-mm-diameter disc from a 7-day-old culture of the pathogen was placed on the surface of the mixed medium in 9-cm-diameter Petri dishes. The plates were incubated at 25°C ± 2. Growth rate (mm/hr) was assessed daily.

In this experiment the fungicide Ridomil Gold was used at the recommended dose of 1kg/fed. The tea tree-based formulations with the four concentrations (0.5, 1, 2 & 3%) of the pure essential oil were tested in this study under nursery conditions adopting recommended husbandry practices in raising tomato plants. The plants were grown in 7kg soil obtained from a heavy infected sick plot, the soil was placed in 9 inch diameter plastic pots. In each pot 6 seeds were sown. Thinning was done after two weeks and three seedlings were left in each pot. The plants were kept in the nursery with four replicates and were sprayed 45 days after sowing date two times and four times for the fungicide and the essential oil respectively, with intervals of 15 days. The experimental design adopted was Complete Block Randomized Design. The efficacy of fungicide and essential oil were assessed weekly in terms of disease incidence. Marketing yield of each replicate was recorded at harvesting, 90-110 days after sowing.

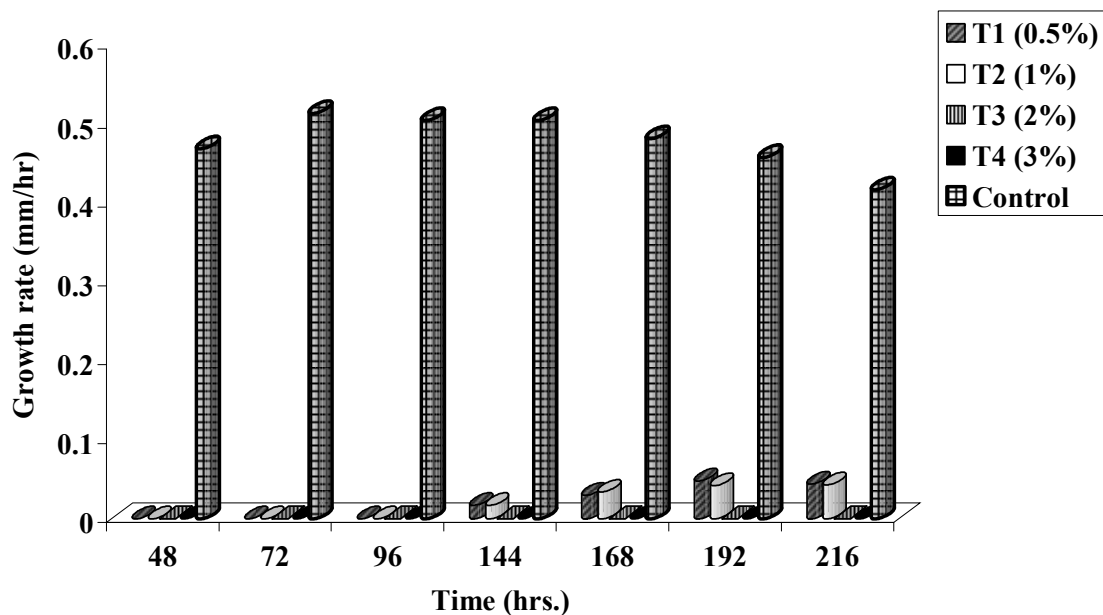
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\*Food and Agriculture Organization Corporate Statistical Database

## Results and Discussion

As shown in figure 1 the highest growth rate was obtained daily from the negative control. The use of four concentrations of the tea tree oil resulted in the same growth rate (0.00 mm/hr) in the first, second and third readings. *Alternaria* sp. which was grown on the medium supplemented with 0.5% and 1% of Melaleuca oil started to grow 144hrs after incubation (Plates 1, 2 & 3). The growth patterns in the both treatments (T1 0.5% and T2 1%) after 144hrs of incubation were quite similar especially in the last reading (Figure 1). Statistical analysis revealed that there were significant differences in the last reading after 216 hours of incubation between the negative control treatment and treatment 1 (0.5%) and treatment 2 (1%) in one hand and the other two treatments (2% & 3%) in the other hand. No significant differences were detected between the treatments 1 (0.5%) and 2 (1%) neither between the treatments 3 (2%) and 4 (3%). These findings have clearly proved that tea tree essential oil has a highly suppressive effect against *Alternaria* sp. and can be used as a successful inhibitory agent

against tomato early blight pathogen. These results are similar to findings of Carson et al. (2006) and Gustafson et al. (1998) in the inhibition effect of this oil against Gram- positive and negative bacteria, yeast and fungi.



**Figure 1: Average of growth rate (mm/hr) of *Alternaria* sp. treated with different concentrations of tea tree essential oil**

The results obtained from the pots (*in vivo*) experiment revealed that the untreated tomato plants (negative control) showed the highest percentage level of disease incidence (27.08%) as shown in figure 2. When the plants were treated with the concentrations 0.5 and 1% of the tea tree oil, the

disease incidence were 26.06 and 25.70% respectively. When the plants were sprayed with 2% of the oil the disease intensity decreased to 15.50%. While the concentration 3% of the oil decreased the percentage of the disease intensity to 12.50%. When tomato plants were treated with the fungicide Ridomil Gold a disease intensity percentage of 17.88% was recorded. The statistical analysis showed that there were no significant differences between tea tree oil treatments EOa (0.5%), EOb (1%) and the fungicide treatment. Also, there were no significant differences between the negative control treatment and the solvent control treatment. As well, no significant differences were detected between the tea tree oil treatments EOc (2%) and EOd (3%). When the negative control treatment and the solvent control treatment were put in a group and the oil treatments EOa and EOb and the fungicide treatment were put in another group and the the oil treatments EOc and EOd were put in a third group there were significant differences between these groups. The successful translation of *in vitro* activity of this oil into *in vivo* activity in the plant has been strongly proved by these findings, which matched with results reported by Caolotanski *et al.* (2002). Also these results showed that this oil is more powerful than the recommended fungicide Ridomil Gold. The highest yield of 826.50 ton/ha was obtained from the fungicide treatment. This yield decreased by 28.09% when the plants were treated with the concentration 3% of the tea tree oil. Whereas the yield from plants sprayed with the concentration 1% of the tea tree oil decreased only by 0.16%, and when the tomato plants sprayed with 2% of the essential oil the reduction percentage of the yield was 27.51% (Figure 3 and Table 1). Statistical analysis revealed that there were no significant differences between the treatments. Tomato plants which were treated with the fungicide produced the highest yield although the lowest disease intensity was obtained from tea tree oil 3% and this result clarified that even plants which were treated with the fungicide were infected but they were tolerant enough to produce the highest yield.



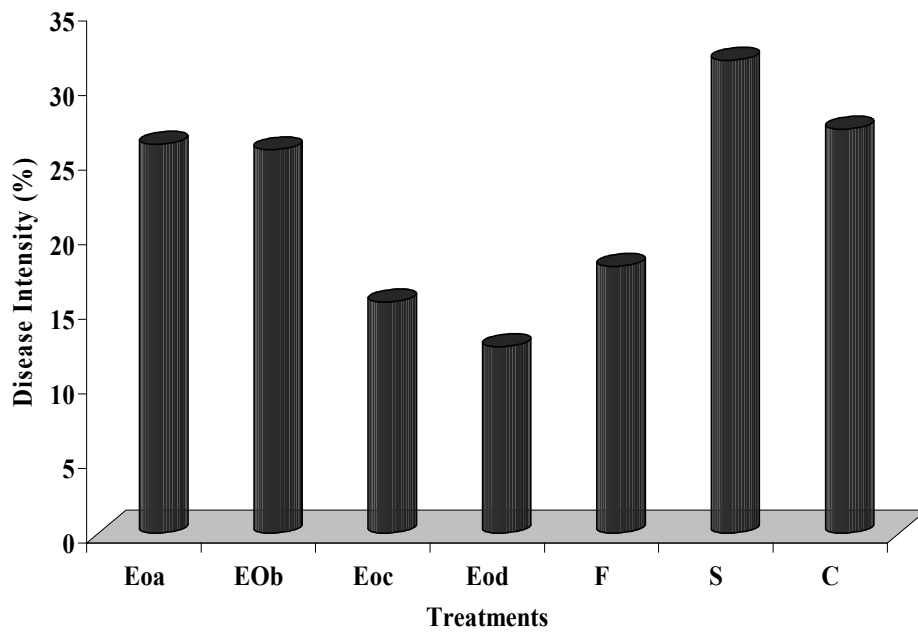
**Plate 1: Growth of *Alternaria* sp. untreated and treated with the concentration 0.5% of tea tree oil**



**Plate 2: Growth of *Alternaria* sp. treated with the concentration 1% of tea tree oil**



**Plate 3: Growth of *Alternaria* sp. treated with the concentration 3% of the oil**



**Figure 2: Disease intensity of tomato cultivar Peto 86 treated with the fungicide Ridomil Gold and different concentrations of tea tree essential oil**

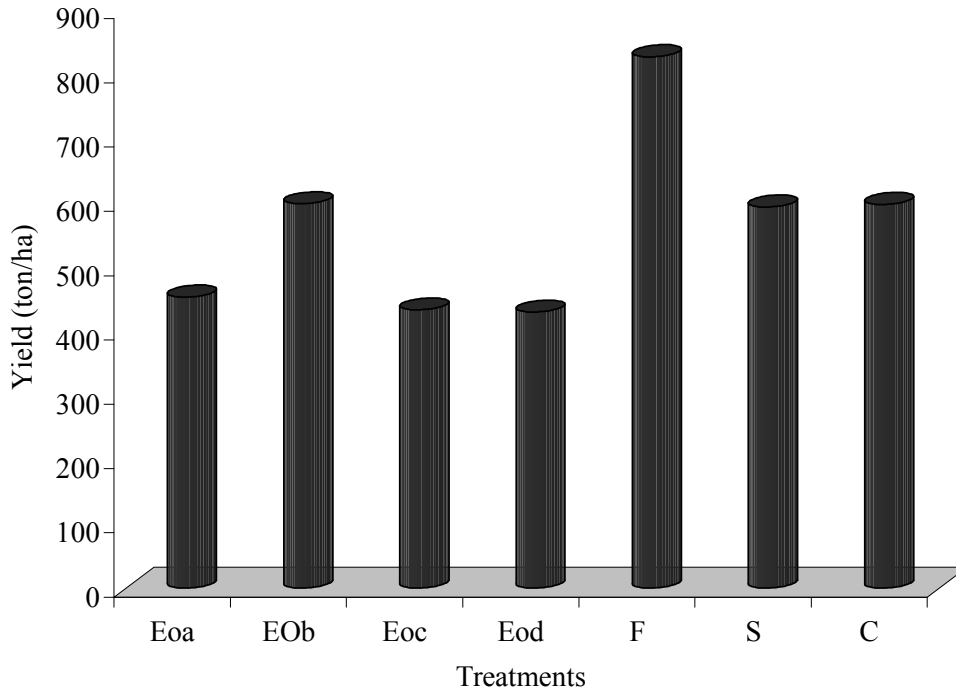


Figure 3: Marketing yield (ton/ha) of tomato cultivar Peto 86 treated with the fungicide Ridomil Gold and defferent concentrations of tea tree essential oil

**Table 1: Means of yield (ton/ha) of tomato cultivar Peto 86 treated with the fungicide Ridomil Gold and different concentrations of tea tree essential oil**

Treatments	Replicate 1	Replicate II	Replicate III	Replicate IV	Mean	% Change
<b>EOa</b>	236.0	307.2	516.6	750.3	452.53 <sup>ab</sup>	-24.21
<b>EOb</b>	872.5	583.4	294.4	641.9	598.05 <sup>ab</sup>	+0.16
<b>EOc</b>	525.5	834.4	54.2	317.3	432.85 <sup>ab</sup>	-27.51
<b>EOd</b>	146.2	793.9	416.7	315.6	429.35 <sup>ab</sup>	-28.09
<b>F</b>	649.4	924.3	1000.5	731.8	826.50 <sup>a</sup>	+38.42
<b>S</b>	160.5	1086.1	515.9	609.7	593.05 <sup>ab</sup>	-0.68
<b>C</b>	544.8	771.1	697.8	374.7	597.10 <sup>ab</sup>	Control

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