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## Different plant extracts against the early blight (*Alternaria solani*) on tomato (*Solanum lycopersicum*)

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### Abstract

*Alternaria solani*, the causal agent of early blight disease, is one of the most destructive pathogens of *Solanum lycopersicum* and reduces productivity by approximately 80%. The aim of this study was to assess the efficiency of three different plant extracts to control *Alternaria solani* on tomato under field conditions. A randomized complete block design (RCBD) was used with four replications and four treatments, namely *Allium sativum* L., *Carica papaya*, *Azadirachta indica*, and control (Water). *Allium sativum* L. (Garlic), *Carica papaya* (Paw paw) and *Azadirachta indica* (Neem) extracts were observed to be more effective on disease severity and disease incidence during week four with a significant difference ( $P < 0.01$ ) compared to the control treatment. The plot treated with *Carica papaya* showed the highest total yield, followed by *Azadirachta indica* and *Allium sativum* L. (1052.8 g, 639.8 g, and 566.8 g, respectively) with significant differences ( $p < 0.01$ ) while the control treatment produced 265.0 g. The application of plant extracts revealed a significant reduction of early blight disease as well as an increased total yield of tomatoes compared to the control treatment.

**Keywords:** *Alternaria solani*, *Allium sativum* L., *Carica papaya*, *Azadirachta indica*, Phytochemical compounds

## Introduction

Tomato (*Solanum lycopersicum*) is one of the most important economic crops and is a widely consumed vegetable. It belongs to the *Solanaceae* family and is susceptible to (Foolad et al., 2008) different types of pathogens. Early blight is the causative agent of *Alternaria solani* (*A. solani*), which is one of the most destructive fungal diseases on tomatoes (Foolad et al., 2008; Roy et al., 2019) and causes yield losses up to 80% (Khan et al., 2012; Pandey et al., 2020). The conidia of *A. solani* survive under the host debris and its thick wall enables this pathogen to survive in adverse climatic conditions. Temperatures ranging from 8-32 °C are suitable for the mycelia of *A. solani* to germinate (Kemmitt, 2002). This pathogen can infect the leaf, stem, and fruit of tomato plants (Foolad et al., 2008; El-Kholy et al., 2021) by entering tissues through stomata or wounds (Kemmitt, 2002). To combat early blight disease in tomatoes, synthetic fungicides are commonly applied (Akila and Sathiy, 2012; Mugao et al., 2021). Although chemical substances are effective agents and prevent the pathogen's development, it poses a life-threatening risk to the lives of non-target organisms in the environment (Martinez, 2012; Yoon et al., 2013; Ritika and Utpal, 2014). Furthermore, *A. solani* may be resistant to synthetic fungicides, which may leave residue inside tomato fruits. Currently, the global trend toward sustainable agricultural practices encourages reducing the application of chemical substances. Therefore, alternative methods of controlling the pathogen without having detrimental impacts on the environment are taken into consideration. The majority of organic pesticides degrade quickly, leave no hazardous residues on crops and have no risk to the non-target organism (Kimani, 2014). They present a safe alternative for controlling fungi and mycotoxins in food and feed as a result (Iram et al., 2015; Prakash et al., 2020). Several plants produce different groups of chemical compounds that offer protection against phytopathogens (Kagale et al., 2004; Goussous et al., 2010; Sallam, 2011; Romanazzi et al., 2012; Das et al., 2020). These consist of phenolic compounds such as flavonoids, phytosterols, carotenoids, tocopherols, terpenoids, alkaloids, saponins, tannins, aromatic acids, glucosinolates, carotenoids, essential oils, chlorophyll and organic acids (Adebo and Gabriela Medina-Meza, 2020; Loi et al., 2020; Righini et al., 2021). Applying plant extracts is one strategy to inhibit fungal spore development on host plants (Sharma and Kumar, 2009). Naik et al. (2020) reported that aqueous extracts of *Azadirachta indica*, *Ocimum sanctum* and *Zingiber officinale* at 10% concentration limit the development of *A. solani* spores accounting for 64.24%, 58.62% and 57.32% inhibition, respectively. Mohana and Raveesha (2007) demonstrated that the antifungal activities of *Decalepis hamiltonii* extract, which was able to inhibit mycelial growth of various species such as *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Drechslera* spp. and *Alternaria* spp. Thus, this research investigates the efficacy of plant extracts from three different plant species against *A. solani* under field conditions.

## Materials and Methods

### 1. Treatments and experimental designs

Extracts from the plant species *Allium sativum* L., *Carica papaya*, *Azadirachta indica* were selected for the study. Then, the plant materials were washed for 2-3 minutes before drying. A randomized complete block design (RCBD) with four replications and four treatments was utilized for this experiment.

### 2. Isolation and inoculation pathogen

Infected tomato leaves and fruits exhibiting symptoms of early blight disease were collected from fields in Kandal province, Cambodia. A diseased 5 mm portion of the leaf was cut and placed into sterile, distilled water followed by 0.1% sodium hypochlorite (NaOCl) and then rinsed with distilled water again. The leaf segments were transferred to potato dextrose agar (PDA) culture media and placed in room temperature ( $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) (Parvin et al., 2021). After mycelial development, spores were isolated under the microscope and verified according to the morphology and reproductive structure of *A. solani*. Once confirmed, the spores of *A. solani* were cultured again to purify the pathogen. Cultured spores were adjusted to  $5 \times 10^6$  conidia/ml after 15 days and used to inoculate 30-day-old tomato plants using 20 ml per plant. After inoculation, plants were covered with polyethylene bags for 48 hours to maintain a high humidity condition. Then, the plastic bags were removed and plants were maintained under field conditions.

### 3. Pathogenicity testing

Ten days after inoculation using the methods described in the previous section the leaves of the tomato plants turned yellow and developed dark ring spots that later turned into necrotic areas. Leaves of tomato showing symptoms of *A. solani* infection were used to re-isolate the pathogen in the plant pathology laboratory, Faculty of Agronomy at the Royal University of Agriculture. The pathogen was cultured on potato dextrose agar and the morphology of sporulation was characterized using the same technique previously described. The spores of *A. solani* was used to inoculate in the experiment once the pathogen was confirmed.

### 4. Extract preparation

The plant materials, namely *Allium sativum* L., *Carica papaya*, and *Azadirachta indica* were dried in the oven for 1 day at  $70^{\circ}\text{C}$ . Leaves were then ground in an electric grinding machine to find the powder. The 40g of powder from each plant was soaked for 24 hours in 200 ml of 80% ethanol (Baka and Rashad, 2016). Next, the suspension was filtered through Whatman's filter paper and each extract was diluted to 30% concentration using distilled water and centrifuged for 15 minutes. Plant extracts were applied by spraying four times at 15-day intervals starting one week after plants were inoculated

## 5. Disease severity and disease incidence

Two weeks after inoculation disease severity (DS) and disease incidence (DI) were recorded and plant extracts were applied (Sallam, 2011). Symptom severity and disease rates of *A. solani* infection was scaled from 0-9 according to Latha et al. (2009): 0 = Healthy, 1 = 1-5%, 2 = 6-10%, 3 = 11-25%, 5 = 26-50%, 7 = 51-75%, 9 = > 75% of leaf area infected with early blight symptoms.

## Results

The result of disease severity on tomato plants from week 1 to week 4 after spraying the plant extracts is presented in Table 1. There was no significant difference between disease severity during weeks 1 2, but weeks 3 and 4 demonstrated significantly different levels of disease severity ( $p < 0.01$ ). Regarding results from week 1, plants sprayed with extract from *Allium sativum* displayed the highest rate of disease severity (15.50%), followed by control (15.00%), *Azadirachta indica* (13.25%) and *Carica papaya* (9.50%). Conversely, the control had the highest amount of disease severity in week 2 with 62.50%, followed by *Allium sativum* L., *Azadirachta indica* and *Carica papaya* with 43.75%, 37.50%, 0 and 31.25%, respectively. For weeks 3 and 4, the control plants exhibited significantly higher disease severity results ( $p < 0.01$ ) compared to plants receiving extract treatments, while there was no significant difference between extract treatments. The severity of disease observed on control plants was by far the highest at 57.50% when compared to plants treated with extracts from *Allium sativum* L. (23.75%), *Azadirachta indica* (20.75%), and *Carica papaya* (18.25%). During week 4, control treatment still demonstrated the highest percentage of disease severity at 70.75%, followed by *Allium sativum* L. (25.75 %), *Azadirachta indica* (24.25 %) and finally *Carica papaya* (19.00%).

**Table 1** Effect of different plant extract on disease severity of early blight disease on tomato measured in percentage.

Treatments	Week 1	Week 2	Week 3	Week 4
Control	15.00	62.50	57.50 <sup>a</sup>	70.75 <sup>a</sup>
<i>Allium sativum</i> L.	15.50	43.75	23.75 <sup>b</sup>	25.75 <sup>b</sup>
<i>Carica papaya</i>	9.50	31.25	18.25 <sup>b</sup>	19.00 <sup>b</sup>
<i>Azadirachta indica</i>	13.25	37.50	20.75 <sup>b</sup>	24.25 <sup>b</sup>
LSD (P=0.05)	0.7612 <sup>ns</sup>	0.3242 <sup>ns</sup>	0.0022 <sup>**</sup>	0.00 <sup>**</sup>

**Note:** The value in the table followed by the different letters indicate significant difference among treatments according to the last significant difference test  $p < 0.01$  and  $p < 0.05$  using LSD (\*) =  $p < 0.05$ , (\*\*) =  $p < 0.01$ , ns= non-significant.

Disease incidence results presented in Table 2, which shows no significant difference all treatments in weeks 1 and 2. In week 1, tomatoes sprayed with *Allium sativum* L. displayed the highest percentage of disease incidence at 37.50%, whereas control, *Carica papaya* extract, and *Azadirachta indica* extract treatments were all 31.25%. The percentage of disease incidence in the control treatment increased to 62.50% by the second week, while *Allium sativum* L., *Azadirachta indica* and *Carica papaya* extract treatments showed disease incidence levels of 43.75%, 37.50% and 31.25%, respectively. By weeks 3 and 4, however, there were significant difference at  $p<0.05$  and  $p<0.01$ , respectively. Control treatment plants had 87.5% of disease incidence by week 3, which was significantly higher than plant treated with extracts from *Carica papaya* (37.50%) and *Azadirachta indica* (50.00%). Control plants had 100.00% disease incidence by week 4, while *Carica papaya* (T2) had the lowest disease incidence percentage (37.50%) followed by *Azadirachta indica* (62.50%) and *Allium sativum* L. (68.75%).

**Table 2** Effect of different plant extracts on disease incidence of early blight disease on tomato measure in percentage.

Treatments	Week 1	Week 2	Week 3	Week 4
Control	31.25	62.50	87.50 <sup>a</sup>	100.00 <sup>a</sup>
<i>Allium sativum</i> L.	37.50	43.75	56.25 <sup>ab</sup>	68.75 <sup>b</sup>
<i>Carica papaya</i>	31.25	31.25	37.50 <sup>b</sup>	37.50 <sup>c</sup>
<i>Azadirachta indica</i>	31.25	37.50	50.00 <sup>b</sup>	62.50 <sup>bc</sup>
LSD (P=0.05)	0.944 <sup>ns</sup>	0.3294 <sup>ns</sup>	0.0385 <sup>*</sup>	0.0058 <sup>**</sup>

**Note:** The value in the table followed by the different letters indicate significant differences between treatments according to the least significant difference test  $p<0.01$  and  $p<0.05$  using LSD (\*) =  $p<0.05$ , (\*\*) =  $p<0.01$ , ns= non-significant.

Table 3 indicates that there was no significant difference in days to between 50% flowering. The control treatment took more days to flower at 40.50 days, followed by *Azadirachta indica*, *Allium sativum* L. and *Carica papaya* at 40.00, 39.75 and 39.00 days, respectively. However, of the number of days it took for 100.00% of the plants in each treatment to flower significant different at  $p\leq 0.01$  which the control treatment proved the highest with 49.75 days to flower, followed by *Allium sativum* L. (44.50 days), *Azadirachta indica* (42.25 days) and *Carica papaya* (41.00 days). The height of plants treated with *Carica papaya* extract (99.688 cm) were significantly greater than those of other treatments. There was no significant difference between the plant heights of *Allium sativum* L. and *Azadirachta indica* extract treatment groups at 87.875 cm and 85.625 cm, respectively. Control treatment plants were significantly shorter than all other treatments at 75.370 cm. Fruit weight showed no significant differences between all treatments. The *Carica papaya* extract treatment group demonstrated the greatest fruit weight at 35.332 g followed by *Azadirachta indica*, *Sativum* L. and the control treatment with 32.347 g, 31.387 g and 28.033 g, respectively. In addition, the total yields exhibited significant differences at  $p\leq 0.01$ . The plot treated with *Carica papaya* extract showed a significantly

higher highest total yield (1,071.3 g) than other treatments, while *Azadirachta indica* (639.8 g) and *Allium sativum* L. (566.8 g) extract treatment plots were not significantly different from one another. The plot containing plants in the control treatment group had a significantly lower yield compared to plots that underwent extract treatments (265.0 g).

**Table 3** Effect of different plant extracts on days of 50% and 100% flowering, plant height, fruit weight, and total yield.

Treatments	50% F (days)	100% F (days)	PH (cm)	FW (g)	Total Yield (g)
Control	40.50	49.75 <sup>a</sup>	75.375 <sup>c</sup>	28.033	265.0 <sup>c</sup>
<i>Allium sativum</i> L.	39.75	44.50 <sup>b</sup>	87.875 <sup>b</sup>	31.387	566.8 <sup>b</sup>
<i>Carica papaya</i>	39.00	41.00 <sup>c</sup>	99.688 <sup>a</sup>	35.332	1071.3 <sup>a</sup>
<i>Azadirachta indica</i>	40.00	42.25 <sup>bc</sup>	85.625 <sup>b</sup>	32.347	639.8 <sup>b</sup>
LSD (P=0.05)	0.6670 <sup>ns</sup>	0.0011 <sup>**</sup>	0.0025 <sup>**</sup>	0.0772 <sup>ns</sup>	0.0007 <sup>**</sup>

**Note:** The value in the table followed by the different letters indicate significant differences among treatments according to the least significant difference test  $p < 0.01$  and  $p < 0.05$  using LSD (\*) =  $p < 0.05$ , (\*\*) =  $p < 0.01$ , ns= non-significant. 50% and 100% F= Percentage of flower, FW: Fruit weight, PH= Plant height.

## Discussion

The present study illustrated that the application of plant extracts has a significant influence on disease severity, disease incidence, plant height, time to 100% flowering and total yield compared to the control treatment. Several studies have reported the effectiveness of various plant extracts in reducing pathogen development. Latha et al. (2009) reported that the antimicrobial activity of plant extracts induced the growth of *A. solani* mycelium approximately 87%. Plant extracts reduced fungal development by destroying the membrane and inducing the synthesis of lipids, proteins and nucleic acids (Harris et al., 2001; Bayan et al., 2014). The antifungal compounds of plant extracts had the inhibition of fungal mycelium growth (Anusha, 2003). The chemical compounds attributed to the antifungal activity of plant extract had various groups such as coumarins, pyrones, steroids, phenolics and phenol acid, flavonoids, isoflavonoids, alkaloids and other compounds (Jantasorn et al., 2016). The presence of llicin in garlic extracts exhibited antifungal activity that inhibited the development of fungal spores (Satya et al., 2005) and prevented fungal growth by disrupting the membrane and prohibiting synthesis of lipids, proteins and nucleic acids (Bayan et al., 2014). Tegegne et al. (2008) reported there were various types of tetra terpenoids and phenolics in neem extract that were antagonistic to the *Alternaria solani* mycelium. According to the result, the extract from *Carica papaya* was the most effective against *A. solani* development and also increased the total yield compared to other plant extracts and control treatments. Many researchers have reported *Carica papaya* leaf enriches phytochemical compounds which enable it to inhibit the spore development of pathogens,

however, these compounds can be different according to the choice of solvent substances. Ethanol is more efficient at dissolving bioactive compounds than water (Baka and Rashad, 2016) and organic solvents (Lengai and Muthomi, 2018). Baskaran et al. (2012) reported that the ethanolic plant extracts retained the presence of alkaloids, carbohydrates, saponins, glycosides, proteins, amino acids, phytosterol, flavonoids and terpenoids. Noshad et al. (2018) reported that *Cariaca papaya* leaf extract exhibited antimicrobial activity and secondary metabolites which were effective against various fungal diseases. The secondary metabolites play an essential role in improving plants resistance to fungal diseases. Flavonoid compounds have been identified as an effective antifungal agents against a vast array of disease pathogens (Salazar-Aranda et al., 2015; Yang et al., 2017; Mohotti et al., 2020) and serve vital roles in the plant such as cell signaling, plant growth, UV photo-protection, reproduction and transfer of auxin (Buer and Muday, 2004). With regards to alkaloids, it is known to have a wide range of antimicrobial activity which plays a major role in plant physiology and host-plant resistance (Dheeb, 2015). Apart from the secondary metabolites, *Carica papaya* leaf also contains nutrients such as protein, phosphorus, Vitamins B and E, calcium and sodium (Tewari et al., 2014). The composition of nutrients in papaya leaf has also been shown to significantly improve the yield and development of tomatoes (Singh et al., 2000).

## Conclusion

The study illustrates that three types of plant extracts, namely *Allium sativum* L., *Carica papaya*, *Azadirachta indica* can reduce the development of *A. solani* on tomatoes compared to the control treatment. In this case, the application of *Carica papaya* leaf extract had antifungal properties that led to significant improvements in multiple parameters compared to control conditions and treatments with the other two plant extract in some cases and was the most effective method to control *A. solani*. Therefore, this preliminary study warrants further investigation by isolation and characterization of specific compounds from plant extracts.

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