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# Evaluation of Essential Oils Against Potato Late Blight (*Phytophthora infestans* (Mont.) de Bary) at Holleta, Ethiopia

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ABSTRACT

This study was conducted to evaluate essential oils from *Croton macrostachyus* Hochst. ex Delile, *Eucalyptus globulus Labill.*, *Allium sativum* L., *Cymbopogon citratus* (DC.) Stapf, *Cymbopogon martini* (Roxb.) W.Watson, *Rosmarinus officinalis* L. and *Thymus schimperi* Ronniger using four concentrations (l mL, 2 mL, 3 mL, 4 mL) and three sprays against potato (*Solanum tuberosum* L.) late blight disease. Two improved potato varieties were planted using randomized complete block design with factorial combinations in three replications. Analysis of disease incidence means at 53 and 60 days after planting showed highly significant differences between varieties and among sources of essential oils. Furthermore, the combined effect of essential oils and potato varieties on disease severity showed significant differences. Disease severity was maintained from 25% to 48% (*Jalene*); 13% to 30% (*Gudene*) with essential oils while it was maintained between 92% and 95% (*Jalene*); 37% to 38% (*Gudene*) for control plants. The lowest disease percentage of disease severity was obtained on plants treated with *A. sativum* for *Jalene* variety while on plants sprayed with *C. macrostachyus*, *E. globulus* and *T. schimperi* for *Gudene* variety. The lowest disease progress rate 0.198/day and 0.162/day as well as values of area under disease progress curve 228% days and 93% days were obtained for varieties *Jalene* and *Gudene*, respectively. Maximum reduction in mean disease severity was exhibited by garlic for varieties *Jalene* (28.3%) and *Gudene* (16.3%), as compared to the control (93.3% for *Jalene* and 37.5% for *Gudene*). Therefore, results indicated the potentials of essential oils to minimize effects of potato late blight disease. Communities involved in potato production could use the current results as base line information to find alternative management options for late blight disease which has no effect on human health and environment. However, further investigation is required on essential oils affordability and availability to farmers across different locations.

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## Introduction

Potato (*S. tuberosum*) has been produced in more than 160 countries in the world. It is the third most important crop in terms of consumption after wheat and rice (FAOSTAT, 2021). It provides high productivity and generates more food per unit area as well as per unit time for ensuring long-term food security than major cereals (Devaux et al., 2014). It contains all major and minor nutrients approximately in the same proportion in which they are required in balanced diet (Camire et al., 2009). Moreover, the ability to grow potatoes in a wide range of climates and their adoption by a broad range cultures has increased potato consumption worldwide (King and Slavin, 2013). The population of the world has been increasing and therefore, the global potato production needs to be increased to achieve food security (Devaux et al., 2020).

According to Pankhurst (1964), potato cultivation started in Ethiopia at 1858. Since then, potato has gradually become a staple crop for 155 years in which almost every household allocate a land fragments every year for achieving food security. Therefore, it is grown in many parts of the country with increasing by almost ten-fold in production area from 30,000 ha during 1970s to over 296, 000 ha in 2016. Despite its potential, however, the productivity remains low in Ethiopia due to late blight disease occurrence, among others (Gildemacher et al., 2009a). Late blight, caused by oomycete fungus *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases of potato worldwide which results in yield losses with estimated global costs of €9 billion per year (related to control and losses) (Haverkort et al., 2016).

It may cause destruction of the plant in 7-10 days when disease incidence is high and plants are unprotected (Nowicki et al., 2013). It occurs in areas of potato production and farmers failed to produce potato during the main rainy season without fungicide application (Shiferaw et al., 2011). For example, major losses in yield due to late blight were reported in Ethiopia (Bekele and Eshetu, 2008; Demissie, 2019). Late blight incidence without fungicides applications was reported as 76.25% (for white flowered cultivar) and 82.5% (in the case of cultivar *Agazer*) while for improved varieties like *Gudene* and *Jalene* as 58% and 50%, respectively, (Habetamu et al., 2012).

Hence, late blight is very common and widespread in the country wherever potato is grown with varying degrees of severity which can cause losses up to 100% (Ahmed et al., 2015). To overcome the constraints in potato production, research institutes have released different resistant improved varieties with major emphasis to wide adaptability, high tuber yield and late blight resistant (Getachew et al., 2016). Furthermore, fungicides are used as essential element in late blight management programs, despite the development of improved cultural practices, disease forecasting models and cultivation of resistant varieties (Teresa et al., 2014; Haesaert et al., 2015). However, due to unstable nature of *Phytophthora infestans* the resistances of released varieties could not stay for longer duration (Shiferaw et al., 2011). Due to these facts, farmers in Ethiopia like other potato growers are seen using fungicides as a preferred controlling strategy for late blight disease, among others. A fungicide as preferred method was reported by farmers of Shahemene (82-94%) and West Shewa (93-99%) to control late blight disease (Daniel et al., 2021). However, on the one hand, *P. infestans* has become resistant to many fungicides, as they have faster regeneration times, different metabolic pathways and high evolutionary potential than true fungi (Hung et al., 2015; Qin et al., 2016). On the other hand, chemical application is not always effective against pathogens and it has led to environmental pollution, pathogen resistance and increased risk for human health (De Curtis et al., 2010; Haverkort et al., 2014; Anna et al., 2015; Lamichhane et al., 2018). Even if there is no study which indicates the potential of health hazards due to fungicide application in Ethiopia, several authors reported the probability of high health risk in developing countries due to fungicide applications (Yanggen et al., 2004; Atreya et al., 2011). Due to this, it is timely endeavor for searching eco-friendly late blight disease management options that have no impacts on human health. Essential oils as alternative measures to fungicides that are likely to achieve sustainable control of late blight posing less severe challenges to environment and minimizing the risks associated with emerging fungicide-resistant strains of *P. infestans* (Lee et al., 2008; Bi et al., 2012; Majeed et al., 2015). Plant-based natural compounds like essential oils and plant extracts have been well-studied on many target pathogens (Rahman et al., 2010; Parveen et al., 2014; Amini et al., 2016; Dewitte et al., 2018; Mohammed et al., 2019; Mohammed et al., 2020; Sevindik et al., 2020). Different researches have also reported different quantities of essential oils to inhibit late blight disease. However, there is no optimum recommendation across different countries. Hence, evaluation of essential oils against potato late blight is important for finding alternative solutions in Ethiopia. Using essential oil to control plant disease has been investigated in different countries; however, it is not tested for potato in Ethiopia. Therefore, the main objectives of this study are: 1) to identify effects of essential oils against late blight, 2) to find optimum quantity at three frequencies against late blight symptoms. The research aims to find alternative management options for potato late blight disease which is eco-friendly and has no effects on human health.

## Materials and Methods

### *Description of the Study Area*

The field experiment was conducted at Holleta Ethio-AgriCEFT farm which is located at 37 km west of the capital city of Ethiopia, Addis Abeba. The area has an altitude of 2391 m a.s.l and known to have bimodal rainfall distribution which occurs in the two major seasons known as *Belg* (short rainy season from February to April) and *Meher* (long rainy season from June to September). The average annual rainfall is 1152 mm along with 7°C and 22°C annual minimum and maximum temperatures, respectively.

### *Experimental Materials*

Potato varieties *Gudene* (CIP-386423.13; relatively resistant to late blight) and *Jalene* (CIP-384321.19; susceptible) were planted on June 23, 2020 at Ethio-AgriCEFT farm. *Gudene* and *Jalene* are cultivars with wide-range of environmental adaptation and known to have been released in 2006 and 2002, respectively (Gebremedhin et al., 2008). Potato seed materials of the two cultivars were obtained from Holleta Agricultural Research Center, Ethiopia. Plants for sources of essential oil were selected based on the knowledge of their ethnobotanical uses and their previously demonstrated antifungal and antibacterial properties. Therefore, the plant material for extraction of essential oils was collected from Ethio-AgriCEFT farms located at Holleta and West Gojam (Liebir farm). Leaves of rosemary (*R. officinalis*) and thymus (*T. schimperi*) were collected from Ethio-Agriceft farm at Holleta while leaves of croton (*C. macrostachyus*) and eucalyptus (*E. globulus*) were collected near Holleta EthioAgriCEFT farm. Moreover, garlic (*A. sativum*) bulbs were obtained from farmers at Holleta while leaves of lemon grass (*C. citratus*) and palmarosa (*C. martini*) were obtained from Liebir farm. The identities of these species were authenticated at the National Herbarium of Ethiopia using the taxonomic literature, sample authenticated species and through assistance of taxonomic experts. All essential oils were extracted from fresh leaves using the method of hydrodistillation for 3hrs. However, fresh cloves were used for extraction of essential oil from garlic using similar methods. It is a conventional method which does not use organic solvents, but uses water and/or steam for extraction of the essential oils (Silva et al., 2005; Azmir et al., 2013). Then the essential oil was collected, dehydrated by $Na_2SO_4$ and stored at 4ºC in the refrigerator until used. The amount of essential oils obtained per 1000 g plant material used were 12.0 mL, 11.0 mL, 1.3 mL, 6.7 mL, 6.5 mL, 5.4 mL and 8.5 mL for croton, eucalyptus, garlic, lemongrass, palmarosa, rosemary and thymus, respectively.

***Experimental Design and Treatments***

The experiments were designed to determine the effects of essential oils in the management of late blight disease using potato varieties. The experimental units were laid down in randomized complete block design with factorial combinations in three replications. The spacing between plants and ridges were 40 cm and 70 cm, respectively. Each replication consisted of 8 plots in which 8 plants were planted in each plot. Four randomly selected plants were used from each plot for treatments.

Extracted essential oils from croton, garlic, eucalyptus, lemon grass, palmarosa, rosemary and thymus were applied using hand sprayer with four different quantities (1 mL, 2 mL, 3 mL, 4 mL per 150 mL water each) together with a control (without essential oil). The first application of essential oils was commenced as soon as first symptoms were identified on the leaves at 46 days after planting (DAP) and subsequent spraying were continued for consecutive three weeks within seven days intervals. In order to avoid the interference of one treatment to other, two plants were used as a border between treatments and plastic cover was used during spraying.

***Assessments of Late Blight Incidence and Severity***

Following identification of the first late blight symptom on the leaves, each plant within each plot was visually assessed for incidence (number of plants infected) and disease severity (percentage of leaves affected by *P. infestans*) within seven days intervals. Number of plants that showed symptoms of late blight was counted and the percentage of disease incidence (PDI) were calculated according to the formula by Wheeler (1969).

$$\text{PDI (\%)}=\frac{\text{Number of diseased plants}}{\text{Total number of plants inspected}}\times 100$$

Disease severity data was recorded using percent scale of Shouong et al. (2007). The severity grades were converted into Percentage Severity Index (PSI) according to the formula by Wheeler (1969).

$$\text{PSI (\%)}=\frac{\sum \text{individual numerical ratting}}{\text{TNPA}\times\text{MSS}}\times 100$$

Where;

TNPA: Total number of plants assessed

MSS: Maximum Score in the scale

***Area under Disease Progressive Curve***

To quantify of the disease development over time, the area under disease progress curve (AUDPC) was evaluated for each treatment by recording the disease severity at seven days intervals commencing at the first symptoms and lasting up to 74 DAP (days after planting). The effect of types and quantities of essential oils on disease severity data was integrated into area under disease progress curve (AUDPC), as described by Campbell and Madden (1990).

$$\text{AUDPC}=\sum_{i=1}^{n-1}\frac{(x_{i+1}+x_i)\,(t_{i+1}-t_i)}{2}$$

Where n is the total number of assessments, $t_i$ is the time of the $i^{th}$ assessment in days from the first assessment days, $x_i$ is percentage of disease severity at $i^{th}$ assessment. AUDPC was expressed in %-days because the severity (x) was expressed in percent and time (t) in days. The rates of disease progress in time were determined by recording the severity of late blight at seven days interval from the first appearance of disease symptoms (46 DAP) to the maximum disease severity values of susceptible unsprayed (without essential oils) plants recorded at 67 DAP.

***Disease Progress Rate***

Linear logistic, ln[(X/1-X)] and Gompertz, -ln[(X)] models (Campbell and Madden 1990) were compared for the estimation of disease progress rate (r) from each treatment and the logistic model was found fit to the data. The goodness of fit of the models was tested based on the values of the coefficient of determination ($R^2$). The transformed data of disease severity were regressed over time to determine the model. The model was then used to determine the disease progress rate and then subjected to further statistical analysis. Therefore, an estimate of the rate is obtained from the following formula:

$$r=\frac{\ln\left(\frac{X_f}{1-X_f}\right)-\ln\left(\frac{X_i}{1-X_i}\right)}{t}$$

Where r is disease progress rate, $X_i$ is initial disease severity, $X_f$ is final disease severity and t is the duration of the epidemic, ln refers to natural logarithm.

***Data Analysis***

Multivariate analysis and linear regression were analyzed using SPSS software. Furthermore, correlation analysis was performed to determine the association between the disease parameters (PSI, PDI, AUDPC, r).

## Results

Multivariate analyses indicated the existence of highly significant ($P<0.05$) differences between varieties, sources of essential oils and among concentration levels. The results indicated that essential oils inhibited the symptoms that occurred due to late blight pathogen. However, the essential oils from lemongrass and palmarosa caused wilting symptoms after essential oil application within a week and then wilting symptoms progress following second applications. Therefore, the data from lemongrass and palmarosa were not included in data analysis since plants lost their leaves. During application of essential oils, it was also noticed that essential oils have the potential to repel insects and for use as herbicides which trigger further investigation to verify the current observation. In order to explain the effects of essential oils against late blight symptoms, the results obtained from days to first symptom appearance, disease incidence and severity were analyzed in subsequent parts.

***Days to First Disease Symptom Appearance***

The first symptoms of late blight appeared on unsprayed susceptible potato variety (*Jalene*). Water-soaked spots on the edge of leaves marked the commencement of late blight symptoms in which there was variation in first disease symptom appearance between varieties. At 46 DAP (days after planting) symptoms were observed for succeptible potato variety and at 53 DAP for moderately resistant variety (*Gudene*). Disease appearance at 53 DAP, 60 DAP and 67 DAP showed significant differences (P<0.05) among sources of essential oils and quantities as well as between varieties.

Figure 1. Photograph showing potato leaves with symptom of necrosis after treatment with essential oil (Photo courtesy Daniel Wondimu, Holetta, Ethiopia, 2020).

The days for commencement of disease incidence for Gudene variety was delayed due to their nature of moderately resistant levels to late blight. Following applications of essential oils, symptoms of late blight either maintained as it was or increased in smaller rate and then remains as necrosis relative to unsprayed plants at all levels for both varieties as indicated in Figure 1 as an example. Therefore, essential oils showed the ability to hinder the growth of the pathogen on the leave. Moreover, plants sprayed with essential oils from croton, eucalyptus, garlic, rosemary and thymus showed thick and healthy leaves relative to the control plants (without essential oils) as indicated from Figure 2a to f.

***Disease Incidence***

In both varieties, the effects of essential oils on late blight disease incidence showed similar results. It was assesed four times (at 46, 53, 60 and 67 days after planting). At first evaluation, 67% and 33% of the plants were found infected in control plants for *Jalene* and *Gudene* plants, respectively (Table 1). There after proportion of infected plants pogressed with increase in day after planting, reaching to a mximum (100%) at 53 and 60 DAP for varieties *Jalene* and *Gudene*, respectively. During disease evaluation of *Jalene* variety, high disease incidence (100%) was found in plants without essential oil and on plants treated with rosemary and thymus while disease incidence value of 67% was found on plants treated with garlic followed by plants treated by both croton and eucalyptus (75%) at 53 DAP (Table 2). Similarly, during disease evaluation of *Gudene* variety, high disease incidence (100%) was found on plants without essential oil and on plants treated with rosemary and thymus while disease incidence value of 67% found on plants treated with both eucalyptus and garlic followed by plants treated with croton (84%) at 60 DAP (Table 2). Multivariate analysis tests showed that there was highly significant difference (P<0.05) between varieties, among types of essential oils and non significant difference among quantities. Furthermore, analysis of disease incidence means at 53 DAP and 60 DAP showed highly significant differences (P<0.05) between varieties and among types of essential oils (Table 3).

Table 1. Rating scale for the assessment of late blight disease (Shutong et al. 2007)

| Disease severity rating grade | Disease incidence % | Level of resistance/Susceptibility |
|---|---|---|
| 0 | 0 | No disease lesion |
| 1 | 10 | Small lesions with area of <11% on whole leaflet |
| 3 | 10 to 20 | Lesion area between 10% and 21% on whole leaflet |
| 5 | 20 to 30 | Lesion area between 20% and 31% on whole leaflet |
| 7 | 30 to 60 | Lesion area between 30% and 61% on whole leaflet |
| 9 | 60 | Lesion area above 60% of whole leaflet |

Table 2. Disease incidence means (%) for essential oils applications

| Variety | Dates | Croton | Eucalyptus | Garlic | Rosemary | Thymus | Control |
|---|---|---|---|---|---|---|---|
| Jalene | 46 DAP | 67 | 67 | 67 | 67 | 67 | 67 |
| | 53 DAP | 75 | 75 | 67 | 100 | 100 | 100 |
| | 60 DAP | 100 | 100 | 100 | 100 | 100 | 100 |
| | 67 DAP | 100 | 100 | 100 | 100 | 100 | 100 |
| Gudene | 46 DAP | 0 | 0 | 0 | 0 | 0 | 0 |
| | 53 DAP | 0 | 0 | 0 | 0 | 0 | 33 |
| | 60 DAP | 84 | 67 | 67 | 100 | 100 | 100 |
| | 67 DAP | 100 | 100 | 100 | 100 | 100 | 100 |

DAP=days after planting, application of essential oils commenced at 46 DAP after the data of disease incidence taken. Data at 46 DAP showed prior to essential oils application.

Table 3. Analysis of disease incidence means (%) within seven days interval

| Source | Incidence at | Mean square | F | Sig. |
|---|---|---|---|---|
| Varieties | 46 DAP | 16.67 | 120.05 | .000 |
| | 53 DAP | 23.36 | 240.29 | .000 |
| | 60 DAP | 0.69 | 10.00 | .002 |
| | 67 DAP | 0.01 | 1.00 | .320 |
| Types of essential oils | 46 DAP | 0.17 | 1.25 | .292 |
| | 53 DAP | 0.37 | 3.77 | .004 |
| | 60 DAP | 0.16 | 2.32 | .049 |
| | 67 DAP | 0.01 | 1.00 | .422 |
| Quantities | 46 DAP | 0.01 | 0.05 | .985 |
| | 53 DAP | 0.03 | 0.29 | .836 |
| | 60 DAP | 0.01 | 0.13 | .940 |
| | 67 DAP | 0.01 | 1.00 | .396 |

(a) Treated with croton (b) Treated with eucalyptus (c) Treated with garlic

(d) Treated with rosemary (e) Treated with thymus (f) Control

Figure 2. Photographs showing evaluation of essential oils from croton (a), eucalyptus (b), garlic (c), rosemary (d) and thymus (e) relative to plants without essential oil (f) against late blight symptoms on Gudene potato variety (Photo courtesy Daniel Wondimu, Holetta, Ethiopia, 2020). The data from lemongrass and palmarose were excluded since plants lost their leaves after spraying.

***Disease Severity***

The combination effect of essential oils and potato varieties on disease severity showed a significant difference (P<0.05) among the types of essential oils used whereas no significant difference was observed among the quantities applied. Moreover, multivariate analysis of disease severity data per seven days interval indicated significant difference for the period of essential oil applications (Table 4). The lowest PSI (percentage of severity index) (55.6%) was found on plots treated with garlic followed by eucalyptus and thymus (73.3%) for *Jalene* variety whereas the lowest value of PSI (55.6%) for *Gudene* variety was found on plants treated with croton, eucalyptus and thymus followed by garlic (66.7%) at 67 DAP. At all treatments, highly significant differences (P<0.05) in PSI were obtained among plots treated with all levels of essential oil in the moderately resistant variety (*Gudene*). At 67 DAP, a reduction in late blight severity was found in both varieties at different quantities applied relative to control plants. Among these essential oils, maximum reduction in mean disease severity for *Jalene* variety was exhibited by garlic (28.3%) followed by rosemary (36.3%), eucalyptus (41.3%) and thymus (42.3%) while for *Gudene* variety maximum reduction in disease severity was exhibited by garlic (16.3%) followed by thymus (21.7%), croton (22.9%) and eucalyptus (23.8%) as compared to control (93.3% for *Jalene* and 37.5% for *Gudene*).

***Evaluation of Essential Oils Quantity on Late Blight Symptoms on Potato Varieties***

Four concentrations of essential oil (1 mL, 2 mL, 3 mL and 4 mL) were examined to study their effects on the symptoms of *P. infestans* as seen on potato leaves under field condition. They showed better protection for late blight disease symptoms relative to plants grown without essential oil for both varieties. For *Jalene* variety at 74 DAP, the minimum disease severity (33%) was obtained from plants sprayed with garlic essential oil at 1 mL followed by rosemary (45%) while the maximum disease severity (93%) was obtained from plants without addition of essential oil (Figure 3a). At 2 mL, the minimum disease severity (28%) was obtained for plants sprayed with garlic essential oil followed by rosemary (35%) and then by thymus (47%); however, disease severity of 92% was obtained for plants grown without essential oil (Figure 3b).

At 3 mL, the minimum disease severity was 27% for plants sprayed with garlic essential oil followed by rosemary (35%) and then by croton (37%); however, disease severity of 95% was obtained for plants without essential oil (Figure 3c). At 4 mL, the minimum disease severity was 25% for plants sprayed with garlic essential oil followed by both eucalyptus and rosemary (30%); however, disease severity of 93% was obtained for plants without essential oil (Figure 3d). Thus results showed that all quantities of essential oils showed lower value of disease severity relative to the control.

Table 4. Analysis of disease severity means (%) within seven days interval

| Source | Severity at | Mean square | F | Sig. |
|---|---|---|---|---|
| Varieties | 46 DAP | 416.84 | 120.05 | .000 |
| | 53 DAP | 2541.84 | 375.41 | .000 |
| | 60 DAP | 5196.01 | 446.70 | .000 |
| | 67 DAP | 8326.56 | 599.51 | .000 |
| Types of Essential oils | 46 DAP | 4.34 | 1.25 | .292 |
| | 53 DAP | 345.73 | 51.06 | .000 |
| | 60 DAP | 1459.34 | 125.46 | .000 |
| | 67 DAP | 4518.64 | 325.34 | .000 |
| Quantities | 46 DAP | .17 | .05 | .985 |
| | 53 DAP | 20.08 | 2.97 | .036 |
| | 60 DAP | 42.30 | 3.64 | .016 |
| | 67 DAP | 236.75 | 17.05 | .000 |

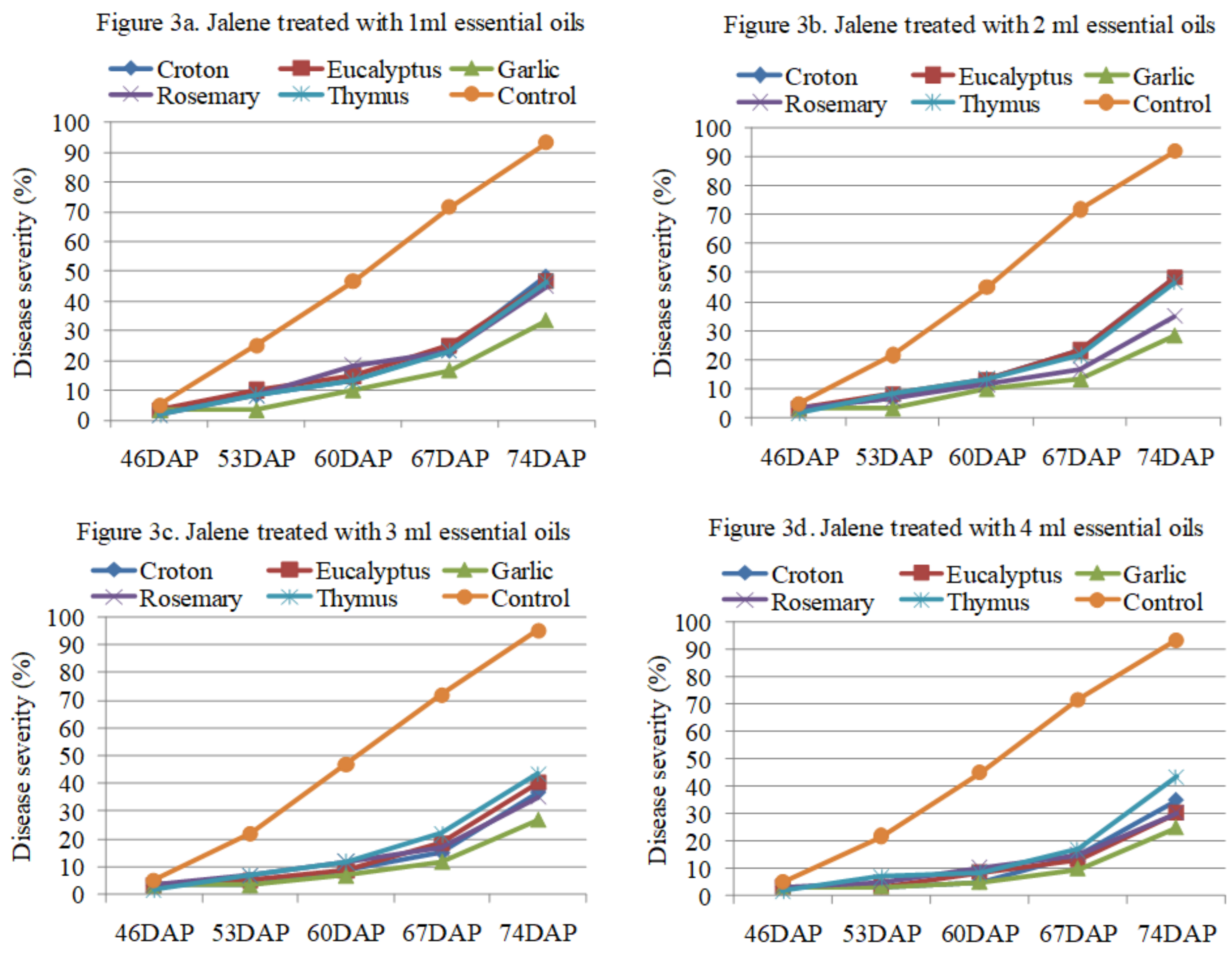


Figure 3. Temporal late blight (P. infestans) disease progress starting from 46 DAP (days after planting) up to 74 DAP for Jalene potato variety treated with different amounts of essential oils (1 mL, 2 mL, 3 mL, 4 mL) from croton, eucalyptus, garlic, rosemary, thymus in comparison with control (a to d).

When applications of essential oils were interrupted after three sprays at 67 DAP, the plants showed the disease severity increment from 15% to 27% for *Jalene* potato variety. However, the disease severity increments from 20% to 23% were found on plants without essential oil at 74 DAP. The disease severity was maintained between 25% and 48% on plants sprayed with essential oils while the disease severity was between 92% and 95% for plants without essential oil following the commencement of the first disease symptoms up to 74 DAP (Figure 3a to d).

For *Gudene* variety at 74 DAP, the maximum disease severity (38%) was obtained for plants without essential oils at all quantities. At 1 mL, the minimum disease severity (20%) was obtained from garlic essential oil followed by *Thymus* (22%) and then by *Eucalyptus* (27%) (Figure 4a). At 2 mL, the minimum disease severity was 17% for plants sprayed with garlic essential oil followed by thymus (22%) and then by both croton and eucalyptus (23%) (Figure 4b). At 3 mL, the minimum disease severity was 15% for plants sprayed with garlic followed by both *Eucalyptus* and *Thymus* (22%) (Figure 4c). At 4 mL, the minimum disease severity (13%) was obtained for plants sprayed with garlic followed by both croton and rosemary (18%) (Figure 4d).

When applications of essential oils were interrupted after three sprays at 67 DAP, plants showed the disease severity increment from 10% to 17% for *Gudene* potato variety. However, the disease severity from 13% to 17% was obtained on plants without essential oil at 74 DAP. Therefore, disease severity was maintained between 13% and 30% on plants sprayed with essential oils while the disease severity between 37% and 38% was found for plants without essential oil following the commencement of the first disease symptoms up to 74 DAP (Figure 4a to d).

Thus, the results indicated that the effects of essential oils quantities on disease severity varied between varieties. Furthermore, almost all essential oils showed a decreasing trend in disease severity as the amount of essential oils increased from 1 mL to 4 mL.

***Disease Progress Rate (r)***

Comparisons of disease progress rate (r) among the types of essential oils and the quantities of essential oils used were based on logistic model. For *Jalene* variety, disease progress rate was highest on plants without essential oil (0.589/day) while the minimum disease progress rate was found on plants sprayed with 4 mL rosemary (0.198/day) followed by plants sprayed with 2 mL and 3 mL garlic with disease progrss rate of 0.200/ day and 0.203/day, respectively (Table 5). In comparison with the type of essential oils, the minimum disease progress rate was found on plants sprayed with rosemary (0.198/day) followed by garlic (0.200/day). For *Gudene* variety, disease progress rate was highest (0.235/day) on plants sprayed with 4 mL garlic. Whereas minimum disease progress rate (0.162/day) was found on plants sprayed with 2 mL eucalyptus (Table 6). In comparison with the types of essential oils, the minimum disease progress rate was found on plants sprayed with eucalyptus (0.162/day) followed by croton (0.174/day) for *Gudene* variety.

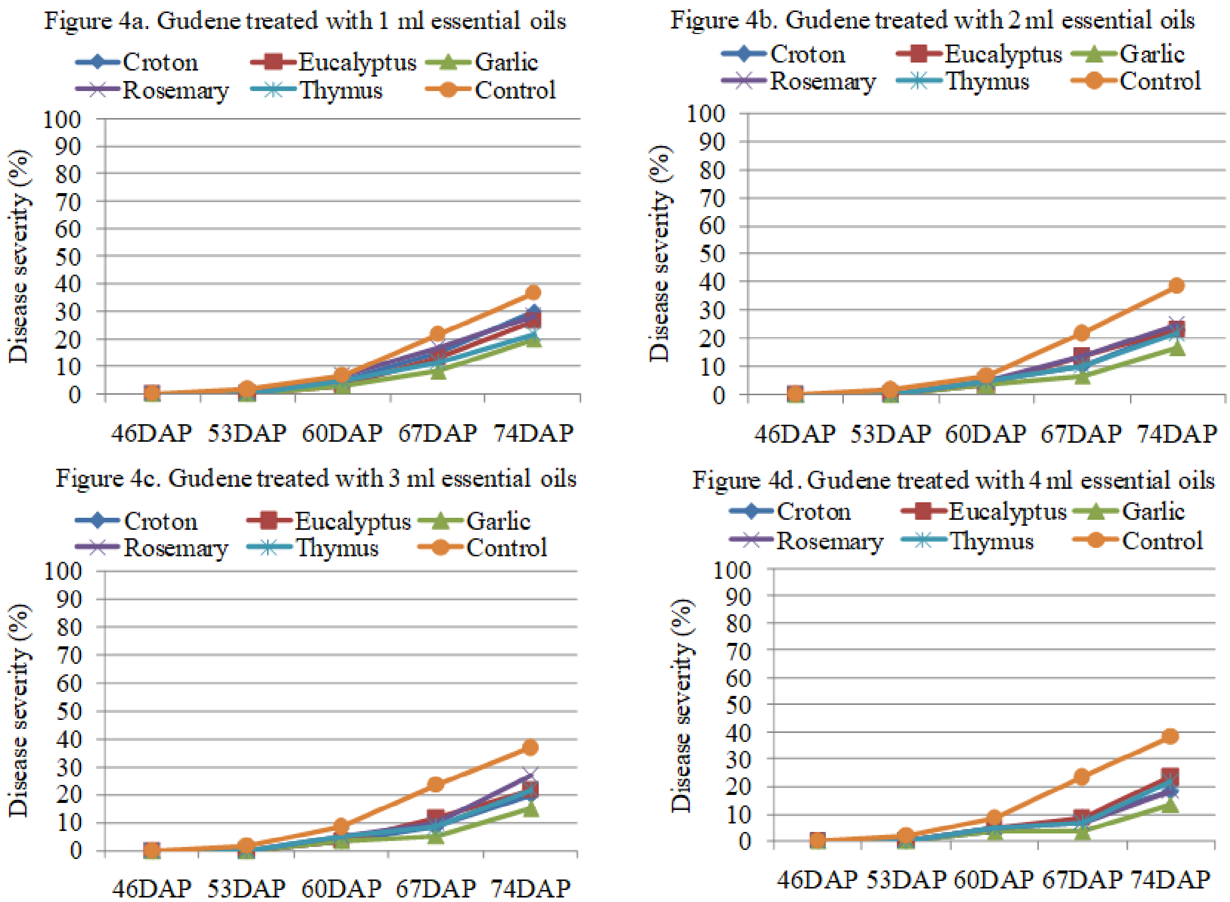


Figure 4. Temporal progress of late blight (P. infestans) starting from 46 DAP (days after planting) up to 74 DAP for Gudene potato variety (a to d) with different amounts of essential oils (1 mL, 2 mL, 3 mL, 4 mL) from croton, eucalyptus, garlic, rosemary, thymus in comparison with the control.

Table 5. Area under disease progress curve (AUDPC) in %-days and disease progress rate per day (r) for Jalene potato variety

| Source of essential oil | Quantity (mL) | AUDPC up to 74 DAP | Rank | r at 74 DAP | Rank |
|---|---|---|---|---|---|
| *C. macrostachyus* Croton | 1 | 496 | 17 | 0.262 | 17 |
| | 2 | 484 | 16 | 0.262 | 17 |
| | 3 | 327 | 6 | 0.239 | 11 |
| | 4 | 298 | 4 | 0.230 | 8 |
| *E. globulus* Eucalyptus | 1 | 525 | 20 | 0.245 | 14 |
| | 2 | 496 | 17 | 0.262 | 17 |
| | 3 | 373 | 9 | 0.239 | 10 |
| | 4 | 292 | 3 | 0.211 | 4 |
| *A. sativium* Garlic | 1 | 338 | 8 | 0.233 | 9 |
| | 2 | 298 | 4 | 0.200 | 2 |
| | 3 | 257 | 2 | 0.203 | 3 |
| | 4 | 228 | 1 | 0.209 | 4 |
| *R. officinalis* Rosemary | 1 | 513 | 19 | 0.243 | 13 |
| | 2 | 379 | 10 | 0.219 | 6 |
| | 3 | 379 | 10 | 0.219 | 6 |
| | 4 | 327 | 6 | 0.198 | 1 |
| *T. shimperi* Thymus | 1 | 484 | 15 | 0.252 | 15 |
| | 2 | 473 | 14 | 0.260 | 16 |
| | 3 | 438 | 13 | 0.241 | 12 |
| | 4 | 379 | 10 | 0.353 | 20 |
| Control (Water) | 1 | 1348 | 24 | 0.545 | 22 |
| | 2 | 1307 | 21 | 0.511 | 21 |
| | 3 | 1330 | 23 | 0.589 | 24 |
| | 4 | 1313 | 22 | 0.545 | 22 |

Table 6. Area under disease progress curve (AUDPC) in %-days up to 67 DAP and disease progress rate per day (r) for Gudene potato variety

| Source of essential oil | Quantity (mL) | AUDPC up to 74 DAP | Rank | r at 74 DAP | Rank |
|---|---|---|---|---|---|
| *C. macrostachyus* Croton | 1 | 245 | 19 | 0.198 | 14 |
| | 2 | 187 | 13 | 0.196 | 13 |
| | 3 | 152 | 6 | 0.183 | 9 |
| | 4 | 145 | 4 | 0.174 | 4 |
| *E. globulus* Eucalyptus | 1 | 210 | 17 | 0.188 | 8 |
| | 2 | 198 | 15 | 0.162 | 1 |
| | 3 | 181 | 11 | 0.164 | 2 |
| | 4 | 175 | 10 | 0.217 | 21 |
| *A. sativium* Garlic | 1 | 152 | 7 | 0.189 | 10 |
| | 2 | 128 | 3 | 0.185 | 6 |
| | 3 | 111 | 2 | 0.203 | 17 |
| | 4 | 93 | 1 | 0.235 | 24 |
| *R. officinalis* Rosemary | 1 | 263 | 20 | 0.186 | 7 |
| | 2 | 216 | 18 | 0.190 | 11 |
| | 3 | 198 | 15 | 0.221 | 22 |
| | 4 | 146 | 5 | 0.201 | 16 |
| *T. shimperi* Thymus | 1 | 193 | 14 | 0.164 | 2 |
| | 2 | 181 | 11 | 0.182 | 5 |
| | 3 | 169 | 9 | 0.204 | 19 |
| | 4 | 158 | 8 | 0.231 | 23 |
| Control (Water) | 1 | 338 | 21 | 0.201 | 15 |
| | 2 | 344 | 22 | 0.211 | 20 |
| | 3 | 362 | 23 | 0.193 | 12 |
| | 4 | 368 | 24 | 0.203 | 18 |

***Area under Disease Progress Curve (AUDPC)***

The cumulative AUDPC values up to 74 DAP were calculated from mean disease severity values in which they showed variation between varieties (Figure 5). There was no significant difference among the quantities of essential oils (1 mL, 2 mL, 3 mL and 4 mL) whereas there was highly significant difference in the value of AUDPC among types of essential oils for both varieties. For *Jalene* variety, the maximum value of AUDPC (1330% days) was found on plants without essential oil while the minimum value (228% days) was found on plants sprayed with 4 mL garlic essential oil (Table 5). For *Gudene* variety, the maximum value of AUDPC (368% days) was found on plants without essential oil while the minimum value (93% days) was found on plants sprayed with 4 mL garlic essential oil (Table 6). In both varieties, the maximum values of AUDPC were found on plants without essential oil (control) while the minimum values were found on plants sprayed with garlic.

***Correlation Analysis***

The correlation analysis among percentage of severity index (PSI), percentage of disease incidence (PDI), area under disease progress curve (AUDPC) and disease progress rate (r) showed variability between varieties. The correlation among disease parameters was varied for both varieties. For *Jalene* variety, PSI showed weak association with AUDPC (0.157) and r (0.162) (Table 7). However, very strong association was observed between AUDPC and r (0.966). Moreover, there was negligible association between PSI and PDI, PDI and AUDPC as well as between PDI and r. For *Gudene* variety, PSI showed moderate association with AUDPC (0.423) while weak association between PSI and r, PDI and AUDPC and r (Table 8). However, there was negligible association between PSI and r, PDI and AUDPC as well as between PDI and r.

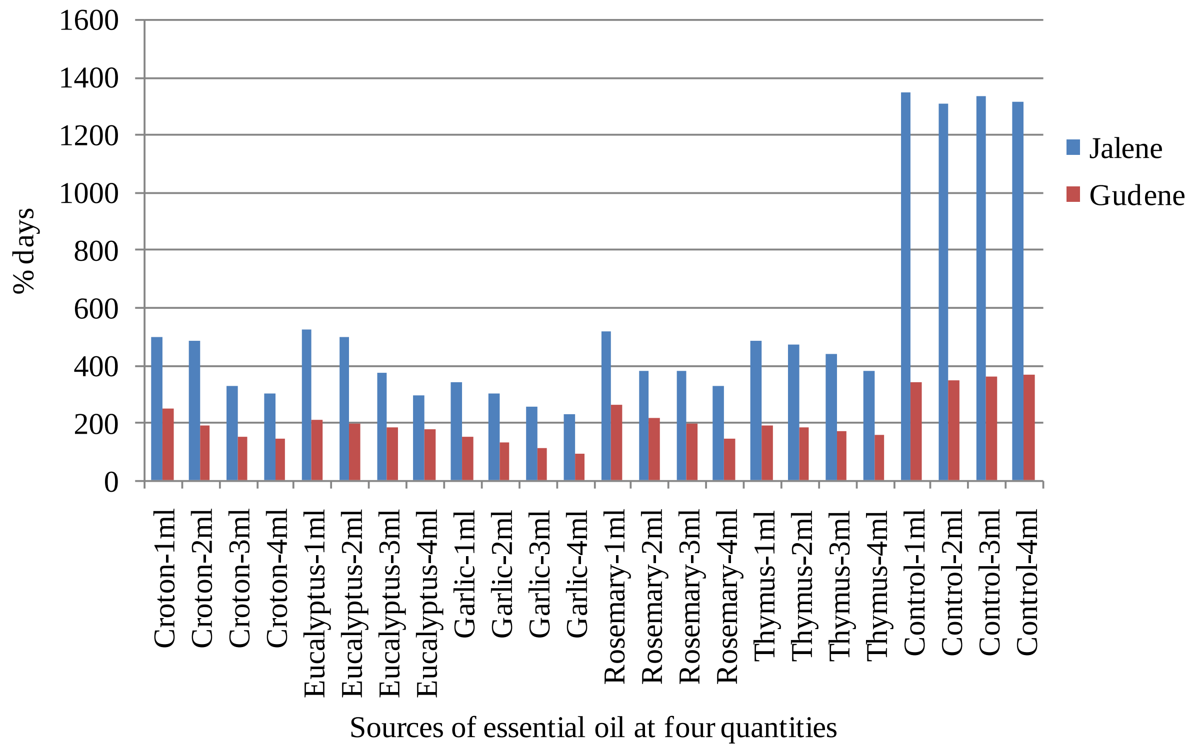


Figure 5. Comparison of the values of area under disease progress curve (AUDPC) for Jalene and Gudene potato variety for different essential oils at four quantities (1 mL, 2 mL, 3 mL, 4 mL) against late blight disease relative to plants without essential oil (control).

Table 7. Correlation among disease parameters for Jalene potato variety

| | PSI | PDI | AUDPC | r |
|---|---|---|---|---|
| PSI | 1 | | | |
| PDI | 0.0 | 1 | | |
| AUDPC | $0.157^{NS}$ | 0.0 | 1 | |
| r | $0.162^{NS}$ | 0.0 | $0.966^{*}$ | 1 |

$^{NS}$= no significant difference , $^{*}$ significant difference

Table 8. Correlation among disease parameters for Gudene potato variety

| | PSI | PDI | AUDPC | r |
|---|---|---|---|---|
| PSI | 1 | | | |
| PDI | 0.0 | 1 | | |
| AUDPC | $0.423^{*}$ | 0.0 | 1 | |
| r | $0.056^{NS}$ | 0.0 | $-0.215^{NS}$ | 1 |

$^{NS}$= no significant difference , $^{*}$ significant difference

**Discussion**

Late blight, caused by *P. infestans* has been controlled using fungicides. However, application of fungicides raised the issue of health and environmental hazards which demands alternative management options. Essential oil and its major component had antifungal activity against plant pathogenic fungi (Plaza et al., 2004; Angelini et al., 2006; Terzi et al., 2007). In the current study, it was evidenced that essential oils from croton, garlic, eucalyptus, rosemary and thymus minimize the symptoms of potato late blight disease which indicates their antifungal properties. Essential oils from *E. globulus*, *T. schimperi* and *R. officinalis* are recognized as a promising antifungal agent due to the presence of 1,8-Cineol, carvacrol and α-Pinene at high concentration, respectively (Pinto et al., 2006; Vilea et al., 2009; Awol et al., 2016). Similarly, essential oils from *C. macrostachyus* and *A. sativum* are recognized as a promising antifungal agent due to the presence of linalool and diallyl trisulfide compounds, respectively (Yinebeb et al., 2010; Satyal et al., 2017). Therefore, the current results provide baseline information for potential field application of essential oils.

***Days to first Symptoms Appearance and Disease Incidence***

Effects of essential oils on late blight pathogen progress depend on varieties. This may be due to the nature of their disease resistance levels in which susceptible variety showed the symptoms earlier. However, the values of disease incidence were similar for both varieties in which the maximum value of incidence obtained within two disease assessments following the commencement of first symptom. The highest values of disease incidence (100%) were found in control plants. This result is in line with the previous reports in which the maximum disease incidence of 91.5% reported for unsprayed *Jalene* plants (Ashenafi et al., 2017; Admasie et al., 2021). Similarly, the maximum disease incidence of 80% reported for susceptible local potato cultivar (Shiferaw & Tesfaye, 2018).

***Disease Severity***

Disease severity index is a number summarizing large information on disease severity (Chaube and Singh, 1991) to determine the effect of treatments for disease control (Nsabiyera *et al*., 2012). Therefore, the data of disease severity and its index were used to explain the effect of essential oils on late blight disease symptoms. For both varieties, essential oils showed the potential of minimizing the disease severity. However, the values for the disease severity were higher for the susceptible variety (*Jalene*) with the same amount and frequencies. This may be due to the requirements of more number of essential oil applications since previous reports indicated that susceptible variety requires more fungicide spraying frequencies (Kirk et al., 2001). Therefore, the current study showed that the response of disease severity to essential oil applications varies according to potato variety resistance to late blight disease. But, it is indicated that all essential oils were able to minimize occurrence of disease severity relative to the control plants for both varieties. This may be due to inhibitory effects of bioactive compounds in essential oils (Rahman et al., 1991; Soylu et al., 2006; Bi et al., 2012).

***Disease Progress Rate***

The current study revealed that essential oils could minimize late blight disease progress rate. The maximum disease progress rate was found on plants without essential oils for both varieties at all quantities which indicated that late blight disease progress was faster on plants without essential oils. Moreover, it was faster on *Jalene* variety relative to *Gudene* variety. This variation may be due to the variable resistance of varieties and their responses to the types of essential oils used. Similar results were reported during fungicide applications for controlling of late blight disease by other researchers (Shiferaw and Tesfaye, 2018; Admasie et al., 2021).

***Area under Disease Progress Curve***

Area under disease progress curve (AUDPC) is generally used to compare among treatments (Xu, 2006) and to evaluate the resistance of plant species to a pathogen (Mikulova et al., 2008). Jerger (2004) indicated that comparison of AUDPC among treatments is the most commonly used tool for evaluating practical disease management strategies. The current results revealed that the value of AUDPC varied with potato varieties and types of essential oils used. It means that a response of individual varieties to application of essential oils is variable. Thus, values of AUDPC include initial disease severity, the rate parameter and the duration of the epidemics which determines the final disease severity (Madden et al., 2008). Hence, the values of AUDPC used to evaluate the effect of essential oils on late blight disease progress and to select potential plants for sources of essential oil. During the current study the values of AUDPC were 1330% days and 368% days on plants without essential oils for *Jalene* and *Gudene* varieties, respectively. Lower value of AUDPC was found relative to the values reported as 1725.57% days and 1395.33% days for untreated *Jalene* and *Gudene* potato varieties, respectively (Admasie et al., 2021). Moreover, the values of AUDPC for untreated plants on *Jalene* and *Gudene* varieties reported as 2457.5% days and 480% days, respectively (Ashenafi et al., 2017). Similar authors reported that the value of AUDPC as 442.5% days and 268.5% days for *Jalene* and *Gudene* potato varieties sprayed with fungicides, respectively. Therefore, the values of AUDPC may vary for similar potato variety depends on disease severity data for specific experiment. In line with previous reports, the plants treated with essential oils showed lower AUDPC values relative to plants without essential oils except for rosemary essential oil. The results obtained indicated that overall disease progress was significantly influenced by essential oils for both varieties.

Different researchers have reported antifungal potential of essential oils from *E. camaldulensis* against *Fusarim solani* (Bashir & Tahira, 2012), *T. vulgaris* against *Botrytis cinerea* (Vitoratos et al., 2013), *Thyme* spp. against *F. solani* (Tejeswini et al., 2014), *E. globulus* against *Alternaria solani* (Tomazini et al., 2017) in tomato. Moreover, it is reported that essential oil from garlic effectively inhibited *B. cinerea* in-vitro grown on media (Daniel et al., 2015). This confirms that the potential of essential oils in effectively reducing the late blight disease symptoms. Similarly, the essential oils studied in the current experiments showed the inhibitory effects for late blight disease symptom. However, the tendency of disease progress increment was observed following the interruption of third spray. This result suggested the need for additional essential oil applications. Furthermore, it has been demonstrated through many studies that the response of a specific phytopathogenic fungus in contact with essential oil was highly variable from one essential oil to another. For example, *B. cinerea* is inhibited by essential oil from black caraway and fennel, but not from peppermint (Aminifard and Mohammadi, 2012). Based on the current results, essential oils from thymus, garlic, croton and eucalyptus could be suggested as potential alternative options to control late blight disease. However, the quantities used in the current study didn't show significant difference in contrary with the previous findings which stated that inhibition of essential oils against *P. infestans* growth were quantity dependent (Soylu et al., 2006). Therefore, further investigation on effects of essential oils quantities is required.

The capacity of essential oils to inhibit fungal growth has been reported (Da Cruzet et al., 2013; Sivakumar and Bautista-Banos, 2014). The compounds in essential oils may have several invasive targets for inhibition of different physiological processes, such as attacking cell walls and cell membranes that result in affecting the permeability and release of intracellular constituents, in addition to interfering with membrane functions (Al-Reza et al., 2010). The properties of essential oil components may help the oil to penetrate the plasma membrane, affect enzymatic activities and deregulate cell wall synthesis (Rasooli et al., 2006). Therefore, essential oils may be promising natural substitutes to synthetic fungicides in organic production systems (Dewitte et al., 2018; Han et al., 2019).

## Conclusion

Late blight may cause destruction of the whole plant in just a few days unless it is protected. The current study revealed that essential oils could reduce symptoms due to late blight disease. Especially, essential oils from garlic, thymus, croton and eucalyptus showed promising potential to combat late blight disease Thus, the results provide information on the potential of essential oils found in widely cultivated and wild plants as effective botanical treatments of the potato late blight in the field. However, the use of these compounds needs further investigation across different locations and varieties. Moreover, since the compositions of extracted essential oils depend on the methods of extraction, different methods of essential oil extraction should be tested. Since Ethiopia has hundreds of endemic plant species that can be used as potent sources of essential oils, it will be a good opportunity to develop alternative management options to control late blight disease on potato and others crop attacked by this disease. However, its affordability and availability by farmers should be investigated.

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