

Different Plant Extracts against *Phytophthora infestans* (Mont.) de Bary in Tomato *in Vitro*

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Abstract

The objective of this study was to evaluate the efficacy of plant extracts in managing late blight disease in tomato, *in vitro*. Crude extracts were from *Plectranthus barbatus*, *Tephrosia vogelii*, *Sphaeranthus suaveolens* and *Lantana camara*. These were compared with commercial formulations Otiva fungicide and untreated as negative control. Their effectiveness was determined by measuring the inhibition zone of the mycelial growth of the pathogen recorded in triplicate at 48 hours and 72 hours. The results showed significant differences ($P \leq 0.001$) among the extracting solvents on percentage inhibition of *Phytophthora infestans*. Methanol was superior in inhibiting the growth of mycelial growth of *P. infestans* as compared with ethyl acetate. Furthermore, all plants tested showed antifungal activity against *P. infestans*. The *P. barbatus*, *L. camara* and *S. suaveolens* were comparable with the commercial fungicide in inhibiting the growth of *P. infestans*. In this study, *T. vogelii* extract showed poor results in inhibiting the mycelial growth of *P. infestans* as compared with other plant extracts. Also, it was observed that, there were significant ($P < 0.05$) interactive effects between solvent and plant extracts and between incubation time and plant extracts.

Keywords

Tomato, Late Blight Disease, Plant Extracts, Inhibitionpercentage

1. Introduction

Tomato is one of the most important vegetables in the world grown for its different use, fresh market and processing industries [1]. Nutritionally, the crop is a

major source of lycopene powerful anti-oxidant, vitamins such as vitamin A, B, C, D, E, K and mineral nutrients mainly potassium and phosphorus [2]. Tomato grown for fresh tomato is used in making different products such as salads, sauces, stews and puree among others while the processed tomato is only for value addition such as pastes. In tropical Africa, tomato is a major source of income due to its growth cycles; that is, most varieties of tomato have a shorter maturity period and can be harvested more than four times a year [3]. In Tanzania, tomatoes are grown commercially mainly by small scale farmers as a source of income and livelihood [4].

Despite its economic importance, tomato is susceptible to a wide range of diseases such as bacteria [5], fungi [6], viruses [7] and nematodes [8] among others which adversely affect quality, quantity and profitability. Of these diseases, the fungal disease like late blight disease caused by *Phytophthora infestans* (Mont.) de Bary is among the economically important diseases which reduce the quality and quantity of the tomato yield and the losses can go up to 100% [6] [9]. The disease causes loss in terms of reduced yield, poor quality of fruits and diminished storability [10]. The late blight disease-causing-pathogen is seriously deadly to tomato due to its biology (sexual and asexual reproduction), host range (more than 20 hosts), dispersal mechanism and persistence for a long period of time in the soil [11]. In Tanzania, the average tomato yield is 17.5 tons/ha which is far below the global average yield of 33.6 tons/ha.

Farmers rely on the use of synthetic fungicides to manage late blight diseases of tomato by a combination of protective and curative synthetic fungicides yet the problem is still the challenge [12] [13]. In an effort to meet market demands of tomato, farmers have resorted to continuous use of synthetic pesticides. However, there is a growing concern about toxicity of synthetic pesticides due to retention of their residues in the food products [14]. Synthetic fungicide has negative effects on the environment because of its poor biodegradability leading to pollution, health hazards to the farmers, toxicity to non-target natural enemies, and loss of biodiversity among others [12]. In addition, in the market there is the issue of maximum residue levels (MRLs) of pesticides to the vegetables which disqualify the product with respect to quality standards or requirements in the market [15]. Therefore, integrated crop management such as the use of cultural practices (rogueing), biological practices (fungus) and chemical fungicides as the last option is important in the cropping systems for disease management [16]. Therefore, introduction of bio-pesticides in tomato production systems will reduce the risks associated with the use of synthetic chemicals [16].

Bio-pesticides are non-toxic, easily biodegradable, and safe to non-targets and natural enemies and do not retain residues in the food products [17]. Moreover, some plants with fungicidal activity can inhibit *Phytophthora infestans* making them even better alternatives to synthetic pesticides for sustainable agriculture [15]. The objective of this study was to evaluate the *in vitro* activity and effectiveness of selected plant extracts on *P. infestans* infecting tomato.

2. Materials and Methods

2.1. Isolation and Maintenance of *Phytophthora infestans* of Tomato

Leaves of tomato with infected symptoms of late blight disease were collected from different fields in Iringa and Arusha regions of Tanzania, and used to isolate the pathogen causing late blight disease. In the laboratory the tissues of tomato leaves sample were washed with 2% sodium hypochlorite and 70% ethanol then rinsed with sterilized distilled water for 5 to 10 minutes, followed by blotting of excess moisture with a sterile blotting paper. The clarified V-8 agar (to make 1 litre, V8 juice 200 ml, agar 20 g, and CaCO₃ 2 g in 800 ml of dH₂O) amended with Rifampicin and rye agar was used as isolation medium. The pH of the isolation medium was 5.76. The medium was autoclaved at 121 °C and 15 psi for 15 minutes, allowed to cool to about 45 °C thereafter it was amended with antibiotics (2.5% Pimaricin aqueous solution and 20 mg/L Rifampicin SV sodium salt). Rifampicin was dissolved in small volume of ethanol then mixed with distilled water. Antibiotics were filtered by using ultrafilters-microfilters (Sartorius, Minisart® steril 600 kPa max.) before being added to the growth medium. After amendments, 25 - 30 mL of the medium was poured to each of the Petri dishes and allowed to solidify in the laminar hood. Each sample was divided into 1 cm segments whereby 4 segments were placed equidistantly on each of five Petri dishes (9 mm diameter) containing V8 agar amended with antibiotics. The inoculated plates were incubated at 20 °C and observations were made on 3rd day whereby white colonies (cotton-like mycelia) characterized by water-like droplets were observed. The isolated fungi, *Phytophthora infestans* was purified by sub-culturing them onto molten rye agar and incubated at room temperature. Polymerase Chain Reaction (PCR) method used in the identification of the *P. infestans*. The primers that target the Internal Transcribed Spacer (ITS) with 600 bp were used. PINF (CTCGCTACAATAGGAGGGTC) Forward and ITS5b (GGAAGTAAAAGTCGTAACAAGG) reverse primer was used for PCR-based identification of fungal isolates based on procedure by [18]. The PCR premix was made up of 8.5 µl of water, 0.5 µl of PINF forward, 0.5 µl of ITS5b reverse and 3 µl of DNA and, was added mixed in 1.5 ml eppendorf tube.

2.2. Collection of Plant Samples

Five important medicinal plants were selected from different field in Tanzania which has antimicrobial and antifungal activity with guiding principle of different researchers worldwide [13]. The plants collected included *Tephrosia vogelii*, *Plectranthus barbatus*, *Sphaeranthus suaveolens* and *Lantana Camara* as described in **Table 1**. The parts of the plant collected are only leaves as shown in **Figure 1**. Plant leaves, collected were air-dried (25 °C - 27 °C) for 10 days.

2.3. Screening Bioassay Preparation of Plant Extract

Screening of plant extract was conducted in laboratory conditions. For this purpose, 10 g of different plant powder was macerated with 100 ml of distilling water.

Table 1. Uses or ailments treated of selected medicinal plants.

Name of plants	Family	Local name	Uses
<i>Tephrosia vogelii</i>	Fabaceae	Vogeli tephrosia/ Tchieuc	- It controls larval stages of mosquitoes and soft-bodied insects and mites - Dried leaves protect stored legume seeds from bruchids
<i>Plectranthus barbatus</i>	Lamiaceae	Coleous, makandi	- It is economically important with horticultural, medicinal and food uses and also is used in the antimicrobial activities [19]
<i>Sphaeranthus suaveolens</i>	Asteraceae	Sphaeranthus Indicus (General name)	- Leaves used as traditional medicine as anti-malaria, antibacterial and antifungal agents [20] - Whole plant is drunk as a cough relaxant [20]
<i>Lantana camara</i>	Verbenaceae	Lantana	- Inhibitory and stimulatory biochemical interactions between plants - Essential oil of Lantana camara has antibacterial activity against all the bacterial strains [21]

**Figure 1.** Pictures of selected plants whose extracts were evaluated for *P. infestans* mycelial inhibition.

The exudation of biochemicals was kept overnight and biomass was filtered by using of standard What man No. 1 filter paper. Inhibition of different extracts to the pathogen (Poison food technique) was also determined in lab conditions. Plant extract at 3 mL of each stock solution was added in V8 juice media pour sterilized petri plates. The disc of 5 mm diameter of different fungi 3 days old culture was placed at the centre of Petri plates. Three replicates were kept for each treatment and incubated at room temperature. After the three days inoculation, radial growth of mycelium was measured and compared with the results of control. The following formula of inhibition percentage was applied for each fungus in treatment.

$$\text{Inhibition percentage} = \frac{\text{Colony diameter without extract} - \text{Colony diameter with extract}}{\text{Colony diameter without extract}} \times 100\%$$

2.4. Extraction and Formulation of Crude Extracts from Plant Samples

Crude extracts from plant samples were extracted using the modified method [22] [23]. Plant sample materials were blended and for each sample 1 kg of dried extract was dissolved in 1 Litre of methanol and ethyl acetate respectively in the ratio of (1:1 wt/vol, dry powder/solvent) followed by soaking for 48 hrs. Then, the concentrate transferred from round flask to universal bottle and passed to nitrogen to remove the extraction solvent and the extract were kept to cool place.

2.5. Evaluation of Antimicrobial Activity of Crude Plant Extracts

Screening of the crude plant extracts for antimicrobial properties was done following modified procedures described by [22] [23]. V8 juice medium was prepared and cooled. Plant extracts were then incorporated at a ratio of (1 mL extract: 30 mL medium) per petri dish that means 3 mL of the dissolved extract were added to of 90 mL of molten V8 juice media, mixed and then poured equally into three 10 cm³ petri dishes. After the media had set, 5 mm agar discs cut from 14 day old fungal pathogen cultures were placed at the centre of the plate and incubated at room temperature. Positive control had media amended with Otiva as chemical fungicide and negative control plates had media not amended with plant extracts. Observations were made at 48 and 72 hours after planting and antifungal activity was determined after measuring the fungal colony radial growth using the following formula shown at 2.2.

3. Data Analysis

Growth inhibition (*GI*) was calculated as per the following formula:

$$GI = \frac{(A - B)}{A} \times 100\%$$

where *A* is the radial diameter of fungus growing on the control plate; and *B* is the radial diameter of fungus growing on the experimental plate.

All experiments were conducted in triplicates and the data collected on inhibition of mycelial growth of *Phytophthora infestans* were analysed using by 3-way ANOVA (Analysis of variance) using a statistical software STATISTICA. The Fisher's least Significance Difference (LSD) was used to compare treatment at P = 0.05 level of significance.

4. Results

4.1. Effects of Incubation Time on Percentage Inhibition of *P. infestans*

After 5 mm diameter of the pathogen being placed to the centre of the petri dishes containing media and plant extracts the observation as made for 48 hours and 72 hours. There was no significance difference in incubation time on inhibiting the growth of the pathogen (**Table 2**).

4.2. Effects of Plant Extracts on Percentage Inhibition of *P. infestans*

The result from **Table 2** showed that there was a significance different ($P \leq 0.001$) among the plant extracts on the growth of *P. infestans*. However, all plants showed antifungal activity against *Phytophthora infestans*. From the tests of plant extracts, *Plectranthus barbatus* (94 ± 1.0) and *Lantana camara* (91 ± 2.4) were the best of all plant treatments and these were statistical similar to the positive control (97 ± 0.0) **Table 2**. This was followed by *Sphaeranthus suaveolens* (80 ± 3.4). In this study, *Tephrosia vogelii* extract showed poor results in inhibiting

Table 2. Descriptive statistics results.

TREATMENT	% INHIBITION
SOLVENT	
Methanol	56 ± 5.4a
Ethyl acetate	55 ± 5.2b
INCUBATION TIME	
48 Hours	56 ± 5.1a
72 Hours	56 ± 5.5a
PLANT EXTRACTS/TREATMENT	
Negative control	0 ± 0.0d
Positive control	97 ± 0.0a
<i>Tephrosia vogelii</i>	16 ± 3.5c
<i>Plectranthus barbatus</i>	94 ± 1.0a
<i>Sphaeranthus suaveolens</i>	80 ± 3.4b
<i>Lantana camara</i>	91 ± 2.4a
3-WAY ANOVA (F VALUE)	
Solvent	33.36***
Incubation Time	0.69ns
Plant Extracts	5257.82***
Solvent and Incubation Time	1.17ns
Solvent and Plant Extract	19.72***
Incubation Time and Plant Extract	28.95***
Solvent and Incubation Time and Plant Extract	1.32ns

Each value is a mean ± standard error of three replicates, *** = significant at $P \leq 0.001$ and ns = not significant. Means within the same column followed by the same letter(s) are not significantly different at ($P = 0.05$) from each other using Fishers Least Significant Different (LSD) test.

the mycelial growth of *P. infestans* (16 ± 3.5) as compared with other plant extracts. The negative control did not inhibit (0 ± 0.0) the mycelial growth of *P. infestans*.

4.3. Interactive Effects between Solvent and Plant Extracts

Interactive effectiveness of the solvent and the plant extracts on inhibition of mycelial growth of the *P. infestans* was observed to be significantly different ($P \leq 0.001$). It was revealed that, the combination of solvent and plant extracts strongly inhibited the growth of mycelia of *P. infestans* (Figure 2, Figure 3). In methanolic plant extracts to all of the three plants *P. barbatus*, *L. camara* and *S. suaveolens* inhibited the mycelial growth of the pathogen from 80% and above except *T. vogelii* which inhibited the growth of the pathogen below 18% as shown in Figure 4. In ethyl acetate plant extraction, the inhibition percentage of

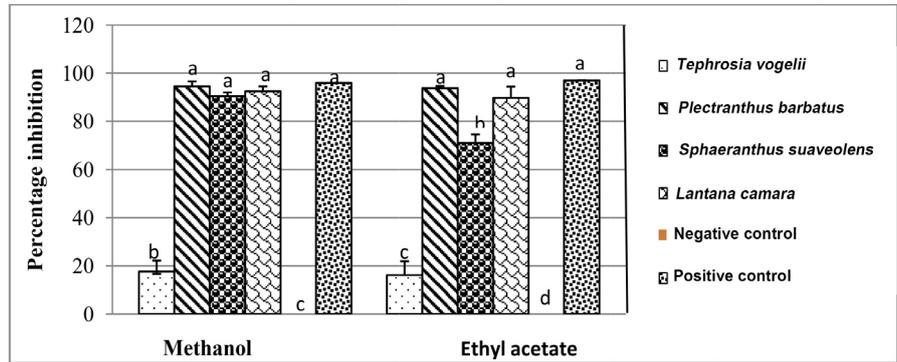


Figure 2. Interaction between the solvent and the plant extract in inhibiting the growth of *P. infestans*.

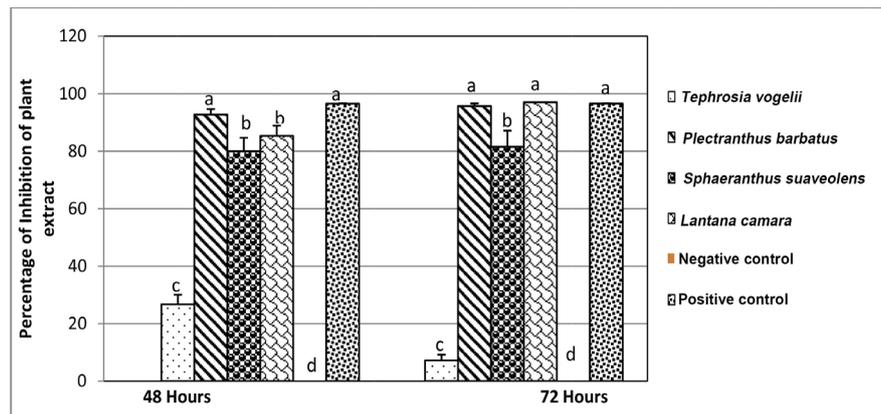


Figure 3. Interaction between the Incubation time and the plant extract in inhibiting the growth of *P. infestans*.

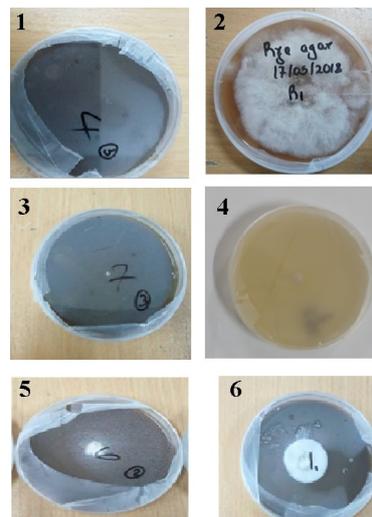


Figure 4. Assessment of the activity of plant extracts in the inhibition of *P. infestans* mycelia growth using Food Poison Technique. Where 1 = Positive control, Otiva chemical fungicide, 2 = Negative control, untreated, 3 = *Plectranthus barbatus*, 4 = *Lantana camara*, 5 = *Sphaeranthus suaveolens*, 6 = *Tephrosia vogelii*.

was observed to be statistically similar with that of methanolic extract except that the inhibition by *S. suaveolens* was lowered to 70%.

4.4. Interactive Effects between Incubation Time and Plant Extracts

The interactive effect of incubation time and plant extracts was observed and it was found to be significantly different ($P \leq 0.001$). The combination of the incubation time and plant extracts strongly inhibited the growth of mycelia of the *P. infestans*. Our results revealed that, the three plant extracts, *Plectranthus barbatus*, *Lantana camara* and *Sphaeranthus suaveolens* were the best on inhibiting the mycelial growth of *P. infestans* and statistically similar between 48 hours and 72 hours. However, the performance of *T. vogelii* in inhibiting the growth of the *P. infestans* significantly decreased at 72 hours as compared with 48 hours as shown in **Figure 4**.

5. Discussion

5.1. Effects of Methanol and Ethyl Acetate Extracting Solvent on Percentage Inhibition of *P. infestans*

The nature of chemical solvent used in extracting the active compounds from the plants may dictate the quality of extracts yielded [24] [25] [26] [27]. In this study, methanol and ethyl acetate as polar and nonpolar solvent were used in extracting and preparing the crude extracts from the tested plants. Results from **Table 2** reveals that, the methanolic plant extract was the best and significantly inhibited the mycelial growth of *P. infestans*. Similar to our results, the methanol leaf extract of *L. camara* reported to have better inhibition to tested fungal activities [28]. Also, [19] also reported that crude extracts of *P. barbatus* had the lowest MIC values against the test microbes compared to the other this is because the methanol was not used as the extracting solvent. From our study, methanol crude extract was effective in controlling the activities of *P. infestans* as compared with ethyl acetate extracts. This means that methanol was effective in the extraction of active polar compounds from the plant which were more effective than the nonpolar compounds extracted by ethyl acetate [29].

5.2. Effects of Plant Extract on Percentage Inhibition of *P. infestans*

The results of the study showed that all plant extract have active compounds with inhibitory effects to the growth of *P. infestans* as compared with the negative control. Different plant parts are known to produce secondary metabolites such as phenolic/flavonoids and terpenes/monoterpenes which inhibit pathogen growth [30] [31] [32].

Our findings revealed that the *Plectranthus barbatus*, *Lantana camara*, and *S. suaveolens* crude extracts inhibited the mycelial growth of the *P. infestans* and these were statistically similar with the commercial chemical fungicide. Such results are very promising and may be used in the future to develop potential can-

didate pesticide in controlling *P. infestans* in tomato and potato. Research evidence has shown the presence of different active compounds with antifungal and antibacterial properties in *Plectranthus barbatus* [19], *Lantana camara* [33] and *Sphaeranthus* species [20].

T. vogelii extract from this study was poor in inhibiting the mycelial growth of *P. infestans*. Despite of the poor inhibition of the *T. vogelii* on *P. infestans* from this study, other studies have reported the great potential in controlling insect pests in the field and storage [28].

The interactive effects between the extraction solvents and plant crude extracts showed that ethyl acetate significantly lowered the inhibition ability of *S. suaveolens*. Furthermore, the interactive effects between incubation time and plant extracts revealed that, the performance of *T. vogelii* in inhibiting the growth of *P. infestans* was significantly decreased at 72 hours of incubation. Further studies must be conducted to establish the mechanism of the interactive effects observed in our findings.

6. Conclusion

This study shows that, there is a potential for developing natural fungicides based on plant-derived products for late blight control. The *Plectranthus barbatus*, *Lantana Camara* and *Sphaeranthus suaveolens* plant extracts were effective as a commercial synthetic pesticide in reducing the growth of *Phytophthora infestans* and hence can be used alone as an alternative to chemical fungicide. *T. vogelii* extract was not efficiency on in hitting the mycelial growth of the pathogen, and hence it is not advocated in this purpose.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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