Antifungal Activities of Sweet Basil (Ocimum basilicum L.) Aqueous Extract Against Sclerotium rolfsii, Causal Agent of Damping-Off on Tomato Seedling

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ABSTRACT

The study aims to evaluate the effectiveness of sweet basil aqueous extract against Sclerotium rolfsii in-vitro and damping-off on tomato seedling in-vivo. The sweet basil leaves were obtained from a commercial market in Los Banos, Philippines. The leaves were rinsed by water and then air dried. The dried leaves were ground using a domestic blender and 10 grams of this material was extracted using 100 ml of sterile distilled water (1:10 w/v) and 0.01 ml absolute methanol. The mixture was kept for 48 hours at room temperature. The solution was strained by a Whatman filter paper No. 1, then the extract was stored at 4°C. The effectiveness of sweet basil extract was determined in-vitro by measuring the mycelial growth inhibition of S.rolfsii and in-vivo by the percentage of disease incidence on tomato seedlings. The result showed that sweet basil extract was effective to inhibit the mycelial growth (33.35%). However, the effectiveness of water extract of sweet basil was considered ineffective for reducing disease incidence on the inoculated tomato seedlings, i.e. 46.67%-60%, similar to benomyl after the inoculation. Further investigation is needed to find out an effective formula of O. basilicum leaf extract which is stable and prolonged persistence for controlling S. rolfsii.

INTRODUCTION

Tomato is one of important vegetables and consumed by almost people around the world. It is grown worldwide, either in tropical and temperate zone. Tomato is consumed in diverse ways, such as; dishes, salads, sauces, and drinks. It is also used as raw material of food industries, such as tomato ketchup, tomato sauce, and seasoning in instant noodle. According to the Food and Agriculture Organization (FAO), tomato is one of the eighth most valuable agricultural products worldwide. The top five of tomato producer countries are China, India, USA, Turkey, and Egypt. Otherwise, in Southeast Asia, Indonesia is one of the most tomato producer countries with the annual average production of 877,729 tones (FAO, 2016).

Damping-off of tomato seedling, caused by Sclerotium rolfsii, is considered as a major constraint in tomato production (Punja, 1988). S. rolfsii is a soil-borne pathogen which has wide host range, including tomato, groundnut, bean, peas, carrot, cotton, wheat, potato, maize, and garpevines (El-Nagar et al, 2013; Keyser et al, 2017; Rangarani et al, 2017. It occurs world wide in the tropics, sub-tropics, and other warm temperate regions (Punja, 1985). It causes damping-off on seedling, while infection on reproductive stage causes southern blight disease (De Curtis et al, 2010; Mullen, 2001; Flores-Moctezuma et al, 2006). Initial inoculum of S.rolfsii can be hyphae of infected tomato tissues and germinating sclerotia. Direct penetration occurs when hyphae contact with basal stem, root, bulb, fruit or leaf tissues. Disease starts with a small,
According to Colpas et al. (2009), aqueous extract due to antimicrobial compounds contained in Basil. Several fungicides have been reported effective against \textit{S. rolfsii} (Keyser et al, 2017; Rangaranzi et al, 2017; Vineela et al, 2017). However, to date, environmental and food safety issues must be considered to choose a method for controlling the disease. More environmental-friendly efforts should be made to control the disease by using botanical pesticides. Various studies have been carried out to seek potent botanical pesticides which have antimicrobial compounds. Carović-Stanko et al (2010) reported that basil plant in genus \textit{Ocimum} has to be used as botanical pesticide. The genus is an aromatic plant which commonly used in culinary and medicine (Simon et al, 1999). The plant is widely distributed in the world either cultivated or grow wild.

In medical and agricultural fields, basil extract has been reported to inhibit the growth of fungal pathogens, namely \textit{Enterococcus sp.}, \textit{Listeria sp. }, \textit{Staphylococcus sp.}, \textit{Aspergillus sp.}, \textit{Escherichia coli}, and \textit{Fusarium sp.}. Carović-Stanko et al., 2010; Kocic-Tanackov et al, 2008; Bhardwaj, 2012; Carović-Stanko et al., 2010; Damboleana et al., 2010; Kocic-Tanackov et al., 2011; Piyo et al, 2009; Kumar et al, 2010). This is due to antimicrobial compounds contained in Basil. According to Colpas et al. (2009), aqueous extract of \textit{Ocimum gratissimum} induced the production of phytoalexins in soybean cotyledons and sorghum mesocotyls and also induced systemic resistance in cucumber to \textit{Colletotrichum lagenarium}, reflected by reduction in disease incidence and an increase in chitinase production. Moreover, previous study reported that aqueous extract of \textit{O. basilicum} significantly reduced the early blight incidence on tomato, caused by \textit{Alternaria solani}, under greenhouse and field condition (Nashwa et al, 2012). In addition, Abdollahi et al (2011) reported that essential oil of \textit{O. basilicum} completely inhibit the growth of \textit{Rhizopus stolonifer}, a post-harvest fungal pathogen, in vapour phase method.

Regarding to the potent utilization of sweet basil on disease control, the study was conducted to evaluate the effectiveness of sweet basil aqueous extract against \textit{Sclerotium rolfsii} under in-vitro condition and damping-off on tomato seedling, caused by \textit{Sclerotium rolfsii}, under greenhouse condition.

### MATERIALS AND METHODS

#### Isolation and Cultivation of \textit{Sclerotium rolfsii}

This research was conducted from January to June 2016. \textit{Sclerotium rolfsii} was isolated from an infected tomato plant on farm field of Bureau of Plant Industry - National Seed Quality Control Services (NSQCS) - Region 4, Los Banos, Laguna, Philippines (14°10'33.3"N; 121°13'31.6"E). Isolation and cultivation of \textit{S. rolfsii} was conducted in the Laboratory of Plant Pathology at Institute of Weed, Entomology, and Plant Pathology, University of the Philippines Los Banos. Infected vascular tissue was cut into small pieces and sterilized by using 0.5 % sodium hypochlorite solution for 3-5 minutes, then rinsed twice in sterilized distilled water and blot dried with a sterile tissue paper. The cut tissues were inoculated on 15 ml potato dextrose agar medium (PDA) in petri dishes for 7-10 days at room temperature. Then the mycelia of \textit{S. rolfsii} that grow on the media were transferred to other PDAs for purification for another 5 - 7 days. The mycelia was identified as \textit{S. rolfsii} based on its mycelial and sclerotial characters (Barnett et al, 1972). Afterwards, the desired pathogen was determined by pathogenicity test. Preparation of mycelia and sclerotial bodies for \textit{in vitro} and \textit{in vivo} assays was carried out by culturing pure cultures on PDAs. Then, harvesting of mycelia and sclerotial body was carried out at the time of treatment. The pathogenicity of each fungal mycelia isolates and sclerotial body were preliminarily assessed on 21days-old tomato seedlings from 5 replications.

#### Tomato Seedling Preparation for Bioassay

Seedlings of tomato were grown in the greenhouse of Institute of Weed, Entomology, and Plant Pathology, University of the Philippines Los Baños. The seeds were sown in seedling trays which were filled by standard horticultural potting mix. Fourteen days old seedlings were moved into individual pot. Plant materials were maintained by standard cultural practice for tomato plant. Twenty one-days-old tomato seedlings were used for \textit{in-vivo} bioassay experiment.

#### Extraction of Sweet Basil

\textit{Ocimum basilicum} L. leaves were obtained from a commercial market in Los Banos, Philippines and extracted in the Laboratory of Plant Pathology at Institute of Weed, Entomology, and Plant Pathology, University of the Philippines Los Baños. Leaves were...
rinsed by water to remove dust particles and then air dried. Extraction of sweet basil was based on the method of Wong, Leong, & William Koh (2006) with some modifications. Dried leaves were ground using a domestic blender and 10 g of this material was extracted using 100 ml of sterile distilled water (1:10 w/v) and 0.01 ml absolute methanol. The mixture was allowed to stand for 48 hours at room temperature. The solutions were strained by a Whatman filter paper No. 1 to get aqueous extract to be used for analysis without further treatment. The aqueous extract was then stored at 4°C under refrigerator.

**Preparation of Plant Extract Medium for Different Concentration**

The standard stock solutions of plant leaves extract were made with the rate of 1 ml aqueous extract/1 ml sterilized distilled water. This formed-standard plant extracts were made in aqueous medium of 25%, 50%, 75%, 100% concentrations.

**In vitro Antifungal Assay**

The antifungal activities of *Ocimum basilicum* L. leaves extracts were evaluated against mycelia and sclerotic body of *S. rolfsii* by using agar dilution technique (Valencia, Castro, Pascual, & Magdalita, 2011) in the Laboratory of Plant Pathology at Institute of Weed, Entomology, and Plant pathology, University of the Philippines Los Baños. The experiment was arranged in a completely randomized design with six treatments and three replications for each type of inoculum. The treatments were distilled water as negative (-) control, *Ocimum basilicum* L. extracts with the concentrations of 25%, 50%, 75%, 100% and fungicide (active ingredient Benomyl) with 300 ppm as positive (+) control.

A five mm diameter mycelial disc of *S. rolfsii* was taken from 3 – 4 days old mycelial culture attached on the tape and then placed 1 – 2 mm distance from the base of stem on the surface of potting mix having 100% concentration of plant extract, sterilized distilled water as negative control, and 300 ppm of Benomyl as positive control. Five replications were maintained for each treatment. For the treatment before inoculation, the solutions were drenched right before inoculation with volume per pot 100 ml. While, for the treatment after inoculation, the solutions were drenched 24 hours, 48 hours, and 72 hours after inoculation with volume per pot 100 ml for each application. All these pots were stored in a greenhouse. The observations were carried out during 10 days after inoculation.

**Statistical Analysis**

The effectiveness of *O. basilicum* L. leaves extract under *in-vitro* evaluation was determined by measuring the percentage of mycelial growth inhibition (MGI), according to the following formula:

\[
MGI (\%) = \left[ \frac{d_c - d_t}{d_c} \right] \times 100
\]

where: \(d_c (\text{mm})\) = mean colony diameter of pathogen at the negative control; \(d_t (\text{mm})\) = mean colony diameter of pathogen at the evaluated treatments (Yahyazadeh, Omidbaigi, Zare, & Taheri, 2008).

Damping-off incidence on tomato seedlings in *in-vivo* experiment were calculated using the following formula:

\[
\text{Disease incidence (\%) = } \left[ \frac{\text{Amount of infected plant in treatment}}{\text{Total amount of plant in treatment}} \right] \times 100
\]
Values of mycelial growth inhibition under *in-vitro* evaluation and disease incidence on tomato seedling were submitted to Analysis of Variance (Anova) using statistical tool SPSS software version 22 and means were compared by Duncan’s Multiple Range Test (DMRT) at *P* 0.05.

**RESULTS AND DISCUSSION**

**Effect of Sweet Basil Aqueous Extract on *In-vitro* Growth of *Sclerotium rolfsii***

The sweet basil extract exhibited the mycelial growth of *S. rolfsii* on PDA (Fig. 1). Fig. 1 gave the growth of fungal pathogen *S. rolfsii* on PDA during 60 hours incubation, while Table 1 showed the diameter of mycelium and inhibitory effect (%) of sweet basil extract on mycelial growth of *S. rolfsii* after 60 hours incubation.

The growth of *S. rolfsii* mycelium from mycelial inoculum started to grow after 24 hours in all treatments. These conditions inferred that, all treatment did not delay the growth of *S. rolfsii* at 24 hours. After 60 hours incubation, the mycelial growth inhibition under all leaves extract concentrations were significantly lower than the control treatments (Table 1). Moreover, the highest mycelial growth inhibition was observed under leaves extracts treatments with the concentration of 100%. The lower mycelial growth suppression was observed on the treatments of 25, 50, and 75% with insignificant differences. The sweet basil leaves extract with the concentration of 100% gave highest percentage of growth inhibition among the applied treatments. While other concentration of leaves extract treatments showed lesser effectivities with negligible differences with controls.

**Table 1. Effect of sweet basil (*Ocimum basilicum*) aqueous extract on *Sclerotium rolfsii* growth from mycelial inoculum after 60 hours inoculation under room temperature**

<table>
<thead>
<tr>
<th>Plant Extract (w/v %)</th>
<th>Diameter of Growth (mm)</th>
<th>Growth Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (negative control)</td>
<td>89.00 a</td>
<td>0.00 a</td>
</tr>
<tr>
<td>25</td>
<td>82.33 ab</td>
<td>7.45 b</td>
</tr>
<tr>
<td>50</td>
<td>75.33 b</td>
<td>15.34 b</td>
</tr>
<tr>
<td>75</td>
<td>75.17 b</td>
<td>15.49 b</td>
</tr>
<tr>
<td>100</td>
<td>59.33 c</td>
<td>33.35 c</td>
</tr>
<tr>
<td>300 ppm Benomil (positive control)</td>
<td>78.83 b</td>
<td>11.53 b</td>
</tr>
</tbody>
</table>

Remarks: Means in the same column followed by the different letters differ significantly under DMRT (*α* ≤ 5%)

Sweet basil aqueous extract tested at various concentrations also showed the capacity to inhibit the growth of *S. rolfsii* derived from the sclerotial body. Fig. 2 shows that mycelial growth of *S. rolfsii* can be observed at 24 hours after sclerotial body was inoculated in PDA media of all treatments.

![Fig. 1. The mycelium growth of Sclerotium rolfsii grown on PDA containing various concentrations of sweet basil (Ocimum basilicum) aqueous extracts during 60 hours incubations.](image)
This suggests that the sweet basil extract tested was unable to delay the growth of *S. rolfsii* during 24 hours after inoculation of sclerotial body. After 48 hours inoculation, sweet basil aqueous extract did not affect sclerotial body of *S. rolfsii* as indicated by mycelial growth from the sclerotia treated with 25-50% of the extract. However, at higher concentrations, i.e. 75 and 100%, the mycelial growth were slightly suppressed, similar to that of benomyl treatment (Table 2).

Table 2. Effects of sweet basil (*Ocimum basilicum*) extract on *Sclerotium rolfsii* growth from sclerotial body inoculum after 96 hours inoculation

<table>
<thead>
<tr>
<th>Plant Extract (w/v %)</th>
<th>Diameter of Growth (mm)</th>
<th>Growth Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (negative control)</td>
<td>90.00 a</td>
<td>0.00 a</td>
</tr>
<tr>
<td>25</td>
<td>87.83 a</td>
<td>2.41 ab</td>
</tr>
<tr>
<td>50</td>
<td>87.67 a</td>
<td>2.59 ab</td>
</tr>
<tr>
<td>75</td>
<td>77.33 b</td>
<td>14.07 c</td>
</tr>
<tr>
<td>100</td>
<td>81.00 ab</td>
<td>10.00 bc</td>
</tr>
<tr>
<td>300 ppm Benomil (positive control)</td>
<td>72.50 b</td>
<td>19.44 c</td>
</tr>
</tbody>
</table>

Remarks: Means in the same column followed by the different letters differ significantly under DMRT (α≤ 5%).

Antimicrobial activity of *O. basilicum* could be related to composition of main compounds, especially phenolic compounds (Nychas, 1995). The antifungal compounds contained in *O. basilicum* are linalool, methyl-cavicil (estragol), camphor, and eugenol (Abdollahi, Hassani, Ghosta, Meshkatalsadat, & Shabani, 2011; Carović-Stanko et al., 2010; Dambolina et al., 2010; Danesi et al., 2008; Hussain, Anwar, Hussain Sherazi, &Przybylski, 2008; Kocic-Tanackov, Dimic, Levic, Tanackov, &Tuco, 2011; Shirazi, Gholami, Kavoosi, Rowshan, &Tafsiry, 2014; Vieira et al., 2014). Nychas (1995) found that phenolic compound in essential oil of Ocimum play an important role on denaturation of enzyme which control spore germination. Furthermore, those antifungal compound affected on inhibition of early fungal development e.g. spore germination, germ tube growth and/or appressorium formation, and inhibited mycelial growth (Amini, Farhang, Javadi, & Nazemi, 2016; Oxenham, Svoboda, & Walters, 2005; Sethi, Prakash, Chandra, Punetha, & Pant, 2013). However, Hasegawa, Tajima, Toi, & Sugimura (1997) suggested that the antifungal activity of essential oil or extract of herbs has to be investigated separately against a particular fungal pathogen. Synergistic and antagonistic effect of certain minor compounds in mixture have to be considered (Daferera, Ziegas, &Polissiou, 2003; Velluti, Sanchis, Ramos, Egido, &Marin, 2003).
Effect of Sweet Basil Aqueous Extract to Damping-Off Incidence, Caused by *Sclerotium rolfsii*, on Tomato Seedling

The effect of sweet basil extract on the damping-off incidence on tomato seedling was investigated. The extract was prepared by boiling fresh sweet basil leaves with water. The extract was then applied to the tomato seedlings before and after inoculation with the damping-off fungus. The results showed that the extract was effective in reducing damping-off incidence, with the highest reduction observed when the extract was applied before inoculation.

Table 3. The effect of sweet basil (*Ocimum basilicum*) extract, distilled water and 300 ppm Benomyl with the application after and before inoculation to disease incidence of damping-off on tomato seedling.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After inoculation</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>66.67a</td>
</tr>
<tr>
<td><em>Ocimum basilicum</em> extract</td>
<td>60.00a</td>
</tr>
<tr>
<td>Benomyl</td>
<td>40.00a</td>
</tr>
<tr>
<td>Before inoculation</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>66.67a</td>
</tr>
<tr>
<td><em>Ocimum basilicum</em> extract</td>
<td>46.67a</td>
</tr>
<tr>
<td>Benomyl</td>
<td>46.67a</td>
</tr>
</tbody>
</table>

Remarks: Means in the same column followed by the different letters differ significantly under DMRT (α≤ 5%)

Some studies state that leaf extract of *O. basilicum* completely inhibit fungal plant pathogen, such as: *Botrytis fabae* (Oxenham, Svoboda, & Walters, 2005), *Fusarium spp* (Dambolena et al., 2010), *Rizoctonia solani* (Sethi, Prakash, Chandra, Punetha, & Pant, 2013), and *Phytophthora spp* (Amini, Farhang, Javadi, & Nazemi, 2016). In this study, *O. basilicum* aqueous extract completely inhibit mycelial growth of *S. rolfsii in-vitro*. However, the effective concentration of the aqueous extract to *S. rolfsii* is ≥ 75%. This study also reveals that *O. basilicum* aqueous extract on the selected concentration is not effective in reducing the damping-off incidence on tomato seedlings under greenhouse condition. Factor that restricts efficacy of botanical pesticide is short persistence of phytochemical which are caused by rapid biodegradation as well as rapid release (Pavela, 2014). Furthermore, advance investigation is needed to reveal an effective formula of *O. basilicum* leaf extract which is stable and prolonged persistence to control damping-off incidence on tomato seedlings.

CONCLUSION AND SUGGESTION

The effective concentration of *O. basilicum* aqueous extract (≥ 75% w/v) completely inhibit mycelial growth of *S. rolfsii under in vitro* conditions. The leaves extracts were not effective in reducing the damping-off incidence on the inoculated tomato seedlings. Further investigation is needed to find out the an effective formula of *O. basilicum* leaf extract which is stable and prolonged persistence to control damping-off incidence on tomato seedlings.

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