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, subtropics, 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,
water-soaked lesion on the basal stem of plant. The lesion expands rapidly and gridles the stem. Within 2-4 days after infection, symptoms of soft rot are usually apparent (Mullen, 2001).

Several fungicides have been reported effective against S. rolfsii (Keyser et al, 2017; Rangarani 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 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 Enterococcus sp., Listeria sp., Staphylococcus sp., Aspergillus sp., Escherichia coli, and Fusarium sp. (Bansod et al, 2008; Bhardwaj, 2012; Carović-Stanko et al., 2010; Dambolena 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 Ocimum gratissimum induced the production of phytoalexins in soybean cotyledons and sorghum mesocotyls and also induced systemic resistance in cucumber to Colletotrichum lagenarium, reflected by reduction in disease incidence and an increase in chitinase production. Moreover, previous study reported that aqueous extract of O. basilicum significantly reduced the early blight incidence on tomato, caused by Alternaria solani, under greenhouse and field condition (Nashwa et al, 2012). In addition, Abdollahi et al (2011) reported that essential oil of O. basilicum completely inhibit the growth of Rhizopus stolonifer, a post-harvest fungal pathen, 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 Sclerotium rolfsii under in-vitro condition and damping-off on tomato seedling, caused by Sclerotium rolfsii, under greenhouse condition.

**MATERIALS AND METHODS**

**Isolation and Cultivation of Sclerotium rolfsii**

This research was conducted from January to June 2016. 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 Baños, Laguna, Philippines (14°10'33.3"N; 121°13'31.6"E). Isolation and cultivation of S. rolfsii was conducted in the Laboratory of Plant Pathology at Institute of Weed, Entomology, and Plant Pathology, University of the Philippines Los Baños. 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 S. rolfsii that grow on the media were transferred to other PDAs for purification for another 5 - 7 days. The mycelia was identified as 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 in vitro and 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 in-vivo bioassay experiment.

**Extraction of Sweet Basil**

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 sclerotal 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 the concentration of 300 ppm as positive (+) control.

Standard stock solutions of plants leaves extract 25, 50, 75, 100% concentrations were prepared separately by adding the required quantity of plants extract to the molten PDA medium. One set was made without plant extract and kept as negative control. All these were poured into sterilized Petri plates. Petri plates with plant extract were mixed gently before the medium solidify. A five mm diameter mycelial disc of *S. rolfsii* was taken from 3 – 4 days old mycelial cultures and centrally inoculated onto PDA medium in each of the petri plates containing different leaves extracts concentrations and control under aseptic conditions. All these petriplates were incubated at room temperature. The diameter of the colony was measured in two directions and average was recorded. Observations were carried out until mycelia fully growth in the negative control plates at 60 hours after inoculation.

**In vivo Antifungal Assay**

In vivo antifungal assay was conducted in the greenhouse of Institute of Weed, Entomology, and Plant Pathology, University of the Philippines Los Baños. The experiment was arranged a factorial design with two factors and five replications. First factor was time of leave extracts application after (A) and before (B) mycelia inoculation; and the second factor was antifungal agents, i.e. 100% *Ocimum* extract, distilled water as negative (-) 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: \(d_c\) (mm) = mean colony diameter of pathogen at the negative control; \(d_t\) (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:

\[
\text{Disease incidence} = \frac{\text{Amount of infected plant in treatment}}{\text{Total amount of plant in treatment}} \times 100
\]
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 \( P \leq 0.05 \).

**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

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

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

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-url)
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

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

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-caviicol (eugenol), camphor, and eugenol (Abdollahi, Hassani, Ghiata, Meshkatalsadat, & Shabani, 2011; Caroš-Stanko et al., 2010; Dambolela et al., 2010; Danesi et al., 2008; Hussain, Anwar, Hussain Sherazi, &Przybylski, 2008; Kocic-Tanackov, Dimic, Levec, 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, &Marín, 2003).
Effect of Sweet Basil Aqueous Extract to Damping-Off Incidence, Caused by *Sclerotium rolfsii*, on Tomato Seedling

The effect of sweet basil extract concentration 100% (1:10 w/v), distilled water and 300ppm Benomil to damping-off disease incidence on tomato seedling were ranged from 40% to 66.67% for the application after inoculation and 46.67% to 66.67% for the application before inoculation (Table 3). There were not statistically different between treatments, either on before and after inoculations. However, based on this study, application before inoculation has lower disease incidence than after inoculation. Sweet basil extract has a potential to reduce disease incidence 30% and 10 % in application before and after inoculation respectively.

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.

| Treatment                  | Disease incidence (%) |
|----------------------------|-----------------------|
| After inoculation          |                       |
| Distilled water            | 66.67a                |
| *Ocimum basilicum* extract | 60.00a                |
| Benomil                    | 40.00a                |
| Before inoculation         |                       |
| Distilled water            | 66.67a                |
| *Ocimum basilicum* extract | 46.67a                |
| Benomil                    | 46.67a                |

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

The antifungal activities of *O. basilicum* could be related to chemical compounds contained in the aqueous extract. Sanni, Onyeyili, & Sanni (2008) reported that saponin and alkaloids are the most abundant chemical constituent present in aqueous extract of *O. basilicum*, while flavonoids, cardiac glycosides, terpenes and steroids were present in medium quantity. In addition, tannins and carbohydrates present in low quantity. However, flavonoids and tannins could be antifungal compounds which are known to possess antimicrobial activities (Narayana, Reddy, Chaluvadi, & Krishna, 2001). Flavonoids is phenolic compound with one carbonyl group which is synthesized by plant in response to microbial infection and often found effective in vitro as antimicrobial substance against various pathogens (Gurjar, Ali, Akhtar, & Singh, 2012).

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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