Weeds and Herbs as Biofumigants Against *Sclerotium rolfsii* Sacc. causing Stem and Root Rot of Robusta Coffee Seedlings

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ARTICLE INFORMATION

Article History:
Received: 12/17/2016
Received in revised form: 5/26/2016
Accepted: 11/2/2016

Keywords:
biofumigation, Robusta coffee, Sclerotium stem and root rot, *Sclerotium rolfsii*

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ABSTRACT

The study explored the properties of weeds and herbs as biofumigants against stem and root rot of robusta coffee seedlings. All the 10 test biofumigants used significantly reduced population density (cfu/g of soil) of S. rolfsii (Sr) after one month of biofumigation. Delayed symptom of stem and root rot (SRR) disease was manifested by Ceylon spinach- biofumigated soil at 19 days after transplanting (DAT) while the untreated plants were infected earliest at 8 DAT. Ceylon Spinach - and Lemongrass - biofumigated plants exhibited the lowest and comparable percentage infection (PI) of 40.00 and 35.00, respectively. Similar trend was observed on percentage severity infection (%SI). All biofumigants exhibited significantly higher percentage degree of control (%DC) compared to the untreated control. Ceylon Spinach and Lemongrass biofumigants were rated very effective (VE) while the other treatments were rated effective (E) except wild sunflower which was rated moderately effective (ME). Biomass of Ceylon Spinach and Lemongrass were the cost- effective biofumigants to manage SRR of Robusta coffee seedlings in the nursery.

Introduction

Coffee ranks second among the world's legally-traded commodities and there were estimated 25 million coffee growers around the world, mostly are small-scale farmers (Global Exchange Organization, 2011). Philippines is one of the few countries in the world where all these four coffee variants exist: Arabica, Robusta, Excelsa and Liberica (Philippine Coffee Board, 2012). *Coffea Arabica (*Arabica) and *Coffea canephora (*Robusta) are the two most prevalent varieties of coffee in the country wherein Robusta accounts for 75% of the country's total production and it is estimated that around 300,000 Filipinos depend on the coffee industry (Department of Agriculture – HVCDP, 2013).

Coffee-like any other crop is vulnerable to disease attacks like coffee rust, and root or stem rot. Stem and root rot (SRR) reduced yield by 10-25% and could be responsible for plant losses of about 42-62% (Singh & Chand, 1972).

Coffee seedlings are very susceptible to infection and die quickly unlike older plants which gradually girdled with lesions and die. Infected tissues show symptoms of paleness, being brown, and soft; but not watery (Punja, 1985).

The continuous use of chemical pesticides and fertilizers especially in agricultural settings in the production of crops like coffee has created a variety of ecological problems. To maintain the same level of crop production due to increase in world population, farmers and horticulturists have come to increasingly rely on chemically-synthesized fertilizers and chemical pesticides (Varshovi, 2005).

Recently, biofumigation was introduced as management option against soil-borne microorganisms such as fungi, bacteria, and nematode. It is also an alternative to soil fumigant (methyl bromide, formaldehyde). Biofumigation is a process by which naturally occurring biocides are released when tissues of brassica plants are decomposed in the soil and suppress soil borne pests and pathogens of solanaceous and other crops including the root knot nematodes (NOMIARC R&D Team, 2011).

Purpose of the Research

This study aimed to evaluate the efficacy of weeds and herbs as biofumigants against *SRR* of coffee seedlings to determine which among the treatments were most effective in reducing inoculums density of the target pathogen in biofumigated soils and to find out the cost-efficacy of test treatments usage on a thousand Robusta coffee seedlings as eradicative treatments against *S. rolfsii (Sr)* causing *SRR* of coffee seedlings.

Methodology

The study was conducted at the Department of Plant Pathology, College of Agriculture, University of Southern Mindanao, Kabacan, Cotabato (Manabogan & Jover 2014).

Experimental Design

The *in vivo* experiment was laid-out in Randomized Complete Block Design (RCBD) with all the 13 treatments replicated four times (Figure 1).

Figure. 1. Modified screen house experimental set-up laid-out in Randomized Complete Block Design (RCBD) with all the thirteen (13) treatments replicated four (4) times.

The following treatments were produced by decomposing biomass of weeds and herbs at 5 kg per m^2 of soil (Figure 2).

T_1- Silver bush (*Peperomia pellucida)*
T_2- Wild Sunflower (*Tithonia diversifoli)*
T_3- Ceylon Spinach (*Talinum fruticosum)*
T_4- Gotu kola (*Centella asiatica)*
T_5- Jute (*Chorchorus olitorius)*
T_6- Wild eggplant (*Solanum torvum)*
T_7- Thai basil (*Ocimum basilicum)*
T_8- Genovese basil (*Ocimum basilicum)*
T_9- Holy basil (*Ocimum basilicum)*
T_{10}- Lemongrass (*Cymbopogon citrullu)*
T_{11}- Rose Balsam (*Impatiens balsamina)* [Non-Brassicaceous Check]
T_{12}- Cabbage Wastes (*Brassica oleracea)* [Brassicaceous Check]
T_{13}- Inoculated/ Untreated Control

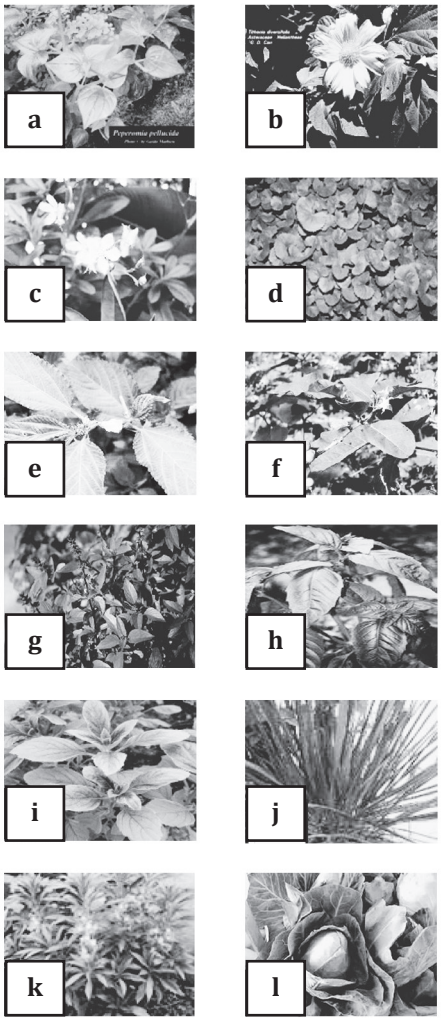

Figure 2. Raw materials or biomass used as test biofumigants: a) Silver Bush, b) Wild Sunflower, c) Ceylon Spinach, d) Gotu Kola, e) Jute Plant, f) Wild Eggplant, g) Thai Basil, h) Genovese Basil, i) Holy Basil, j) Lemongrass, k) Rose Balsam, l) Cabbage Wastes.

Preparation of Culture Media

Potato Dextrose Agar (PDA) was prepared following the standard procedure (Riker & Riker, 1936).

Collection of Diseased Specimen

Sclerotium root-rot infected seedlings showing typical external (Figure 3a) and internal (Figure 3b and 3c) symptoms were collected in the coffee nursery and were brought to the plant pathology laboratory for diagnosis. Microscopic examination was done to establish the association of the causal pathogen.

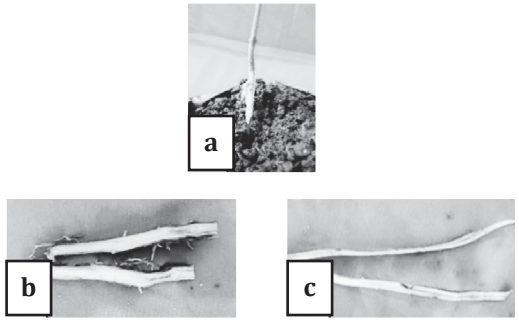

Figure 3. Coffee stem showing typical symptom of stem rot disease, girdled with white string-like mycelium of Sr (a) and longitudinally- sectioned root (b) and stem (c) of coffee seedlings showing symptom of VD.

Isolation of the Pathogen

Sr was isolated by planting sclerotial bodies from the infected coffee seedlings onto the PDA medium using a flamed transfer needle and was incubated under room temperature. Subsequent transfers from the initial isolation were done to obtain pure culture of the pathogen (Figure 4).

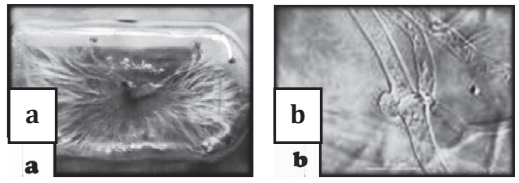

Fig. 4. Pure culture S. rolfsii showing whitish to brown spherical Sclerotia on PDA (a) and hypha (400x) as seen under the microscope (b).

Establishment of Infested Plots

Before the incorporation of the raw materials of the test biofumigants, the test pathogen was seeded into the plots (1m x 1m) by broadcasting 100 sclerotial bodies/ plot.

Preparation and Incorporation of Test Treatments

After 14 days of incubation, raw materials of the test biofumigants were collected, finely chopped/shredded and their biomass were incorporated into the infested soil at the rate of 5 kg/m^2, applied with water and were allowed to decompose for a month after which biofumigated soil media of the different test treatments were dispensed at 1 kg/polyethylene bag ("5 x 6").

Planting and Management of the Test Plants

Five month-old Robusta coffee seedlings were planted in biofumigated soil dispensed in polybags. Each seedling was carefully transplanted to the prepared planting medium. Standard cultural management for coffee seedlings was employed.

Pre- and Post- Biofumigation Assessment of Population Density (PD) of Sr (cfu/g soil)

Dilution-pour plate technique was employed to determine the PD *(*cfu/g soil) of *Sr* both from infested and biofumigated soil. A 300 g soil sample was gathered per plot then a gram of soil was taken from the composite sample, diluted in 9 ml SDW. A series of dilution was done up to 10^6 dilution. The diluted suspension at 10^4 was used to determine the colony count in four replicated plates after a 24-hr incubation. The PD (cfu/g of soil) was assessed and computed.

Data Collection and Analysis

Pre- (initial) and post- (final) biofumigation PD (cfu/g soil) were done by dilution-pour plate technique using a gram of soil from a 300 g composite soil sample from various infested plots two weeks after pathogen infestation and one month after biofumigation, respectively. PD was calculated by using the following formula:

$$PD = \text{Mean colony count} \times 10^4$$

Where: 10^4– refers to the level of dilution where single colony counts were made.

Percentage Population Reduction/ Increment (%PR/I). Based on pre- and post- biofumigation PD counts the possible reduction (R) or increment (I) of PD of the test pathogen was determined using the following formula:

$$\textbf{\% Population R/I} = \frac{\text{Initial Mean PD} - \text{Final Mean PD}}{\text{Initial Mean PD}} \times 100$$

Number of days to symptom appearance (DSA). This count was taken as the number of DSA of the typical symptoms of the target disease first appeared.

Percentage infection (PI). This percentage was assessed on the plants exhibiting vascular discoloration and computed by using the formula:

$$\textbf{PI} = \frac{\text{No. of plants infected}}{\text{Total no. of plant samples}} x100$$

Percentage Severity Infection (%SI). This percentage was gathered three (3) months after biofumigation, stems and roots of sample plants were sectioned longitudinally and the severity infection was assessed based on vascular discoloration (VD) using the following arbitrary scale:

Scale	Description
0	No VD
1	1-10% of stem & root with VD
3	11-20% of stem & root with VD
5	21-30% of stem & root with VD
7	31-40% of stem & root with VD
9	41% and above of stem & root with VD

Based on scale, % SI was computed as follows:

$$\textbf{\% SI} = \frac{0n_0 + 1n_1 + 3n_3 + 5n_5 + 7n_7 + 9n_9}{9\,(N)} \times 100$$

Where:

On_0...+ $9n_9$ = refer to the number of plants showing the scale 0, 1, 3, 5, 7, and 9, respectively.

N = refers to the total number of the test plants

9 = refers to the highest rating scale

Degree of Efficacy (DE). This measure was based on the % SI on longitudinally - sectioned stem and roots of coffee using the following arbitrary scale:

Disease Severity (%)	Degree of Efficacy (DE)
0-10	Very Effective
11-20	Effective
21-30	Moderately Effective
31-40	Less Effective
41 and above	Not Effective

*Percent degree of control (%DC).*This was computed using the formula as shown below:

$$\%DC = \frac{\%DI\ Untreated - \%DI\ Treated}{\%\ DI\ Untreated} x\ 100$$

Degree of Efficacy (DE). This measure was based on computed %DC of the various test treatments against the target disease using the following arbitrary scale:

%DC	Degree of Efficacy
1 – 20	Not Effective
21 – 40	Less Effective
41 – 60	Moderately Effective
61 – 80	Effective
81 – 100	Very Effective

Cost-efficacy Analysis. This measure was determined on test treatments usage for a thousand biofumigated coffee seedlings.

Results and Discussion

Pre- (Initial) and Post- (Final) Biofumigation PD (cfu/g soil)

No significant differences were observed in pre-biofumigation population density (PD) of *Sr* with means ranging from 10.25 x 10^4 to 21 x 10^4 (cfu/g of soil) indicating comparable pathogen population before application of treatments (Figure 5, 6, and 7).

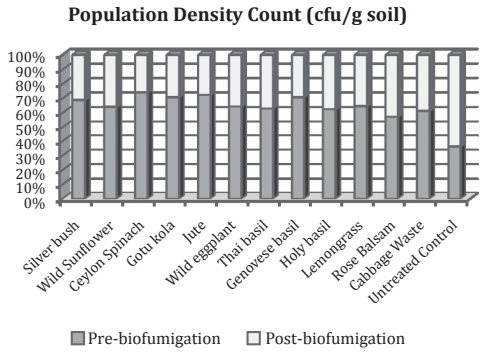

Figure 5. PD count (cfu/g soil) of Sr two weeks after pathogen infestation and one month after biofumigation.

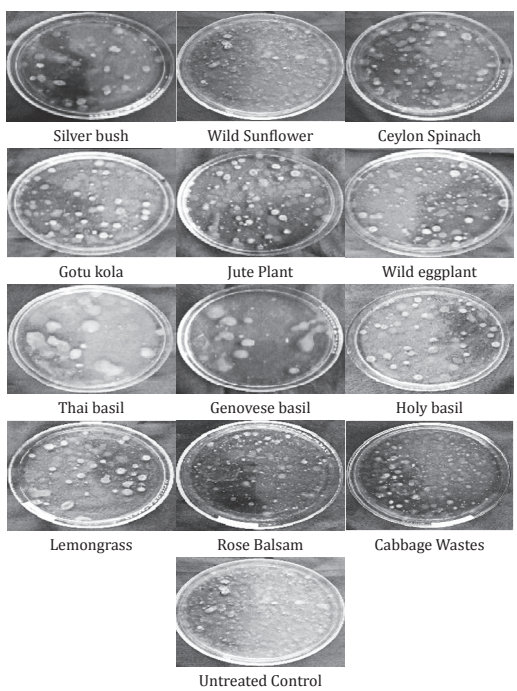

Figure 6. Pre-biofumigation PD counts (cfu/g soil) of Sr.

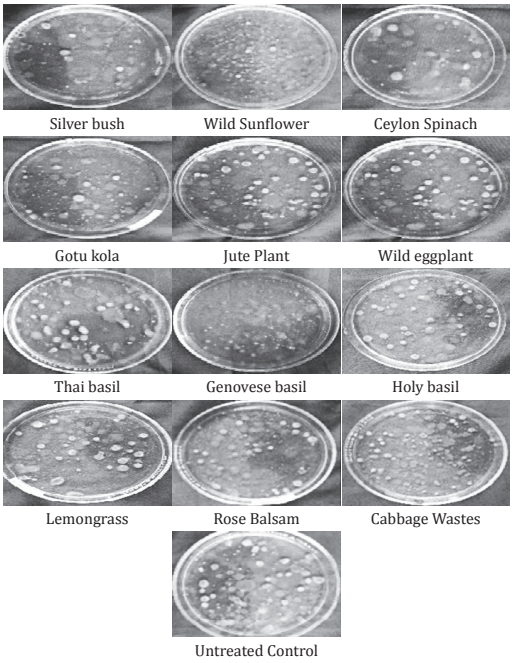

Figure 7. Post-biofumigation PD counts (cfu/g soil) of Sr.

However, after one month of biofumigation, population count of *Sr* was significantly reduced in all biofumigated soil with means ranging from 4.75 x 10^4 to 8.50 x 10^4 with corresponding %PR means ranging from 21.79 × 10^4 to 62.44 × 10^4. These measures were significantly lower compared to the untreated control plots which showed the highest mean of 24.25×10^4 cfu/g soil after one month of incubation.

Similar %PR in all test treatments were observed in Rose balsam (non-brassicaceous check) and Cabbage wastes (brassicaceous check). Only the untreated control soil showed %PI of 10.00 × 10^4 cfu/g soil hence, a nil reduction of PD of *Sr* (Figure 8).

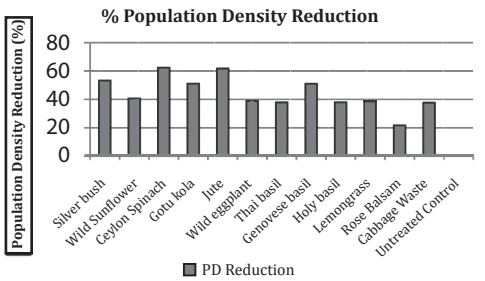

Figure 8. Population Density Reduction (%) of Sr one month after biofumigation.

Numerically however, higher %PR of PD was recorded in biofumigated soil with five weeds (Ceylon Spinach, Jute, Silver Bush, Gotu Kola and Wild Sunflower) and one herb (Genovese Basil). This result could be attributed to the biocidal compounds present in the test plants.

This finding lends support to the report of Aja et al. (2012) that leaves of Ceylon Spinach contain appreciable amount of flavonoids, alkaloids, saponins, among others and low level of toxicants like tannins as well as substantial amount of bioactive compounds while Gotu Kola biomass contain asiaticoside, that work to stimulate skin repair and strengthen skin, hair, nails and connective tissue (Kartnig, 1988). Chemical analysis of the leaf of Wild Sunflower yielded sesquiter penelactones, e.g. tagitinin which possess insecticidal properties (Obafemi & Sulaimon, 2006). Both Jute seeds and leaves are important in folklore medicine due to chorchorin, chorchotoxin, and chorcosularin in the seeds and capsularin in the leaves (Stuart Exchange Organization, 2015). Genovese Basil contains phenolic acid (Tarchoune et al., 2012).

The results imply that the different test biofumigants after one month of biofumigation could significantly reduce the fungal population present in the soil. This finding corroborates the findings of the study conducted by NOMIARC R&D Team (2011) that biofumigation is a management option against soil-borne microorganisms such as fungi, bacteria, and nematode. It is also an alternative to chemical soil fumigant (methyl bromide, formaldehyde). Likewise, it is a process by which the soil borne pests and pathogens of solanaceous and other crops including the root knot nematodes are suppressed by naturally occurring biocides released in the soil when tissues of brassica plants were decomposed on the soil.

Days to Symptom Appearance of SRR in Coffee Seedlings

All test plants in biofumigated soil exhibited delayed symptom of infection with means ranging from 10.00 to 19.75 days after transplanting (DAT) of coffee seedlings as compared to the untreated control which manifested infection earliest at 8.25 DAT. Among the test treatments, Ceylon Spinach and Silver Bush outperformed the effects of the checks (Cabbage wastes and Rose Balsam) in delaying appearance of the disease.

This result implies that Ceylon Spinach as biofumigant exhibited significantly longer stem and root rot- delaying capacity on coffee seedlings while both Wild Sunflower and Holy Basil exhibited significantly earlier rotting on coffee seedlings but were all showing delayed symptom appearance of the disease as compared to the untreated control.

PI and % SI of SRR of coffee seedlings

PI of SRR of coffee seedlings were significantly lower in biofumigated soils compared to the untreated soil (Table 1, Figures 9 and 10). The lowest PI of the disease was recorded in Lemongrass-biofumigated soil (35.00%) and Ceylon Spinach- biofumigated soil (40.00%) which were both significantly lower than the effect of both checks (Rose Balsam and Cabbage wastes) at 55.00% and 52.50%, respectively.

Application of biomass of weeds and herbs as test biofumigants exerted significant reduction in the incidence of number of test plants showing typical rotting symptom, resulting to reduced PI and % SI after three months biofumigation with similar effects exerted by both Rose Balsam (non-brassicaceous check) and Cabbage Waste (brassicaceous check).

Table 1.

Mean number of DSA, PI, %SI and %DC Based on Longitudinally- Sectioned Stem with SRR of Coffee Seedlings caused by Sr and DE of Test Biofumigants Three Months after Application.

Treatments	DSA[1/]	PI[1/]	%SI [1/]	%DC[1/]	DE[2/]
Silver bush	18.50^{a}	60.00^{cd}	22.10^{g}	72.60^{c}	E
Wild Sunflower	10.00^{f}	72.50^{b}	36.66^{b}	54.87^{h}	ME
Ceylon Spinach	19.75^{a}	40.00^{e}	9.72^{i}	87.73^{a}	VE
Gotu kola	14.25^{b}	62.50^{bcd}	29.17^{cde}	63.94^{efg}	E
Jute	13.00^{bcd}	57.50^{cd}	30.83^{cd}	61.80^{fg}	E
Wild eggplant	14.50^{b}	60.00^{cd}	23.06^{fg}	71.48^{cd}	E
Thai basil	10.50^{ef}	67.50^{bc}	31.67^{c}	60.83^{fg}	E
Genovese basil	14.00^{bc}	55.00^{d}	26.39^{defg}	67.36^{cde}	E
Holy basil	10.00^{f}	60.00^{cd}	32.22^{bc}	60.15^{g}	E
Lemongrass	14.00^{bc}	35.00^{e}	15.28^{h}	81.08^{b}	VE
Rose Balsam [Non-Brassicaceous Check]	12.00^{de}	55.00^{d}	27.49^{cdef}	66.00^{def}	E
Cabbage Waste [Brassicaceous Check]	12.50^{cd}	52.50^{d}	24.72^{efg}	69.37^{cde}	E
Untreated Control	8.25^{g}	95.00^{a}	80.83^{a}	0.00^{i}	NE

[1/] Means with common letter superscripts in a column are not significantly different at 1% level, Tukey's Test.

Department of Plant Pathology, College of Agriculture, USM, Kabacan, Cotabato. 2014.

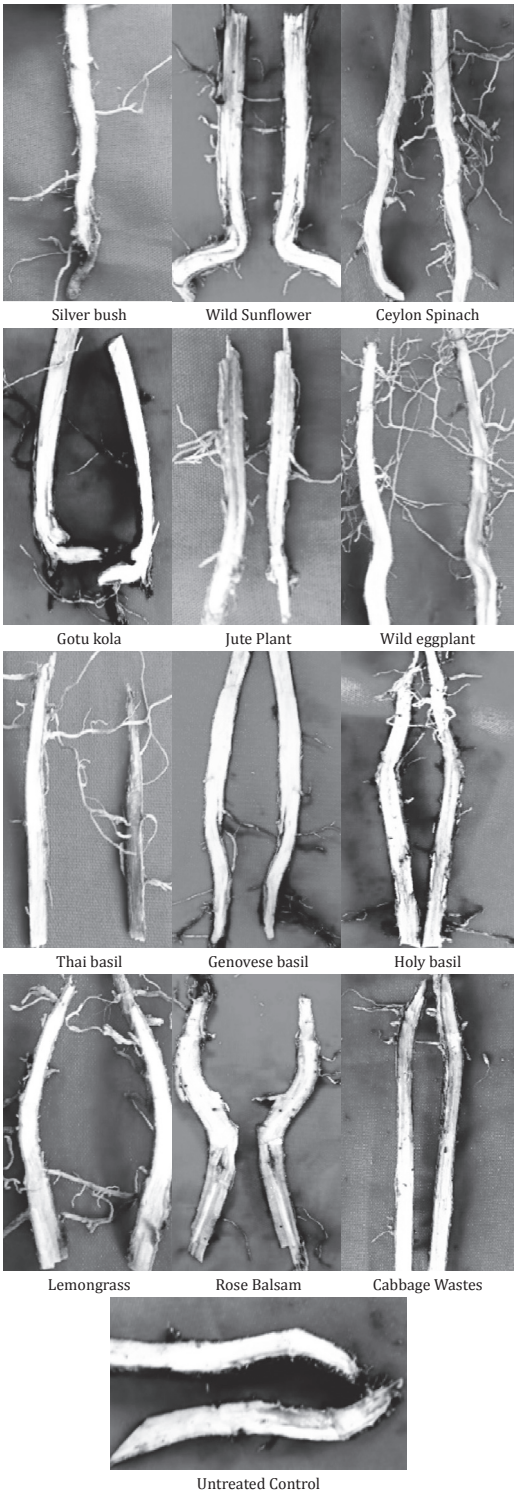

Figure 9. Longitudinally-sectioned roots of coffee seedlings showing various disease intensity levels of SRR based on % VD.

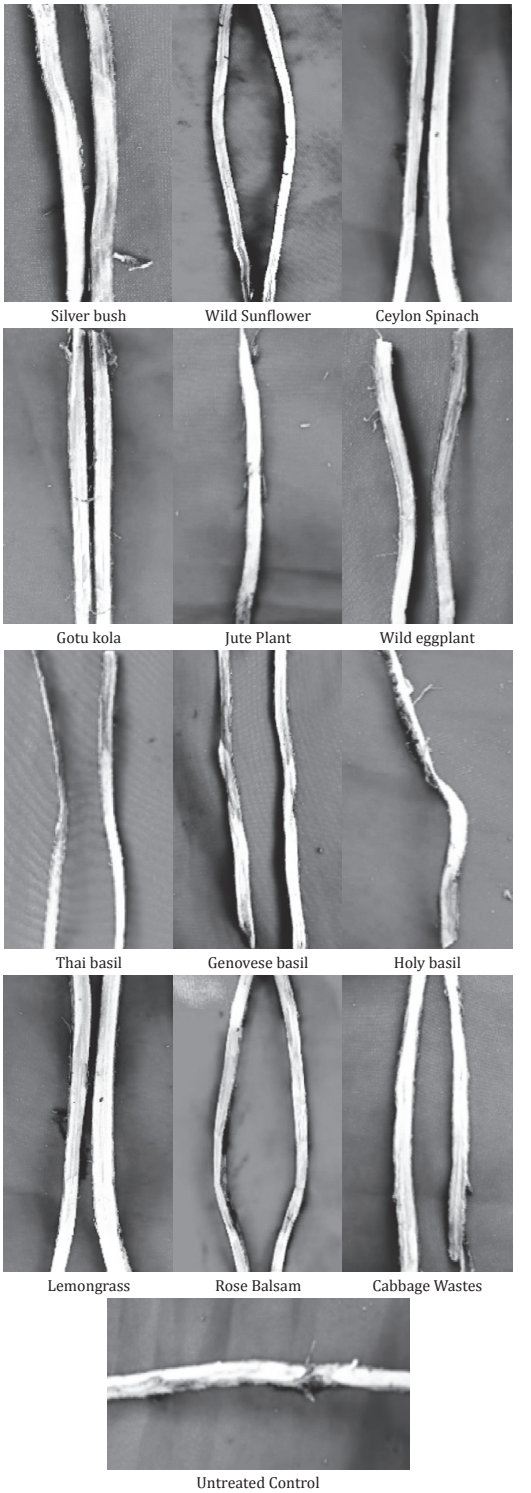

Figure 10. Longitudinally-sectioned stems of coffee seedlings showing various disease intensity levels of SRR based on % VD.

The aforementioned results conform to the findings of Fayzalla et al. (2009) that the common fungal pathogens to soybean causing damping-off, root rot and wilt diseases were controlled by biofumigation which is used as a means to control many diseases by biocidal compounds (mainly ITCs) released from glucosinolates in mustard seed meal, which is hydrolyzed during incorporation in the soil.

Moreover, Ceylon Spinach-treated plants showed the lowest % SI mean of SRR (9.72%) followed by Lemongrass (15.28%) which were both significantly lower than the effects of both checks (Cabbage wastes and Rose Balsam) with respective means of 24.72% and 27.49%.

Of the 10 test biofumigants, Ceylon Spinach-biofumigated soil effected the lowest disease severity of SRR of coffee seedlings (9.72%) based on % VD of both stems and roots. This biofumigation process was followed by the effect of silver bush (22.10%), Wild Eggplant (23.06%) and Genovese Basil (26.39%). While the non-biofumigated soil afforded the highest %SI of SRR at 80.83%.

This result implies that of the 10 treatments tested, 2 treatments (Ceylon Spinach and Lemongrass) demonstrated the highest effect in reducing disease intensity of SRR in coffee seedlings.

The aforecited results lend support to the findings of Aja et al. (2012) that leaves of Ceylon Spinach contain an appreciable amount of flavonoids, alkaloids, saponins, among others and low level of toxicants like tannins and substantial amount of bioactive compounds. Lemongrass contain vitamin A and natural citral that help prevent the growth of some bacteria and yeast.

Percentage DC and DE

Consequently, a marked higher %DC of SRR was observed among the different treatments. Ceylon Spinach- and Lemongrass- biofumigated soil afforded the highest %DC of the target disease at 87.73% and 81.08%, respectively.

Percentage DC exerted by both Ceylon Spinach and Lemongrass were even higher than the effects of Cabbage wastes (brassicaceous check) and Rose Balsam (non brassicaceous check). All other treatments like Silver Bush, Gotu Kola, Wild Eggplant and Genovese Basil exerted comparable %DC with the two biofumigant checks. Likewise, Jute, Thai Basil and Holy Basil exhibited %DC of SRR similar to the effect of Rose Balsam (non- brassicaceous check).

These results suggest that aside from brassicas, other plants like weeds and herbs can also be utilized as biofumigants to manage the target disease due to their reported biocidal contents.

Of the 10 biofumigant treatments, two treatments, Ceylon Spinach and Lemongrass were rated very effective (VE), seven treatments (Silver Bush, Gotu Kola, Jute, Wild Eggplant, Thai Basil, Genovese Basil and Holy Basil) were found effective (E) and wild sunflower was moderately effective (ME) against the test pathogen.

The findings demonstrated the efficacy of weeds and herbs similar to brassicaceous plants as potential and effective sources of biofumigants against *Sr* in reducing infection and severity of SRR on test plants used in this study. Biofumigation was an effective method to control soilborne pathogen like *Sr* using plants that similarly emit antifungal or biocidal compounds.

These findings support earlier report of Abragan et al. (2006) that biofumigation is a management strategy to minimize soilborne diseases like bacterial wilt of potato. It is a process of utilizing compounds (ITCs) excreted by the test plants upon the degradation of the tissues.

Table 2

Cost-efficacy analysis for a thousand seedlings usage of test treatments against Sr causing SRR disease of coffee seedlings. USM, Kabacan, Cotabato. 2014.

PARTICULARS	TREATMENTS												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A. Inputs (Php)													
Planting materials	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
Fertilizers	318	318	318	318	318	318	318	318	318	318	318	318	318
Treatments	0	0	0	0	0	0	100	100	100	50	0	50	0
B. Labor													
Hauling	290	290	290	290	290	290	290	290	290	290	290	290	290
Weeding	290	290	290	290	290	290	290	290	290	290	290	290	290
Watering	290	290	290	290	290	290	290	290	290	290	290	290	290
Planting	290	290	290	290	290	290	290	290	290	290	290	290	290
Fertilization	290	290	290	290	290	290	290	290	290	290	290	290	290
Total Cost (Php)	3, 268	3, 268	3, 268	3, 268	3, 268	3, 268	3,368	3,368	3,368	3,318	3,268	3,318	3,268

ASSUMPTIONS (Input Costs): *Seedlings (coffee) P1.50/seedlings×1000= P1, 500.00 Fertilizer (Urea) – PhP 1275/bag. Recommended rate/seedling:300g. Labor cost: 290/day. Rate/treatment (Php/kg): SB-0, WS-0, CS-0, GK-0, JP-0, WE-0, TB-20, GB-20, HB-20, LG-5, RB-25, CWs-5*

LEGEND:

T_1- Silver Bush

T_2- Wild Sunflower

T_3- Ceylon Spinach

T_4- Gotu Kola

T_5- Jute

T_6- Wild Eggplant

T_7- Thai Basil

T_8- Genovese Basil

T_9- Holy Basil

T_{10}- Lemongrass

T_{11}- Rose Balsam [Non-Brassicaceous Check]

T_{12}- Cabbage Waste [Brassicaceous Check]

T_{13}- Untreated Control

Cost-efficacy Analysis

Table 2 shows the cost-efficacy analysis of treatments usage per 1000 coffee seedlings using the ten test biofumigants against *Sr* causing SRR rot disease of coffee seedlings three (3) months after planting.

Analysis shows that the total production costs of treatments usage ranged from P3, 268.00 to P3, 368.00. The use of test biofumigants showed that they are not only cheap sources of eradicative control agents for SRR disease of coffee seedlings but safe and effective as well as environment- and people- friendly antifungal control agents.

Conclusion and Recommendations

The results of the study showed that other than brassicas, biomass of weeds and herbs can also be utilized as biofumigants against *Sr*, a soilborne pathogen of coffee seedlings in the nursery.

Population count of *Sr* was significantly reduced from 21.79×10^4 to 62.44×10^4 (cfu/g soil) after one month of biofumigation due to the biocidal compounds released by the test plants upon decomposition in the soil. Two test biofumigants, (Ceylon Spinach and Lemongrass) a weed and herb, respectively, afforded higher %DC compared to the two standard biofumigant checks (Cabbage wastes and Rose Balsam). Other weeds like Silver Bush, Gotu Kola, Wild Eggplant and

Jute as well as other herbs like Genovese Basil, Thai Basil and Holy Basil also showed comparable reduced PD with the non-brassicaceous checks (Cabbage Wastes and Rose Balsam).

Among the cost- effective treatments, biomass of Ceylon Spinach and Lemongrass were the excellent biofumigants in managing SRR of coffee seedlings in the nursery caused by *Sr.*

In conclusion, biomass of weeds and herbs as biofumigants used in this study seemed to possess antifungal or biocidal properties which were released during the biofumigation process thus, were capable to significantly reduce the incidence and severity infection of SRR disease of Robusta coffee seedlings. The use of non–brassicaceous plants such as weeds and herbs is therefore considered a cheap, safe and readily available substitute or alternative to the use of chemical soil fumigants which are relatively more expensive for the eradicative control of soilborne pathogen like *Sr.*

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