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Integrated Effect of Soil Solarization and Biofumigants on Soil-Borne Pathogens in Tomato

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A b s t r a c t

An experiment was conducted to evaluate the integrated effect of soil solarization and biofumigation on root-rot and root-knot diseases of tomato caused by *Sclerotium rolfsii* and *Meloidogyne incognita*. In vitro screening of mustard and marigold leaf extracts identified mustard as the most effective biofumigant, inhibiting radial growth (76.33%) and sclerotia formation (79.60%) of *S. rolfsii*. Selected biofumigant plants (mustard and marigold) were cultivated, chopped, and incorporated into the soil. The susceptible tomato variety 'Moneymaker' was used in field trials, where treatments included soil solarization and biofumigation alone or in combination. Among treatments, the combined application (T4) significantly reduced pre- and post-emergence seedling mortality, with the lowest disease incidence (25.37%) and severity (27.76%). Root-knot nematode severity was also lowest in solarized mustard-amended soil (4.37), followed by solarized marigold-amended soil (5.28). Egg mass production and galling index were considerably reduced under combined treatments. These results indicate that integrating soil solarization with biofumigation provides superior control of *S. rolfsii* and *M. incognita* compared to individual treatments.

Keywords: Solarization, Biofumigation, *Sclerotium rolfsii*, *Meloidogyne incognita,* Mustard and Marigold**.**

Introduction

Soil-borne diseases are among the most economically significant challenges faced by farmers and nursery producers worldwide. These pathogens can persist in the soil for extended periods as resistant structures such as chlamydospores, sclerotia, thick-walled conidia, hyphae, eggs, cysts, or in crop residues and infected plant roots (Raaijmakers et al., 2009). Under favorable environmental conditions, pathogens like nematodes, *Rhizoctonia* spp., *Phytophthora* spp., *Sclerotinia* spp., *Pythium* spp., *Verticillium* spp., and *Fusarium* spp. can cause severe yield losses—up to 50–75% in crops including wheat, cotton, maize, vegetables, fruits, and ornamentals (Baysal and Kabir, 2018). Tomato and brinjal are particularly vulnerable to these pathogens throughout their growth stages (Baysal-Gurel et al., 2012). Disease diagnosis is often complicated due to overlapping symptoms such as damping-off, root blackening, canker, root rot, root-knot, wilting, stunting, and yellowing (Astrom, 1998). Consequently, managing these diseases becomes even more difficult. While conventional control strategies largely rely on synthetic chemical fungicides and fumigants, their overuse has led to environmental degradation, human health risks, destruction of beneficial soil microorganisms, aquatic toxicity, and even ozone layer depletion. Due to these concerns, several hazardous chemicals are being restricted or banned globally, prompting researchers to seek safer, sustainable alternatives.

Among emerging eco-friendly strategies, biofumigation combined with soil solarization has shown promising results in managing a broad spectrum of soil-borne pathogens. Solarization involves covering moist soil with transparent polyethylene sheets during hot periods, trapping solar energy to raise soil temperatures to levels that eliminate or suppress pathogens, weed seeds, and pests (Stapleton, 2000). In addition to its direct thermal effect, solarization alters the physical and chemical properties of soil, enhances nutrient availability, and improves crop productivity (Katan and Devay, 1991). Biofumigation, on the other hand, involves incorporating certain plant residues—especially from the Brassicaceae family—into the soil, where they decompose and

release biologically active volatile compounds such as isothiocyanates and ammonia under anaerobic conditions (Angus et al., 1994). These compounds have known biocidal and biostatic properties that suppress soil pathogens. When used together, solarization and biofumigation not only enhance each other's effectiveness but also reduce soil stress and promote microbial antagonism, leading to improved disease management and soil health.

Considering the above, the present study was designed to evaluate the individual and combined effects of soil solarization and biofumigation using mustard and marigold residues against two major soil-borne pathogens—*Sclerotium rolfsii* and *Meloidogyne incognita*—affecting tomato, both under in vitro and in vivo conditions.

Materials & Methods

Seed collection and planting of tomato seedlings

Tomato (Moneymaker) seeds were collected from the local market in Dumki, Patuakhali. The potting medium was prepared in a 2:1:1 ratio of loamy soil, organic compost and sand to ensure optimal growth conditions. Seeds were treated with captan 70% WP (2g/kg seeds). After that, the treated seeds were sown 0.5 cm deep in pots and maintained at 20-25 ◦C with adequate sunlight for germination. Regular irrigation, mulching and pest management were implemented to support healthy seedling development.

Experimental Environment

Soil temperature was recorded by soil thermometer (V tech probe thermometer) placed at average depths of 5-25 cm in solarized and non-solarized soil.

Table 1. Maximum soil temperatures (◦C) in solarized and non-solarized plots during the application period of October 2023 -April 2024

Depth (cm)	Solarized soil (◦C)	Non-solarized soil (◦C)
5	32.5	28.9
15	28.3	24.6

Collection, isolation and preservation of *Sclerotium rolfsii*

A pure culture of *Sclerotium rolfsii* was obtained from the rhizosphere and rhizoplane of tomato (*Solanum lycopersicum*), carrot (*Daucus carota*), and brinjal (*Solanum melongena*). Samples exhibiting characteristic root-rot symptoms were chosen from infected fields. The fungal colonies were cultured on Potato Dextrose Agar (PDA) and identified using the standard key by Barnett and Hunter (1972). The pure culture of *S. rolfsii* was maintained on PDA slants at 10°C in a refrigerator as a stock culture for future use.

Preparation of suspension of *Sclerotium rolfsii*

A 15-day-old pure culture with sclerotia formed structure within (around 100 sclerotia) per gram of soil was used for suspension preparation by scraping off the PDA media plate and crushing through a grinder machine, thus making a suspension mixed with water (1:2).

Collection of *Meloidogyne incognita* from infected tomato root

The population of *Meloidogyne incognita* was used in the experiment, collected from nematode-infected tomato roots from Dumki Upazilla of Patuakhali District. Egg masses and nematodes of J_2 were picked up and inoculated into young seedlings of tomato. Sub-culturing was done subsequently by inoculating new tomato seedlings with egg masses.

Nematode extraction and inoculation

Nematodes from roots were extracted by the Baermann funnel technique as described by Baermann (1917). Then the nematodes were counted under a stereo microscope.

Mature egg mass and J_2 of nematode (*Meloidogyne incognita*) was collected from severely galled roots of tomato. Juveniles (J_2) were extracted through the Baermann funnel technique.

Each tomato seedling was inoculated with approximately 1000 J_2 by using a micropipette, making holes in the soil.

In vitro screening of Mustard and Marigold leaf extract against *S. rolfsii*

This experiment was conducted to evaluate the effect of leaf extract of M*ustard* and Marigold against *S. rolfsii* in culture media. PDA medium was separately amended with the required amount of plant extract after sterilization when it cooled (40° C) under aseptic conditions. Control treatment was maintained by pouring PDA medium without plant extract. A mycelial disc of 5 mm diameter from three days old culture of *S. rolfsii* was cut with a sterilized cork-borer and placed in the centre of plant extract-amended PDA petridishes separately. The petridish having PDA alone (control) was inoculated in the same manner. All plates were incubated in the dark at 25°C until the mycelium of *S. rolfsii* covered the whole plate in control treatment. The inhibitory effect of all the plant extracts against the mycelial growth and sclerotial formation of *S. rolfsii* was tested at 20% and 50% concentrations under *in-vitro* conditions by using the food poison technique (Nene and Thapliyal, 1979). The radial growth and number of sclerotia per plate were assessed**.**

The percent inhibition of the radial growth and sclerotia formation was calculated as described by Sundar *et al.* (1995).

$$\% \text{ Inhibition of mycelial growth} = \frac{X-Y}{X} \times 100$$

Where, X =Mycelial growth of pathogen in absence of leaf extract

Y =Mycelial growth of pathogen in presence of leaf extract

$$\% \text{ Inhibition of sclerotia formation} = \frac{X-Y}{X} \times 100$$

Where, X = Selerotial formation of pathogen in absence of leaf extract

Y = Selerotial formation of pathogen in presence of leaf extract

Treatments (For *S. rolfsii* and *M. incognita*)

Treatments	*S. rolfsii*	Treatments	*M. incognita*
T_0	Uninoculated field (Control-1).	T_0	Solarized soil. Tomato seedlings were transplanted under solarized soil in pot
T_1	*S. rolfsii* inoculated field (Control-2)	T_1	Non-solarized soil. Tomato seedlings were grown in non-solarized (shaded) conditions in pot
T_2	*S. rolfsii* inoculated field + Biofumiga	T_2	Solarized soil with mustard incorporated soil. Tomato seedlings were grown in non-solarized (shaded) conditions and

	nt (Mustard)		the soil was incorporated by mustard plant (1-month-old)
T_3	*S. rolfsii* inoculated field + Solarized soil	T_3	Solarized soil with marigold incorporated soil. Tomato seedlings were grown in non-solarized (shaded) conditions and the soil was incorporated by marigold plant (1-month-old)
T4	*S. rolfsii* inoculated field + Biofumigant (Mustard) + Solarized soil	T_4	Non-solarized soil with mustard incorporated soil. Tomato seedlings were transplanted under shed condition and the soil was incorporated by mustard plant (1- month-old)
		T_5	Non-solarized soil with marigold incorporated soil. Tomato seedlings were transplanted under shed condition and the soil was incorporated by marigold plant (1-month-old)

Counting of galls, egg masses and J_2

The number of egg mass/root systems was counted following Holbrook *et al.* (1983). The roots were soaked in "PhloxineB for 13 min. The roots were observed, and egg masses were counted with a magnifying glass. The egg masses were picked with forceps, and the eggs were separated from viscous materials carefully by using two sterile needles on a slide, then observed under a microscope, and the eggs were counted. Egg per root system was counted; Juvenile was collected following the white head tray method. Pot soil was mixed thoroughly, and 100 g soil was weighed and put it on the sieve that was on a bowl filled with water. On the sieve, there was tissue paper that touched the water level in the bowl. After 3 days, the water from the bowl and the juveniles were counted under the microscope.

Data recording

Data on germination, number of healthy plants, and infected plants were recorded during the growing period. When the root rot symptoms appeared within (bi-weekly), five plants in each plot were randomly selected and uprooted carefully, washed with water, and checked individually and disease severity was rated on a 0-4 scale (0=No symptoms, 1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100% of tomato root covered with lesions).

In case of root-knot,

The disease incidence and disease severity were assessed by the following formula:

$$\text{Percent Disease Incidence} = \frac{\text{Number of diseased plants}}{\text{Number of total plant observed}} \times 100$$

$$\text{Percent Disease Severity} = \frac{\text{Sum of all disease rating}}{\text{Total no. of rating} \times \text{maximum disease grade}} \times 100$$

Statistical analysis

Data were analyzed statistically by using the SPSS computer software package (Version 16). The treatment means were compared following Duncan's Multiple Range Test (DMRT)(Gomez and Gomez, 1984).

Results

Results describe the effect of mustard and marigold leaf extract at 20% concentration and 50% concentration on radial growth of *S. rolfsii* and effects of solarization and biofumigation on root-knot nematode *Meloidogyne inognita* and root-rot fungus *Sclerotium rolfsii* at different parameters.

In vitro effect of mustard and marigold leaf extract (20% and 50%) on radial growth and sclerotia formation of *S. rolfsii*

The effect of leaf extract of mustard and marigold in reducing the radial growth and sclerotia formation of *S. rolfsii* was presented in Table 1. Significantly, the highest inhibition (76.33%) of mycelial growth of *S. rolfsii* was observed at 50% concentration of mustard leaf extract, followed by 20% concentration (60.07%). On the contrary, marigold leaf extract inhibited the mycelial growth by 54.63% and 37.25%, at concentrations of 50% and 20%, respectively. In the case of *sclerotia* formation by *S. rolfsii*, the highest *sclerotia* formation inhibition was (79.60%) recorded at 50% mustard leaf extract significantly followed by 20% concentration (63.61%), however, sclerotium formation was found to be statistically similar in the marigold treated plate by 50% (49.69%) and 20% (41.11%) concentration. These results indicated that mustard leaf extract was significantly superior to other leaf extracts in reducing the radial growth and *sclerotia* formation.

Table 1. Effect of *Mustard and Marigold* leaf extract on inhibition of radial growth and *sclerotia* formation of *S. rolfsii in vitro*

Biofumigants	Concentration (%)	% inhibition over control	
		Radial growth	Sclerotia formation
Mustard	20	60.07 ^{b}	63.61 ^{b}
	50	76.33 ^{a}	79.60 ^{a}
Marigold	20	37.25 ^{d}	41.11 ^{c}
	50	54.63 ^{c}	49.69 ^{c}

* Data were recorded after two weeks of treatments. Different letters indicate the significant differences based on 1-way ANOVA with Tukey's HSD test. Means within the same column followed by a common letter(s) are not significantly different (*P=0.05)*

Effect of biofumigant crop (Mustard) and solarized soil for the management of *S.rolfsii*

Among the different treatments, including biofumigation and soil solarization either individually or in combination, treatment T4 appeared to be superior in reducing the pre and post-emergence mortality of tomato caused by S. rolfsii (Table 2). Among the treatment combinations, the highest total seedling mortality (40.67%) was recorded in treatment T1. On the contrary, significantly the lowest total seedling mortality was observed at treatment T4 (10.83%), followed by T2 (15.04%) and T3 (19.92%), respectively. The results of the current study suggest the superiority of the combined approach of soil solarization + mustard for the management of S. rolfsii over individual treatments, either mustard-incorporated soil or soil solarization.

Effect of the severity of root rot

Disease incidence and severity of root rot disease of tomato were significantly influenced by single-component or combined application of biofumigant and solarized soil (Table 3). The lowest disease incidence (25.37%) and severity (27.76 %) of root rot were found in the treatment ($T_{4)}$,

Table 2. Effect of soil solarization and biofumigation on tomato seedling mortality caused by *S. rolfsii* in the field

Treatments	% mortality		
	Pre-emergence	Post-emergence	Total
Uninoculated (T_0)	8.33	9.00	17.33 [b]
S. rolfsii inoculated (T_1)	25.00	15.67	40.67 [a]
S. rolfsii+Mustard (T_2)	10.57	4.47	15.04 [c]
S. rolfsii+Solarization (T_3)	12.25	7.67	19.92 [b]
S. rolfsii+ Mustard+ Solarization (T_4)	8.33	2.50	10.83 [d]

* Different letters indicate the significant differences based on 1-way ANOVA with Tukey's HSD test. Means within the same column followed by a common letter(s) are not significantly different (*P=0.05*).

where solarized soil and biofumigant were used in integration. On the contrary, significantly the highest disease incidence (54.36%) and severity (76.34%) of root rot were observed in the T_2 treatment, where the tomato was transplanted in the *S. rolfsii* inoculated soil without any other amendment. Even, % disease incidence and severity were also observed to be low in the *S. rolfsii* and mustard-treated soils (T2). Results indicated that soil solarization and combination with biofumigant were effective in reducing root rot disease incidence and severity of tomato.

Table 3. Effect of the soil solarization and biofumigation on the incidence and severity of root rot disease of tomato in the field

Treatments	% Disease incidence	% increased (+) or decreased over control	% Disease severity
Uninoculated (T_0)	47.79 [b]	0.00	65.70 [b]
S. rolfsii inoculated (T_1)	54.36 [a]	13.24	76.34 [a]
S. rolfsii+Mustard (T_2)	34.24 [bc]	26.97	42.34 [d]
S. rolfsii+Solarization (T_3)	41.23 [bc]	17.10	53.70 [c]
S.rolfsii+Mustard+Solarization (T_4)	25.37 [c]	44.34	27.76 [e]

*Data were recorded after forty five days of treatments. Different letters indicate the significant differences based on 1-way ANOVA with Tukey's HSD test. Means within the same column followed by a common letter(s) are not significantly different (*P=0.05*)

Effect of severity on root-knot

The combined effect of solarization and biofumigant treatment had a **s**ignificantly positive effect on reducing the severity of root-knot diseases caused by *M. incognita* (Table 4). Biofumigation had a very significant effect on the reduction of the severity index of root-knot diseases of tomato. Significantly, the highest disease severity was (14.59%) observed in non-solarized soil and then solarized soil (11.28%) alone. On the contrary, the lowest disease severity was recorded after the combination of solarization with mustard-incorporated soil (4.37%), followed by marigold-treated soil (5.28%), suggesting that incorporation of the biofumigant plant to the soil along with solarization might have a highly disease suppressive effect on to plant (Table 4).

Table 4. Effect of the soil solarization and biofumigation on disease severity of root-knot disease of tomato in the field

Soil treatments	Disease severity
	Root-knot nematode
Solarized soil	11.28 [b]
Non- solarized soil	14.59 [a]
S +Mustard	4.37 [d]
S +Marigold	5.28 [d]
NS+ Mustard	6.01 [d]
NS +Marigold	8.26 [c]

Severity of root-root nematode (*Meloidogyne* spp.) used a 0-4 index scale. 0=Healthy;1=1-25%; 2=26-50%; 3=51-75%; 4=76-100% diseased. S=solarized soil and NS=non-solarized soil.

* Data were recorded after sixty days of treatments. Different letters indicate the significant differences based on 1-way ANOVA with Tukey's HSD test. Means within the same column followed by a common letter(s) are not significantly different (*P=0.05*)

Effect on the intensity of egg masses and galling index

The development of egg masses per 10 g of soil varied with different treatments on tomato roots, by root-knot nematode, *M. incognita*. Plants grown in solarized and mustard-incorporated soil produced significantly a smaller number of egg masses (3.12) followed by solarized and marigold-incorporated soil (3.83) compared to all other treatments and control (Table 5). Soil treatments viz. non-solarized & mustard and non-solarized & marigold, the tomato roots produced a similar number of egg masses but these were more as compared to solarized and mustard or marigold incorporated soils. In addition, the formation of gall on tomato roots, by root-knot nematode, *M. incognita* was reduced when the plants were grown in soil amendments. The minimum galling index (2.75) was recorded in solarized and mustard-incorporated soil followed by the solarized and marigold-treated soils (3.02). Even, the galling index was also significantly low in comparison to solarized soil alone. These results indicate that solarization combined with different biofumigants are better in controlling the soil-borne nematode, *Meloidogyne incognita.*

Table 5. Effect of different treatments on **t**he development of egg mass and formation of galling index

Soil treatment	Egg masses /10g of soil	Galling index
Solarized soil	7.23 [b]	6.27 [a]
Non- solarized soil	10.93 [a]	6.80 [a]
S+Mustard	3.12 [d]	2.75 [d]
S+Marigold	3.83 [d]	3.02 [cd]
NS+ Mustard	4.93 [c]	3.17 [cd]
NS +Marigold	5.10 [c]	4.12 [b]

* Data were recorded after sixty days of treatment. Different letters indicate the significant differences based on 1-way ANOVA with Tukey's HSD test. Means within the same column followed by a common letter(s) are not significantly different (*P=0.05*)

Discussions

Root-knot disease caused by *Meloidogyne incognita* and Sclerotium rot caused by *Sclerotium rolfsii* are both significant soil-borne diseases responsible for major yield losses in tomato cultivation. When these pathogens interact, they may form a disease complex that further exacerbates crop damage and can even overcome plant resistance mechanisms. To address this challenge, the present study aimed to evaluate the efficacy of eco-

friendly, cost-effective management strategies, specifically soil solarization and biofumigation using mustard and marigold, against *S. rolfsii* and *M. incognita* under both in vitro and in vivo conditions. Initial in vitro assays demonstrated that mustard leaf extract (50%) was significantly more effective than marigold in reducing both radial colony growth and sclerotia formation of *S. rolfsii* (Table 1). This efficacy is likely due to the high glucosinolate (GSL) content in mustard, a characteristic of Brassicaceae plants, which upon hydrolysis release bioactive isothiocyanatescompounds known for their biocidal properties (Fahey et al., 2001; Sarwar et al., 1998). Based on these results, mustard was selected as the primary biofumigant for subsequent field applications. Field results revealed that the integration of soil solarization and mustard-based biofumigation significantly reduced seedling mortality caused by *S. rolfsii* compared to either treatment alone (Table 2). These findings are in agreement with previous studies that demonstrated enhanced disease control through the integration of antagonists and organic amendments (Begum & Bhuiyan, 2006; Adandonon et al., 2006; Rahman et al., 2012; Bhuiyan & Sen, 2013). Moreover, the combined approach proved effective in reducing both the incidence and severity of root-rot disease in tomato (Table 3). This aligns with the findings of Baysal-Gurel et al. (2019), who reported a 30–35% reduction in *Rhizoctonia* root rot when solarization was combined with biofumigation using cover crops. Regarding *M. incognita*, significant reductions were observed in disease severity (Table 4), egg mass production, and galling index (Table 5) under the integrated management strategy. The incorporation of biofumigant crops, particularly those from the Brassicaceae family, is known to release volatile isothiocyanates during decomposition, which suppress nematode populations (Shaban et al., 2011). Similarly, Moura et al. (2012) reported successful control of *P. lycopersici* and nematode infections through combined use of soil solarization and organic amendments such as cabbage residues and sheep manure. Soil solarization alone also demonstrated effectiveness by raising soil temperatures to levels lethal to many pathogens including nematodes, fungi, and weed seeds. This not only improved plant health but also led to significant yield increasesranging from 25% to 432% in crops like broad beans, onions, tomatoes, and clover in various soil types (Abdel-Rahim et al., 1988).

Overall, the study confirms that the integration of soil solarization and biofumigation provides a synergistic and sustainable approach to managing complex soil-borne diseases in tomato, offering an effective alternative to chemical controls.

Conclusion

Both soil solarization and biofumigation have proven to be effective and environmentally sustainable strategies for managing root-rot and root-knot diseases in tomato. Biofumigation, in particular, demonstrates strong potential for suppressing a range of soil-borne pathogens, especially when integrated into existing cropping systems. Its compatibility with other control methods, such as soil solarization or the use of resistant cultivars, makes it a valuable tool within integrated pest management (IPM) programs for both conventional and organic horticulture. Continued research and refinement of these techniques will help optimize their application across diverse crops, soil types, and climatic conditions, ultimately supporting improved crop productivity and environmental health.

Conflict of interest

The authors affirm that they have no financial or other conflicts of interest that could affect their decision to publish this work.

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