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# EFFECT OF SOIL SOLARIZATION AND BIOFUMIGATION ON STEM ROT DISEASE OF POTATO CAUSED BY *Sclerotium rolfsii*

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## Abstract

The role of soil solarization and biofumigation on stem rot disease of potato caused by *Sclerotium rolfsii* was investigated through the present study conducted during July 2015 to June 2016. Soil solarization was done by covering soil with transparent polyethylene sheet for four weeks. Appropriate antifungal biofumigant was selected by *in vitro* screening of cabbage, cauliflower, mustard and broccoli leaf extracts against a virulent isolate of *S. rolfsii.* Mustard leaf extract was found to be the most effective in inhibiting the radial growth (78.79%) and sclerotia formation (83.13%) of *S. rolfsii*. Selected biofumigant plant was grown, chopped and incorporated into the soil. The stem rot susceptible potato variety Cardinal was used as host plant. Soil solarization and biofumigant were applied alone or in combination to manage the stem rot disease of potato in the field. Among the different treatments, combined application of soil solarization and biofumigation ($T_5$) was appeared to be superior in reducing the pre- and post-emergence mortality of potato seedlings. The lowest disease incidence (26.67%) and plant disease index (PDI) (29.86%) were found in the treatment $T_5$ followed by that of $T_3$ (Biofumigant). Maximum increase (121.67%) of potato yield was also found in plots treated with combined use of soil solarization and biofumigation followed by that of biofumigant (83.02%) over control. The results suggested that combined use of soil solarization and biofumigation is the most effective for the management of *S. rolfsii* compared to the individual treatment either with soil solarization or biofumigation.

**Keywords:** Soil solarization, biofumigation, *Sclerotium rolfsii*, stem rot disease, yield of potato.

## Introduction

Potato (*Solanum tuberosum* L.) ranks next to rice and wheat in terms of production and internal demand in Bangladesh. It is used primarily as vegetable and as nutritional food. In Bangladesh, 471.06 thousand hectares of cultivable land is under potato cultivation and the country produced 9.25 million tons of potato in 2014-2015 with an average yield 19.64 t /ha (BBS, 2015). Among the various factors attributed to low yield of potato, vulnerability of the crop to disease is important one. In Bangladesh, so far as many as 57 diseases have been recorded in potato (Hossain *et al.,* 2008). Among all the pathogens, the soil borne fungi like *Rhizoctonia solani* and *S. rolfsii* cause serious yield loss of potato. *S. rolfsii* Sacc. (telomorph: *Athelia rolfsii)* is a well-known polyphagous soil borne plant pathogenic fungus (Aycock, 1966). This fungi can produce severe stem rot of potato in many potato growing areas

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in Bangladesh, where it is known to cause up to 60 percent reduction in tuber yield (Haque and Khan, 1977). The fungus infects potato plants at the collar region. Grayish brown, slightly sunken spots having 20-30 mm in diameter appear on the stem. Most of the cases first symptom associated with *S. rolfsii* are usually yellowing and wilting of leaves following stem rot infection (Mullen, 2001). This sclerotia forming pathogen is difficult to control through cultural practices or traditional chemical treatment. Chemical compounds have been used as seed treating fungicide and sometimes effective to control soil borne pathogens but their non-judicious application has favored the development of pathogens resistance to fungicides and polluted the environment, simultaneously imbalance the ecology. Soil solarization by solar heat and biofumigation by *Brassica* spp. are considered effective and environmentally safe methods for the control of soil-borne pests, including phytopathogens and weeds (Smolinska *et al.*, 2003; Kapoor, 2013). Solarization elevates soil temperature to a level that is detrimental to soil borne pests and pathogens. The microbial processes induced by solarization may also contribute to disease control (Katan, 1981). Soil solarization helps improve soil structure and increase the availability of nitrogen (N) and other essential plant nutrients (Elmore *et al.*, 1997). Biofumigation by *Brassica* spp. is an alternate method for plant disease suppression. In *Brassica* spp. contain different kinds of natural biocidal compounds, such as glucosinolate (Matthiessen and Shackleton, 2005). After decomposing of *Brassica* tissues, the volatile biocidal compounds (mainly isothiocyanates) can be released which help suppress soil borne pathogens, such as fungi, bacteria, and nematodes (Smolinska *et al.*, 2003). Larkin and Griffin (2007) observed that *Brassica* spp. and barley reduced 20-56% inoculum levels of *R. solani* in greenhouse tests. On the contrary, radish, rapeseed and also Indian mustard reduced 40-83% potato seedling disease in control environmental condition. Therefore, the present study was undertaken to assess the effect of biofumigation with *Brassica* spp. against *S. rolfsii* under *in vitro* condition, to observe the soil solarization effect alone or in combination with biofumigant in controlling *S. rolfsii* and also to observe the significance in the improvement of plant growth and yield of potato.

## Materials and Methods

### Collection, isolation and preservation of *Sclerotium rolfsii*

*Sclerotium rolfsii* was isolated from the rhizosphere and rhizoplane of potato (*Solanum tuberosum*), tomato (*Solanum lycopersicon*), chilli (*Capsicum frutesence*), carrot (*Daucus carrota*), and brinjal (*Solanum melongena*). The specimens which had typical symptoms of stem rot were selected from infected fields. The fungal isolates were isolated following standard method (Mian, 1995). The fungal colonies were grown on PDA and identified by following standard key (Barnet and Hunter, 1972). The pure culture of *S. rolfsii* was preserved by using PDA slants at 10°C in refrigerator as stock culture for further use.

### Preparation of inoculum of test pathogen

Inoculum of the *S. rolfsii* was prepared with autoclaved moist wheat grains in 500 ml Erlenmeyer flask separately. Before using, wheat grains were soaked in water for 12 h.

After soaking excess water was drained out, water soaked grains were poured into 500 ml Erlenmeyer flask separately. Mycelial discs of 5 mm diameter were cut from the edge of three days old PDA cultures in petri dishes. Five to seven mycelial discs were added to autoclaved wheat grains in the different flasks and incubated at 25° C for 21 days. Flasks were shaken by hand at 2-3 days interval for proper colonization. The colonized wheat grains were air dried for two days and stored at 4° C for further study.

**Pathogenicity test**

The pathogenicity of *S. rolfsii* isolates were done by soil infestation method in pot culture under the shade condition. Each earthen pot was filled with 1.0 kg sterilized soil. Inoculum of *S. rolfsii* were thoroughly mixed with sterilized soil at the rate of 20 g/kg soil. A control was maintained where no inoculum was added in sterilized soil. Three pieces of potato seed tuber were transplanted in each pot. Disease development was observed regularly and were recorded at 15 to 30 days after transplanting to pre- and post-emergence seedling mortality. The causal agents of pre-emergence seedling mortality were confirmed after re-isolation of the pathogen from un-germinated tubers.

***In vitro* screening of *Brassica* spp. against *S. rolfsii***

An experiment was conducted to evaluate the effect of leaf extract of *Brassica* spp. against *S. rolfsii* in culture media. Fresh parts of the test plants namely- cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), mustard (*Brassica nigra*), and broccoli (*Brassica oleracea* var. *italica*) were collected and washed thoroughly in clean water. Hundred grams of each washed samples were grinded in mortar and pestle by adding equal amount (100 ml) of sterilized distilled water (1:1 W/V) and boiled at 80° C for 10 min in a hot water bath. The grinded material was filtered through muslin cloth followed by filtering through sterilized what man No. 1 filter paper and treated as standard plant extract (100%). Each of the mixtures was diluted to 10, 20, 30, and 40% concentrations by adding proper volumes of sterilized water. The inhibitory effect of all the plant extracts against the mycelial growth and sclerotial formation of *S. rolfsii* were tested at 10, 20, 30, and 40% concentrations under *in-vitro* conditions by using food poison technique (Nene and Thapliyal, 1979). PDA medium was separately amended with required amount of plant extract after sterilization when it cooled (40° C) under aseptic condition. Control treatment was maintained by pouring PDA medium without plant extract. Mycelial disc of 5 mm diameter from three days old culture of *S. rolfsii* was cut with sterilized cork-borer and placed in the center of plant extract amended PDA petri-dishes separately. The petri-dish having PDA alone (control) were inoculated in the same manner. All plates was incubated in the dark for 25°C until the mycelium of *S. rolfsii* covered the whole plate in control treatment. The radial growth and number of sclerotia of 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 and application methods**

$T_1$ = Uninoculated field (Control-1). No inoculation of pathogen in the field where tubers were transplanted.

$T_2$ = *S. rolfsii* inoculated field (Control-2). Soil was inoculated with wheat grain inoculum of *S. rolfsii* @ 90 g/m$^2$ three weeks before tubers transplantation.

$T_3$ = *S. rolfsii* inoculated field + Biofumigant. Soil was inoculated with wheat grain inoculum of *S. rolfsii* @ 90 g/m$^2$ and simultaneously mustard seeds were sown. After four weeks when the mustard plant was about 4 inches height, it was chopped and incorporated into the soil. Then, the treated plot was kept at least four weeks for proper decomposition and biofumigation.

$T_4$ = *S. rolfsii* inoculated field + Solarized soil. Soil was inoculated with wheat grain inoculum of *S. rolfsii* @ 90 g/m$^2$ and then, it was kept three weeks without disturbing of the soils for proper growth and development of test pathogen. Before four weeks of tubers transplantation, the treated plot was covered with 100 µm thickness transparent polyethylene sheet for increasing soil temperature.

$T_5$ = *S. rolfsii* inoculated field + Biofumigant + Solarized soil. Soil was inoculated with wheat grain inoculum of *S. rolfsii* @ 90 g/m$^2$ and simultaneously mustard seeds were sown. After four weeks, when the mustard plant was about 4 inches height, it was chopped and incorporated into the soil for proper decomposition and biofumigation. At the same time, the treated plot was covered with 100 µm thickness transparent polyethylene sheet at least far four weeks for increasing soil temperature.

**Cultivation of potato in the field**

Land was prepared by using a tractor driven disc plough, rotavator and harrow. The experiment was laid out in the Randomized Complete Block Design (RCBD) with three replications. The unit plot size was 3 m × 2 m where row to row distance was 40 cm and plant to plant distance 25 cm. Standard dose of organic and chemical fertilizer was applied (Chowdhury and Hassan, 2013). Then, potato tuber variety Cardinal was transplanted in the field on 5 December 2015. Tubers were transplanted in rows uniformly at a depth of 2.5 centimeter. Weeding, mulching, ,and irrigation were done in the experimental field whenever necessary.

**Data recording**

Data on germination, number of healthy plants and infected plants were recorded during the growing period. Fifteen plants in each plot were randomly selected and uprooted carefully, washed with water, checked individually and disease severity was rated on 0-4 scale (0=No symptoms, 1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100% of potato stolon and tuber covered with lesions). The disease incidence and disease severity were assessed by the following formula (Rahman *et al.*, 2013):

After harvested, tuber weight in each plot was recorded and tuber yield per hectare was calculated by following formula (Razaq *et al.*, 2015).

**Statistical analysis**

Data were analyzed statistically by using the MSTAT-C and Statistix 10 computer program. The treatment means were compared following Duncan's Multiple Range Test (DMRT) and data were transformed into square root whenever it was necessary (Gomez and Gomez, 1984).

**Results and Discussion**

**Isolation of *S. rolfsii***

Eight isolates of *S. rolfsii* named as PS 1 to PS 8 were isolated from potato, tomato, chilli, carrot, and brinjal.

**Pathogenicity test of the isolates of *S. rolfsii***

Individually, eight isolates of *S. rolfsii* were tested in pot experiment to select the most virulent isolates causing seedlings mortality of potato. The results of the pathogenicity test of *S. rolfsii* against potato seedlings are presented in the Table 1. All the tested isolates of *S. rolfsii* were found pathogenic on potato seedlings causing 8.33 to 100% seedling mortality. The highest seedling mortality (100%) was observed with the isolate PS 4 followed by the isolate PS 7 (91.67%), PS 5 (83.33%), and PS 3 (75.00%). Significantly, the lowest total seedling mortality (8.33%) was observed with the isolate PS 2. The isolate PS 4 was selected for further experiment.

***In vitro* effect of mustard, cabbage, cauliflower and broccoli leaf extract on radial growth and sclerotia formation of *S. rolfsii***

The effect of leaf extract of mustard, cabbage, cauliflower, and broccoli in reducing the radial growth and sclerotia formation of *S. rolfsii* are presented in the Table 2. Significantly, the highest (78.89%) inhibition of mycelial growth of *S. rolfsii* was observed at 40% concentration of mustard leaf extract followed by 30% concentration (60.74%). On the contrary, cabbage, cauliflower, and

**Table 1. Pathogenicity test of *S. rolfsii* against potato seedlings**

| Isolates of *S. rolfsii* | Mortality (%) | | |
|---|---|---|---|
| | Pre-emergence | Post- emergence | Total (%) |
| PS 1 | 50.00 | 16.67 | 66.67 b-d* |
| PS 2 | 8.33 | 0.00 | 8.33 e |
| PS 3 | 41.67 | 33.33 | 75.00 a-c |
| PS 4 | 75.00 | 25.00 | 100.00 a |
| PS 5 | 50.00 | 33.33 | 83.33 ab |
| PS 6 | 33.33 | 16.67 | 50.00 cd |
| PS 7 | 83.33 | 8.33 | 91.67 ab |
| PS 8 | 16.67 | 25.00 | 41.67 d |

*Means within same column followed by common letter(s) are not significantly different (*P=0.05*)

**Table 2. Effect of *Brassica* spp. leaf extract on inhibition of radial growth and sclerotia formation of *S. rolfsii in vitro***

| *Brassica* spp. | Conc. % | % inhibition | |
|---|---|---|---|
| | | Radial growth | Sclerotia formation |
| Mustard | 10 | 33.33 ef* | 41.60 i |
| | 20 | 44.07 d | 50.61 g |
| | 30 | 60.74 b | 63.11 c |
| | 40 | 78.89 a | 83.13 a |
| Cabbage | 10 | 21.11 h | 25.75 l |
| | 20 | 30.00 fg | 36.48 j |
| | 30 | 37.78 e | 43.37 h |
| | 40 | 49.63 c | 60.18 d |
| Cauliflower | 10 | 19.63 h | 22.47 m |
| | 20 | 27.41 g | 34.90 k |
| | 30 | 38.15 e | 44.19 h |
| | 40 | 53.33 c | 58.54 e |
| Broccoli | 10 | 18.52 h | 25.20 l |
| | 20 | 29.26 fg | 37.09 j |
| | 30 | 48.52 cd | 52.53 f |
| | 40 | 63.70 b | 69.06 b |
| Control | | 90.00 mm | 488 |

*Means within same column followed by common letter(s) are not significantly different (*P=0.05*)

broccoli leaf extract inhibited the mycelial growth by 49.63, 53.33, and 63.70%, respectively, at 40% concentration. The lowest inhibition of *S. rolfsii* was recorded 18.52% at 10% conc. of broccoli leaf extract. In case of sclerotial formation of *S. rolfsii*, significantly the highest (83.13%) inhibition was achieved at 40% mustard leaf extract followed by broccoli leaf extract (69.06%) at same concentration. Results indicated that mustard leaf extract was significantly superior to all others leaf extract in reducing the radial colony growth and sclerotial formation. Thus mustard was selected as biofumigant producer in the field experiment for management of *S. rolfsii*.

**Effect of biofumigant and solarized soil for the management of *S. rolfsii***

**Effect on pre- and post-emergence mortality**

Significantly, the highest total seedling mortality of 41.67% was recorded in control-2 treatment ($T_2$) where potato tuber were transplant in the *S. rolfsii* inoculated soil without any other amendment. The non-inoculated field accounted the second highest seedling mortality, treatment $T_1$ (control-1) tubers were transplanted in un-inoculated field. On the contrary, significantly lower total seedling mortality was observed at treatment $T_5$ (10.83%) followed by $T_3$ (15.00%) and $T_4$ (18.33%), respectively. Among the different treatments including

biofumigation and soil solarization either individual or in combination, treatment $T_5$ appeared to be superior in reducing the pre- and post-emergence mortality of potato caused by *S. rolfsii* (Table 3). The results of the current study suggest the superiority of combined approach of soil solarization and biofumigation for management of *S. rolfsii* over individual treatment by biofumigant or soil solarization. Control of seedling mortality and other seedling diseases of different crops was achieved through the integration of antagonist with diverse organic amendments by different investigators (Urbasch, 1984; Stapleton and De Vay, 1986; Begum and Bhuiyan, 2006; Adandonon *et al.*, 2006; Rahman *et al.*, 2012; Bhuiyan and Sen, 2013) by findings of the present study are in confirmatory with these results.

**Effect of biofumigant and solarized soil on disease incidence and severity**

Disease incidence and severity of stem rot disease of potato was significantly influenced by single component or combined application of biofumigant and solarized soil (Table 4). The lowest disease incidence (26.67%) and severity (29.86 %) of stem rot were found in $T_5$ where solarized soil and biofumigant were used in integration. On the contrary, significantly the highest disease incidence (55.56%) and severity (78.24%) of stem rot were observed in the $T_2$ (control-2) treatment where potato was transplanted in the *S. rolfsii* inoculated soil without any other amendment. Results indicated that soil solarization and biofumigant alone or in combination were effective in reducing stem rot disease incidence and severity of potato. The results agreed with the finding of Subbarao and Hubbard (1996), Rahman *et al.* (2013), Chandel & Sharma (2014). They reported the effectivity of soil solarization and biofumigation in reducing population density of sclerotia forming fungi and their diseases in different crops.

**Effect of biofumigant and solarized soil on yield of potato**

Significantly, the highest yield (15.14 t/ha) was recorded in the plot where soil solarized by polythene mulch and biofumigation by *Brassica nigra* (mustard) were applied ($T_5$) in the field (*S. rolfsii* inoculated field + Biofumigant + Solarized soil) followed by $T_3$ (*S. rolfsii* inoculated field + Biofumigant) and $T_4$ (*S. rolfsii* inoculated field + Solarized

**Table 3. Effect of soil solarization and biofumigation on potato seedling mortality caused by *S. rolfsii* in the field**

| Treatments | Mortality % | | |
|---|---|---|---|
| | Pre-emergence | Post-emergence | Total |
| $T_1$ =Uninoculated field (control-1) | 13.33 | 10.00 | 23.33 b |
| $T_2$ =*S. rolfsii* inoculated field (control-2) | 25.00 | 16.67 | 41.67 a |
| $T_3$ =*S. rolfsii* inoculated field + Biofumigant | 11.67 | 3.33 | 15.00 cd* |
| $T_4$ = *S. rolfsii* inoculated field + Solarized soil | 11.67 | 6.67 | 18.33 bc |
| $T_5$ = *S. rolfsii* inoculated field + Biofumigant + Solarized soil | 8.33 | 2.50 | 10.83 d |
| CV | | | 15.22 |

*Means within same column followed by common letter(s) are not significantly different (*P=0.05*)

**Table 4. Effect of the soil solarization and biofumigation on incidence and severity of stem rot disease of potato in the field**

| Treatments | Disease incidence % | % Increased (+) or decreased over control-1 | Disease severity (PDI) |
|---|---|---|---|
| $T_1$ =Uninoculated field (control-1) | 48.89 ab* (6.99) | 0.00 | 66.80 b (8.17) |
| $T_2$ =*S. rolfsii* inoculated field (control-2) | 55.56 a (7.45) | +13.64 | 78.24 a (8.85) |
| $T_3$ =*S. rolfsii* inoculated field + Biofumigant | 35.56 bc (5.96) | 27.27 | 43.33 d (6.58) |
| $T_4$ = *S. rolfsii* inoculated field + Solarized soil | 40.00 bc (6.33) | 18.18 | 54.80 c (7.40) |
| $T_5$ = *S. rolfsii* inoculated field + Biofumigant + Solarized soil | 26.67 c (5.16) | 45.45 | 29.86 e (5.46) |
| CV | 17.67 | | 9.69 |

*Means within same column followed by common letter(s) are not significantly different (*P=0.05*)
Figures within the parentheses are squire root transformed (X+0.5) values.

soil), respectively (Table 5). On the contrary, significantly the lowest yield (3.78 t/ha) was recorded in the treatment $T_2$ (control-2) where tuber pieces were planted in *S. rolfsii* inoculated field without any other amendment. In treatment $T_1$, potato tuber yield was recorded as 6.83 t/ha, where inoculation of *S. rolfsii* or other amendment of soil were not performed. The maximum (121.67%) yield was observed in treatment $T_5$ over control-1followed by $T_3$ and $T_4$, respectively. But yield decreased by 44.66% in treatment $T_2$ over control-1. The treatment $T_2$ plot was inoculated with the test pathogen as a result disease infestation rate was very high. The natural biofumigant contains various kinds of biocidal compounds (mainly isothiocyanates) which are supported to control soil borne diseases. Isothiocyanates can be released from glucosinolates compound in mustard seed meal, when it was degraded into the soil (Shaban *et al*., 2011). In addition, soil solarization technique can increase soil temperature to such level that may kill many disease causing pathogens, such as nematodes, fungi, bacteria, and weed seeds and seedlings. It also speeds up the breakdown of the organic materials in the soil and increases the amount of soluble nutrients, such as nitrate, ammonium, calcium, magnesium, and potassium in the soil. It improved plant growth and increased the yield over control (non-solarized soil) by 25-432% in broad beans, onions, tomatoes and clover in various type of soils (Abdel-Rahim *et al.*, 1998). Several volatile bio-toxic compounds are released when organic matter is heated and they may augment the biocidal activity of the soil. It has been observed that plants grow faster when grown in solarized soil in comparison to non-solarized soils (Kapoor, 2013). Combining solarization with biofumigation and compost amendments the re-introduction of biocontrol agents such as *Trichoderma* spp. and *Bacillus* spp. may be more effective than either treatment alone *in* controlling soil borne disease. Populations of these two microbial antagonists increase relative to other microorganisms in solarized soil (Stapleton and DeVay, 1986). On the

**Table 5. Effect of the soil solarization and biofumigation on yield of potato in the field**

| Treatments | Yield t/ha | % yield increased or decreased (-) over control-1 |
|---|---|---|
| $T_1$ =Uninoculated field (control-1) | 6.83 d | 0.00 |
| $T_2$ =*S. rolfsii* inoculated field (control-2) | 3.78 e | - 44.66 |
| $T_3$ =*S. rolfsii* inoculated field + Biofumigant | 12.50 b | 83.02 |
| $T_4$ = *S. rolfsii* inoculated field + Solarized soil | 10.94 c | 60.18 |
| $T_5$ = *S. rolfsii* inoculated field + Biofumigant + Solarized soil | 15.14 a | 121.67 |
| CV | 6.29 | |

contrary, *T. harzianum* produces a large number of chemicals to solubilize rock phosphate, Zn, $Mn^{4+}$, $Fe^{3+}$, and $Cu^{2+}$ and increase iron availability and enhance iron uptake which might be contributed in increasing yield of potato (Altomare *et al.*, 1999). The solubilization and chelating abilities of *T. harzianum* may also be influenced in increasing yield of potato (Harman, 2000). The findings of the present investigation are in agreement with the findings of other researchers (Chellemi *et al.*, 1994; Kirkegaard and Sarwar, 1998; Charron *et al.*, 1999; Harvey *et al.*, 2002; Bhuiyan and Sen, 2013).

## Conclusion

*Brassica nigra* (mustard) leaf extract was appeared to be highly effective in inhibiting the growth and sclerotia formation of *S. rolfsii* in *in vitro* trial. Combined use of soil solarization and biofumigation provided the best control measure for pre- and post-emergence seedling mortality and stem rot disease of potato caused by *S. rolfsii*. Treatment not only suppresses with the growth and development of the pathogen but also helps to increase the growth and yield of potato.

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