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N Kiran Kumar

Assistant Professor of Plant Pathology, College of Agriculture, V.C. Farm, Mandya, Karnataka, India

T Narendrappa

Professor of Plant Pathology, College of Agriculture, UAS (B), GKVK, Bengaluru, Karnataka, India

VB Sanath Kumar

Associate Professor of Plant Pathology, College of Agriculture, V.C. Farm, Mandya, Karnataka, India

In vitro evaluation of cruciferous plant extracts against *Rhizoctonia solani* causing sheath blight in rice

N Kiran Kumar, T Narendrappa and VB Sanath Kumar

Abstract

Sheath blight of rice caused by *Rhizoctonia solani* has become a major constraint in rice production in India. The efficacy of cruciferous plant extracts were assessed against *R. solani* in laboratory conditions. Inhibitory effect of few cruciferous plant extracts were tested by poisoned food technique. At 1:1 dilution the highest inhibition of 51.1% was observed in cabbage and the least inhibition of 21.93% over control was observed in case of radish. At 1:100 and 1:1000 dilutions none of the plants extracts were effective against *R. solani*. At 1:1 dilution only cabbage was found effective. The bio-fumigant property of the same cruciferous plants was tested against *R. solani* by following sealed Petri plate technique. At 2 days after incubation all the treatments showed inhibitory effect. Mustard completely inhibited the growth of the pathogen whereas cauliflower showed least inhibition with 44.8 mm radial growth of the pathogen. At 4 days after incubation only mustard and radish showed the inhibitory effect where mustard showed 100% inhibition on the growth of the pathogen.

Keywords: Rice, sheath blight, *Rhizoctonia solani*, mustard, bio-fumigant, plant extract

1. Introduction

Rice (*Oryza sativa* L.) is second most important cereal and the staple food for more than half of the world's population. Rice is the most prominent crop of India as it is the staple food for most of the people of the country. In India rice crop is being cultivated in an area of 44.10 m ha, with production of 105.3 m tonnes and productivity of 2.38 tonnes ha⁻¹ and in Karnataka it occupies an area of 4.08 m ha, production of 12.8 m tonnes and productivity of 3149 kg ha⁻¹ (Indiastat, 2015) [3].

Sheath blight is one of the major biotic constraints that affects rice production in India and is considered economically important disease of rice in the world (Lee and Rush 1983; Webster and Gunnell 1992) [4,12]. The disease is caused by *Rhizoctonia solani* Kuhn (teleomorph: *Thanatophorus cucumeris* (Frank) Donk). The Sheath blight is becoming most destructive, being second only to rice blast disease among the rice diseases reducing rice productivity, (Ou, 1985) [5]. The incidence of rice sheath blight disease has increased in recent years, because of the unavailability of resistant cultivars or any other suitable economic disease management measures. The yield losses due to this disease is reported to range from 5.2 to 50%, depending on environmental conditions, crop stages at which the disease appears, cultivation practices and cultivars in India (Rajan, 1987; Sharma and Teng, 1996) [6,7].

The management of sheath blight through fungicide application is the most common approach among the farmers. Because of the disadvantages of using the fungicides, it has become necessary to adopt eco-friendly approaches for enhancing crop yield and better crop health. The use of biological methods for the management of this disease is scarce. It is necessary to evaluate the biological methods including use of bioagents, bio-fumigants and botanicals to manage the disease effectively to avoid resistance developments in pathogen and minimize the fungicidal residues for ecological sustainability. In view of the importance of the crop and seriousness of the sheath blight disease an investigation was taken to find the effect of cruciferous plant extracts against *R. solani*.

Correspondence

N Kiran Kumar

Assistant Professor of Plant Pathology, College of Agriculture, V.C. Farm, Mandya, Karnataka, India

Material and Methods

Selection of cruciferous plants

Cruciferous plants mentioned in table 1 were selected to screen for their inhibitory effect on *R. solani* causing sheath blight of rice. The present investigation was undertaken during 1016 at Department of Plant Pathology, College of Agriculture, Mandya, Karnataka state.

Procedure for water extract

The leaves of the cruciferous plants noted in table 5 were used for the purpose of extraction. 50g of leaves were taken and cut into small pieces under aseptic condition. The sample was put into waring blender containing 50ml sterilized distilled water at a ratio 1:1 (water: plant material). The sample was spun at low speed for 10-15 minutes in a warring blender till the material formed to fine texture. The blended material was then squeezed through a sterilized muslin cloth so as to get a crude liquid extract. The crude extract was filtered through Whatman no 1 filter paper followed by sterilized Seitz filter. The sterilized filtrate was collected in sterilized glass tubes and was kept at 4 °C in a refrigerator for further use.

Table 1: List of Cruciferous plants used to test against *R. solani* causing rice sheath blight

| S. No | Common name | Botanical name |
|-------|-------------|-----------------------------------------------|
| 1. | Mustard | <i>Brassica juncea</i> |
| 2. | Radish | <i>Raphanus sativus</i> |
| 3. | Cabbage | <i>Brassica oleracea</i> var. <i>capitata</i> |
| 4. | Cauliflower | <i>Brassica oleracea</i> var. <i>botrytis</i> |

In vitro evaluation of cruciferous plant extract

Water extract of four cruciferous plants at dilutions 1:1, 1:10, 1:100 and 1:1000 were screened against *R. solani* by following poisoned food technique.

Poisoned food technique

Potato dextrose agar (PDA) medium was prepared in flasks sterilized. Different concentrations of plant extracts (1:1, 1:10, 1:100 and 1:1000) were prepared by mixing with medium with constant stirring. The medium was then poured into sterilized Petri plate and allowed to solidify. A disc of 5 mm diameter of the pathogen grown on a solid medium was cut with the help of a sterilized cork borer and placed aseptically in the centre of the Petri plate containing medium. The plates were incubated at 27±1°C. After required period of incubation i.e., when the growth in control plate recorded 90 mm in diameter, the radial growth of the pathogen was measured and

the percent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947) [11].

$$I = \frac{C-T}{C} \times 100$$

Where,

I = per cent inhibition

C = growth in control

T = growth in treatment

In vitro evaluation of bio-fumigant cruciferous plants

The test was carried out by the sealed Petri plate technique described by Dennis and Webster (1971) [1]. Two Petri plate bases (90mm) were sealed together by an adhesive tape with the bottom plate containing macerated plant material and the upper plate containing 20ml of solidified PDA medium. The set up was left undisturbed overnight to allow the medium on the upper plate to absorb any volatile compounds produced, if any, by the macerated plant material. The upper PDA plates after the required exposure to the macerated plant material was carefully removed and inoculated with 5 mm disc of the pathogen. Three replications were maintained for each treatment. The plates were incubated at 27±1°C. After required period of incubation i.e., when the growth in control plate recorded 90 mm in diameter, the radial growth of the pathogen was measured and the percent inhibition of growth of the pathogen was calculated.

Results and discussion

In vitro evaluation of cruciferous plant extracts against *R. solani*

The extracts obtained from commonly cultivated cruciferous plants viz., radish, cauliflower, cabbage and mustard were tested for their inhibitory effect against *R. solani*. The water extracts with 4 dilutions viz., 1:1, 1:10, 1:100 and 1:1000 were used in the study. The radial growth of the pathogen was recorded when the growth of the pathogen was 90 mm in the untreated control by following poisoned food technique. At 1:1 dilution all the extracts showed inhibitory effect. The highest per cent inhibition was recorded in cabbage (51.1%) followed by cauliflower (27.8%) and mustard (23.03%). The least inhibition at 1:1 dilution was noticed in case of radish (21.93%). Only cabbage (21.38%) showed the inhibitory effect at 1:10 dilution. None of the plants showed inhibitory effect against the pathogen at 1:100 and 1:1000 dilutions (Table 2 and Fig. 1).

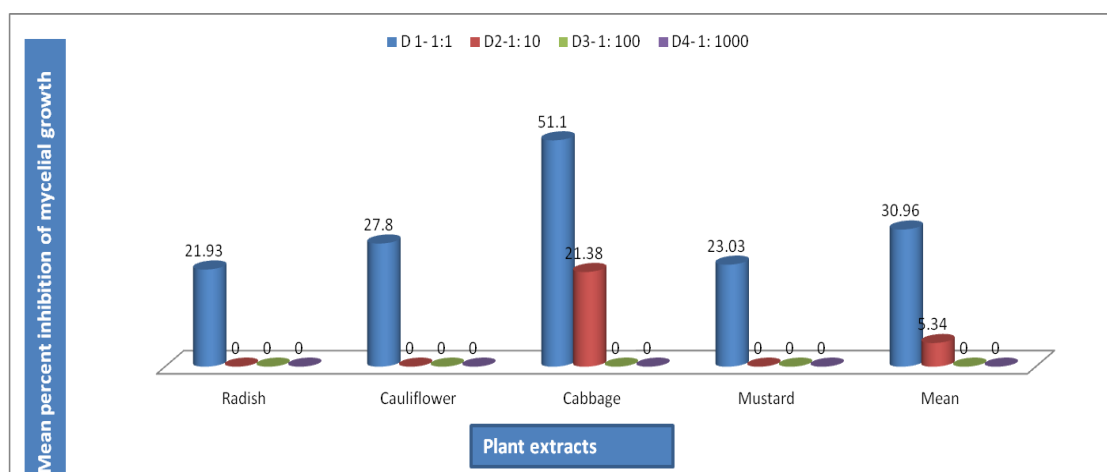


Fig 1: *In vitro* evaluation of cruciferous plant extracts against rice sheath blight pathogen *R. solani*

Table 2: *In vitro* evaluation of cruciferous plant extracts against rice sheath blight pathogen *R. solani*

| Dilution | Mean percent inhibition of <i>R. solani</i> mycelia growth | | | | |
|----------|------------------------------------------------------------|-------------------|-------------------|---------------|---------------|
| | Plant extracts | | | | |
| | Radish | Cauliflower | Cabbage | Mustard | Mean |
| 1:1 | 21.93 (27.91) | 27.80 (31.81) | 51.10 (45.62) | 23.03 (28.67) | 30.96 (33.50) |
| 1:10 | 0.00 (0.00) | 0.00 (0.00) | 21.38 (27.53) | 0.00 (0.00) | 5.34 (6.88) |
| 1:100 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| 1:1000 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | | Plant extract (P) | Concentration (C) | P X C | |
| | S.Em ± | 0.10 | 0.10 | 0.20 | |
| | C.D. (0.01) | 0.38 | 0.38 | 0.76 | |

Figures in parenthesis are arcsine transformed values Sinha *et al.* (2009) ^[8] tested ten botanicals under *in vitro* conditions against *R. solani* and found that garlic and ginger extracts recorded 100% inhibition followed by neem (70%), *Pongamia glabra*, *Prosopis juliflora* and *Thevetia peruviana*. Upma Dutta and Kalha (2011) ^[10] evaluated nine botanicals and found Drek extract was most effective inhibiting 46.5% of mycelial growth of *R. solani* followed by Bhang, Onion, Tulsi, Bael, Paanch phooli, Cury leaves, Congress grass and Eucalyptus. Srinivas *et al.* (2014) ^[9] reported that among thirteen plant extracts tested against *R. solani*, garlic extract was most effective in inhibiting the growth of fungus followed by *Calotropis sp.* at 10% concentration.

In vitro* evaluation of bio-fumigants against *R. solani

Investigation was carried out to study the effect of bio-fumigants plants viz., mustard, radish, cabbage and

cauliflower for their inhibitory effect against Mandya isolate of *R. solani* by producing volatile inhibitory compounds. The test was carried out by sealed Petri plate technique by measuring the radial growth of the pathogen in the treatments when the growth in the control plate was recorded 90 mm in diameter. At 2 days after incubation all the treatments showed inhibitory effect. Mustard completely inhibited the growth of the pathogen whereas cauliflower exhibited least inhibition with 44.8 mm radial growth of the pathogen. Radish and cabbage showed 78.33% and 64.88% inhibition over control. At 4 days after incubation only mustard and radish showed the inhibitory effect whereas cabbage and cauliflower were ineffective in inhibiting the growth of the pathogen. There was no growth of the pathogen were observed in case of mustard with 100% inhibition whereas 38.6 mm growth with 57.11% inhibition was observed in case of radish (Table 3).

Table 3: *In vitro* evaluation of bio-fumigants against rice sheath blight pathogen *R. solani*

| Treatment | Bio fumigant plant | Mean mycelia growth of <i>R. solani</i> (mm) | | | |
|-----------|--------------------|----------------------------------------------|--------------|------|--------------|
| | | Days after incubation | | | |
| | | 2 | % inhibition | 4 | % inhibition |
| T1 | Mustard | 0.0 | 100.00 | 0.0 | 100.00 |
| T2 | Radish | 19.5 | 78.33 | 38.6 | 57.11 |
| T3 | Cabbage | 31.6 | 64.88 | 90.0 | 0.00 |
| T4 | Cauliflower | 44.8 | 50.22 | 90.0 | 0.00 |
| T5 | Control | 90.0 | | 90.0 | |
| | S.Em ± | 0.98 | | 1.05 | |
| | C.D. (0.01) | 3.02 | | 3.22 | |

The soil bio-fumigants are rich in glucosinolates and are used for control of soil borne pathogens. Biocidal effects are attributed to volatile allyl isothiocyanate released upon hydrolysis of glucosinolates. The present finding was in accordance with Handiseni *et al.* (2016) ^[2] who evaluated bio-fumigation activity of nine *Brassicaceae* plants and to other related species *in vitro* and it was found that all plants evaluated significantly suppressed the mycelial growth of *R. solani*. Further, mustard provided the highest mycelial inhibition while sunhemp and Chinese cabbage had the least suppressive effect.

The present investigation revealed that cruciferous plants are effective in suppressing *R. solani*. Hence these cruciferous bio-fumigant plants could be exploited in the management of rice sheath blight disease in severely infested fields by rotating with rice and incorporating the crop refuse after the harvest.

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