*International Journal of Bio-resource and Stress Management* 2016, 7(4) Special:628-635 DOI: 10.5958/0976-4038.2016.00097.X

**Full Research Article**

# Morphological, Physiological and Nutritional Studies on *Rhizoctonia solani* and Their Effect on Seed Germination in Rice

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**Article History**

Manuscript No. ARISE 134
Received in 11th May, 2016
Received in revised form 28th July, 2016
Accepted in final form 1st August, 2016

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***Keywords***

*R. solani*, sclerotia, growth pattern, pusa basmati, tetep

**Abstract**

The sample showing disease (sheath blight) symptoms in rice were collected from six states Gujarat, Karnataka, Pantnagar, Uttar Pradesh, Haryana and Punjab. The isolates (Gujarat, Karnataka, Pantnagar, Uttar Pradesh, Haryana and Punjab) of *R. solani*, were isolated on PDA plates were incubated for 5 days at 28±1 °C. Colony colour, growth pattern, and diameter growth showed great diversity in all the isolates. Based on the colony pigmentation, all the isolates were assigned into 4 groups: dark brown, dirty white, milky white and Yellowish brown. Isolates were grown on five different media; PDA was found the best media for growth and development in comparison to others. The maximum growth was found in two isolates, Gujarat and Karnataka (90 mm or 89.07) after 72 hrs. The maximum number of sclerotia was recorded in Gujarat isolate (125.33) followed by Karnataka (75.67) on Czapek's dox agar while the minimum number of sclerotia was observed in Pant Nagar (6.33) on yeast extract agar medium. The maximum weight of sclerotia was recorded in Gujarat isolate (194.02 mg) followed by Karnataka (186.79 mg) on PDA medium. The fourteen days old broth culture of oat meal broth of different isolate of *R. solani* significantly reduced the radical length and plumule length of the germinated seeds of Pusa Basmati-1 and Tetep as compared to check. Tetep showed the less reduction in germination per cent than Pusa Basmati-1, known as moderately resistant cultivar. The effect of Gujarat isolates was found the highly pathogenic than other isolates on seed germination of both the cultivar.

## 1. Introduction

*Rhizoctonia solani* Kuhn is an important soil borne pathogen causing diseases such as collar rot, root rot, banded blight, sheath blight, web blight etc. in vegetables and some cereals like rice and maize (Santha Kumari and Rehmath Niza, 2005; Rajput and Harlapur, 2014). Rice sheath blight disease is an important fungal disease of rice, which has become a major constraint to rice production during the last two decades (Kobayashi et al., 1997). The emergence of *R. solani* as an economically important rice pathogen has been attributed to the intensification of rice-cropping systems with the development of new short-statured, high-tillering, high-yielding varieties, high plant densities, and an increase in nitrogen fertilization (Gangopadhay and Chakrabarti, 1982; Ou, 1985).

High temperature (22 °C to 35 °C) and high relative (RH) humidity are favorable for sheath blight development. Mycelial growth and sclerotia formation are at its higher at 25 °C to 30 °C and 80 to 95% RH are optimal for disease development. Soil type may also influence disease development (Kannaiyan and Prasad, 1978), disease severity is higher in sandy clay loam than In clayey or sandy soils. Mycelial growth and sclerotia formation are optimum at pH 6.0–7.0 and no growth at pH 3.0 and 9.0. This pathogen can survive in soil for many years by producing small (1–3 mm diameter), irregular-shaped, brown to black structures in soil and on plant tissues. *Rhizoctonia solani* also survives as mycelium by colonizing soil organic matter as a saprophyte, particularly as a result of plant pathogenic activity (Ghaffar, 1988). The sclerotia present in soil and or on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack a wide range of food and fibre crops. The pathogen is transported in infected soil or through movement of diseased plants or bean pods (Wallwork, 1996). Because of a high degree of diversity in pathogenicity and morphology

as well as in cultural, physiological characteristics the species complex of *R. solani* has been classified in 13 anastomosis groups (AGs) based on hyphal anastomosis reaction Parmeter et al. (1969). The purpose of this study was to investigate the behavior of *R. solani* under two rice cultivar and description of cultural characteristics and physiology of different isolates of *R. solani.*

## 2. Materials and Methods

### *2.1. Survey and sampling*

Naturally infected rice plants, showing disease (sheath blight) symptoms, were collected from six different states namely Gujarat, Karnataka, Pantnagar, Uttar Pradesh, Haryana and Punjab during *kharif* season, July 2014.

### *2.2. Isolation of fungi*

The infected parts of plant were firstly washed with tap water and then surface sterilized with 2% sodium hypochlorite solution for 2 min. Isolation procedures were carried out according to the method described by Rangaswami and Mahadevan (2004) on potato dextrose agar medium (potato: 200 g, dextrose: 20 g, agar: 20 g, distilled water: 1 litre). In cultural studies; mycelial discs of 5 mm diameter from 3 days old cultures of each isolates were transferred into the center of sterilized different culture media and plates and incubated for 5 days at 28±1 °C.

### *2.3. Observation of different characters of fungus on different media*

The basic cultural characteristics such as Radial growth (mm), number of sclerotia, weight of sclerotia, and growth of mycelium at different temperature was recorded on five different media i.e. three synthetic media (Czapek's agar and Richard's agar), one semi-synthetic medium (PDA), one non-synthetic medium (yeast extract agar) and one natural medium (Oat meal agar medium) after 72 hrs of inoculation at 28±1 °C. The colony colour was determined with the help of Munsell's soil colour chart. Every growth parameter of the each isolate was measured with three replications.

### *2.4. Pathogenic effect of broth culture of fungi on seed germination*

In other physiological experiment, The Oat Meal broth medium was prepared according to Gaskill (1968) and 200 ml medium taken into the conical flask and inoculated with 5 mm disc of the each isolates. Inoculated flasks in triplicates were incubated for 14 days at 28±1 °C in shaker. After incubation period mycelial mat and sclerotia were filtered through Whatman filter paper. Therefore, culture filtrate was used for phytotoxic effect on seed germination of two rice varieties Pusa Basmati-1 and Tetep. The healthy seeds of rice varieties Pusa Basmati-1 (susceptible) and Tetep (resistant) were surface sterilized with 2% sodium hypo chloride solution for 2 minute and kept for soaking in culture filtrate for overnight. The 30 soaked seeds of each varieties were taken out and placed in sterilized wetted blotter paper and kept for seed germination. Along with sterilized distilled water soaked seeds were kept as check. Observations were recorded after ten days of sowing. Germination Percent over control was calculated by applying the simple mathematical formula.

### *2.5. Statistical analysis*

The statistical analysis was done through two factorial ANOVA. The two-way analysis of variance (ANOVA) is an extension of the one-way ANOVA that examines the influence of two different categorical independent variables on one continuous dependent variable.

## 3. Results and Discussion

Colony colour, growth pattern, and diameter growth showed great diversity in all the isolates. Based on the colony pigmentation, all the isolates were assigned into 4 groups: dark brown, dirty white, milky white and Yellowish brown. Two isolates Haryana and Punjab were found Yellowish brown, two isolates Pant Nagar, Uttar Pradesh, India were found Dirty White, One isolate Gujarat was found milky white and one isolate Karnataka was found Dark brown. Lal and Kandhari (2009) while studying variability of *R. solani* isolate found six isolates as light brown, five isolates were found yellowish brown, four isolates were whitish brown in colour, six isolates were dark brown and four isolates were very pale brown. Sunder et al. (2003) had also reported that colony colour ranged from brown, light brown, dark brown and yellowish brown. The results are also supported by Hoa (1994), reported that sclerotial colour ranged from brown, light/dark brown, black brown, chocolate brown, salmon and dark salmon. The formation of sclerotia was observed in the petridish and classified into three groups: peripheral, scattered and ring at periphery. One isolate was found in ring at, two isolates were found scattered and three were found peripheral (Figure 1: a, b, c, d, e and f). Singh et al. (1990) reported the sclerotial formation in the same manner i.e. central, peripheral or scattered.

The mycelium length, mycelium sclerotia$^{-1}$ weight and no. of Sclerotia were recorded after 72 hrs of inoculation. Isolates were grown on five different media. PDA was found the best media for growth and development in comparison to others. Two isolates (Gujarat and Karnataka) covered the whole plates (90 mm or 89.07) in 72 hrs (Table 1). Figure 2(a) and 2(c) showing the effect of media on the radial growth of isolates. While Figure 2(b) and 2(d) showing the radial growth strain wise. Singh et al. (1974) reported that PDA was found best for radial growth. The maximum number of sclerotia was recorded in Gujarat isolate (125.33) followed by Karnataka (75.67) on Czapek dox agar. The minimum no. of sclerotia was observed in Pant Nagar (6.33) on yeast extract agar Medium (Table 2). Figure 3(a) and 3(c) showing the effect of media on the no.

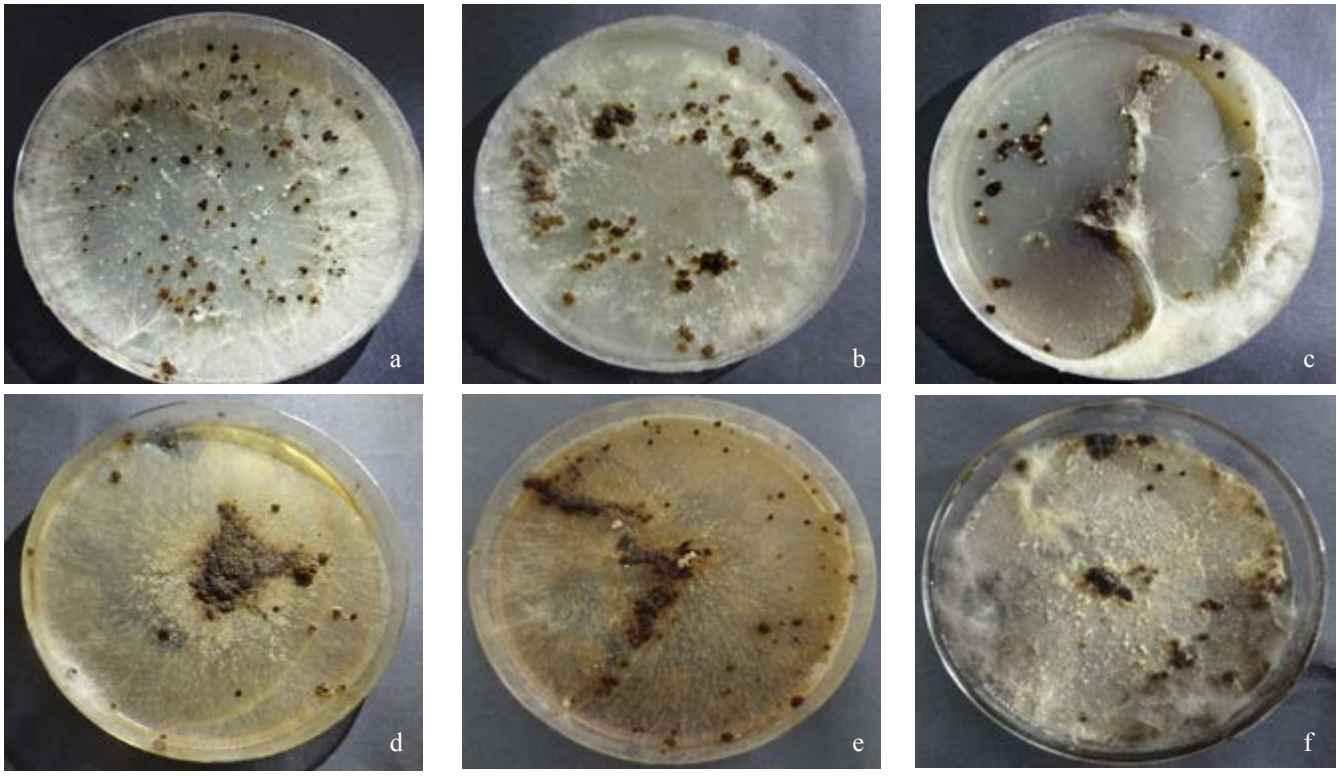


Figure 1: Different strains of *Rhizoctonia solani*: a: Gujarat; b: Karnataka; c: Pantnagar; d: Haryana; e: Punjab; f: Uttar Pradesh

Table 1: Effect of culture medium on radial growth (mm) of different isolates of *R. solani* at 28±1 °C after 72 hrs of incubation period

| Culture medium | | Pantnagar | Karnataka | Gujarat | Uttar Pradesh | Haryana | Punjab |
|---|---|---|---|---|---|---|---|
| Oat meal | mean | 72.50 | 66.27 | 80.23 | 73.71 | 76.65 | 75.80 |
| | SD | 0.79 | 1.23 | 1.27 | 1.20 | 0.91 | 1.81 |
| Potato dextrose | mean | 87.90 | 89.07 | 90.00 | 88.30 | 83.60 | 88.00 |
| | SD | 0.79 | 0.45 | 0.00 | 0.62 | 0.44 | 0.62 |
| Richard's agar | mean | 58.27 | 74.33 | 72.57 | 72.23 | 64.57 | 66.17 |
| | SD | 5.06 | 1.07 | 0.95 | 1.22 | 0.90 | 0.95 |
| Czapek's dox Agar | mean | 8.27 | 71.93 | 72.67 | 79.97 | 81.93 | 65.57 |
| | SD | 0.86 | 1.69 | 1.81 | 1.55 | 1.70 | 0.83 |
| Yeast extract | mean | 35.93 | 18.33 | 35.90 | 35.47 | 26.70 | 30.47 |
| | SD | 0.60 | 0.67 | 1.44 | 2.39 | 1.08 | 0.57 |
| Source of isolates | mean | 67.17 | 63.99 | 70.27 | 69.93 | 66.69 | 65.20 |
| | SD | 19.26 | 24.90 | 19.01 | 18.82 | 21.84 | 19.87 |

of sclerotia media wise while Figure 3(b) and 3(d) showing the number of sclerotia strain wise. The maximum weight of sclerotia was recorded in Gujarat isolate (194.02 mg) followed by Karnataka (186.79 mg) on PDA medium. The minimum weight of sclerotia was recorded in Pant Nagar isolate (12.24 mg) followed by Karnataka (16.87 mg) on Yeast extract agar medium (Table 3). Figure 4(a) and 4(c) showing the effect of media on the weight of sclerotia media wise while figure 4(b) and 4(d) showing the weight of sclerotia strain wise. Tiwari and Khare (2002) reported that Czapek's Dox Agar was best for sclerotial production. Sclerotial colour grouped into two: dark brown and light brown and sclerotial formation pattern grouped in excellent, good and fair. Sinha and Ghufran (1988) reported that more variations were found in the sclerotia number, weight and colour.

The fourteen days old broth culture of oat meal broth of

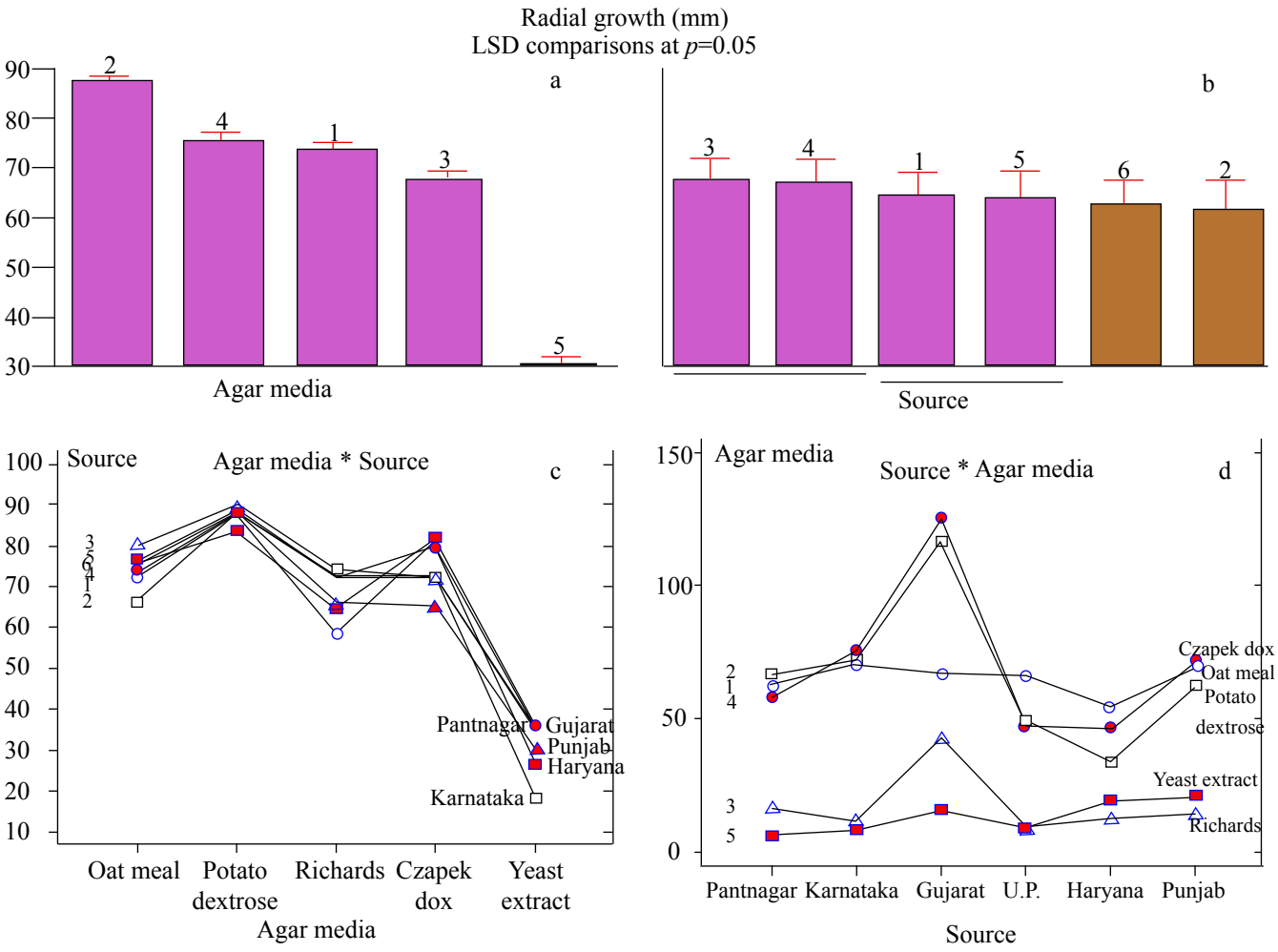


Figure 2: Graph showing the effect of culture medium on radial growth (mm) of different Isolates of *R. solani* at 28±1 °C after 72 hrs of incubation period. 2(a): Media; 2(b): Strains; 2(c): Radial Growth media wise; 2(d): Radial Growth strain wise

Table 2: Effect of culture medium on number of sclerotia of different isolates of *R. solani*

| | | Pantnagar | Karnataka | Gujarat | Uttar Pradesh | Haryana | Punjab |
|---|---|---|---|---|---|---|---|
| Oat meal | mean | 62.67 | 70.33 | 67.00 | 66.00 | 54.33 | 69.67 |
| | SD | 2.08 | 1.53 | 1.00 | 2.65 | 2.52 | 1.53 |
| Potato dextrose | mean | 66.33 | 72.00 | 116.67 | 48.67 | 33.67 | 62.33 |
| | SD | 2.52 | 2.65 | 2.52 | 2.52 | 4.73 | 2.52 |
| Richards | mean | 16.67 | 11.33 | 43.33 | 8.67 | 13.00 | 14.67 |
| | SD | 1.53 | 2.52 | 4.04 | 1.53 | 2.00 | 3.51 |
| Czapek dox | mean | 58.00 | 75.67 | 125.33 | 47.33 | 46.67 | 71.33 |
| | SD | 3.00 | 1.53 | 2.52 | 2.08 | 3.51 | 1.53 |
| Yeast extract | mean | 6.33 | 8.67 | 16.00 | 9.00 | 19.00 | 21.00 |
| | SD | 1.53 | 1.53 | 3.00 | 2.65 | 2.00 | 2.00 |
| Source of isolates | mean | 42.00 | 47.60 | 73.67 | 35.93 | 33.33 | 47.80 |
| | SD | 26.21 | 31.89 | 43.51 | 23.98 | 16.50 | 25.68 |

different isolate of *R. solani* significantly reduced the radical length and plumule length of the germinated seeds of Pusa Basmati-1 and Tetep as compared to check. (Table 4a) In Pusa Basmati-1, the maximum reduction in radical length

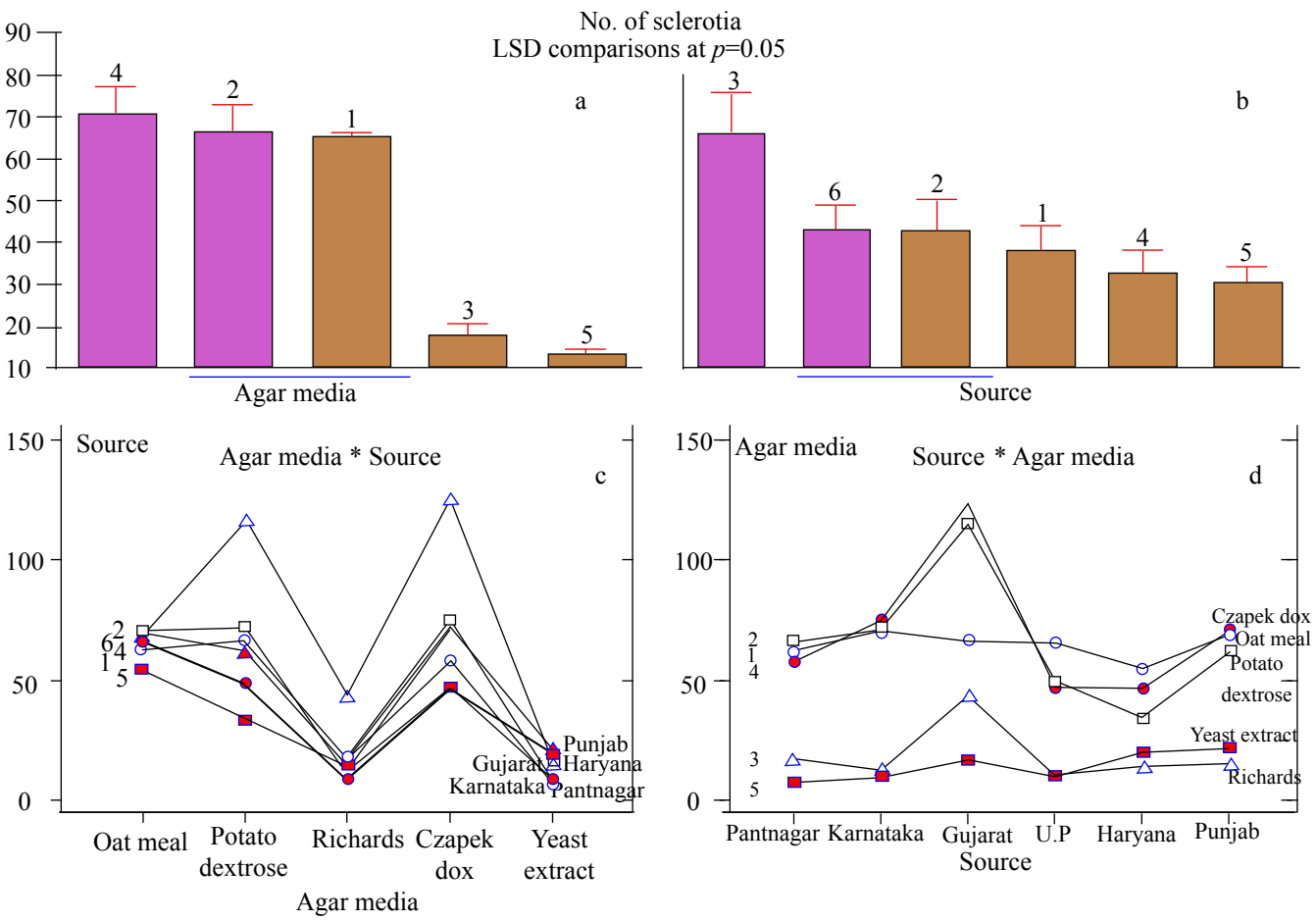


Figure 3: Graph showing the effect of culture medium on number of sclerotia of different isolates of *R. solani*.3(a): Media; 3(b): Strains; 3(c): Number of sclerotia media wise, 3(d): Number of sclerotia strain (isolates) wise

Table 3: Effect of culture medium on Sclerotia weight of different isolates of *R. solani*

| | | Pantnagar | Karnatka | Gujrat | U.P. | Haryana | Punjab |
|---|---|---|---|---|---|---|---|
| Oat meal | mean | 131.30 | 150.82 | 168.26 | 159.96 | 133.64 | 156.34 |
| | SD | 1.93 | 1.48 | 1.91 | 1.22 | 1.25 | 1.40 |
| Potato dextrose | mean | 114.01 | 186.79 | 194.02 | 161.85 | 180.15 | 150.80 |
| | SD | 1.79 | 1.17 | 1.38 | 1.57 | 1.00 | 1.57 |
| Richards | mean | 46.26 | 32.59 | 88.53 | 12.54 | 19.51 | 22.37 |
| | SD | 2.83 | 0.62 | 1.50 | 1.47 | 2.19 | 2.29 |
| Czapek dox | mean | 102.68 | 113.25 | 153.04 | 121.93 | 81.20 | 138.91 |
| | SD | 2.39 | 2.63 | 2.09 | 2.39 | 1.81 | 2.67 |
| Yeast extract | mean | 12.24 | 16.87 | 26.02 | 21.05 | 27.16 | 25.64 |
| | SD | 1.24 | 1.80 | 1.68 | 0.92 | 1.89 | 1.24 |
| Source of isolates | mean | 81.30 | 100.06 | 125.98 | 95.47 | 88.33 | 98.81 |
| | SD | 46.38 | 68.28 | 63.05 | 68.17 | 63.81 | 63.52 |

(in compare to check 6.77 cm) was recorded in Gujarat (1.30 cm) followed by Pantnagar (1.40 cm) and Uttar Pradesh (1.97 cm). In Tetep cultivar (in compare to check 7.57 cm) the maximum reduction in radical length was also recorded in Gujarat (2.23 cm) isolates followed by Pantnagar (2.57 cm) and Uttar Pradesh (2.83 cm). Verma (1973) also reported that phytotoxic metabolites produced by soybean isolate of *R. solani*, inhibited radical elongation, showed seedling root rot

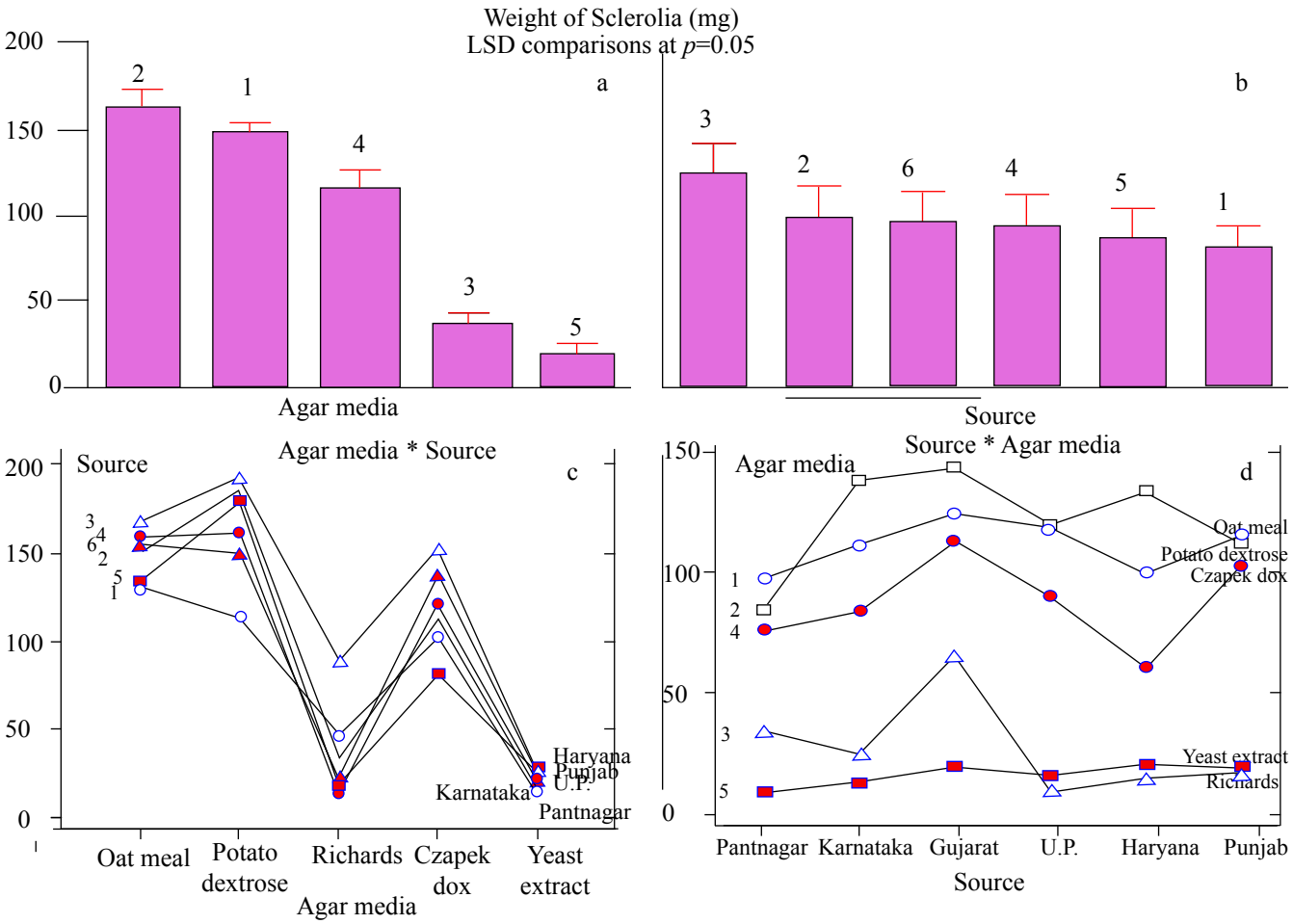


Figure 4: Graph showing the effect of culture medium on Sclerotia weight of different isolates of *R. solani*. 4(a) Media; 4(b): Strains; 4(c): Weight of Sclerotia media wise, 4(d): Weight of Sclerotia strain (isolate) wise

yellowing the shredding of Cotyledons and leaves. Jain and Thapliyal (1980) observed that *R. solani* isolated from soybean leaf produced non-host specific toxic metabolites, capable of producing leaf symptoms, supporting the present investigation. The organism produced more phytotoxic metabolites in the medium on which it grow, which causes inhibition on seed germination and inhibition of root elongation.

In Pusa Basmati-1, the maximum reduction in plumule length was recorded in Gujarat isolate (3.53 cm) in comparison to check (12.27 cm) followed by Uttar Pradesh (4.07 cm) and Pantnagar (4.37 cm). In Tetep cultivar, the maximum reduction in plumule length was recorded in Gujarat isolate (5.13 cm) in comparison to check (14.53 cm) followed by Pantnagar (5.80 cm) and Uttar Pradesh (6.30 cm). Figure 5(a) and 5(c) showing the effect of Oat meal broth media on Pusa Basmati-1 and Tetep while Figure 5(b) and 5(d) showing the effect of all (six) isolates on seedling germination per cent. Table 4(b) showing the Overall % Reduction in germination of seedling of Pusa Basmati-1 and Tetep. Similarly, Sherwood and Lindburg (1962) reported that pectinolytic and cellulytic enzymes

Table 4(a): Effect of oat meal broth culture filtrate isolates of *R. solani* on seed germination of rice cultivars (Pusa Basmati-1 and Tetep)

| Isolates | Seedlings length after 10 days of sowing (cm) | | | |
|---|---|---|---|---|
| | Radical length | | Plumule length | |
| Pantnagar | 1.40 | 2.57 | 4.37 | 5.80 |
| Karnataka | 3.47 | 4.07 | 6.57 | 8.07 |
| Gujarat | 1.30 | 2.23 | 3.53 | 5.13 |
| Uttar Pradesh | 1.97 | 2.83 | 4.07 | 6.13 |
| Haryana | 2.33 | 3.60 | 5.50 | 6.30 |
| Punjab | 2.20 | 3.40 | 5.30 | 7.07 |
| Check | 6.77 | 7.57 | 12.27 | 14.53 |
| SEm± | 0.38 | | | |
| CD (*p*=0.05) | 1.12 | | | |

produced by the isolate of *R. solani*, to be phytotoxic for seed germination. In this experiment, the maximum germination per cent reduction in all isolates was observed in Pusa Basmati in

Table 4(b): Overall % reduction in germination of seedling of both cultivar

| | Varieties | | Pantnagar | Karnataka | Gujarat | U.P. | Haryana | Punjab | Check |
|---|---|---|---|---|---|---|---|---|---|
| % Reduction in length | Tetep | Mean | 33.92 | 53.74 | 29.52 | 37.44 | 47.58 | 44.93 | 100.00 |
| | | SD | 2.75 | 6.78 | 7.28 | 5.50 | 4.77 | 6.06 | 7.28 |
| | Pusa Basmati-1 | Mean | 39.91 | 55.50 | 35.32 | 42.20 | 43.35 | 48.62 | 100.00 |
| | | SD | 2.06 | 3.79 | 2.42 | 2.10 | 2.06 | 2.61 | 4.48 |
| | Source of isolates | Mean | 36.91 | 54.62 | 32.42 | 39.82 | 45.46 | 46.78 | 100.00 |
| | | | 3.49 | 5.01 | 5.80 | 4.55 | 4.02 | 4.63 | 5.40 |

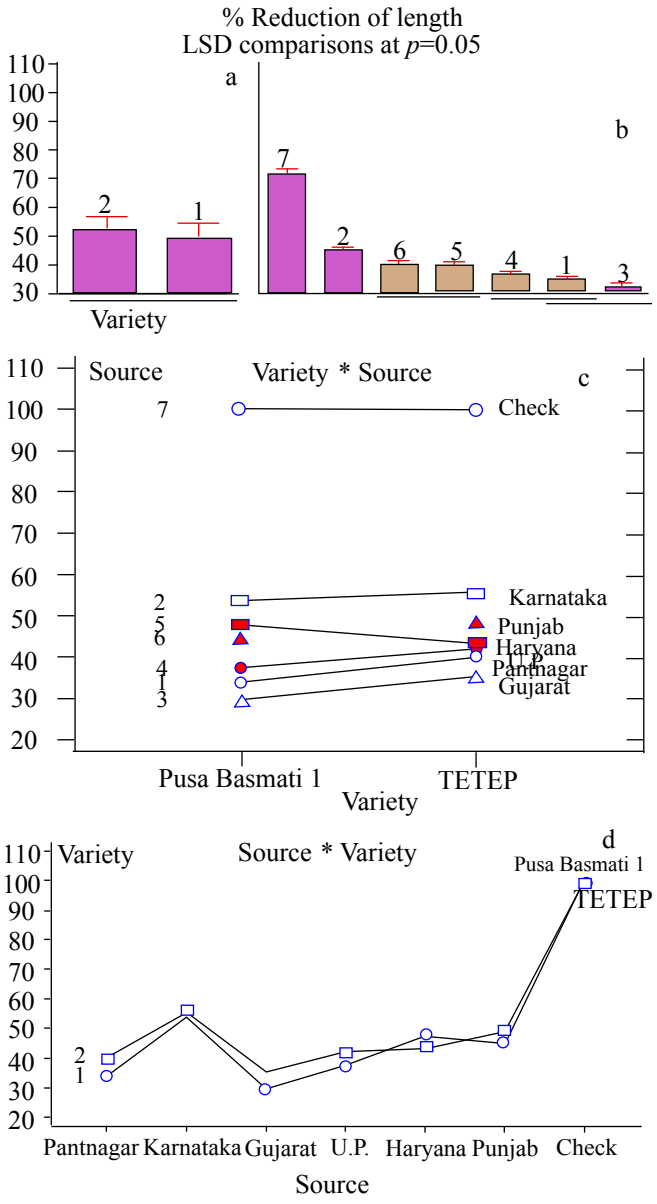


Figure 5 : Graph showing the effect of Oat meal broth culture filtrate isolates of *R. solani* on seed Germination of Rice cultivars (Pusa Basmati-1 & Tetep;. 4(a): Varieties; 4(b): Strains; 4(c): Reduction in germination % variety wise; 4(d): Reduction ingermination % strain (isolate) wise

comparison to Tetep. So Tetep is moderately resistant cultivar while the Pusa Basmati-1 is susceptible cultivar. While the effect of Gujarat isolates was found the highly pathogenic than other isolates on both the cultivar.

## 4. Conclusion

Different isolates of *Rhizoctonia solani* showed the wide variability between each other. The pathogenic effect of all isolates on Pusa Basmati-1 and Tetep also showed the more variability. Tetep showed the less reduction in germination percentage than Pusa Basmati-1. So Tetep is moderately resistant cultivar while Pusa Basmati is susceptible one.

## 5. References

Gangopadhay, S., Chakrabarti, N.K., 1982. Sheath blight on rice. Review of Plant Pathology 61, 451–460.

Ghaffar, A., 1988. Soil borne disease research centre, Final Research report. Department of Botany University of Karachi, 75270, Pakistan, 111.

Gaskill, J.O., 1968. Breeding for *Rhizoctonia* resistance in sugarbeet. Journal of American Society of Sugarbeet Technology 15, 107–119.

Hoa, T.T.C., 1994. Characterization and pathogenicity of *Rhizoctonia solani* Kuhn isolates from different rice zone and management of sheath blight of rice. PhD Thesis, Indian Agricultural Research Institute, New Delhi, 12, 122.

Jain, R.K., Thapliyal, P.N., 1980. Toxic metabolites from *Rhizoctonia solani* Kuhn: Production and possible role in pathogenesis. Indian Journal of Experimental Biology 18, 316–318.

Kannaiyan, S., Prasad, N.N., 1978. Studies on the viability of sclerotia of *Rhizoctonia solani* Kuhn in soil and water. Madras Agricultural Journal 65, 741–742.

Kobayashi, T., Mew, T.W., Hashiba, T., 1997. Relationship between incidence of rice sheath blight and primary inoculum in the Philippines: Mycelia in plant debris and sclerotia. Annals of Phytopathological Society of Japan 63, 324–327.

Lal, M., Kandhari, J., 2009. Cultural and morphological

variability in *Rhizoctonia solani* isolates causing sheath blight of rice. Journal of Mycology and Plant Pathology 39(1), 77–81.

Ou, S.H., 1985. Rice Diseases. 2nd Edition, Common sheath blight of rice. International Rice Research News letter 6, 14.

Parmeter, J.R., Sherwood, R.T., Platt, W.D., 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*, Phytopathology 59, 1270–1278.

Rangaswami, G., Mahadevan, A., 2004. Disease of crop plants in India. Prentice-Hall of India Private Limited Publisher, New Delhi, India, 507.

Rajput, L.S., Harlapur, S.I., 2014. Status of banded leaf and sheath blight of maize in North Karnataka. Karnataka Journal of Agricultural Sciences 27(1), 82–84.

Santha Kumari, P., Rehumath Niza, T.J., 2005. Propiconazole-A New Fungicide for Sheath Blight of Paddy, Karnataka Journal of Agricultural Sciences 18(3), 833–835.

Sinha, B.B.P., Ghufran, S.M., 1988. Physiological studies on five isolates of sheath blight of rice caused by *R. solani* Kuhn. Journal of Research-Rajendra Agricultural University 6, 61–67.

Singh, R., Shukla, T.N., Dwivedi, R.P., Shukla, H.P., Singh, P.N., 1974. Studies on the soybean blight caused by *Rhizoctonia solani*. Indian Journal of Mycology and Plant Pathology 4, 101–103.

Singh, S.K., Satyanarayan, K., Reddy, A.P.K., 1990. Studies on morphology growth habit, *hyphal anastomosis* and virulence pattern of five isolates of sheath blight pathogen of rice. Indian Phytopathology 43, 368–371.

Sherwood, R.T., Lindberg, C.G., 1962. Production of a phytotoxin by *Rhizoctonia solani.* Phytopathology 52, 586– 587.

Sunder, B., Kataria, H.R., Satyavir, O.P., Sheoran, O.P., 2003. Characterization of *Rhizoctonia solani* associated with root collar$^{-1}$ rots and blights. Indian Phytopathology 27–33.

Tiwari, A., Khare, M.N., 2002. Conditions inducing imperfect and perfect stages in *Rhizoctonia solani* causing diseases of mungbean. Journal of Mycology and Plant Pathology 32(2), 176–180.

Verma, H.S., 1973. *Rhizoctonia* aerial blight of soybean symptomotology, etiology and chemical control. M.Sc. Thesis, Govind Ballabh Pant University of Ariculutre and Technology, Pantnagar, 58.

Wallwork, H., 1996. Cereal root and crown diseases. Kondinin Group, Perth, Australia.