Materials
And
Methods
3.0 Site Description

The experimental site was located in and around Balrampur district headquarter which is situated in Shivalik foothills of Himalayas near international boundary of Nepal at 27°-54° N latitude and 82°-49° E longitude at an elevation of 137 metre above mean sea level. Balrampur enjoys sub-tropical semi-humid climate having mean annual rainfall of 100 cm. Approximately 75% of total rainfall is received during June to September by S-W monsoon. The mean maximum and minimum temperatures ranged from 24-39 and 16.8-27° C respectively during the period of investigation. The site experienced 55 cm of rainfall during 2007. The meteorological data of Balrampur has been condensed in appendix I.

3.1 Observation on symptoms

The symptoms of the disease were studied during their development in the rice field as well as in the pots in
3.2 **Isolation of the pathogen**

Rice plants exhibiting characteristics symptoms of sheath blight were collected from the rice field situated headquarter. Infected sheath was cut into small piece of 2-3mm size, with the help of sterilized blade. After surface sterilization with mercuric chloride (HgCl₂) solution (0.1%), the infected pieces were thoroughly washed thrice with distilled water. These pieces were transferred in petriplates containing Potato Dextrose Agar medium, (P.D.A.), under aseptic conditions. The inoculated slants were incubated in BOD incubator at the 28 ± 1°C, superficial growth was subcultured on freshly prepared slants.

3.3 **Purification and Maintenance of the culture**

The fungus was purified by hyphal tip method. The pure culture thus obtained were maintained by subculturing
it at every 30 days interval on PDA and preserved in the refrigerator.

3.4 Identification of the fungal culture

The fungal culture was identified on the basis of their cultural and morphological characteristic. Slides were prepared in cotton blue and examined under compound microscope, for morphological characteristics of the fungus.

3.5 Pathogenicity test

The pathogenicity was proved under glass house conditions using highly susceptible rice cultivar "Pant Dhan-4". Twenty one days old seedlings were transplanted in 20 cm plastic pots. The pots were irrigated regularly to have high moisture condition, At maximum tillering stage, the plants were inoculated by placing infected rice stem pieces having mycelium and sclerotia of the pathogen, at the centre of the hill. Immediately after inoculation plants were placed in moist chamber for 48-72 hours.
Typical symptoms of the disease appeared after 4-5 days of inoculation. Re-isolation of the pathogen was clone from the infected sheath showing typical symptoms of the disease. The pathogenicity test was repeated once more to confirm the results. The fungus was purified by hyphal tip culture method on PDA and found to be identical with the fungus used for inoculation.

3.6 Selection of medium

Eleven media viz., B-R medium, Brown's Agar, Flentze's soil extract Agar, Potato Dextrose Agar, Water Agar, Soybean Decoction Sucrose Agar, V 8 juice Agar, Richard's Agar, PDA + Rice leaf extract Agar were screened to observe the growth and sclerotal production of the fungus and to select a specific medium supporting highest growth and sclerotal production of the fungus.

i. B-R Agar medium

Dipotassium hydrogen phosphate (K₂HPO₄) 696 mg
Potassium chloride (KCl) 149 mg
Zinc sulphate (ZnSO$_4$, 7H$_2$O)  
Thiamine hydrochloride  
Ammonium nitrate (NH$_4$NO$_3$)  
Magnesium sulphate (MgSO$_4$, 7H$_2$O)  
Ferrous sulphate (FeSO$_4$, 7H$_2$O)  
Mangnese sulphate (MnSO$_4$, H$_2$O)  
Glucose (C$_6$H$_{12}$O$_6$)  
Agar  
Distilled water  

**ii. Brown's Agar**

Dipotassium hydrogen phosphate (K$_2$HPO$_4$)  
Asparagine  
Magnesium sulphate (MgSO$_4$, 7H$_2$O)  
Glucose (C$_6$H$_{12}$O$_6$)  
Agar  
Distilled water
### iii. Flentze's soil extract Agar

- Dipotassium hydrogen phosphate ($\text{K}_2\text{HPO}_4$): 0.2 g
- Dried yeast: 0.1 g
- Soil extract: 1000 ml
- Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$): 1 g
- Agar: 25 g
- Distilled water: 1000 ml

### iv. Potato Dextrose Agar (PDA)

- Potato: 200 g
- Dextrose ($\text{C}_{6}\text{H}_{12}\text{O}_{6}$): 20 g
- Agar: 20 g
- Distilled water: 1000 ml

### v. Water Agar

- Dextrose ($\text{C}_{6}\text{H}_{12}\text{O}_{6}$): 20 g
- Agar: 20 g
- Distilled water: 1000 ml
vi.  **Potato Dextrose Agar (PDA)**

Potato  
Marmite yeast extract  
Dextrose \((C_6H_{12}O_6)\)  
Agar  
Distilled water  

<table>
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<tr>
<td>Potato</td>
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<tr>
<td>Marmite yeast extract</td>
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<tr>
<td>Dextrose ((C_6H_{12}O_6))</td>
<td>20 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20 g</td>
</tr>
<tr>
<td>Distilled water</td>
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vii.  **Rice Polish Agar**

Rice Polish  
Agar  
Distilled water  

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<td>Rice Polish</td>
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<tr>
<td>Agar</td>
<td>17 g</td>
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<td>Distilled water</td>
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viii.  **Soybean decoction sucrose Agar**

Soybean seeds  
Sucrose \((C_{12}H_{22}O_{11})\)  
Agar  
Distilled water  

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<tr>
<td>Soybean seeds</td>
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<tr>
<td>Sucrose ((C_{12}H_{22}O_{11}))</td>
<td>20 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20 g</td>
</tr>
<tr>
<td>Distilled water</td>
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ix.  **V-8 Juice Agar**

V-8 juice  

<table>
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<tr>
<td>V-8 juice</td>
<td>10 g</td>
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Calcium carbonate (CaCO) 2.0 g
Glucose (C₆H₁₂O₆) 2.0 g
Yeast extract 2.0 g
Agar 20 g
Distilled water 1000 ml

x. Richard's Agar

Potassium nitrate (KNO₃) 10 mg
Potassium di-hydrogen phosphate (KH₂PO₄) 5 g
Magnesium sulphate (MgSO₄, 7H₂O) 2.5 mg
Ferric chloride (FeCl₃) 0.02 g
Sucrose (C₁₂H₂₂O₁₁) 50 g
Agar 20 g
Distilled water 1000 ml

xi. PDA + Rice leaves extract Agar

Leaf extract @ 2% 500 g
Leaves 50 g
Potato 100 g
Agar 20 g
Dextrose (C₆H₁₂O₆) 10 g
Distilled water 1000 ml

All the media were adjusted to pH 7.6 and sterilized at a pressure of 15 lbs/inch² for 20 minutes.

3.7 Maintenance of pH

The desired pH values for all the different media were maintained by adding required amount of 0.1N HCl or 0.1N NaOH in the media with the help of digital meter.

3.8 Sterilization of petriplates and other glass wares

Cleaned petriplates and other glass wares were sterilized in hot air oven at 180°C for 60 minutes.

3.9 Pouring and inoculation

Previously sterilized petriplates of 90 mm diameter were used for pouring the medium. Eighteen to twenty ml
sterilized melted but cooled medium was aseptically poured in each petriplate. The plates were shaken uniformly and the medium in the plate was allowed to solidify. After solidification of the medium in plates, these were inoculated by placing a 5 mm mycelial disc cut from the margin of the 3 days old culture of the test fungus. The mycelial disc was kept in such a manner that the mycelial portion of the disc touched the surface of the medium. Inoculated petriplates were incubated at 28±1 °C for 3 days. Each treatment was replicated 3 times.

3.10 Measurement of mycelial growth and development of sclerotia

The radial growth of the fungus was measured in millimeters at the interval of 24 hrs up to 3 days. For the formation of sclerotia the plates were incubated at 28±1°C for 15 days. The sclerotia were counted at the intervals of 3 days till 15th day.
3.11 Survival of *Rhizoctonia solani*

Experiments were carried out under laboratory and glasshouse conditions to record the length of survival of *R. solani* in culture medium and in crop debris. Different methods used in the present investigation are given below:

3.11.1 Survival of *R. solani* in culture medium

The culture of *R. solani* was maintained on Potato Dextrose Agar slants at 25 ± 1°C. At monthly intervals, the survival of the fungus was ascertained by sub-culturing the fungus on PDA slants and development of sclerotial bodies. The observations were recorded on the new growth of the fungus on medium.

3.11.2 Survival of *R. solani* in crop debris having mycelium

The experiments were conducted under laboratory and glass house conditions to ascertain the role of plant debris in
survivability of *R. solani*. The experiments were conducted under following sets of conditions.

3.11.2.1 Survival of *R. solani* on crop debris buried in soil and stored at room temperature

In the first set of experiment infected rice leaf sheath of variety "Pant-Dhan-4" showing disease symptoms were collected from field during November, 2006. The infected leaf sheath were cut into small pieces of approximately 4 to 5 cm size and then placed in to nylon bags and buried in natural soil at field condition. They were stored at room temperatures, where the maximum temperature recorded upto 41°C (during May and June) and minimum 16°C (during December and January). Isolation of the fungus from these leaf sheath pieces was done at monthly intervals on PDA medium starting from December 2006 to May 2007. The infected bits were picked out from treatment and washed under tap water to remove soil particles. They were then sterilized with 0.1% mercuric chloride solution and plated on PDA
to observe the mycelial growth of the fungus. The number of infected pieces yielding colonies was recorded and percentage recovery from infected pieces (percent survival) was calculated.

3.11.2.2 Survival of *R. solani* in crop debris stored at different temperature

In the second set of experiment, infected leaf sheath pieces (4-5 cm size) filled in nylon bags were buried in natural soil in plastic pots and then incubated at different temperatures i.e., 0, 10, 28 and 40°C. Three replications were maintained for each treatments. Isolation of the fungus from leaf sheath pieces was done at the interval of 30 days, to ascertain the survival to pathogen. Percent leaf sheath pieces yielding colonies of the fungus was recorded.

3.11.23 Survival of *R. solani* in crop debris having mycelium buried in different types of soil

In this experiment, the diseased crop debris were buried in soils of different texture, i.e., sandy loam, clay loam and
tarai/local soil. Infected leaf sheath pieces kept in small nylon bags were buried in different types of soils, filled in plastic pots. These pots were kept in glasshouse. At monthly intervals the pieces were taken out and isolation were made on PDA medium as described earlier to ascertain the percent survival of the fungus.

3.11.2.4 Survival of R. solani in crop debris having mycelium buried in soil at different depth

In this set of experiment, survival of the fungus in diseased rice plant debris buried at different depths in soil was studied. Infected leaf sheath pieces (4 to 5 cm size) showing disease symptoms, were placed in nylon bags and buried at different depths, viz., 2.5, 5.0, 10.0, 15.0 and 20.0 cm deep in plastic pots (20 cm size) filled with natural soil. Three replications were maintained for each treatments. These pots were kept in glasshouse. At monthly intervals, the pieces were taken out and isolation were made on PDA medium to ascertain the percent survival of the fungus.
3.11.2.5 Survival of *R. solani* in crop debris having mycelium placed in soil at different moisture regimes

In this set of experiment, infected leaf sheath pieces (4-5 cm size) were kept in small nylon bags and buried in soil having different moistures conditions i.e. dry, field and submerged condition at 10 cm deep. Moisture levels of each pot were maintained by periodic addition of water. Three replications were maintained for each treatments. Isolation was made at monthly intervals by the method mentioned earlier.

3.11.2.6 Survival of *R. solani* in crop debris having mycelium buried in soil amended with different organic amendments and green manures

Infected leaf sheath pieces kept in small nylon bags were buried in soil amended with different organic amendments and green manures. At first, the plastic pots were filled with natural soil and amended with different organic amendments/green manures. The organic substances used for soil amendments
were oil cakes of Neem (Azadirachta indica), Mustard (Brassica campestris) and Castor (Ricinus communis), Rice (Oryza sativa), FYM (Farm Yard Manures) and Green manures, Neem (Azadirachta indica), Dhaincha (Sesbania aculeata), Sanai (crotalaria juncia) and leaves of legumes. Organic amendments were mixed with the soil @ 1% (w/w) and green manures were used @ 2%. Three replications were maintained of each treatment with suitable control (unamended soil). After incorporation of organic amendments, the proper moisture was maintained by periodic addition of tap water. Isolation was made at monthly intervals following the method described earlier.

3.11.2.7 Survival of R.solani in crop debris having mycelium buried in soil amended with Nitrogen, Phosphorus and Potash

The infected leaf sheath pieces in small nylon bags were buried in unsterilized soil amended with N, P and K in different combinations. Plastic pots were filled with unsterilized soil and treated with NPK fertilizers. Urea, Single Super Phosphate and
Muriate of Potash were added to the soil at 0.05 percent level. The treatments included N, P, K, NP, NK, PK, NPK and control. Methods for the isolation of the fungus from infected leaf sheath pieces were same as described earlier.

3.11.3 Survival of sclerotia of *R. solani* in soil

The experiments were conducted under laboratory and glasshouse conditions to ascertain the role of sclerotia (dormant structure of the fungus which can survive in adverse conditions) in survivability of *R. solani*. Sclerotia of uniform size were collected from 15 days old culture of fungus grown on PDA medium. Studies on the survival of sclerotia of *R. solani* in culture and in soil stored at room temperature, different temperature, in different soil types, at different depths, at different moisture regimes, amended with different organic amendments and green manures and amended with NPK fertilizers, were conducted under laboratory and glasshouse conditions following the methods described earlier.
3.12 Statistical Analysis

The data so recorded for each character were subjected to analysis with the help of computer. Overall differences were subjected to 'F' test at 5% level of significance as suggested by Cochran and Cox (1959). In case of significant results, critical difference at 0.5% level of probability was calculated for testing the significance between the two treatment means.