Integrated Management of Foot Rot of Lentil Using Biocontrol Agents under Field Condition

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The efficacy of cowdung, Bangladesh Institute of Nuclear Agriculture (BINA)-biofertilizer, and Bangladesh Agricultural University (BAU)-biofungicide, alone or in combination, was evaluated for controlling foot rot disease of lentil. The results exhibited that BINA-biofertilizer and BAU-biofungicide (peat soil-based *Rhizobium leguminosarum* and black gram bran-based *Trichoderma harzianum*) are compatible and have combined effects in controlling the pathogenic fungi *Fusarium oxysporum* and *Sclerotium rolfsii*, which cause the root rot of lentil. Cowdung mixing with soil (at 5 t/ha) during final land preparation and seed coating with BINA-biofertilizer and BAU-biofungicide (at 2.5% of seed weight) before sowing recorded 81.50% field emergence of lentil, which showed up to 19.85% higher field emergence over the control. Post-emergence deaths of plants due to foot rot disease were significantly reduced after combined seed treatment with BINA-biofertilizer and BAU-biofungicide. Among the treatments used, only BAU-biofungicide as the seed treating agent resulted in higher plant stand (84.82%). Use of BINA-biofertilizer and BAU-biofungicide as seed treating biocontrol agents and application of cowdung in the soil as an organic source of nutrient resulted in higher shoot and root lengths, and dry shoot and root weights of lentil. BINA-biofertilizer significantly increased the number of nodules per plant and nodules weight of lentil. Seeds treating with BAU-biofungicide and BINA-biofertilizer and soil amendment with cowdung increased the biomass production of lentil up to 75.56% over the control.

Keywords: Integrated management, foot rot, lentil, biocontrol agents, field condition

Different phytopathogenic soilborne as well as seedborne fungi are responsible for disease development of pulses, which attack plants during seedling to maturity stages and are more destructive at the seedling stage [8]. Foot rot (causal agents *F. oxysporum* and *S. rolfsii*) is considered as an important and destructive disease of pulses in almost all legume-growing countries of the world [1].

Control of the soilborne pathogens *F. oxysporum* and *S. rolfsii* with chemicals is practically difficult. On the other hand, indiscriminate use of chemicals causes environmental pollution and health hazards [9]. Nowadays, integrated Disease Management (IDM) is very much popular for controlling plant diseases. There are several tactics within IDM, among them biological control being one of the most important tactics [18]. *Trichoderma* may be used as an ecofriendly biocontrol agent in this regard. The biocontrol agent *Trichoderma* has the potential to protect seedlings against several plant pathogenic fungi. *Trichoderma* spp. have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers [28]. *Trichoderma harzianum* has been reported to be effective in controlling seed- and soilborne diseases of different crops, namely legumes and vegetables [2, 6, 12, 20, 24, 26]. The use of antagonistic bacteria as a biological...
control means may provide a great alternative for plant pathologists [11]. Hossain et al. [11] also reported prominent antagonistic effect of *Rhizobium* against foot and root rot pathogens (*F. oxysporum* and *S. rolfsii*) of pulses. This antagonist also increased the percentage of seedling emergence, plant height, fresh weight, and vigour index [12, 22]. It has been reported that rhizobial strains have significant effect in reducing the severity of foot and root of chickpea [16]. *Rhizobium* spp. and *Trichoderma* sp. are compatible and have combined effects in controlling the fungi *F. oxysporum* and *S. rolfsii*, which caused the root rot of lentil [21]. Application of cowdung and manures in the soil is aimed to supply nutrients to the crops, creating a positive environment of inducing disease resistance to the plant. As a result, plants may recover from the disease or be resistant to disease, or overcome the disease epidemic [14, 17].

Considering the above facts the present study was undertaken to find out the effect of cowdung, BINA-biofertilizer (peat-based *R. leguminosarum*), and BAU-biofungicide (organic substrate-based *T. harzianum*), either alone or in combination on foot rot disease of lentil under field conditions.

### Materials and Methods

**Collection of BINA-Biofertilizer**

Peat-based biofertilizer was collected from the Soil Microbiology Laboratory of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. The composition of the biofertilizer was peat soil and *Rhizobium leguminosarum*. The *R. leguminosarum* was firstly collected from the nodules of legume plants. After collection, *Rhizobium* broth was prepared. Sterilized 500 g peat soil was poured in a polythene bag and inoculated with a previously prepared 5 ml broth of *R. leguminosarum* (10^11 CFU/ml) and mixed thoroughly for proper distribution. Then the materials were incubated for 7 days at 25 ± 2°C. After 7 days of incubation, it was ready for use. The material can be stored up to 6 months at 22 ± 1°C for future use.

**Collection of BAU-Biofungicide**

BAU-biofungicide was collected from the Disease Resistance Laboratory, Department of Plant Pathology, BAU, Mymensingh, Bangladesh. BAU-biofungicide was invented from a naturally occurring fungus, *Trichoderma harzianum* growing on an organic substrate (black gram bran). The *T. harzianum* was firstly collected from the rhizosphere of a lentil field. The isolated fungi were cultured on potato dextrose agar (PDA) at 28 ± 2°C. Then 50 g of black gram bran was moistened with 1.5 ml of water and sterilized in an autoclave. After cooling, the sterilized substrate was inoculated with previously prepared 7-day-old culture of *T. harzianum* (four to five 1 cm block of *T. harzianum*). After 7 days of incubation, it was ready for use. The material can be stored up to 6 months at 22 ± 1°C for future use.

**Collection of Seeds of Pulse**

Seed samples of lentil variety BINA Masur-1 were collected from the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. The collected seeds were kept in a paper bag and stored in the refrigerator at 5–7°C for one month for subsequent studies.

**Seed Treatment**

Required amounts of seeds were taken in a beaker and a few drops of water were added for moistening the seed surface uniformly to allow maximum adherence of BAU-biofungicide on the whole surface of seeds. Seeds were treated with BAU-biofungicide at 2.5% weight of seeds until the whole surface of the seeds were coated, where 2.5% biofungicide contained 10^8 cells/ml [19]. For seed coating with biofertilizer, seeds were initially moistened with water. Then the seeds were thoroughly mixed with biofertilizer (at 2.5% of seed weight) where the biofertilizer contained 10^8 *Rhizobium* cells/ml formulations. The inoculant-coated seeds were placed in a cool and dry place under shade for drying. The treated seeds were kept in paper bags and stored in the refrigerator at 5–7°C for one month for subsequent studies.

In the present study, the following treatments were used:

- Control
- Cowdung
- BINA-biofertilizer
- BAU-biofungicide
- Cowdung + BINA-biofungicide
- Cowdung + BAU-biofungicide
- BINA-biofertilizer + BAU-biofungicide
- Cowdung + BINA-biofertilizer + BAU-biofungicide

Cowdung (5 t/ha) was mixed with the soil during the final land preparation as per treatment specification [15].

**Field Experiment**

**Seed sowing.** The field experiment was conducted in a randomized block design with 3 replications. The size of the individual plot was 2 m × 1 m, and the spaces between the plots and blocks were 1 m and 1 m, respectively. Treated seeds were sown (35 kg/ha) in lines about 2.0 cm in depth and the seeds were immediately covered with soil. Two times of weeding were preformed, one after 25 days and another 40 days after sowing. No plant protecting chemicals (insecticides or fungicides) were applied in the field.

**Determination of foot rot disease.** The experimental plots were inspected routinely to observe the foot rot disease on plant. In case of complexity to identify the disease, symptoms-bearing plants were collected from the field using polythene bag and brought to the Disease Resistance Laboratory, Department of Plant Pathology, BAU, Mymensingh. From the infected plants, the fungi were isolated following tissue planting methods [4]. After incubation, the fungi that grew over potato dextrose agar (PDA) were purified by the hyphal tip culture method. The isolated fungi were identified as *F. oxysporum* and *S. rolfsii* according to reference mycology books and manuals [3, 5, 25]. The pure cultures of the fungi were preserved in PDA slants at 4°C in the refrigerator as stock culture for future use.

**Data collection and analysis.** Data on different parameters (*viz.*, germination, post-emergence death of plants, plant stand, shoot length, root length, dry shoot weight, dry root weight, number of nodule/plant, weight of nodule/plant, and biomass) were taken. Five plants were randomly selected and uprooted carefully from each plot for recording data. Data were expressed as the means ± standard errors. The results were analyzed using the SPSS statistical package (ver. 16, SPSS Inc., Chicago, IL, USA) and Microsoft excel program.