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# Physiological Study and both *in vitro* and *in vivo* Antifungal Activities against *Stemphylium botryosum* causing Stemphylium Blight Disease in Lentil (*Lens culinaris*)

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**Abstract:** The experiment was carried out to study and the evaluation of six fungicides and an antagonist both *in vitro* and *in vivo* condition against stemphylium blight disease of lentil caused by *Stemphylium botryosum*. The investigation was undertaken at Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh during September 2007 to April 2008 using completely randomized design in lab condition and completely randomized block design in filed condition. The colony color of *S. botryosum* was greenish brown, irregular margin and velvety texture was found on PDA medium. The suitable temperature and pH for the maximum colony growth was recorded at 25°C and 6.0, respectively. The maximum (100%) germination of conidia was found at 25°C after 6 h of incubation. From the *in vitro* test, six fungicides have the potentiality to inhibit the radial mycelial growth at a lower (500 ppm) concentration except Agrimyl (Mancozeb+Metalaxyl) whereas it inhibited radial colony growth at higher (2000 ppm) concentration. In field condition the minimum disease score (1.0) was recorded from Iprosan 50 WP treated plot and the highest (4.75) was found in control plot. Among the six fungicides Iprosan from the iprodione group gave the best performance. The highest root length (9.48 cm), shoot length (44.6 cm), number of branches plant$^{-1}$ (9.25), number of pods plant$^{-1}$ (39.10), thousand grain weight (21.08 g) and grain yield (1271.00 kg ha$^{-1}$) was obtained from Iprosan 50 WP.

**Key words:** Germination, pH, Temperature, *Trichoderma harzianum*, Lentil, *Stemphylium*

## INTRODUCTION

Lentil (*Lens culinaris* Medik.) is one of the oldest and the most important pulse grain legume crops in terms of both area (134,700 ha) and production (115,300 t) and the highest consumer preference and the total consumption (BBS, 2005). This crop has been grown mainly as an inexpensive source of high quality protein in human diets, especially in West Asia. Lentil being an important pulse crop of Asia-Pacific region (which covers about 53% and produces

Forty nine percent of world's lentil (Sarker *et al*., 2004). Lentil has been cultivating in Bangladesh since long back the crop is found to grow everywhere.

Now a day the production of the crop is decreasing every year due to some biotic and abiotic factors. Among the two groups disease under biotic factors is the major constraint for lentil production all over the country. So far 15 pathogens causing 17 diseases of lentil have been reported in Bangladesh (Huq and Khan, 2007a). Among the diseases stemphylium blight caused by *S. botryosum* is a serious threat to lentil cultivation. Stemphylium blight disease of lentil has been reported in Bangladesh, Egypt, Syria and the USA (Bayaa and Erskine, 1998).

The disease already gained much more importance and 80-92.35% crop loss has been reported by Bakr and Ahmed (1992). In neighboring country India, the intensity of the disease was 82.55% and the loss was recorded as 93.4% (Singh *et al*., 1990).

Environmental temperature plays an important role to develop this disease. The prevalence of warm temperature (>25°C) and wetness duration longer than 24 h favors the appearance, development and spread of the Stemphylium blight disease in South East Asia (Erskine and Sarker, 1997). A wide range of pH (6-8) also favors the radial growth of *S. botryosum* (Huq, 2003). However, few studies have been investigated for the detail study of the pathogen *S. botryosum* (Saha *et al*., 2010; Huq and Khan, 2008; Huq and Khan, 2007b).

Hence, due to importance of this disease concentration need to be paid and try to shed new focus on the causal agent of this disease especially of its physiological and nutritional requirements for the growth and development which will help to manage the disease

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efficiently. In view of the above facts the present research work was undertaken with the following objectives-to know the morphological growth pattern, physiological requirements of the *S. botryosum* pathogen and also evaluation of six fungicides both *in vitro* and *in vivo* condition.

## MATERIALS AND METHODS

**Experimental site:** The experiment was carried out at laboratory and field of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during September 2007 to April 2008. The experiment was conducted in Completely Randomized Design (CRD) having 5 replications for each treatment in lab condition.

**Collection, isolation and identification of *S. botryosum*:** The plant samples showing stemphylium blight infection were collected from the infected field of lentil blight. Isolation and identification and sensitivity to fungicides were done using standard protocols before going to set the field experiment.

**Purification:** Purification was done using single spore isolation technique. Spore suspension was prepared from the 15 days old culture of *S. botryosum*. One or two drops of spore suspension were poured onto a Petri plate containing Water Agar (WA) the drop of suspension was rubbed onto WA with the help of a sterilized glass rod then the plate was observed under a compound microscope with 10 x magnification. After locating a single spore it was focused into centre. One of the microscope objectives was replaced with a specially made spore cutter. Then the spore was cut with the spore cutter. The cutting agar block was taken and placed onto a petri plate containing PDA media and incubated at 25°C for 5 days then observed confirmed. The purified spore was kept in a refrigerator at 4°C as a slant culture for further use.

**Cultural and morphological characteristics of *S. botryosum*:** The cultural and morphological characteristics of *S. botryosum* were observed on PDA medium after 6 days of incubation on the basis of colony color, texture, margin, conidial color, size and color of conidiophores.

**Scanning Electron Microscope (SEM) study of *S. botryosum*:** A double sided adhesive carbon cement tape was attached on an aluminum SEM stub. A loop full of *S. botryosum* pure culture (sporulating plate, 12 days old culture) was taken out with the help of a tungsten loop and gently placed onto the adhesive carbon cement tape. Then aluminium SEM stubs were placed in a platinum coater (Model: JEOL JFC-1600, Auto fine coater) and provided 10 mA current flow and 5±0.5 Pa pressure at 10 sec to make the test samples conductive. After coating the samples then was placed into the SEM (Model: JEOL JSM-6490 LA, Analytical Scanning Electron Microscope) for obtaining the image. For getting a clear SEM image working distance, spot size and accelerating voltage was maintained (40, 12 and 10 Kv in high vacuum condition).

**Physiological studies of *S. botryosum***

**Influence of temperature:** Seven different levels of temperature viz., 5, 10, 15, 20, 25, 30 and 35°C were studied for its impact on radial colony growth of *S. botryosum*. Sixteen milliliter of PDA medium were poured into the Petri plates using media dispenser having 5 replications for each temperature and autoclaved at 121°C for 30 min at 15 psi and then taken out and shifted into the clean bench for solidification. Five millimeter diameter mycelial disc were cut from the periphery of 5 days old culture of *S. botryosum* and inoculation was done and the plates were placed in an incubator in respective temperature level.

**Influence of pH:** Seven different pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were studied in this experiment. The different level of pH were maintained using 0.1 N NaOH or 0.1 N HCl and the other protocol as same as stated under temperature.

**Influence of incubation time and temperature on conidia germination of *S. botryosum*:** For the test of conidia germination of *S. botryosum,* conidia were harvested from 12 days old culture. Conidial suspension (at least 50 conidia per low microscopic field) was prepared using glass slide in sterilized distilled water. Conidial suspension was evenly spread over each of six well slides. At least three blotter papers placed on each glass Petri dishes and it made moistened using sterile water. The slides were placed into centre of the labeled six glass Petri-dishes. Observations were made after 2, 4, 6, 8, 12 and 24 h of incubation. The number of germinated conidia was counted as percentage of the total number of conidia observed. The conidia were considered as a germinated when the germ tube was developed at least half of the width of the conidia. Conidia germination was determined by evaluating at least 50 conidia. The observations were made in a 10x microscopic field under compound microscope.

***In vitro* antifungal activity against *S. botryosum*:** For the test of antifungal activity of six different fungicides namely, Iprosun 50 WP, Edcuzeb 80 WP, Proud 25 EC, Rovral 50 WP, Emivit 50 WP and Agrimyl were studied

*in vitro*. The required amount of fungicides was weighed using electric balance to get the proposed concentration such as 500, 1000, 1500 and 2000 ppm. The required concentrations of tested fungicides and sterilized distilled water were added to the conical flasks containing double strength PDA medium to achieve the proposed concentration with 5 replications. The other method of this experiment was same as mentioned earlier under temperature experiment. Inoculated plates were incubated at 25°C with 12 h light and dark phase alternatively and then examined after 7 days of incubation.

Per cent inhibition of the radial mycelial growth of *Stemphylium botryosum* was calculated on the basis of the following formula:

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

Where:

I = Per cent of growth inhibition
C = Average diameter (mm) of fungal colony in control treatment
T = Average diameter (mm) of fungal colony in fungicides treated PDA medium

**Field experiment:** The research experiment was undertaken during the period from mid October, 2007 to April 2008. The experiment was carried out in randomized completely block design (RBD) having four replications. The land was well pulverized in mid October, 2007. Unexpected plants and others material were removed from the experimental field. Fertilizers were applied during final land preparation as per recommendation. The treatments were applied to the plots in a random selection in each block. The plot size was 2×3 $m^2$. Eight treatments including control were used in this experiment viz., iprosun, edcuzeb, proud, rovral, emivit, agrimyl, antagonist (*Trichoderma harzianum*) and control (sterile water). Six chemicals and their trade name, active ingredient (ai) and applied concentration are shown in the Table 1.

**Sowing of lentil seeds:** BARI Moshur-1 (Utfala) seeds were used in this experiment. The lentil seeds were sown in furrows maintaining 20 cm distance to another. The furrows were covered with the soil after sowing of seeds. The line to line distance also maintained as 20 cm with continuous sowing of seeds between the lines. The lentil seeds were sown in the afternoon on November 12, 2007.

Table 1: List of fungicides with their trade name, active ingredient and applied concentration to the experimental field of lentil

| Trade name of fungicides | Active ingredient (ai) | Concentration of applied product (%) |
|---|---|---|
| Iprosun 50 WP | Iprodione | 0.2 |
| Edcuzeb 80 WP | Mancozeb 80 WP | 0.2 |
| Proud 25 EC | Propiconazole 25 EC | 0.2 |
| Rovral 50 WP | Iprodione | 0.2 |
| Emivit 50 WP | Copper oxychloride 50 WP | 0.2 |
| Agrimyl | Mancozeb + Metalaxyl | 0.2 |

**Anti-fungal chemicals and antagonist application:** The experimental plots were monitored regularly to notice any symptoms of stemphylium blight disease infection. Spraying of tested fungicides was applied when first disease was appeared. At least three sprays were done at 10 days of interval. The experimental plots were sprayed through Knapsack sprayer as required volume. Spore suspension of $10^6$ spore $mL^{-1}$ was prepared by adding sterile water and sprayed into the concerned plot.

**Intercultural operations:** Intercultural practices were done to get the experimental field in hygienic condition for the crop growth of lentil with less competition. Weeding was done two times during the critical stage of lentil plant growth. Simply light irrigation was provided after each weeding and excess water was removed through well drainage system from the research plot to make safe the crop safe from stagnant water.

**Data recording on disease score of stemphylium blight:** The disease score of stemphylium blight of lentil was recorded at 65 days after sowing of lentil seeds. The plants were selected randomly and at least 10 plants were taken in each plot for recording the disease score following a rating scale (0-5 scale) and which were designated by Bakr and Ahmed (1992). Data recording on yield and yield contributing parameters of lentil were root length, shoot length, number of branches $plant^{-1}$, number of pods $plant^{-1}$, 1000 seed weight and yield kg $ha^{-1}$. Root and shoot length of lentil plant were measured in centimeter with a centimeter scale. Data were recorded as the average of 10 plants selected at random from the inner rows of each treated plot.

The number of branches $plant^{-1}$ and number of pods $plant^{-1}$ was counted from the average of 10 plants selected at random from the each plot. Ten plant selected unbiased from the inner rows of lentil plot and the number of pods $plant^{-1}$ was calculated manually. Thousand seeds were counted by a seed counter and weight taken through digital balance (0.001 g). Grain yield of lentil kg $ha^{-1}$ was calculated by converting the weight of plot yield into hectare and was expressed in kg.

**Data analysis:** All data were analyzed statistically using MSTAT-C computer package program. Treatment mean were compared using Duncan's Multiple Range Test (DMRT) at 5% levels of significance.

## RESULTS

### Laboratory experiment

**Cultural and morphological features of *S. botryosum*:** The fungal pathogens varied in their cultural characteristics. *Stemphylium botryosum* colony showed greenish brown color, irregular shape and velvety type texture. Conidia were brown in color and most of them are oblong round at the ends, muriform and constriction at the middle of the conidia, length and breadth varied from 8-15 and 3-8 μm, respectively. The mean length 10.48 μm and breadth 4.78 μm was observed. The conidiophores were brown and the terminal swelling in nature. Conidia and conidiophores of *S. botryosum* are shown in Fig. 1a and b.

**Scanning electron microscopic study of *S. botryosum*:** Single conidium form by swelling the tip of the conidiophore, conidia polyspermic, dotted, pited structure observed under SEM and shown in Fig. 2a-c.

### Influence of physiological requirements

**Influence of temperature (°C):** The influence of temperature on radial mycelial growth of *Stemphylium botryosum* is presented in Table 2, the maximum radial mycelial growth was found at 25°C followed by 20°C and the lowest radial growth was found at 5°C preceded by 10°C. It was clearly showed that with the increasing of temperature level radial growth increases up to 25°C but decreases after 25°C. The suitable temperature for the fungal pathogen of *Stemphylium botryosum* was 25°C.

Table 2: Influence of temperature on radial mycelial growth of *Stemphylium botryosum*

| Temperatures (°C) | Radial mycelial growth (mm) |
|---|---|
| 5 | 10.7e |
| 10 | 18.0d |
| 15 | 33.8bc |
| 20 | 35.4b |
| 25 | 48.2a |
| 30 | 30.0c |
| 35 | 20.0d |
| CV (%) | 11.26 |


Fig. 1: *Stemphylium botryosum*. (a) Conidia and (b) Conidia on vesicular tip of conidiophore

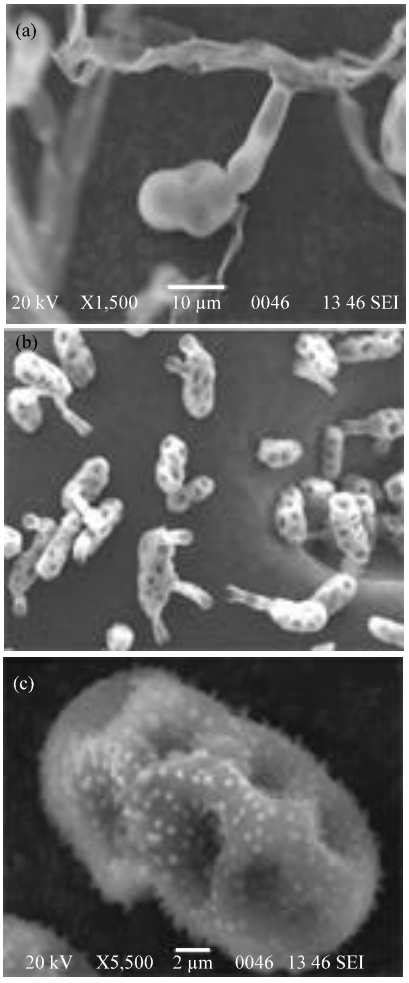


Fig. 2: Scanning electron micrographic (SEM) view of *S. botryosum* (a) Single conidia form on a single conidiophore, (b) Conidia polyspermic and (c) Dotted surface of conida

**Influence of pH:** Results on effect of varied pH on the radial colony growth of *S. botryosum* are presented in Table 3; the pathogen grew well at a wide range of pH. In the present research excellent radial mycelial growth was observed at pH range 6.0 to 7.5. The effect of pH 6.0 and 6.5 were statistically identical and the best growth (31.50 mm) was noted at pH 6.0 followed by 31.25 mm at pH 6.5. Among the pH level the lowest (16.50 mm) radial mycelial growth was recorded at pH 4.5 preceded by 18.00 mm at pH 5.0. From the test of varied pH against *Stemphylium botryosum* radial growth was increased up to pH 6.5.

**Influence of incubation period and temperature on conidia germination:** The conidia of *Stemphylium botryosum* germinated over a wide range of temperatures (5 to 35°C) and presented in Fig. 3, the conidia were polyspermic and produced several germ tubes and it's depending on incubation period and temperature. Generally it was observed that per cent germination of conidia increased with increased temperature level and incubation period. After 24 h of incubation maximum number of germinated conidia were observed for all the temperature levels. At least 12% conidia germinated after 2 h of incubation at 25°C, whereas no germination was recorded at 5 and 10°C. The impact of the rate of germination of conidia increased as temperature above 30°C. However, the slowest and fasted germination response was observed at 5°C and 25°C. There were differences in number of germination after 4 h of incubation between 25°C (86%) and 30°C (19%) but after 6 h of incubation all conidia were germinated. The quickest 100% conidia germination was

Table 3: Influence of pH on radial mycelial growth and sporulation of *Stemphylium botryosum*

| pH | Radial mycelial growth (mm) |
|---|---|
| 4.5 | 16.50e |
| 5.0 | 18.00de |
| 5.5 | 19.00d |
| 6.0 | 31.50a |
| 6.5 | 31.25a |
| 7.0 | 27.50b |
| 7.5 | 25.00c |
| CV (%) | 4.84 |

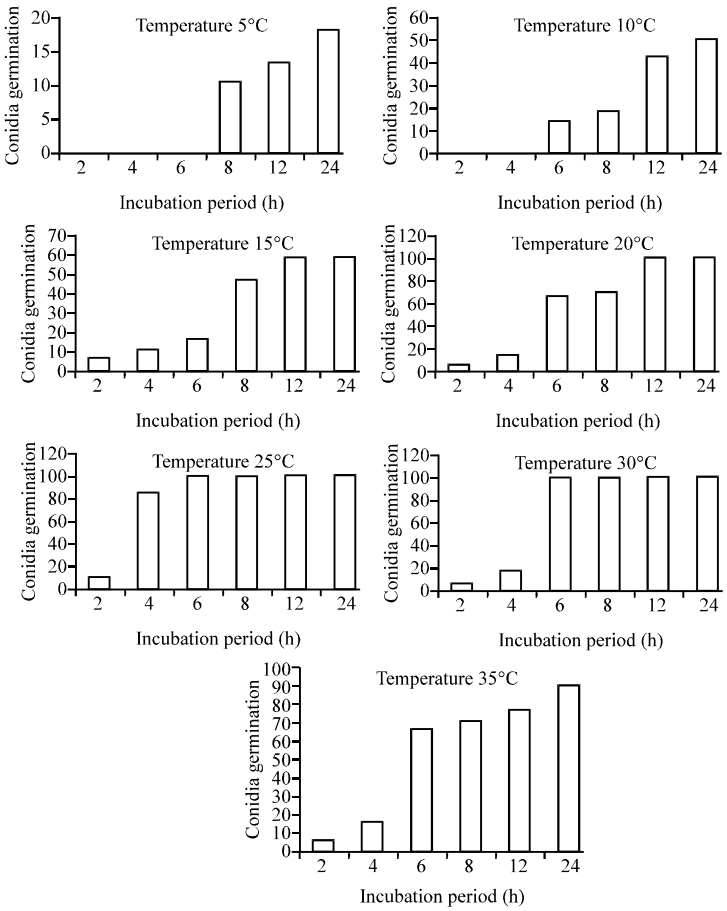


Fig. 3: Influence of incubation period and temperature on conidia germination of *S. botryosum*

noted after 6 h of incubation at 25 and 30°C. Although, the number of germination of conidia differed at 4 h of incubation between 25 and 30°C but there was no differences after 6 h of incubation between them. However it is concluded that the optimum temperature for the germination of *Stemphylium botryosum* conidia 25°C or close by rather than 30°C after 6 h of incubation period.

***In vitro* anti-fungal activity:** All the fungicides inhibited radial mycelial growth significantly over the control at different concentrations and shown in Table 4, all the fungicides and at a least concentration (0 ppm) inhibit the mycelial growth 100% except Agrimyl. The fungicides Agrimyl also inhibit the colony growth over control. The colony growth cumulatively decreases with the increases of Agrimyl concentrations. The highest colony growth (45.50 mm) was observed from the control preceded by Agrimyl (32.75 mm) at lower concentration (500 ppm). From the *in vitro* test of all fungicides showed the equal performance over control except Agrimyl fungicide.

**Field experiment**

**Fungicidal effect on yield attributes of lentil:** All the tested antifungal chemicals reduced the disease score and significantly increased plant growth parameters and yield of lentil compared to control and presented in Table 5 and 6.

**Disease score:** The lowest disease score was observed in plots sprayed with Iprosun 50WP followed by Edcuzeb 80WP which are indicating that it's have the highest reducing disease capability. There were not statistically different among the disease score of lentil plant infection in the plots treated with three fungicides of Proud 25EC, Rovral 50 WP and Emivit 50 WP, respectively and other two treated plot spraying with Agrimyl and antagonist had no significant difference between them (Table 5).

**Root length (cm):** The highest (9.48 cm) root length was produced in plot treated with Iprosun and the lowest (4.44 cm) in control treated plot. Fungicides of Rovral,

Table 4: Antifungal activity against radial mycelial growth and per cent inhibition of *Stemphylium botryosum*

| Treatments | Concentrations (ppm) | Radial mycelial growth (mm) | Inhibition of radial mycelial growth (%) |
|---|---|---|---|
| Iprosun 50 WP | 500 | 0.00e (0.71) | 100 |
| (Iprodione) | 1000 | 0.00e (0.71) | 100 |
| | 1500 | 0.00e (0.71) | 100 |
| | 2000 | 0.00e (0.71) | 100 |
| Edcuzeb 80 WP | 500 | 0.00e (0.71) | 100 |
| (Mancozeb) | 1000 | 0.00e (0.71) | 100 |
| | 1500 | 0.00e (0.71) | 100 |
| | 2000 | 0.00e (0.71) | 100 |
| Proud 25 EC | 500 | 0.00e (0.71) | 100 |
| (Propiconazole) | 1000 | 0.00e (0.71) | 100 |
| | 1500 | 0.00e (0.71) | 100 |
| | 2000 | 0.00e (0.71) | 100 |
| Rovral 50 WP | 500 | 0.00e (0.71) | 100 |
| (Iprodione) | 1000 | 0.00e(0.71) | 100 |
| | 1500 | 0.00e (0.71) | 100 |
| | 2000 | 0.00e (0.71) | 100 |
| Emivit 50 WP | 500 | 0.00e (0.71) | 100 |
| (Copper oxychloride) | 1000 | 0.00e (0.71) | 100 |
| | 1500 | 0.00e (0.71) | 100 |
| | 2000 | 0.00e (0.71) | 100 |
| Agrimyl | 500 | 32.75b (5.72) | 28.02 |
| (Mancozeb+Metalaxyl) | 1000 | 23.50c (4.85) | 48.35 |
| | 1500 | 22.75c (4.77) | 50.00 |
| | 2000 | 16.75d (4.09) | 63.19 |
| Control (plain water) | | 45.50a | - |
| CV (%) | | 3.05 | |

Number within the similar letters do not differ significantly at 5% level of significantly according to Duncan's Multiple Range Test DMRT; Figures within the parenthesis are square root transformed values

Table 5: Influence of anti-chemicals and antagonist on disease score and plant growth parameters of lentil

| Treatments | Disease score | Root length (cm) | Shoot length (cm) | Number of branches plant$^{-1}$ | Number of pod plant$^{-1}$ |
|---|---|---|---|---|---|
| Iprosun 50 WP | 1.00e | 9.48a | 44.6a | 9.250a | 39.10a |
| Edcuzeb 80 WP | 1.50de | 8.35b | 42.55b | 7.900b | 32.95b |
| Proud 25 EC | 1.75cd | 7.90bc | 40.55c | 6.750c | 29.60b |
| Rovral 50 WP | 2.25c | 7.05cd | 40.22cd | 6.100cd | 23.20c |
| Emivit 50 WP | 2.25c | 6.85d | 39.30cd | 5.250de | 19.90c |
| Agrimyl | 3.00b | 6.30d | 38.67d | 4.950ef | 19.60c |
| *T. harzianum* spore suspension | 3.00b | 6.00d | 36.43e | 4.000fg | 16.00d |
| Control | 4.75a | 4.44e | 36.15e | 3.30g | 12.75d |
| CV (%) | 17.62% | 9.59% | 2.74% | 11.86% | 9.99% |

Table 6: Influence of anti-chemicals and antagonist on yield attributes of lentil

| Treatments | Thousand grain weight (g) | Yield (kg ha$^{-1}$) |
|---|---|---|
| Iprosun 50 WP | 21.08a | 1271.00a |
| Edcuzeb 80 WP | 20.02a | 1108. 00b |
| Proud 25 EC | 17.77b | 1080. 00b |
| Rovral 50 WP | 16.38c | 1028. 00bc |
| Emivit 50 WP | 16.05c | 1018. 00bc |
| Agrimyl | 15.80c | 930.00cd |
| *T. harzianum* spore suspension | 15.15cd | 892.40d |
| Control | 14.23d | 666.20e |
| CV (%) | 4.58% | 6.74% |

Emivit, Agrimyl and *T. harzianum* had no significant different from each other but root length significantly increased over control plot (Table 5).

**Shoot length (cm):** Plot sprayed with Iprosun and Edcuzeb produced the highest shoot length 44.61, 42.55 cm, respectively and the lowest in control and antagonist treated plot, while shoot length in plots treated with Proud, Rovral and Emivit did not differ significantly from each other (Table 5).

**Number of branches plant$^{-1}$:** The number of branches plant$^{-1}$ varied significantly due to application of fungicides over control. The highest number of branches plant$^{-1}$ was recorded in plot sprayed with Iprosun (9.25) followed by Edcuzeb (7.90) and Proud (6.75) and the lowest in control (3.00) preceded by antagonist sprayed (4.00) plot (Table 5).

**Number of pod plant$^{-1}$:** The number of pods plant$^{-1}$ as influenced by the different fungicides application (Table 5). The maximum pods plant$^{-1}$ was obtained from the plot sprayed with Iprosun (39.10) followed by Edcuzeb (32.95) and Proud (29.60) both were statistically identical. The minimum pods plant$^{-1}$ was recorded from the plot treated with control (12.75) preceded by antagonist (16.00) and these are significantly not differed from each other. The comparatively moderate number pods plant$^{-1}$ was obtained from the Rovral, Emivit and Agrimyl treated plots and all theses were statistically similar.

**Thousand grain weight (g):** Thousand seeds weight also influenced by the application of fungicides and weight was increased over control (Table 6). The fungicides Iprosun and Edcuzeb influenced equally on the thousand grain weight and the highest 21.08, 20.02 g, respectively was recorded from them. The lowest seed weight in control treated plot and the remaining plots treated with other fungicides gave the statistically similar results.

**Yield (kg ha$^{-1}$):** Tremendous effect of fungicides was noticed on the crop yield of lentil and yield kg ha$^{-1}$ considerably increased compared to control treated plot (Table 6). The maximum lentil yield (1271.00 kg ha$^{-1}$) was obtained from the plot treated with Iprosun and minimum from the control plot while yield was 666.20 kg ha$^{-1}$. The applying with four fungicides of Edcuzeb, Proud, Rovral and Emivit gave the statistically same results.

## DISCUSSION

In the present investigation stemphylium blight of lentil caused by *Stemphylium botryosum* showed considerable yield loss due to appearing of remarkable symptoms on lentil plants. The pathogen of *Stemphylium botryosum* colony showed greenish color, irregular shape and velvety texture on PDA medium. The dimension of conidia 10.48×4.78 μm was observed. This finding is well supported by Hosen *et al*. (2009) who found that *Stemphylium botryosum* varied in their colony color, texture, margin, shape and also size of conidia on PDA medium while worked on four isolates (MIH -1 to MIH -4) and measured the conidia of *Stemphylium botryosum* as 13.33-16.04×6.46-9.17 μm.

*Stemphylium botryosum* was greatly influenced by physiological requirements such as temperature (°C) and pH. The pathogen grew well at a varied range of temperature and pH. The highest radial colony growth was found at 25°C followed by 20°C and the lowest at 5°C. Radial growth increases upto 25°C and decreases over 25°C. The findings agreed with Hosen *et al*. (2009) they found that optimum temperature for the radial mycelial growth was 25°C.

Incubation time and temperature had the significant role in conidia germination. The conidia germinated with a wide range of temperature (°C). After 4 h of incubation no germination was observed at temperature 5 and 10°C whereas little germination was recorded rest of temperatures. Short period (4 h) of incubation maximum (85.71) percentage of conidia germination was noted at 25°C followed by 30°C. Hundred percent of conidia germination was obtained from the temperature 25 and 30°C after 6 h of incubation. Mwakutuya (2006) was observed that the percentage of conidia germination increased with temperature and incubation period increased and noted that the maximum rate of germination were 30°C followed by 25°C after 20 h of incubation and the impact of the rate of conidia germination increased as temperature above 15°C and generally the lowest and fated response was at 5 and 20°C, respectively. From the

results it is clearly noted that suitable temperature for germination of conidia of *S. botryosum* between 25-30°C.

The luxuriant radial growth was noted at pH 6.0 followed by pH 6.5. The lowest radial growth was recorded at pH 4.5. It was appeared that the higher range of pH is required for the radial mycelial growth of *Stemphylium botryosum*. Huq (2003) reported that the best was observed in pH 6.0 followed by pH 7.0 but Rajani *et al*. (1991) found that optimum pH was 5.5 while working on *Stemphylium lycopersici*. Padhi and Synder (1954) reported that the optimum being pH 5.5 which gave the luxuriant mycelial dry weight and the maximum sporulation was noted recorded at pH 5.4.

From the *in vitro* test of fungicides radial mycelial growth inhibited significantly over the control. All the fungicides retarded radial colony growth of *Stemphylium botryosum* and no growth was observed at all except Agrimyl. The maximum growth was noted in control Petri-plates followed by Agrimyl at lower concentration (0 ppm). Huq (2003) reported that Rovral 50 WP was the most effective fungicides among the others and there was no growth was recorded at higher concentration (2000 ppm). Hosen *et al*. (2009) evaluated six fungicides and found that Rovral 50 WP from the iprodione group was the best fungicides in respect of reducing the radial colony growth of *S. botryosum* among the others at a lower concentration (500 ppm).

Management of plant diseases successfully achieved through application of chemical fungicides. All the tested fungicides reduced the disease score and noticeable increased plant growth parameters and yield of lentil in comparison to treated with control plot. The yield of lentil was enhanced sharply through the application of fungicides. The lowest disease score was achieved in plots sprayed with Iprosun 50WP followed by Edcuzeb 80WP and the highest in control plot preceded by *Trichoderma harzianum* treated plot. Root length, shoot length number of branches plant$^{-1}$ and numbers of pod plant$^{-1}$ were found maximum in treated with Iprosun 50 WP treated plots followed by Edcuzeb 80WP and Rovral 50 WP. The highest grain yield was recorded from the Iprosun treated plot followed by Edcuzeb treated plot and both were showed identical. The highest grain yield of lentil was recorded from the Iprosun treated plot and the lowest in the treated of with control plots. Bakr and Ahmed (1992) reported that disease score was lowest in plots treated with Rovral 50 WP @ 0.2% and it's indicating the highest disease reducing capability than rest of three fungicides and also found that plots sprayed with Rovral produced the highest seed yield followed by others while the lowest yield in control plots. Sardar (2005) reported that the lowest disease was obtained fro the Rovral 80WP with Tilt 250 EC treated plots. From the finding of others researcher Rovral 50WP was the most effective fungicides in reducing the disease score and increasing the yield of lentil but the present research work showed that Iprosan 50WP from the iprodione group was the most effective fungicides in controlling the disease severity and increasing the yield of lentil.

## REFERENCES

BBS, 2005. Statistical year book of Bangladesh for 2005. Ministry of Planning, Government of Peoples Republic of Bangladesh, Dhaka, Bangladesh.

Bakr, M.A. and F. Ahmed, 1992. Development of Stemphylium blight of lentil and its chemical control. Bangladesh J. Plant Pathol., 8: 39-40.

Bayaa, B. and W. Erskine, 1998. Diseases of Lentil. In: The Pathology of Food and Pasture Legumes, Allen, D.J. and J.M. Lenne (Eds.). CAB International, Wallingford, United Kingdom, pp: 786.

Erskine, W. and A. Sarker, 1997. Lentil: The Bangladesh breakthrough. ICARDA Caravan, 6: 8-10.

Hosen, M.I., A.U. Ahmed, J. Zaman, S. Ghosh and K.M.K. Hossain, 2009. Cultural and physiological variation between isolates of *Stemphylium botryosum* the causal of stemphylium blight disease of lentil (*Lens culinaris*). World J. Agric. Sci., 5: 94-98.

Huq, M.I., 2003. Epidemiology and management of stemphylium blight of lentil. Ph.D. Thesis, Department of Botany, University of Dhaka, Bangladesh.

Huq, M.I. and A.Z.M.N.A. Khan, 2007a. Effect of sowing dates on the incidence of *Stemphylium blight* of lentil during 1998-2001. Bangladesh J. Sci. Ind. Res., 42: 341-346.

Huq, M.I. and A.Z.M.N.A. Khan, 2007b. Efficacy *in-vivo* of different fungicides in controlling *Stemphylium blight* of lentil during 1998-2001. Bangladesh J. Sci. Ind. Res., 42: 89-96.

Huq, M.I. and A.Z.M.N.A. Khan, 2008. Epidemiology of *Stemphylium Blight* of lentil. Bangladesh J. Sci. Ind. Res., 43: 513-520.

Mwakutuya, E., 2006. Epidemiology of stemphylium blight of lentil (*Lens culinaris*) in Saskatchewan. M.Sc. Thesis, University of Saskatchewan, Saskatchewan.

Padhi, B. and W.C. Snyder, 1954. Stemphylium leaf spot of lettuce. Phytopathology, 44: 175-180.

Rajani, V.V., P.P. Rawel and R.R. Khandar, 1991. Cultural studies on *Stemphylium lycopercisi* causing leaf spot of tomato. Ind. J. Mycol. Pant. Pathol., 21: 34-42.

Saha, G.C., A. Sarker, W. Chen, G.J. Vandemark and F.J. Muehlbauer, 2010. Inheritance and linkage map positions of genes conferring resistance to *Stemphylium blight* in lentil. Crop Sci., 50: 1831-1839.

Sardar, M.M., 2005. Chemical control of *Stemphylium blight* disease of lentil. M.Sc. Thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh

Sarker, A., W. Erskine, M.A. Bakr, M.M. Rahman, M.A. Afzal and M.C. Saxena, 2004. Lentil Improvement in Bangladesh-A Success Story of Fruitful Partnership between the Bangladesh Agricultural Research Institute and International Center for Agricultural Research in the Dry Areas. APAARI Publication, Thailand, Bangkok.

Singh, B.P., S.P. Singh and A. Mohammad, 1990. Economic efficacy of different fungicides for the control of leaf spot of Cauliflower. Ind. Phytopathol., 43: 207-209.