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# Integrated Approach for the Management of New Threat *Stemphylium botryosum* walr Causing Blight of Lentil (*Lens culinaris* Medik)

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**Abstract**

Efforts were made to study integrated disease management for lentil stemphylium blight caused by *Stemphylium botryosum* Walr at GLRP, Rampur, Chitwan, Nepal during 2011/12 and 2012/13 using CRD in laboratory (in-vitro), RCBD in field (in-vivo) and screening host resistance genotypes. Over years, botanicals *Acorus calamus* L. and *Zanthozylum armatum* DC, fungicides Mancozeb and Krilaxyl and antagonist *Trichoderma viridae* were effective for disease control and yield increment. *Acorus calamus* L. at higher dose (8% W/V) and Krilaxyl even at lower (500 ppm) on potato dextrose agar (PDA) checked the pathogen growth completely in-vitro. On 5$^{th}$ day of incubation period, mycelium growth of the pathogen was collapsed by fungal antagonist *T. viridae*. The mycelial growth inhibition percent of *Z. armatum* DC (8% W/V) and Mancozeb (2000 ppm) on PDA was 31.17 and 55.94 respectively. In field, botanicals were sparingly effective for a short period. The Percent disease control (PDC) was higher in *A. calamaus* L. (46.60%) and *Z. armatum* DC (46.26%) compared to unsprayed plot. The higher percent yield increase (PYI) was obtained from Krilaxyl (71.22%) and Mancozeb (55.31%) and @ 2 gm/litre of water over control. The lower Percent Disease Index (PDI) was observed in Krilaxyl (36.00) and Mancozeb (37.35). The PDC and PYI were higher in *T. viridae* (PPD isolate) i.e. 42.14% and 58.80% respectively. Out of 58 genotypes, in screening nursery, RL-28, ILL 10134, RL-44, FLIP 2008-7L, NR-2001-71-3, NR-2001-71-4, ILL 7657, ILL 2437, ILL 7349, RL 23, RL 25, RL 47, RL 62, ILL 10856 were found resistant to the disease.

**Key words:** Lentil, *Stemphylium botryosum*, botanicals, fungicides, antagonist, resistant genotypes

## Introduction

Lentil (*Lens culinaris* Medik) is the most important and highly commercialized pulse among the grain legumes in terms of both area (206522 ha), production (226931 mt) and productivity (1099 kg/ha) which shares almost 62% of total area and 65% of total production of pulses and rates the highest consumer preference in Nepal (MOAD, 2013). Lentil is one of the oldest cultivated crops and has been a major food source of many civilizations for more than 8000 years (Oplinger et al. 1990). It is rich in protein and carbohydrate, and crop residues are used as animal feed. In the developing world it is often referred to as “poor man’s meat” because of its high protein content and easy accessibility by the lower economic class. Like many other pulses, it is rich in cholesterol-lowering soluble fiber and high in folate, a valuable functional food in the human diet (McVicar et al. 2005). The national lentil yield level, at present, is far below than potential yield. There are various biotic and abiotic yield limiting factors in lentil of which diseases and poor crop management are important ones. Lentil plants are affected by wide range of pathogen with fungal diseases being the most important. Among the diseases, Stemphylium blight caused by *Stemphylium botryosum* Walr is becoming a serious threat to lentil cultivation. Stemphylium blight disease of lentil has been reported in Bangladesh, Egypt, Syria and the USA (Bayaa and Erksine, 1998). It was first reported during 1993 in Nepal. This disease has become widespread throughout major lentil growing areas of the country (Bayaa et al. 1998). *Stemphylium botryosum* causes leaf blight on lentil that can result in large scale defoliation of plants. Preliminary studies in Bangladesh and India estimated yield losses of 62% and total crop failure

have been reported in some cases where the disease defoliated the crop in the early pod setting stage (Bakr, 1991, Erksine and Sarker, 1997). In recent years, *Stemphylium* has been observed increasingly in lentil fields in Banke, Bardia, Rupandehi, Chitwan, Nepalgunj, Makwanpur, Bara, Parsa and Rautahat districts of Nepal (Joshi, 2006). In view of the above facts, the present research work was undertaken with the objective of evaluation of botanicals, fungicides and bio-control agents both under *in-vitro* and *in-vivo* condition and screening of host resistance genotypes to manage the disease.

## Materials and Methods

### Experimental site

The experiment was carried out at laboratory and field of National Grain Legume Research Program, Rampur, Chitwan. The temperature, moisture, relative humidity and rainfall of the site were measured during experiment period.

### Lentil variety

A Stemphylium blight susceptible variety Shital was used for the experiment.

### Pathogen

Isolate of *Stemphylium botryosum* Walr (7 day old PDA) culture maintained in laboratory condition isolated from diseased sample of lentil under asceptic condition at PPD, Khumaltar, Kathmandu. Some plates with the pathogen were maintained in the laboratory for judging the climatic factor effects and rest for the management techniques both in vitro and in vivo.

### Botanicals

Five different botanicals were selected for the evaluation. They were Timur (*Xanthoxylum armatum* Dc.), Neem (*Azadirachta indica* A.Juss), Bojho (*Acorus calamus* L.), Titepati (*Artimisia indica* Willd.) and Van Phanda Kanda (*Lantana camera* L.)

### Fungicides

Five different fungicides were selected for the evaluation. They were SAAF (Carbendazim 12%+ mancozeb 63% WP), Krilaxyl (Metalaxyl 8%+ Mancozeb 64% WP), Bavistin (Carbendazim 50% WP), Blitox-50 (Copper oxy chloride 50% WP), and Dithane M-45 (Mancozeb 75% WP)

### Biological control agents

Five different commercially available and laboratory isolated biological control agents were selected for the evaluation. They were *Trichoderma viridae* (PPD Isolate), *Trichoderma viridae* (commercial product), *Trichoderma harzianum* (PPD isolate), *Trichoderma harzianum* (commercial product) and *T. Koningii* (PPD isolate) .Commercially available biological control agents' formulation was cultured in selective media.

### Potato dextrose agar (PDA)

Commercially available Potato Dextrose Agar (HIMEDIA, REF MO96-500G) was used to conduct In Vitro experiment at laboratory. 39 gm of PDA was weighed and suspended in 1 litre of distilled water, heated to boiling to dissolve the medium completely and sterilized in autoclave at 15 psi for 15 minutes at $121^0$C in the laboratory of NGLRP Rampur, Chitwan. The standard formula of the product was [Potatoes infusion from 200gm/lt, Dextrose 20gm/lt , Agar 15 gm/lt and final pH (at $25^0$C) $5.6 \pm 0.2$ ].

### In-vitro test

Five different botanicals, fungicides and bio-control agents were evaluated on the growth of *S. botryosum* Walr at different concentrations by poisoned food technique in the laboratory condition. The concentration was maintained to 2%, 4%, 6% and 8% W/V in case of botanicals while 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm for fungicides. Different quantities of tested botanicals and fungicides were added to flask separately containing sterile molten PDA medium before its solidification to achieve the proposed concentrations, then rotate gently for 2 minutes to ensure equal distribution of the concentration and poured into sterilized petridishes (9cm diameter) @ 20 ml / plate. Strepto-penicillin (Bistrepen-V) (250 mg per litre) was added to the medium at the time of pouring to prevent bacterial contamination. The plates were inoculated and incubated with three replications in incubator. Three plates was considered as one experimental unit and replicated 3 times. Each experiment was carried out in completely randomized design (CRD). The diameter of the colony was measured and percent of mycelial growth inhibition was calculated in different incubation dates. The antagonist effect of bio-control agents were evaluated against *S. botryosum* Walr in dual culture technique. Four millimetre diameter of *Stemphylium botryosum* Walr of one week old culture and above stated bio control agents were cut by cork borer and picked up with the help of inoculating needle and placed apart on solidified PDA medium in an equal distance comprising 3 replications and incubated as stated earlier. Observations were recorded by measuring the

average radial growth of *S. botryosum* on 1, 2, 3 and 4th days of incubation.

**Inoculation**

Four millimetre diameter of *S botryosum* Walr of one week old culture was cut by cork borer and picked up with the help of inoculating needle and placed onto the centre of the plate. The plate contained PDA amended plant extract and the cut piece was kept upside down for better contact of pathogen to the media. The plates were incubated at incubator at $30^0$C for up to 25 days.

**Observation**

The colony diameter (cm) of the pathogen was determined by measuring the average radial growth on different incubation dates. Average radial growth was recorded by using a measuring scale from the lower view of the petri-plates. Mycelial growth inhibition percent was calculated by following formula.

Mycelial growth inhibition (%) = [(dc-dt) / dc] × 100

Where dc = average diameter of fungal colony in the control

dt = average diameter of fungal colony in the treatment group

**In-vivo test (Experiment under field condition)**

The three different experiments to test the botanicals, fungicides and bio-control agents were conducted under natural epiphytotic condition and laid out in a piece of land following RCB design with four replications (Zaman et al. 1982) at GLRP, Rampur, Chitwan, Nepal during 2012/13. The unit plot size was 4m x 1.5m with 25 cm row to row spacing. A susceptible genotype Shital was sown in November 23 for all 3 experiments. In case of botanicals, 5 above mentioned botanicals with concentration (8% W/V) and one control comprised six treatments. Similarly for fungicides also, 5 fungicides @ 2gm/lt of water and one control comprised six treatments. Experimental design for bio-control agents comprised treatments as *Trichoderma viride*a (PPD isolate), *Trichoderma harzianum* (PPD isolate) and *T. Koningii* (PPD isolate) were applied @ 150gm /6 $m^2$ plot soil application mixing with rice husk during field preparation. While *Trichoderma viride*a (Nicoderma 1% WP - 2× $10^6$ CFU/g), *Trichoderma harzianum*-Dextrose base (CFU - $10^9$/g) (commercial preparation) were applied @ 0.6 g per litre of water starting from vegetative stage subsequent three sprays was done at 10-15 days interval. All experiments were kept under constant observation. First spray was given just after the appearance of disease symptom in the field. Three sprays were given at an interval of 15 days. Data was recorded before every spray using 1-9 scoring scale from 25 randomly tagged plants /plot. Similarly PDI was computed according to the formula and calculation was based on the final data record at 15 days after the last spray. Percent Disease Control (PDC) was calculated on the basis of the formula developed by Shivankar and Wangikar, 1993.

**Observation**

The disease data was recorded from 25 randomly tagged plants/plot on the basis of 1-9 scoring scale (Morrall and Mckenzie, 1974).

1= No lesion visible (Highly resistant)
3= Few scattered lesions, usually visible after careful searching (Resistant)
5= Lesions common on plants and easily observed but defoliation and/ or damage not great, or in only one or two patches in plot (Moderately resistant)
7= Lesions very common and damaging (Susceptible)
9= Lesions extensive on all plants, defoliation and drying branches, and killing of some plants (Highly susceptible).

Percent Disease Index (PDI) was computed on the basis of recorded data according to the formula (Wheeler, 1969)

Early Plant Stand (EPS) and Final Plant Stand (FPS) were recorded by the scale developed by International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

1- 90% or more = very good
2- 80-89% = good
3- 70-79% = acceptable
4- 60-69% = poor
5- Less than 60% = very poor

Data was recorded on yield and yield attributes after necessary sun drying. Yield increase over the control was calculated. The temperature, moisture, relative humidity and rainfall were measured during experiment period.

**Screening of lentil genotypes for *Stemphylium* blight resistance**

The experiment was conducted during winter season of 2012/13 in rod row design. The plot size was 2m long two rows for each genotype. The total

lentil genotypes were 58. Susceptible and resistant checks were repeated after every 12 test genotypes. The early plant stand, disease score, final plant stand and yield data were recorded from randomly tagged plants of plot as described earlier.

**Data analysis**

All data were analyzed statistically using MSTAT-C computer package program. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at 1 and 5% levels of significance. The correlation among percent yield increased over control and percent disease control was calculated.

**Results**

**1. In-vitro test**

**1.1 Effect of botanicals on mycelial growth inhibition percent of *S. botryosum***

Botanical extracts except *Artimisia indica* and *Azdirachta indica* at a lowest dose also inhibit the mycelial growth. The botanical *Acorus calamus* at highest dose was able completely to check the growth of pathogen (table 1). *Zanthoxylum armatum,* and *Lantana camera* showed better result with the increase in concentration. But in case of *Artimisia indica* and *Azdirachta indica* as the concentration increased, the growth of the *S. botryosum* was also increased (Figure 4). The mycelial growth inhibition percent of *Zanthoxylum armatum,* and *Lantana camera* @8% on PDA was 31.17 and 24.56 respectively (table 2).

**Table 1:** Effect of different botanicals incorporated PDA on the growth of *S. botryosum* in 20th day of incubation period at 30 $^0$C temperature on incubator during 2011/12

| Treatments | Mean colony diameter (cm) | | | | |
|---|---|---|---|---|---|
| | ***Zanthoxylum armatum*** | ***Artimisia indica*** | ***Azadirachta indica*** | ***Acorus calamus*** | ***Lantana camera*** |
| **2% on PDA** | 7.67 $^{b\dagger}$ | 7.57 $^{c}$ | 8.37 $^{bc}$ | 4.00 $^{b}$ | 7.00 $^{bc}$ |
| **4% on PDA** | 7.33 $^{b}$ | 8.50 $^{ab}$ | 8.47 $^{ab}$ | 2.00 $^{c}$ | 7.23 $^{bc}$ |
| **6% on PDA** | 6.67 $^{c}$ | 8.07 $^{abc}$ | 8.67 $^{ab}$ | 0.40 $^{d}$ | 7.33 $^{b}$ |
| **8% on PDA** | 5.83 $^{d}$ | 8.67 $^{a}$ | 8.93 $^{a}$ | 0.40 $^{d}$ | 6.80 $^{c}$ |
| **Control** | 8.47 $^{a}$ | 7.87 $^{bc}$ | 7.87 $^{c}$ | 8.50 $^{a}$ | 8.47 $^{a}$ |
| **F-Test** | ** | * | * | ** | ** |
| **LSD (≤0.01)** | 0.66 | 0.75 | 0.55 | 0.12 | 0.51 |
| **CV%** | 5.08 | 5.05 | 3.61 | 1.46 | 3.79 |

$^{\dagger}$ Means of 3 replication. Means in column with same superscript is not significantly different by LSD $_{(P<0.01)}$.
PDA- Potato Dextrose Agar

**Table 2:** Percent inhibition of *S. botryosum* by different plant extracts incorporated PDA over control at 30$^0$C temperature on incubator during 2011/12

| Treatments | Mycelial growth inhibition percent | | |
|---|---|---|---|
| | ***Z. armatum*** | ***A. calamus*** | ***L. camera*** |
| **2% on PDA** | 9.44 | 52.94 | 17.35 |
| **4% on PDA** | 13.46 | 76.47 | 14.64 |
| **6% on PDA** | 21.25 | 95.29 | 13.46 |
| **8% on PDA** | 31.17 | 95.29 | 24.56 |
| **Control** | - | - | - |

Z – *Zanthozylum*, A-*Acorus*, L-*Lantana*

**1.2 Effect of fungicides on mycelial growth inhibition percent**

All fungicides even at a lowest dose also inhibited radial mycelial growth of the pathogen significantly over the control at different concentrations and shown in Table 3. Krilaxyl and Blitox-50 were able completely to check the growth of pathogen even in the lowest dose (500 ppm) while SAAF, Mancozeb and Bavistin showed better result with the increase of concentration (Figure 1). The mycelial growth inhibition percent of SAAF, Mancozeb and Bavistin @2000 ppm were 68.79, 55.94 and 47.18 respectively.

### 1.3 Effect of bio-control agents on mycelial growth inhibition percent

The mean colony diameter of the antagonist is significantly higher than the pathogen *S. botryosum* Walr in each day of incubation period at $30^0$C temperature on incubator. On $5^{th}$ day of incubation period, all fungal antagonists growing over *S. botryosum* with *S. botryosum* mycelium collapsed except T. viridae (commercial) and *T. Koningii* (PPD isolate) (Table 4,5).

**Table 3:** Effect of different fungicides incorporated PDA on the growth of *S. botryosum* in $17^{th}$ day of incubation period at 30 $^0$C temperature on incubator during 2011/12

| Treatments | Mean colony diameter (cm) | | | | |
|---|---|---|---|---|---|
| | **SAAF** | **Krilaxyl** | **Bavistin** | **Blitox-50** | **Mancozeb** |
| **500 ppm on PDA** | 4.57 $^{b\dagger}$ | 0.40 $^{b}$ | 5.40 $^{b}$ | 1.27 $^{b}$ | 5.17 $^{c}$ |
| **1000 ppm on PDA** | 3.33 $^{c}$ | 0.40 $^{b}$ | 4.40 $^{b}$ | 0.40 $^{c}$ | 6.40 $^{b}$ |
| **1500 ppm on PDA** | 3.47 $^{c}$ | 0.40 $^{b}$ | 4.33 $^{b}$ | 0.40 $^{c}$ | 3.83 $^{d}$ |
| **2000 ppm on PDA** | 2.60 $^{c}$ | 0.40 $^{b}$ | 4.40 $^{b}$ | 0.40 $^{c}$ | 3.67 $^{d}$ |
| **Control** | 8.33 $^{a}$ | 8.33 $^{a}$ | 8.33 $^{a}$ | 8.50 $^{a}$ | 8.33 $^{a}$ |
| **F-Test** | ** | ** | ** | ** | ** |
| **LSD (≤0.01)** | 0.87 | 0.34 | 1.17 | 0.48 | 1.10 |
| **CV%** | 7.53 | 6.50 | 8.43 | 8.49 | 7.76 |

$^{\dagger}$ Means of 3 replication. Means in column with same superscript is not significantly different by LSD $_{(P<0.01)}$.
PDA- Potato Dextrose Agar, ppm- parts per million

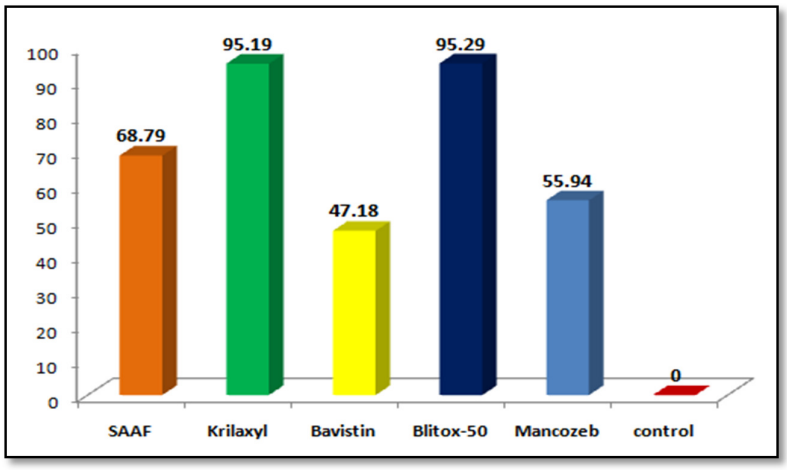


Figure 1. Antifungal activity against Mycelial Growth Inhibition (MGI) Percent of *Stemphylium botryosum* at 2000 ppm on PDA over control incubated for $30^0$C during 2011/12.

**Table 4:** Effect of bio-control agents on the growth of *S. botryosum* in different incubation period at 30 $^0$C temperature on incubator

| Bio-control agents | Mean colony diameter (cm) on PDA | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2nd day | | 3rd day | | 4th day | | 5th day | |
| | Bca | *St. b* | Bca | *St. b* | Bca | *St. b* | Bca | *St. b* |
| ***T. viridae* (PPD isolate)** | 5.00$^{b\dagger}$ | 0.50$^{b}$ | 7.63$^{a}$ | 0.50$^{d}$ | 8.46$^{ab}$ | 0.50$^{c}$ | 9.00$^{a}$ | 0.50$^{c}$ |
| ***T. viridae* (commercial)** | 3.70$^{c}$ | 0.83$^{a}$ | 5.40$^{b}$ | 1.00$^{a}$ | 6.53$^{c}$ | 1.00$^{a}$ | 7.60$^{b}$ | 1.00$^{a}$ |
| ***T. harzianum* (PPD isolate)** | 5.06$^{b}$ | 0.73$^{a}$ | 7.60$^{a}$ | 0.73$^{bc}$ | 8.53$^{a}$ | 0.73$^{b}$ | 9.00$^{a}$ | 0.73$^{b}$ |
| ***T. harzianum* (commercial)** | 5.53$^{a}$ | 0.66$^{ab}$ | 7.50$^{a}$ | 0.66$^{cd}$ | 8.37$^{b}$ | 0.66$^{bc}$ | 9.00$^{a}$ | 0.66$^{bc}$ |
| ***T. Koningii* (PPD isolate)** | 2.5$^{d}$ | 0.50$^{b}$ | 4.40$^{c}$ | 0.93$^{ab}$ | 5.07$^{d}$ | 1.00$^{a}$ | 5.97$^{c}$ | 1.20$^{a}$ |
| **F-Test** | ** | ** | ** | ** | ** | ** | ** | ** |
| **LSD (≤ 0.01)** | 0.18 | 0.20 | 0.22 | 0.20 | 0.14 | 0.22 | 0.14 | 0.22 |
| **CV%** | 1.67 | 11.98 | 1.25 | 10.10 | 0.78 | 10.98 | 0.64 | 10.44 |

$^{\dagger}$ Means of 3 replication. Means in column with same superscript is not significantly different by LSD (P<0.01). Bca – Bio-control agents, *St. b* – *Stemphylium botryosum* , PPD (Plant Pathology Division, NARC), T. - *Trichoderma*

**Table 5:** Antagonistic activity against radial mycelial growth and percent inhibition of *Stemphylium botryosum* at incubator (30$^0$C) during 2011/12

| Bio-control agents | MCD (cm) of *St. b* on PDA (5th Day) | MGI % |
|---|---|---|
| ***T. viridae* (PPD isolate)** | 0.50$^{\dagger}$ | 80 |
| ***T. viridae* (commercial)** | 1.00 | 60 |
| ***T. harzianum* (PPD isolate)** | 0.73 | 71 |
| ***T. harzianum* (commercial)** | 0.66 | 74 |
| ***T. Koningii* (PPD isolate)** | 1.20 | 52 |
| **Control (only PDA)** | 2.50 | - |

$^{\dagger}$ Means of 3 replication. *St. b* – *Stemphylium botryosum*, MCD- Mean Colony Diameter, MGI- Mycelial Growth Inhibition, PPD- Plant pathology Division, PDA- Potato Dextrose Agar T- *Trichoderma*

**2. In-vivo test**

**2.1 Efficacy test of botanicals, fungicides and bio-control agents against Stemphylium blight of lentil**

**Botanicals**

All botanicals had significant effect (P≤0.05) on Percent Disease Index (PDI), Grain Yield and Hundred Seed Weight (HSWT) over control. The Percent Disease Control (PDC) was higher in *Acorus calamus* (46.60) and *Xanthoxylum armatum* (46.26) compared to control plot. The higher percent Yield increase was obtained from *Acorus calamus* treated plot (91.50) over control (Table 6).

**Table 6:** Effect of botanicals on disease severity and yield performance of lentil at Rampur, Chitwan during 2012/13

| Treatments Water extract (8 % conc$^n$) | EPS (%) | DS (1-9 scale) | PDI | FPS (%) | YIELD (kg/ha) | HSWT (gm) | PDC % | % YI |
|---|---|---|---|---|---|---|---|---|
| *Xanthoxylum armatum* (Timur) | 85.00$^{\dagger}$ | 4.00$^{de}$ | 35.55$^{d}$ | 79.00$^{ab}$ | 972.78 $^{a}$ | 1.77 $^{a}$ | 46.26 | 88.67 |
| *Artimisia indica* (Titepati) | 87.50 | 6.00$^{bc}$ | 49.28$^{bc}$ | 82.00 $^{a}$ | 680.53 $^{b}$ | 1.54 $^{a}$ | 25.50 | 32.00 |
| *Azadirachta indica* (Neem) | 83.75 | 6.50$^{ab}$ | 53.78$^{b}$ | 77.50$^{b}$ | 668.00 $^{b}$ | 1.54 $^{a}$ | 18.70 | 29.60 |
| *Acorus calamus* (Bojho) | 86.25 | 3.00$^{e}$ | 35.33$^{d}$ | 82.00 $^{a}$ | 987.39 $^{a}$ | 1.88 $^{a}$ | 46.60 | 91.50 |
| *Lantana camera* (Vanphanda ) | 86.25 | 5.00$^{cd}$ | 42.53$^{c}$ | 81.00 $^{ab}$ | 697.23 $^{b}$ | 1.64 $^{a}$ | 35.71 | 35.22 |
| Control | 78.75 | 7.50$^{a}$ | 66.15$^{a}$ | 71.00 $^{c}$ | 515.62 $^{c}$ | 1.43$^{a}$ | - | - |
| F-Test | NS | ** | ** | ** | ** | * | | |
| LSD (≤0.05) | | 1.054 | 6.78 | 3.83 | 142.69 | 0.05 | | |
| CV% | 5.32 | 13.11 | 9.55 | 3.23 | 12.56 | 2.22 | | |

$^{\dagger}$ Means of 4 replication. Means in column with same superscript is not significantly different by DMRT (P<0.05). EPS – Early Plant Stand per plot, DS – Disease severity, PDI- Percent Disease Index (25 plants), FPS – Final Plant Stand/plot, YIELD- Grain yield, HSWT- Hundred Seed Weight, PDC- Percent Disease Control, YI- Yield Increase, NS- Not Significant, ** - Highly significant, Conc$^n$ - Concentration

**Relationship between PDC and yield increase over the control**

In 2012-2013 the higher PDC and corresponding yield was given by *Acorus calamus* (8% W/V) followed by *Xanthoxylum armatum* (8% W/V). A positive linear correlation between PDC and PYI was observed during 2012/13. Equation Y = 1.871X -7.736 and $R^2$ = 0.857 gave the best fit.

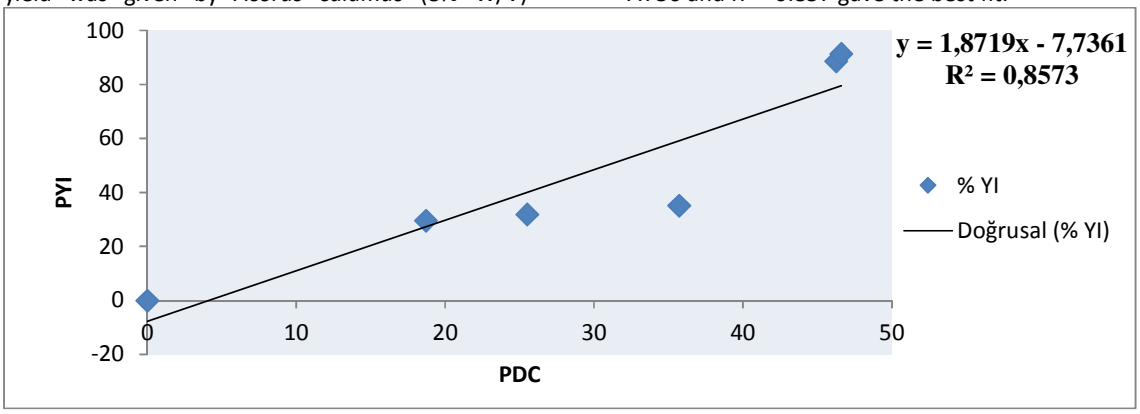


Figure 2: Relationship between PDC and PYI of botanicals used in Stemphylium blight management experiment at Rampur, Chitwan during 2012/13

**Fungicides**

All fungicides had effect over the control in reducing the disease during 2012/13. Among fungicides tested Krilaxyl followed by Mancozeb with the concentration of 2 gm/lt of water found most effective and differed significantly from the other fungicides tested. The lower PDI was obtained from the plot treated with Krilaxyl (36.00%) and Mancozeb (37.35%) compared to control (72.00%). The higher crop yield was recorded from the plot treated with Krilaxyl (1008.00 Kg/ha) followed by Mancozeb (914.30 Kg/ha) and SAAF (853.80 Kg/ha). The Percent Disease Control (PDC) was higher in krilaxyl treated plot (50.00%) followed by Mancozeb (48.13 %) when compare to control. The higher Percent yield increase was obtained from Krilaxyl treated plot (71.22%) followed by Mancozeb (55.31%) over control (Table 7).

**Table 7:** Influence of fungicides on disease severity and yield performance of lentil at Rampur, Chitwan during 2012/13

| Treatments (Conc$^n$ :2 gm/lt) | EPS (%) | DS (1-9scale) | PDI | FPS (%) | YIELD (kg/ha) | HSWT (gm) | PDC % | % YI |
|---|---|---|---|---|---|---|---|---|
| SAAF | 87.50 † | 4.00 $^{bcd}$ | 51.97 $^{c}$ | 81.25 $^{ab}$ | 853.80 $^{c}$ | 1.74 $^{a}$ | 27.82 | 45.03 |
| Krilaxyl | 90.00 | 3.00 $^{d}$ | 36.00 $^{d}$ | 85.00 $^{a}$ | 1008.00 $^{a}$ | 1.75$^{a}$ | 50.00 | 71.22 |
| Bavistin | 87.50 | 6.00 $^{b}$ | 57.15 $^{b}$ | 80.00 $^{ab}$ | 822.50 $^{d}$ | 1.56 $^{c}$ | 20.63 | 39.71 |
| Blitox-50 | 87.50 | 5.50 $^{bc}$ | 51.53 $^{c}$ | 78.75 $^{b}$ | 805.80 $^{d}$ | 1.64 $^{bc}$ | 28.43 | 36.88 |
| Mancozeb | 87.50 | 3.50 $^{cd}$ | 37.35 $^{d}$ | 81.25 $^{ab}$ | 914.30 $^{b}$ | 1.73 $^{ab}$ | 48.13 | 55.31 |
| Control | 81.25 | 8.00 $^{a}$ | 72.00 $^{a}$ | 70.00 $^{c}$ | 588.70 $^{e}$ | 1.24 $^{d}$ | - | - |
| **F-Test** | NS | * | ** | * | ** | ** | | |
| **LSD (≤0.05)** | 6.44 | 1.93 | 2.66 | 5.07 | 19.81 | 0.09 | | |
| **CV%** | 4.92 | 25.65 | 3.46 | 4.24 | 1.58 | 3.77 | | |

† Means of 4 replication. Means in column with same superscript is not significantly different by DMRT (P<0.05). EPS – Early Plant Stand per plot, DS – Disease severity, PDI- Percent Disease Index, FPS – Final Plant Stand/plot, YIELD- Grain yield, HSWT- Hundred Seed Weight, PDC- Percent Disease Control, YI- Yield Increase, NS-Not significant, *- Significant, ** - Highly significant .

During 2012/13, the higher yield increase with corresponding PDC was recorded from Krilaxyl followed by Mancozeb with the concentration of 2 gm/lt of water. The yield obtained was correlated positively with PDC which was linear and could be

shown by the equation y = 1.224x + 5.645 and coefficient of regression $R^2$ = 0.909 (Figure 3)

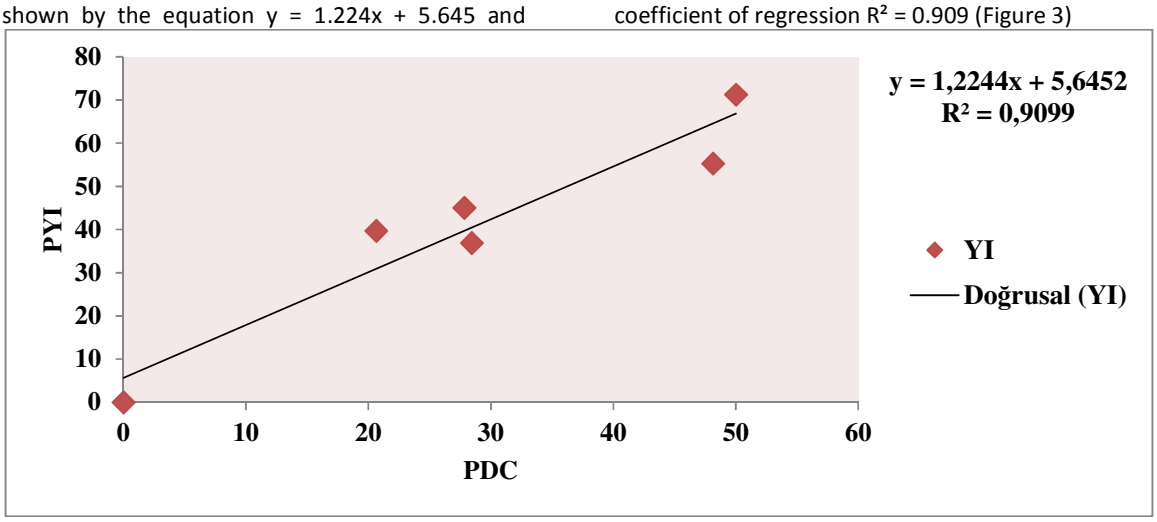


Figure 3: Relationship between PDC and PYI of fungicides used in Stemphylium blight management experiment at Rampur, Chitwan during 2012/13

**Bio-control agents**

The Percent Disease Control (PDC) was higher in *T. viridae* (PPD isolate) treated plot (42.14%) followed by *T. harzianum* (PPD isolate) (38.04%) when compared to control. The higher Percent yield increase was obtained from *T. viridae* (PPD isolate) treated plot (58.80 %) followed by *T. viridae* (Commercial) (54.82%) and T. harzianum (PPD isolate) (47.18%) over control (Table 8).

Table 8. Table 8: Influence of bio-control agents on disease severity and yield performance of lentil at Rampur, Chitwan during 2012/13

| Treatments | EPS (%) | DS (1-e) | PDI | FPS (%) | YIELD (kg/ha) | HSWT (gm) | PDC % | % YI |
|---|---|---|---|---|---|---|---|---|
| *T. harzianum* (PPD Isolate) | 88.75 † | 4.50 c | 44.33 c | 78.75 a | 924.76 bc | 1.64 ab | 38.04 | 47.18 |
| *T. viridae* (PPD isolate) | 88.75 | 3.50 c | 41.40 c | 81.25 a | 997.83 a | 1.71 a | 42.14 | 58.80 |
| *T. viridae* (Commercial) | 90.50 | 4.50 c | 45.23 c | 82.50 a | 972.78 ab | 1.68 a | 36.79 | 54.82 |
| *T. harzianum* (Commercial) | 85.00 | 6.50 b | 56.48 b | 78.75 a | 862.14 c | 1.59 b | 21.06 | 37.21 |
| *T. konengii* (PPD Isolate) | 87.50 | 6.00 b | 53.55 b | 80.00 a | 878.84 c | 1.60 b | 25.16 | 39.87 |
| Control | 81.25 | 8.00 a | 71.55 a | 67.50 b | 628.34 d | 1.41 c | - | - |
| F-Test | NS | ** | ** | ** | ** | ** | | |
| LSD (≤0.05) | | 1.46 | 5.78 | 3.79 | 63.19 | 0.06 | | |
| CV% | 5.27 | 17.57 | 7.36 | 3.22 | 4.78 | 2.45 | | |

Means of 4 replication. Means in column with same superscript is not significantly different by DMRT (P<0.05). T. - *Trichoderma* EPS – Early Plant Stand per plot, DS – Disease severity, PDI- Percent Disease Index, FPS – Final Plant Stand/plot, YIELD- Grain yield, HSWT- Hundred Seed Weight, PDC- Percent Disease Control, YI- Yield Increase, NS- Not Significant, ** - Highly significant .

In 2012/13 the higher PDC and corresponding yield was given by *T. viridae* (PPD isolate). The percent yield increase showed positive correlation with PDC which was linear and exhibited the equation Y = 1.322X + 3.672 and regression of coefficient $R^2$ = 0.952 gave the best fit (Figure 4).

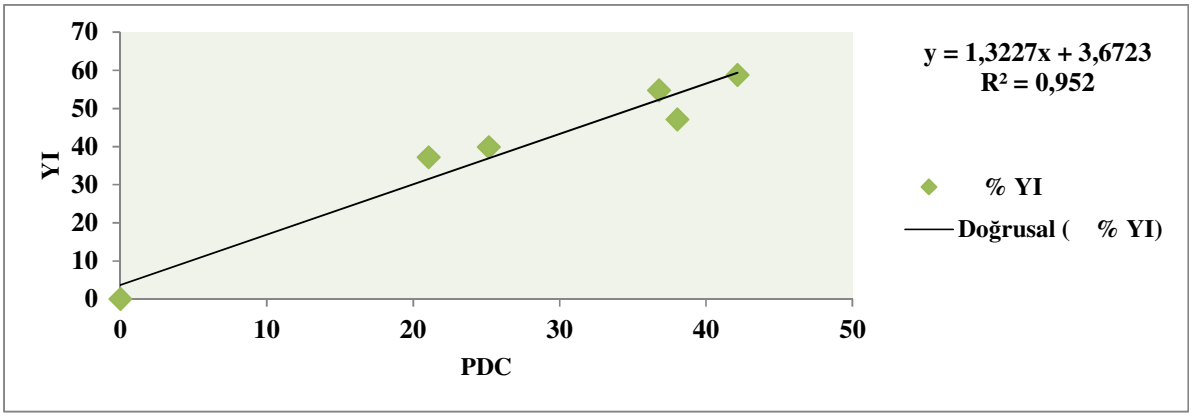


**Figure 4:** Relationship between PDC and PYI of bio-control agents used in Stemphylium blight management experiment at Rampur, Chitwan during 2012/13

**Stemphylium blight screening Nursery 2012/13**

Crop season of 2012/13 was affable for Stemphylium blight disease development. Disease severity was very high in Rampur condition. In case of Rampur, out of 58 genotypes including M. Bharati and Sagun as a check, FLIP2008-7L(3000 kg/ha), NR-2001-71-4(2134 kg/ha), RL-28(1864 kg/ha), RL-62(1823 kg/ha), ILL7657(1785 kg/ha), NR-2001-71-3(1764 kg/ha) and ILL 10856(1760 kg/ha), ILL 10134 (1561 kg/ha), RL-44 (1518 Kg/ha), ILL 7349 (1516 kg/ha) RL 25 (1510 kg/ha), RL 47 (1507 kg/ha) ILL 2437 (1450 Kg/ha) and RL 23 (1003 Kg/ha) (Table 9)were resistant to the disease.

**Discussion**

Phongpaichit et al. (2005) investigated that an antimicrobial guided one of methanol extract of *A. calamus* containing β-asarone as a major component and found that the β-asarone fraction showed high antifungal activity against *M. gypseum, T. rubrum* and *P. marneffei* and had moderate activity against *C. albicans* and *C. neoformans.* Findings of current study is in agreement with the report of Deena and Thoppil (2000) described the essential oil of *Lantana camera* containing ß-caryophyllene, geranyl acetate, terpinyl acetate, bornyl acetate and limonene remarkably inhibited the growth of many tested bacteria and fungi. P.aeruginosa, A.niger, F.solani, C.albicans appeared as the most sensitive ones. Fungicides Krilaxyl and Mancozeb were able completely to check the growth of pathogen even in the lowest dose while SAAF, Blitox-50 and Bavistin showed better result with the increase of concentration. This result hence is in full agreement with the report of Hosen et al. (2009) who investigated that all the fungicides tested gave significant reduction in mycelia growth over control. Among the fungicides Rovral 50WP (Iprodione) was found to be the most effective fungicide to retard the mycelia growth of *Stemphylium botryosum*. The higher Percent yield increase was obtained from Krilaxyl and Mancozeb treated plot. This result is in agreement with the report of Huq and Khan, (2007) who did an experiment with seven different fungicides Rovral 50WP @ 0.2 % was noted as the most effective fungicide followed by Dithane M-45 @ 0.2 % and Tilt 250EC @ 0.05 %. Gharti et al. (2008) also found that 2-3 sprays of mancozeb 75 WP @ 2.5 g /l or carbendazim 50 WP @ 2g/l also have been found effective to control Stemphylium blight. The natural control of several bio-control microorganisms belonging to *Trichoderma*, *Pseudomonas* and *Bacillus* genera are detected (Weller et al. 2002, Guo et al. 2004 and Huang et al. 2005). Antagonists *T. viridae* and *T. harzianum* (both PPD isolate and Commercial preparation) are effective to suppress the *S. botryosum* on incubator at $30^0$C temperature. This result is agrees with the findings of (Hosen, 2011) who found that *T. harzianum* revealed to be an effective antagonist against *B. cinerea* and *S. botryosum* in dual culture technique. The growth of the antagonist become dark green and could not be re-isolated from any part of the over grown petriplates. *Trichoderma* Spp has proved to be useful in the control of phytopathogens affecting different crops (Benitez et al. 2004 and Soytong et al. 2005). The mechanism of Trichoderma and Bacillus action on pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Cal et al. 2004)

**Table: 9** Stemphylium blight severity in lentil genotypes tested in Stemphylium blight screening nursery at Rampur during 2012/13.

| EN | Cultivars | EPS | DF | DM | PHT | P/P | S/P | GY (gm/plot) | GY (kg/ha) | HSWT (g) | STB (1-9 scale) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ILL-6260 | 1.00 | 82.00 | 122.00 | 35.00 | 48.00 | 1.40 | 132.70 | 1327.00 | 1.70 | 5.00 |
| 2 | RL-38 | 2.00 | 62.00 | 108.00 | 29.00 | 26.00 | 1.50 | 125.50 | 1255.00 | 2.40 | 5.00 |
| **3** | **RL-28** | **2.00** | **63.00** | **108.00** | **37.00** | **19.00** | **1.60** | **186.40** | **1864.00** | **2.80** | **3.00** |
| **4** | **ILL10134** | **2.00** | **81.00** | **133.00** | **42.00** | **103.00** | **1.80** | **156.10** | **1561.00** | **2.20** | **3.00** |
| **5** | **RL-44** | **2.00** | **69.00** | **111.00** | **33.00** | **37.00** | **1.80** | **151.80** | **1518.00** | **2.10** | **3.00** |
| 6 | ILL-10068 | 3.00 | 71.00 | 121.00 | 29.00 | 26.00 | 1.70 | 89.30 | 893.00 | 1.90 | 5.00 |
| 7 | FLIP-2008-9L | 4.00 | 68.00 | 130.00 | 41.00 | 31.00 | 1.60 | 18.60 | 186.00 | 1.90 | 9.00 |
| **8** | **FLIP2008-7L** | **3.00** | **87.00** | **133.00** | **37.00** | **66.00** | **1.90** | **300.00** | **3000.00** | **2.20** | **3.00** |
| 9 | ILL-9934 | 1.00 | 62.00 | 111.00 | 32.00 | 57.00 | 1.80 | 104.20 | 1042.00 | 2.50 | 5.00 |
| 10 | ILL-6021 | 2.00 | 79.00 | 131.00 | 32.00 | 33.00 | 1.60 | 108.20 | 1082.00 | 1.70 | 5.00 |
| C1 | M.BHARATI | 4.00 | 88.00 | 132.00 | 39.00 | 85.00 | 1.60 | 70.00 | 700.00 | 2.40 | 5.00 |
| C2 | SAGUN | 3.00 | 88.00 | 128.00 | 40.00 | 81.00 | 1.90 | 118.20 | 1182.00 | 1.60 | 5.00 |
| **11** | **NR-2001-71-3** | **2.00** | **79.00** | **133.00** | **36.00** | **71.00** | **2.00** | **176.40** | **1764.00** | **1.80** | **3.00** |
| **12** | **NR-2001-71-4** | **2.00** | **81.00** | **134.00** | **38.00** | **81.00** | **2.10** | **213.40** | **2134.00** | **2.00** | **3.00** |
| 13 | NR-2001-71-7 | 3.00 | 63.00 | 111.00 | 35.00 | 50.00 | 1.90 | 110.50 | 1105.00 | 2.90 | 7.00 |
| **14** | **ILL7657** | **3.00** | **88.00** | **133.00** | **36.00** | **68.00** | **2.00** | **178.50** | **1785.00** | **1.70** | **3.00** |
| 15 | ILL2565 | 3.00 | 87.00 | 131.00 | 45.00 | 66.00 | 1.90 | 75.60 | 756.00 | 1.70 | 5.00 |
| **16** | **ILL2437** | **3.00** | **87.00** | **128.00** | **41.00** | **79.00** | **1.90** | **145.00** | **1450.00** | **1.60** | **3.00** |
| **17** | **ILL7349** | **3.00** | **88.00** | **133.00** | **34.00** | **65.00** | **1.80** | **151.60** | **1516.00** | **2.10** | **3.00** |
| 18 | ILL10045 | 3.00 | 63.00 | 133.00 | 32.00 | 54.00 | 1.90 | 122.40 | 1224.00 | 2.10 | 5.00 |
| 19 | ILL10065 | 5.00 | 79.00 | 131.00 | 35.00 | 63.00 | 2.00 | 39.30 | 393.00 | 2.80 | 9.00 |
| 20 | ILL10638 | 3.00 | 108.00 | 128.00 | 38.00 | 43.00 | 1.90 | 39.20 | 392.00 | 2.20 | 9.00 |
| C1 | M.BHARATI | 3.00 | 88.00 | 127.00 | 39.00 | 88.00 | 2.00 | 49.70 | 497.00 | 2.20 | 7.00 |
| C2 | SAGUN | 3.00 | 79.00 | 128.00 | 35.00 | 65.00 | 2.00 | 145.30 | 1453.00 | 1.80 | 3.00 |
| 21 | X39S-666 | 2.00 | 79.00 | 129.00 | 35.00 | 91.00 | 1.80 | 134.40 | 1344.00 | 1.90 | 5.00 |
| 22 | ILL7538 | 4.00 | 86.00 | 129.00 | 37.00 | 63.00 | 2.10 | 127.00 | 1270.00 | 1.70 | 5.00 |
| 23 | RL-20 | 2.00 | 63.00 | 111.00 | 32.00 | 31.00 | 1.50 | 74.10 | 741.00 | 2.50 | 7.00 |
| 24 | RL-21 | 2.00 | 61.00 | 114.00 | 31.00 | 31.00 | 1.90 | 55.70 | 557.00 | 2.40 | 7.00 |
| 25 | RL-22 | 1.00 | 63.00 | 110.00 | 27.00 | 26.00 | 1.90 | 81.90 | 819.00 | 3.40 | 7.00 |
| **26** | **RL-23** | **2.00** | **89.00** | **128.00** | **31.00** | **62.00** | **1.70** | **100.30** | **1003.00** | **2.20** | **3.00** |
| **27** | **RL-25** | **2.00** | **67.00** | **106.00** | **28.00** | **27.00** | **1.60** | **151.00** | **1510.00** | **2.00** | **3.00** |
| 28 | RL-95 | 3.00 | 88.00 | 133.00 | 37.00 | 72.00 | 1.90 | 88.10 | 881.00 | 1.90 | 7.00 |
| 29 | RL-94 | 2.00 | 61.00 | 111.00 | 35.00 | 44.00 | 1.80 | 73.40 | 734.00 | 2.50 | 7.00 |
| 30 | RL-85 | 3.00 | 62.00 | 113.00 | 39.00 | 35.00 | 2.00 | 100.80 | 1008.00 | 2.70 | 5.00 |
| C1 | M.BHARATI | 5.00 | 89.00 | 122.00 | 29.00 | 33.00 | 1.70 | 21.70 | 217.00 | 2.90 | 9.00 |
| C2 | SAGUN | 2.00 | 86.00 | 118.00 | 35.00 | 39.00 | 2.00 | 86.70 | 867.00 | 1.80 | 7.00 |
| 31 | RL-84 | 2.00 | 61.00 | 113.00 | 33.00 | 32.00 | 1.80 | 76.40 | 764.00 | 2.20 | 7.00 |
| 32 | FLIP-2006-99 | 1.00 | 86.00 | 133.00 | 33.00 | 33.00 | 2.00 | 64.50 | 645.00 | 1.50 | 7.00 |
| 33 | RL-78 | 1.00 | 61.00 | 110.00 | 39.00 | 33.00 | 2.00 | 94.50 | 945.00 | 2.10 | 5.00 |
| 34 | RL-77 | 1.00 | 61.00 | 108.00 | 31.00 | 21.00 | 1.90 | 111.60 | 1116.00 | 2.00 | 5.00 |
| 35 | RL-76 | 2.00 | 79.00 | 128.00 | 31.00 | 35.00 | 1.80 | 48.10 | 481.00 | 2.60 | 9.00 |
| 36 | RL-74 | 2.00 | 88.00 | 128.00 | 34.00 | 28.00 | 1.90 | 67.30 | 673.00 | 1.50 | 5.00 |
| 37 | RL-73 | 1.00 | 61.00 | 111.00 | 34.00 | 37.00 | 1.90 | 126.00 | 1260.00 | 2.00 | 5.00 |
| 38 | RL-72 | 1.00 | 61.00 | 113.00 | 38.00 | 25.00 | 1.70 | 96.60 | 966.00 | 2.00 | 5.00 |
| 39 | FLIP-2009-59L | 2.00 | 87.00 | 133.00 | 34.00 | 62.00 | 1.90 | 47.90 | 479.00 | 1.50 | 7.00 |
| **40** | **RL-47** | **1.00** | **74.00** | **121.00** | **34.00** | **29.00** | **1.90** | **150.70** | **1507.00** | **1.90** | **3.00** |
| C1 | M.BHARATI | 3.00 | 87.00 | 131.00 | 32.00 | 50.00 | 2.00 | 31.40 | 314.00 | 1.70 | 9.00 |
| C2 | SAGUN | 1.00 | 86.00 | 131.00 | 33.00 | 41.00 | 1.70 | 73.60 | 736.00 | 2.30 | 5.00 |
| 41 | RL-55 | 2.00 | 89.00 | 130.00 | 35.00 | 64.00 | 1.70 | 93.50 | 935.00 | 1.30 | 5.00 |
| 42 | RL-58 | 1.00 | 61.00 | 113.00 | 36.00 | 28.00 | 1.70 | 43.40 | 434.00 | 1.80 | 7.00 |
| 43 | RL-60 | 1.00 | 63.00 | 106.00 | 33.00 | 22.00 | 1.70 | 103.40 | 1034.00 | 2.00 | 5.00 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 44 | RL-61 | 1.00 | 60.00 | 108.00 | 32.00 | 30.00 | 1.50 | 67.00 | 670.00 | 3.40 | 7.00 |
| **45** | **RL-62** | **1.00** | **63.00** | **121.00** | **43.00** | **52.00** | **2.00** | **182.30** | **1823.00** | **2.00** | **3.00** |
| 46 | RL-69 | 1.00 | 61.00 | 113.00 | 36.00 | 49.00 | 1.70 | 146.60 | 1466.00 | 2.10 | 5.00 |
| 47 | RL-70 | 3.00 | 61.00 | 111.00 | 32.00 | 57.00 | 1.80 | 143.30 | 1433.00 | 2.20 | 5.00 |
| 48 | ILL-8009 | 2.00 | 63.00 | 128.00 | 36.00 | 63.00 | 2.10 | 136.60 | 1366.00 | 1.80 | 5.00 |
| 49 | ILL-8010 | 2.00 | 86.00 | 132.00 | 33.00 | 37.00 | 2.20 | 44.70 | 447.00 | 1.40 | 7.00 |
| 50 | ILL10853 | 2.00 | 86.00 | 133.00 | 35.00 | 67.00 | 1.80 | 14.50 | 145.00 | 1.20 | 9.00 |
| **51** | **ILL10856** | **2.00** | **90.00** | **128.00** | **36.00** | **59.00** | **1.90** | **176.00** | **1760.00** | **1.60** | **3.00** |
| C1 | M.BHARATI | 2.00 | 90.00 | 129.00 | 26.00 | 36.00 | 1.90 | 46.60 | 1419.00 | 2.10 | 5.00 |
| C2 | SAGUN | 1.00 | 86.00 | 121.00 | 31.00 | 49.00 | 1.90 | 138.50 | 939.00 | 1.80 | 7.00 |
| 52 | RL-51 | 3.00 | 61.00 | 110.00 | 31.00 | 33.00 | 1.70 | 84.90 | 849.00 | 2.60 | 7.00 |
| 53 | RL-41 | 1.00 | 74.00 | 106.00 | 26.00 | 32.00 | 1.30 | 86.80 | 868.00 | 1.50 | 7.00 |
| 54 | RL-42 | 1.00 | 61.00 | 106.00 | 28.00 | 30.00 | 1.70 | 96.00 | 960.00 | 2.00 | 7.00 |
| 55 | RL-43 | 1.00 | 61.00 | 108.00 | 28.00 | 30.00 | 1.80 | 86.40 | 864.00 | 2.00 | 7.00 |
| 56 | RL-40 | 1.00 | 68.00 | 108.00 | 30.00 | 43.00 | 1.80 | 106.80 | 1068.00 | 1.50 | 5.00 |
| C1 | M.BHARATI | 1.00 | 82.00 | 122.00 | 35.00 | 48.00 | 1.40 | 132.70 | 1327.00 | 1.70 | 5.00 |
| C2 | SAGUN | 2.00 | 62.00 | 108.00 | 29.00 | 26.00 | 1.50 | 125.50 | 1255.00 | 2.40 | 5.00 |

EPS- Early Plant Stand, DF- Days to Flowering, DM- Days to Maturity, PHT- Plant Height in cm, P/P- Pod per Plant, S/P- Seed per pod, GY- Grain Yield, gm- Gram, Kg- Kilogram, ha- hectare, HSWT (g)- Hundred Seed Weight in Gram, STB- Stemphylium Blight score

Genotypes namely, ILL FLIP2008-7L, NR-2001-71-4, RL-28, RL-62, ILL7657, NR-2001-71-3, ILL 10856, ILL 10134, RL-44, ILL 7349, RL 25, RL 47, ILL 2437, and RL 23 resistant to Stemphylium blight in Stemphylium blight screening nursery. This finding is in Agreement with the report of GLRP, 2012 that genotypes like RL-23, RL-25, FLIP 2008-7L, ILL 4402, ILL 6467, ILL 10856, ILL 2437, and ILL 7657 showed field tolerance to Stemphylium blight at Rampur.

**Conclusion**

Stemphylium blight caused by *Stemphylium botryosum* Walr is widespread throughout major lentil growing areas of the country and become key yield limiting factor in lentil. From the present study, it is concluded that *Acorus calamus* L. and *Zanthozylum armatum* DC from the botanicals, fungicides Mancozeb and Krilaxyl and antagonist *Trichoderma viridae* have the potentials to suppress the radial colony growth of *Stemphylium botryosum* Walr and also timely application was effective *in vivo* for Stemphylium blight disease control and yield increment. Genotypes ILL FLIP2008-7L, NR-2001-71-4, RL-28, RL-62, ILL7657, NR-2001-71-3, ILL 10856, ILL 10134, RL-44, ILL 7349, RL 25, RL 47, ILL 2437, and RL 23 also added some additional new sources of resistance and included in breeding programme to develop Stemphylium blight resistant lentil varieties.

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