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**Research Article**

# Efficacy of fungicides in management of Downy mildew disease of Cucumber (*Cucumis sativus* L.) under open field conditions, in Dhading district of Nepal

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## ABSTRACT

Downy mildew (*Pseudoperonospora cubensis*) is one of the most important disease of cucumber under open field conditions. Hence, the experiment was conducted to study the efficacy of fungicides in management of downy mildew disease of cucumber (*Cucumis sativus* L.) under open field in Chauradi-7 Dhading during spring season, 2019. Bhaktapur local variety of cucumber was used for the study. The experiment was laid out in single factor randomized complete block design with four replications. Three different bio-fungicides; *Trichoderma viride* + *Trichoderma harzianum*, *Bacillus subtilis*, *Verticillium lecanii* and one chemical fungicide; Krilaxyl (metalaxyl 8% + mancozeb 64%) were used as treatments. Normal water spray served as control. It was observed that bio-fungicides had insignificant effect in controlling the downy mildew. Pathological observation such as disease incidence percentage, severity percentage and AUDPC in bio-fungicides treated plots were not significantly different from water-sprayed control plots. Similarly, biometric attributes such as number of fruit, length and diameter of fruit, weight of fruit were also non-significant. Krilaxyl resulted significant effect in controlling downy mildew, providing maximum disease reduction (37.48%-50.72%) with lowest disease incidence (1.25-61.25%) and lowest value of AUDPC (138.7). Krilaxyl treated plots had higher number of fruit (6), longer length of fruit (17.15-18.95), higher diameter of fruit (4.16- 4.45 cm) and higher weight of fruit (677 - 759 g). Hence, chemical fungicide Krilaxyl was found better for the management of downy mildew disease in open field cucumber cultivation. Further, researches on bio-fungicides, especially of native strains are required for best result.

**Keywords:** Bio-fungicide, cucumber, downy mildew, fungicide

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## INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most economically important cucurbit vegetable and it is the fourth most cultivated vegetable around the world (Plader *et al*., 2007; Innark *et al*., 2013). Also, it is one of the most prized vegetable owing to its excellent flavour, varied usefulness, texture and medicinal value (Sebastian *et al.*, 2010).

Downy mildew caused by *Pseudoperonospora cubensis*, is the major disease followed by powdery mildew (*Sphaerotheca fuliginea*) during spring and rainy season (Singh & Banyal, 2011). Downy mildew is the most widely distributed disease of cucurbitaceous crops (Labeda & Cohen, 2011). Due to recurrent outbreaks of downy mildew over the past decades, it has emerged to be the most destructive disease and created problem both in the western nations as well as in east and south-east Asia, while accounting for the annual yield losses of up to 80% in Europe and USA (Lebeda & Schwinn, 1994).

Downy mildew is one of the most prevalent and ubiquitous disease that reduce production affecting both yield and quality (Ahamad, 2000). The disease is controlled in commercial cucurbit crops by frequent application of fungicides but intensive use of fungicides is regarded undesirable for both environmental reasons and for the risk of the development of resistance by pathogen. Heavy use of fungicide has resulted in the development of pathogen population resistant to fungicides and has raised public concerns over contamination of foods and environment (Chen *et al*., 2007). In this context, biocontrol approaches can be an eco-friendly management strategy for controlling plant disease (Heydari & Misaghi, 2003; Bharathi *et al*., 2004; Sharaki *et al*., 2008). Among biocontrol agents (BCA), antagonistic bacteria *Bacillus* spp. and *Pseudomonas* have been found to be effective in controlling several diseases (Collins & Jacobson, 2003; Heydari & Misaghi 2003; Shahraki *et al*., 2008, 2009). For biological control, mycoparasite *Trichoderma* have been found effective in enhancing growth and resistance in pearl millet (Raj *et. al*, 2005). Downy mildew has been successfully controlled by *Bacillus* spp. and *Trichoderma viride* + *Trichoderma harzianum* (Abd-El-Moity *et al*., 2003).

The study focuses on effectiveness of fungicides (biological and chemical) in management of downy mildew disease of cucumber in open field conditions, in Nepal.

## MATERIALS AND METHODS

### Experimental location

The experiment was carried out in the commanding area of Prime minister Agriculture Modernization Project (PMAMP), Project Implementation Unit, Vegetable zone-Dhading, at Charaudi-7, Benighat Rorang Rural-Municipality, Dhading. Geographical location is found to be 27˚48’ N latitude and 84˚45’ E longitude with an elevation of 487m above sea level (Kayastha & Smedt, 2009). The research site lies in the sub-tropical zone of Nepal. The texture of soil in study area is sandy loam. Research was conducted during the month of December to May.

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**Experimental Design**

The experiment was conducted in randomized complete block design (RCBD) with five treatments, including control and each treatment was replicated four times. Each plot contained four rows and each row accommodated five plants.

**Treatment details**

| S. N. | Name of treatment | Dosage | Concentration | Source |
|---|---|---|---|---|
| 1. | *Trichoderma viride* + *Trichoderma harzianum* | 10 g/L | 1×10^8 cfu/g | Phytocare Intl Pvt.Ltd, Nepal |
| 2. | *Bacillus subtilis* | 2 mL/L | 1×10^8 cfu/mL | Peak Chemical Industries, Siliguri, India |
| 3. | *Verticillium lecanii* | 2 mL/L | 1×10^8 cfu/mL | Peak Chemical Industries, Siliguri, India |
| 4. | Krilaxyl | 2g/L | (Metalaxyl 8%+ Mancozeb 64%) | Krishi Rasayan Pvt. Ltd, India |
| 5. | Water spray (Control) | _ | _ | _ |

All the treatments were applied as seedling dipping during transplantation and later as foliar spray at every 10 days interval, after transplantation.

**Plant material**

Bhaktapur-local variety of cucumber was used. The seeds were sown under protected conditions in poly-bags of size 10×12 cm. One seed per poly bag was sown and kept inside the poly house. Regular watering was carried out as required. After complete germination of the seed, seedlings at 5 leaf stage were transplanted.

**Weather data**

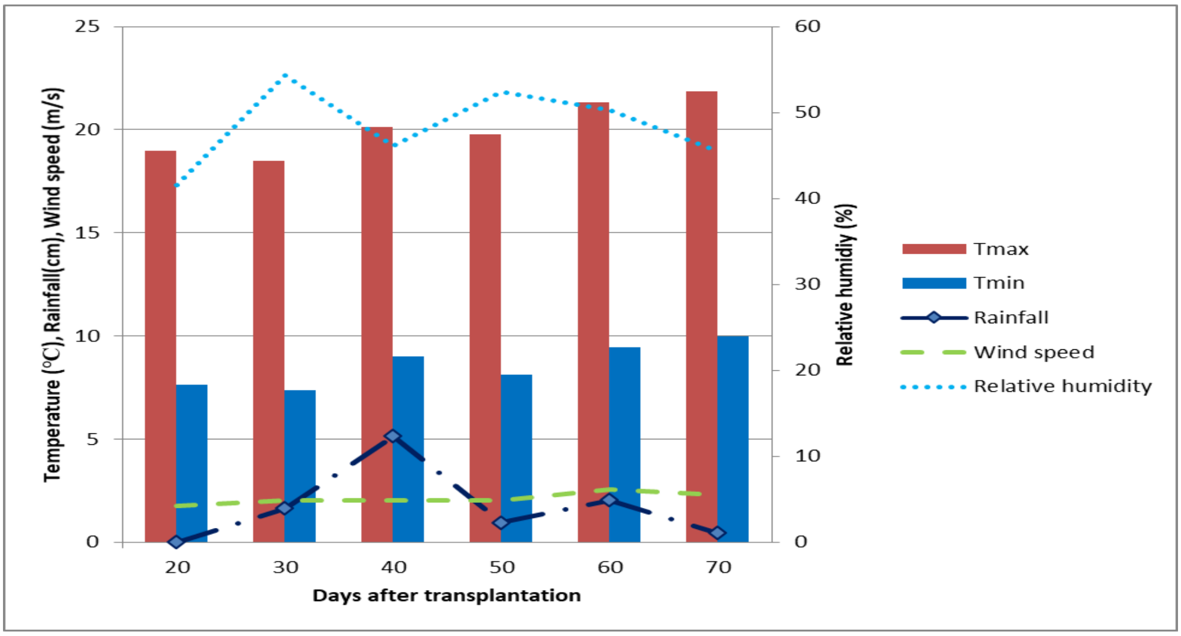


**Figure 1. Weather data of the experimental site, Charaudi-7, Benighat Rorang Rural-Municipality, Dhading, during 10th Jan to 4th May, 2019**

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The experimental site received rainfall of 95 cm at 50 days after transplantation (DAT) and 44 cm at 70 DAT. The temperature also progressed gradually during the experimentation period. Other climatic parameters are depicted in figure 1.

### Crop management

The field was ploughed twice followed by planking to attain good tilth. Then, silver plastic mulch was laid throughout the experimental field. Seedling with poly bags were thoroughly dipped into different fungicidal suspension solution for about 30 minutes and transplanted into pits belonging to concerned treatment plots with soil intact on 20th January, 2019. After transplantation, different fungicidal treatments were sprayed at 10 days interval.

### Measurement of data

### Pathological observations

1. Disease incidence

Total number of plants affected by disease in a plot was recorded. For this, all plants from each of the plots were observed and calculated as below (Shrestha *et al*., 2019).

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plant assessed}} \times 100$$

2. Percentage disease severity

To record disease severity, five plants from middle rows of each plot were selected at random and tagged before the appearance of the disease. Up to 10 leaves of the tagged plants from top to bottom were observed. The disease was graded on the basis of area of leaves covered by downy mildew lesions. The grades were assigned numerical ratings proportional to the diseased area (Table 1). Based on numerical rating/score observed, the per cent disease severity (PDS) was recorded by following McKineey (1923) as cited in (Bhagwanrao, 2014).

$$\text{PDS} = \frac{\text{Sum of observed ratings}}{\text{Number of leaves observed} \times \text{maximum score}} \times 100$$

### Severity scale of downy mildew of cucumber

| Score | Severity |
|---|---|
| 0 | No symptoms |
| 1 | 1-10 % leaf area affected |
| 2 | 11-25 % leaf area affected |
| 3 | 26-50 % leaf area affected |
| 4 | 51-75 % leaf area affected |
| 5 | Above 76 % leaf area affected |

(Bhagwanrao, 2014)

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**Biometric observation**

1. Number of fruits per plant

The number of fruit per plant was recorded as the average of number of fruits produced by all sample plants at marketable stage at various date.

2. Fruit weight per plant

Weight of fruits at each harvest from sample plants was taken.

3. Diameter of fruit per plant

Sum of diameter of each fruit harvested from sample plant was obtained and divided by total number of fruits to calculate the average diameter of fruit per plant.

4. Length of fruit per plant

Sum of length of each fruit harvested from sample plant was obtained and divided by total number of fruits to calculate the average length of fruit per plant.

**Statistical analysis**

Various data recorded on pathological and biometric parameters were arranged and compiled using Microsoft Excel 2010. The percentage data were transformed according to Arcsine transformation formula as and when required. Then, Data were subjected to ANOVA test by using R-3.6.2 and the significant treatment means were compared by using DMRT in accordance to Gomez and Gomez (1984). Means were compared by least significance difference (LSD) for treatment difference (Gomez & Gomez, 1984; Shrestha, 2019).

## RESULTS

**Pathological observations**

**1. Disease incidence**

No significant effects of fungicide in downy mildew incidence percentage were observed at 62 DAT. At 65 DAT, the effect was significant at which lowest downy mildew incidence (61.25%) was observed in plots treated by Krilaxyl followed by *Bacillus subtilis* treated plots (72.50%) and *Verticillium lecanii* treated plots (73.75%). The effect of Trichoderma was not significantly different from control (Table 2).

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**Table 1. Effect of fungicides on disease incidence percentage (%) of cucumber, in Dhading, 2019**

| Fungicides | Incidence percentage (%) | |
|---|---|---|
| | 62 DAT | 65 DAT |
| *Trichoderma viride* + *Trichoderma harzianum* | 8.75 (14.784) | 85.00 (68.48ab) |
| Bacillus subtillis | 3.75 (8.16) | 72.50 (59.28bc) |
| *Verticillium lecanii* | 2.50 (5.09) | 73.75 (63.75bc) |
| Krilaxyl | 1.25 (3.711) | 61.25 (51.57c) |
| Control (water spray) | 3.75 (6.1777) | 95.00 (80.46a) |
| Sem (±) | 4.35 | 5.30 |
| LSD (0.05) | _ | 15.5 |
| CV % | 66.5 | 15.7 |
| F-test | NS | * |
| P value | 0.448 | 0.015 |

## 2. Disease severity

The effect of fungicides on disease severity percentage was significant at 65 and 68 DAT while it was insignificant at 62 DAT (Table 3). Krilaxyl treated plots had the lowest disease severity percentage (22.89%) at 65 DAT and (34.70%) at 68 DAT. Among the biocontrol agents, *Bacillus subtilis* treated plots had low severity percentage but statistically insignificant and at par with *Trichoderma viride* + *Trichoderma harzianum*, *Verticillium lecanii* and even with control at both date of observation which has been presented in table as below.

**Table 2. Effect of fungicides on disease severity percentage (%) of cucumber in Dhading, 2019**

| Fungicides | Percentage severity (%) | | |
|---|---|---|---|
| | 62 DAT | 65 DAT | 68 DAT |
| *Trichoderma viride* + *Trichoderma harzianum* | 19.79 (26.37) | 31.04 (33.84b) | 47.43b |
| Bacillus subtillis | 17.63 (24.73) | 30.02 (33.18b) | 53.57b |
| *Verticillium lecanii* | 15.54 (23.08) | 30.00 (33.21b) | 50.87b |
| Krilaxyl | 11.97 (20.19) | 22.89 (28.57a) | 34.70a |
| Control (water spray) | 21.15 (27.25) | 33.90 (35.59b) | 56.95b |
| Sem (±) | 1.57 | 0.93 | 3.74 |
| LSD (0.05) | _ | 2.88 | 11.52 |
| CV % | 12.9 | 5.7 | 15.3 |
| F-test | NS | ** | * |
| P value | 0.053. | 0.003 | 0.011 |

*Sem: Standard error of means. LSD: Least significant difference. CV: Coefficient of variation.*

*Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance. Mean in parenthesis is arcsine transformed value by using formula =asin(sqrt(data/100))*180/PI() in excel. NS= Non-significant, *=significant at 5% probability level, **= significant at 1% probability, ***=significant at 0.1% probability*

## 3. Disease reduction percentage

The effect of fungicides on disease control percentage was significant at 62, 65, 68 DAT. Krilaxyl significantly controlled disease which was maximum (43.92 – 50.72%). Effect of bio-fungicides was at par with one another.

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**Table 3. Effect of fungicides on disease control (%) of cucumber in Dhading, 2019**

| Fungicides | Disease control (%) | | |
|---|---|---|---|
| | 62 DAT | 65 DAT | 68 DAT |
| *Trichoderma viride + Trichoderma harzianum* | 19.37 b | 25.82 b | 24.20 b |
| Bacillus subtillis | 29.14 ab | 29.00 b | 17.72 b |
| *Verticillium lecanii* | 37.74 ab | 27.94 b | 19.87 b |
| Krilaxyl | 50.72 a | 37.48 a | 43.92 a |
| Control (water spray) | 0 c | 0 c | 0 c |
| Sem (±) | 4.46 | 3.35 | 3.50 |
| LSD (≤0.05) | 13.76 | 10.32 | 10.78 |
| CV % | 31.6 | 32.1 | 28.8 |
| F-test | ***. | ***. | *** |
| P value | <0.001 | <0.001 | <0.001 |

*Sem: Standard error of means. LSD: Least significant difference. CV: Coefficient of variation.*

*Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance. NS= Non-significant, *=significant at 5% probability level, **= significant at 1% probability, ***=significant at 0.1% probability*

### 4. Area under disease progress curve (AUDPC)

The effect of fungicides on progress of disease was highly significant as compared to water-sprayed control (Table 5, Fig. 2). Krilaxyl significantly slowed the disease progress with lowest AUDPC value (138.7) in comparison to maximum value of AUDPC in water-sprayed control (218.9). The AUDPC values between the bio-fungicides and the water-sprayed control were not significantly different.

**Table 4. Effect of fungicides on area under disease progress curve (AUDPC) of downy mildew of cucumber, in Dhading, 2019**

| Fungicides | (AUDPC) |
|---|---|
| *Trichoderma viride + Trichoderma harzianum* | 193.9b |
| Bacillus subtillis | 196.9b |
| *Verticillium lecanii* | 189.6b |
| Krilaxyl | 138.7a |
| Control (water spray) | 218.9b |
| Sem (±) | 10.14 |
| LSD (0.05) | 31.25 |
| CV % | 10.8 |
| F-test | ** |
| P value | 0.002 |

*Sem: Standard error of means. LSD: Least significant difference. CV: Coefficient of variation.*

*Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance. NS= Non-significant, *=significant at 5% probability level, **= significant at 1% probability, ***=significant at 0.1% probability*

The disease progress curve in plot treated with Krilaxyl showed slow disease progress as compared to control while disease progress was at par for *Trichoderma viride + Trichoderma harzianum, Bacillus subtilis, Verticillium lecanii* treated plot with control. This result has been illustrated in figure below.

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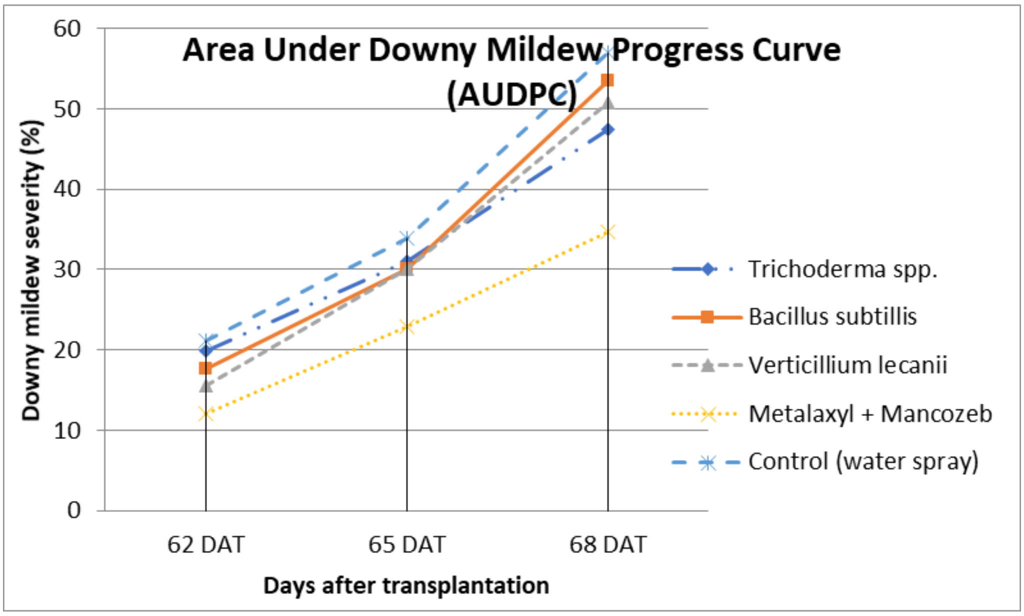


**Figure 2. Area Under Disease Progress Curve (AUDPC) showing trend of disease development**

**Biometric observation:**

**1. Number of fruit per plant**

Effect of fungicide on average number of fruits per plant of cucumber was insignificant at 58 DAT but was found to be highly significant at 62, 65 and 68 DAT (Table 6). Average number of marketable fruits from plot treated with Krilaxyl was highest in all dates 62, 65 and 68 DAT. The number of fruits in bio-fungicides treated plots were not significantly different from water-sprayed control plots.

**Table 5. Effect of fungicides on average number of fruit per plant of cucumber, in Dhading, 2019**

| Fungicides | Average number of fruit per plant | | | |
|---|---|---|---|---|
| | 58 DAT | 62 DAT | 65 DAT | 68 DAT |
| *Trichoderma viride + Trichoderma harzianum* | 1.34 | 4.63b | 3.55b | 1.68b |
| *Bacillus subtilis* | 1.25 | 4.83b | 3.48b | 1.78b |
| *Verticillium lecanii* | 1.20 | 4.80b | 3.55b | 1.70b |
| Krilaxyl | 1.59 | 5.93a | 5.43a | 5.25a |
| Control (water spray) | 1.40 | 4.58b | 3.43b | 1.60b |
| Sem (±) | 0.1020 | 0.1435 | 0.2213 | 0.1324 |
| LSD (0.05) | _ | 0.44 | 0.68 | 0.37 |
| CV % | 15.1 | 5.8 | 11.5 | 9.83 |
| F-test | NS | *** | *** | *** |
| P-value | 0.131 | <0.001 | <0.001 | <0.01 |

*Sem: Standard error of means. LSD: Least significant difference. CV: Coefficient of variation.*

*Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance. NS= Non-significant, *=significant at 5% probability level, **= significant at 1% probability, ***=significant at 0.1% probability*

**2. Average length of fruits per plant**

Effect of fungicide on average length of fruit per plant was found statistically highly significant at different dates of observation (Table 7). For all dates (58, 62, 65 and 68 DAT),

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the average length of fruit per plant was found highest in Krilaxyl treated plots while the bio-fungicides treated plots wre found to effect insignificantly when compared with water-sprayed control.

**Table 6. Effect of fungicides on average length of fruit per plant of cucumber in Dhading, 2019**

| Fungicides | Average length of fruit per plant | | | |
|---|---|---|---|---|
| | 58 DAT | 62 DAT | 65 DAT | 68 DAT |
| *Trichoderma viride + Trichoderma harzianum* | 17.45bc | 11.73b | 10.80b | 11.40b |
| *Bacillus subtilis* | 17.42bc | 11.85b | 10.95b | 11.30b |
| *Verticillium lecanii* | 16.35c | 11.28b | 10.80b | 11.05b |
| Krilaxyl | 18.86a | 18.95a | 18.42a | 17.15a |
| Control (water spray) | 17.51b | 11.48b | 10.48b | 11.10b |
| Sem (±) | 0.376 | 0.447 | 0.265 | 0.251 |
| LSD (0.05) | 1.16 | 1.38 | 0.817 | 0.773 |
| CV % | 4.29 | 6.84 | 4.4 | 3.97 |
| F-test | ** | *** | *** | *** |
| P value | 0.009 | <0.01 | <0.01 | <0.01 |

*Sem: Standard error of means. LSD: Least significant difference. CV: Coefficient of variation.*

*Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance. NS= Non-significant, *=significant at 5% probability level, **= significant at 1% probability, ***=significant at 0.1% probability*

### 3. Average diameter of fruit per plant

Effect of fungicides on average diameter of fruit per plant was found insignificant at 58 DAT while it was highly significant at 62, 65 and 68 DAT (Table 8). For all the observed dates, only Krilaxyl had significant effect on average diameter of fruit per plant. The bio-fungicides bio-fungicides had no significant effect when compared with water-sprayed control.

**Table 7. Effect of fungicides on average diameter of fruit per plant of cucumber, in Dhading, 2019**

| Fungicides | Average diameter of fruit per plant | | | |
|---|---|---|---|---|
| | 1st reading | 2nd reading | 3rd reading | 4th reading |
| *Trichoderma viride +Trichoderma harzianum* | 3.98 | 3.06b | 2.88c | 2.68b |
| *Bacillus subtilis* | 4.05 | 3.17b | 2.95bc | 2.73b |
| *Verticillium lecanii* | 3.95 | 3.05b | 3.13b | 2.53b |
| Krilaxyl | 4.16 | 4.45a | 4.29a | 4.5a |
| Control (water spray) | 3.98 | 3.25b | 3.10bc | 2.63b |
| Sem (±) | 0.0510 | 0.0934 | 0.0736 | 0.1197 |
| LSD (0.05) | _ | 0.288 | 0.227 | 0.369 |
| CV% | 2.54 | 5.5 | 4.51 | 7.95 |
| F-test | NS | *** | *** | *** |
| P value | 0.051 | <0.01 | <0.01 | <0.01 |

*Sem: Standard error of means. LSD: Least significant difference. CV: Coefficient of variation.*

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### 4. Weight of a single fruit per plant

Effect of fungicide on average weight of a single fruit per plant was significant on all dates of observation (Table 9). Krilaxyl treated plots produced significantly higher average weight of a single fruit per plant at 62, 65 and 68 DAT. The bio-fungicides had no significant effect.

**Table 8. Effect of fungicide on average weight of a single fruit per plant of cucumber, in Dhading, 2019**

| Fungicides | Average weight of a single fruit per plant | | | |
|---|---|---|---|---|
| | 58 DAT | 62 DAT | 65 DAT | 68 DAT |
| *Trichoderma viride +Trichoderma harzianum* | 569.00b | 539.50b | 472.75b | 462.25bc |
| *Bacillus subtilis* | 563.75b | 546.25b | 477.75b | 469.25b |
| *Verticillium lecanii* | 530.50b | 536.50b | 476.25b | 465.75b |
| Krilaxyl | 683.25a | 759.50a | 677.00a | 677.00a |
| Control (water spray) | 569.50b | 546.50b | 478.50b | 452.00c |
| Sem (±) | 16.18 | 12.47 | 15.91 | 3.98 |
| LSD (0.05) | 49.8 | 38.4 | 49 | 12.2 |
| CV % | 5.55 | 4.26 | 6.16 | 1.57 |
| F-test | *** | *** | *** | *** |
| P value | <0.01 | <0.01 | <0.01 | <0.01 |

*Sem: Standard error of means. LSD: Least significant difference. CV: Coefficient of variation.*

*Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance. NS= Non-significant, *=significant at 5% probability level, **= significant at 1% probability, ***=significant at 0.1% probability*

### Correlation of disease severity with climatic parameters

The climatic data recorded during the observation dates are summarized in table 10. All the climatic parameters were found to be highly correlated with each other (Table 11). As the days progressed, severity of disease increased. Disease severity was found to be highly and positively correlated with maximum temperature and wind speed while negatively correlated with humidity and minimum temperature. No correlation was found between severity and rainfall. Positive correlation showing increase in one weather parameter (independent variable) causes increase in disease severity (dependent variable). Negative correlation showing increase in weather parameter (independent variable) cause to decrease in disease severity (dependent variable).

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**Table 9. Downy mildew disease severity and climatic parameters at different dates of observation, Dhading, 2019**

| Days (DAT) | Severity | Rainfall | Humidity | Tmax | Tmin | Windspeed |
|---|---|---|---|---|---|---|
| 62 | 21.15 | 1.11 | 53.13 | 20.15 | 10.95 | 1.97 |
| 65 | 33.9 | 0.01 | 45.17 | 21.37 | 9.9 | 2.4 |
| 68 | 56.95 | 1 | 41.64 | 23.1 | 10.03 | 2.45 |

**Table 10. Correlation of downy mildew disease severity with climatic parameters as the days progressed, Dhading, 2019**

| | Days | Severity (%) | Rainfall (mm) | Relative Humidity (%) | Tmax ($^0$C) | Tmin ($^0$C) | Windspeed (m/s) |
|---|---|---|---|---|---|---|---|
| Days | 1 | | | | | | |
| Disease Severity | 0.986483 | 1 | | | | | |
| Rainfall | -0.09078 | 0.07363 | 1 | | | | |
| Humidity | -0.97611 | -0.92731 | 0.304999 | 1 | | | |
| Tmax | 0.995056 | 0.99788 | 0.008574 | -0.9497 | 1 | | |
| Tmin | -0.80364 | -0.69526 | 0.665611 | 0.91375 | -0.74056 | 1 | |
| Windspeed | 0.9095 | 0.829087 | -0.49656 | -0.9781 | 0.863715 | -0.9783 | 1 |

## DISCUSSION

Downy mildew occurred in the experimental field was due to natural source of inoculum (sporangia disseminated by wind and rainfall) which coincided the time when fruits were setting and some of the fruits were harvest stage. The disease progress got favored due to daily evening rainfall 50-70 DAT. Water soaked lesions were observed on very few border plant at 58 DAT as initial stage of disease infection.

As the disease started late and was sporadic at establishment phase, the incidence percentage was not significant at 62 DAT. Later with sufficient disease inoculum produced in the field and favorable climatic conditions created both the disesase incidence and severity increased and showed significant effects of the treatments, especially by the chemical fungicide, Krilaxyl (metalaxyl 8% + manozeb 64%).Such effectiveness (69% disease reduction) of chemical was also reported by Zhan-Bin Sun *et al*. (2013). Gupta and Shyam (1996) found metalaxyl + mancozev most effective in reducing sporangial number and disease progress when compared to other five fungicides viz., cymoxanil, cymoxanil + mancozeb, captan, chlorothalonil and cuprous oxide.

In the present study we did not find *Trichoderma viride* + *Trichoderma harzianum*, *Bacillus subtills* and *Verticillium lecanii* effective as bio-fungicides. This might be due to fact that biocontrol agents require optimum humidity and thermal requirement for their effectiveness while average relative humidity was recorded as 45.78% during experiment duration. Similar argument was reported by Belanger and Avis (2001) who suggested that relative humidity requirement of biocontrol agents are not reproducible in the field as in the greenhouse. So, biocontrol agents are more effective in greenhouse system than in field. Paulitz and Belanger

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(2001) also reported unfavorable environment in the field as a reason for failure or inconsistent performance of biocontrol agents.

Observation of biometric parameters such as number of fruits, length of fruits, diameter of fruits, weight of fruits were intended to establish relevancy of the fact that disease free or healthy plant produce high and quality produce and so was the result from Krilaxyl treated plot. Krilaxyl could better control the disease so these parameters were seen significantly higher than the water-sprayed control and the ineffective bio-fungicides treated plots. According to Prabhudesai (1992), though the fruits are not attacked by the pathogen, the fruit quality is affected resulting in reduced export potential of the fruit. The present results clearly showed that an increase in disease severity was found correlated with reduction in yield. As reported by Takahashi *et al*. (1977), products of photosynthesis moved from healthy tissues to downy mildew lesions and were incorporated in to mycelia and sporangia of this fungus. This caused low quality of fruit as fruit do not attain potential length and diameter. Nasir *et al*. (2015), variation in number of fruits and fruit yield was due to damaging foliage. Also, downy mildew decreases flower set and fruit development by killing the foliage (Kristkova *et al*., 2009). The results of our experiment also showed that plots with more disease severity had lesser diameter, shorter length, lower number and lower weight of fruits.

As the days progressed, severity of disease increased. Disease severity was found to be highly and positively correlated with maximum temperature and wind speed while negatively correlated with humidity and minimum temperature. No correlation was found between severity and rainfall. Contrary to this result, disease severity was been found to be positively correlated with all climatic parameter. All these parameters contributed positively to disease severity as studied by Mahrishi and Siradhana (1984); Huang *et al*. (1989). The climatic parameters and severity observation recorded during the experiment can't generalize their relationship. The result is strictly based on the experiment.

## CONCLUSION

Krilaxyl (Metalaxyl 8% + mancozeb 64%), a commonly recommended fungicide for the management of downy mildew disease, is still working under open field conditions. The bio-fungicides (*Trichoderma viride* + *Trichoderma harzianum*, *Bacillus subtilis* and *Verticillium lecanii*) did not control the disease significantly in the present study. Further researches on biofungicides, especially of native strains are required.

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### Author's contributions

- Shailesh Pandit conducted the experiment and recorded data, analyzed and prepared the final manuscript.
- Sramika Rijal, Krishna Hari Dhakal supervised the experiment.

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- Hira Kaji Manandhar guided and revised the article for the final approval of the version to be published.
- Sandesh Bhandari and Sushma Paneru helped during data observation.

**Conflict of Interest**

The authors declare no conflicts of interest regarding publication of this manuscript.

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