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# Determination of susceptible growth stage and efficacy of fungicidal management of Curvularia leaf spot of maize caused by *Curvularia lunata* (Wakker) Boedijn

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## Abstract

Maize is an important food security crop along with rice and wheat globally. Losses caused by biotic stresses in maize are substantial and Curvularia leaf spot is important among them. Further management of Curvularia leaf spot is done primarily through chemicals, therefore an attempt was made to evaluate the efficacy of commonly used systemic and non-systemic fungicides against the pathogen, and most susceptible growth stage for disease development was identified. In vitro evaluation of four systemic and four non-systemic fungicides was done at different concentrations, for checking the growth of pathogen. The data revealed that Carboxin (at 25 ppm) completely inhibited growth of pathogen. Further among non-systemic fungicides treatment of Mancozeb showed maximum growth inhibition (98. 24% at 200 ppm). Under glass house conditions mancozeb was found to be more effective than Carboxin for controlling the disease severity. Further to determine the time of application of fungicides, developmental stage most susceptible to *Curvularia lunata* was studied. Three growth stages (Knee height stage, Silking stage and Tasseling stage) were compared. Maximum disease index and severity was found at Silking stage (47% and 53.75%, respectively) followed by Tasseling stage (42.5% and 18.4%, respectively) and Knee height stage (37% and 30%, respectively), indicating that the disease progresses with the maturity of the plant and is maximum at the Silking stage. Results suggest that susceptible maize varieties may give higher yield with the optimisation of the time of application of the fungicides and higher economic and environmental gains can be achieved with judicious use of fungicides.

**Keywords**: *Curvularia lunata*, maize, developmental stages, fungicide, economic management

## Introduction

Increase in *per capita* income and rise in the standard of living has led to change in the feeding habits of the people worldwide. Presently, corn (*Zea mays* L) is the world's third leading crop after wheat and rice grown in different agro-ecologies of the world (Directorate of Maize Research, 2012). The crop has high economic value as both grain and fodder crop. Maize is easily processed, has high fiber content and is cheaper than other cereals (IITA, 2001) further low cost of cultivation and high nutrient content adds to economic value. Production of maize is limited by many constraints including the diseases, worldwide, about 9% yield losses have been estimated in maize due to diseases (Khokhar et al, 2014). Curvularia leaf spot caused by *Curvularia lunata* (Wakker) Boedijn, is one of the most destructive foliar disease of the crop (FuHua et al, 2004). This pathogen is seed and soil borne causing disease prevalent in the hot, humid maize growing areas and causes approximately 10-60% yield losses due to loss of photosynthetic region of the crop and upto 33.4% losses in grain number (DingFa et al, 1999).The pathogen can cause damage from seedling to harvesting stage of maize plant. Many strategies has been developed for controlling the Curvularia leaf spot in maize including management strategies based on chemicals, bio-control agents and integrated management of the disease (Kumar and Tomar, 2005). But most common and effective method is use of chemicals for the management of Curvularia leaf spot (Patil et al, 2001; Prajapati et al, 2003).

This disease is present at all the developmental stages of maize plant while reproductive stage is most susceptible to the pathogen (Akonda et al, 2015). Further, this disease have the ability to develop into epiphytotics under favourable environmental conditions on susceptible developmental stage of maize which hampers development of ears and grains formation by decreasing photosynthetic ability (FaChao et al, 1998).The pathogens overwintered on diseased crop residues left in the field therefore, multiple cultural practices can be used for the prevention of disease but are region depended and therefore are not a vi-

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able option (Hou et al, 2013). Therefore, there is need to optimise the time of application of the fungicides as susceptibility of the crop to the pathogen is determined by the age of the plant (Moratelli et al, 2015). Considering the lacuna in understanding of interaction of growth stages of the crop and application of fungicides for disease management present research work was designed. The study aims at determining the effective fungicide for management of *C. lunata* among commonly used fungicides, and effect of fungicide application on diseases management at different growth stages of the crop. In addition this study may help in better understanding of timely application of fungicides at different growth stages for improved results.

## Materials and Methods

***Isolation and maintenance of pure culture of Curvularia lunata***

Pure culture of *C. lunata* was isolated from infected leaves of susceptible local variety Gaurav collected from Norman E. Borlaug Crop Research Centre, GB Pant University of Agriculture and Technology, Pantnagar using single spore isolation technique (Anon 1943, 1947; Leslie and Brett, 2006). Culture was preserved on Potato Dextrose Agar (PDA) slants at 4°C and was maintained with repeated sub culturing at 15 days interval.

***In vitro screening of fungicides against the pathogen***

*in vitro*, the efficacy of different fungicides against *C. lunata* was studied by poisoned food technique (Shravelle, 1961). The fungicides viz., Chlorothalonil, Carboxin, Carbendazim, Metalxyl, Thiram, Mancozeb, Copper oxychloride and Iprodion (Supplementary Table 1) were evaluated against the test fungus at the concentration of 25, 50, 100, and 200 ppm and PDA was used as control. The colony diameter was measured. The percent inhibition in growth was determined with the help of mean colony diameter and calculated by using the following formula:

inhibition (%) = X - Y / X ×100

where X = colony diameter in control; Y = colony diameter in treated medium.

***Determination of effect of developmental stages on susceptibility to Curvularia lunata***

Seeds of susceptible maize variety Gaurav were procured from Tarai Development Corporation (TDC), Pantnagar, Uttarakhand, India. Glass house experiments were conducted to study the effect of plant age on development of Curvularia leaf spot, seeds were sown by staggered sowing at 20 days interval, so as to obtain knee height stage, silking stage and Tasseling stage for simultaneous inoculation at one time. Ten seeds were sown per pot, and placed at about 2-3 cm deep. The seeds were then properly covered with soil. After two weeks of germination, thinning of plants was done and 5-6 plants were maintained. Weeding and other agronomic practices were followed as recommended for maize crop. The plants were spray inoculated with *C. lunata* spores @ $10^6$ spores $ml^{-1}$ then after 48 hrs, Carboxin and Mancozeb (which were screened out in laboratory) were sprayed to assessed for the management of *C. lunata*. Disease severity was observed from 3 days untill 18 days after pathogen inoculation based on a 1-5 disease rating scale (Payak and Sharma, 1983) (Supplementary Table 2) and further PDI (Percent Disease Index), rate of infection (R-value) and area under disease progress curve (AUDPC) were calculated as follow:

PDI = (X / Y × Z) ×100

where X = sum of all individual ratings; Y = total number of sample assessed; Z = maximum rating.

Infection rate were calculated for weekly interval by using following formula (Vanderplank, 1963).

$$r = \frac{2.3}{t_2 - t_1} \log \frac{x_2}{x_1}$$

where r = apparent rate of infection; $X_1$ = PDI at time $T_1$; $X_2$ = PDI at time $T_2$; $t_2$ - $t_1$ = time interval in days between two observations.

The area under disease progress curve (AUDPC) was calculated by using the formula as suggested by Wilcoxson et al (1975):

$$AUDPC = \sum_{i=1}^{k} \frac{1}{2}(S_i + S_{i+1})(T_i - T_{i-1})$$

where $S_i$ = PDI at the end of time i; k = number of successive evaluations; $T_i - T_{i-1}$ = time interval between two evaluations i and i - 1 of the disease.

***Statistical Analysis***

Data was analysed using statistical software STPR-2 following CRD (Completly Radomized Design) and Duccan's Multiple Range Test (DMRT) at P < 0.5.

## Results

***Effect of fungicides on growth of the test fungus***

Curvularia leaf spot of maize cause's substantial losses in maize productivity and high yield losses are reported. An experiment was conducted to evaluate the efficacy of different systemic viz., Chlorothalonil, Carboxin, Carbendazim, Metalxyl and non-systemic/contact fungicides viz., Thiram, Mancozeb, Copper oxychloride and Iprodion in controlling the in vitro growth of the pathogen. Poisoned food technique was adopted to determine the relative efficacy and optimum concentration of different fungicides in controlling in vitro growth of *C. lunata*. Growth inhibition was calculated in terms of percentage inhibition in diameter growth after 15 days of inoculation. Results of the study are presented in Figure 1 and Supplementary Table 3 and revealed that Carboxin and Mancozeb were found most effective in inhibiting the growth of the fungus at all the concentrations. Further these fungicides were used under glass house conditions to assess their efficacy against *C. lunata* at different

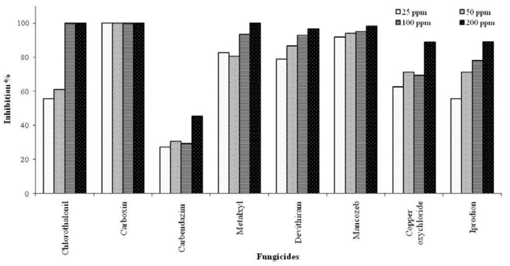


Figure 1- Inhibition in radial growth of *Curvularia lunata*. Effect of different fungicides viz., Chlorothalonil, Carboxin, Carbendazim, Metalxyl, Thiram, Mancozeb, Copper oxychloride and Iprodion on per cent inhibition in radial growth of Curvularia lunata. The different color bars shows different concentrations (25 ppm, 50 ppm, 100 ppm, and 200 ppm) of fungicides used.

development stages of maize plant.

***Determination of effect of developmental stages on susceptibility to Curvularia lunata***

Disease incidence and disease severity were recorded from third day after inoculation to eighteenth day after inoculation and the data (Table 1) revealed that the disease severity was maximum at silking satge as compare to the knee height stage and tasseling stage. The change in developmental stage causes change in level of infection. Generally the infection was lower at early developmental stage than at late developmental stages. The maximum disease index and severity was observed at silking stage (47% and 53.75%, respectively) followed by Tasseling stage (42.5% and 18.4%, respectively), while lowest at knee height stage (37% and 30%, respectively), indicating that the disease progresses with the maturity of the plant and is maximum at the silking stage, after which again decline at Tasseling stage. Further rate of infection (R-value) and AUDPC (A-value) was calculated and represented in Table 2. Infection rate was comparable at silking and Tasseling stage (0.47 and 0.49, respectively) and were more than knee height stage (0.37). Among the developmental stage maximum AUDPC (150) was observed at silking stage followed by knee height (82.5) and Tasseling stage (50.6) which means that silking stage is most sensitive among the three developmental stages. A-value and R-value depicts that disease progress from knee height to silking stage then starts

Table 1 - Disease severity on last day of experiment.

| S.No. | Treatment | Knee height* | Silking* | Tasseling* |
|---|---|---|---|---|
| 1 | Vitavex | 24.52b | 43.56b | 41.07b |
| 2 | Mancozeb | 7a | 19.37a | 24.05a |
| 3 | Check | 30b | 53.75b | 31.25ab |

* figures followed by same alphabet are not significantly different according to DMRT, SPSS 16.

decreasing to Tasseling stage (Figure 2). The result signifies that the reproductive stage (silking) is more prone for disease development.

On comparing the treatment of fungicides for controlling the disease, among the treatment Mancozeb was found effective at all the developmental stages in comparison to vitavax and control. Mancozeb was found most effective at knee height stage where minimum disease index and severity (13.5 and 7) was observed followed by silking stage (19.5 and 19.3) and tasseling stage (20.5 and 24). This suggests that mancozeb is more effective in controlling the disease compared to Carboxin. Similar trend was observed in disease progress (Figure 3) which depicts that the disease increase in slow rate at knee height stage. It suggests that mancozeb treatment is most effective at knee height stage for the better management of the disease. Further R-value and A-value were calculated and similar trend was observed which is represented in Figure 4.

## Discussion

Though at present plant disease management strategies based on biological means is favoured, use of chemicals is unavoidable considering their efficacy and rapid control of pathogen under consideration. *C. lunata* is a destructive pathogen of maize and its rapid and effective control is inevitably needed. Further only few disease management strategies based on biological means are reported (Chaube et al, 2002; Lal et al, 2006). Thus considering the importance of chemical based management of the pathogen, in the present study eight fungicides were evaluated under laboratory condition against *C. lunata*. All the fungicides were found effective in all the concentration (25 ppm, 50 ppm, 100 ppm, and 200 ppm). Similar observation was reported by Choudhary et al (2011). Carboxin was found most effective followed by Mancozeb and Metalxyl while Carbendazim was found to be least effective in controlling *in vitro* growth of

Table 2 - Efficacy of fungicide tested under glass house conditions.

| Developmental Stages | Treatments | DI | DS | R-Value | AUDPC |
|---|---|---|---|---|---|
| Knee Height Stage | Vitavex | 18.5 | 24.525 | 0.373957 | 63.0375 |
| | Mancozeb | 13.5 | 7 | 0.860513 | 24 |
| | Check | 37 | 30 | 0.28187 | 82.5 |
| Silking Stage | Vitavex | 23 | 43.5625 | 0.086141 | 125.775 |
| | Mancozeb | 19.5 | 19.375 | 0.474329 | 65.85 |
| | Check | 47 | 53.75 | -0.04999 | 150 |
| Tasseling Stage | Vitavex | 19.5 | 41.075 | 0.120049 | 98.4 |
| | Mancozeb | 20.5 | 24.05 | 0.38255 | 80.5875 |
| | Check | 42.5 | 18.40625 | 0.495366 | 50.67188 |

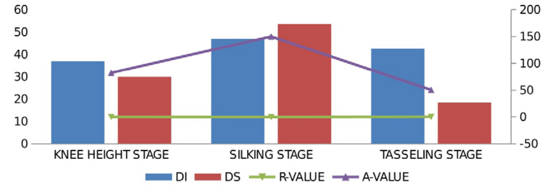


Figure 2 - Effect of different developmental stages on disease development. Rate of increase and decrease in disease developmental parameters i.e. disease index (DI), disease severity (DS), rate of infection (R-Value), and area under disease progress curve (A-Value) at different disease developmental stages i.e. knee height stage, silking stage and tasseling stage.

the pathogen at all concentrations. Similar studies were conducted by Sumangala et al (2008), they suggested that among the three non-systemic fungicides used Mancozeb was found most effective in inhibiting the growth of the fungus. Magie (1954) found Manzet and Zineb effective against *C. lunata*. Johnston et al (1957) reported control with Tricholoromethyl arenethio sulphonates. Dithiocarbamates and copper fungicides were found most effective in reducing the growth of *C. lunata* under *in vitro* conditions (Muis et al, 2008; Aktar et al, 2014). Such chemicals either inhibit germination, growth and multiplication of the pathogen or are deleterious to the pathogen (Sharma 2006; Banyal et al, 2008; Pan et al, 2010).

The two fungicides (Mancozeb and Carboxin) screened from the laboratory studies were further tested under glass house at different developmental stages (Knee height, silking, and Tasseling stage). There was no significant difference in disease severity in case of vitavex treatment as compare to control in all the three developmental stages. Disease severity was minimum in plants treated with mancozeb compare to control (Table 1). Infection rate represents the increase or decrease of disease per unit time. It is a sensitive indicator of disease progress, which is influenced by several host, pathogen and environmental factors (Chiarappa et al, 1972). Infection rate and AUDPC was obtained which shows in control plants the disease severity was increasing from third day after inoculation to eighteenth day after inoculation in all the developmental stages viz., knee height stage, silking stage and Tasseling stage (Supplementary

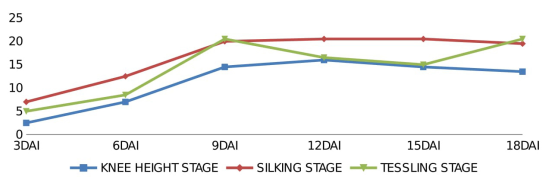


Figure 3 - Effect of Mancozeb treatment on Disease Progress curve at different developmental stages. The effect on disease progress from the third day after inoculation (DAI) to the eighteenth day after inoculation (DAI) at different developmental stage i.e. knee height stage, silking stage and tasseling stage.

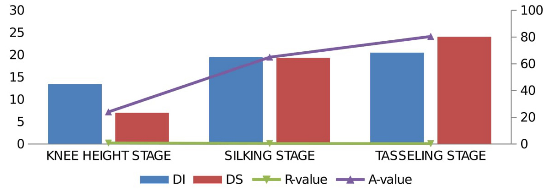


Figure 4 - Effect of Mancozeb treatment on Disease development at different developmental stages. Effect on disease developmental parameters i.e. disease index (DI), disease severity (DS), rate of infection (R-Value) and area under disease progress curve (A-Value) at different disease developmental stages i.e. knee height stage, silking stage and tasseling stage.

Figures 1, 2, and 3). While in treated plants the disease severity increases initially and then there was gradual decrease in disease severity.

Disease severity was gradually increasing from knee height stage to silking stage and then gradually decreases from silking stage to Tasseling stage. The studies suggest that disease severity was high during silking stage and gradually decreases during Tasseling stage. This may be due to the decrease in host tissue (Gregory et al, 1978). Similar observations were recorded by Choudhary et al (2011), they reported that maize cultivars which were prone to infection by *C. lunata*, were artificially inoculated and it was found that ten-days old plants of both cultivars were found immune as no disease developed. However, 20 and 40-days old plants developed 24.3% to 48.4% disease severity. Similar trends have been observed in different hosts other than maize with regard to disease development with respect to host age.

Results of this study suggest that the interaction of host and pathogen occurs in late stage, and also it may be possible for disease to appear after the knee height stage and increase in faster rate. Thus control practices should be applied at late knee height stage or before silking stage for effective disease management. Further, pathogen is reported to be favoured by moist and warm conditions; therefore use of effective management strategies may give highest returns in these conditions. In absence of resistant varieties use of fungicides can give profitable control of the pathogen in high yielding cultivars by applying chemicals at most susceptible stage of the crop. The study conducted provide an insight into the relationship between different growth stages and disease development by the pathogen, Further examination of the interaction of the pathogen and host are needed for devising better management strategies and understanding dynamics of disease development by the pathogen.

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