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***Research Article***

# EFFECT OF MEDIA, PH AND TEMPERATURE ON GROWTH AND SPORULATION OF *CERCOSPORA ZEAE-MAYDIS*, THE CAUSAL AGENT OF GREY LEAF SPOT OF MAIZE

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## ABSTRACT
The effects of media, pH and temperature on growth of Cercosporazeae-maydis, the cause of grey leaf spot of maize were investigated. Four media, Potato Dextrose Agar (PDA), CzapeckDox Agar (CDA), Green Corn Leaf Decoction Agar (GCLDA and Vegetable Juice Agar (V-8JA) were evaluated for their effects on growth and sporulation of the pathogen. Different temperature regimes and pH values were also tested for their effect on the growth of the pathogen. The variables used were colony diameter and spore concentration using haemocytometer. There was significant difference between growth and sporulation on different media with the best growth on PDA and GCLDA, and the best sporulation on GCLDA. There was significant difference between growths at different temperatures. The best growth and sporulation were observed at 25º C and total inhibition at 30º C. The pathogen grew best on pH 6 and the least growth occurred at pH 9.

***Key Words:*** *Cercosporazeae-Maydis, Effect, Media, Temperature, Ph, In-Vitro*

## INTRODUCTION
*Cercospora* leaf spot or grey leaf spot (GLS) of maize (*Zea mays* L.) caused by *Cercosporazeae-maydis* Tehon and Daniels first reported in Western Kenya in 1995 (Kung'u and Boa, 1997) has been reported to be wide spread in the maize growing zones in most districts in Kenya (Kinyua, 2004). Grey to tan rectangular lesions develops along the length of the leaf. These lesions coalesce to completely blight the leaves of susceptible maize cultivars. Severe infection develops from tassel ling stage of maize growth interfering with development due to less photosynthate production resulting less grain and thus leading to yield losses. Severe yield loss is expected if infection occurs early in the season and favourable conditions prevail during infection (Smit and Ward, 1997). The survey conducted on severity of infection of the disease revealed that the disease occurred at varying levels of severity in most growing areas in Kenya (Ajanga, Oduor and Simmons, 2000; Kinyua, 2004). Although the disease is causing severe yield lossesupto 60% (Murithi and Gathama, 1998) no work in the country is noticeable in literature on the factors influencing growth and sporulation of the pathogen. *Cercosporaspp* are known to grow slowly and sporulate sparsely in commonly used media. Beckman and Payne (1983) reported that routine techniques for obtaining sporulation in *Cercosporazeae-maydis* were unsuccessful. A good sporulation of the pathogen *in-vitro* is prerequisite for studying the disease development and to screen resistant cultivars under green house conditions and in the field as well. The present paper therefore, evaluates the effect of media, pH and temperature *in-vitro* on growth and sporulation of *C. zeae-maydis.*

## MATERIALS AND METHODS
### *Isolation of the Pathogen*
Fresh GLS – infected maize leaves (*Cercosporazeae-maydis*) were collected from randomly selected farmers' fields in the four districts ( Kakmega, Bungoma, Busia and Vihiga) in the Western province. These materials were preserved at 5ºC for subsequent use. Single well-separated lesions were examined for sporulation under a stereomicroscope. If conidia were not observed, infected leaf with good lesions was kept in moist chamber at 25° C for sporulation. A good sporulation occurred when lesions were

incubated under moist chamber. Conidial suspension was poured and evenly spread on plain agar. Single germinated conidium from plain agar was transferred onto freshly prepared GCLDA (Green Corn Leaf Decoction Agar) and incubated at 25º C. The culture of the pathogen obtained on GCLDA was stored at 4º C for subsequent use.

***Effect of Media***

Growth and sporulation of *C. zeae-maydis* were determined on four solid media, Czapekdox agar (CDA), Green corn leaf decoction agar (GCLDA), Potato dextrose agar and V8 juice agar (V8JA). Two mm mycelia discs were cut from 14 days old culture of *C. zeae-maydis*, and one disc was placed aseptically onto each sterile Petri-plate containing 20 ml of the medium. Each plate was replicated thrice. The plates were incubated at 25° C, and average colony diam. on each medium was determined in mm every week for one month. Ten ml of sterile water was added to the culture at the end of the fourth week, shaken well and conidia dislodged with a glass slide. The conidia suspension was taken out and its conc. was determined using haemocytometer.

***Effect of Temperature***

The effect of temperature on growth and sporulation was investigated on GCLDA. Inoculated plates were incubated at 15° C, 20º C, 25° C and 30º C. The plates were replicated three times at each temperature. Average colony growth in diam. (mm) was determined at one week interval for four week. Conidial conc. was determined as described earlier.

***Effect of pH***

The effect of pH on growth and sporulation was investigated also on GCLDA. The pH of the medium was adjusted to 5, 6, 7, 8, and 9 using 1MHCL and 1MNaOH prior to autoclaving. Inoculation and determination of growth and conidial conc. were done as described before. The experiment was repeated thrice at each pH.

**RESULTS AND DISCUSSIONS**

The result on the effect of different types of media on growth and sporulation of *C. zeae-maydis* presented in Table 1 show that there was significant difference (P=0.05) between growth on the four media investigated with the highest vegetative growth on PDA followed by GCLDA and V8JA. The lowest growth occurred on CDA. A good sporulation was obtained on GCLDA followed by V8JA, and sparse sporulation on PDA and CDA. *Cercospora* species are very slow growers and known to sporulate sparsely in artificial media. Beckman and Payne (1983) failed to obtain sporulation of *C. zeae-maydis* on commonly used media. Our study shows that despite the pathogen grew slowly, attaining 24 mm of growth in a month of incubation, but varied degree of sporulation occurred in all the media with maximum sporulation on GCLDA and V8JA. Both the media contained nutrients which favored sporulation. It seems that there is genetic variation amongst the isolates of the pathogen. *Cercospora*species are known to grow and sporulate better on media which contain host and vegetable extracts (Alabi, Naqvi and Ekundayo, 1971).

The result on the effect of temperature on growth and sporulation presented in Table 2 show that there was steady increase in growth from 15º C - 25° C. Thebest growth and maximum sporulation of the pathogen occurred at 25º C. Lowest growth without any sporulation was observed at 15° C, and the growth was completely inhibited at 30° C. This is in disagreement with Beckman and Payne (1983) who reported that the pathogen grew well at 30º C.The disease development was also reported to be favoured by a temperature range of 20º C to 25° C (Beckman and Payne, 1983; De Nazareno, Lipps and Madden, 1992; Rupe, Siegel and Hartman, 1982) which coincided with growth at this temperature range in our studies *in vitro*.

In a column means followed by the same letter are not significantly (*P = 0.05*) different from each other according to Least Significant Difference (LSD) test.

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Results on the effect of pH on growth and sporulation are presented in Table 3. There was significant differences (P=0.05) between growths at different pH. The best growth and good sporulation occurred at pH 6 and 7. Poor growth with fewer spores was observed at pH 5 and very little growth with no sporulation at pH 8 and 9. This indicated that the pathogen has a very narrow pH range for growth and sporulation and thrives best under slightly acidic to neutral pH.

**Table 1: Effect of different types of media on growth and sporulation of *C. zeae-maydis*during 4 weeks of incubation at 25°C.**

| Media | Mean Colony Diameter at One Week's Interval | | | | Sporulation (Per ml) |
|---|---|---|---|---|---|
| | 1st Week | 2nd Week | 3rd Week | 4th Week | |
| **Czapekdox Agar** | 9.33 | $14.00^{b}$ | $17.00^{c}$ | $20.33^{c}$ | 0.6 x 10 |
| **Green Corn Leaf Decoction Agar** | 7.67 | $18.67^{a}$ | $24.67^{a}$ | $24.67^{ab}$ | $2.4 \times 10^{3}$ |
| **Potato Dextrose Agar** | $8.67^{b}$ | $13.00^{b}$ | $20.67^{b}$ | $27.00^{a}$ | 1.6 x 10 |
| **Vegetable Juice Agar** | $12.67^{a}$ | $12.67^{b}$ | $17.33^{c}$ | $23.00^{b}$ | $2.4 \times 10^{2}$ |
| **% CV** | | | 8.93 | | |
| **LSD** | | | 0.8741 | | |

**Table 2: Growth of *C. zeae-maydis* on green corn leaf decoction agar during 4 weeks of incubation at different temperatures.**

| Temperature | Mean Colony Diameter (mm) at One Week's Interval | | | | Sporulation (Per ml) |
|---|---|---|---|---|---|
| | 1st Week | 2nd Week | 3rd Week | 4th Week | |
| **15°C** | $6.00^{b}$ | $10.33^{b}$ | $14.33^{b}$ | $16.67^{c}$ | Nil |
| **20°C** | $8.33^{a}$ | $12.33^{ab}$ | $15.00^{b}$ | $20.00^{b}$ | 2 x 10 |
| **25°C** | $9.67^{a}$ | $14.33^{a}$ | $20.66^{a}$ | $26.67^{a}$ | $3 \times 10^{3}$ |
| **30°C** | $6.00^{b}$ | $5.00^{c}$ | $5.00^{c}$ | $4.00^{d}$ | Nil |
| **% CV** | | | 11.46 | | |
| **LSD** | | | 0.8036 | | |

**Table 3: Growth of *C. zeae-maydis* on green corn leaf decoction agar at different pH during 4 weeks of incubation at 25°C.**

| pH | Mean colony diameter (mm) at one week's interval | | | | Sporulation ( per ml) |
|---|---|---|---|---|---|
| | 1st week | 2nd week | 3rd week | 4th week | |
| 5 | $7.00^{ab}$ | $8.33^{c}$ | $12.00^{c}$ | $13.67^{c}$ | 2.0 x 10 |
| 6 | $8.66^{a}$ | $14.66^{a}$ | $20.33^{a}$ | $26.33^{a}$ | $2.8 \times 10^{3}$ |
| 7 | $7.33^{ab}$ | $10.66^{b}$ | $15.33^{b}$ | $22.00^{b}$ | 2.3 x 10 |
| 8 | $7.33^{ab}$ | $8.67^{c}$ | $10.67^{c}$ | $11.67^{d}$ | Nil |
| 9 | $6.00^{b}$ | $7.33^{c}$ | $6.67^{d}$ | $6.67^{c}$ | Nil |
| CV% | 10.29 | | | | |
| LSD | 0.6873 | | | | |

In a column means followed by the same letter are not significantly (*P = 0.05*) different from each other according to Least Significant test.

## REFERENCES

**Ajanga SG**, **Oduor M and Simmons S (2000).** Map showing the severity of infection by Grey Leaf spot on maize by districts in Kenya. *Kenya Agricultural Research Institute,* Kakamega, Kenya.

**Alabi RO, Naqvi ZHS and Ekundayo AJ (1971).** Growth and sporulation of *Cercosporaarachidicola* Hori in pure culture. *Western African Journal of Science* **19**(1) 21–26.

**Beckman PM and Payne GA (1982).** External growth, penetration and development of *Cercosporazeae-maydis*in corn leaves.*Phytopathology* **72** (3) 810–815.

**Beckman PM and Payne GA (1983).** Cultural techniques and conditions influencing growth and sporulation of *Cercosporazeae-maydis*lesion development in corn leaves.*Phytopathology* **73**(2) 286–289.

**Kinyua ZM (2004).** Genetic structure, virulence, characteristics and survival of *Cercospora*populations causing maize grey leaf spot in Kenya. Ph.D. Thesis, School of Biological Sciences, University of London.

**Kung'u JN and Boa ER (1997).** Kenya checklist of fungi and bacteria on plants and other substrates. *Kenya Agricultural reseach Institute* 96.

**Murithi LM and GathamaSK (1998)**. Grey leaf spot of maize, a new disease on increase. Crop Protection News letter, November issue.

**De Nazareno NRX, Lipps PE and Madden LV (1992).** Survival of *Cercosporazeae-maydis*in corn residues in Ohio. *Plant Disease* **76**(6) 560-563.

**Rupe JC, Siege lMR and Hartman JR (1982).** Influence of environment and plant maturity on grey leaf spot of corn caused by *Cercosporazeae-maydis*. *Phytopathology* **72**(6) 1587–1590.

**Smit E and Ward JMJ (1997).** Grey leaf spot of maize. ARC-Grain Crops Institute, RSA.