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*Full Length Research Paper*

# Comparison of two fungicide in the control of black Sigatoka (*Mycosphaerella fijiensis* Morelet), in banana cv. 'Dwarf giant ' (AAA), Teapa, Tabasco, México

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**The study was carried out in September 2016, at the Experimental Station GRAND MEND MEXICO, S.A. of C.V (GMM), Tabasco, Mexico, with the objective of evaluating the efficacy of different doses of Azoxystrobin+Tebuconazole (Azo-Teb) in the control of black Sigatoka (*Mycosphaerella fijiensis* Morelet) in banana cv. Dwarf Giant (AAA). The trial was performed on a randomized complete block design consisting of four treatments and four replicates, where two fungicides, Azo-Teb at 350, 400 and 500 mL ha$^{-1}$ and Tridemorph (Calixin 86 OL®) 500 mL ha$^{-1}$ were applied. The damage on the youngest diseased leafs (DYDL), percentage of diseased leaves (PDL), weighted average of infection (PPI) and the fungicides efficacy were evaluated. The (PDL) for Azo-Teb at 400 and 500 mL ha$^{-1}$ was 32.54 and 19.27 % respectively, and lower at Tridemorph with 12.87 %. In the DYDL the first symptoms were observed in positions 3.3 and 3.45, when the dose of 500 mL ha$^{-1}$ of Azo-Teb and Tridemorph respectively was used. The PPI, after applying Azo-Teb and Tridemorph to 500 mL ha$^{-1}$, was 0.29 and 0.17 % respectively, with no differences between them. After the third Azo-Teb evaluation at 500 mL ha$^{-1}$ it offered adequate protection against black Sigatoka with an efficiency of 91.04 %, like the commercial fungicide Tridemorph that obtained 94.8 %. Therefore, it is recommended to apply the formulation Azo-Teb in the maximum dose for control of black Sigatoka.**

**Keywords:** efficacy, azoxystrobin, tebuconazole, tridemorph

## INTRODUCTION

The Musaceae, including bananas and plantains, are the fourth most important food crop economically, after rice, wheat, and maize (Churchill, 2011).

The gross volume of global banana exports rea a record of 17 million ton., 6.1% above the 2012 level (FAO, 2015). Increase given mainly by the growing exports of Latin

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America and the Caribbean, which passed from 12.5 to 13 million tons, despite the bad results of Ecuador. Data corresponding to 2012 show an increase in the participation of Central America and Mexico with increased from 29.4% to 20.8% (FAOSTAT, 2014).

According, Churchill (2011), the causative agent of the Black Sigatoka-SN (*Mycosphaerella fijiensis* Morelet, anamorph *Pseudocercospora fijiensis* (Morelet) Deighton), is qualified as the most destructive member of the "Sigatoka complex", which includes the yellow Sigatoka-SA (*M. musicola* R. Leach ex. J.L. Mulder, anamorph *P. musae* (Zimm.) Deighton) and the Foliar spot Eumusae (*M. eumusae* Crous and Mour., anamorph *P. eumusae* Crous and Mour.). Also, the Sigatoka complex is considered the most destructive disease for banana and plantain crops, since, when attacking the leaves, it generates a rapid deterioration of the foliar area, decreases the photosynthetic capacity of these and reduces the quality and quantity of the fruit, by inducing the premature ripening of the clusters (Patiño, 2000; Bornacelly, 2009).

Hidalgo et al., (2006) reported that the net photosynthetic rate foliar (Fn; µmol de $CO^2$ reduced by $m^2$ $s^{-1}$), declined with the increase in the percentage of severity (Fn = 6.85 – 0.15 X; $R^2$ = 0.72) and the disease stage (Fn = 8.36 – 1.62 X; $R^2$ = 0.60).

Gómez et al., (2017) evaluated as a biological variable indicative of the condition of severity of the disease in the plantation (DYDL) and determined the models that were adjusted for weeks 45 and 47, showed strong spatial dependence; the model of the week 46, moderate space dependence and model of the week 43, null space dependence.

Rodriguez and Cayon (2008) stated that in leaves where grade four of severity was observed, according to, the scale of Stover modified by Gauhl (1989), there was a decrease in the photosynthetic rate close to 30%. Chillet et al. (2009) and Castelán *et al.* (2012) reported the reduction of green fruit life (6.1 to 13.8 days) in plots where the disease was not controlled, compared to plots where control was performed (24.7 to 28 days); as well as a lower fruit weight (40%) (Castelán et al., 2012). In addition, Kema (2006) and Rey and Mira (2010) assert that disease control accounts for between 20 and 30% of production costs.

For the control of black Sigatoka have been used contact fungicides like alquilenbisditiocarbamatos and chlorothalonil, also systemic inhibitors of mitosis like benzimidasole, inhibitors of the biosynthesis of ergosterol (mainly triazoles and morfolinas), inhibitors of the transport of electrons in mitochondria QoI's (estrobilurinas and oximino acetates), inhibitors of the production of enzymes in the pathogen (anilinopyrimidinas) and resistance inducers, which have generally been used in oil and water emulsion or directly in oil (Pérez, 2006).

In Mexico, the most used fungicides are mancozeb and chlorothalonil (preventive), and systemic ingredients of the groups benzimidasole, triazoles, estrobirulinas, and anilopirimidinas. In Tabasco, the chemical management of the black Sigatoka requires 48 to 52 applications of fungicides per year, mainly of mancozeb, propiconazole and tridemorph, therefore there is risk of development of resistance of the pathogen to these agrochemicals (FRAC, 2010).

The fungicides application in addition, to increasing production costs has generated reports of environmental pollution, human health, and resistance due to the large number of chemicals that are deposited in the banana orchards (Orozco et al., 2001).

Considering the high consumption of fungicides for the control of this disease, efforts are made to achieve formulations more effectively or similar that can be used in mixtures or alternations within a sustainable management in banana plantations.

Therefore, the aim of the study was to determine the biological efficacy of three doses of Azoxystrobin + Tebuconazole and a commercial fungicide (Tridemorph), for the control of black Sigatoka (*Mycosphaerella fijiensis* Morelet) in banana plants CV. ' Dwarf giant ' (AAA) in Teapa, Tabasco, Mexico.

## MATERIALS AND METHODS

### Plant materials

The work was carried out during the month of September 2016 at the Grand Mend Mexico Experimental Station, S.A. de C.V (GMM), Tabasco, México, located geographically at 17 ° 37 ' 18.90 de Latitude Norte, 93 ° 02 ' 04.93 of west longitude and at a height above the average level of the sea of 20 m, located in the town of Juan Aldama, municipality of Teapa, Tabasco, Mexico.

The cultivation of bananas used was 'dwarf giant ' (Musa AAA), Sub-group Cavendish) (Simmonds and Shepherd, 1955; Simmonds, 1962).

### Chemical treatments

The experimental design used was complete random blocks with five treatments and four replicates. The experimental unit consisted of a row of 5 plants with 2.5 m of separation (12.5 m) per 2.5 m wide (31.25 m2 per plot) and 125 m2 per treatment. The total area was 625 $m^2$ and the useful plot was the entire experimental unit (Table 1).

Each treatment was added 6.0 L $ha^{-1}$ of oil (Paroil) + Inex-A to 1% (V/V). Three applications were performed at 7day

**Table 1**. Treatments and doses evaluated in the control of the black Sigatoka (*M. fijiensis*) in banana plants cv. 'Dwarf giant ' (AAA)

| Treatments | | Active ingredient (a.i) Formulated product (g ha$^{-1}$) | | | Doses (F.P) mL ha$^{-1}$ |
|---|---|---|---|---|---|
| | | Azoxystrobin | Tebuconazole | Tridemorph | |
| **1.** | Azo-Teb $^{CS}$ | 42.0 | 70.0 | | 350 |
| **2.** | Azo-Teb $^{CS}$ | 48.0 | 80.0 | | 400 |
| **3.** | Azo-Teb $^{CS}$ | 60.0 | 100.0 | | 500 |
| **4.** | Tridemorph $^{CE}$ | | | 430 | 500 |
| **5.** | Absolute witness | - | - | - | - |

(CE) concentrated emulsifiable, (CS) concentrated suspension, (a.i) active ingredient, (F.P) product dosage formulated. (Azo-Teb)=Azoxystrobin + Tebuconazole, Tridemorf = (Calixin 86 OL®). Absolute witness (treatments without fungicide).

**Table 2**. Scale proposed by Gauhl (1989) to assess the severity of the black Sigatoka (M. fijiensis) in banana plants cv. 'Dwarf giant ' (AAA)

| Category | Foliar damage (%) |
|---|---|
| 0 | Healthy leaf |
| 1 | Up to 10 spots per leaves |
| 2 | Less than 5% of diseased leaf area |
| 3 | 6 to 15% of the diseased leaf area |
| 4 | From 16 to 33% of the diseased leaf area |
| 5 | From 34 to 50% of diseased leaf area |
| 6 | More than 50% of diseased leaf area |

intervals, using a motorized sprinkler for the application of ULV fruit (Solo®), calibrated for a 160.0 L ha$^{-1}$ water flow.
A pre-evaluation was carried out (prior to the application of the treatments) and three evaluations every 7 days.
The following parameters were analyzed:

**Damage on the youngest diseased leafs (DYDL)**

It is the first sheet counting from top to bottom that has at least 10 spots (Grade 1) (table 2). This leaf is a good indicator of the level of evolution and intensity of the disease. We calculated the number of leaves with grade 1 of the scale, added and then divided by the number of plants sampled by experimental unit (Almodóvar et al., 2006;) Foure and Ganry, 2008).

**Percentage of diseased leaves (PDL)**

The percentage was obtained by dividing the total number of leaves in each grade between the total number of leaves and then multiplying by 100.

**Weighted average of infection (PPI)**

For the calculation of this parameter, the percentage of the leaf in each degree was multiplied, by the value of the degree of the scale of Stover modified by Gauhl (1989), divided by 100 (table 2) as indicated in the formula:

$$PPI = \sum \frac{(\%\ \text{leaves in each grade} \times \text{repetitive grade})}{100}$$

**Fungicides effectiveness**

The weighted average of infection was calculated using the Abbott formula of 1925, modified by Henderson and Tilton (1955).

$\%\ Ef = [1 - (PPIa \div PPId) * (PPTc \div PPIb] * 100$

PPIa = Weighted average of infection in pree-valuation
PPIb= Weighted average of infection in the treatment after the application
PPIc= Weighted average of infection in the witness before treatment
PPId= Weighted average of infection in the witness after treatment

**Statistical analysis**

The data obtained in each evaluation for the different variables were subjected to an analysis of variance and the Tukey mean comparison test with a significance level of 5% using the statistical analysis package SAS ® 8.02 (SAS, 1996).

**Table 3.** Younger leaf disease caused by black Sigatoka, according to the treatments in banana plants cv. 'Dwarf giant ' (AAA).

| Treatments | Doses (F.P) mL $ha^{-1}$ | PRE-EVALUATION | 7 DDA | 14 DDA | 21 DDA |
|---|---|---|---|---|---|
| **Azo-Teb** CS | 350 mL | 1.25 a | 1.45 bc | 1.25 c | 1.95 c |
| **Azo-Teb** CS | 400 mL | 1.50 a | 1.60 b | 1.90 b | 2.55 b |
| **Azo-Teb** CS | 500 mL | 1.40 a | 1.75 ab | 2.40 b | 3.30 a |
| **Tridemorph** CE | 500 mL | 1.25 a | 2.05 a | 3.05 a | 3.45 a |
| **Absolute witness** | - | 1.55 a | 1.10 c | 1.10 c | 1.10 d |

Different letters in columns differ significantly with Tukey's test with a level of significance ($p<0.05$). DDA: Days after the first application. Absolute witness (treatments without fungicide).

**Table 4.** Percentage of leaves diseased according the treatments and doses in the cycle, in plants of banana CV. ' Dwarf giant ' (AAA).

| Treatments | Doses (F.P) mL $ha^{-1}$ | PRE-EVALUATION | 7 DDA | 14 DDA | 21 DDA |
|---|---|---|---|---|---|
| Azo-Teb CS | 350 mL | 52.06 a | 50.02 b | 50.46 c | 42.10 c |
| Azo-Teb CS | 400 mL | 52.48 a | 46.98 ab | 38.26 b | 32.54 bc |
| Azo-Teb CS | 500 mL | 53.96 a | 45.03 a | 31.98 ab | 19.27 ab |
| Tridemorph CE | 500 mL | 56.19 a | 43.36 a | 25.05 a | 12.87 a |
| Absolute witness | - | 54.11 a | 58.05 c | 78.80 d | 91.0 d |

Different letters in columns differ significantly with Tukey's test with a level of significance ($p<0.05$). DDA: Days after the first application. Absolute witness (treatments without fungicide).

## RESULTS AND DISCUSSION

### Damage on the youngest diseased leaf (DYDL)

During the pre-evaluation, the diseased youngest leaf (DYDL) was observed in the 1.39 position. After treatments application was observed the protective effect exerted by fungicides on the incidence of black Sigatoka, it was detected that from the second evaluation began to register the first streak-symptoms in the leaf 3.05 when treatment with Tridemorph (500 ml $ha^{-1}$) was applied, with significant differences respect to the rest of the treatments. Corroborating the reports of Pérez (2006) and Brent and Hollomon (2007), about the most used morpholines in banana cultivation were tridemorph and fenpropimorph, applied in oil or water - oil emulsion.

After three applications, the best treatment proved to be Azo-Teb (500 ml $ha^{-1}$), as it showed the youngest diseased leaf in position 3.30, without statistical differences with the treatment Tridemorph (500 ml $ha^{-1}$), which showed the first symptoms in the 3.45 leaf. In the untreated control the damage was kept present on leaf 1.10 (Table 3).

The results show that if a suitable application program is maintained, a greater number of leaves are achieved free of damage, which allows to reach the harvest with 8 to 10 functional leaves (Pérez, 2006).

### Percentage of diseased leaves (PDL)

The application program started with a percentage of sick leaves of about 53.76%.

At 7 days after first application (DAA), it oscillated between 50 and 58%. From this moment, the values began to descend by the protective activity of the fungicides, to the 21 (DAA) where a decrease of (PDL) occurred, with better performance in the treatment of Azo-TEB, (500 ml $ha^{-1}$) with 19.27% and Tridemorph in equal doses with 15.99%, without statistical differences between them, but with the rest of the treatments and the control that reached 91% of damaged leaves (Table 4).

The differences in percentage of diseased leaves (PDL), in the middle and low doses of Azo-Teb, could be interpreted as a deficiency of the formulation, however, it is important to point out that, at the beginning prior to the fungicide applications, *M. fijiensis* was already present in field with an incidence of up to 58%. Which corroborates what was raised by Almodóvar et al. (2006) who considered that the application of fungicides should be

**Table 5**. Weighted average of infection (PPI) according (Abbott, 1925, modified by Henderson and Tilton,1955), efficacy of fungicides and doses applied in plants of banana cv. ' Dwarf giant ' (AAA).

| Treatments | Doses (F.P) mL ha$^{-1}$ | PRE EVAL. | 7 DDA | | 14 DDA | | 21 DDA | |
|---|---|---|---|---|---|---|---|---|
| | | PPI | PPI | Ef. | PPI | Ef. | PPI | Ef. |
| Azo-Teb $^{CS}$ | 350 mL | 0.96a | 0.86 b | 41.83b | 0.91c | 61.62b | 0.76c | 75.79b |
| Azo-Teb $^{CS}$ | 400 mL | 1.01a | 0.69a | 55.64a | 0.58b | 76.75a | 0.47bc | 85.77b |
| Azo-Teb $^{CS}$ | 500 mL | 0.99a | 0.61a | 59.99a | 0.45a | 81.60a | 0.29ab | 91.04a |
| Tridemorph $^{CE}$ | 500 mL | 1.00a | 0.65a | 57.79a | 0.42a | 82.29a | 0.17a | 94.80a |
| Absolute witness | - | 1.00a | 1.54c | - | 2.47d | - | 3.27d | |

Different letters in columns differ significantly with Tukey's test with a level of significance ($p<0.05$). DDA: Days after the first application.

done in a timely manner, before the fungus infects the primary leaves.

According, Guzmán and Romero (1997), the fungicide Azoxystrobin, has the ability to move through the interior of plant in systemic way, through the sap and has infection control, decreasing the percentage of diseased leaves.

In correspondence, Martínez et al. (2012), in banana plantations in Tabasco, determined the susceptibility of isolates of *M. fijiensis* to Tridemorph by the reduction of the percentage of diseased leaves, given the preventive and curative action that blocks the agent.

**Weighted average of infection (PPI)**

At the start of the application program, a 0.99% PPI was detected, without difference between treatments.

The lowest values of PPI were reached at 21 (DDA), highlighting the treatments with Tridemorph and Azo-Teb at the highest dose, 500 ml ha$^{-1}$, with 0.17 and 0.29 respectively, without difference between them, but with the rest of the treatments (Table 5).

The reduction of this biological index is indicative of the decrease in the severity of the disease in the field (Hernández et al., 2001). Values were below 2 in all treatments, which expresses a low rate of damage, and adequate management of the disease in these treatments, compared with the witness who maintained values greater than 1 from the beginning, reaching the end with a weighted average of infection 3.27.

**Fungicides effectiveness**

Efficacy of fungicides was in correspondence with the PPI observing a better result at 14 DDA with 50.7 and 82.29 % at high doses of Azo-Teb and Tridemorph respectively. But its effect was better at 21 DDA, that is after three applications where the treatment with Tridemorph achieved a 94.80 % and Azo-Teb (500 ml ha$^{-1}$) obtained 91.04 %, without statistical differences between them, but yes with the rest of the treatments.

This evidences that the Azo-Teb formulation is a good fungicide for the control of black Sigatoka in the field, with results very similar the commercial witness Tridemorph.

To respect some authors (Frac, 2010), have stated that Tridemorph is an important component in strategies to avoid resistance with fungicides from the groups of benzimidasole, estrobirulinas and triazoles. Meanwhile, others add that they have an excellent efficacy in mixtures with triazoles to 75% of the doses of both active ingredients (Pérez, 1993).

The efficacy of the Azo-Teb formulation could be increased and especially with the lowest doses, if applied with a lower inoculum pressure in the initial stage. This would reinforce the idea that the best products should be applied in a timely and alternating manner, together with other measures establishing an adequate handling of black sigatoka in the field.

The differences in the fungicides efficacy, in the handling and control of black Sigatoka, allow to recognize that is a critical factor limiting the success of control of this pathogen, by having to increase the doses or frequency of the sprays on bananas and bananas (Mena and Couoh, 2015).

**CONCLUSIONS**

A reduction in the percentage of diseased leaves (PHE) was obtained by applying Tridemorph 500 ml ha$^{-1}$ (15.99

%), similar formulated Azoxystrobin + Tebuconazole (400 and 500 ml $ha^{-1}$ with 32.54 and 19.27% respectively.

In the DYDL the first symptoms corresponded to positions 3.3 and 3.45, without differences when applying the dose of 500 ml $ha^{-1}$ of formulated Azoxystrobin + Tebuconazole and Tridemorph.

The weighted average of infection (PPI) had a considerable decrease, after three applications of the fungicides Azoxystrobin + tebuconazole and Tridemorph to (500 ml $ha^{-1}$) with 0.29 and 0.17% respectively.

It was found that formulated Azoxystrobin + Tebuconazole applied to 500 ml $ha^{-1}$, offers adequate protection against black Sigatoka (*M. fijiensis*) with efficacy of 91.04%, without difference with the commercial witness (Tridemorph) who achieved 94.8% in equal doses.

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