VALIDATION OF PRODUCT MBS-11 (CM ARMOR) IN CHIAPAS

2016

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Before the coffee leaf rust appeared, the disease caused no significant economic damage to production, although the cultivated varieties present high susceptibility to various pathogens; in some cases, circumstantial epidemics were recorded in certain years or in certain localities, which caused damage to coffee production. Such records are always associated with atypical weather conditions, improper handling of the coffee plantation or nutrition problems of coffee trees (Almeida,1986).

However, rust damage manifests in severe defoliation in coffee plants that results in significant reductions in production, depending on the severity of the attack (Macías, 2001).

Currently Chiapas has an index of severe mortality due to the amount of fungus infestation, the failure to find a solution that controls the fungus. Beneficiaries are abandoning their plots or prefer to destroy them to plant other crops resistant to coffee leaf rust fungus without knowing that that would lead to not having quality coffee that does not present organoleptic characteristics as flavor, color, aroma, and acidity.

1.1 Pollution

The attack of the coffee plant rust begins with the release of pseudo spores, the most important reproductive structure of this fungus, which can linger year after year in this state. These spores germinate between 3 and 12 hours. This serves as a kind of tube germination progresses beginning when a drop of water finds an open stoma on the lower leaf surface. Immediately, between the intercellular spaces, the mycelium begins to develop and organs called Haustoria appear, whereby, rust penetrates the inside of the cells and starts to feed on the leaf tissue. Between 10 and 15 days after the start of the attack as the leaf tissue necrotizes, it is possible to see yellowish spots turning brown. The emergence of new pseudo spores can take place within 15 days, more or less, although the incubation period depends on weather conditions.

1.2 DEVELOPMENT OF FUNGUS

On the undersides of these spots, an orange powder appears, when touched it feels like rust, consisting of several hundred thousand pseudo spores, with the help of the wind, the rain, the passage of animals and people or transfer of vegetative material, among others. They are distributed by the same coffee tree leaves, neighboring coffee trees and nearby coffee plantations.

The most susceptible to attack by Roya leaves are the young leaves. This immediately slows down the development of the coffee tree. It is precisely these leaves that are starting their period of full physiological activity that provide the most nutrients to the plant. When attacked by fungus and between 10% and 30% is necrotic tissue, they are no longer functional. Also, the fungus produces ethylene, the leaves age and fall prematurely. If the attack is severe, the plant reduces its growth, fruits do not grow and this generates large economic losses.

2. OBJETIVE:

The biological effectiveness of the product "MBS-11" for controlling coffee rust (Hemileia vastatrix) was evaluated in the field. The main function of MBS-11 is crop protection, through a nanometer protective layer, which prevents the development of fungi, viruses and bacteria.

3. APPROACH

Healthy plants (asymptomatic) Geisha coffee are sampled in order to determine and evaluate the transmission of the coffee leaf rust fungus, Hemileia vastatrix. The test was conducted at 940 meters under extensive conditions (field), in the town of [redacted] Chiapas. Two lots of healthy plants (asymptomatic), one protected by MBS-11 and the other without treatment, provided by the company [redacted] and applied by foliar, allowing them to dry before introducing the experimental design with the plants infected with rust. We looked for suitable climatic conditions for propagation. Later, the development conditions of the fungus were partially monitored during the necessary period until sporulation

Photo 1. *Healthy plants (Asymptomatic) used in the different lots*

RESPONSIBLE: [redacted]

MUNICIPALITY OF ESTABLISHED VALIDATION:

[redacted]

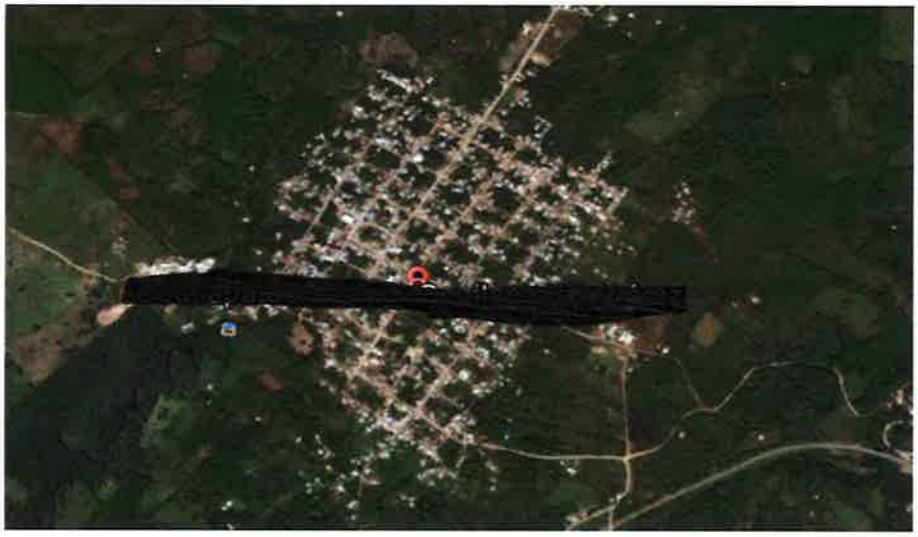
LOCATION OF ESTABLISHED VALIDATION:

[redacted]

HEIGHT: 940 m.s.m.n

Latitude	16 grados	**Minutes**	51	**Seconds**	22
Longitude	93 grados	**Minutes**	24	**Seconds**	39

SKETCH OF VALIDATION LOCATION



PLANT DATA:

FIRST VALIDATION LOT

- **VARIETY:** Geisha
- **NURSERY:** [redacted]
- **AGE:** 12 months
- **COMMUNITY:** [redacted]
- **MUNICIPALITY:** [redacted]
- **OWNER:** [redacted]
- **START DATE OF VALIDATION**: 07/06/2016
- **FINAL DATE OF VALIDATION:** 10/06/2016

SECOND VALIDATION LOT

- **VARIETY**: Bourbon
- **NURSERY**: nursery throughout seedbed collection
- **AGE**: 12 months
- **COMMUNITY**: [redacted]
- **MUNICIPALITY**: [redacted]
- **OWNER**: [redacted]
- **START DATE OF VALIDATION:** 09/19/2016
- **FINAL DATE OF VALIDATION**: NOT DETERMINED

DELIVERABLES:

- An end of the "Assessment of biological effectiveness of the product" MBS-11 "for controlling coffee rust (Hemileia vastatrix)" report.

- Final scientific report

4. MATERIALS AND METHODS

COMMON AND COMMERCIAL PRODUCT NAME:

Trade name: **"MBS-11"**

Common name: **CHAPE ANTIMICROBIAL**

Composition of commercial product **"MBS-11":**

An inert nanometer layer composed of colloidal silicon, which mechanically protects all surfaces against microorganisms such as fungi, viruses and bacteria. The MBS-11, in concentrated form, is composed of 90% colloidal silicone and 10% deionized water.

Said protective layer MBS-11, does not destroy biological or chemical reactions, but acts through mechanical contact with the microorganism. The protective layer prevents contact with the protected surface and therefore prevents infection.

FORMULA AND CONCENTRATION: 100 mL. Of "MBS 11" in 60,000 dilution mL. deionized water. The doses of each treatment were delivered by [redacted] abeled for specific use.

STUDY SITE: The analysis of the above treatments was conducted in the laboratory of [redacted] located in [redacted] Chiapas.

PHENOLOGICAL STATE: We evaluated [this product] with completely healthy plants (asymptomatic) free of any contamination, infestation or disease as well as no previous treatments or use of chemical products.

TIME AND FORM OF APPLICATIONS: For purposes of the test, the manual application was applied with a micro sprinkler type spray or fog nozzle. The leaves were sprayed liberally, noting that the underside is completely covered with the product. The application was applied at the time of day with the highest temperature, when the foliage and / or leaf is free of excess moisture, making sure [there would be] no rain in the next 75 minutes after the application was applied. Application intervals will be according to the timetable.

5. EXPERIMENTAL DESIGN

In the experimental site, two blocks of coffee plants are selected; treatments in each block were set as described:

BLOCK 1 (LOT 1) VARIETY (GEISHA)	BLOCK 2 (LOT 2) VARIETY (GEISHA)
GREEN PLANTS= 20 SAMPLES YELLOW RIBBION PLANTS= 20 SAMPLES	GREEN PLANTS= 20 SAMPLES

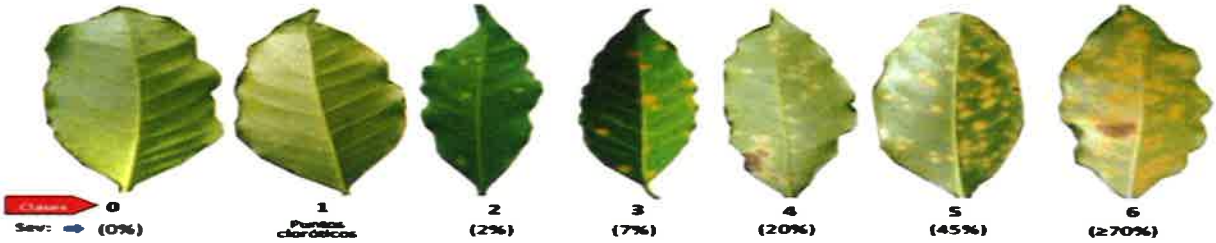
DESCRIPTION:

ABSOLUTE WITNESS: GREEN PLANTS WITH APPLICATION OF MBS-11.

INFESTATION WITNESS: YELLOW PRODUCT "MBS-11" dose 1 (was applied once at the start of the test to green ribbon plants.)

NOTE: NO OTHER PLANTS WERE COLLECTED BECAUSE THE SEVEN AREAS OF STATE PRESENTS AN INDEX OF MORTALITY AND HIGH CONTAMINATION.

INDEX OF CONTAMINATION



SOURCE: [redacted]

6. EVOLUTION OF THE FIELD RESULTS ARE:

BLOCK 1 (FIELD LOT 1) VARIETY (GEISHA)

START DATE OF VALIDATION: 07/06/2016

DATE OF FIRST MONITORING: 07/27/2016

Photography 2. *Installation and monitoring of samples in field of green plants treated with the MBS-11 product without the presence of rust fungus*

***Photography 3**. Results of yellow colored untreated samples in the a lot where a level of rust fungus infestation is detected around 1-2%*

DATE OF SECOND MONITORING: 09/19/2016

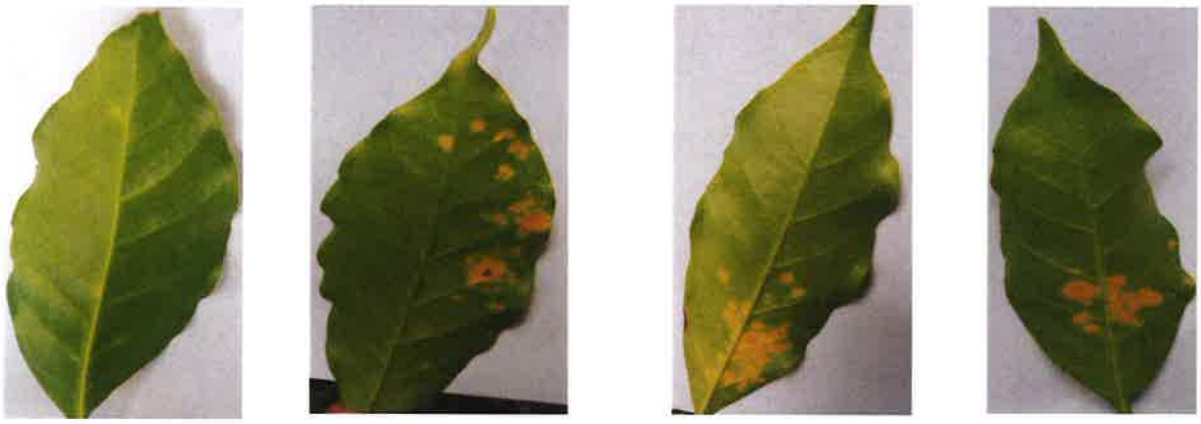
Photography 4. Results of green samples treatment with the lot where there is still no presence of fungus

Photography 5. *Results of yellow colored untreated samples in the same lot, where an increased level of rust fungus infestation is detected around 5-7%*

DATE OF FINAL MONITORING: 10/06/2016

Photography 6. *Results of green colored samples treated with MBS-11 in the a lot, which is not detected or an infestation of rust*

Photography 7. *Results of yellow colored untreated samples in the same lot, where an increased level of rust fungus infestation is detected around a 7-45%*



Photography 8. *Results of yellow colored untreated samples in the same lot, where an increased level of rust fungus infestation is detected in the leaves around a 7-45%*

BLOCK 2 (LOT 2[redacted], VARIETY (GEISHA)

START DATE OF VALIDATION: 07/06/2016

DATE OR FIRST MONITORING: 07/27/2016

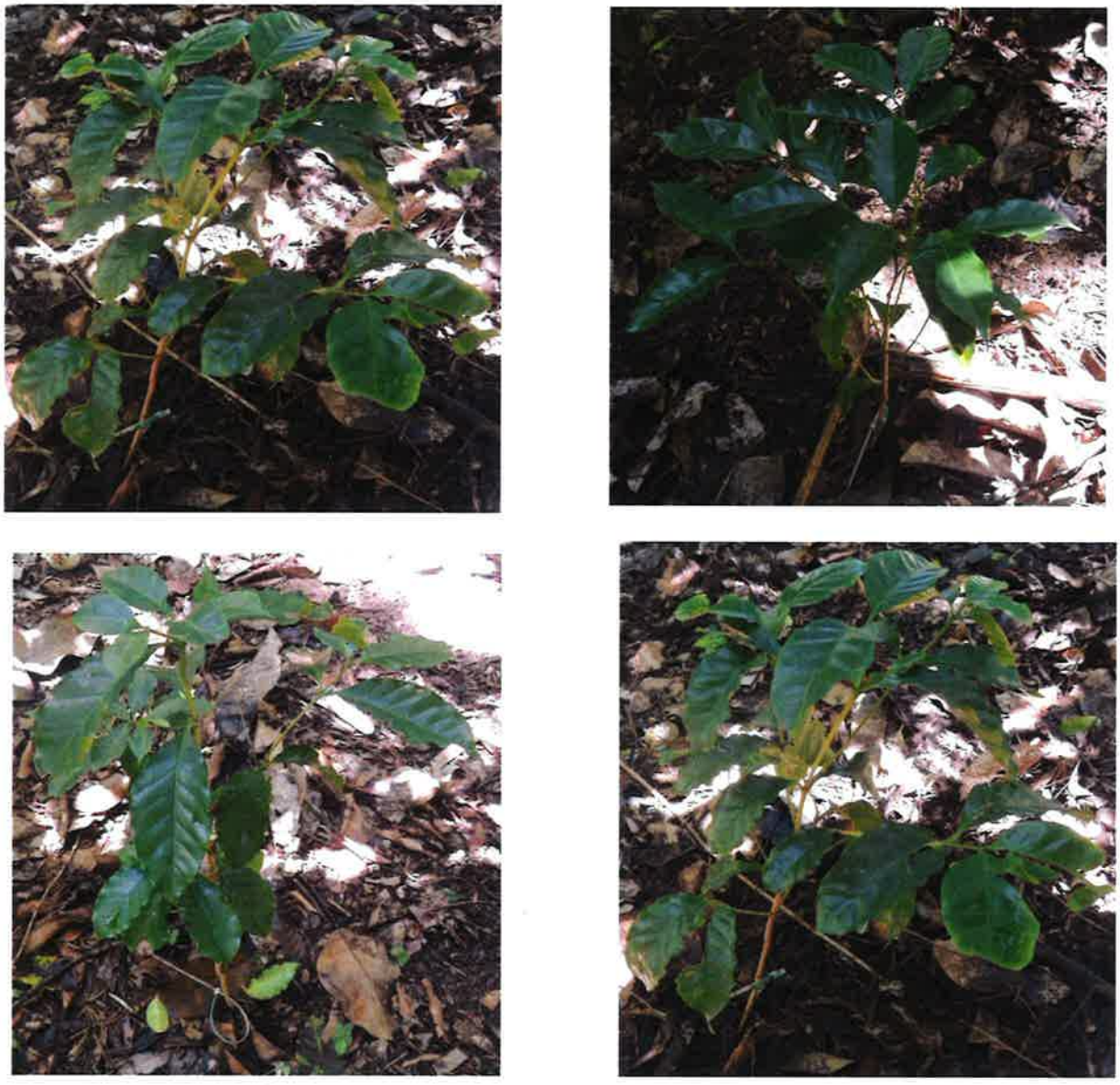
Photography 9. *Monitoring two blocks where all samples were treated with the MBS-11 product under conditions with mature plants and seedlings infested within the same site, having a null result of infestation is the control plants*

DATE OF SECOND MONITORING: 09/19/2016




Photograph 10. *Monitoring two blocks where all samples were treated with the MBS-11 product under conditions infested with adult plants and seedlings within the same site, having a null result of infestation is the control plants*

DATE OF FINAL MONITORING: 10/06/2016



***Photograph 11**. Monitoring two blocks where all samples were treated with the MBS-11 product under conditions infested with adult plants and seedlings within the same site, having a null result of infestation is the control plants*

7. CONCLUSIONS

For approximately the past 5 years, the state of Chiapas has been going through a disastrous crisis in the coffee sector because of the damage caused by coffee leaf rust. It is urgent to control or eradicate the problems of this disease that is present in the state. The MBS-11 product is the alternative for the country due to its high effectiveness which allows us to claim that this product can save the varieties that are not resistant to rust, which provide organoleptic quality. The validation observes a gap growth of rust fungus on treated coffee plants, giving a result considered effective to repel the fungus. Given the results obtained in the field, it should be mentioned that [this product] not only repels fungi, the plants have improved in appearance as noted by the presence of nitrogen in the leaves, a higher photosynthetic cycle in the coffee plants and increase in their vigor and quality.

The quality index of the product achieves a value of 10 (on a scale of 0 to 10) considered because of the protection noted on the coffee plant throughout the validation phase and without a doubt is the best alternative to save coffee varieties that were devastated by rust production that left many of the small coffee producers without production.

8. Reccomendations

The application of the product MBS-11 in the field should be applied once a month at a dose of 18 ml in 10 Lt of water for greater efficiency and control of leaf rust and continue appropriate cultural practices such as pruning, fertilization based on micro and macro essential nutrients and constant monitoring of plots.

9. ANNEX

9.1 Laboratory Report

To isolate Hemileia vastatrix, samples were collected from plants with symptoms of the disease caused by coffee leaf rust. These plants with symptoms of the disease were moved to the research laboratory of [redacted] located at [redacted] meters. When the plants were placed in the laboratory, the leaves most affected based on the impact of the fungus were taken [to be used] (Figure 1).

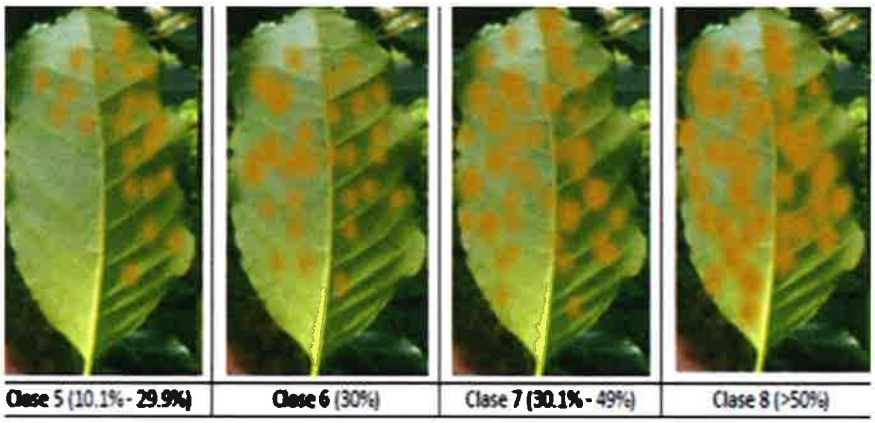


Figure 1. Reference of levels of damage on the coffee tree leaves caused by Hemileia vastatrix are used for this sample

9.2 Isolation of Hemileia Vastatrix

Procedures based on the utilization of plant traps or baits were evaluated to catch the fungus. Later, the fungus was isolated in selected mediums to identify the species Hemileia vastatrix. The plant traps were made with chopped leaves were the most utilized in the determination of the existence of spores of the fungus. To prepare the traps, 25 grams of sick leaves were weighed and then suspended in 100 mL of distilled water; the suspension was shaken and was poured into glass jars (diameter 1.5 x 6.5 cm high) with a screw top and bakelite cap of 5 to 7 mL. The glasses were incubated under laboratory conditions (30 ° C) for 10 days, periodically observed by electron microscopy ( ) according to (2016). After the third day, the determination of the presence or absence of fungus spores [is observed].

9.3 Inoculation and incubation of Hemileia vastatrix

The fungus is isolated in flasks with distilled water in order to remove the excess water that is filtered through filter paper (Whatman 2: 8 .mu.m), then it was inoculated in a medium of Potato Dextrose Agar (PDA) and Honey peptone Agar (HPA) according to Cruz et al. (2012), finally they incubated at 28 ° C for 20 days to allow the development and sporulation of the fungus.

After 20 days of incubation 1cm squared of PDA and HPA were transferred with mycelium Hemileia vastatrix to a flask 500mL with liquid honey. Later, it went to an incubator at 28 ° C at a relative humidity of 65% for 15 days.

9.8 Experiments Design

To design the experiments, coffee plants of the Geisha species were used. For this purpose, a completely randomized design was used with a total of 40 plants for 2 treatments, 20 plants per treatment. Such distribution is identified below:

Table 1. Experimental distribution

Treatments	Total of Samples Coffee Plants
Plant +Biological Reactive control	20
Plants + + Infection (PI)	20
Total	40

A variance analysis for each variable will be done through the statistical software program InfoStat, considering sources of variation as a crop system, t 9.9 Determination of the occurrence of coffee rust the three treatments and repetitions; with a comparison of Tukey ($p \leq 0.05$).

9.9 Determination of the occurrence of coffee rust

The study was conducted to know the rates of occurrence where they found rust on coffee plantations and also to know the different areas where more or less damage occurs. An existing report on the delimitation and geographical situation of the location was used, [which also outlined] sub locations. Sampling was determined as follows: First the area is traversed and leaf samples were collected for each treatment, each treatment with 20 assessed coffee plants, trying to cover the entire area.

Three branches are chosen for each plant, corresponding to the low, middle and upper parts of the plant, collecting 9 coffee leaves per plant and 180 leaves per treatment. The percentage of the rate of occurrence was calculated by dividing the number of evaluated leaves by the number of leaves affected by rust. To analyze the data collected, a

procedure recommended by the institution SENASA of Peru 2003 was used. It is represented below:

Determining the occurrence of coffee rust is as follows: Number of leaves with rust / total sampled leaves * 100

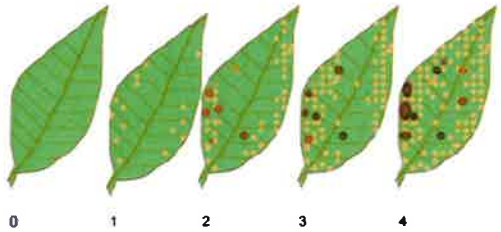
9.10 Determination of the severity of coffee rust

Three branches were randomly selected from each plant, taken from the low, middle and upper parts of the plant. Collecting 9 coffee leaves per plant, trying to cover [each part] of the plant, for a total [of] 180 leaves per treatment. The observed symptoms determined the severity of the disease, according to the percentage of damage on the leaf. To analyze the data collected in the field and the determination of the variables of the study, the procedure recommended by the institution of SENASA of Peru, which is presented below, was used. Table 2 shows the severity scale in each leaf according to the symptoms observed.

Table 2. Scale to determine the degree of infection or damage by Hemileia vastatrix

Grade	Symptoms
0	Healthy or without visible symptoms
1	Visible symptoms of about 1% to 5% of the whole area of the plant
2	The spots start touching each other and occupy about 6% to 20% [of the plant]
3	The leaves start necrotizing with about 21% to 50% affected
4	More than 50% of the area of the leaf is affected

Figure 2. *Degree and Severity scale of Hemileia vastatrix*



Determining the severity of coffee leaf rust was done with the formula:

SEV = (N0 * 0) + (N1 * 1) + (N2 * 2) + (N3 * 3) + (N4 * 4) (100) N * 4

Where: N0 = # Leaves with value 0 on the scale. N1 = # Leaves with value 1 scale. N2 =

Leaves with value 2 of the scale. N3 = # Leaves with value 3 scale. N4 = # pages with the scale value 4

9.11 Validation

In the first instance, IMAGE TOOL 3Æ0 (UTHSCSA, 2010) software was used to classify lesions based on size and shape. Finally, the preparation of standard area diagram (SAD) was done with the software program QUANT establishing a set of diagrams of the severity of the disease, which clearly indicate the levels of disease severity at 10, 20, 40 and 80%.

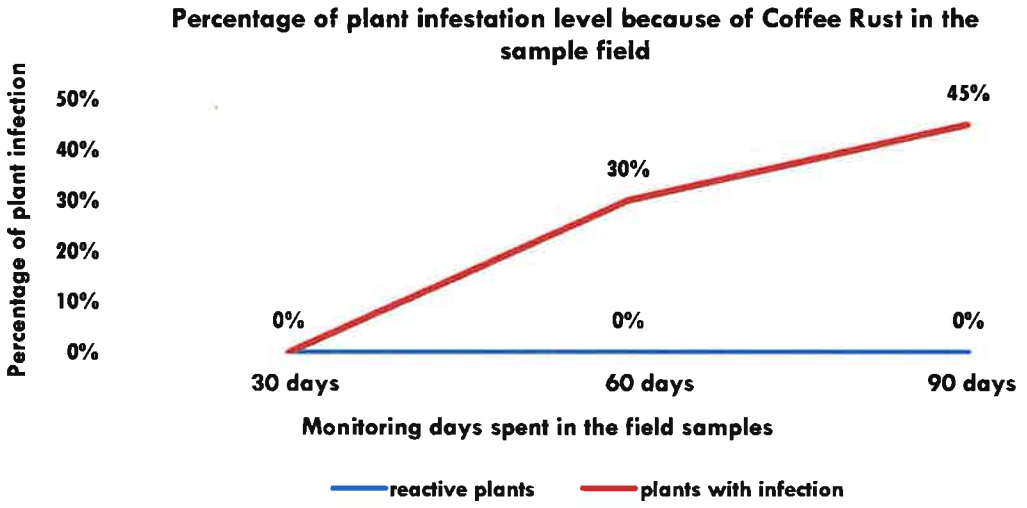


Figure 4. The monitoring is based on comparative levels of plants with the product and plants without the product with rust infection during three stages of 15 days. For each stage where the occurrence of rust infection is quite noticeable, the product strength was effective in obtaining a 100% plant health, taking into account that the samples were taken homogeneously and randomly.

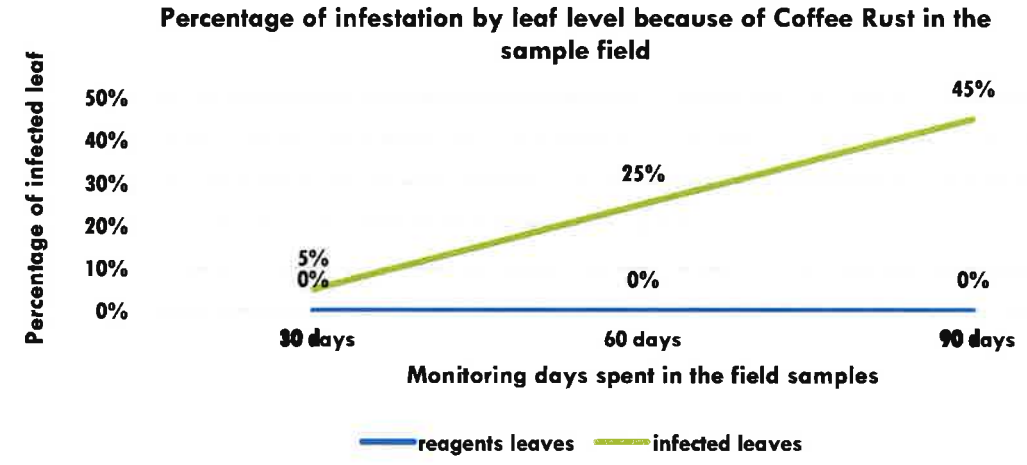


Figure 5. In this evaluation two types of variables are taken in two ways to measure the applied product to which the results are 100%

9.4 Counting Hemileia vastatrix by optical density

To verify the colorimetric changes induced for crop growth, the optical density (OD) of the crop was determined.
To all the tubes 6 mL of Hemileia vastatrix was added in a logarithmic growth phase. Readings were taken with a λ to 450 nm. 3 replicates per treatment (Meneses 2001) were analyzed.

9.5 Experiment Location

The experiment location is located in [redacted] The Municipality of the State of Chiapas, Mexico, with a latitude of [redacted] t also recognizes a temperature of 20 ° C - 25 ° C, a luminous intensity of 2.5 plugs / ft and a relative air humidity of 95%.

9.6 Plant material

The plants used for the experiment were coffee plants of the Geisha species both with opposite, oval or oblong dark green colored leaves at an age of 12 months.

9.7 Disseminating Hemileia vastatrix in the field

After incubation and a mold count using the spectrophotometer method, a plant is sprayed with a concentration of 1x106 CFU / ml in order to achieve high sporulation between plants.

10. REFERENCIAS

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