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Agroecological management of anthracnose (*Colletotrichum gloeosporioides*) in the flowering and fruiting stages of mango *(Mangifera indica)*

Manejo agroecológico de antracnosis (*Colletotrichum gloeosporioides*) en las etapas de floración y fructificación del mango *(Mangifera indica)*

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Abstract

Anthracnose is the main disease affecting the mango crop, especially during the flowering and fruit set stages. The objective of the research is to evaluate the efficacy of three agroecological products of mineral and biological origin copper sulfate pentahydrate, sulfocalcic broth and *Bacillus subtilis* for the control of the fungus *Colletotrichum gloeosporioides* in the flowering and fruiting stages of the Tommy Atkins variety of mango. A randomized complete block design (RCBD) was used, with 5 treatments and 4 replicates. The treatments with the highest degree of affectation were the absolute control T5 and *Bacillus subtilis* T2 with a percentage higher than 70% for the variables described; on the other hand, the treatments with the least affectation were copper sulfate pentahydrate T1, sulfocalcic broth T3 and

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the conventional control T4. The treatments copper sulfate pentahydrate T1 and sulfocalcic broth T3 obtained a similar result of efficacy in the control of anthracnose to that obtained with the conventional control T4 of synthetic origin. Of the three agroecological products evaluated, copper sulfate pentahydrate and sulfocalcic broth achieved a better efficacy result than *Bacillus subtilis*. Therefore, the application of copper sulfate pentahydrate and sulfocalcic broth is recommended as a viable alternative for the control of anthracnose in Tommy Atkins mango.

Keyword: Consumption, feeding, organoleptic, treatment, analysis.

Resumen
La antracnosis es la principal enfermedad que afecta al cultivo de mango especialmente durante las etapas de floración y amarre de frutos. El objetivo de la investigación es evaluar la eficacia de tres productos agroecológicos de origen mineral y biológico sulfato de cobre pentahidratado, caldo sulfocalcico y *Bacillus subtilis* para el control del hongo *Colletotrichum gloeosporioides* en las etapas de floración y fructificación del mango variedad Tommy Atkins. Se usó el diseño estadístico por bloques completos al azar (DBCA), con 5 tratamientos y 4 repeticiones. Los tratamientos con mayor grado de afectación fueron el testigo absoluto T5 y *Bacillus subtilis* T2 con un porcentaje mayor del 70% para las variables descritas, por otro parte, los tratamientos con menor afectación fueron sulfato de cobre pentahidratado T1, caldo sulfocalcico T3 y el testigo convencional T4. Los tratamientos sulfato de cobre pentahidratado T1, y caldo sulfocalcico T3 obtuvieron un similar resultado de eficacia en el control de antracnosis al obtenido con el testigo convencional T4 de origen sintético. De los tres productos agroecológicos evaluados, el sulfato de cobre pentahidratado y el caldo sulfocalcico alcanzaron un mejor resultado de eficacia que *Bacillus subtilis*. Por consiguiente, se recomienda la aplicación de sulfato de cobre pentahidratado y caldo sulfocalcico como alternativa viable en el control de antracnosis en el cultivo de mango variedad Tommy Atkins.

Palabras clave: Consumo, alimentación, organoléptico, tratamiento, análisis.

Introduction

Mango is one of the finest tropical fruits and appreciated by consumers worldwide, this fruit is native to India, it is believed that it began to be cultivated 2,000 years before Christ, although it is assumed that it was already known long ago. Some botanists estimate that this plant was domesticated by man 6000 years ago (Jara, 2011). "Mango cultivation in Ecuador had its beginnings in 1980 and today it is one of the most important export products that contribute significantly to the GDP and sustainability of the national economy" (Merino and Tandazo, 2015).

This species is mainly developed in the province of Guayas, with an approximate area of 7,700 hectares registered in full production, and of which approximately 6,500 are dedicated to export. The remaining, are dedicated to other local markets, Andean pact or to the production of juices and fruit concentrate (Fundación Mango Ecuador, 2019).

The productive areas for export contemplate the Tommy Atkins varieties which represents 65%, followed by the Kent variety with 17% and the Atahulfo variety with 11%, according to data from the Mango Foundation, the country exports on average about 11.4 million boxes of mango, generating income of $42 million dollars to the country (Farinango, 2018).

Among the main problems that hinder the good development and yield of mango cultivation in Ecuador are the presence of pests and diseases, the main problem being the disease caused by the fungus *Colletotrichum gloeosporioides,* commonly known as anthracnose, which according to Santos-Villalobos et al. (2011), due to its high incidence and severity, significantly reduces crop yields by up to 90%.

To control anthracnose, growers currently use multiple applications of fungicides formulated with different chemical ingredients that pollute the environment and put at risk the sustainability of pest management in mango cultivation, since every year there are increases in the prices of agrochemicals. In this context, the objective of this research was to evaluate the efficacy of three agroecological products for the control of anthracnose (*Colletotrichum gloeosporioides*) in the flowering and fruiting stages of the Tommy Atkins variety of mango.

Materials and methods

The present research was carried out at "El Clavo N° 2" farm, located in the province of Guayas, Canton Colimes, Km 86 of the Palestina - Balzar road. The plant material used was Tommy Atkins, whose methodology consisted of implementing the experimental design of completely randomized blocks with five treatments, with three replications. Three agroecological products were used: copper sulfate pentahydrate, sulfocalcic broth and *Bacillus subtilis* to measure efficacy results against a conventional control and an absolute control. The degree of severity and incidence of the disease was determined by direct observation, according to the previously established scale of affectation and recorded in a field record. The number of experimental plots was a total of 20, in which Tukey's comparative means were used with a 5% probability.

The area of the experimental unit was 11345 m2, the number of treatments 5 and 4 replications. With a planting spacing of 9m X 7m. with a planting density of 159 plants per ha.

Table 1. *Description of treatments*

TREATMENTS	DOSAGE P.C/lt of water	Lt of water/plt	N° of trees x treatment.	Total lts of water x treatment.	Total commercial product	P.C/ha 159 trees (9 x 7)	Frequency of application
T1: Copper Sulfate	2.5 cc	4	36	144 lts	360 cc	1.6 lts/ha	1-15-30-45-60 days
T2: *Bacillus subtilis*	6 gr	4	36	144 lts	864 g	3.8 kg/ha	1-15-30-45-60 days
T3: Sulfocalcic Broth	10 cc	4	36	144 lts	1,440 cc	6.36 lts/ha	1-15-30-45-60 days
T4: Witness Conv. (Tebuconazole)	1.25 cc	4	36	144 lts	180 cc	0.8 lts/ha	1-15-30-45-60 days

T5: Absolute witness.	NA	NA	NA	NA	NA	NA	NA

Trial management

The application of the products was carried out once the flower buds were swollen, that is, in the bud stage. The products were applied five times with motorized backpack pumps at 15-day intervals and the data was recorded every 14 days after each application and ended at the time of harvesting the fruit. At flowering time, the total number of trees was counted and with the help of a technical sheet, the basic flowering date was recorded for each experimental unit in the blocks when they had between 5 and 10% of inflorescence.

Number and length of flower panicles (n)

When the plantation reached the basic flowering stage, 20 flower panicles were selected at random from each central tree located within the 20 experimental plots that made up the study area, according to the four cardinal points, taking into account the most productive area of the tree, which corresponds to the level of the middle third to the bottom of the tree crown, and the unit of measurement to determine the length of the panicles was expressed in linear centimeters.

Degree of affectation of floral panicle and fruit (%)

The direct observation method was used to measure the degree of damage to flower panicles, based on a modified reference scale from 0 to 6, as detailed in Table 2, and for fruits, a reference scale described in Table 3 was used, also valued from 0 to 6, considering the presence of disease symptoms and damage caused to the plant tissue.

Table 2. *Levels, degree of severity and percentage of damage to panicles*

Degree of damage	% of area affected
0	Healthy panicle
1	less than 5% of the affected area
2	6 - 15 % of the affected floral area
3	16 - 25 % of the damaged area (1/8)
4	26 - 50 % of the damaged area (1/4)
5	51 - 70 % of the affected area
6	100% of the affected area

Table 3. Levels, degree of severity and percentage of damage to panicles

Degree of damage	% of area affected
0	Completely healthy fruit
1	Signs of infection spots smaller than 2 mm2
2	less than 5% of the damaged area

3	up to 12.5% of the damaged area (1/8)
4	up to 25 % of the damaged area (1/4)
5	up to 45 % of the affected area
6	more than 45 % of the affected area

The percentage of incidence (%) was determined according to the presence of the disease in the experimental unit and the sample unit (number of panicles and diseased fruit) in 20 panicles selected from the central tree of each experimental plot for each treatment.

The formula to evaluate the percentage of incidence in floral panicles was the following:

$$\boldsymbol{Incidencia}\ (\boldsymbol{I}) = \frac{Número\ de\ paniculas\ florales\ \ enfermos}{Número\ total\ de\ paniculas\ florales\ \ evaluados}\ x\ 100$$

The formula for evaluating the percentage of incidence in fruit was as follows:

$$\boldsymbol{Incidencia}\ (\boldsymbol{I}) = \frac{Número\ de\ frutos\ enfermos}{Número\ total\ de\ frutos\ evaluados}\ x\ 100$$

The severity percentage (%) was established according to the degree of disease affectation in the experimental unit and the unit of sample damage in panicles and fruits. In 20 panicles selected from the central tree of each experimental plot per treatment.

The data collected in the field were analyzed and transformed using the following formula (Anculle and Álvarez, 2006).

The formula to evaluate the percentage of severity in flower panicles was the following:

$$\boldsymbol{Severidad}\ (\boldsymbol{IS})(\%) = \frac{\sum(\ N°\ de\ panículas\ evaluados\ x\ cada\ grado\ )}{N°\ \ de\ panículas\ evaluador\ x\ grado\ mayor}\ x\ 100$$

The formula to evaluate the percentage of severity in fruit was the following:

$$\boldsymbol{Severidad}\ (\boldsymbol{IS})(\%) = \frac{\sum(\ N°\ de\ frutos\ evaluados\ x\ \ cada\ grado\ )}{N°\ \ de\ frutos\ evaluador\ x\ grado\ mayor}\ x\ 100$$

The number of fruits for each treatment (n) at the time of harvesting the central tree of each experimental plot, the total number of fruits obtained for each treatment was recorded.

Fruit weight (kg) was recorded in each experimental unit within each plot where the treatments were applied, diameter and weight were recorded and the result was expressed in kilograms.

Yield (kg/ha) was evaluated by taking the average fruit weights obtained in each treatment and expressed in kilograms per hectare.

Result

This research work evaluated the efficacy of three agroecological products based on mineral and biological extracts (Copper Sulfate Pentahydrate, Sulfocalcic Broth and *Bacillus subtilis*) in comparison with a conventional control (Tebuconazole) and an absolute control (without application) for the control of the fungus *Colletotrichum gloeosporioides* during the flowering and fruiting stages in the Tommy Atkins mango crop:

During the time of evaluation of the disease, the characteristic symptoms were only evident during the flowering stage with the presence of necrotic spots of different shapes and sizes that caused the drying and death of the epidermal tissue of the floral panicles. Based on the percentage of incidence and degree of severity of the fungus *Colletotrichum gloeosporioides* in this research, it was determined that the treatments with the highest degree of affectation were the absolute control (T5) and *Bacillus aubtilis* (T2) with a percentage higher than 70% for the variables evaluated; on the other hand, the treatments with the least affectation were copper sulfate (T1), sulfocalcic broth (T3) and the conventional control (T4). Therefore, the statement by Monteon et al, (2018) that anthracnose damage is more accentuated during the flowering and fruit set stages, and that the level of damage may increase under conditions of high relative humidity, which is a particular characteristic of the area evaluated during the dry season.

Table 4. *Colletotrichum gloeosporioides* incidence

Treatments	DAY 1	DAY 15	DAY 30	DAY 45	DAY 60
Bacillus subtilis	0	20,00 A	41,25 A	56,25 A	68.75 AB
Sulfocalcic broth	0	12,50 A	35,00 A	47,50 A	50.00 BC
Copper Sulfate Pentahydrate	0	18,75 A	33,75 A	35,00 A	37,50 C
Absolute Witness (No Application)	0	16,25 A	36,25 A	60,00 A	80,00 A
Conventional Witness (Tebuconazole)	0	12,50 A	37,50 A	46,25 A	46.25 BC
CV	**-**	**35,47**	**39,02**	**21,49**	**14,90**
Significance level	**-**	**ns**	**ns**	**ns**	*****

*Means with a common letter are not significantly different (p > 0.05), according to Tukey's test at 5% significance. *=significant **=highly significant; ns: not significant.*

The treatments copper sulfate pentahydrate (T1) and sulfocalcic broth (T3) obtained a similar efficacy result in the control of anthracnose to that obtained with the conventional control (T4) of synthetic

origin. As expressed by Mitchell (2017), the use of copper sulfate in the preparation of mineral broths that are applied to plants before the time when the pathology is expected, has been one of the products that has generated the best results in agricultural production, since when it comes into contact with the pathogen it inhibits its development. In addition, Aguilar (2016) maintains that sulfocalcic broth is a potent and effective product for the control of pathogens that cause diseases in agricultural crops.

Table 5. *Percent Severity of Colletotrichum gloeosporioides*

Treatments	DAY 1	DAY 15	DAY 30	DAY 45	DAY 60
Bacillus subtilis	0	17,50 A	38,54 A	54,17 A	61.25 AB
Sulfocalcic broth	0	9,17 A	29,79 A	38,33 A	37.71 BC
Copper Sulfate Pentahydrate	0	12,92 A	28,33 A	28,75 A	29,17 C
Absolute Witness (No Application)	0	15,00 A	30,63 A	52,92 A	69,38 A
Conventional Witness (Tebuconazole)	0	8,13 A	31,25 A	35,21 A	35.21 BC
CV	**-**	**37,92**	**41,48**	**23,86**	**19.23**
Significance level	**-**	**ns**	**ns**	**ns**	*****

Means with a common letter are not significantly different (p > 0.05).

The study allowed determining that the best average results of fruit yields were obtained in the treatments copper sulfate pentahydrate (218.25) followed by the conventional control (213.50) and sulfocalcic broth (202.50), On the contrary, the treatments with the lowest average yields were *Bacillus subtilis (*167.00) and absolute control (149.50), which differs with Farinango (2018) who points out that in Ecuador the average yields of a mango tree range from 100 to 150 fruits.

Table 6. *Yield (Kg / Ha)*

TREATMENT	N	Yield (Kg/ha)	
Copper Sulfate Pentahydrate	4	12356,69	A
Conventional Witness (Tebuconazole)	4	12091,61	A
Sulfocalcic Broth	4	11654,62	AB
Bacillus subtilis	4	9040,53	BC
Absolute Witness (No Application)	4	8768,84	C
C.V	11,61		
Significance level	*		

Means with a common letter are not significantly different (p > 0.05).

Table 7. *Number of fruits per treatment*

TREATMENT	N	No. Fruits harvested per treatment	
Copper Sulfate Pentahydrate	4	218,25	A

Conventional (Tebuconazole)	**Witness**	4	213,50	A
Sulfocalcic Broth		4	202,50	A B
Bacillus subtilis		4	167,00	B C
Absolute Witness (No Application)		4	149,50	C
C.V		9,64		
Significance level		*		

Means with a common letter are not significantly different (p > 0.05).

The results corresponding to the variables weight, diameter and length of the harvested fruits determined that none of the five treatments exceeded 400 gr of average weight, being the absolute control treatment with the least amount of fruits the one that reached the highest average weight with 368.30 gr. However, the treatment copper sulfate pentahydrate with a weight of 356.245 gr obtained the highest averages of diameter with 8.91 cm and length with 10.51 cm, which meets the requirements demanded by international markets. In agreement with Bajaña and Mora (2003), who state that depending on the destination market, the ideal weight of fruit for export fluctuates between 250 and 750 g, and depending on the size, 5 to 12 fruits can be placed in 4 kg boxes.

As a result of the variables of total fruit yield in kg/ha and its transformation to number of boxes/ha of 4 kg for which it was considered in the latter case a discard of 20% of the total weight for the rejection that is generated both in the field and in the packing plant, it was determined that the treatments with the highest average yield did not exceed 12,400 kg/ha obtaining as the highest average conversion an amount of 2471.34 boxes of 4 kg. Therefore, Farinango (2018), who maintains that mango production in Ecuador fluctuates in the range of 10 to 15 MT/ha, is accepted.

The analysis of the cost-benefit results of this research determined statistically that of the three agroecological treatments, copper sulfate pentahydrate (T1) and sulfocalcic broth (T3), the latter with a lower investment cost than the conventional control, obtained a similar control and a similar production to that of the chemical treatment, Therefore, the hypothesis of Rebolledo and Martinez (2013) is accepted when indicating that the use of agroecological products for the control of anthracnose in mango cultivation does not influence yield and fruit weight, allowing to maintain the quality and production equivalent to those obtained in conventional systems.

Conclusions

Copper sulfate pentahydrate, sulfocalcic broth and the conventional control, achieved a better efficacy result than Bacillus subtilus, for the control of the fungus Colletotrichum gloeosporioides causing the anthracnose disease in the mango crop, similarly with higher productivity, the same treatments, with an average production range of 2300 to 2470 boxes per hectare. Demonstrating that the use of agroecological products becomes viable alternatives for mango production under sustainable agriculture criteria, the economic benefit is representative to adapt to extensive crops.

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