

Reduction in the development of coffee orange rust with homeopathic mixture

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Abstract

Orange rust (*Hemileia vastatrix* Berk. & Broome) is the main disease of coffee cultivation, and given the need to reduce the losses it causes, ecological alternatives have been implemented for its control and under this concept this research aimed to quantify the effect of four homeopathic preparations and 0.5% Bordeaux mixture in the severity, foliar infestation and defoliation caused by this fungus, the experiment was carried out in the East Regional Center of the Autonomous University Chapingo in Huatusco, Veracruz, Mexico, from September to November of 2014, where the homeopathic plants were sprayed every week for 60 d, with four repetitions/trees per treatment in a randomized complete block design. The Bordeaux mixture at 0.5% was applied twice for 60 days and the control trees were not applied. At 60 d the severity was recorded in 10 leaves per tree and at 30 and 60 d the foliar infestation and defoliation were quantified. The results showed that the severity was not affected at 60 d or the foliar infestation at 30 d, the plant polypharm (mixture of 72 anti-stress homeopathic preparations and diseases, nutrients, pests and plants of agricultural importance)/212 Centesimal Hahnemanniana, 49.75% decreased the defoliation at 30 d and the homeopathic mixture reduced 26.65 and 45.06% foliar infestation and defoliation at 60 d. The homeopathic mixture allows to protect the plant rather than to manage or eliminate the pathogen, it is an ecological and organic alternative to solve the current contingency of coffee rust.

Keywords: *Hemileia vastatrix*, agrohomeopathy, alternatives, biorational.

Reception date: June 2019

Acceptance date: August 2019

Introduction

The cultivation of coffee (*Coffea arabica* L.) has been affected by orange rust in the last seven years, causing an ecological and economic imbalance in Chiapas, Oaxaca, Puebla and Veracruz, Mexico, mainly (Avelino and Rivas, 2014). It is a fungus that invades the leaf and causes defoliation of the tree, diminishing the yield and the physical and sensorial quality of the grain (Medina-Meléndez *et al.*, 2016).

The control has been carried out mainly with the application of Bordeaux mixture, copper oxychloride and systemic organosynthetic fungicides, such as cyproconazole (Lutz, 2012); however, there is a growing demand from society to produce healthy food without compromising the environment, which forces us to seek ecological alternatives that do not affect the environment, the crop and that the harvest is innocuous, do not affect the health and economy of the producer, that avoid technological dependence and that propitiate technological self-sufficiency.

Among several alternatives, homeopathy (Ruiz, 2015) has stood out as a phytosanitary option to manage the fungus and promote better biological aptitude in the crop. The mixture of white arsenic, sulfur and silicon earth to the 6 Centesimal Hahnemannian (CH) and nosode (homoeopathic preparation of the same phytosanitary problem that you want to control) to the 30 CH, applied four times to the foliage, diminishes the spots of rust, but it also induces growth in apices, improves vegetative structure and reduces defoliation (Leonel and Barros, 2013).

The biweekly aspersion of potassium iodide at 200 CH and nosode at 200 and 204 CH inhibits, at 18.79-77.16, 13.39-58.28% and 17.6-44.18, respectively, the development of rust pustules from 1 to 2.5 months, according to Rodríguez-Hernández *et al.* (2014), who also point out that the application of nosode to 6, 7 and 8 CH protects the leaves and the coffee plantations are recovered 30 days after spraying in Simojovel, Chiapas. However, the effect of homeopathic products on the severity and foliar infestation of rust or on defoliation of the crop has not been evaluated.

The objective of this research was to evaluate the spraying of four homoeopathic preparations and 0.5% Bordeaux mixture to the foliage and its effect on the development of coffee orange rust and defoliation, by quantifying the severity, foliar infestation and fallen leaves, after four and eight applications of homeopathic and two sprays of Bordeaux mixture.

Materials and methods

This experiment was carried out from September to November 2014 in the experimental plots of the Regional Center of the East (CRUO) of the Autonomous University Chapingo (UACH), in Huatusco, Veracruz, Mexico, within a commercial plantation of coffee trees in the productive stage of the Garnica variety, where 24 plants of 10 years of age were selected, with 65.46% foliar infestation of orange rust on an area of 2 700 m², 90 m long and 30 m wide, on a slope under the cultivation system with shade at levels lower than 40%, where the main cultural tasks that have been carried out each year have been: pruning in the month of August, fertilization once a year with fresh cow dung, weeding and application of Bordeaux mixture twice a day year.

The homeopathic evaluated were: nosode of rust *H. vastatrix* at 200 CH (Nos-roya 200 CH), coffee polypharm (combination of 12 homeopathic preparations, of 1 mite species, 5 of fungi, 4 of insects, 1 of lichen and 1 of nematode) to 200 CH (PFC 200 CH), plant polypharm (mixture of 72 homeopathic preparations of agricultural importance, where 4 species of mites were integrated, 6 antistressants, 4 species of bacteria, 1 of terrestrial crustacean, 12 of fungi, 19 of insects, 2 of mollusks, 1 of nematode, 20 nutrients and 3 species of plants) to 212 CH (PFP/212 CH) and the mixture of these.

The Nos-roya 200 CH was elaborated with the fungus *H. vastatrix*, collected from coffee leaves with the presence of pustules in the month of October 2013 in the community of Jalapilla, Xicotepec de Juarez, Puebla, Mexico; located at 500 masl in the coordinates of longitude 20° 22' 22" and latitude -97° 54' 54". The PFC 200 CH is a mixture of homeopathic preparations of various species of mites, fungi, insects, lichen and nematodes, at 200 CH each, whose description of its content is specified in Ruiz (2015). The PFP/212 CH contains the dynamizations of bacteria, fungi, insects, mites, molluscs, nematodes and plants such as pepper (*Capsicum annum* L.), quimaphila (*Chimaphila umbellata* (L.) WPC Barton) and mercadela (*Calendula officinalis* L.) to 200 CH, in addition to anti-stress and major and minor elements to 204 CH (Ruiz, 2015).

Of each homeopathic preparation, 0.03 mL was used and diluted in 1 L of water contained in a 1.5 L plastic container and after succussing, agitating the liquid with ascending and descending movements in a forceful and continuous way for 2 min, mixed in a sprayer backpack with 4 L of water. In the homeopathic mixture 0.03 mL of Nos-roya 200 CH, PFC 200 CH and PFP/212 CH were used and diluted in 1 L of water, then succumbed, diluted and sprinkled.

In the preparation of the 0.5% Bordeaux mixture (Bordeaux mixture 0.5%), 50 g of copper sulphate was diluted in 5 L of water and it was added to 50 g of hydrated lime diluted in 5 L of water and sprinkled on the foliage. The application was made to the foliage of 24 plants selected at random in four blocks, which represented four repetitions. Homeopathics were sprayed eight times a week for 60 days, starting on September 8 and ending on October 30, and Bordeaux mixture 0.5% was applied twice during the 60 d; September 8 and 18.

In each plant three branches were selected, from the lower, middle and upper strata, and in the upper and lower branches three leaves were marked and in the middle stratum four were marked, with 10 leaves marked by tree, 240 in the whole experiment. To these, prior to the first application, 1 d before, they were quantified severity, foliar infestation and defoliation. The severity was measured at 0 and 60 d by the damage scale of SENASICA (2013), which ranges from 0 to 6; indicating 0= no visible symptoms, 1= hardly any chlorotic spots, 2, 3, 4, 5 and 6 when there are 2, 7, 20, 45 and $\geq 70\%$ of leaf area affected by the fungus, respectively.

The initial severity was multiplied by 100 and divided by the final severity and after subtracting 100, the net development of the rust in percentage was obtained in that period of time. The foliar infestation (number of leaves with orange rust on the branch) was determined at 0, 30 and 60 d by counting the leaves with presence of pustules, which was multiplied by 100 and divided among the total leaves in each branch selected for a percentage, as mentioned by CENICAFE (Montes *et al.*, 2011). Then, the initial infestation multiplied by 100 and was divided between the final infestation (at 30 and 60 d) and after subtracting 100, the net increase of the infestation was obtained, in percentage for the 30 and 60 d.

The defoliation consisted of counting the leaves on each selected branch at 0, 30 and 60 d and in comparison to the initial reading, the defoliation percentage was calculated; the number of leaves at 30 and 60 d multiplied by 100 and was divided by the number of initial leaves, and 100 was subtracted to obtain the defoliation percentage for that time.

To the percentages of development of severity, foliar infestation and defoliation, tests of the experimental assumptions regarding homogeneity of variances were made with the Levene test (Steel and Torrie, 1986) and normality of the errors with the modified test of Shapiro Wilks (Royston, 1982), with significance levels of $p \leq 0.05$ and analysis of variance was carried out with Friedman's nonparametric test (Márquez, 2008), making multiple comparisons of the averages of the ranges with the InfoStat program (Balzarini *et al.*, 2008), version 2015.

Results and discussion

The severity at 60 d after the application of the treatments is observed in Table 1, where it is found that the experiment started with an average of 13.10% within the range of 10.93 to 18.24% (together with the control) and at the end from this time the average increased to 60.17%, with variation from 57.33 to 62.95%, this indicates that the average increase in severity at 60 d was 47.07% with respect to the initial date, and specifically 48.62% in the control, which is the real increase in severity in the experiment.

Table 1. Severity of *H. vastatrix* in coffee cultivation 60 days after the application of the treatments.

Treatments	Severity (%)		Growth
	Initial	60 d	60 d
Bordeaux mixture 0.5%	10.93	57.6	(88.18)* 4.75**b
Nos-roya 200 CH	11.61	61.65	(81.7) 2.75 ab
Homeopathic mixture	12.02	58.55	(73.85) 1.75 a
PFC 200 CH	11.48	62.95	(88.87) 4.75 b
PFP/212 CH	18.24	57.33	(77.34) 3 ab
Control	14.33	62.95	(84.12) 4 ab

* = average of the treatments in percentage; ** = Average ranges. Averages of ranges with the same letter in development are similar statistically in their effect with the Friedman test.

The advance of the severity was not homogeneous, because it started with different intensity and there was a different effect of the treatments. The normal development of the disease, observed in the control of 84.12% at 60 d, was statistically similar to that of the treatments; however, Bordeaux mixture 0.5% and PFC 200 CH increased the severity by 4.82 and 5.64% and with the homeopathic mixture, the development of normal severity was reduced by 12.2%.

In this way, it is observed that when the coffee leaves have 10.93% to 18.24% severity in September, as in the conditions of Huatusco, Veracruz, the homeopathic products and the Bordeaux mixture 0.5% do not stop the development of the severity of the rust in November, at 60 d, time in which only 12.2% reduce the advance of this, as in the case of the homeopathic mixture applied every 8 d for two months.

The leaf infestation of the rust, number of infested leaves of the total leaves in the branch sampled, registered at 30 and 60 d after the application of the treatments is observed in Table 2, where it is shown that at the beginning of the experiment this it was 65.46% on average, with variation from 59.14 to 74.55% between treatments, and at 30 and 60 d the average foliar infestation was 89.53 (83.42-98.36) and 87.08 (72.12-98.33)%, respectively. With this, it is verified that the infestation increased in 24.07 and 21.62% at 30 and 60 d, while the control, the infestation increased in 21.03 and 30.98% in the same times, with respect to the initial average.

Table 2. Foliar infestation caused by *H. vastatrix* in the coffee culture at 30 and 60 d after the first application of homeopathic and Bordeaux mixture 0.5%.

Treatments	Infestation (%)			Growth (%)	
	Initial	30 d	60 d	30 d	60 d
Bordeaux mixture 0.5%	59.14	98.36	80.73	(39.93)* b	(10.14) 3.83** b
Nos-roya 200 CH ^l	64.58	89.6	94.84	(28.93) ab	(31) 3.96 b
Homeopathic mixture	74.55	83.42	72.12	(8.16) a	(-26.12)1.92 a
PFC 200 CH	66.14	85.11	88.21	(23.26) ab	(25.31) 3.17 ab
PFP/212 CH	61.04	92.36	88.28	(35.39) ab	(25.57) 4.13 b
Control	67.35	88.38	98.33	(21.29) ab	(30.84) 4 b

*= average of the treatments in percentage; **= average ranges. Averages of ranges with the same letter in development are similar statistically in their effect with the Friedman test.

The control did not always have the greatest infestation and the treatment that started with the greatest infestation was not the most damaged, on the contrary, it had the least infestation.

The development of foliar infestation at 30 d of the application had no statistical difference with the control; nevertheless, it increased up to 87.55% with Bordeaux mixture 0.5% and it was reduced 61.67% with the application of the homeopathic mixture. At 60 d, the infestation increased 30.98%, as observed in the control, which was statistically similar to Bordeaux mixture 0.5%, Nos-roya 200 CH, PFC 200 CH and PFP/212 CH, but significantly decreased 26.65% with the application of the homeopathic mixture.

Foliar infestation decreased at 60 d, compared to 30 d, in Bordeaux mixture 0.5%, homeopathic mixture and PFP/212 CH because leaf formation was induced and the proportion of infested leaves decreased; nevertheless, these young leaves were infested in Bordeaux mixture 0.5% and PFP/212 CH and the percentage of infestation increased again, not showing significance, unlike the coffee plants sprinkled with the homeopathic mixture, where the new leaves did not have the same infestation, therefore the percentage of foliar infestation was reduced to be negative.

The defoliation registered at 30 and 60 d after the application of the treatments is shown in Table 3, where it is evident that at 30 d this was 32.03% on average and ranged from 9.60 to 56.98%. All the treatments reduced the defoliation, being the best PFP/212 CH, with 49.75% with respect to the control. At 60 d of the beginning of the experiment the average defoliation was of 56.04%, with variation from 41.27 to 75.11%, obtaining reduction in all the treatments, being better the homeopathic mixture, which reduced in 45.06% the defoliation with respect to the control.

Table 3. Defoliation (%) in coffee cultivation at 30 and 60 d after the first application of homeopathic and Bordeaux mixture 0.5%.

Treatments	30 d	60 d
Bordeaux mixture 0.5%	(43.93) * 3.75 ** ab	(55.78) 3.13 ab
Nos-roya 200 CH ¹	(9.6) 3.67 ab	(42.12) 3.88 ab
Homeopathic mixture	(18.68) 3 ab	(41.27) 2.46 a
PFC 200 CH	(34.68) 3.25 ab	(59.16) 3.38 ab
PFP/212 CH	(28.35) 2.88 a	(62.83) 3.71 ab
Control	(56.98) 4.46 b	(75.11) 4.46 b

* = average of the treatments in percentage; ** = average ranges. Averages of ranges with the same letter in development are similar statistically in their effect with the Friedman test.

The homeopathic mixture reduced the defoliation because the tree with this treatment retained leaves, in comparison to the control, even in those with high severity, the leaves fell, but the plant emitted new leaves in greater quantity than other treatments that also formed leaves, as in the case of Bordeaux mixture 0.5% and PFP/212 CH but that did not show statistical significance.

In the trees where the Nos-roya 200 CH was applied, the number of initial leaves was lower, and the proportion of fallen leaves with the new leaves was less than 50%, which indicated little recovery. The application of the homeopathic mixture every 8 d during 60 d diminished the development of the severity of the coffee orange rust at 60 d (although not significantly), the damage in the leaves was minor and by not harming them the plant was strengthened, resistance to coffee was induced and reduced infestation (without statistical difference at 30 d and with significance at 60 d), since the new foliage did not present orange pustules and in turn, defoliation decreased at 60 d, since the leaves most affected fell and only those where the damage was not so severe remained in the branch, thus helping the plant to recover faster, stimulating the generation of new leaves, which led to greater photosynthesis that was reflected in the yield, producing more load and to continue like this, the application of the treatment until the harvest would be expected better results.

There are other homeopathic mixtures that have also shown an effect in the control of insects and pathogens. Rodríguez-Hernández *et al.* (2014) state that when using the nosode of coffee rust *H. vastatrix* to 3 CH mixed with *Hypothenemus hampei* (Ferrari) and sodium sulfate at 3 CH, there is a control on orange rust and coffee berry borer (*H. hampei*). Leonel and Barros (2013) point out that the mixture of white arsenic, sulfur and silicon earth at 6 CH plus coffee rust *H. vastatrix* at 30 CH reduces the defoliation in coffee due to a higher sprouting of leaves.

The development of leaves at the end of the treatment, as observed with the homeopathic mixture, is an indicator that homeopathy strengthens the plant, as has Tichavsky (2009) in tomato, with an indirect and diverse effect on the pathogen. A sample of this is the use of PFP/212 CH that with four sprays in 30 d induced more foliage; nevertheless, chlorotic spots and orange pustules were observed in the leaves, which could be an indicator that the plant was strengthened, but no resistance was induced against the pathogen (Erdmann *et al.*, 2011) in the same way at 60 d, in the PFP/212 CH most of the initial leaves fall and the new shoots remain in the branch, which are mostly infested with rust.

In this same period, the Nos-roya 200 CH did not stop or increase the severity, the infestation increased and the defoliation decreased, because at 30 d there was more foliage, but the proportion of leaves with chlorotic spots and pustules was higher; symptom that lasted until 60 d.

The same effect of infested leaves was observed with the PFC 200 CH, where the number of leaves with chlorotic spots and pustules increased from the first 30 d, in addition to increasing the damage development in the leaves at 60 d, not obtaining the results expected in comparison with Honorato (2012), who reported that with the use of the polypharm of lettuce, they controlled various pests and diseases and obtained better vigor and quality in the crop. In coffee, there was less recovery of the plant and the defoliation was in the same proportion as the plants where no applications were made.

Based on the above, it would be expected that with the passage of time the plants sprinkled with the PFC to the 200 CH and the Nos-roya 200 CH would have greater damage, since these preparations showed no control over the fungus *H. vastatrix*, even the first homeopathic stimulates (without significance) the development of severity; nevertheless, it is not a reason to affirm in a general way that nosodes do not work; although with the spraying of these homeopathic the expected results were not obtained.

Ruiz (2015) states that in the case of the nosodes the symptoms can be corrected by the most similar, taking secretions directly from the diseased organs, which was not done in this way for the PFC 200 CH and the Nos-roya 200 CH, since the samples were taken from a different plot to where the experiment was developed; for the preparation of the first homeopathic rust was used from a plot in Jalapilla, Puebla, at an altitude of 500 meters above sea level, it is possible that the material from Huatusco is different and more resistant, since it was worked at a height of 1 344 meters above sea level, where previously this disease was not developed (Medina-Meléndez *et al.*, 2016).

The practices carried out in the plot where the sample was taken for the preparation of the homeopathic should also be considered, since having selected leaves with rust that had a treatment or a beneficial microorganism that could parasitize the phytopathogenic fungus, the nosode could have stimulated the survival of the fungus, obtaining an effect contrary to the expected, although this is only a hypothesis, in this sense it is recommended to make the preparation for each region and update it constantly, due to the rapid mutation of rust races, besides using diseased leaves of plants that have not been treated before.

Another cause that can be considered to explain why this nosodes have no effect on the control of the fungus, is that the power used has not been adequate, in a similar investigation, Rodríguez-Hernández *et al.* (2014) reported that with the Nos-roya at 6, 7 and 8 CH the plant recovers at 30 d of its sprinkling, at 204 CH it inhibits the growth of the pustule, while at 200 CH it has little effect in the control of the fungus.

Other nosodes used for the control of fungi that have also shown no effect on the crops are the nosode of the hypericum rust (*Melampsora hypericorum* (DC.) J. Schröt.) to 30 Hahnemannian Decimal applied to plants hypericum without odor (*Hypericum inodorum* Mill.), which, although it increases the disease, there is less defoliation (Erdmann *et al.*, 2011), the nosodes of *Alternaria*

solani (Ell. and Mart.) Jones and Grout, in different potencies they have no effect on spore germination and the growth of *A. solani* (Carneiro *et al.*, 2006), which could be due to the influence of the environment and the genotype of the plant.

Another treatment that was used was Bordeaux mixture 0.5%, with two aspersions in 60 d, which increased, but without statistical significance, the infestation, since the number of leaves with presence of pustules was greater from the first 30 d and the 60 d this one decreased as well as the defoliation, because the new leaves and their proportion with respect to the number of leaves with pustules was lower; nevertheless, the advance in severity was not stopped, on the contrary, a stimulus of foliar damage was observed in the young leaves, while with homeopathic barely a few chlorotic spots appeared, indicating advanced symptoms of the disease.

Homeopathic preparations, which also affect phytopathogenic viruses (Rodríguez *et al.*, 2013), stimulate the production of secondary, semiochemical metabolites that improve the physiological state of the plant, induce resistance and favor multiple biological interactions that restore the vital energy of the plant (Teixeira *et al.*, 2009) and reduce the incidence and damage of pathogens and pests (Bonato, 2007).

Based on the previous analysis, it is observed that the effect of the homeopathic preparations in the coffee rust is variable, which depends on the power used, the number of applications and the mixture with other homeopathic, it is also evident that its effect is seen more clearly at 60 d with weekly applications, where rust seemingly is not eliminated, but homeopathy allows it to not develop normally, thus affirming that homeopathic dynamises have the potential to replace the use of Bordeaux mixture, under certain circumstances (Modolon *et al.*, 2012) Teixeira, which is one of the most recurrent alternatives for the protection of coffee against this pathogen (Lutz, 2012; Barrera, 2013), homeopathy is a biorational alternative that does not kill the fungus (Ruiz, 2015), but activates the resistance system in the plant, due to the stimulation of its metabolism (Leonel and Barros, 2013).

In this way, the homeopathic mixture decreases infestation and defoliation, which can be potentiated with the application of other homeopathics in the detoxification of the soil, the nutrition of the plant and other pathogens and various pests or to protect the crop from the attack of other fungi, such as silicon earth, and mites, nematodes or aphids, such as staphisagria (Meneses, 2017). In addition to being integrated into a cultural management that involves pruning, shade control and rejuvenation of the plantation, among other measures such as biofertilization and management of spontaneous plants to protect the crop mainly, to avoid attack and damage and to influence the proliferation of the pathogen.

Conclusions

The homeopathic products, nosode of rust *H. vastatrix*, PFC 200 CH and PFP/212 CH, and Bordeaux mixture 0.5%, do not diminish the development of the severity at 60 d.

No treatment diminishes the development of the infestation at 30 d and at 60 d the homeopathic nosode of rust *H. vastatrix*, PFC 200 CH, PFP/212 CH, and Bordeaux mixture at 0.5% are not effective either.

Homeopathic, nosode de rust *H. vastatrix* and PFC 200 CH, and Bordeaux mixture 0.5% do not reduce defoliation at 30 and 60 d, application of PFP/212 CH, does not reduce leaf fall at 60 d, however, had a reduction of 49.75% in defoliation at 30 d.

The homeopathic mixture reduces the development of coffee orange rust infestation by 26.65% and also reduces defoliation by 45.06% at 60 d with eight weekly applications.

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