PHYTOTOXICITY OF MINERAL, VEGETABLE OILS AND FOLIAR FERTILIZERS IN COFFEE SEEDLINGS

Leandro Alvarenga Santos[1], Edson Ampélio Pozza[2], Paulo Estevão de Souza[3]

(Recebido: 08 de junho de 2016; aceito: 15 de agosto de 2016)

ABSTRACT: The objective of this study was to evaluate the toxicity in coffee seedlings caused by mineral oil, vegetable and foliar fertilizer, applied in different radiation intensities. In the first experiment not observed symptoms of phytotoxicity in the control plants and sprayed with foliar fertilizer. Two experiments to study the phytotoxic action of oils and foliar fertilizers were performed. The first was evaluated phytotoxic effects of mineral oil, vegetable oil and foliar fertilizer in different application times. In the second experiment evaluated the intensity of radiation in the occurrence of toxicity symptoms caused by both oils. At the end of the second experiment samples were taken to perform scanning electron microscopy. Plants sprayed 12:00 h had higher incidence and severity of injuries to both oils in relation to others (9:00 and 17:00h). In the light intensity experiment proved that the increase of light intensity from 80 to 320 µmol/m²/s promoted an increase in symptoms of phytotoxicity. In the analysis of lesions by scanning electron microscopy, deformations on the leaf surface caused by mineral oil and also for vegetable oil were observed.

Index terms: Adjuvants, magnifier effect, scanning electron microscopy, *Coffea arabica*.

FITOTOXIDEZ DE ÓLEOS MINERAL, VEGETAL E ADUBO FOLIAR EM MUDAS DE CAFÉ

RESUMO**:** *Objetivou-se, neste trabalho, avaliar a fitotoxidez em mudas de cafeeiro causadas por óleo mineral, vegetal e adubo foliar, aplicados em diferentes intensidades de radiação. No primeiro experimento não se observou sintomas de fitotoxidez nas plantas da testemunha e nas pulverizadas com o adubo foliar. Foram realizados dois experimentos para o estudo da ação fitotóxica de óleos e adubos foliares. No primeiro foram avaliados os efeitos fitotóxicos do óleo mineral, óleo vegetal e de adubo foliar em diferentes horários de aplicação. No segundo experimento foi avaliada a intensidade da radiação na ocorrência de fitotoxidez causada por ambos os óleos. Ao final do segundo experimento amostras foram retiradas para realizar microscopia eletrônica de varredura. As plantas pulverizadas às 12:00h apresentaram maiores incidência e severidade das lesões para ambos os óleos em relação as demais (09:00 e 17:00h). No experimento de intensidade luminosa verificou que o aumento da intensidade luminosa, de 80 para 320 µmol/m²/s promoveu aumento nos sintomas de fitotoxidez. Na análise das lesões através da microscopia eletrônica de varredura, deformações no limbo foliar causadas por óleo mineral e também por óleo vegetal foram observadas.*

Termos para indexação: *Adjuvantes, efeito lupa, microscopia eletrônica de varredura, Coffea arabica.*

1 INTRODUCTION

Pesticide application has become indispensable in many farming systems and an important factor in increasing the productivity and income of the producer. To ensure the best performance and optimize the application is used adjuvants and nutrients mixed with pesticide solution (CUNHA; ALVES, 2009). According to Jursík et al. (2013) the pesticidal activity is often increased by adjuvants, which sometimes are not contained in the product formulation.

Mineral and vegetable oils are the most commonly used adjuvants. They reduce losses from hydrolysis, photodegradation, volatilization, drift, and rainwater thus improving pesticide performance. Mineral oils are derived from petroleum and have been used for controlling plant diseases and phytophagous for over 100 years. They are formulated predominantly with paraffin hydrocarbon fractions with different chain length sand branches (MENDONÇA; RAETANO; MENDONÇA, 2007). Vegetable oils have varying proportions of fatty acids such as oleic, linoleic, and linolenic (HARKER, 1992). However, although useful for improving control these adjuvants may cause phytotoxicity when improperly applied. Other use of mineral oil is the insect pest control, Sridharana, Shekhar and Ramakrishnan (2015) found that mineral oil in combination with either neem oil or *Pongamia glabra* Vent., seed oil is efficacious in checking the whitefly population; Furthermore, it is not phytotoxic to okra.

Another compound that can cause phytotoxicity is foliar fertilizer. According to

[1]Universidade Estadual do Centro Oeste - UNICENTRO - Departamento de Agronomia/DEAGRO - Rua Simeão Camargo Varela de Sá, 03 - Vila Carli - 85.040-080 - Guarapuava - PR- leandro.alvarenga.s@hotmail.com
[2,3]Universidade Federal de Lavras/UFLA - Departamento de Fitopatologia/DFP - 37.200-000 - Lavras - MG - eapozza@gmail.com, pauleste@ufla.br

Vargas et al. (2015), application of high amounts of nutrients can cause phytotoxicity. According to the author this effect is related to nutrient source, nozzle and spray volume, as well as spraying time. Application time and consequently radiation intensity are reported as being primarily responsible for symptoms associated with phytotoxicity, defined in practice as "Magnifier Effect".

Thus, our aim was to evaluate the phytotoxicity of coffee seedlings caused by mineral oil, vegetable oil, and foliar fertilizers applied at different radiation intensities and study its effect at cellular level through scanning electron microscopy.

2 MATERIAL AND METHODS

Two experiments were conducted to study the phytotoxicity of oils and foliar fertilizers.

Experiment I: Phytotoxicity of mineral and vegetable oils

The first experiment was carried out in the field with coffee seedlings (*Coffea arabica* L.) cultivar Mundo Novo379/19. The experiment consisted of twelve treatments (Table 1) in a randomized complete block design, having four replicates with four plants per replication. Analysis of variance was conducted in a factorial 4x3 design. Factor 1 was the nature of the product applied and factor 2 was the application time. The treatment was based on the differences in product composition and application time.

We tested mineral oil having 56.5% aliphatic hydrocarbon, a vegetable oil composed of 72% methyl ester of soybean oil, and a foliar fertilizer containing 6% nitrogen and 14% soluble Phosphorus (P_2O_5).

After being sprayed, seedlings were kept outdoors until assessment day (Table 2).

The coffee seedlings were evaluated seven days after spraying. We assessed incidence and severity of damage on their leaf blades. We performed a joint analysis of weather data in both experiments with repetition. As there was no significance, the data represented an average of the experiments. Data was subjected to analysis of variance by factorial F test. Significant variables in the F test were subjected to the *Scott-Knott clustering algorithm* at 0.05 significance level with Sisvar® software.

Experiment II: Radiation intensity

Seedlings were placed in a growth chamber equipped with metal halide lamps capable of emitting photosynthetically active radiation. Different light intensities were obtained with different distances from the light source.

We used a split-plot randomized block design. Light intensities of 80, 100, 160, 240 and 320 µmol/m²/s were arranged in plots, while subplots received either the control, the vegetable oil, or the mineral oil with 3 replicates consisting of one seedling each.

The growth chamber was maintained at a constant temperature of 20ºC, relative humidity above 80%, and a 12-hour photoperiod. Seedlings were sprayed with mineral or vegetable oil at the same doses of the previous experiment.

Data was collected one week after application. We evaluated incidence and severity of damage in the leaves. Incidence was assessed by the percentage of damaged leaves to total leaves of the plants in each plot. Assessment of severity proceeded as in Experiment I. At the end of evaluation we collected samples for scanning electron microscopy of the symptoms found in the leaves.

We made a joint analysis of weather data in both experiments with repetition. As there was no significance, the data represented the average of the experiments. Data was subjected to analysis of variance in subplots. Significant variables in the F test were subjected to regression model adjustment using SISVAR® software (FERREIRA, 2011).

The leaves were processed and samples prepared according to standard protocol Electron Microscopy Laboratory of UFLA. Subsequently, we used a LEO Evo 40 microscope for finding damaged symptoms or any change in the cuticle or parenchymal structures.

3 RESULTS AND DISCUSSION

Experiment I: Phytotoxicity of mineral and vegetable oils

The F test exhibited significant interaction between treatments for both incidence and severity. There was no phytotoxicity in either control plants or in plants sprayed with foliar fertilizer. We found higher incidence and severity of damage for both oils at 12pm with a significant difference from the other application times (Table 3).

TABLE 1 - Experimental treatments to assess phytotoxic effects on coffee seedling sat different application times. Lavras, MG. 2014

	Nature of Product	Dosage	Application time
1.	Control	-	9am
2.	Control	-	12pm
3.	Control	-	5pm
4.	Foliar fertilizer	0.025%	9am
5.	Foliar fertilizer	0.025%	12pm
6.	Foliar fertilizer	0.025%	5pm
7.	Mineral oil	0.5%	9am
8.	Mineral oil	0.5%	12pm
9.	Mineral oil	0.5%	5pm
10.	Vegetable oil	0.5%	9am
11.	Vegetable oil	0.5%	12pm
12.	Vegetable oil	0.5%	5pm

TABLE 2 - Weather conditions on the day of application of mineral and vegetable oils. Lavras, MG. 2013/2014

Weather variables	Application time		
	9pm	12pm	5pm
Temperature	22.8 °C	30.4 °C	25.5 °C
Precipitation	0 mm	0 mm	0 mm
Relative humidity	72%	66%	70%
Radiation	1200 µmol/m^2/s	2200 µmol/m^2/s	1100 µmol/m^2/s

TABLE 3 - Incidence and severity of phytotoxicity in coffee plants subjected to application of mineral oil, vegetable oil and foliar fertilizer at different times. Lavras, MG. 2014.

Application Time	Incidence*				Severity*			
	Control	Foliar Fertilizer (0.25%)	Mineral oil (0.5%)	Vegetable oil (0.5%)	Control	Foliar Fertilizer (0.25%)	Mineral oil (0.5%)	Vegetable oil (0.5%)
	------------------ % ------------------				------------------ % ------------------			
9am	0.00 Aa	0.00 Aa	18.7 Ab	12.5 Ab	0.00 Aa	0.00 Aa	4.6 Ab	3.02 Ab
12pm	0.00 Aa	0.00 Aa	65.12 Cc	47.57 Bb	0.00 Aa	0.00 Aa	9.67 Bb	9.77 Bb
5pm	0.00 Aa	0.00 Aa	27.63 Bc	10.45 Ab	0.00 Aa	0.00 Aa	4.77 Ac	2.3 Ab
CV (%)	29.47				59.01			

Measures followed by the same uppercase letter in the column and lower case in the row belong to same cluster by the Scott-Knott test at 5%probability.

At 12pm both mineral and vegetable oils showed higher incidence of phytotoxicity symptoms, 65%, 12%, and 47.57% respectively. Lower incidence with vegetable oil is due to its higher stability as described by Harker (1992). Thus, as unsaturated fractions of mineral oil are reactive they tend to cause lesions on leaf surface. Cuillé and Blanchet (1958) studied 84 different types of oils in maize plants and found that these unsaturated fractions can cause variations in damage intensity regarding physicochemical properties, which vary with dosage and environmental conditions.

The different application times are significantly related to incidence and severity of toxicity. The highest rates, 65.12% incidence and 9.77% severity occurred at 12 pm at the highest temperature (30.4°C) and radiation exposure (2200 µmol/m^2/s). This result can be explained by oil exposure to greater light intensity producing acidic compounds, which are responsible for phytotoxicity due to disruption of cellular membranes (HODGKINSON; JOHNSON; SMITH, 2002).

Another case of phytotoxicity of oil is lemongrass oil in seed germination and seedling length of *Parthenium hysterophorus* L., by the authors Paudel and Gupta (2008). According to these authors, the oil reduced seed germination and seedling growth. This reduction can be explained by the loss of respiratory activity, which affects the synthesis of macromolecules (BATISH et al., 2007). Jursík et al. (2013) observed increased cytotoxic capability flumioxazin when used with sunflower oil as adjuvant. In the study it was found highest phytotoxicity (72%), reduction of net photosynthesis (by 80%) and stomata conductance of sunflower.

Experiment II: Light intensity

No significant interaction was found between different types of oil and light intensity. However, increasing the light intensity from 80 to 320µmol/m²/s increased phytotoxicity symptoms, with incidence values rising from around 5% to 45% in the leaves (Figure 1).

Several authors regarded exposure to light as the decisive factor of oil phytotoxicity. However, information on behavior of damage incidence in relation to increase in light intensity is still lacking in available literature.

Only the first degree of injury severity scale occurred because light intensity produced by metal halide lamps in the controlled environment (growth chamber) was lower (360 µmol/m^2/s) than light intensity of full sunlight (2200 µmol/m^2/s). Surface damage was possibly caused by cell membrane degradation, which is traditionally associated with acidic compounds in the oil. Such compounds are formed due to exposure of unstable molecules to light after spraying.

Scanning electron microscopy allowed us to observe leaf deformations caused by mineral and vegetable oil. Treatments with mineral oil had extended deformations (Figure 2 - A) while treatments with vegetable oil had deeper ones (Figure 2 - F).

Such deformations may be due to changes in membrane permeability caused by the oil, leading to plasma extravasation into intercellular spaces. According to Currier (1951), leakage of cellular contents into intercellular spaces due to oil phytotoxicity causes turgor loss and darkening of leaves.

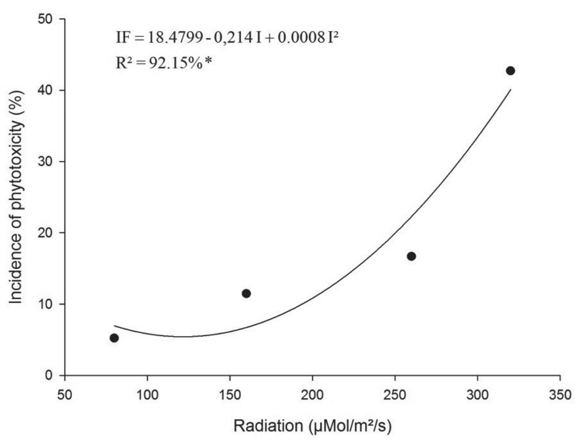

FIGURE 1 - Incidence of leaves with phytotoxicity damage in relation to radiation. Lavras, MG. 2014.

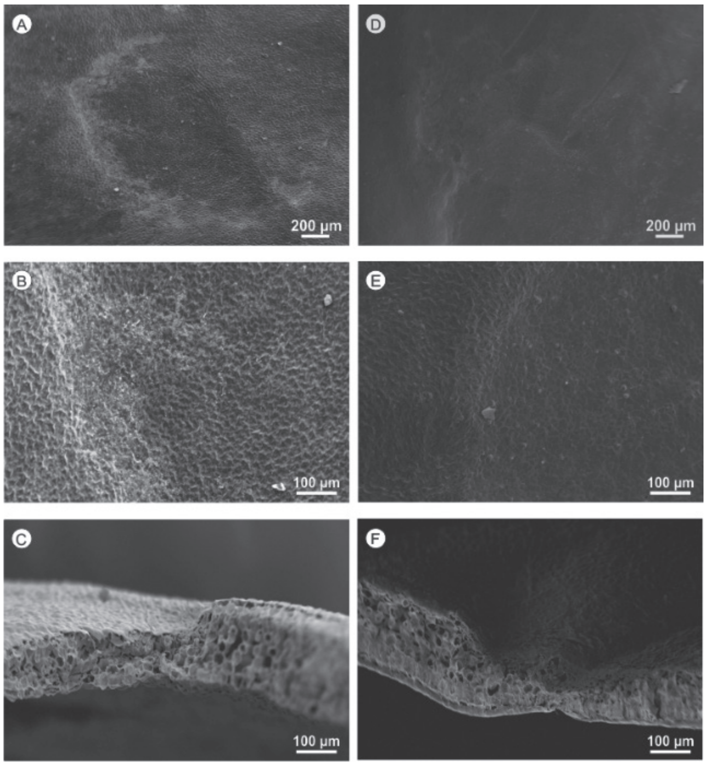

FIGURE 2 - Scanning electron microscopy of coffee leaves with damage caused by mineral and vegetable oil. A –Injury caused by mineral oil. B-Detail of change in leaf surface relief due to phytotoxicity of mineral oil. C-Depth of lesion on leaf surface caused by phytotoxicity of mineral oil. D- Injury caused by vegetable oil. E-Detail of change in surface relief due to phytotoxicity of vegetable oil. F-Depth of leaf surface injury caused by phytotoxicity of vegetable oil. Lavras, MG. 2013.

Sant'Anna-Santos et al. (2007) reported formation of hollows in leaves caused by toxicity symptoms in a study of phytotoxic effects of fluoride in *Magnolia ovata* (A.St.-Hil.) Spreng. . In the same line of research, Sant'Anna-Santos and Azevedo (2010) assessed the effects of fluoride contamination in vegetables and found that *Ocimum basilicum* L, *Petroselinum crispum* (Mill.) Fuss and *Brassica oleracea* var. *acephala* DC also showed changes in the cuticular relief and stomatal structure.

These results help clarify the magnifying glass effect, widely known by farmers but little studied up to now.

4 CONCLUSION

Mineral and vegetable oils can cause tissue injury of coffee plants.

The phytotoxic effect of these compounds is directly related with the environmental conditions at application time, particularly with the incident radiation.

The highest intensity of phytotoxicity of both oils occurred at 12pm, when radiation incidence and temperatures were higher.

The higher the light intensity the higher the phytotoxicity caused by mineral oils.

Phytotoxicity causes hollows in leaves due to changes in cell membrane of coffee plants.

5 REFERENCES

BATISH, D. R. et al. Alternative control of littleseed canary grass using eucalypt oil. **Agronomy for Sustainable Development**, Paris, v. 27, n. 3, p. 171-177, 2007.

CUILLE, J.; BLANCHET, B. Low-volume spraying of tropical fruits: oil-based spray products with special reference to their phytotoxicity. **Publication Exchage Service**, Washington, v. 45, p. 53-65, 1958.

CUNHA, J. P. A. R. da; ALVES, G. S. Características físico-químicas de soluções aquosas com adjuvantes de uso agrícola. **Interciência**, Caracas, v. 34, n. 9, p. 655-659, 2009.

CURRIER, H. B. Herbicidal properties of benzene and certain methyl derivatives. **Hilgardia**, Gainesville, v. 19, p. 383-406, 1951.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, Lavras, v. 35, n. 6, p. 1039-1042, nov./dez. 2011.

HARKER, K. N. Effects of various adjuvants on sethoxydim activity. **Weed Technology**, Washington, v. 6, p. 865-870, 1992.

HODGKINSON, M. C.; JOHNSON, D.; SMITH, G. Causes of phytotoxicity induced by petroleum-derived spray oil. In: BEATTIE, D. M. et al. (Ed.). **Spray oils beyond 2000**. Sydney: University Western Sydney, 2002. p. 170-178.

JURSÍK, M. et al. Effect of different adjuvants on phytotoxicity of flumioxazin to sunflower in different growth stages. **Romanian Agricultural Research**, Bottom, v. 30, p. 365-372, 2013.

MENDONÇA, C. G. de; RAETANO, C. G.; MENDONÇA, C. G. de. Tensão superficial estática de soluções aquosas com óleos minerais e vegetais utilizados na agricultura. **Engenharia Agrícola**, Jaboticabal, v. 27, p. 16-23, 2007.

PAUDEL, V. R.; GUPTA, V. N. P. Effect of some essential oils on seed germination and seedling length of Parthenium hysterophorous L. **Ecoprint: An International Journal of Ecology**, Kathmandu, v. 15, p. 69-73, 2009.

SANT'ANNA-SANTOS, B. F.; AZEVEDO, A. A. Toxicidade e acúmulo de flúor em hortaliças nas adjacências de uma fábrica de alumínio. **Acta Botânica Brasílica**, Porto Alegre, v. 24, p. 952-963, 2010.

SANT'ANNA-SANTOS, B. F. et al. Utilização de parâmetros morfoanatômicos na análise da fitotoxidez do flúor em folhas de *Magnolia ovata* (A. St.-Hil.) Spreng. (Magnoliaceae). **Revista Árvore**, Viçosa, v. 31, p. 761-771, 2007.

SRIDHARAN, S.; SHEKHAR, K. C.; RAMAKRISHNAN, N. Bioefficacy, phytotoxicity, and biosafety of mineral oil on management of whitefly in okra. **International Journal of Vegetable Science**, Philadelphia, v. 21, n. 1, p. 28-35, 2015.

VARGAS, V. P. et al. Maize leaf phytotoxicity and grain yield are affected by nitrogen source and application method. **Agronomy Journal**, Madison, v. 107, n. 2, p. 671-679, 2015.