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Effects of Fungicides for Non Target Fungi *Alternaria cassiae*

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Abstract**—** *The fungicides are used to control of pathogenic fungi in several tilth but they can affect negatively the microorganisms diversity of soil. The aim of this research was to evaluate the toxicity and environmental risk of tebuconazoles: captan, tebuconazole and the mixture chlorothalonil + propamocarb hidrochloride for fungi Alternaria cassiae. Each fungicide were performed three experiments in completely randomized design with three repetitions and the growth was evaluated daily. Inhibition concentration (IC50;7d) of tebuconazole was 3.49 mg L^{-1}, the captan was 47.36 mg L^{-1} and of mixture chlorothalonil + propamocarb hidrochloride, 64.04 mg L^{-1}. Tebuconazole is classified as moderately toxic and sensitivity, captan, low toxicity and sensitivity and the mixture, non toxic and insensitive but only captan showed possibility of adverse effect for A. cassiae.*

Keywords**— soil, microorganism, xenobiotics, pesticide.**

I. INTRODUCTION

The fungicides have been using control pathogenic fungi of plants and some of them are non specific and can affect the abundance of non target microorganisms (Stenersen 2004; Zhang *et al*., 2016). Besides, as azoles can affect the ergosterol biosynthesis, may be studied its effects for non target organisms present in the application area (Dijksterhuis *et al*., 2011).

As one of them, Tebuconazole is a systemic triazole classified by USEPA as carcinogenic for human beings (Konwick *et al*., 2006; Hu *et al*., 2007) and toxic for aquatic organisms (Yu *et al*., 2013). Captan is from carboximide group, non systemic, wide range and inhibits the spores germination, growth and the oxygen uptake (Boran *et al*., 2012). Propamocarb is a carbamate practically non toxic and 97% is degraded by aquatic microorganisms in 35 days and chlorothalonil is a derivative of benzoic acid, considered moderately toxic and is degraded in two metabolits in two weeks (Teather *et al*., 2001).

The soil microbiota is responsable by decomposition of organic residues and by nutrient cycling and exercises influency in the organic matter transformation and in the carbon and mineral nutrient stocking (Zilli *et al*., 2003). Fungi are part of this microbiota and they are affected negatively by chemical intake according to Botelho e Monteiro (2011) who verified that inseticides, herbicides and maturers used in the manegement of sugar cane cause toxic effects for *Metarhizium anisopliae* and *Beauveria bassiana*.

Alternaria spp. is an ascomyce from Dematiaceae Family, Order Hyphomycetes and produces conidia as dictyospores (Rotem, 1994). *Alternaria cassiae* has wide geographic distribution in the tropical and subtropical region, as Asia (Jurair and Khan, 1960), North America (Walker and Boyette, 1982) and South America (Figueiredo *et al*., 1992). This fungi has a big importancy in the agroecosystem dynamic, especially, in the biological control of *Senna obtusifolia* (L.) (Pitelli *et al*., 2007) and it can be a good option as bioherbicide (Boyete *et al*., 2012). Besides, *Alternaria* is natural inhabitant of soil submited at organic or conventional manegement and for this it is exposed at pesticides (Prade *et al*., 2007).

Therefore, the evaluation of the pesticides effect for non target fungi is very important because the soil with unbalance fungal content can presents problems with decomposition and mineralization rate, quantity and nutrient variety, water retention capacity and erosion resistance (Stahl e Parkins, 1996).

In this context the ecotoxicology has been using as a tool to estimate the effects for non target organisms, comunities, populations, animals or vegetables, terrestrial or aquatics (Cairns e Niederlehner, 1995). According to Zagatto e Bertoletti (2008) the ecotoxicology can evaluate the damage occurred in the several ecosystems after the exposure and also to predict the future impacts of chemicals.

Thus, the risk evaluations may be more necessary to contemplate the principal agriculture patrimony, the soil

health and fertile. The aims of this research were to estimate the toxicity of captan, tebuconazole, clorotalonil + chloridrate propamocarb and to evaluate the environmental risk for fungi *Alternaria cassiae*.

II. MATERIAL AND METHODS

Cultivation of fungi

The stain of *A. cassiae* was from Laboratory of Biological Control of Weed (CENARGEN/EMBRAPA), Brazil.

For the cultivation it was used the medium PDA (potato-dextrose-agar) that after autoclaved at 121ºC, during 15 min, was spilled in plates and expected until solidificaton. After, it was disposed a disk from a fungi colony (6.5 mm diameter) in the center of plate, from the growth active region of colony. The plates were incubated in at 25ºC and photoperiod of 12 hours.

Toxicity of fungicides for fungi

The fungicides were captan (Captan® SC), tebuconazole (Orius® 250EC) and the mixture clorotalonil + chloridrate propamocarb (Tattoo® C).

First of all, the sensibility of fungi was evaluated with the reference substance potassium dicromate, being IC50;7d, 65.97 ± 9.48 mg L^{-1} (coefficient of variation 14.38%).

Previous tests were performed and it was determinade the interval of concentrations that caused zero and 100% of inhibition in the fungi growth according IBAMA (1987), with some adaptations.

In the definitite assays the concentrations used were: captan, 1.0, 10.0, 50.0, 90.0, 130.0 and 170.0 mg L^{-1}; tebuconazole, 1.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg L^{-1}; and clorotalonil + chloridrate propamocarb, 25.0, 50.0, 100.0, 150.0, 200.0, 250.0 and 300.0 mg L^{-1} and the control. Three definitive tests were performed each one in completely randomized design with three replicates each concentration.

The fungicides were added in the medium, spilled in the plates and after solidification a disk 6.5 mm diameter of *A. cassiae* was disposed centrally. The plates were sealed and kept in the 25ºC, and photoperiod of 12 hours, for seven days.

The evaluation of growth halo was daily according two axis perpendicular. The inhibition concentration (IC50;7d) were estimated by Trimmed Spearman-Karber software (Hamilton *et al*., 1977).

The toxicity was classified according to Edgington *et al*., (1971): IC50 < 1.0 mg L^{-1}: high toxicity and sensitivity; 1.0 < IC50 < 10.0 mg L^{-1}: moderate toxicity and sensitivity; 10.0 < IC50 < 50.0 mg L^{-1}: low toxicity and sensitivity and IC50 > 50.0 mg L^{-1}: non toxic and insensitive.

The environmental risk was estimated according to Urban e Cook (1986) which use the estimated environmental concentration (EEC), which is the higher and lower concentration each fungicide recommended and IC50;7d each fungicide found in the toxicity tests *in vitro.* Thus, these data resulte in a quotient (Q): without adverse effect ($Q \leq 0.1$); possibility of adverse effect ($0.1 \leq Q \leq 10$) and probability of adverse effect ($Q > 10$).

III. RESULTS

IC50;7d of tebuconazole was 3.49 mg L^{-1}; captan, 47.36 mg L^{-1} and of mixture clorotalonil + chloridrate of propamocarb, 64.04 mg L^{-1} (Table 1) and they were classified as moderately toxicity and sensitivity; low toxicity and sensibility and non toxic and insensitive, respectively.

Table.1: Inhibition Concentration 50% (IC50;7d) (mg L^{-1}), upper limit (UL) and lower limit (LL) of fungicides for A. cassiae.

		Captan	
Tests	**LL**	**IC50;7d**	**UL**
1	48.44	60.00	74.57
2	37.81	44.98	53.53
3	25.99	37.00	52.79
Average	**37.41**	**47.36**	**60.29**
		Tebuconazole	
Tests	**LL**	**IC50;7d**	**UL**
1	2.95	3.98	5.38
2	2.95	4.14	5.81
3	1.33	2.37	4.23
Average	**2.41**	**3.49**	**5.14**
		Clorotalonil + Chloridrate de Propamocarb	
Tests	**LI**	**CE50;7d**	**LS**
1	51.06	66.73	87.22
2	52.58	80.04	121.84
3	30.59	45.37	67.32
Average	**44.74**	**64.04**	**92.12**

LL: lower limit; UL: upper limite; IC50 – inhibition concentration 50%

Captan caused 8% of inhibition with 1.0 mg L^{-1} and 81% with 170.0 mg L^{-1}. Tebuconazole caused 26% with 1.0 mg L^{-1} and 83% with 50.0 mg L^{-1}, but there was a stabilization trend from 20.0 mg L^{-1}. Mixture, caused 36% with 25.0 mg L^{-1} and 73%, 300.0 mg L^{-1} (Figure 1).

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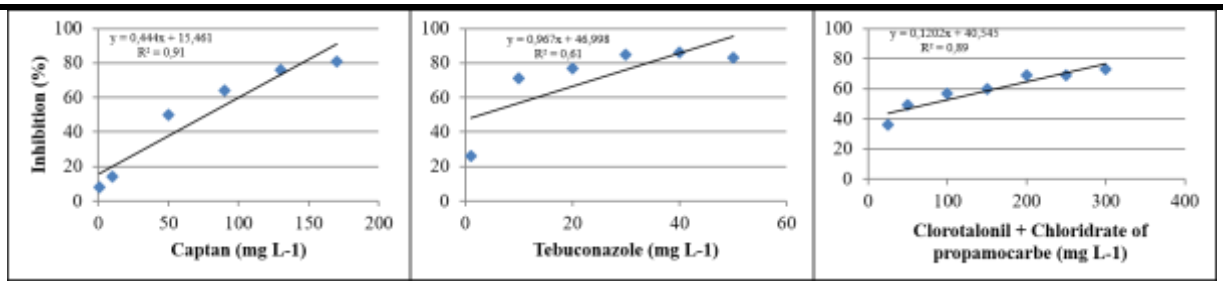

Fig.1: Relation of concentration effect of fungicides for Alternaria cassiae.

In relation to environmental risk, captan has possibility of adverse effect, therefore tebuconazole and the mixture don´t cause any adverse effect for fungo *A. cassiae*, independent of the dosage, according to Urban and Cook (1986) (Table 2).

Table.2: Environmental risk of fungicide for Alternaria cassiae.

Fungicides	**EEC (mg m^{-2})**	**Q=EEC/IC50**	**Classification**
Captan	12.00	0.25	PAE
	16.80	0.35	PAE
Tebuconazole	0.075	0.02	AAE
	0.150	0.04	AAE
Chlorothalonil + Chloridrate Propamocarb	1.80	0.03	AAE
	2.70	0.04	AAE

ECC: estimate environmental concentration; PAE: possibility adverse effect (0.1≤Q≤10); AAE: any adverse effect (Q≤0.1)

IV. DISCUSSION

The influence of fungicides for soil microorganisms depends of many factors between the physical properties, biochemical soil and fungicide concentration (Vyas, 1988). Fungicide effects for microorganisms envolve modification of availability and transformation of nytrogen, as nitrification and desnitrification (Chen *et al*., 2001) and consequentely in the soil quality. Then, the soil quality affects your potential, productivity and global sustainable of the agroecosystem and become necessary your study to secure the decisions for the better use of this resource (Sposito and Zabel, 2003).

Tebuconazole was the more toxic for *A. cassiae* (3,49 mg L^{-1}) and its toxicity is associated with inhibition of ergosterol synthesis, one component of fungal membrane and responsable by fluidity regulation, activity and distribution of integral proteins and control of celular cycle (Bard *et al*., 1993). This characterize the ergosterol biosynthesis way as essential for fungal growth (Alcazar-Fuoli *et al*., 2008). Tebuconazole also was toxic with low concentrations for *Colletotrichum gloeosporioides*, IC50 < 1.0 mg L^{-1}, which causes diseases in papaya (Tavares and Souza, 2005) and it showed high patogenecity *in vitro* for *Lasiodiplodia theobromae,* IC50 of 0.42 mg L^{-1} (Locatelli *et al*., 2015).

Captan offers risk for *A. cassiae* and it can unbalance the fungal content in the soil. Others agrochemicals used in the sugar cane (insecticides aldicarbe and fipronil, herbicides diuron and clomazone+ametryn and maturers etil-trinexapac and sulfometurom-metilic) were toxic for fungi *Metarhizium anisopliae* and *Beauveria bassiana* afecting the biological control by these fungi (Botelho and Monteiro, 2011).

Tebuconazole was more toxic that others but it doesn´t cause risk for this funghi because the EEC is much lower that IC50. According Carraschi *et al* (2015), the closer the recommended dose of the insecticide thiameothoxan is from the lethal concentration, the greater the possibility of it causing an adverse effect for a bioindicator. This relation also was verified with capatan and the mixture for *A. cassiae*.

Beside the fungi, the bacterial biomas also can be affected by agrochemicals use. According Widenfalk *et al*. (2008) captan doesn´t cause negative effects in the microbial biomass using the dosage permited and according to Milenkovski *et al*. (2010) only was observed some effect when used larger doses than found in the environment. Tebuconazole decrease the bacterial biomass in the litter after chronic exposure (six weeks, 20.0 µg L^{-1}) (Artigas *et al*., 2012). About clorotalonil, Xiaoqiang *et al*. (2008) described that the inhibitory effect by clorpirifós for soil organisms is increased when it is associated with clorotalonil.

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V. CONCLUSION

Tebuconazole causes moderately toxicity, captan, low toxicity and the mixture is considered non toxic for *A. cassiae,* the funghi non target, but only captan represents the possibility of adverse effect for this organism.

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