

## Antifungal Effects of Paraquat and Glyphosate on *Rhizoctonia solani* (Kühn) in Potato *in vitro* Condition

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### Abstract

Potato is one of the main crops worldwide. In this research, antifungal activity *in vitro* of paraquat and glyphosate were evaluated for *Rhizoctonia solani* control. *R. solani* was identified from potato tubers collected out from open markets in Saltillo, Coahuila, Mexico. Two types of herbicides were applied: paraquat and glyphosate, at four different dosage treatments of: 10, 100, 1 000 and 10 000 µL. One 5 mm diameter PDA disc with *R. solani* mycelium was placed at the center of the Petri dish, with a radial registry fungal every 24 h for 192 h. Pathogen was identified by morphological criteria and the data was evaluated randomly with a factorial arrangement, on which, herbicides represented factor A and dosage treatments were represented by factor B. Thus experimental design had two levels for factor A and five levels for factor B with six replications. The results were analyzed by the SAS version 9.1 statistical program, the mean separation with the Tukey test ( $p=0.05$ ). Glyphosate achieved inhibition of *R. solani* by 35.5882% and paraquat up to 80.0399%. Results reveal the importance of the need for more studies of these herbicides as fungicides. High concentrations of paraquat (10 000 µL) inhibits *R. solani*, and glyphosate does not affect *R. solani* mycelium development at low dosages (10 and 100 µL) and inhibits it at higher doses (10 000 µL).

**Keywords:** concentration, crop, fungi, herbicide, inhibition, dosage

### 1. Introduction

Potato (*Solanum tuberosum* L.) is one of the four crops of greater importance in the world, just after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.), with a production of 368 168 914 t in 2018 and in Mexico was 1 802 592 t (FAOSTAT, 2020), however, potato will be one of the key crops in reducing hunger and food security across the globe (Hussain et al., 2018). *R. solani* cause widespread soil-borne diseases is responsible for causing significant economic losses in many important field and horticultural crops all over the world (Grosch et al., 2004; Elsharkawy et al., 2014). Furthermore, is responsible for potato losses of 70% (Carling et al., 1989; Wilson et al., 2007), if you take that 70% loss, during that cycle, 1 261 814.4 t was lost in Mexico and 257 718 239.8 t worldwide.

Pesticides are chemical products used in agriculture to protect crops from pests (WHO, 2020). Glyphosate [N-(phosphonomethyl) glycine] is a broad-spectrum herbicide and the more is one of the more frequently used herbicides worldwide (Vila-Aiub et al., 2008; Duke & Powles, 2008; Duke, 2018), in forestation, aquatic weed control and agriculture (Bártoli et al., 2012), since its introduction in 1974 by the Monsanto company (now Bayer) with the commercial product Roundup (Woodburn, 2000). This herbicide disables, the enzyme 5-enolpyruvylshikimate-3- synthetize phosphate, which prevents the union of metabolite phosphoenolpyruvate in the active site of the enzyme (Carrera & Carreras, 2011; Salazar & Aldana, 2011). Paraquat [Dichloride of N, N'-dimethyl-4, 4'-bipyridylum] is a non-selective contact herbicide, in 1969 was introduced to Mexico and to this day, despite its high toxicity, it is one of the more used compounds for the control of broadleaf and pasture weeds (Hernández & Martínez, 2006).

*R. solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is necrotrophic pathogen, with little effective resistance in crop plants (Nikrafter et al., 2013), causing diseases to a wide range of hosts, affecting aerial and subterranean parts (Agrios, 2004); additionally, it produces a significant reduction in the plants strength and the production of tuberculous in potato crops (Cedeño, 2001). *R. solani* consists of 14 anastomosis

groups (AG 1-13 and BI), which have been divided based on hyphal anastomosis behavior, cultural morphology, host range, pathogenicity and other characters (Ogoshi, 1976; Carling et al., 1994, 1999, 2002).

*R. solani* is one of the most important soil pathogen fungi of the world, in cultivated and not cultivated soil, causing diseases in several crops as potato, beans, tomato, among others (Montealegre et al., 2003; Meza-Moller et al., 2007). Studies have shown effects of herbicides on plant pathogens *in vitro*, as the inhibition of growth and reproduction of the pathogen (Katan & Eshel, 1973). Due the above mentioned, the objective of this research was to evaluate the antifungal activity of glyphosate and paraquat *in vitro* for *R. solani* control.

## 2. Materials and Methods

### Pathogen Isolation and Identification

Potato tubers with *R. solani* (sclerotia) symptoms were collected from open markets in Saltillo, Coahuila, Mexico, 1 cm cuts with healthy and sclerotia and tissue, disinfected with 2% sodium hypochlorite for 5 min, and washed two times with sterile distilled water for 5 min. Four cuts equidistant manner in were placed in Petri dishes with PDA kept at  $28 \pm 2$  °C. *R. solani* identification was determined following Sneha et al. (1991) keys. Purification was done through a spearhead and isolations were kept at  $6 \pm 2$  °C.

### Used Herbicides

Glyphosate and paraquat were the herbicides used in 10, 100, 1 000 and 10 000 µL concentrations, in addition, a control treatment (herbicide free) (Tab. 1).

Table 1. Herbicides and treatments in study

Treatments	Paraquat		Glyphosate	
	µL	Herbicide (mL)	µL	Herbicide (mL)
I	10	0.0027	10	0.0020
II	100	0.0276	100	0.0245
III	1 000	0.2762	1 000	0.2450
IV	10 000	2.7620	10 000	2.4500
V*	0	0	0	0

V\*= Control (Herbicide free)

### PDA preparation with several herbicide dose levels

Several herbicide dosage levels in PDA culture medium (200 and 300 mL of PDA in glyphosate and paraquat respectively), were added once the sterilized environment was set at  $38 \pm 2$  °C, poured into Petri dish in a 20 mL average per plaque.

### Herbicide effectiveness

Dhingra & Sinclair (1985) food poisoning technique was used placing 5 mm diameter PDA discs with *R. solani* mycelium, at the center of the Petri dish with contaminated PDA, kept at a  $26 \pm 2$  °C for eight days.

### Evaluation

Mycelial growth was measured daily with the support of a vernier pointing to the four cardinal points (N, S, E, W), during 192 h. Data collected was evaluated randomly in a factorial arrangement, factor A for herbicides and factor B the treatments, with a two level in factor A and five levels for factor B, with six replications per treatment. Results were analyzed through the statistical computer program SAS version 9.1 (SAS Institute, 2002), and mean separation using the Tukey test ( $p=0.05$ ).

## 3. Results and Discussion

The purified colonies showed a dark brown mycelial growth, this pathogen displayed a mature mycelium forming 90° angle, branches, moderately wide and sclerotia hyphae, characteristic of *R. solani*, results that agree with to Nicoletti et al., (1999), Abd-Elsalam et al., (2009), Meza-Moller et al., (2011), D áz-N ñera et al., (2014), Ajayi et al., (2017) and Misawa et al., (2018). According to statistical analysis ( $p<0.0001$ ), with the resulting coefficient of variation of 3.5053, there is a statistical difference between herbicides used; glyphosate with a 2.73.75 radial growth and paraquat with 0.8483 (Tab. 2), the Petri dish radio was 4.25 cm (100%), in general the glyphosate achieved *R. solani* inhibition by 35.5882% and paraquat up to 80.0399%.

Paraquat and glyphosate are two of the more used herbicides in the world (James & Krattiger, 1996), and in Mexico the glyphosate continues to be used without any restriction or repercussion (agricultural, urban and

gardening use), among other highly dangerous herbicides (2,4-D, alachlor, atrazine, diuron, oxifluorfen and linuron). In 2013, 31,195 t of herbicides were applied in Mexico (Arellano-Aguilar & Von, 2016). Currently, in the world, glyphosate is the active ingredient with more than 750 generic products (UITA, 2020), and in Mexico the glyphosate “faena” its cost per 1 L varies from \$90.00 in Colima to \$325.00 in Culiacan, Sinaloa (SNIIM, 2020).

Table 2. General mean of glyphosate and paraquat with effect on the growth of *R. solani*

Herbicide	Radial growth (cm)	Ag	Inhibition mean (cm)
Glyphosate	2.7375 ± 2.0531	a	1.5125
Paraquat	0.8483 ± 1.6463	b	3.4017

Ag= Statistical aggrupation, equal letters are not statistically different according to Tukey test (p=0.05).

Table 3 shows difference among treatments; however, according to Tukey a 0.05 test, not significant, although, difference with observed on both herbicides at 10 000 µL resulted in a higher *R. solani* inhibition (Fig. 1). Castaño (1986) reported paraquat a low dosage (12.5, 25 and 50 µL) had an inhibiting effect against *R. solani*, and glyphosate did not present an inhibiting effect. Mensin et al. (2013) reported paraquat achieved mycelium growth inhibition for nematophagous fungi *Arthrobotrys oligospora* (80%), *A. conoides* (100%), *A. musiformis* (100%) and *A. thaumasiium* (100%) at the lowest evaluated concentration (575 mg/L). The fungal growth inhibition by glyphosate was due probably to the blocking of enzyme 5-enolpyruvylshikimate-3-phosphate (EPSPS) in the shikimic acid pathway that affect the amino acid synthesis (Franz et al., 1997), and paraquat has a marked effect on the growth rate (Wilkinson & Lucas, 1969a), respiration rate (Wilkinson & Lucas, 1969b), spore germination (Wilkinson & Lucas, 1969c) and sporulation (Gareth & Williams, 1971).

In this research, higher dosages gave better results (10 000 µL), results obtained are opposed those to Vargas et al. (2002) who reported the lowest glyphosate dosage accomplishing inhibition of *R. solani* growth. (2.500 mg/L) and 300 mg/L (highest dosage) concentration, which had no effect on *R. solani* growth. Harikrishnan & Yang (2001) found no negative effect of glyphosate on vegetative growth of several *R. solani* isolates and anastomosis groups, however, inert ingredients in several formulations are suggested to be responsible of effect of glyphosate in the inhibiting mycelial growth and spore germination (Morjan et al., 2002).

Table 3. Data of glyphosate and paraquat treatments against *R. solani*

Herbicide	Treatment	Rep	Mean	Standard deviation	Ag
Glyphosate	1	6	4.07142857	0.02249339	a
	2	6	4.08000000	0.02091650	a
	3	6	1.49583333	0.14527273	a
	4	6	0.01666667**	0.03027650	a
	5	6	4.02500000	0.06892024	a
Paraquat	1	6	0.11666667	0.10206207	a
	2	6	0.01250000	0.03061862	a
	3	6	0.02083333	0.03322900	a
	4	6	0.00833333**	0.02041241	a
	5	6	4.08333333	0.03027650	a

\*\*Treatment with more antagonistic effect against *R. solani*. Treatments: T1= 10 µL, T2= 100 µL, T3= 1 000 µL, T4= 10 000 µL and T5= Control; Rep= Replicates; Ag= Statistical aggrupation, equal letters are not statistically different according to Tukey test (p=0.05).

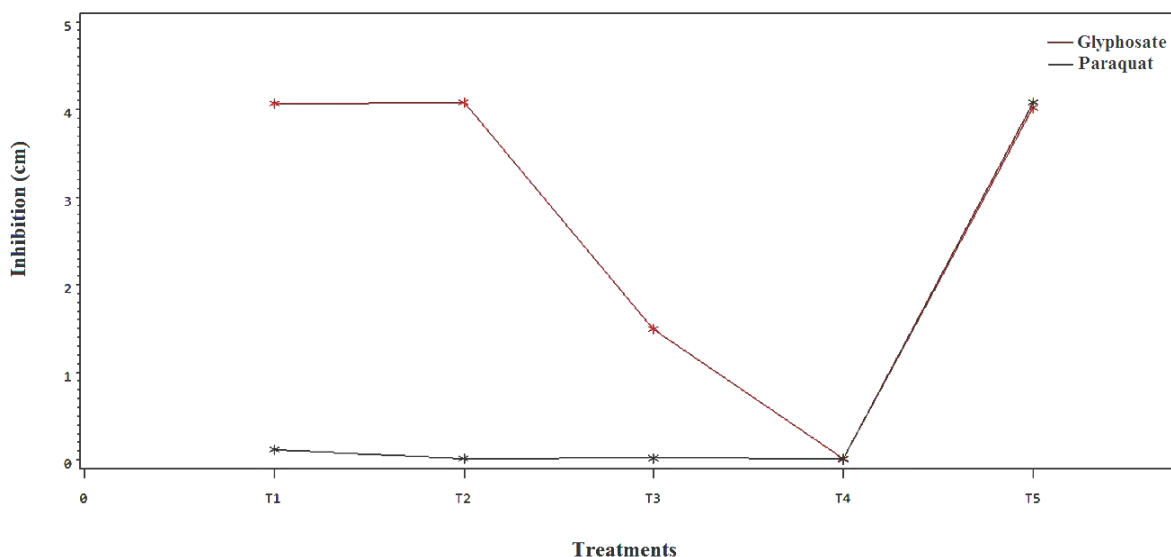


Figure 1. Inhibition of *R. solani* with the different treatments of paraquat and glyphosate. Treatments: T1= 10  $\mu$ L, T2= 100  $\mu$ L, T3= 1 000  $\mu$ L, T4= 10 000  $\mu$ L and T5= Control.

#### 4. Conclusions

High concentrations of paraquat inhibits *R. solani* (10 000  $\mu$ L).

Glyphosate does not affect *R. solani* mycelium development at low dosages (10 and 100  $\mu$ L) and inhibits it at higher doses (10 000  $\mu$ L).

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#### Author Contributions

JLAV organized the manuscript, wrote the manuscript and edited it, and contributed to the application of statistical techniques. ASA conceptualized and formulated the general objectives of the research. MaEGC supervised the execution of research activities. CTR responsible for preservation of data, and annotations in the investigation.

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