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NOTES AND COMMENTS

Antifungal activity *in vitro* of propolis solutions from Argentina against two plant pathogenic fungi: *Didymella bryoniae* and *Rhizotocnia solani*

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Didymella bryoniae (Auersw.) Rehm and *Rhizoctonia solani* Kühn cause some of the most destructive fungal plant diseases in the southern Pampas Region of Argentina. The gummy stem blight caused by *D. bryoniae* affects melon crops, damaging leaves and fruits (Fiori *et al.,* 2000). *R. solani* is a well-known species complex which causes seedling death in several crops (Nicoletti *et al.,* 1999).

Chemical control of fungi has increased the productivity and quality of various crops, but abuse of chemical products has caused soil pollution and harmful effects on human beings (Fiori *et al*., 2000). The replacement of synthetic fungicides by natural products, particularly those which are of plant origin such as propolis, could present advantages such as efficacy and low toxicity (Agüero *et al.,* 2010).

Propolis is an extremely complex resinous material gathered by honey bees from various plant sources. The antibacterial and antifungal activities are the most popular and the most extensively investigated biological actions of propolis. However, the use of propolis for control of phytopathogens has been poorly assayed (Quiroga *et al*., 2006). In this context, we studied the possible use of propolis as a biological alternative for control of phytopathogenic fungi. The objective of this work was to evaluate the effect of an alcoholic propolis solution against *D. bryoniae* and *R. solani* colony growth *in vitro* by the mycelial growth test.

The propolis (poplar-type) was collected from the southern Pampas region in Argentina. Sensory, chemical and physical characteristics of the sample were determined by Luis Maldonado in the Experimental station of INTA EEA Famaillá (Tucumán, Argentina). The propolis sample was ground into small pieces and sieved through a 2 mm mesh before the extraction. The alcoholic propolis solution was prepared by dissolving 20 g of propolis per 200 ml of 96 % ethanol, agitated at 40±2°C for 18 h and filtered with Whatman Nº 40 paper. In order to concentrate this propolis solution, it was agitated for 72 h under the same conditions, and then stored at 4ºC in the dark until used. The final concentration of propolis soluble compounds was determined (Bedascarrasbure *et al*., 2006). Phytopathogenic fungi were obtained from Plant Phytopathology (Universidad Nacional del Sur, Argentina). *D. bryoniae* was isolated from fruits of *Cucurbita moschata* (squash) while *R. solani* was from *Allium* (onion) roots. They were grown at 22ºC on potato dextrose agar (PDA) (potato 200 g, agar 13 g, dextrose 20 g and water to 1l) and stored at 4ºC.

The antifungal activity of the alcoholic propolis solution was assayed *in vitro* by the mycelial growth test. A propolis treatment (called P) and two different control treatments (C1 and C2) were performed. Propolis alcoholic solution (P) and alcohol 96º (C1) were both dropped in separated plastic Petri dishes (1.5 ml/dish) and left in a laminar flow chamber until total evaporation. Then, 15 ml of PDA were carefully poured into the dishes. Another treatment called C2, which consisted of only PDA, was added. Once the medium solidified, circles of mycelium of 10 mm diameter were obtained from colonies with 72 h of incubation and were placed in the centre of the plates. Plates were incubated in the dark for 30 days at 22ºC. The colony growth of *Didymella bryoniae* and *Rhizoctonia solani* was measured daily. The experimental design was completely randomized with four replicates per treatment. Multiple comparisons were performed with a one way analysis of variance. Least significant difference (Fischer LSD) was used for comparison of means.

The propolis was composed of granulated fragments of soft consistency. The aroma of the sample mainly corresponded to "soft resinous". The flavour was sweet and the predominant colour was yellow / yellowish brown. The propolis composition showed an average value of 63.38 % of total resins, 24.37 % of total phenolic compounds, 7.65 % of total flavonoids and 3.27 % of impurities

Table 1. Effect of propolis solution on myceliar growth of *D. bryoniae* and *R. solani* over 30 days. Portions of mycelium of 10 mm diameter were streaked onto the agar. Incubation was carried out in darkness for 30 days at 22ºC. P: propolis (12.78 % of propolis soluble compounds in ethanol 96 % plus potato dextrose agar); C1: Control 1 (ethanol 96 % plus potato dextrose agar) and C2: Control 2 (potato dextrose agar). *Myceliar diameters in mm [1]are the mean of 4 determinations ± SD. Means with different letters differ significantly at $p \leq 0.01$ according to the Fisher LSD.

Fungus	Treatment	Days			
		7	10	17	30
D. bryoniae	P	15.6*±2.4[1]a	32±3.7b	67.9±3.1b	81.4±0.5a
	C1	50.9± 0.5b	72.5±0.4c	85±0c	85±0b
	C2	51.6±2.1b	71.9±2.0c	85±0c	85±0b
R. solani	P	14.9±2.7a	27±3.2a	56.9±4.1a	80.6±2.4a
	C1	66.4±0.8c	85±0d	85±0d	85±0b
	C2	67.7±1.3c	85±0d	85±0d	85±0b

(beeswax and other debris). UV spectrograms showed that the propolis extract analysed displayed a maximum absorbance range between 270 and 315 nm, with a main absorption peak at 294 nm which is indicative of an important biological activity (Sosa López *et al.,* 2003). It is interesting to notice that physicochemical and sensory characteristics previously described were consistent with data recorded from other propolis samples from the Pampas Region of Argentina (Bedascarrasbure *et al.,* 2006). The propolis sample characteristics and the abundance of poplar trees within the foraging area indicate that the main resin origin was *Populus* sp.

Table 1 shows the fungal growth in mm measured by day. The final propolis soluble compounds concentration in the alcoholic solution was 127.83 mg/ml (12.27 %). Both plant pathogenic fungi were sensitive to this tested concentration. The mean diameters of *D. bryoniae* and *R. solani* colonies in the propolis treatment were significantly smaller ($p \leq 0.01$) than in their respective controls until the end of the experiment. The colonies of *R. solani* in both control treatments covered the whole surface of the dish at day 10, and those of *D. bryoniae* at day 17, whereas none of the colonies reached the edge of the Petri dishes in the propolis treatment at day 30 (Figs 1a,b). Myceliar growth stopped developing in the propolis treatment on day 27 for *Didymella* and on day 28 for *Rhizoctonia* and could not reach the Petri dish diameter (85 mm diameter) as was the

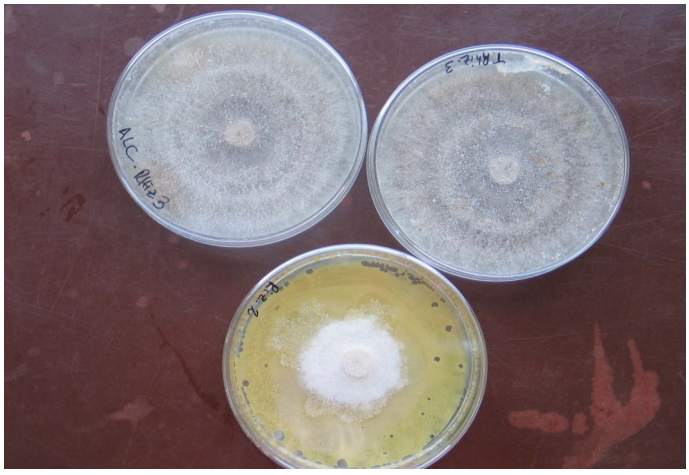

Fig. 1a. Inhibitory effect of propolis solution on *R. solani* growth at day 9. Three treatments were compared: propolis (P) (12 % in ethanol 96 % plus PDA); Control 1 (C1) (ethanol 96 % plus PDA); and Control 2 (C2) (PDA). Small portions of mycelium of 10 mm diameter were streaked onto the agar. Incubation was carried out in darkness at 22ºC.

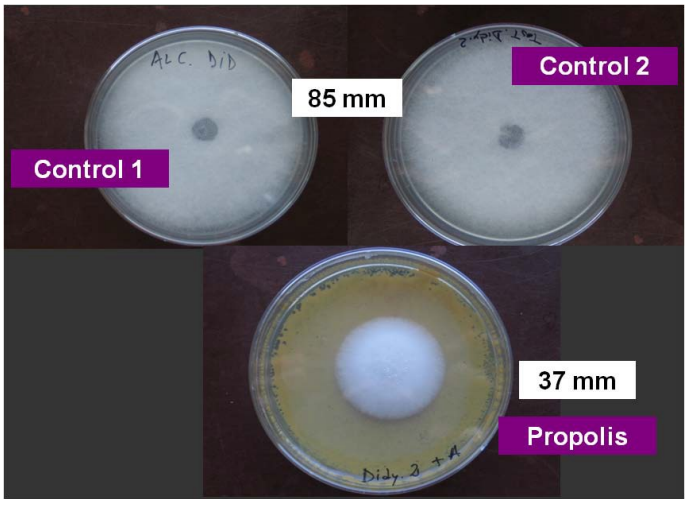

Fig. 1b. Inhibitory effect of propolis solution on *D. brioneae* growth at day 12. Three treatments were compared: propolis (P) (12% in ethanol 96% plus PDA); Control 1 (C1) (ethanol 96% plus PDA); and Control 2 (C2) (PDA). Small portions of mycelium of 10 mm diameter were streaked onto the agar. Incubation was carried out in the darkness at 22ºC.

case for both controls. The mycelial growth test revealed that growth of both fungi was inhibited within the first week in the propolis treatment compared to the controls. This is particularly important because this period is crucial in crop development (Agrios, 1996).

Many other researchers also found promising results with propolis for control of fungal pathogens using the same *in vitro* technique. Pineda *et al*. (2010) evaluated antifungal property of *Apis mellifera* propolis on isolates of *Colletotrichum gloeosporioides* that affect fruits of avocado (*Persea americana*), papaya (*Carica papaya*) and passion fruit (*Passiflora edulis*) from Venezuela. Quiroga *et al.* (2006) determined the antifungal and cytotoxic activities of partially purified propolis extract on plant pathogenic fungi such *Aspergillus niger*, *Fusarium* sp. and *Macrophomina* sp. Chaillou y Nazareno (2009) evaluated antimycotic activity of different propolis samples from Argentina against *Fusarium* sp., *Macropohomina* sp., *Phomopsis* sp., *Aspergillus niger* and *Trichoderma* sp.

In conclusion, we assessed the antifungal activity of an alcoholic propolis solution against two plant pathogenic fungi: *Didymella bryoniae* and *Rhizotocnia solani* by the mycelial growth test. Propolis showed a fungistatic effect on the growth of both plant pathogenic fungi. Further investigations of *in vivo* studies are necessary to make sure propolis has the potential to effectively control fungal diseases under field conditions.

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References

AGRIOS, G N (1996) Fitopatología. Segunda Edición. Editorial Limusa México, 838 pp. ISBN 968-18-5184-6

AGUERO, M B; GONZALEZ, M; LIMA, B; SVETAZ, L; SANCHEZ, M; ZACCHINO, S; EGLY FERESIN, G; SCHMEDA-HIRSCHMANN, G; PALERMO, J; WUNDERLIN, D; TAPIA, A (2010) Argentinean propolis from *Zuccagnia punctata* Cav. (Caesalpinieae) exudates: phytochemical characterization and antifungal activity. *Journal of Agricultural and Food Chemistry*, 58: 194-201. http://dx.doi.org/10.1021/jf902991t

BEDASCARRASBURE, E; MALDONADO, L M; FIERRO MORALES, W; ALVAREZ, A R (2006) *Propóleos. Caracterización y normalización de propóleos argentinos. Revisión y actualización de composición y propiedades*. Ediciones Magna; Buenos Aires, Argentina. 218 pp.

CHAILLOU, L L; NAZARENO, M A (2009) Bioactivity of propolis from Santiago del Estero, Argentina, related to their chemical composition. *LWT - Food Science and Technology,* 42: 1422-1427. http://dx.doi.org/10.1016/j.lwt.2009.03.002

FIORI, A C G; SCHWAN-ESTRADA, K R F; VIDA, J B; SCAPIM, C A; CRUZ, M E S; PASCHOLATI, S F (2000) Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae. Journal of Phytopathology,* 148: 483-487. http://dx.doi.org/10.1046/j.1439-0434.2000.00524.x

NICOLETTI, R; LAHOZ, E; KANEMATSU, S; NAITO, S; CONTILLO, R (1999) Characterization of *Rhizoctonia solani* isolates from tobacco fields related to anastomosis groups 2-1 and BI (AG 2-1 and AG BI). *Journal of Phytopathology,* 147: 71-77. http://dx.doi.org/10.1046/j.1439-0434.1999.147002071.x

PINEDA, J; PRINCIPAL, J; BARRIOS, C; MILLA, D (2010) Propiedad fungistática *in vitro* de propóleos sobre tres aislamientos de *Colletotrichum gloeosporioides. Zootecnia Tropical,* 28(1): 83-91.

QUIROGA, E N; SAMPIETRO, D A; SOBERO, J R; SGARIGLIA, M A; VATTUONE, MA (2006) Propolis from the northwest of Argentina as a source of antifungal principles. *Journal of Applied Microbiology*, 101: 103–110.

SOSA LÓPEZ, A; SUBOSKY, M J; MAIDANA, J F; CASTILLO, A (2003) Organoleptic and physical characteristics of propolis from northeastern Argentina. *Spanish Journal of Agricultural Research,* 1(2): 37-40.