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Verbascum thapsus: Antifungal and phytotoxic properties

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ABSTRACT

Verbascum thapsus (Schrophulariaceae) better known as Mullein is a medicinal plant used in the treatment of inflammatory diseases, asthma, spasmodic cough, diarrhea, and other pulmonary problems. The objective of this study was to evaluate the effect of *V. thapsus* extracts on fungal plant pathogens growth and seed germination. *Verbascum thapsus* leaves were treated with n-hexane, chloroform, methanol, cold and warm water to obtained the extracts. Antifungal activity was observed mainly in the methanol extract (1000 µg mL^{-1}) against *Fusarium graminearum* and *Macrophomina phaseolina*. Furthermore, results show that the hexane, cold and warm aqueous extract favored or had no toxic effect on *Lycopersicon esculentum* and *Triticum aestivum* seeds germination and growth, while the chloroform and methanol extracts affected negatively the germination.

Keywords: Natural product, *Verbascum thapsus*, Antifungal, Phytotoxic.

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Introduction

Crops are easily infected by phytopathogenic fungi around the world, and fungal diseases are hard to control with out the use of synthetic fungicides. However, the application of large quantities of chemicals in agriculture has the potential to exert toxic effects on humans and wildlife as well as to causes environmental pollution (Nguyen *et al*., 2009). For that reasons, the discovery of new antifungal agents against fungal plant pathogen with less toxic effects is desirable. Natural products obtained from plants are an attractive alternative for disease control in agricultural crops since they can be degraded by one or other organism.

Verbascum thapsus (Scrophulariaceae) is a medicinal plant popularly known as "common mullein". It has been used for the treatment of inflammatory diseases, asthma, spasmodic coughs, diarrhea and other pulmonary problems. Although it is native from Europe and Asia, it was introduced in America several times (Turker and Camper, 2002). This plant is reported to be active against influenza virus (Mehrotra *et al*, 1989), bovine herpes virus type 1 (McCutcheon *et al*., 1995), bacteria (Turker and Camper, 2002), fungi (McCutcheon *et al*., 1994) and against mosquito larvae (Gross and Werner, 1978).

In spite of the numerous studies made of mullein, antifungal activity against plant pathogens has not been investigated. The objective of this research was first, to study the effect of *V. thapsus* extracts on fungal plant pathogen growth and secondly, the effect on germination of *Lycopersicon esculentum* and *Triticum aestivum*, both species affected by some of the pathogens under study.

Experimental

Preparation of plant extracts

Five extracts were obtained form leaves of *Verbascum thapsus* collected in the mountainous region of the Córdoba province, Argentina in May 2008. The plant was identified by Ing. Luis del Vitto, professor in the Area of Botany of the Universidad Nacional de San Luis. The plant material was powdered and successively extracted for 48 h at room temperature with n-Hexane (HE), Chloroform (CE) and Methanol (ME). To obtain Cold Aqueous Extract (CAE), plant material was extracted with water at 4ºC (48 h). In addition, the extracted material was left to soak in distilled water at 70ºC in order to obtain the Warm Aqueous Extract (WAE). Extracts were concentrated to dryness and dissolved in Dimethyl Sulfoxide (DMSO) to give a concentration of 100 mg mL^{-1}.

Antifungal assay

The assay was performed against the plant pathogens of economical importance in agriculture as *Fusarium graminearum, Fusarium solani, Fusarium verticillioides, Macrophomina phaseolina* and *Sclerotium rolfsii.* The activity was study by the agar dilution method. It is based on the hyphal radial growth rate of fungi in the presence and absence of the plant extract, according with reported procedures (Tegegne *et al*., 2008). Extracts were added to molten Potato Dextrose Agar (PDA) to obtain a final concentration of 1000 µg mL^{-1} and then pour into the Petri dishes (9.0 cm in diameter). A 4 mm diameter plug of actively growing fungi, taken from PDA plates was placed onto the centre of Petri dishes; treatments were incubated at 25ºC. Experiments were repeated 3 times in triplicate. Parallel controls were included by mixing DMSO with PDA medium. Sensitivity of each fungal species to each tested extracts was calculated as percentage of mycelial growth inhibition, according to the formula described by Pandey *et al*., (1982): (dc-dt)/dc x 100, where dc = average diameter of the fungal colony of the negative control and dt = average diameter of the fungal colony treated with the extract.

Phytotoxicity assay

A bioassay based on germination, radicle and epicotil growth of *Lycopersicon esculentum* Mill. (tomato) and *Triticum aestivum* (wheat), were used to study the allelopathic effects of *V. thapsus* extracts when applied at a concentration of 1000 µg mL^{-1}. Seeds were surface-disinfected and then placed on Petri dishes (20-40 seeds per dish) containing a layer of Whatman filter paper on cotton, which had previously been impregnated with 20 ml of extract solution dissolved in distilled water or 1% DMSO (control). Dishes were then incubated at 25ºC for 3 days for *T. aestivum* and 7 days for *L. esculentum* (Basile *et al*., 2000). Three replicates were carried out for each assay. The number of germinated seeds was determined according to the 2 mm radicle extrusion criterion. Radical and epicotyl growth was measured in twenty germinated seeds. Data were compared in a descriptive manner and analyzed by using analysis of variance (ANOVA). The Dunnett (bilateral) *post hoc* test was applied to make comparisons between the means at $P<0.05$.

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Results and discussion

The extracts yield from *V. thapsus* leaves were: HE: 0.32%, CE: 0.32%; ME: 1.50%; CAE: 14% and WAE: 10%. Those extracts were subjected to antifungal and phytotoxic activity.

Table 1 showed the results of the extracts against the 5 plant pathogenic fungi tested. The ME was the most active to reduce fungal growth. All five fungi were sensitive to ME, but *F. graminearum* and *M. phaseolina* were the most, both with a high inhibition (56%) while *S. rolfsii* was inhibited in 49%. Otherwise *Fusarium solani* and *F. verticillioides* were inhibited in 29% and 36% respectively.

Table 1: Antifungal activity of *V. thapsus* extracts (1000 µg ml^{-1}) against plant pathogenic fungi. Agar dilution method

Plants Extracts	*Fungal Strains*				
	Fg[a]	Fs[b]	Fv[c]	Mp[d]	Sr[e]
Hexanic	+	-	±	+	+
Chloroformic	++	-	±	+	±
Methanolic	++	+	+	++	++
Cold Aqueous	-	-	-	-	+
Warm Aqueous	-	-	-	-	-

Fg[a]*: F. graminearum;* Fs[b]*: F. solani;* Fv[c]*: F. verticillioides;* Mp[d]*: M. phaseolina;* Sr[e]*: S. rolfsii.* The inhibition was reported as (-) < 10% growth inhibition, (±) between 10 and 20%, (+) between 20 and 40%, (+ +) between 40 and 80%, and (+ + +) >80%. (*n* = 9)

In addition, HE and CE had similar behavior against the fungi tested and showed slight mycelial inhibition against *F. graminearum*, *F. verticillioides*, *M. phaseolina* and *S. rolfsii*. Both aqueous extracts were completely inactive against the fungi tested.

Has been reported that various plant extracts exert *in vitro* antifungal activity at different levels against phytopathogenic fungi. These plants include *Alpinia galanga*, *Cinnamomum cassia*, *Glycyrrhiza uralensis*, *Zingiber officinale* (Nguyen *et al*., 2009). In Turkey, two *Verbascum* species (*V. lasianthum* and *V. pterocalycinum*) were studied in their antifungal activity against three *Colletotrichum* species isolated from strawberry. The authors identify the saponins ilwensisaponin A and C from *V. pterocalycinum* var. *mutenseas*, as responsible for the activity. Since the genus is the same, it can be suggestive that similar compound present in *V. thapsus* may be responsible for the antifungal behavior of the ME (Tatli *et al*., 2003).

The effect of *V. thapsus* extracts on germination and both, radicle and epicotyl length on *L. esculentum* seeds is shown in **Table 2**, and **Table 3** enlists the activity on *T. aestivum*.

Table 2: Phytotoxic activity of *V. thapsus* extracts (1000 µg ml^{-1}) on *L. esculentum* seeds.

Treatments	Germination (%)	Epicotyl Length (mm)	Inhibition (%)	Radicle length (mm)	Inhibition (%)
Control	100	33		51	
Hexanic extract	100 n.s.[a]	39 n.s.[a]	+ 18	62 *[b]	+ 21
Chloroformic extract	94 *[b]	8 *[b]	75	18 *[b]	64
Methanolic extract	97 n.s.[a]	5 *[b]	85	28 *[b]	45
Cold Aqueous extract	99 n.s.[a]	40 n.s.[a]	+ 21	49 n.s.[a]	4
Warm Aqueous extract	97 n.s.[a]	48 *[b]	+ 45	54 n.s.[a]	+ 6

n.s.[a] : not significative. **b*: statistically different from control (Dunnett bilateral test, *P<0.05*).

Table 3: Phytotoxic activity of *V. thapsus* extracts (1000 µg ml^{-1}) on *T. aestivum* seeds.

Treatments	Germination (%)	Epicotyl Length (mm)	Inhibition (%)	Radicle length (mm)	Inhibition (%)
Control	100	17		29	
Hexanic extract	100 n.s.[a]	20 n.s.[a]	+ 16	30 n.s.[a]	+ 3
Chloroformic extract	100 n.s.[a]	10 *[b]	40	24 n.s.[a]	16
Methanolic extract	100 n.s.[a]	8 *[b]	49	20 *[b]	30
Cold Aqueous extract	100 n.s.[a]	17 n.s.[a]	1	32 n.s.[a]	+8
Warm Aqueous extract	100 n.s.[a]	16 n.s.[a]	7	30 n.s.[a]	+1

n.s.[a] : not significative. **b*: statistically different from control (Dunnett bilateral test, *P<0.05*).

Among the parameter analyzed on plants growth, the germination was the less vulnerable, only *L. esculentum* exhibit significant differences when it was treated with the CE. Chloroform and ME affected most epicotyl and radicle length in

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both crops, but *L. esculentum* was the most affected. On the other hand, HE appeared to stimulate epicotyl and radicle growth in both crops, while WAE stimulated only *L. esculentum*. CAE did not show any phytotoxic effect on the germination of the seeds.

The results obtained are consistent with those reported by other authors who informed that fractions or compounds isolated from plants can modulate positive or negatively the development of other plants (Basile *et al*., 2000).

Phytochemicals reports inform that leaves of *V. thapsus* are rich in iridoid glycosides, flavonoids, phenylethanoid glycosides and saponins, and they may be responsible for the biological activities (Tatli and Akdemir, 2004).

Conclusions

The used of plant products for the management of plant diseases have achieved greater significance in recent years due to its readily available nature, antimicrobial activity, easy biodegradability, lowed phytotoxicity, besides inducing resistance in host. In this study, *V. thapsus* ME showed to posses high antifungal and low phytotoxic properties on wheat seeds.

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