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Antifungal activity of *Datura metel* L. organic and aqueous extracts on some pathogenic and antagonistic fungi

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The present study was undertaken to assess the *in vitro* antifungal activity of petroleum ether, chloroform, methanol and aqueous extracts of *Datura metel* L. leaves and flowers, against two *Trichoderma* species (*Trichoderma harzianum* and *Trichoderma viride*) and three *formae speciales* of *Fusarium oxysporum*, that is, *Fusarium oxysporum* f. sp*. melonis*, *Fusarium oxysporum* f. sp*. lycopersici* and *Fusarium oxysporum* f. sp*. tuberosi*. Radial growth of the pathogen on PDA plates was recorded after 3 to 7 days of incubation at 25°C. Results revealed an important antifungal activity of *D. metel* leaf and flower aqueous extracts at all concentrations tested (1, 2, 3 and 4%) against pathogenic fungus. However, the extent of response to extracts was species specific. In fact, the highest inhibition (69%) of *T. viride* mycelial growth was observed with flower aqueous extracts. Indeed, *F. oxysporum* f. sp*. melonis* was found to be more susceptible than the other fungal species to organic extract; radial growth inhibition varied from 24 to 76% and from 31 to 76% in the presence of leaf and flower organic extracts, respectively. In addition, the radial growth of *F. oxysporum* f. sp*. Lycopersici* was reduced by all organic extracts. The fungicidal activity of leaf extracts was more effective against *F. oxysporum* f. sp*. tuberosi* as compared to flower extracts. Both *Trichoderma* species were less sensitive to *D. metel* organic extracts than *Fusarium* species. Our findings showed that *D. metel* extracts can be used as potential source of fungicides to control the phytopathogenic fungi tested.

Key words: Antifungal activity, aqueous and organic extracts, *Datura metel*, *Fusarium, Trichoderma,* radial growth.

INTRODUCTION

Recently, the development of biopesticides has been focused on as a viable pest control strategy. Allelopathy is considered to be one of the promising options for sustainable pest management (Khanh et al., 2007; Zahid et al., 2012). A lot of work has been done on allelopathic plant extracts for their antifungal properties as natural alternatives for plant fungal disease control (Bajwa et al., 2003, 2004, 2006; Hao et al., 2010). Organic (Bajwa et al., 2008; Shafique and Shafique, 2008) and aqueous extracts (Bajwa et al., 2001) of many allelopathic plants are known to exhibit antifungal properties. Allelochemicals act by reducing the germination of spores and the mycelial growth of pathogenic fungi (Bajwa et al., 2003). In Tunisia, wild plants are widespread and represent a very

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rich and characteristic flora (Pottier-Alapetite, 1979, 1981). Many of them such as *Euphorbia macroclada*, *Euphorbia bougheii*, *Euphorbia striatella*, *Euphorbia serrata*, *Euphorbia virgata*, *Euphorbia fortissimo* and *Euphorbia cooperi* are used to control root infecting fungi including *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria solani* and *Verticillium dahliae* (Gundidza et al., 1992; Gundidza and Kufa, 1993; Shaudat and Siddiqui, 2002; Al-Mughrabi, 2003). Another plant such as *Datura metel* L., a Solanaceous species, is a sub-glabrous shrubby herb that exists throughout the world (Rajesh, 2002) and is widely distributed in Tunisia. This species has not hitherto been the subject of biological and chemical investigation in Tunisia, except the recent work of Bellila et al. (2011) which was more focused on the cytotoxic activity of with a-nolides isolated from Tunisian *D. metel*. However, this plant when collected from other countries was found to be a potential source of several biologically active metabolites. In previous studies, it was reported that *D. metel* parts contained two anticholinergic alkaloids namely hyoscyamine and scopolamine (Chopra et al., 1956). It is known for its use in fever with catarrh, cerebral complications, diarrhea, skin diseases, antiseptic, animal bites, anti helmenthic and in herpetic diseases, and also has healing potential on burn wounds (Priya et al., 2002). Moreover, different *D. metel* extracts were reported to have hypoglycemic (Krishna Murthy et al., 2004) and antimutagenic properties (Reid et al., 2006). *D. metel* is also known for its larvicidal activity against the third instars larvae of *Culex quinque fasciatus* (Chakkaravarthy et al., 2011), antibacterial activity against burn pathogens (Gnanamani et al., 2003) and antifungal activity against various plant pathogens (Rajesh, 2002; Dabur et al., 2004; Kagale et al., 2004). Crude as well as ethanolic extracts of some plants including *Datura* sp. have been tested by many authors for their effectiveness against several plant pathogenic fungi *in vitro*, in glasshouses, and also under field conditions (Reimers et al., 1993; Bambawole et al., 1995; Prithiviraj et al., 1996; Sarma et al., 1999). Indeed, *D. metel* leaf extract has been reported to exhibit plant virus inhibiting properties (Singh and Verma, 1981). They have also been assayed against spore germination of *Alternaria alternata*, *Drechslera halodes* and *Helminthosporium speciferum* (Srivastava and Srivastava, 1998). Therefore, the aim of this investigation was to assess the *in vitro* antifungal activity of aqueous and organic extracts of *D. metel* leaves and flowers. This allelopathic plant species was tested, as potential source of natural biologically active substances, against three phytopathogenic and two antagonistic fungi.

MATERIALS AND METHODS

Fungal agents used

Pure culture of two *Trichoderma* species (*Trichoderma harzianum* and *Trichoderma viride*) and three *formae speciales* of *Fusarium oxysporum*, that is, *F. oxysporum* f. sp. *melonis* (FOM), *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *tuberosi* (FOT) infecting melon, tomato and potato, respectively, were obtained from the Laboratory of Plant Pathology of the Regional Center of Research on Horticulture and Organic Agriculture, Chott-Mariem (Tunisia). The isolates were isolated from the diseased samples on potato dextrose agar (PDA), purified and maintained at 4°C until use.

Collection of plant material

Fresh and healthy leaves and flowers of *D. metel* were collected from Tunisian littoral (Monastir, Tunisia). Fresh materials were washed thoroughly with detergent to remove any residual dust. Washed leaves and flowers were dried in an electric oven at 30°C for 72 h and crushed to make powder.

Extracts preparation

Aqueous extract was prepared by soaking thirty grams of dried leaf and flower powder of the test species in 100 ml of sterilized distilled water for 24 h. Extract was filtered through a double layered muslin cloth followed by Whatman No. 1 filter paper and then passed through 0.22 µm micro-filter pore to remove bacteria. Filtrates were preserved at 4°C until use. To avoid any prospective chemical alterations, the extracts were generally used within a week.

Sequential extraction was carried out in organic solvents with rising polarity: petroleum ether, chloroform and methanol. Eighty grams of powder were immersed in the organic solvent for 7 days at room temperature.

Organic extracts were evaporated to dryness under reduced pressure in a rotary evaporator at 45 to 50°C, respectively, to remove the petroleum ether, chloroform and methanol. Samples of 15, 30 and 45 mg were individually dissolved in 2 ml of methanol and then diluted by adding 3 ml of sterilized distilled water to make final volume of 5 ml to give three extract concentrations (3000, 6000 and 9000 ppm). The stock extract was stored at 4°C and used within four days.

Antifungal bioassays

The antifungal activity against the test fungal agents was determined according to the poisoned food technique of Grover and Moore (1962). In fact, PDA medium was prepared and sterilized at 150°C for 30 min in autoclave. Appropriate quantities of aqueous extracts (1.5, 3, 5 and 6.25 ml) and distilled water were added to this medium (40 ml), cooled to 45 to 50°C, to get 1, 2, 3 and 4% (w/v) concentrations of leaf and flower aqueous extracts. The control medium received the same quantity (1.5, 3, 5 and 6.25 ml) of sterile distilled water. Stock solution of organic extracts (5 ml) prepared above at 3000, 6000 and 9000 ppm was added to PDA medium. Control received the same quantity (5 ml) of diluted methanol used as control for all bioassays with organic extracts.

The plant extracts were thoroughly mixed with the medium. Ten milliliters of each medium was poured in each 9 cm diameter sterilezed Petri plate. After solidification, mycelial plugs of 5 mm diameter were taken with a pre-sterilized cork borer from 5 to 7 days old culture of test fungus and were placed in each Petri plate. Each treatment was replicated thrice. Plates were incubated in an incubator at 25 ± 2°C for 3 to 7 days. Fungal radial growth was measured by averaging the two diameters taken from each colony. Percentage growth inhibition of the fungal colonies was calculated by applying the following formula (Khanh et al., 2005):

Growth/inhibition (%) = [(Growth in control – growth in treatment) / growth in control] × 100.

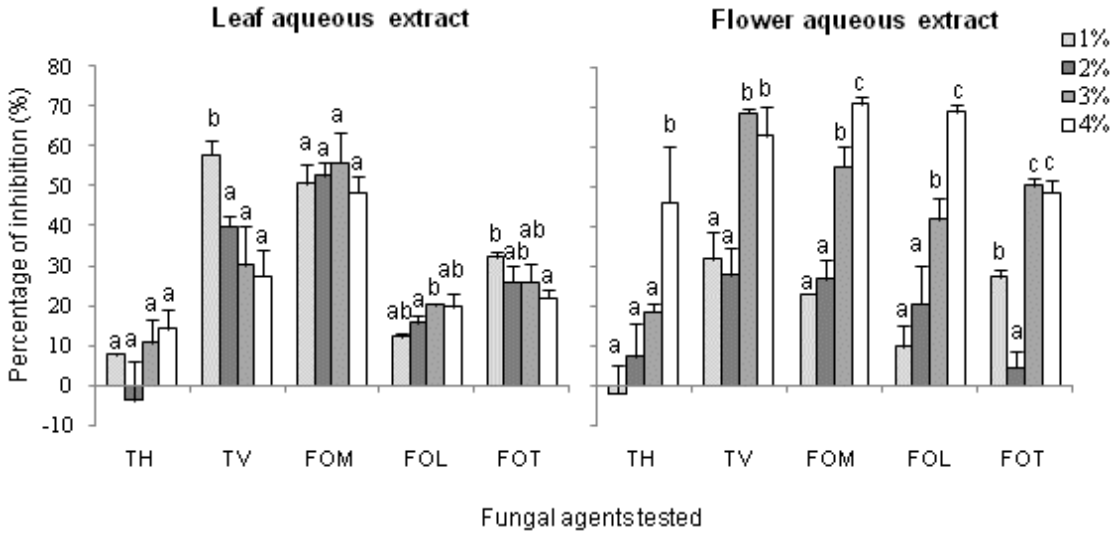

Figure 1. Percentage inhibition of the mycelial growth of fungal agents induced by *D. metel* leaf and flower aqueous extracts tested at different concentrations. For each fungus tested and each extract type, bars (indicating concentrations) affected by the same letters are significantly similar according to Duncan's test at the 0.05 level. TH: *Trichoderma harzianum;* TV: *Trichoderma viride;* FOM: *Fusarium oxysporum* f. sp. *melonis*; FOL*: Fusarium oxysporum* f. sp.*lycopersici;* FOT: *Fusarium oxysporum* f. sp.*tuberosi*; Incubation temperature: 25 ± 2°C; Incubation period: 3 to 4 days.

Statistical analysis

The SPSS statistical methods [PASW Statistics 18] were used to calculate the means, standard errors and standard deviations. Statistical analysis one-way ANOVA was applied to the data to determine differences in the three factors tested (extracts, concentrations and fungi tested, and their interactions) according to a completely randomized factorial design. To check significant differences between the levels of the main factor, Duncan multiple comparison tests at 5% significance were applied.

RESULTS

Effect of *D. metel* aqueous extracts on fungal mycelial growth

Results of this study showed that the different *D. metel* extracts have inhibitory effects on the growth of the test organisms. The potential of aqueous extracts to inhibit growth of pathogenic and antagonistic fungi differed with the origin of the extract, the concentration and the organism itself (Figure 1). On the whole, *F. oxysporum* f. sp. *melonis* was more sensitive to *D. metel* aqueous extracts than *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *tuberosi*; the recorded radial growth inhibition varied from 27 to 71%, depending on extracts and concentrations used. Flower aqueous extracts exhibited more inhibitory effects on the mycelial growth of *F. oxysporum* f. sp. *lycopersici* than on *F. oxysporum* f. sp. *tuberosi* at the highest concentration tested (2, 3 and 4%). The degree of inhibition increased with the increase in the extracts concentrations. Reduction varied between 10 and 69%. However, when exposed to leaf aqueous extracts, at all concentrations, the mycelial growth of these two agents was contrasted; *F. oxysporum* f. sp. *lycopersici* exhibited less sensitivity to tested extract than *F. oxysporum* f. sp. *tuberosi*. The highest radial growth inhibition of about 32% was obtained with leaf aqueous extracts applied at 1% against *F. oxysporum* f. sp. *tuberosi* (Figure 1).

Also, as shown in Figure 1, the mycelial growth of the two *Trichoderma* species tested seems to be affected by *D. metel* aqueous extracts. In fact, the mycelial growth of *T. harzianum* was less affected by *D. metel* leaf and flower aqueous extracts as compared to *T. viride*. The addition of leaf (1%) and flower (2%) aqueous extracts to PDA medium increased the radial growth of *T. harzianum* by 4 and 2%, respectively. Indeed, the highest inhibition of the mycelial growth of this fungus was observed with flower aqueous extracts. The degrees of inhibition increased with increasing extract concentration; the recorded percentage of inhibition ranged from -2 to 46%. The antifungal activity of *D. metel* leaf aqueous extracts against *T. viride* was more pronounced at the lowest concentration (1%). The inhibitory effects of this extract seem to be dependent on the concentration used. In contrast, flower aqueous extracts exhibit their strongest antifungal activity at the highest concentrations (3 and 4%) where the recorded inhibition was about 66% (Figure 1).

Effect of *D. metel* organic extracts on the fungal mycelial growth

The results obtained from bioassays of *D. metel* leaf and

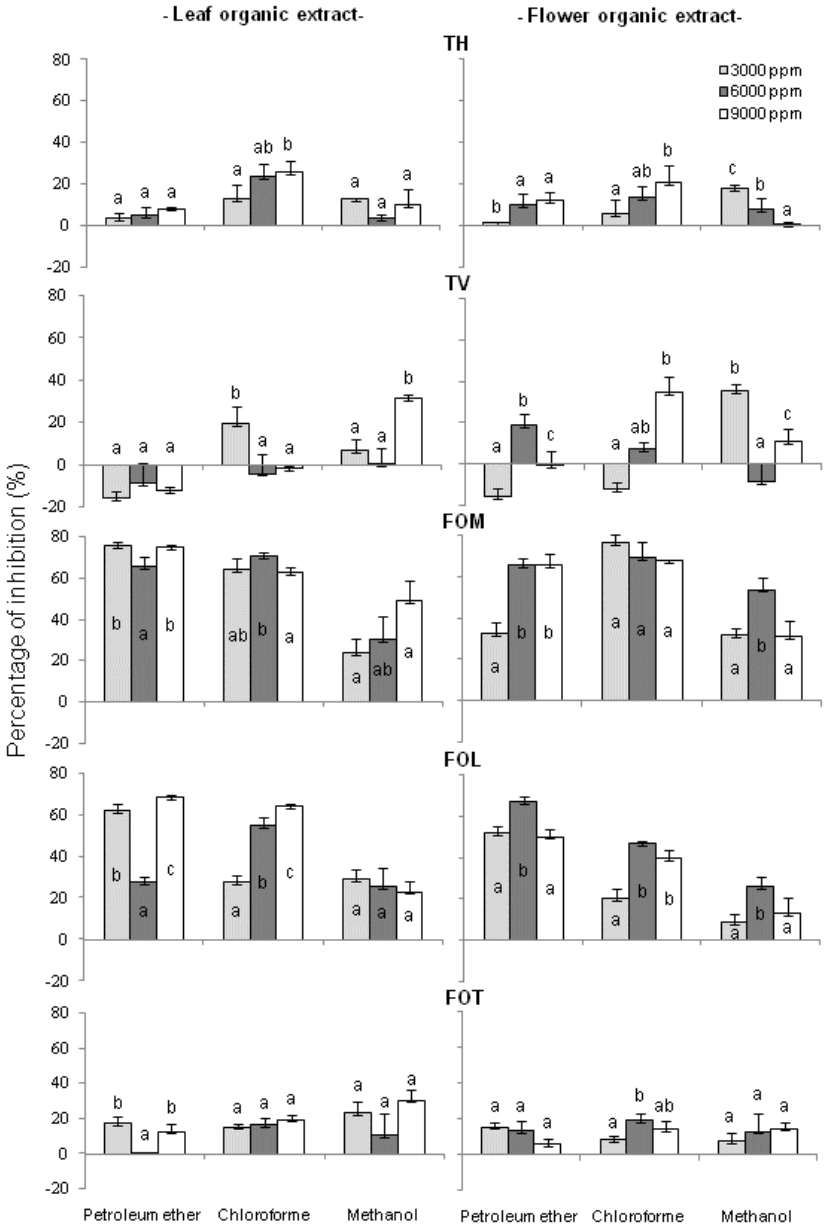

Figure 2. Percentage inhibition of the mycelial growth of fungal agents induced by *D. metel* leaf and flower organic extracts tested at different concentrations. For each fungus tested and each extract type, bars (indicating concentrations) affected by the same letters are significantly similar according to Duncan's test at the 0.05 level. TH: *Trichoderma harzianum;* TV: *Trichoderma viride;* FOM: *Fusarium oxysporum* f. sp. *melonis*; FOL*: Fusarium oxysporum*f. sp.*lycopersici;* FOT: *Fusarium oxysporum*f. sp. *tuberosi*; Incubation temperature: 25 ± 2°C; Incubation period: 3 to 7 days.

flower organic extracts against the target agents are presented in Figure 2. A variable effect of various extracts and concentrations was recorded for the test species. However, the response of the fungal agents to the extracts tested seems to be different depending on the concentrations used.

In fact, the mycelial growth of pathogenic fungus was significantly inhibited with all organic extracts of *D. metel* except petroleum ether leaf extract. It was shown to be ineffective in suppressing the fungal growth of *F. oxysporum* f. sp. *tuberosi* when used at 6000 ppm.

F. oxysporum f. sp. *Melonis* was more affected by the extracts tested than the other fungal agents. In fact, the greatest allelopathic stress (75%) was induced at 3000

ppm by leaf petroleum ether and flower chloroform extracts. Moreover, *F. oxysporum*f. sp. *lycopersici* exhibited important sensitivity to *D. metel* organic extract than *F. oxysporum* f. sp.*tuberosi*; the highest radial growth inhibition (67.5%, in average) was obtained with leaf and flower petroleum ether fractions applied at 9000 and 6000 ppm, respectively. However, *F. oxysporum*f. sp. *tuberosi* appeared to be less sensitive to *D. metel* organic extracts than the other *Fusarium* species. The recorded radial growth inhibition varied from -3 to 30%, depending on extracts and concentrations used.

The mycelial growth of the two *Trichoderma* species seems to be less affected by *D. metel* organic extracts as compared to the three *F. oxysporum formae speciales* tested. *T. harzianum in vitro* growth was significantly inhibited on PDA medium amended with the leaf and flower organic extracts of *D. metel* at all concentrations tested. The highest inhibition of about 26 and 21% was recorded in the presence of leaf and flower chloroform extracts applied at 9000 ppm, respectively. The addition of relatively increasing or decreasing concentrations of *D. metel* leaf and flower organic extracts caused depression or stimulation of fungal growth of *T. viride*. In fact, the addition of the leaf petroleum ether fraction, at all concentrations tested, had enhanced the mycelial growth of this fungus by 9 to 16%. Moreover, leaf chloroform extracts had also improved *T. viride* growth by 3% at the highest concentrations used (6000 and 9000 ppm). However, the radial growth of this fungus was inhibited by 19% with the same extract when used at the lowest concentration (3000 ppm). The addition of flower petroleum ether and chloroform extracts, applied at 3000 ppm also increased the radial growth of *T. viride* by 15 and 12%, respectively. However, leaf and flower methanol extracts applied at 9000 and 3000 ppm exhibited the highest inhibitory (of about 33.5%) effect against *T. viride*.

DISCUSSION

Biocontrol is the safest and economical method of controlling plant pathogens by using extracts of different plant parts (Bajwa et al., 2004; Bajwa and Iftikhar, 2005; Shafique et al., 2011). Presently, the study was conducted to assess the *in vitro* efficacy of aqueous and organic extracts of *D. metel* against three pathogenic and two antagonistic fungi. The results obtained in the present study revealed that generally aqueous extracts of *D. metel* reduced the pathogenic fungal growth in all the test concentrations (1, 2, 3 and 4%). The inhibition of the mycelial growth of the sensitive agent may be attributed to the presence and detrimental effects of allelochemicals on cell division, cell elongation and nutrient uptake (Blake, 1985). The phytochemical analysis of the aqueous extracts of *D. metel* aerial parts reported by Akharaiyi (2011) can support this finding. The results revealed the presence of saponins, flavonoids, tannins, glycosides, phenols, alkaloids, steroids and terpenoids. Saponin has been reported to have antifungal capacities (Sparg et al., 2004). Also, the amount of phenolic and flavonoids content on aqueous extracts of *D. metel* aerial parts was determined by Srivastava et al. (2012). The results showed that the maximum phenolic content was present in flower (19.75 mg/g) and the highest flavonoids content was found in leaves (2 mg/g). Winkelhausen et al. (2005) reported that the phenolic compounds derived from olive pomace hold a good promise as a natural fungicide against common pathogens to crops. Flavonoids have been proven for use against fungal pathogens of man since they have the ability to inhibit spore germination of plant pathogens (Cushnie and Lamb, 2005).

Also, the results of this conceptual study clearly reflect that organic extract of *D. metel* aerial parts (leaf and flower) has inherent ability to induce allelopathic effects on mycelial growth of the target fungal species. In fact, the greatest allelopathic stress (75%) was observed on the mycelial growth of *F. oxysporum* f. sp. *melonis* by leaf petroleum ether and flower chloroform extracts tested at 3000 ppm. Moreover, *T. harzianum in vitro* growth was significantly inhibited on PDA medium amended with the leaf and flower organic extracts of *D. metel* at all concentrations tested (3000, 6000 and 9000 ppm). Satish et al. (2007) reported that *D. stramonium* leaf organic extracts (petroleum ether, benzene chloroform, methanol and ethanol) had suppressed *Aspergillus flavus* growth. This antifungal potential may probably be due to the presence of several phytochemical compounds in the extracts. Phytochemical analysis of the methanolic extracts of *D. metel* aerial parts revealed the presence of five withanolide glycosides namely, daturataturin A, daturametelins H-J and 7, 27-dihydroxy-1-oxowitha-2, 5, 24-trienolide (Ma et al., 2006). In addition, Gupta et al. (1991) identified a hexa cyclic withanolide namely withametelin B which was obtained from *D. metel* leaves. Withanolides are known to exhibit antifungal activities (Choudhary et al., 1995). Similarly, Singh et al. (2001) indicated that *Datura* is a potentially important source of antifungal compounds effective against a wide range of plant pathogenic as well as saprophytic fungi. They reported that the steroid compound isolated from *D. metel* leaves named withametelin, showed antifungal activity at a very low concentration (125 ppm). *D. metel* is well known for tropane alkaloids and with asteroids, yielded several sphingosine derivatives. One of this derivatives was characterized as (4E, 8Z)-1-O-(beta-D - glucopyranosyl) -N- (2'-hydroxyhexadecanoyl)-sphinga-4,8- dienine and it is known for antifungal properties (Sahai et al., 1999). Moreover, Dabur et al. (2004) isolated a new pyrrole derivative from *D. metel* leaves which was characterized as 2-beta-(3, 4-dimethyl-2, 5-dihydro-1H-pyrrol-2-yl)-1'-methy-lethyl pentanoate on the basis of spectral data analyses and chemical reactions and which was endowed with antifungal activity.

Data obtained from our study indicated that the effecttiveness of extracts tested against the target agents was

dependent on the solvent used for extraction and the plant part from which they were obtained. Indeed, different levels of biological activities were recorded when varying solvents were used. The inhibitor effect of leaf organic extract tested at 3000 ppm, on radial growth of *F. oxysporum* f. sp. *melonis* varied with the type of solvent. The highest activity was obtained with petroleum ether (75%) as compared to 64.5 and 24% obtained with chloroform and methanol fractions. The differences in the toxicity of the different extracts could be attributed to the presence of active principles extracted using different solvents which may be influenced by several factors such as the method of extraction, the type of extracting solvent and the time of harvesting plant materials (Nicolls, 1969; Qasem et al., 1996). Also, the variation in antifungal activity of the different extracts may be attributed to their chemical nature. Chemicals may be dissolved in different solvents and thus, a variable activity of extracts of a given plant part may occur by using different solvents as recorded in our study. These findings are in agreement with several other works. In fact, Bajwa et al. (2008) found that the methanolic fraction of *D. metel* exhibited more promising results in suppressing the fungal growth of *Ascochyta rabiei* than aqueous fractions. In the same way, Kagale et al. (2004) have also noted that the methanol extract of *D. metel* leaves exhibited the best suppressive effect (10 to 35% more toxicity) against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae* than their aqueous extract.

On the other hand, antifungal activity of flower aqueous extracts was more pronounced against *T. harzianum* than that of leaf aqueous extract. This difference could be attributed to the presence of variable amounts of bioactive secondary metabolites in the different plant organs. The composition of these secondary metabolites in turn varies from species to species and depends on climatic conditions and the physiological stage of the plant (Pandey, 2007). Lindequist (1992) indicate that the alkaloid content of *D. metel* varies to a great extent depending on the plant part concerned: roots 0.1 to 0.2%, leaves 0.5%, flowers 0.1 to 0.8%, fruits 0.12% and seeds 0.2 to 0.5% alkaloid. Moreover, the antifungal activity of *D. metel* leaf aqueous extracts on the radial growth of *T. viride* was more pronounced at the lowest concentration (1%). The effects of this extract are variable depending on the concentration used. In contrast, the flower aqueous extract exhibited more antifungal activity at the highest concentration (3 and 4%) against the same fungus. Similarly, Fabry et al. (1996) reported that extracts of *Entada abyssinica*, *Terminalia spinosa*, *Harrisonia abyssinica*, *Ximenia caffra*, *Azadirachta indica*, *Zanha africana* and *Spilanthes mauritiana*, tested at different concentrations, had different effects on the radial growth of *Candida* spp. and *Aspergillus* spp.

The response of fungal growth to the allelopathic stress was found to be also dependent on the target fungal species. In fact, *F. oxysporum* f. sp. *melonis* was found to be highly sensitive (75%) to the lower concentrations of leaf petroleum ether and flower chloroform extracts. Also, at the lowest concentration, the addition of flower petroleum ether and chloroform extract had improved the mycelial growth of *T. viride* by 15 and 12%, respectively. For *F. oxysporum*f. sp. *lycopersici*, a growth reduction of 67.5% was obtained with leaf and flower petroleum extracts tested at the higher concentrations (6000 and 9000 ppm). Similarly, with the highest concentration (9000 ppm) of leaf and flower chloroform extracts, the maximum inhibition of 26 and 21%, respectively, was recorded in the case of *T. harzianum.* This variable susceptibility to plant extracts could be due to the inherent differences in the physiological and morphological characteristics of the various species involved (Shaukat et al., 1983). Toxicity is assumed to be associated with the presence of strong electrophilic or nucleophilic system. Action by such systems on specific positions of proteins or enzymes would alter their configuration and consequently, affect their activity (Macias et al., 1992).

According to the previously mentioned results, a strong toxicity of different plant extracts on fungal growth was shown. In the same way, our results showed a strong toxicity of *D. metel* aqueous extract against *T. viride*, which growth was stimulated by different organic fractions. In contrast, the growth of *T. harzianum* was more affected by aqueous than organic extracts. The difference in behavior of *Trichoderma* species could be possibly due to antagonistic effect between allelochemicals. Indeed, different allelopathic compounds in aqueous extracts are separated into different organic fractions. The synergistic and antagonistic effects between allelochemicals were reported in some works. In fact, Tamgue et al. (2011) noted the presence of synergism and antagonism in the essential oil fractions of *Cymbopogon citratus*, *Ocimum gratissium* and *Thymus vulgaris* when tested against *Penicillium expansum*.

It is concluded from our study that aqueous and organic extracts of various *D. metel* organs (leaf and flower) may be developed as biofungicides against some phytopathogenic fungi. In fact, among the tested aqueous extracts, *D. metel* flower extract was proven to be the most effective against all the target agents when used at the highest concentration. However, their use, at a given dose, may adversely influence the growth of some antagonistic fungi as noted with *T. harzianum* and *T. viride*. Therefore, future researches will be focused on their biological activity against other target plant pathogens and their adverse effects on antagonistic microorganisms.

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