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Phytotoxicity of *Daphne Gnidium* L. Occurring in Tunisia

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***Abstract*—**Phytotoxicity of *Daphne gnidium* L. was evaluated through the effect of incorporating leaves, stems and roots biomass into soil (at 12.5, 25, 50g/Kg) and irrigation by their aqueous extracts (50g/L), on the growth of two crops (*Lactuca sativa L.* and *Raphanus sativus L.*) and two weeds (*Peaganum harmala L.* and *Scolymus maculatus L.*). Results revealed a perceptible phytotoxic effect which increased with dose and concentration. At the highest dose, roots and leaves residues was the most toxic and caused total inhibition respectively, for lettuce and thistle seedling growth. Irrigation with aqueous extracts of *D. gnidium* different organs decreased also seedlings length of all test species. Stems extract was more inhibitor on thistle than peganum seedling growth; it induced a significant reduction of 80% and 67%, for, respectively, roots and shoots. Results of the present study suggest that different organs of *D. gnidium* could be exploited in the management of agro-ecosystems.

***Keywords*—**Biomass, *Daphne gnidium* L., phytoxicity, seedling growth

I. Introduction

IN the recent years, indiscriminate use of agrochemicals, for the success of modern agriculture has (i) made our soils sick, (ii) caused environmental pollution, (iii) developed resistance in pest and (iv) toxic residues in our food [1]. However, the application of agrochemicals for a certain period of time may obviously cause a tremendous environmental deterioration, resulting in chemical pollution in soil and reducing the soil fertility. This indicates the new technology is not sustainable over long periods [2]. Therefore, a considerable effort has been put into designing alternative strategies to reduce dependence on synthetic herbicides. Towards these ends, allelopathy could be one of these alternatives: it is a complex interaction among plants including stimulatory as well as inhibitory influence [3] through biochemicals released into the environment either actively or passively [4].

Members of the *Daphne* genus (Thymelaeaceae) have been of interest due to their excellent medicinal values. And one species are taking place in Tunisia: *Daphne gnidium* L. It is an evergreen shrub that grows in the Mediterranean area and can reach 2 m in height. It is a well-known plant with cancer-related ethnobotanical use [5]. The powdered roots of this species have been used in the traditional medicine as an abortifacient and the bark has been used as a diuretic agent and to treat toothache [6] and against hepatitis [7]. Recently, it has been demonstrated that different organic extracts of this plant have antiproliferative effects [5]. This plant is also used to dye the wool and the silk in yellow or in brown [8]. Although, various biological activities have been reported for *D. gnidium* as insecticidal [9], antiinflamatory[10], antibacterial [11], antimycotic [12]-[13] and antioxidant activity [14]. *D. gnidium* appears to be a promising source of natural compounds for organic and conventional agriculture management. Since previous short report revealed the presence of several compounds, including coumarins and flavonoids, in this species [15]-[16].

The present study purpose is to evaluate the phytotoxicity of *Daphne gnidium* different organs (roots, stems and leaves) through testing their residues incorporation in soil and irrigation with their aqueous extracts in pots cultures. Target species are two crops (*Raphanus sativus* L., *Lactuca sativa* L.) and two weeds (*Scolymus maculatus* L., *Peganum harmala* L.).

II. Plant Material

D. gnidium were collected (September 2010) in the area of Latitude 36° 42'27.13" N, longitude 08°40'25.25"E, Northwest region of Tunisia

A. Aqueous Extracts

Fresh *D. gnidium* plants were rinsed and separated into roots, stems and leaves Different organs were then oven-dried at 60°C for 72 h and grinded. Fifty grams of each dried material were soaked in 1 L distilled water at room temperature for 24 h. The extracts were filtered several times and kept at 4°C in the dark until use

B. Powder Incorporation in soil

The nursery trays (7 x 11 grids, each square 3 cm x 3 cm) were filled with sandy soil. Powder of roots, stems and leaves of *D. gnidium* were thoroughly mixed in pot soil (12.5, 25 and 50 g kg-1of soil on dry weight bases). Soil without D. gnidium powder, was the control. Subsequently, the nursery. trays were irrigated with tap water. After that, five pre-germinated seeds per square of target species (lettuce, radish, peganum and thistle) were sown, Nursery trays were placed in growth room at 25°C under 12 h photoperiod for 7 days and then transferred in open sunlight. Pots were irrigated with tap water daily to keep the soil moisture level at field capacity. Plants were

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harvested two weeks after sowing and data regarding root/shoot length were recorded. Treatments were arranged in a completely randomized design with three replications and data were transformed to percent of control for analysis [17].

C. Irrigation With Aqueous Extract

Additional trial was carried out in an incubator set at 25°C with 14/10 h, day/night. Nursery trays (7 x 11 grids, each square 3 cm x 3 cm) were filled with sand soil, five pre-germinated seeds (lettuce, radish, peganum and thistle) were planted per square just under the soil surface then sprayed with distilled water to moisten the soil. Three days later, 5 mL of each extract (roots, stems and leaves) at 50 g L^{-1} were added per square. Distilled water was the control. Treatments were arranged in a completely randomized design with three replications and data were transformed to percent of control for analysis [18].

D. Statistic analyses

The laboratory bioassays and pot culture were conducted in a completely randomized design with three replications. ANOVA and Duncan- tests were performed using PASW statistics 18.0, for Windows program, to analyze treatment differences. The means were separated on the basis of least significant differences at the 0.05 probability level

III. Results

A. Powder Incorporation In Soil

D. gnidium roots, stems and leaves powder was mixed with a soil sample at three doses: 12.5 (dose 1), 25 (dose 2) and 50g Kg^{-1} (dose 3) to test their effects on growth of the four target species (lettuce, radish, peganum and thistle) (Fig.1). Results showed that residue affected seedling growth which was varied with organ kind and target species. Moreover, the phytotoxic effects increased with residues doses and roots are more sensitive than areal parts. At the highest dose, roots and leaves residues were the most toxic and they caused a total inhibition respectively, for lettuce and thistle seedling growth. Leaves residue affected a lot more weeds than crops, their seedlings were inhibited, respectively by 96 % and 77% at the highest dose. However, a respective inhibition of 68% and 87% was recorded for radish and lettuce growth at the same dose. In the same trend, stems residues affected crops and weeds growth by a respective average inhibition of 86% and 90% at the highest dose and roots residue induced an average reduction of 91% for all target species (Fig.1).

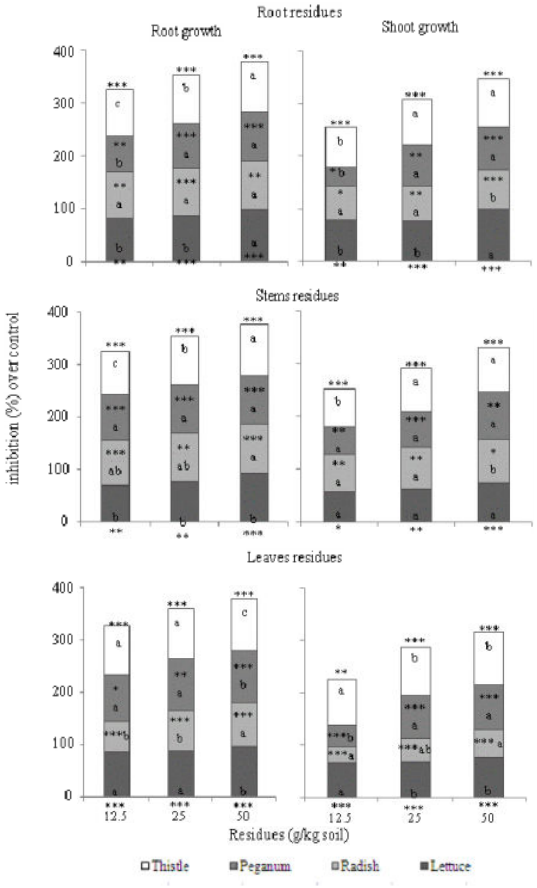

Fig. 1 Inhibitory effects of residues (Roots , Stems, leaves) incorporation of *D. gnidium* at 12.5 , 25 and 50g/Kg , on root and shoot length, expressed in % of the control, of target species, 15 days after germination. Values (N=3±S.E.) Different letters in columns indicate significant differences among concentrations at P<0.05 (LSD test). Significant differences from the control treatment are marked by * with P < 0.05, ** with P < 0.01 and *** with P < 0.001 (*t*-test)

B. Irrigation With Aqueous Extract

Irrigation with roots, stems and leaves aqueous extracts were more or less inhibitory for target species growth. Weeds were more sensitive than crops for all tested extracts, and roots were more sensitive than shoots (Fig.2). Stems extract was more toxic for thistle than peganum, it induced a significant respective reduction of 73.5% and 51%. Roots and leaves extracts induced an average reduction of 67 % for weeds and 61% for crops (Fig.2)

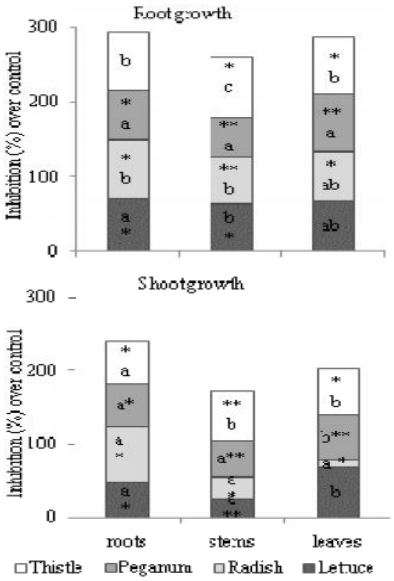

Fig. 2 Effects of aqueous extracts (at 50g/l) of *D.* gnidium root, stems and leave on root and shoot inhibition (%) over control of target species, 15 days after germination. Values (N=3±S.E.) Different letters in columns indicate significant differences among concentrations at P<0.05 (LSD test). marked by * with P < 0.05, ** with P < 0.01 and *** with P < 0.001 (*t*-test)

IV. Discussion

This study was conducted to investigate phytotoxic effect of residues and aqueous extracts of *D. gnidium* different organs, through testing them on two crops (lettuce and radish) and two weeds (peganum and thistle). *D. gnidium* residues and aqueous extracts caused significant inhibition of target seedlings growth. Nevertheless, reduction varied with *D. gnidium* organs, residues dose and target species. The differences in responses may be related to plant allelochemicals which act differently on the receptor plant assayed. Among *D. gnidium* residues, roots and leaves were the most toxic and caused a total inhibition respectively, for lettuce and thistle growth at the highest dose. This indicates that higher dose was more effective possibly due to presence of higher allelochemicals amount [19]. Several studies have shown that different tissues of various allelopathic plants suppressed the test species growth and that the response to phytotoxins is species-dependent. Reference [20] shows that *Passiflora edulis* Sims. aqueous extract strongly inhibited growth of *Lactuca sativa* L., *Raphanus sativus* L. and *Monochoria vaginalis* . Generally, *D. gnidium* induced a stronger toxic effect on weeds than crops growth. It was shows that *Chenopodium murale* L. influence some weeds and crops, indicating the strong suppressive potential on some growth and physiological parameters of test plants [21]. Also, different sensitivity of plant species to phytotoxins depends on their physiological and biochemical characteristics as well as environmental conditions [22]. At certain concentrations, phytotoxins that exhibit negative effect on weed growth might cause less or no inhibition on another weed [23]-[24]. Although, target species roots were stronger affected than shoots and the consistent inhibition of root length reveals that it is a very sensitive indicator of phytotoxicity. These observations agree with the findings of other authors, who reported that root length is the most sensitive and reliable response parameter to allelochemicals [25]-[26]-[27]-[28]-[29]. *D. gnidium* furnished four coumarins (daphnetin, daphnin, acetylumbelliferone, and daphnoretin) and seven flavonoids (apigenin, luteolin, quercetin, orientin, isoorientin, luteolin 7-O-glucoside, apigenin 7-O-glucoside, genkwanin, and 5-O-β-D-primeverosyl genkwanine) [11]. One or some of these chemical components could play a significant role in the phytotoxic effect [30]. It is reported that coumarin, as main and typical component of *S. chamaejasme*, might act as a plant growth inhibitor which can severely inhibit cell growth at low concentrations [31]. However, it is unknown to date what components of *D. gnidium* that acts as allelopathic chemicals. The phytotoxic activity of *D. gnidium* in soil depends on the concentration of active compounds released into soil from residues, even though phytotoxic activity is influenced by soil factors, adsorption on the soil solids and degradation by microorganisms [32]. To show the allelopathic activity, allelochemicals must accumulate and persist at phytotoxic levels and must come in contact with target plant [33].

V. Conclusion

The present study indicates that residues and aqueous extracts of *D. gnidium* from different plant parts showed a phytotoxic effect on crops (lettuce and radish) and weeds (peganum and thistle). The degree of inhibition was largely dependent on the concentration and the plant tissue of *D. gnidium*. This medicinal plant release allelopathic substances which may be accumulated in bioactive concentrations and adversely affect seedling growth of target species. Pot experiments suggest that the observed responses may operate in natural conditions. Nevertheless, more research is needed to isolate and identify the allelochemicals involved, as well as how biotic and abiotic factors influence the effect of *D. gnidium* on representative receptor plants in natural conditions.

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