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EVALUATION OF HERBICIDE POTENTIAL OF SESQUITERPENE LACTONE AND FLAVONOID: IMPACT ON GERMINATION, SEEDLING GROWTH INDICES AND ROOT LENGTH IN *ARABIDOPSIS THALIANA*

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Abstract

Plants produce a vast array of natural products that mediate their interaction with the environment. Artemisinin is important sesquiterpene lactones, mostly isolated from the *Artemisia annua* plant, has a wide range of biological activities, including insecticidal, antibacterial and antifungal, antifeedants, and allelopathic properties. Flavonoids (rutin) have attracted attention, primarily as natural antioxidants, and many are allelopathic agents, commonly present in *Fagopyrum esculentum* Moench. In the present study, phytotoxic effect of artemisinin and rutin on germination and seedling growth of *Arabidopsis thaliana* were tested under controlled bioassays. Total germination % age was reduced in *A. thaliana* after treatment with artemisinin at 10, 20, 40, 80, 160 µM concentration; while maximum reduction in germination %age was observed at highest concentrations of 160 and 80 µM. Rutin at 100, 250, 500, 750 and 1000 µM concentration decreased germination % age in *A. thaliana* but the concentration 1000 µM proved to be most deleterious. Artemisinin at 10, 50, 40, 80, 160 µM concentration inhibited the speed of germination (S) of *A. thaliana*. Similarly, Rutin-delayed the *A. thaliana* "S" at all the concentration tested and maximum inhibition was recorded at 1000 µM concentration. The effect of artemisinin and rutin on radicle length (RL) of *A. thaliana* was concentration dependent. There was a gradual decrease in RL of *A. thaliana* due to rutin at all concentration. Seedling vigour index (SVI) of *A. thaliana* was decreased after treatment with both artemisinin and rutin at all concentration while the maximum reduction was observed at highest concentration tested.

Introduction

Herbicide use has undoubtedly contributed to crop yield increases and the efficiency of production. Nevertheless the extended use of synthetic herbicides and pesticides has produced several ecological problems. Some of those molecules can persist in the ecosystem for long period; not being easily biodegradable and potentially can pose a threat to human health, ecology and environment. An additional problem is that herbicide-resistant weeds have been identified in 21 European countries, with the highest number of resistant biotypes found in France (30), Spain (26), United Kingdom (24), Belgium (18) and Germany (18) (Heap, 2004). The potential for using allelochemicals in weed management has been well documented (Batish *et al.,* 2007a). The role of natural products can be determinant because of their high potential as possible bioherbicides (Duke *et al.,* 2000). Allelochemicals produced and released from the leaves, flowers, seeds, stems, and roots of living or decomposing plant materials into the environment and affect the germination, growth and physiology of neighbouring crops and weeds (Dayan *et al.,* 2009; Hussain *et al.,* 2011a).

The search for allelochemicals and natural phytotoxins is a growing research field, because they have a great potential for controlling noxious weeds and could be used as herbicides in modern agriculture (Singh *et al.,* 2002). Plants produce a vast array of volatiles that mediate their interaction with the environment. A large portion of these volatiles consists of the terpenoids, also known as the isoprenoids because all are synthesized through the condensation of C_5 isoprene units (Pichersky & Gershenzon, 2002). Sesquiterpene lactones (SLs) constitute an interesting and well-documented group of compounds. The monoterpenes and sesquiterpenes are derived from either mevalonate pathway active in the cytosol/endoplasmic reticulum or the plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Rodriguez-Concepción & Boronat, 2002). They present a wide range of biological activities, including insecticidal (Datta & Saxena, 2001), antibacterial (Wedge *et al.,* 2000) and antifungal (Ahmed & Abdelgaleil, 2005). SLs have also been reported as allelopathic agents in many cases (Macias *et al.,* 1999) with high levels of activity, being particularly abundant in plants of the family Compositae (Batish *et al.,* 2001; Macias *et al.,* 2000). Many of the noxious weeds contain SLs as allelochemicals, and their invasive potential could be directly related to their chemical content. Flavonoids have also attracted attention, primarily as natural antioxidants, for the prevention of diseases of advanced-age such as atherosclerosis and cancer, as well as coronary and heart diseases (Verhoeyen *et al.,* 2002). Similarly, flavonoids are necessary for fertility and normal pollen development; they influence auxin transport and play an important function in the interactions between plants and other organisms. Flavonoids are one of many allelopathic agents that plants produce in order to reduce competition. Common buckwheat (*Fagopyrum esculentum* Moench, family Polygonaceae) is one of the plants rich in flavonoids, and also one of the crops with allelopathic potential (Bonafaccia *et al.,* 2003).

Since the mode of action of these compounds is yet unknown, no attempts to establish any further comparison between the mode of action of rutin and the artemisinin are made. Previously, we have reported several phenolics and flavonoids (including rutin) from flowers and phyllodes of invasive *Acacia melanoxylon* R. Br. that are probably involved in the germination, seedling and root growth

inhibition of native herbs (Hussain *et al.*, 2011b). Germination and seedling growth bioassays are widely used for natural product phytotoxicity study (Lottina-Hennsen *et al*., 2006). In the present study, *Arabidopsis thaliana* seeds were treated with artemisinin (SL) and rutin (flavonoid), and the changes in germinating seedlings responses are described here. *A. thaliana* is native to central Asia and has spread throughout Europe and North America through long distance dispersal, caused in large measure by human activities (Vander Zwan *et al.,* 2000; Hoffmann, 2002). It self pollinates to a high degree, creating homozygous lineages in natural populations that can maintain high levels of linkage disequilibrium (Nordborg *et al.,* 2002). The aim of present investigation was to assess the herbicidal potential of artemisinin and rutin on *A. thaliana* germination, emergence, root elongation and seedling growth to better understand their mechanism of action and plant growth inhibition. These processes have been accepted as indirect measures of the effects caused by the allelochemical on their target sites.

Materials and Methods

An experiment was conducted in the growth chamber of Plant Biology and Soil Science Department, Faculty of Biology, University of Vigo, Lagoas-Marcosende Campus, Vigo, Spain during 2011. The objective of this study was to analyze the phytotoxic effect of artemisinin (sesquiterpene lactone) and rutin (flavonoid) on the germination and seedling growth of *Arabidopsis thaliana.* The experiment was arranged in Randomized Complete Block Design (RCBD) with four replications.

Artemisinin and rutin solution preparation: Stock solutions of test compounds (artemisinin and rutin) were prepared in different solvents based on the compound's solubility. Artemisinin was dissolved in acetone. The distilled water (distilled water + Tween 20 (1L/0.1 mL) was added equal to volume of acetone and solution was magnetically stirred and acetone was allowed to evaporate in a rotary evaporator (Rotavapor RE 12: BUCHI Switzerland). Distilled water + Tween 20 (1L/0.1 mL) was added in this solution to make stock solution of 100 mL volume. Distilled water + Tween 20 were added to this stock solution to prepare different concentrations of 10, 20, 40, 80 and 160 µM. The procedure was repeated to prepare "control A" without artemisinin. Rutin (Rutin Hydrate) was dissolved in Dimethyl Sulfoxide (DMSO) and distilled water (distilled water + Tween 20 (1L/0.1 mL) was added equal to volume of DMSO and solution was magnetically stirred and DMSO was allowed to evaporate in rotary evaporator. Distilled water + Tween 20 (1L/0.1 mL) was added in this solution to make stock solution of 100 mL volume. Distilled water + Tween 20 was added to this stock solution to prepare different concentrations (100, 250, 500, 750, 1000 µM) of rutin. The procedure was repeated to prepare "control B" without rutin. The pH of all these chemical solution including control was adjusted to 6.0 with KOH (Martin *et al.,* 2002) (Fig. 1).

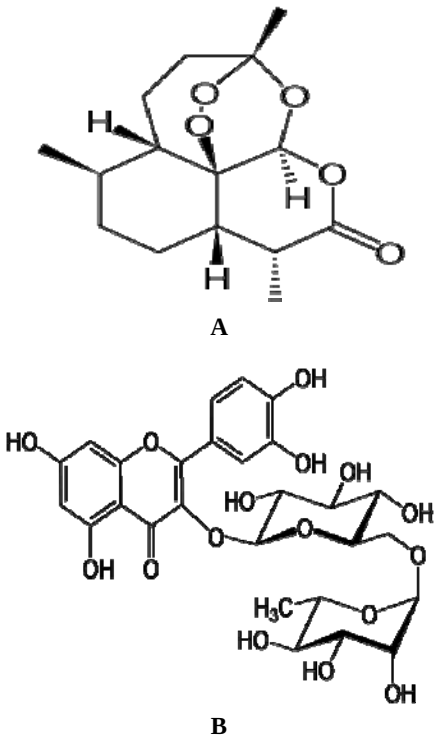

Fig. 1. Chemical structure of tested compounds (A) artemisinin and (B) rutin.

Germination bioassays of *Arabidopsis thaliana*: Seeds of *A. thaliana* L. (Heyn.) ecotype Columbia (Col- 0) were placed in 24-well plates (1.25-cm-diam) having Whatman no.1 paper disks (Maidstone, England). Four seeds of *A. thaliana* were placed in each plate and watered with 200 µl of the test solution (artemisinin or rutin) or controls. The control and test treatments were replicated four times for germination tests. Plates were placed in growth chamber with day/night temperatures of 22 and 20ºC, respectively. Average light intensity was maintained at ~80 µmol m^{-2} sec^{-1} during a 16-hr light cycle.

Root elongation test of *A. thaliana*: Seeds of *A. thaliana* L. (Heyn.) ecotype Columbia (Col- 0) were sterilized for 3 min in two consecutive aqueous solutions of EtOH (50%) and NaOCl (0.5%), both with TritonX-100 (0.01%), washed in auto claved water three times, and vernalized for 48 h at 4ºC in 0.1% agar to favor synchronized germination. For root elongation with nutrients supplied, 24 seeds were placed in square Petri plates with a semisolid media and allelochemical (5 mL/dish) containing 0.5× Murashige and Skoog salts, 1× Gamborg's B5 vitamins, 1% sucrose (w/v), and 2% Gelrite® (w/v) adjusted to pH 6. All treatments and controls were replicated four times. Plates were placed in a cold chamber for 3 d at 4ºC to synchronize germination. The square dishes were then placed vertically in a thermostatically controlled chamber with day/night temperatures of 22 and 20ºC, respectively for root growth. Root length was measured 15 d after artemisinin and rutin exposition. Average light intensity was maintained at ~80 µmol m^{-2} sec^{-1} during a 16-hr light cycle.

Total number of germinated seeds and germination %age: Germination was assessed by counting the number of germinated seeds at 0, 12, 24, 36, 48, 60, 72 and 84 h with a magnifying glass. The data was used to calculate the "total germinated seeds, germination index "G_T" and Speed of germination "S" as described by Chiapusio *et al.,* (1997). The germination vigor index was calculated according to Orchard (1977) by using the equation;

Seedling vigor index (SVI) = [Seedling length (cm) × Germination percentage] ..... (I)

Radicle length measurement: The Petri dishes were placed in cold chamber at 4ºC before measurement of radicle elongation. The radicle length (RL) of *A. thaliana* was measured after 15 days with a measuring scale. Ten seeds were selected at random to measure RL and then averaged. Inhibitory effect of phytochemicals on RL of Arabidopsis was calculated by using the best-fit equation based on the coefficient of determination (r^2), for the different concentration tested.

Statistical analysis: ANOVA was used to detect significant variation in sample means due to treatment effects. Kolmogorov-Smirnov test was used to assure normality, while homogeneity of variance of data was tested using a LEVENE TEST. If this assumption failed ($p<0.05$) then a Kruskal-Wallis rank order nonparametric ANOVA analogue used. After testing normality and homogeneity of variances, all data were analyzed by one-way ANOVA, LSD tests. The regression analysis was performed based on ANCOVA for radicle length data using SPSS 15.0 for Windows.

Results and Discussion

Seedling growth response: Several phytotoxic substances causing germination and growth inhibitions have been isolated from plant tissues and soils. These substances, collectively known as allelochemicals, are usually secondary plant products of main metabolic pathways of plants (Chon & Kim, 2002). Germination and growth bioassays are primary tools for determining phytotoxic activity and may detect potential allelopathic effects under controlled laboratory conditions (Reigosa & Pazos-Malvidos, 2007; Hussain *et al.,* 2008). In the present study, total germination %age was significantly reduced in *Arabidopsis thaliana* (Table 1) after treatment with artemisinin at 10, 20, 40, 80, 160 µM concentration, while maximum reduction in germination was observed at highest concentrations (160 and 80 µM). However, several studies have reported that the response to allelochemicals may be the concentration dependent. Allelochemicals that inhibit the growth of some species at certain concentration might stimulate the growth of the same or different species at different concentrations (Narwal, 1994). It is essential to identify concentration at which each specific response occurs if allelopathic interaction is to be used in weed management programme.

The flavonoids (rutin) at 100, 250, 500, 750 and 1000 µM concentration decreased the germination in *A. thaliana* and concentration 1000 µM proved to be most deleterious that inhibited the *A. thaliana* germination (Table 1). Aqueous extracts and volatile chemicals from some plants inhibit the root and hypocotyls growth, cell elongation and cell division of seedlings (Cruz-Ortega *et al.,* 1998). Saxena *et al.,* (1996) also reported that flavonoids and other secondary compounds usually inhibit seed germination and root growth. Rutin and other flavonoids are UV-B absorbing secondary plant metabolites synthesized in higher plants, mosses, and ferns in order to protect them from the harmful effects of UV-B radiation and disease, pests and other adverse circumstances (Du *et al.,* 2003). The effects of allelochemicals have been studied mostly on seed germination and the suggested mechanisms for its inhibition are the disruption of mitochondrial respiration (Abrahim *et al.,* 2000) through the influence of allelochemicals on glycolysis, Krebs cycle and electron transport.

Table 1. Effect of different concentration of artemisinin and rutin on germination percentage and germination indices (G_T, S) of *Arabidopsis thaliana*.

Allelochemicals	*Arabidopsis thaliana* germination response		
	Germinatio %age	G_T	S
Artemisnin (µM)			
Control	3.50 ± 0.28	87.50 ± 7.21	0.52 ±0.03
10	2.50 ± 0.28*	62.50 ± 7.21*	0.22 ± 0.04*
20	1.25 ± 0.25*	60.25 ± 6.25*	0.01 ± 0.01*
40	1.00 ± 0.00*	25.00 ± 0.00*	0.03 ± 0.00*
80	0.50 ± 0.28*	12.50 ± 7.21*	0.01 ± 0.00*
160	0.75 ± 0.25*	18.75 ± 6.25*	0.05 ± 0.03*
Rutin (µM)			
Control	3.25 ± 0.47	81.25 ± 11.96	0.19 ± 0.07
100	2.00 ± 0.40*	50.00 ± 10.20*	0.07 ± 0.01*
250	1.25 ± 0.47*	31.25 ± 11.96*	0.08 ± 0.03*
500	1.25 ± 0.25*	30.00 ± 6.25*	0.08 ± 0.03*
750	1.75 ± 0.85*	43.75 ± 21.34*	0.12 ± 0.08*
1000	0.75 ± 0.47*	18.75 ± 11.96*	0.02 ± 0.01*

Results represents the mean (± S.E.) of four replicates

*Significant differences with respect to control for $p<0.05$ according to Mann-Whitney U test

Meanwhile, indices of total germination (G_T) in *A. thaliana* decreased following the application of artemisinin at 10, 20, 40, 80, 160 µM concentration. Highest reduction in G_T in *A. thaliana* was recorded after treatment with artemisinin at 160 µM. The rutin decreased the G_T in *A. thaliana* at 100, 250, 500, 750 and 1000 µM concentration but the concentration 1000 µM proved to be most deleterious at which maximum reduction was observed in G_T of *A. thaliana*. Similarly, Reigosa & Pazos-Malvido (2007) reported that four compounds-vanillic acid, *L*-mimosine, juglone, and *trans*-cinnamic acid delayed *A. thaliana* germination at the highest concentration tested. The study of inhibitory or stimulatory effect of allelochemicals is very interesting in agro-ecosystem, especially their role in many weed-crop, crop-crop and weed-weed interaction (Einhellig, 1996). Moreover, the study of natural compounds with biological activity might offer future alternatives to classical pesticides. The chemical nature of allelochemicals can be water-soluble or organic-soluble, but generally those released from plant parts like leaves decay, root exudates are water-soluble compounds (Reigosa *et al.,* 1999a) and those released from flowers are more phytotoxic to other plant species (Hussain *et al.,* 2011b). Speed of germination (S) measures retardation or acceleration in the germination process and has advantageous over G_T, because it is more sensitive indicator of allelopathic effects, which occurred during the germination process. The application of artemisinin at 10, 20, 40, 80, 160 µM concentration inhibited the S of *A. thaliana* (Table 1), while rutin delayed the *A. thaliana* germination speed at all the concentration tested and maximum inhibition was recorded at 1000 µM concentration. Similarly, Bughio *et al*., (2013) reported that that the allelopathy is a concentration dependent process, when the concentration increases the extent of the inhibition also increase.

Effect of artemisinin and rutin on radicle growth: There are clear differences in the results obtained from the effect of different concentration of rutin on the radicle elongation of *A. thaliana* seedlings. The highest radicle length was recorded in the control treatment and then decreased by increasing rutin concentration (Fig. 2). The effect of artemisinin and rutin on root length of *A. thaliana* was concentration dependent. There was a gradual decrease in root length of *A. thaliana* due to rutin at all concentration. The rutin at 1000 µM concentration was more phytotoxic as compared to control that almost completely inhibited radicle length in *A. thaliana* (Fig. 2b). Finally, radicle length was relatively more sensitive to allelochemicals of rutin than germination. These results agree with earlier studies reporting that water extracts of allelopathic plants had more pronounced effects on root growth than on shoot growth (Batish *et al*., 2007b; Teerarak *et al*., 2010). It has been assumed that allelopathic compounds in soil come in contact with roots of test plant and may alter its absorption capacity for water and minerals, cell division and other physiological functions (Majeed *et al*., 2012). One of the suggested explanations for disruption of seedling growth and development during allelopathy stress is modification in mitochondrial respiration leading to decreased supply of ATP for all energy demanding processes (Gniazdowska & Bogatek, 2005).

There was non- significant variation detected due to artemisinin treatment on root length of *A. thaliana* at all concentration (Fig. 2a). However, there was a tedendency of stimulation in radicle growth of *A. thaliana* at lower concentration of artemisinin. Marginal improvement in the radicle length at low concentrations of artemisinin (80 µM) could be the result of detoxification of allelochemical through conjugation, sequestration or secretion of carbohydrates, and oxidation of phytotoxic compounds (Inderjit & Duke, 2003). Similarly, Reigosa *et al.,* (1999b) concluded that certain allelochemicals have a stimulatory effect or no action on various plant species at lower concentration.

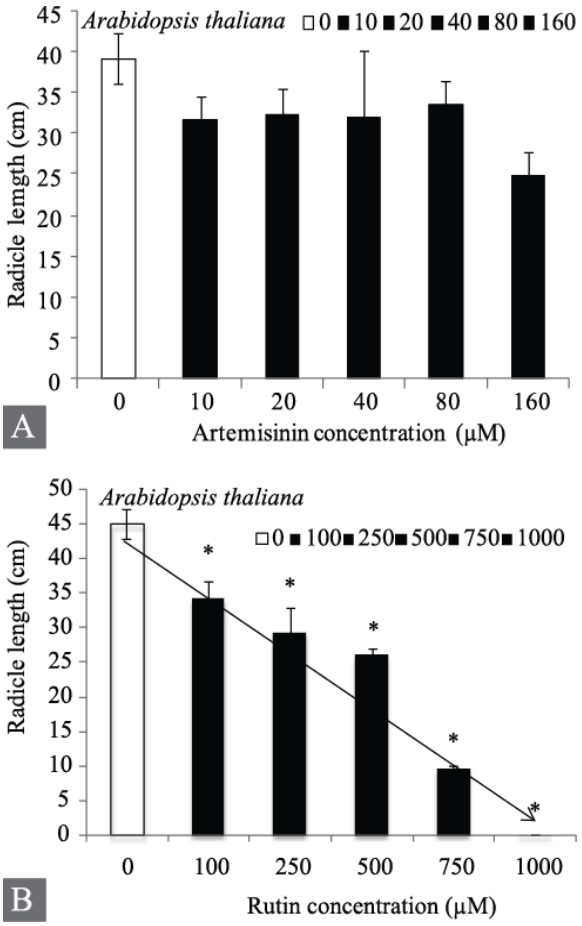

Fig. 2. Effect of artemisinin (0, 10, 20, 40, 80, 160 µM) and rutin (0, 100, 250, 500, 750, 1000 µM) on radicle length of *Arabidopsis thaliana*. Every bar in each graph represents the mean value ± S.E. from four replicates. *Asterisks indicate significant differences at 0.05 probability level with respect to control according to Mann-Whitney test.

Effect of artemisinin and rutin on seedling vigour index (SVI): Seedling vigour index (SVI) was established by Orchad (1977) and its significance seems to be important in the interpretation of allelopathic studies. In germination vigour index studies, *A. thaliana* showed a decreasing trend as concentration of artemisinin increases. The artemisinin at concentration of 80, and 160 µM , *A. thaliana* showed the lowest values of SVI (436.88 and

490) which increased to moderate level (1047.5) at 40 µM and then to the highest level (2023.75) at 10 µM. Therefore, *A. thaliana* showed the highest SVI at 0 µM and lowest value of SVI at 20 µM concentration. The SVI increased to a greater extent as the concentration rose from 0-20 µM, while in further increase in concentration up to 160 µM, the increment was lower. However, *A. thaliana* showed the lowest values of SVI (2.5 and 412.5) following treatment with 750 and 1000 µM concentration of rutin. Meanwhile, *A. thaliana* showed the highest SVI (3555) at 0 µM and lowest value of SVI at 1000 µM concentration. There was a gradual decreasing trend in SVI values in *A. thaliana* from 0 to 1000 µM (Fig. 3).

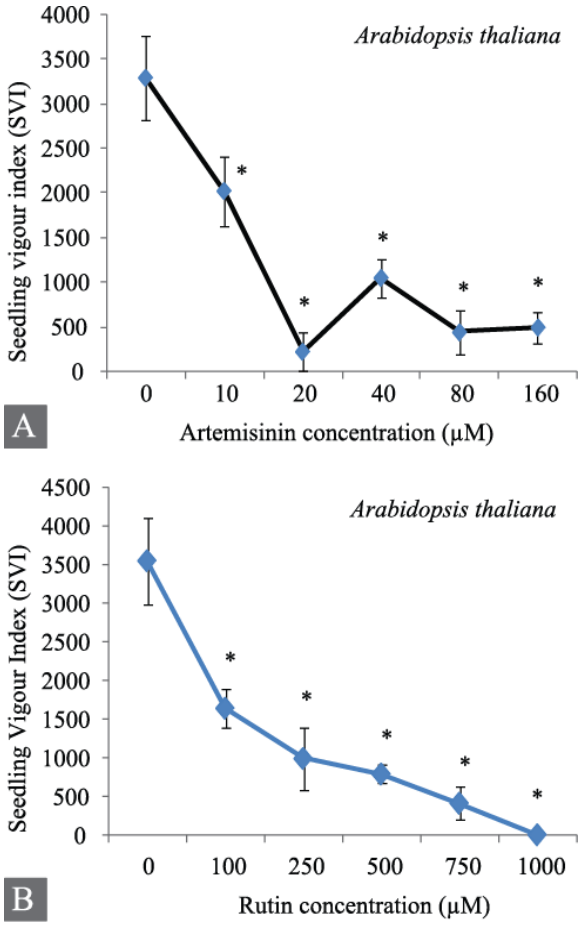

Fig. 3. Effect of (a) artemisinin (0, 10, 20, 40, 80, 160 µM) and (b) rutin (0, 100, 250, 500, 750, 1000 µM) on seedling vigour index (SVI) of *A. thaliana*. Every value in the line graph represents the mean value ± S.E. from four replicates. *Asterisks indicate significant differences at 0.05 probability level with respect to control according to Mann-Whitney test.

Regression analysis and radicle length inhibition: Fig. 4 summarizes the inhibitory effects of the tested compounds on radicle length (RL) of *A. thaliana* using the best-fit equation based on the coefficient of determination (r^2). The graph shows that both the chemicals cause a reduction in RL of *A. thaliana*. With increasing extract concentration, RL decreased and a significant correlation was observed between RL and different concentrations of allelochemicals and the lowest slope of regression trend was obtained in artemisinin extract. This is line with the view that different plant species may vary enormously in their response to potentially allelopathic substances (Baleroni *et al.*, 2000).

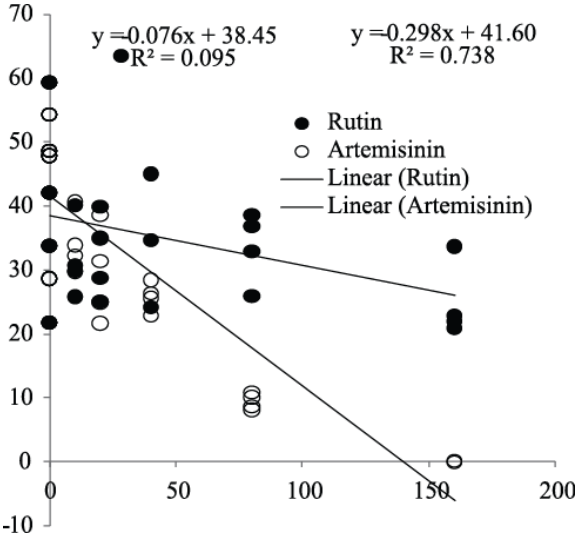

Fig. 4. Regression plots based on coefficient of determination (r^2) using ANCOVA showing effect on radicle length in *A. thaliana* following treatment with artemisinin (0, 10, 20, 40, 80, 160 µM) and rutin (0, 100, 250, 500, 750, 1000 µM) assayed in the presence of nutrients.

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References

Abrahim, D., W.L. Braguini, A.M. Kelmer-Bracht and E.L. Ishii-Iwamoto. 2000. Effects of four monoterpenes on germination, primary root growth and mitochondrial respiration of maize. *J. Chem. Ecol.*, 26: 611-624.

Ahmed, S. M. and S.A.M. Abdelgaleil. 2005. Antifungal activity of extracts and sesquiterpene lactones from *Magnolia grandiflora* L. (Magnoliaceae). *Int. J. Agric. Biol.*, 7: 638-642.

Baleroni, C.S.S., M.L.L. Ferrarese, A.L. Braccini, C.A. Scapim and O. Ferrarese-Filho. 2000. Effects of ferulic and *p*-coumaric acids on canola (*Brassica napus* L. cv. Hyola 401) seed germination. *Seed Sci. Tech.*, 28: 333-340.

Batish, D.R., A.K. Lavanya, H.P. Singh and R.K. Kohli. 2007a. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. *Plant Growth Regul.*, 51:119-128.

Batish, D.R., K. Arora, H.P. Singh and R.K. Kohli. 2007b. Potential utilization of dried powder of *Tagetes minuta* as a natural herbicide for managing rice weeds. *Crop Protection*, 26: 566-571.

Batish, D.R., R.K. Kohli, H.P. Singh and D.B. Saxena. 2001. Allelopathic effects of parthenin-a sesquiterpene lactone, on germination and early growth of mung bean (*Phaseolus aureus* Roxb.). *PGRSA Quatarly*, 29: 81-91.

Bonafaccia, G., M. Marocchini and I. Kreft. 2003. Composition and technological properties of the flour and bran from common and tartary buckwheat. *Food Chemistry*, 80: 9-15.

Bughio, F.A., S.M. Mangrio, S.A. Abro, T.M. Jahangir and H. Bux. 2013. Physio-morphological responses of native *Acacia nilotica* to *Eucalyptus* allelopathy. *Pak. J. Bot*., 45: 97-105.

Chiapusio, G., A.M. Sanchez, M.J. Reigosa, L. Gonzalez and F. Pellisier. 1997. Do germination indices adequately reflect allelochemical effects on the germination process? *J. Chem. Ecol.,* 23: 2445- 2453.

Chon, S.U. and J.D. Kim. 2002. Biological activity and quantification of suspected allelochemicals from alfalfa plant parts. *J. Agron. Crop Sci*., 188: 281-285.

Cruz-Ortega, R., A.L. Anaya, B.E. Hernandez-Bautista and G. Laguna-Hernandez. 1998. Effect of allelochemicals produced by *Sicyos deppei* on seedling root ultrastructure of *Phaseolus vulgaris* and *Cucurbita ficifolia*. *J. Chem. Ecol.,* 24: 2039-2057.

Datta, S. and D.B. Saxena. 2001. Pesticidal properties of parthenin (from *Parthenium hysterophorus*) and related compounds. *Pest Manag. Sci.,* 57: 95-101.

Dayan, F.E., C.L. Cantrell and S.O. Duke. 2009. Natural products in crop protection. *Bioorg. Med. Chem.,* 17: 4022-4034.

Du, J., Z.D. He, R.W. Jiang, W.C. Ye, H.X. Xu and P.H. Paul. 2003. Antiviral flavonoids from the root bark of *Morus alba* L. *Phytochemistry*, 8: 1235-1238.

Duke, S.O., F.E. Dayan, J.G. Romagni and A.M. Rimando. 2000. Natural products as sources of herbicides: current status and future trends. *Weed Res.,* 40: 99-111.

Einhellig, F.A. 1996. Interactions involving allelopathy in cropping systems. *Agron. J*., 88: 886-893.

Gniazdowska, A. and R. Bogatek. 2005. Allelopathic interactions between plants. Multi site action of allelochemicals. *Acta Physiol. Plant.,* 27: 395-407.

Heap, I. 2004. The international survey of herbicide resistant weeds. Internet. February 13, 2004. Available online at: www.weedscience.org

Hoffmann, M.H. 2002. Biogeography of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). *J. Biogeog.,* 29:125-134.

Hussain, M.I., L. Gonzalez and M.J. Reigosa. 2008. Germination and growth response of four plant species towards different allelochemicals and herbicides. *Allelop. J.,* 22: 101-110.

Hussain, M.I., L. Gonzalez and M.J. Reigosa. 2011a. Allelopathic potential of *Acacia melanoxylon* R. Br. on the germination and root growth of native species. *Weed Biol. Manag.,* 11: 18-28.

Hussain, M.I., L. Gonzalez, C. Souto and M.J. Reigosa. 2011b. Ecophysiological responses of three native herbs to phytotoxic potential of invasive *Acacia melanoxylon* R. Br. *Agrofor. Syst.,* 83:149-166. DOI 10.1007/s10457-011-9433-0.

Inderjit, S.O. Duke. 2003. Ecophysiological aspects of allelopathy. *Planta,* 217: 529-539.

Lottina-Hennsen, B., B. King-Diaz, M.I. Aguilar and M.G. Fernandez-Terrones. 2006. Plant secondary metabolities, targets and mechanisms of allelopathy. *In*: *Allelopathy: A physiological process with Ecological implications.* (Eds.): M.J. Reigosa, N. Pedrol and L. González. Springer, Dordrecht: 229-265.

Macías, F.A., D. Castellano and J.M.G. Molinillo. 2000. In the search for a standard bioassay for allelopathic studies of phytotoxicity. Selection of standard target species (STS). *J. Agric. Food Chem.,* 48: 2512-2521.

Macías, F.A., J.C.G. Galindo, D. Castellano and R.F. Velasco. 1999. Sesquiterpene lactones with potential use as natural herbicide models (I): *trans, trans*-Germacranolides. *J. Agric. Food Chem.,* 47: 4407-4414.

Majeed, A., Z. Chaudhry and Z. Muhammad. 2012. Allelopathic assessment of fresh aqueous extracts of *Chenopodium album* l. For growth and yield of wheat (*Triticum aestivum* L.). *Pak. J. Bot*., 44: 165-167.

Martin, T., O. Oswald and I.A. Graham. 2002. Arabidopsis seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon: nitrogen availability. *Plant Physiol.,* 128: 472-481.

Narwal, S.S. 1994. Allelopathy in Crop Production. Scientific Publishers, Jodhpur, India.

Nordborg, M. and J. Bergelson. 1999. The effect of seed and rosette cold treatment on germination and flowering time in some *Arabidopsis thaliana* (Brassicaceae) ecotypes. *Amer. J. Bot.,* 86: 470-475.

Orchard, T. 1977. Estimating the parameters of plant seedling emergence. *Seed Sci. Tech.,* 5: 61-69.

Pichersky, E. and J. Gershenzon. 2002. The formation and function of plant volatiles: Perfumes for pollinator attraction and defense. *Curr. Opin. Plant Biol.,* 5: 237-243.

Reigosa, M.J. and E. Pazos-Malvido. 2007. Phytotoxic effects of 21 plant secondary metabolities on *Arabidopsis thaliana* germination and root growth. *J. Chem. Ecol.,* 33: 1456-1466.

Reigosa, M.J., A.M. Sanchez-Moreiras and L. González. 1999a. Ecophysiological approaches in Allelopathy. *Crit. Rev. Plant Sci*., 18: 83-88.

Reigosa, M.J., X.C. Souto and L. Gonzalez. 1999b. Effects of phenolic compounds on the germination of six weed species. *Plant Growth Regul.,* 28: 83-88.

Rodriguez-Concepción, M. and A. Boronat. 2002. Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids: A metabolic milestone achieved through genomics. *Plant Physiol*., 130: 1079-1089.

Saxena, A., D.V. Singh and N.L. Joshi. 1996. Autotoxic effect of pearl millet aqueous extracts on seed germination and seedling growth. *J. Arid Environ.,* 33: 255-260.

Singh, H.P., D.R. Batish, S. Kaur, H. Ramezani and R.K. Kohli. 2002. Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis*. *Ann. App. Biol.,* 141: 111-116.

Teerarak, M., C. Laosinwattana and P. Charoenying. 2010. Evaluation of allelopathic, decomposition and cytogenetic activities of *Jasminum officinale* L. f. var. *grandiflorum* (L.) Kob. on bioassay plants. *Bioresource Technology.,* 110:5677-5684.

Vander Zwan, C., S.A. Brodie and J.J. Campanella. 2000. The intraspecific phylogenetics of *Arabidopsis thaliana* in worldwide populations. *Systematic Botany*, 25: 47-59.

Verhoeyen, M.E., A. Bovy and G. Gollins. 2002. Increasing antioxidant levels in tomatoes through modification of the flavonoid biosynthetic pathway. *J. Exp. Bot.,* 53: 2099-2106.

Wedge, D.E., J.C.G. Galindo and F.A. Macías. 2000. Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. *Phytochemistry,* 53: 747-757.

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