0971-4693/94 US $ 5.00
Allelopathy Journal 22 (1): 101-110 (2008)
Table: -, Figs : 2

Germination and growth response of four plant species to different allelochemicals and herbicides

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(Received in revised form: June 4, 2008)

ABSTRACT

Laboratory bioassays were conducted to test the phytotoxicity of 6 allelochemicals (ferulic acid, *p*-hydroxybenzoic acid, 2-benzoxazolinone, *L*-mimosine, juglone, *trans*-cinnamic acid), 6 allelochemicals mixture and 2 herbicides (pendimethalin and S-metolachlor) on germination and seedling growth of 4 plants species (*Rumex acetosa* L., *Lolium perenne* L., *Lactuca sativa* L. and *Dactylis glomerata* L.). Data obtained were used to compare four germination indices viz., (total germination index (G_T), speed of germination index (S), accumulated speed of germination index (AS), and coefficient of rate of germination index (CRG). The allelochemicals 2-benzoxazolinone and *trans*-cinnamic acid inhibited the total germination index of *L. perenne* L. at 1 µM concentration. The *L*- mimosine and six allelochemicals mixture (1 µM concentration) inhibited G_T, S, AS, in *L. perenne* and also inhibited G_T, S, AS in *D. glomerata* at 1 µM and 0.1 µM concentration. *L*- mimosine also inhibited the CRG in *R. acetosa* at 0.01 µM and in *D. glomerata* at 0.001 µM concentration. The six allelochemicals mixture (1 µM concentration) inhibited the speed of germination (S) of *D. glomerata* L. while *trans*-cinnamic acid inhibited the S in *R. acetosa* at 0.001 µM concentration. All other allelochemicals at 0.1, 0.01, 0.001 µM concentrations showed non-significant behavior to S all four plant species. Juglone inhibited G_T of *L. perenne* at 1 µM concentration and in *D. glomerata* at 1 µM, 0.01 µM and CRG in *R. acetosa* at 0.01 µM concentration. The application of both herbicides strongly inhibited G_T, S, AS, CRG in *L. perenne* L. and *D. glomerata* at concentration of 10^4 µM. These results indicate that each index led to a different interpretation of allelochemicals effect on germination.

Key Words- Allelochemicals, commercial herbicides, germination inhibition, *Rumex acetosa* L., *Lolium perenne* L., *Lactuca sativa* L., *Dactylis glomerata* L, phytotoxicity, germination indices.

INTRODUCTION

The overuse of synthetic agrochemicals for pest control has increased environmental pollution, unsafe agricultural products and human health concerns (53). Furthermore, herbicide resistant weeds have become major problem in last 20 years (16,18,38,42). FAO (15) Expert Consultation Group on Weed Ecology and Management has expressed great concern about the problem associated with the use of herbicides for

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weed control and has recommended minimizing or eliminating the use of herbicides with alternative strategies like allelopathy.

Allelopathy is an emerging branch of applied science, which studies any process primarily involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of biological and agricultural systems, including positive and negative effects (20). Allelochemicals are synthesized in plants as secondary metabolites. They are present in specialized organs of plants and their amount vary in different plant organs (43), plant variety (11) and have potential as either herbicides or templates for new herbicide classes (21). Allelopathic chemicals are released from plant tissue in many: volatilization from living plant parts (1, 36), root exudates (12); leachates from above-ground parts during rain, fog, or dew (8) and decomposition crop residues/ little by microorganisms (40).

The possible use of secondary metabolites from plants as herbicides has long been discussed (30,44). Although, the physiological and ecological actions of allelochemicals are weaker than commercial herbicides (34). However, these secondary metabolites have ecological advantages like, greater biodegradability (30). Allelochemicals affects many physiological processes in target organisms, including inhibition of; seed germination (50), growth of plant species (33), ion uptake (26), inhibition of electron transport in photosynthesis and respiratory chain (1, 24) and involved in root growth inhibition (32). Some allelochemicals rapidly depolarize the cell membrane, increasing membrane permeability, inducing lipid per oxidation and causing a generalized cellular disruption that ultimately leads to cell death (17,29,56,55).

Bioassays for such studies typically include seed germination, radicle growth and a photosynthetic activity test (19, 3). Attempts to describe germination responses in phytotoxic studies include the use of indices such as speed of germination (48), accumulated speed of germination (14), coefficient of rate of germination (13). More recently, Chiapusio *et al.*, (9) compared four indices and comparisons between the control and treatments at each time of counts for their ability to test physiological hypothesis in allelopathic studies.

This study aimed to investigate the phytotoxic effect of 6 allelochemicals and 6 allelochemicals mixtures compared to 2 herbicides on seed germination of four plants species (*Rumex acetosa* L., *Lolium perenne* L., *Lactuca sativa* L. and *Dactylis glomerata* L). The same data was used to compare four germination indices (G_T, S, AS, and CRG) examined by Chiapusio *et al.*, (9) to discuss their physiological meaning.

MATERIALS AND METHODS

This study investigated potential phytotoxic effects of 6 plant secondary metabolites (ferulic acid, *p*-hydroxybenzoic acid, 2-benzoxazolinone, *L*-mimosine, juglone, *trans*-cinnamic acid), 6 allelochemicals mixture and two herbicides (pendimethalin, S-metolachlor) on germination and seedling growth of *Rumex acetosa* L., *Lolium perenne* L., *Lactuca sativa* L. and *Dactylis glomerata* L. The test species were selected based on utilization capacity, handling and availability for experiment (45) and included mono and dicotyledonous plant species with different physiological responses. The experiment was arranged in Randomized Complete Block Design and replicated thrice

under controlled conditions. The seeds of test plant species (*Rumex acetosa* L., *Lolium perenne* L., *Lactuca sativa* L.) were purchased from Semillas Fito (Barcelona, Spain), and that of *Dactylis glomerata* L. were obtained from Herbiseed (Twyford, England).

Phenolic Compounds and Herbicide Solutions

The phenolic compounds (ferulic acid, *p*-hydroxybenzoic acid, 2-benzoxazolinone, *L*-mimosine, juglone, and *trans*-cinnamic acid) were obtained from Sigma Chemical Company, St. Louis, MO, USA. It should be noted that all phenolic tested in this study are phytotoxins but all phytotoxins are not allelochemicals. The herbicide Pendimethalin (33% p/v) was obtained from Probelt S.A. (Murcia, Spain), while, S-metolachlor (96% p/v) was purchased from Syngenta Agro S.A. (Madrid, Spain). The selection of allelochemicals was based on their allelopathic activity described previously (10, 27, 37, 33, 52). Stock solutions of allelochemicals were made in methanol: water (20:80). The control was prepared with distilled water and methanol in same proportion. Methanol was evaporated in a rotary vapourizer and remaining aqueous solution was adjusted to 10 µM concentration. These stock solutions were diluted to 1, 0.1, 0.01, 0.001 µM. The equimolar mixtures of six phenolic compounds were prepared in 1, 0.1, 0.01, 0.001 µM concentration. The pH of each phenolic solution was adjusted to 6.0 with NaOH (23). The herbicide solutions were prepared in distilled water in 10^4, 10^2, 1, 10^{-2} µM concentrations.

Germination Bioassays

Twenty-five seeds of each species were placed on Whatman 3 MM paper in a 9 cm dia petri dish to which 3 ml of solution was added at the start. An additional one ml of each solution was added every 48 h. Three replicates of each treatment were incubated in a germination chamber with the following germination conditions: *R. acetosa* (day/night temperature: 28/20 ^{0}C and -9/15 h of light and darkness); *L. perenne* (day/night temperature: 25/15 ^{0}C and -12/12 h of light and darkness), *L. sativa* (day/night temperature: 23/17 ^{0}C and -18/6 h of light and darkness) and *D. glomerata* (day/night temperature: 25/20 ^{0}C and -14/10 h of light and darkness) and a relative humidity of 80 %. The light was provided by cool white fluorescent tubes with an irradiance of 35 µmol $m^{-2}sec^{-1}$. The germination was recorded after every 24 h by counting the number of germinated seeds: rupture of seed coats and emergence of radicle ≥ 1mm, (31), until no further seeds germinated. The total germinated seeds (%) were calculated with the cumulative germination data after one week (49). The same data were used to calculate the germination indices [G_T: total germination index, S: Speed of germination; AS: Speed of cumulative germination; CRG: coefficient of germination rate as described by (9)].

Statistical analysis

Data from germination bioassay were analysed using one-way analysis of variance (ANOVA) (39) to compare means of treatments in each species. The Levene test was used, when variance was homogeneous. The LSD and *post hoc* test were used to determine main differences between the treatment means. The Kruscal-Wallis test was applied when variance was not homogeneous. All statistical analysis was done using SPSS (version 14.0) for Windows. Significant differences between treatment means were compared at 5% probability level.

RESULTS AND DISCUSSION

The phenolic compounds tested in this study are well known phytotoxins. The use of germination indices helps in determining the allelochemical effects on the physiological germination process (9). Results showed that each index leads to different interpretations of allelochemical effects on seed germination of four plant species. Thus, two aspects could be discussed: germination capacity (G_T) and germination progress or germination indices (S, AS and CRG).

Total germination index (G_T)

It is most common index to interpret of germination and useful to obtain ecological information in plant succession (22). Allelochemicals *L*- mimosine, juglone, six allelochemicals mixture (1 μM concentration) inhibited the G_T of *L. perenne* (Fig 1 a), while, 2-benzoxazolinone, *L*-mimosine, juglone, *trans*-cinnamic acid, six allelochemical mixture have shown inhibition on G_T at 1 μM concentration in *D. glomerata* (Fig. 1 b). This inhibition is stronger than that reported by Li *et al.,* (28) on seedling germination for *L. sativa* at 10^{-3} M. Similarly, Reigosa and Malvido (33) reported that four compounds (vanillic acid, *L*-mimosine, juglone, and *trans*-cinnamic acid) delayed the *A. thaliana* germination at the highest concentration tested. However, pendimethalin and S-metolachlor strongly inhibited the G_T of *L. perenne* and *D. glomerata* at 10^4 μM concentration, respectively (Fig 1a and b). Allelochemicals and herbicides did not influence G_T in case of *R. acetosa* and *L. sativa* at the same concentration (data not shown). These two plants were not sensitive to low concentrations of test allelochemicals and herbicides in this study.

The allelochemicals *L*-mimosine and six allelochemicals mixture at 0.1 μM concentration significantly inhibited the G_T in *D. glomerata,* but pendimethalin and S-metolachlor herbicides proved most deleterious and strongly inhibited the G_T of *D. glomerata* (100 μM) (Fig 1c). However, juglone is the only molecule that had phytotoxic effect on total germination index in *L. perenne* even at very low concentration (0.01 μM) (Fig 1d). This compound is potent allelochemical (41) and also influences the four germination indices. However, Angela *et al.,* (4) reported that juglone between 10 and 40 μM inhibited the *Lemna minor* growth, chlorophyll content and net photosynthesis.

The phytotoxic effects of molecules were concentration dependent. At 0.001μM concentration, none of the allelochemicals or herbicides (0.01 μM) inhibited the G_T of any plant species. These results agree with Reigosa *et al.,* (34) who concluded that allelochemicals have a stimulatory effect or no action on various weeds at lower concentration. In this study, ferulic acid and *p*-hydroxybenzoic acid did not inhibit total germination at any concentration. The *p*-hydroxybenzoic and ferulic acids did not influence the germination of *Poa annua* L. (51).

Speed of germination index (S)

S measures retardation or acceleration in the germination process (7,25,46,47) and is advantageous over G_T, because it is more sensitive indicator of allelopathic effects

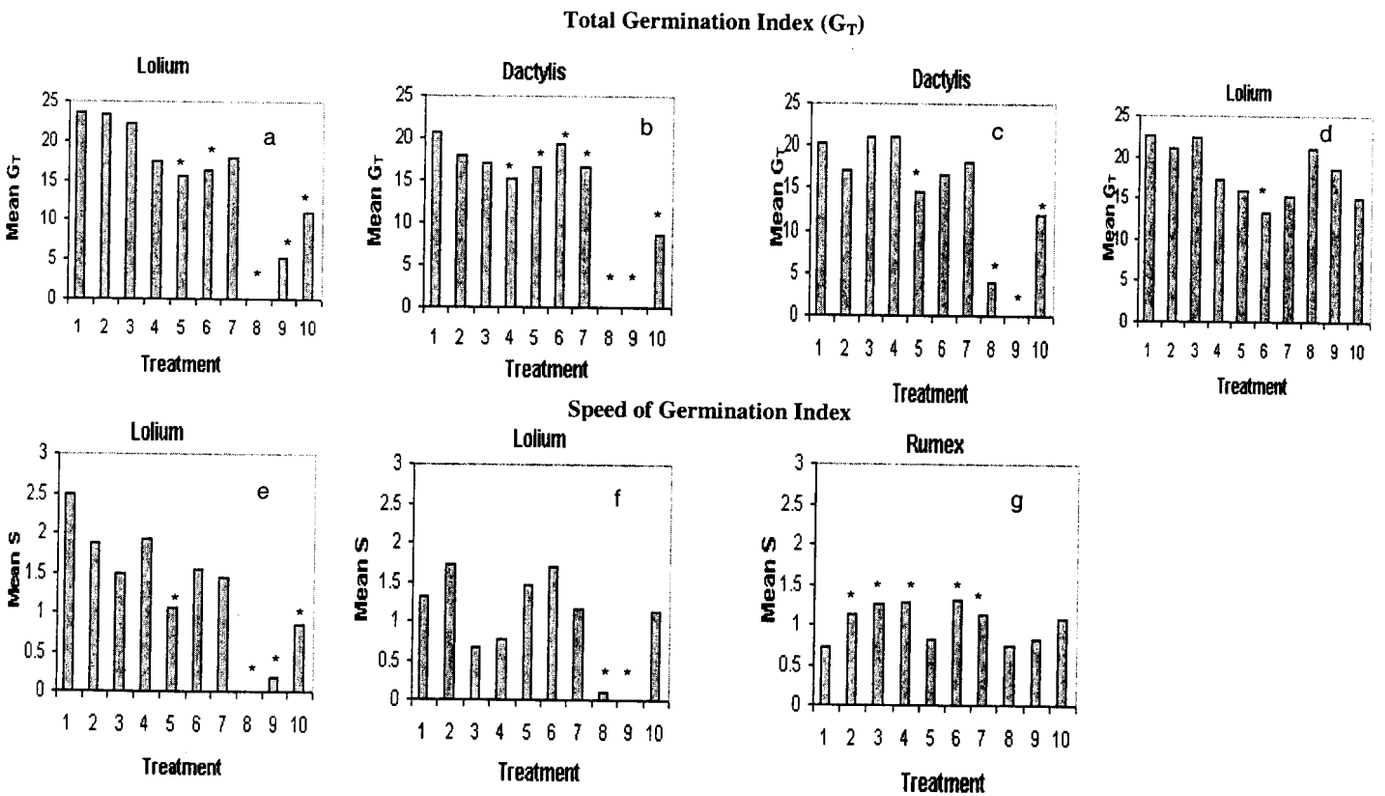

Figure 1.Effect of allelochemicals and herbicides on total germination indices (G_T) and speed of germination (S) of *Lolium perenne*, *Dactylis glomerata* and *Rumex acetosa*. Where as treatments (1= Control, 2 = Ferulic acid, 3 = *p*-Hydroxybenzoic acid, 4 = 2-Benzoxazolinone (BOA), 5 = *L*-Mimosine, 6 = Juglone, 7 = *trans*-Cinnamic acid, 8 = Pendimethalin, 9 = S-Metolachlor, 10 = Six allelochemicals mixture). Concentration (allelochemicals (ALC) 1 μM, herbicides (HBC) 10^{4} μM: a, b, e); (ALC 0.1 μM, HBC 10^{2} μM: c, f) (ALC 0.01 μM, HBC 1 μM: d); (ALC 0.001 μM, HBC 10^{-2} μM: g)

(2), which occurred during the germination process. The application of allelochemicals *L*- mimosine and six allelochemical mixtures (1 μM concentration) inhibited the S of *L. perenne* (Fig 1e). Similarly, vanillic acid, *L*-mimosine, juglone, and *trans*-cinnamic acid—delayed the *A. thaliana* germination at the highest concentration tested (33). However, the pendimethalin and S-metolachlor at 10^4 μM concentration proved most inhibitory to S of *L. perenne* respectively (Fig 1e). All the allelochemicals at 0.1 μM concentration had non-significant effects on S in all four test plant species. However, both herbicides at 100 μM concentration proved most inhibitory to S of *L. perenne* (Fig 1f).

At 0.01 μM concentration, none of allelochemicals or herbicides (1μM) controlled the S of any plant species (data not shown). However, in *R. acetosa* there was significant difference in inhibition of S between the different allelochemicals at 0.001 μM concentration. At this concentration, only *trans*-cinnamic acid was a good inhibitor of S in *R. acetosa* (Fig. 1g). These results are in agreement with the view that different plant species can vary enormously in their response to potentially allelopathic substances (5). All allelochemicals and herbicides have shown non-significant effect on S in *L. sativa* at any concentration (data not shown).

Speed of cumulative germination index (AS)

It involves the cumulative number of germinated seeds at each exposure time. It was adapted from (7,25,47). The application of allelochemicals (*L*- mimosine, juglone, six allelochemical mixtures) at 1 μM concentration inhibited the AS of *L. perenne* (Fig. 2a). However, pendimethalin and S-metolachlor drastically inhibited the AS in *L. perenne* at 10^4 μM concentration (Fig 2a). The six allelochemicals mixture had good inhibitory effect on AS in *D. glomerata* at 0.1 μM (Fig.2b). The pendimethalin and S-metolachlor inhibited AS in *D. glomerata* at 100 μM (Fig 2b). The allelochemicals (ferulic acid, *p*-hydroxybenzoic acid, BOA, juglone and *trans*-cinnamic acid) inhibited the AS at 0.001 μM concentration in *R. acetosa* (Fig 2c). These results are in agreement with literature (33,35,54).

Coefficient of germination rate (CRG)

CRG was established by Bewley and Black (6) and its significance is similar to AS index. The allelochemical BOA (1 μM concentration) inhibited the CRG of *R. acetosa* (Fig. 2d). However, Reigosa *et al.*, (35) reported that growth of *Rumex crispus* was inhibited by the allelochemicals (gallic, ferulic, vanillic, *p*-coumaric, *p*-hydroxybenzoic acid, *p*-vanillin and phenolic mixture) at concentration of 0.01 M. The pendimethalin and S-metolachlor proved most inhibitory CRG in *R. acetosa* at 10^4 μM concentration (Fig 2d). The allelochemical *L*-mimosine inhibited the CRG in *D. glomerata* at 1 μM concentration (Fig 2 e). Similarly, pendimethalin and S-metolachlor herbicide proved most deleterious to CRG in *D. glomerata* at 10^4 μM (Fig 2e) and in *L. perenne* at 10^2 μM (fig 2f) concentration.

The allelochemicals, *L*-mimosine, juglone, *trans*-cinnamic acid and six allelochemicals mixture (0.01 μM) significantly inhibited the CRG in *R. acetosa* (Fig 2g), while, only S-metolachlor inhibited the CRG at 1 μM concentration (Fig 2g). Only *L*-mimosine at 0.001μM concentration inhibited the CRG of *L. perenne* (Fig 2h).

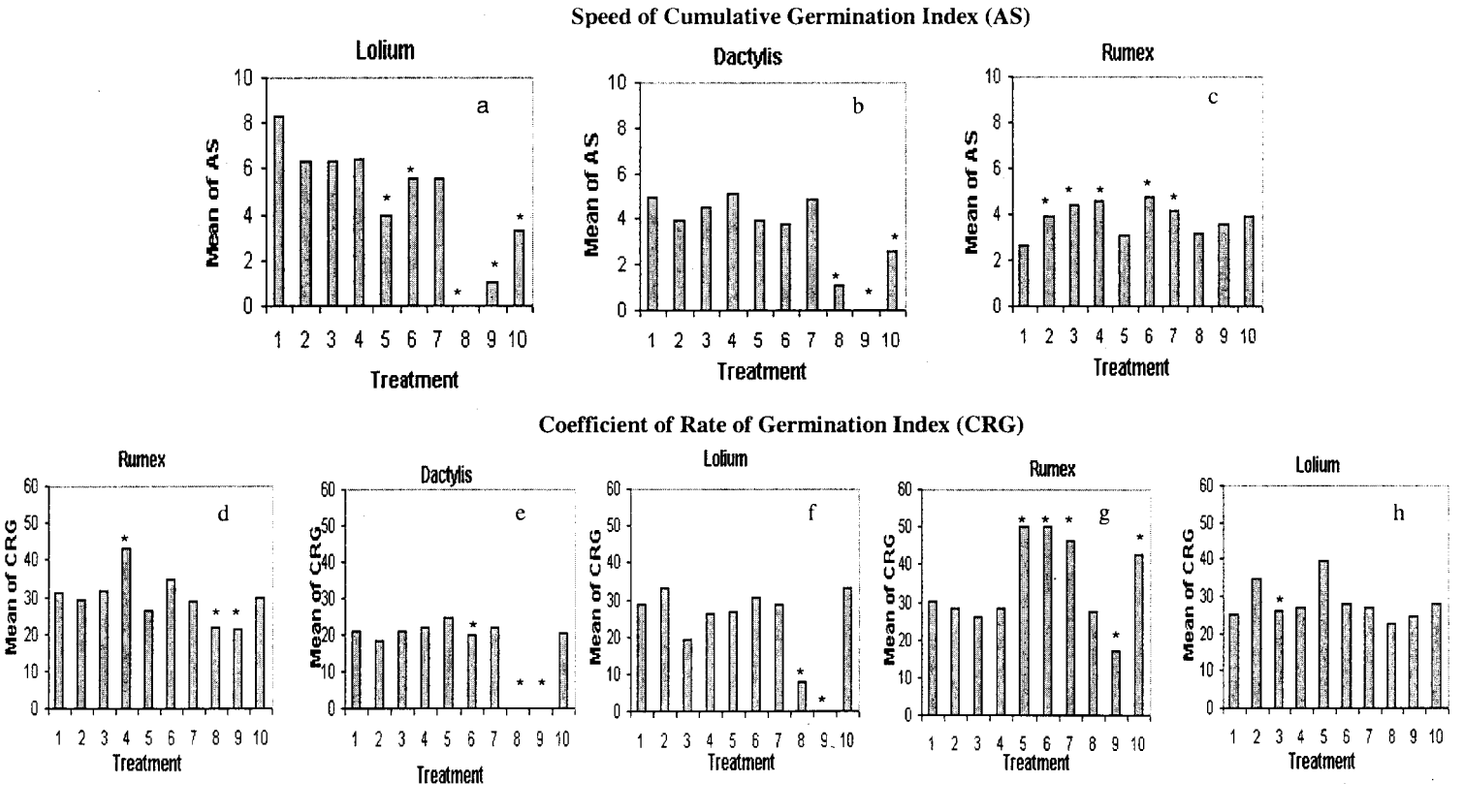

Figure 2.Effect of allelochemicals and herbicides on speed of cumulative germination (AS) and coefficient of rate of germination (CRG) of *Lolium perenne*, *Dactylis glomerata* and *Rumex acetosa*. Where as treatments (1= Control, 2 = Ferulic acid, 3 = *p*-Hydroxybenzoic acid, 4 = 2-Benzoxazolinone (BOA), 5 = *L*-Mimosine, 6 = Juglone, 7 = *trans*-Cinnamic acid, 8 = Pendimethalin, 9 = S-Metolachlor, 10 = Six allelochemicals mixture). Concentration (allelochemicals (ALC) 1 μM, herbicides (HBC) 10^4 μM: a, d, e); (ALC 0.1 μM, HBC 10^2 μM: b, f) (ALC 0.01 μM, HBC 1 μM: g); (ALC 0.001 μM, HBC 10^{-2} μM: c, h).

ACKNOWLEDGEMENTS

We are grateful to Dr. A. Barreiro, C. Bolano, A. Justo, M. Ricart and P. Lorenzo for help in field and laboratory work. We are also thankful to two anonymous reviewers for their constructive and critical comments on the manuscript.

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