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Importance of Germination Indices in Interpretation of Allelochemical Effects

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ABSTRACT

In several fields including allelopathy, germination bioassays are in wide use to assess the effectiveness of designed treatments. Mostly the maximum percentage germination is considered sufficient for interpretation, which depends only on final results. In this study six germination indices i.e. G_T, T_{50}, T'_{50}, S, AS, and CRG were calculated from same data to evaluate their effectiveness in data interpretation. All indices showed different results with slight variations AS found to be most sensitive index where as CRG was least responsive.

Key Words: Germination; Interpretation; Allelochemical

INTRODUCTION

Allelopathy is a process whereby plants provide themselves with a competitive advantage by putting phytotoxins into the near environment (Lamberset *et al.*, 1998). The increasing interest in allelopathy has been driven by the recognition that agro-ecological applications of Allelopathy may provide alternatives to synthetic herbicides for weed control (Wu *et al.*, 1999; Vyvyan, 2002). Laboratory bioassay is the first step to investigate probable involvement of Allelopathy (Foy, 1999). Aqueous extract bioassays have been widely employed to evaluate Allelopathy of a suspected donor species. Extract bioassays are simple, rapid, inexpensive and straightforward. Therefore these can be used preliminarily to determine Allelopathy for weed control. Seed germination and seedling growth has been widely accepted as main parameters to monitor growth responses owing to amendment. Numerous studies also include plant growth bioassays but the effect on germination is often not separated from the effect on growth (Kato-Noguchis *et al.*, 1994). In general, extract bioassays are conducted in petridishes by placing seeds of receiver species on substrata (often filter paper) moistened with aqueous plant extracts of donor species (Wu *et al.*, 1998). The petridishes are placed in an incubator under controlled light and dark periods are regularly checked for their germination, usually up to seven days. Data taken in the end is used to calculate percentage germination, which is mostly served to validate Allelopathy in ecosystems or agrosystems. But this has received great criticism in recent years (Inderjit & Dakshini, 1995). Investigators have proved that percentage germination do not provide sufficient information on the allelopathic potential of the donor plant. It has been found difficult to interpret data from seed germination bioassays to make conclusions about the most bioactive allelochemicals and treatment concentration that can be selected for green house and field studies (Macias *et al.*, 1999). To overcome this dilemma many investigators have used different indices to show allelochemical effects on germination. These indices have been classified in three general types (i) maximum percentage germination, also termed as germination capacity, (ii) germination progress and (iii) shape of germination curve (Chiapusio *et al.*, 1997).

Objectives of the present study were to calculate different germination indices from the same data in order to make comparisons between each of them and to evaluate better ways of using these indices in order to improve the precision in seed germination bioassays.

MATERIALS AND METHODS

Selection of test species. For the study under taken here *Helianthus annuus* L. (var. Suncross-42) and *Triticum aestivum* L. (var. Inqalab 91) were selected as test specie to evaluate their natural allelopathic potential against *Rumex dentatus* L. The selection of allelopathic plants was based on huge body of literature available that confirms their allelopathic characteristics (El-Khatib & Hegazy, 1999; Rizvi & Rizvi, 2000; Dahiya & Narwal, 2003).

Extract preparation. Fresh and healthy leaves of selected varieties of sunflower and wheat were washed thoroughly under running tap water, dried with blotting paper and were cut into small pieces. A 30% w/v stock solution of plant extract was obtained by soaking the crushed plant material in sterilized water for 48 h at room temperature. It was then passed through muslin cloth and finally filtered through Whatman filter paper No. 1. The lower concentrations of 25, 20, 15, 10 and 5% were prepared by adding appropriate quantity of sterilized water in stock solution. The extract was stored at 4°C in pre-sterilized flasks. To avoid contamination and prospective chemical alterations, the extract was ensured to be used within 3-4 days.

Germination test. Ten surface sterilized seeds of *Rumex dentatus* were placed in a Petri dish (9 cm diameter) on double-layered Whatman filter paper No. 1. The filter paper was moistened with 5 mL of leaf extract concentrations (i.e., 5, 10, 15, 20, 25 & 30%) and with distilled sterilized water in the case of control treatment. There were three replicates of each treatment in completely randomized designed. Seeds

were incubated at 20°C±2 and Petriplates were regularly checked for moisture. Germination of seeds was recorded for four days after every 12 h.

Germination indices. Six germination indices i.e. Total germination (also known as final germination percentage) (G_T), Number of days required for 50% of the total number of seeds to have germinated (T_{50}), Number of days for 50 % of the total number of seeds germinated (T'_{50}), Speed of germination (S), Speed of accumulated germination (AS) and Coefficient of the rate of germination (CRG) were selected for this study. The selected indices were calculated as described in Table I.

RESULTS

The leaf water extract of both *Helianthus annuus* and *Triticum aestivum* significantly reduced the seed germination of *Rumex dentatus* when applied in different concentrations. *H. annuus* showed a slight stronger inhibition than *T. estivum.* Pronounced effects were observed at concentrations higher than 20%.

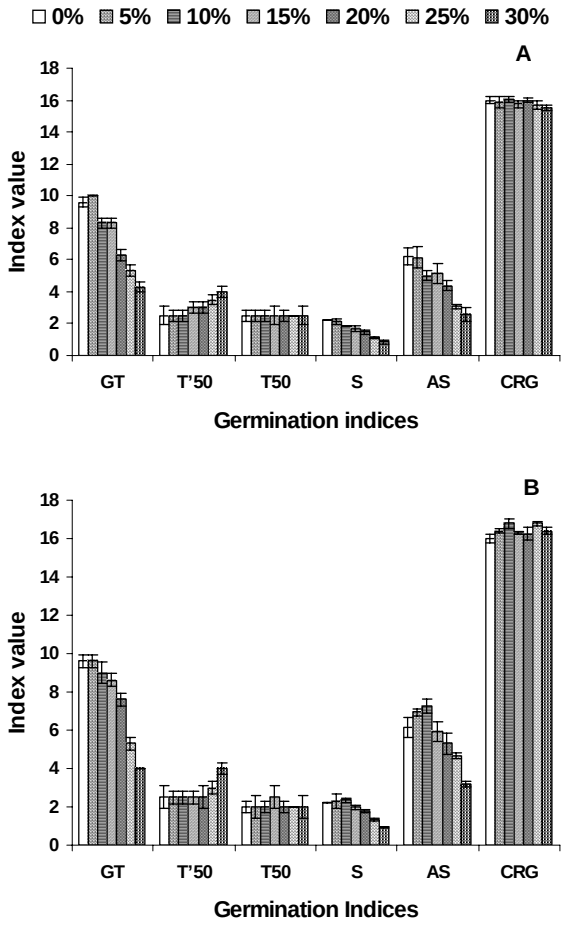
Six germination indices i.e., Total germination (G_T), Number of days required for 50% of the total number of seeds to have germinated (T_{50}), Number of days for 50 % of the total number of seeds germinated (T'_{50}), Speed of germination (S), Speed of accumulated germination (AS) and Coefficient of the rate of germination (CRG) were calculated from same data in the way given in Table I. All the six indices showed different results with low variability. In case of *H. annuus* 20, 25 and 30% concentrations illustrated highly significant reduction in total germination of *R. dentatus*. Where as speed of germination was slightly reduced at above three concentrations. Speed of accumulated germination calculated from the same data showed broader spectrum of sensitivity as 10% aqueous leaf extract showing insignificant effect on total germination and speed of germination significantly reduced the speed of accumulated germination. Coefficient rate of germination was found least sensitive as slightly significant reduction effect was observed at 30% leaf aqueous extract concentration. No significant difference was observed in case of T_{50}, where as difference in T'_{50} increased with increase in concentration (Fig. 1A).

The same trend was observed for all the six germination indices calculated for *T. aestivum*, but with little variations. Aqueous leaf extract of *T. aestivum* tested against *R. dentatus* showed highly significant reduction in total germination and speed of accumulated germination at 25 and 30% concentrations, where as the same concentrations showed slight significant reduction in speed of germination. Coefficient rate of germination was insignificantly affected by all tested concentrations (Fig 1B).

DISCUSSION

The results from the present study reveal that each index leads to different interpretation of aqueous extract effect on *Rumex dentatus.* Germination capacity of tested

Fig. 1. Effect of different concentrations of leaf aqueous extract of H. annuus (A) and T. aestivum (B) on seed germination of R. dentatus



seeds in different treatments was observed and calculated in terms of total germination (G_T), number of days required for 50% of the total number of seeds to have germinated (T_{50}) and number of days for 50% of the total number of seeds germinated (T'_{50}), where as germination rate was discussed in terms of speed of germination (AS) and coefficient of the rate of germination (CRG).

Total germination index is actually the maximum percentage germination, which is a widely used index (Correa *et al.,* 2000). The G_T index only depends on final results, hence it interprets for germination capacity of seeds under any treatment. Therefore G_T index has not been considered enough by various investigators to evaluate the allelochemicals effect on physiological process like germination (Wardle *et al.,* 1991; Haugland & Brandsaeter, 1996). In the present study, aqueous extracts of both *Helianthus annuus* and *Triticum aestivum* showed significant reduction in total germination of tested seeds at concentrations higher than 20%. It does not mean that aqueous extracts affected the germination process only at these concentrations. As in case of *H. annuus* even 10 and

Table I. Formulae to calculate germination indices

Germination index	Formula		References
Total germination (Final germination percentage) (G_T)	$G_T = \frac{[N_T \times 100]}{N}$	N_T: proportion of germinated seeds in each treatment for the final measurement N: Number of seeds used in bioassay	Widely used
T_{50}	Days required for 50% germination of total germinated seeds		Orchard (1977), Josep and Maria (2002)
T'_{50}	Days required for 50% germination of the total seeds		Josep and Maria (2002)
Speed of germination (S)	$S = (N_1 \times 1) + (N_2 - N_1) \times 1/2 + (N_3 - N_2) \times 1/3 + \ldots\ldots\ldots (N_n - N_{n-1}) \times 1/n$	N_1, N_2, N_3, N_{n-1}, N_n: Proportion of germinated seeds observed at first, second, third …… (n – 1), (n) days or hours	Bradbeer (1988), Wardle *et al.* (1991)
Speed of Accumulated germination (AS)	$AS = [N_1/1 + N_2/2 + N_3/3 \ldots\ldots + N_n/n]$	N_1, N_2, N_3, N_n: Cumulative number of seeds which germinate on time 1, 2, 3, ……., N	Bradbeer (1988), Wardle *et al.* (1991), Haugland and Brandsaeter (1996)
Coefficient of the rate of germination (CRG)	$CRG = \frac{[N_1 + N_2 + N_3 + \ldots.. + N_n]}{(N_1 \times T_1) + (N_2 \times T_2) + (N_3 \times T_3) + \ldots\ldots (N_n \times T_n)} \cdot X\ 100$	N_1: number of germinated seeds at time T_1 N_2: number of germinated seeds at time T_2 N_n: number of germinated seeds at time T_n	Bewley and Black (1985)

15% aqueous concentrations showed slightly significant reduction in speed of germination and speed of accumulated germination. Speed of germination considers the number of germinated seeds between two exposure times, where as accumulated germination involves the cumulative number of germinated seeds at each exposure time. T_{50} is the number of days required for emergence of 50% of total germinated seeds, where as T'_{50} is the time when half of the total seeds germinate. T_{50} was found ineffective when different treatments of both *H. annuus* and *T. aestivum* extracts were compared to the control.

Generally, the S and AS showed same results with slight variations. AS found to be more sensitive than S specially in case of *H. annuus*, as it showed the effectiveness of lower concentrations of 10 and 15% in delaying the germination process. This confirms the importance of different germination indices in evaluating the effect of any treatment on seeds.

Coefficient of the rate of germination was found to be least sensitive as it showed only slight significant reduction at 30% aqueous leaf extract of *H. annuus.*

Results supported the hypothesis that data interpretations depends on the choice of germination index and also that one index might not be suitable for precise description of allelopathic effects on the germination process. Comparison of different indices may provide better justification. This will contribute in making allelopathy a more precise science.

REFERENCES

Bewley, J.D. and M. Black, 1985. *Seeds: Physiology of development and Germination.* pp. 367. Plenum Press, New York

Bradbeer, J.W., 1988. *Seed Dormancy and Germination,* pp. 146. Blackie and Son, Glasgow

Chiapusio, G., A.M. Sanchez, M.J. Reigosa, L. Gonzalez and F. Pellissier, 1997. Do germination indices adequately reflect allelochemical effect on the germination process? *J. Chemical Ecol.,* 23: 2445–53

Correa, J.F., I.F. Souza, A.M. Ladeira and M.C.M. Young, 2000. Allelopathic potential of *Eupatorium maximiliani* Schrad. Leaves. *Allelopathy J.,* 7: 225–34

Dahiya, D.S. and S.S. Narwal, 2003. Allelopathic plants. 7. Sunflower (*Helianthus annuus* L.). *Allelopathic J*., 11: 1–20

El–Khatib, A.A. and A.K. Hegazy, 1999. Growth and physiological responses of wild oats to the allelopathic potential of wheat. *Acta Agronomica Hungarica*., 47: 11–8

Foy, C.L., 1999. How to make bioassays for allelopathy more relevant to field conditions with particular reference to cropland weeds. *In:* Inderjit, K.M.M. Dakshini & C.L. Foy (eds.), *Principals and Practices in Plant Ecology: Allelopathic Interactions.* pp. 25–33. CRC Press, Washington

Haugland, E. and L.O. Brandsaeter, 1996. Experiments on bioassay sensitivity in the study of allelopathy. *J. Chemical Ecol.,* 22: 1845–59

Inderjit, and K.M.M. Dakshini, 1995. On laboratory bioassays in allelopathy. *Bot. Rev. (Lancaster),* 61: 29–44

Josep, A.R. and M. Maria, 2002. Seed Germination and Reproductive Features of *Lysimachia minoricensis* (Primulaceae), A Wild–extinct Plant. *Annals of Botany*, 89: 559–62

Kato–Noguchi, H., S. Kosemura, S. Yamamura, J. Mizutani and K. Hasegawa, 1994. Allelopathy of oats. 1. Assessment of allelopathic potential of extract of oat shoots and identification of an allelochemical. *J. Chemical Ecol.,* 20: 309–14

Lambers, H., F.S. Chapin and T.L. Pons, 1998. *Plant Physiologcal Ecology*. p. 540. Springer–verlag, Berlin

Macias, F.A., D. Castellano, J.C.G. Galindo, R.F. Velasco and D. Chinchilla, 1999. Statistical approach for selection of allelochemicals as natural herbicide models. *In:* Abstract book, 2nd World Congress on Allelopathy: Critical Analysis and Future Prospects. Lakehead University, Canada

Orchard, T.J., 1977. Estimating the parameters of plant seedling emergence. *Seed Sci. Tech.*, 5: 61–9

Rizvi, S.J.H. and V. Rizvi, 2000. Genetic variation in allelopathic activity of wheat (*Triticum aestivum* L.) genotypes. *Wheat Information Service*. 91: 25–9

Wardle, D.A., M. Ahmad and K.S. Nicholson, 1991. Allelopathic influence of nodding thistle (*Carduus nutans* L.) seeds on germination and redicle growth of pasture plants. *New Zealand J. Agric. Res*., 34: 185–91

Wu, H., J. Pratley, D. Lemerle, T. Haig and B. Verbeek, 1998. Differential allelopathic potential among wheat accessions to annual ryegrass. Pp. 567–71. *In: Proc. 9th Australian Agron. Conf., Wgga Wagga*, NSW, Australia

Wu, H., J. Pratley, D. Lemerle and T. Haig, 1999. Crop cultivars with allelopathic capability. *Weed Res.*, 39: 171–80

Vyvyan, J.R., 2002. Allelochemicals as leads for new herbicides and agrochemicals. *Tetrahedron,* 58: 1631–46

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