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Allelopathic Impact of Sunflower on Germination Factors of some Agronomic Crops

Dr. Ashima Gakhar
Associate Professor
Department of Botany
KVA DAV College for Women, Karnal
drashimagakhar@gmail.com

Abstract
Sunflower *(Helianthus annuus L.*) contains water-soluble allelo-chemicals that inhibit the germination and growth of other species. The current work investigated alleopathic effects of extracts from the leaves from the plant for various parameters viz a viz percent germination, seed vigour and seedling growth against **fourteen agronomic plants**. Not only that different species responded differently to the same formulation of allelochemicals, but also varied physiological parameters or components thereof showed different response to the same treatment. The experiments were designed to access the degree of susceptibility/ resistance of 14 chosen agronomic crop seeds to germinate under the influence of hydroleachable allelochemics of *Helianthus annus L.* leaves. An attempt was also made to select a few morphological parameters of the seeds that could be suggested as an indicator for predicting the allelopathic response of crop. Seedling growth was affected the most out of all the germination factors studied, followed by speed of germination and germination percentage. As regards to germinability of seeds, the most resistant indicator was found to be *Linum usitatissimum*, of all the species under investigation.

Key Words: *Helianthus annus L.*, allelopathy, allelochemicals, agronomic plants.

Introduction

Allelopathy is a phenomenon in which one plant affects (favorably or adversely) the establishment, growth and performance of other crops of its immediate environment. Several phytotoxic substances causing germination and growth inhibitions have been isolated from plant tissues and soils. These substances, collectively known as allelochemicals or allelochemics, are usually secondary plant products or waste products of main metabolic pathways of plants (**Whittaker, R. H., and P. P. Feeny, 1971; Hall, M. H., and P. R. Henderlon, 1989; Chon, S. U., and J. D. Kim, 2002**). The impact of allelochemicals on agriculture has long been described in the last 40 years. It has been suggested that crops having an allelopathic effect on other crops shouldn't be followed by susceptible ones (**Kausar, 1999**).

The cultivation of sunflower for edible oil and protein is long been into practice. The crop is resistant to drought, and its short duration, makes it a suitable crop if sowing has to be delayed. However, yields of some crops when they follow sunflower are lower than normal, possibly because of inadequate nutrition and chemical inhibition. Sunflower is often grown when rainfall is marginal, and depletion of soil moisture by sunflower may also be a factor although this remains unproven so far **(Kamal, Javed, and Asghari Bano, 2008).** The cultivation of sunflower on large scale with disregard to edaphic and climatic requirements, ecologically speaking, appears an unhealthy sign especially when it has been indicated to exhibit allelopathy (**Kruse, M., Strandberg, M., and Strandberg, B. 2000**). The allelopathic interactions of sunflower in the Indian context of fourteen crops were being studied under the current investigation. The observation parameters included percent germination,

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seed vigour and seedling (radicle and plumule) length of 14 crops under the influence of aqueous leachates from leaves. Also, the morphological characters like seed volume, seed coat thickness and size were measured and the colour, shape and seed texture were observed.

Materials and Methods

The leaves and other plant parts of *Helianthus annus* L. were collected from plants grown in the experimental plots of Botanical Garden, Panjab University, Chandigarh (30° N; 77° E; 280 mean sea level). The healthy, uniform and viable seeds of plants used (viability ensured through 2, 3, 5-triphenyl tetrazolium chloride test) were:

Pulses: *Phaseolus aureus* Roxb. Var. ML-267*, Phaseolus mungo* L. var. Mash 1-1, *Cajanus cajan* DC. Var. AL-15, *Vigna umbellate* Thunb. Var. RBL-1, *Lens esculentum* L. var. LL-56,

Cereals: *Triticum aestivum* L., *Zea mays* L., *Oryza sativa, Avena sativa,*

Oil Seeds: *Brassica campestris* L., *Linum usitatissimum* L., *Hibiscus esculentum*, *Lycopersicum esculentum* L. *and Solanum melongena* L. were procured from Plant breeding Department of Agricultural University, Ludhiana.

Plants of *Phaseolus aureus* Roxb. were raised from seeds sown in earthen ware pots (8 inches diameter) filled with sand and clay (1:1 v/v).

Extraction of Allelochemicals

Aqueous leachates

Freshly collected, surface cleaned, healthy leaves of *Helianthus annus* chopped into pieces were weighed and suspended in pure water in the ratio of 1:1 w/v for 24 hours. The suspension was filtered using bilayer muslin cloth. The filtrate was used as aqueous leachate. The concentration used was selected on the basis of preliminary trial involving wide range of concentrations (0.1 to 1 g fresh weight/mL of water) of leachates on cell survival values of a few representative plant leaves (**Muhammad, Zahir, and Abdul Majeed. 2014**).

Viability Test

Few seeds from each of the fourteen plants under investigation were tested for their viability using 2, 3, 5-triphenyltetrazolium chloride prepared in phosphate buffer of pH 7.4, on the basis of rules prescribed by International Seed Testing Association (ISTA). Appearance of red formazon in the embryo confirmed their viability (**Achhireddy, N. R., and Megh, S.,1984**).

Germination Trial

International **Rules** for Seed Testing (ISTA rules) were followed (**Agarwal, 1980 & Tian, Y., Guan, B., et al 2014**). Uniform, healthy viable seeds of plants under investigation were used. For each treatment 120 seeds of each type were taken. These were presoaked in aqueous leachates, organic components of aqueous leachates and organic extracts for 24 hours. Seeds sown in water served as control. The soaked seeds of each treatment were placed in 6 inches diameter petri dishes. The petri dishes were lined with a wad of thin absorbent cotton and overlined with Whatman number 40 filter paper. These were moistened with treatment solution and 30 seeds were arranged in each petri dish in concentric rings maintaining a proper inter-seed distance. 4 petri dishes were set for each treatment and the set up was maintained in seed germinator for definite temperature (**table1**), relative humidity 74 $\pm$ 1 % and continuous light of 4000 lux for 24 hours daily.

Protrusion of radicle from seed coat signified initiation of germination. Daily observations with hand lens were made in one representative out of the four petri dishes. Observations were made for seven days till no more seeds germinated.

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Seed Characteristics

The observations on various characteristics like seed volume, seed coat thickness, mean of maximum width and length of seeds were measured and colour, shape and surface texture were noticed. The observations on the number of respective seed types that germinated, speed of germination and mean seedling length of treated samples were calculated with respect to that of respective control and presented in a ratio between the treated and control. Out of all the permutation combinations of the various characteristics of the seeds, only data on the ratio seed coat thickness and seed volume was found reasonable and was retained and presented (Nakar et al.,1915)

Table1 Conditions of temperature, substratum and pretreatment to the seeds of various groups under observation for germination

BOTANICAL NAME	TEMPERATURE/ SUBSTRATUM	PRE-TREATMENT
Phaseolus aureus Roxb. Var. ML-267	25 $\pm$3 (BP)	-
Phaseolus mungo L. var. Mash 1-1	25 $\pm$2 (BP)	Diffuse light
Cajanus cajan DC. Var. AL-15	25 $\pm$2 (BP)	-
Vigna umbellate Thunb. Var. RBL-	25 $\pm$2 (BP)	-
Lens esculentum L. var. LL-56	25 $\pm$2 (BP)	Pre-chill treatment
Triticum aestivum L.	20 (BP)	Diffuse light, pre-chill KNO_3
Zea mays L.	25 $\pm$3 (BP)	-
Oryza sativa	25 $\pm$3 (BP)	-
Avena sativa	20 (BP)	Diffuse light, pre-chill KNO_3
Brassica campestris L.	20 $\pm$3 (BP)	Light, pre-chill KNO_3
Linum usitatissimum L.	20 (BP)	Light, pre-chill, pre-dry
Hibiscus esculentum	25 $\pm$2 (BP)	Light
Lycopersicum esculentum L.	25 $\pm$2 (BP)	Light, KNO_3
Solanum melongena L.	25 $\pm$2 (TP)	Light, KNO_3

BP – between the folds of filter paper
TP- top of filter paper

Results

Germination Characteristics

The various extracts from *Helianthus annus* exhibited toxicity towards the investigated agronomic plants. Except the seeds of *Linum usitatissium,* where treated seeds did not show any statistical deviation from that of control, seeds of all other types of plants under test, showed significantly low percent germination over that of respective control.

Linum usitatissium, Phaseolus mungo, Triticum aestivum and Oryza sativa were resistant towards aqueous leachates of *H. annus. Lycopersicum esculentum L., Vigna umbellate, Solanum melongena and Lens esculentum* were susceptible to leachable allelochemics of *H.annus.* The response of different seed types towards different parameters of germination (percent germination, seed vigour and seedling length) was dependent on the ratio of their respective seed coat thickness and seed volume (**table 2**). For different parameters of germination different values of seed coat thickness and seed volume ratio govern the extent of response.

Table 2 Effect of Aqueous Leaf Leachates (0.5 g fresh weight/mL) on the germination, seed vigour, mean seedling length of a few agronomic crop seeds under study.

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BOTANICAL NAME	CONCENTRATION	% GERMINATION	% SEED VIGOUR	MEAN SEEDLING LENGTH (Cm)
Phaseolus aureus Roxb. Var. ML-267	Control T_{50}	100 43.3	100 31.6	15 2.6
Phaseolus mungo L.var. Mash 1-1	Control T_{50}	100 83.29	96 28.6	20.46 9.44
Cajanus cajan DC. Var. AL-15	Control T_{50}	100 40.0	91.5 28.6	13.9 13.72
Vigna umbellate Thunb. Var. RBL-	Control T_{50}	100 20.0	95 18	25.6 17.08
Lens esculentum L. var. LL-56	Control T_{50}	100 27.97	71.6 28	9.23 3.56
Triticum aestivum L.,	Control T_{50}	100 93	100 71.1	12.46 6.25
Zea mays L.	Control T_{50}	100 53.3	92.7 50.1	16.07 12.77
Oryza sativa	Control T_{50}	100 93.10	96.6 45	7.44 4.42
Avena sativa	Control T_{50}	100 0	9.78 0	2.401 0
Brassica camperstris L.	Control T_{50}	100 40	100 12	10.17 5.6
Linum usitatissimum L.	Control T_{50}	100 100	81 72	5.75 0.92
Hibiscus esculentum	Control T_{50}	100 27.28	73.3 18.3	4.476 0.455
Lycopersicum esculentum (L.)	Control T_{50}	100 13.05	21.3 8	7.15 1.18
Solanum melongena L.	Control T_{50}	100 21.43	42.1 3.3	3.38 1.97

Germination in control seeds of:
a) *Avena sativa* = 33.3
b) Lens esculentum = 83.3
c) Hibiscus esculentum = 73.3
d) Lycopersicum = 76.6
e) Solanum melongena = 93.3
In rest of the seeds, it was 100 %.

The speed of germination in each type of seeds was drastically low to that of respective control. Even those seeds, where percent germination was not affected significantly was seen to show reduced seed vigour (**Table 2**). The lengths of radicle and plumule of seedlings of treated seeds were drastically short compared to the respective seeds of control. The difference was more pronounced in case of radicle than the plumule except in *Brassica camperstris L. and Solanum melongena* L. The ratio of treated to control was less than 0.5 in majority of the cases. However, it was more in *Linum usitatissimum* L., *Oryza sativa*, *Zea mays*, *Triticum aestivum* L *and Phaseolus mungo L.*

A mathematical model was employed, whereby 14 seeds under study were arranged in descending order on the basis of their seed coat thickness (SCT) and seed volume (S_{vol}) ratio (**Daizy, 2008**). The ratio of seed germination, seed and mean seedling length between treated and control was plotted against these values of SCT/ S_{vol} (**Table3**, **Fig 1a, 1b, 1c**) and an exponential behavior was observed.

Table 3 Ratio of seed coat thickness (mm) over seed volume (cc) in relation to their germination, seed vigour and mean seedling length over control of various taxa (shown in decreasing order)

BOTANICAL NAME	SCT/Svol	T/ C. G.	T/ CSV	T/ CMSL

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Linum usitatissimum L.	2.28	1	0.8888	0.160
Vigna umbellate Thunb. Var. RBL-	0.833	0.2	0.1894	0.6671
Solanum melongena L.	0.830	0.2143	0.0783	0.5828
Hibiscus esculentum	0.761	0.2728	0.2496	0.1016
Lycopersicum esculentum (L.)	0.700	0.1305	0.3755	0.1650
Oryza sativa	0.686	0.930	0.4658	0.6370
Lens esculentum L. var. LL-56	0.333	0.279	0.3910	0.3856
Avena sativa	0.300	0	0	0
Triticum aestivum L.,	0.228	0.93	0.717	0.5016
Phaseolus mungo L.var. Mash 1-1	0.212	0.8329	0.677	0.4613
Zea mays	0.185	0.533	0.5404	0.7946
Phaseolus aureus	0.105	0.433	0.306	0.6705
Cajanus cajan L.	0.066	0.400	0.306	0.6705
Brassica campestris L.	0.060	0.400	0.12	0.5505

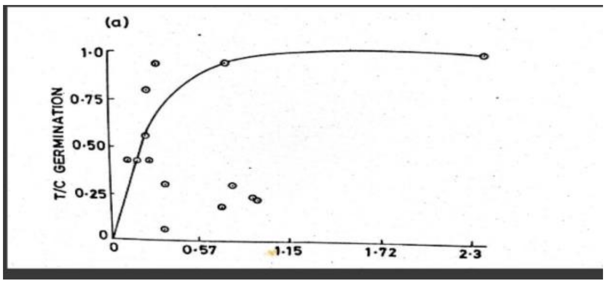

Fig 1a. To show exponential behavior when curve is plotted between T/C values for germination and seed T/ V values of plants under observation.

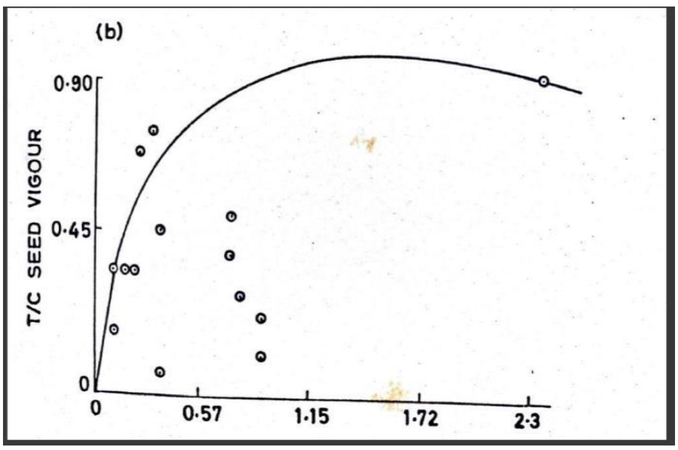

Fig 1b: To show exponential behavior when curve is plotted between T/C values for seed vigour and seed coat thickness/ seed volume, T/ V values of plants under observation

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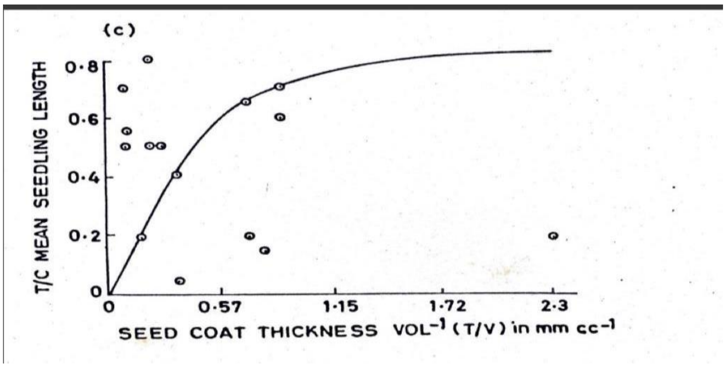

Fig 1c: To show exponential behavior when curve is plotted between T/C values for mean seedling length and T/V values of plants under observation

Based on the depicted exponential behavior, famous mathematical equation was employed to predict the degree of susceptibility/resistance of the seeds against the aqueous leachates.

$y = y_{max}^{(1 - e \wedge -x/xc)}$, where

y represents the value of germination of the treated seeds over that of control, i.e. T/C for germination (T/C G),

y_{max} represents maximum value of germination of treated seeds over control. This is one in this case. x represents seed coat thickness (mm) per unit seed volume (in cc), i.e. SCT/Svol in mm cc^{-1}. This value is different for different seeds.
x_c represents that threshold value of SCT/Svol above which seeds will be expected to show significant change in degree of germination.
By calculating the value of x_c for each of x and ln $\left(\frac{y_{max} - y}{y_{max}}\right)$
the mean was taken for calculating the value of x_c in general. y_{max} was found out to be 0.889; from slope, x_c for seed vigour was calculated to be 0.306, and y_{max} was 0.16 and x_c for seedling length was 1.1325 (**fig 2a, fig 2b, fig 2c**).

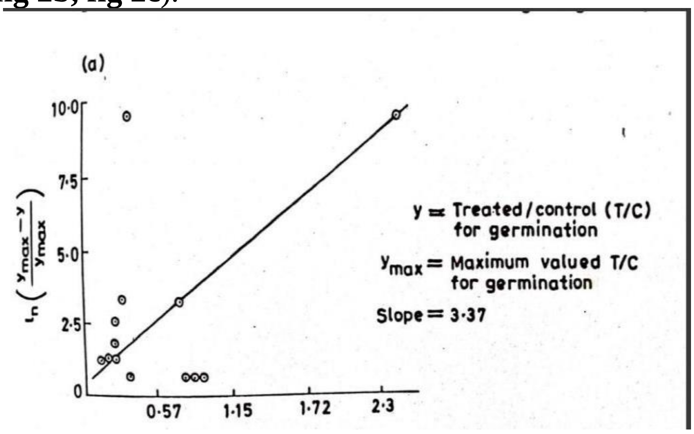

Fig 2a Relationship between seed coat thickness/seed volume represented by x and ln ($y_{max} - y/ y_{max}$) in order to evaluate slope for seed germination.

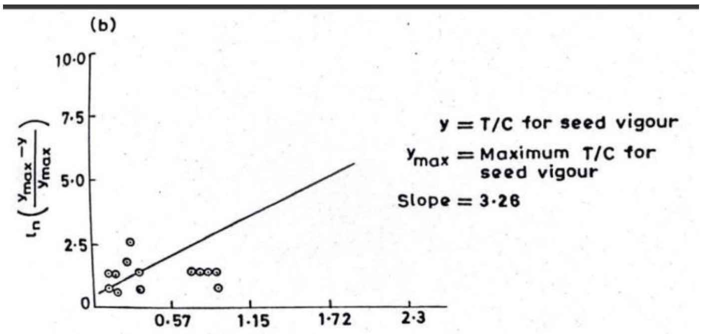

Fig 2b Relationship between seed coat thickness/seed volume represented by x and ln ($y_{max} - y/ y_{max}$) in order to evaluate slope for seed vigour.

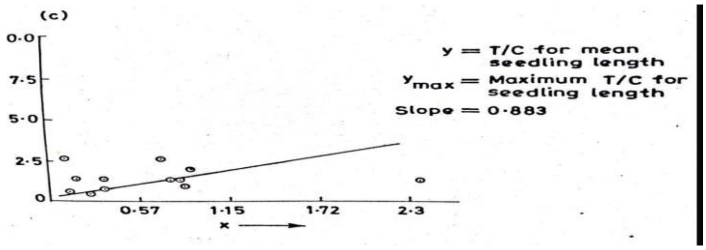

Fig 2c Relationship between seed coat thickness/seed volume represented by x and ln ($y_{max} - y/ y_{max}$) in order to evaluate slope for mean seedling length.

Discussion

It was apparently seen that different seed types responded differently to the allelochemics present in the polar leachates extracted from *H. annus*. The seed germination of *Linum* showed resistance, whereas those of *Lycopersicum, Solanum melongena, Hibiscus esculentum, Vigna umbellate and Lens esculentum were highly susceptible. Oryza sativa, Triticum aestivum and Phaseolus mungo* were least susceptible, while the remaining showed medium susceptibility.

None of the morphological characters of the plants, especially the non-quantifiable ones like colour, shape, texture of the seed surface could reflect any correlation between the character and response. The quantifiable characters, length or breadth or product thereof did not yield any meaningful correlation. Also, the thickness of seed coat (mm) or the volume of seed (cc) independently or their product failed to indicate any trend in response to leachates.

Nevertheless, with an increase in SCT/Svol value, germination percentage and also to some extent seed vigor was seen to rise exponentially (**fig 1a, fig 1b**). The relationship of seedling length with SCT/Svol was more or less irregular (**fig 1c**).

For the result of 14 plant type seeds investigated the critical values for 3 parameters understudy were found to be:

Germination x_c (G): 0.296

Seed Vigour x_c (SV): 0.306

Mean Seedling Length x_c (MSL): 1.1325

The significance of these values under the present set of conditions could not be assessed, as the number of samples was low for prediction with respect to SCT/Svol.

Conclusion

The polar extracts from *Helianthus annus* L. exhibit toxicity, and impair various germination parameters (percent germination, seed vigour and seedling growth). The aqueous leachates showed differential toxicity towards fourteen agronomic plant types tried. In the current study, as regards to germinability of seeds, most resistant indicator was found to be *Linum usitatissimum*, while

Lycopersicum, *Solanum melongena*, *Hibiscus esculentum*, *Vigna umbellate* and *Lens esculentum* were highly susceptible. It was also observed that seedling growth was affected the most out of all the germination factors, followed by speed of germination and germination percentage. The ratio of seed coat thickness (SCT) and seed volume (Svol) of the responding seeds showed a direct relation to the retardation of germination by leachable toxins by sunflower leaves.

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