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A preliminary study on the comparison of inhibitory effects induced by PEG 6000 and *Euphorbia hirta* in two crop plants

İki tarla bitkisinde Euphorbia hirta ve PEG 6000 ile oluşan engelleyici etkilerin karşılaştırması üzerine bir ön çalışma

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ABSTRACT

Euphorbia hirta is commonly found in all tropical regions of the world, as invasive weed with an adverse effect on other plants. The current study features two important aspects: (i), Effects of *E. hirta* roots exudates (0, 50, 75 and 100% concentrations) (ii), Influence of PEG 6000 "Polyethylene glycol" (8, 16 and 40 g/l concentrations) on germination and growth criterion of *Cicer arietinum* (Chick pea) and *Phaseolus vulgaris* (French bean). In this regard osmotic potential of all the solutions have been balanced. It is observed that the root leachates of spurge weed significantly ($p < 0.05$) affected the germination rate while PEG has no positive or negative effect on growth activity. The growth parameters in both crops have altered by spurge weed and PEG solutions while highest inhibition was recorded in root length root exudates (3.5 cm) of *C. arietinum* which is in contrast to PEG (11.97 cm), this might be due to the presence of allelochemicals. The prescribed treatments also raised their pH values, where highest pH (7.9) obtained from 100% spurge weed induction which is relatively a stronger basic nature rather than neutral control samples. Comparative studies of both test crops shows that *C. arietinum* has appeared to be more influential than *P. vulgaris*.-Two-way ANOVA has produced a significant difference ($p < 0.05$) between the effects of both treatments on germination and growth of test crops while within group (concentrations of each treatment) are weakly significant ($p < 0.1$) to each other. This would rather intimates that the inhibition has strongly emerged as an allelopathic response on *Cicer arietinum* and *Phaseolus vulgaris* plants by the induction of spurge weed (*Euphorbia hirta*) root exudates.

ÖZ

Euphorbia hirta, diğer bitkiler üzerinde olumsuz etkiye sahip istilacı bir yabancı ot türü olarak dünyanın bütün tropikal bölgelerinde yaygın olarak bulunmaktadır. Yürütülen çalışmada, *Phaseolus vulgaris* L. (Fasulye) ve *Cicer arietinum* L. (Nohut) çimlenme ve büyüme kriteri üzerine (i) *E. hirta*'nın kök salgılarının etkileri (0, 50, 75 ve %100 konsantrasyon), (ii) PEG 6000'nin (8, 16 ve 40 g/l konsantrasyon) "Polietilen glikol" etkisi olmak üzere iki önemli husus bulunmaktadır. Bu bakımdan bütün solüsyonların osmotik potansiyeli dengelenmiştir. Sütleğen bitkisinin kök salgılarının çimlenme oranını önemli ölçüde ($p < 0.05$) etkilediği, PEG'in ise büyüme aktivitesi üzerinde olumlu veya olumsuz bir etkisinin olmadığı gözlemlenmiştir. PEG solüsyonları ve sütleğen bitkisi her iki üründeki büyüme parametrelerini değiştirmiş, *C. arietinum*'un kök uzunluğunda PEG (11.97 cm)'in aksine en yüksek oranda inhibisyon kök salgıları uygulamasında (3.5 cm) kaydedilmiştir. Bu durum alelokimyasalların varlığından kaynaklanmış olabilir. En yüksek pH (7.9) nötr kontrol örneklerine göre daha güçlü bir bazik yapısı olan %100 sütleğen otu indüksiyonundan elde edilmiş, yapılan uygulamalar ayrıca pH değerlerini de yükseltmiştir. Çalışmadaki her iki test bitkisi karşılaştırıldığında *C. arietinum*'un *P. vulgaris*'ten daha fazla etkilendiği görülmüştür. İki yönlü varyans analizine göre (Two-way ANOVA) her iki uygulamanın test bitkilerinin çimlenme ve büyümesi üzerindeki etkileri grup içerisinde (her bir uygulama konsantrasyonları) az oranda önemli olmasına rağmen ($p < 0.1$) her iki uygulama arasında önemli bir fark ($p < 0.05$) oluşturmuştur. Bu inhibisyonun sütleğen bitkisinin (*Euphorbia hirta*) kök salgılarının *Cicer arietinum* ve *Phaseolus vulgaris* bitkileri üzerine alelopatik etkisine karşı güçlü bir yanıt olarak ortaya çıktığı düşünülmektedir.

1. INTRODUCTION

Plants are the fundamental bodies in agriculture systems that accelerates world's economy, global food demand and socio-economic spectrum. Pakistan's economy also mainly depends upon agriculture however there are certain plants that can produce harmful effects in agricultural environment (Asad et al., 2020). Weeds are one of them. "Pigweed" is a pantropical weed, scientifically termed as *Euphorbia hirta* and widely used as a medicinal herb for the treatment of respiratory tract infections (Alam, 1991). It produces bronchial relaxation for asthma patient and is commonly known as asthma plant (Dalziel, 1937; Kerharo & Adam, 1974). Despite having all these positive characters, *E. hirta* is known to possess allelopathic effect on crops like pea, tomato, wheat, chickpea, cotton, alfalfa, lettuce, ground nut and soybean (Rice,1984).

Allelopathy is a process in which one plant species is affected by another and releases chemicals to compete for their survival (Ridenour & Callaway, 2001; Inderjit & Callaway, 2003). These allelochemicals can also retarded the growth of soil microbes which ultimately reduce soil fertility. Root leachates of *Lollium multiflorum* inhibited the soil microbial activity and soil structure (Ferreira et al., 2017). These are secondary metabolites synthesized inside the plant body and may release out either in the form of root exudates or by volatization etc. They may present in any of the plant organs like roots, leaves, flowers, fruits and buds (Ashrafi et al., 2007). These bio-chemicals can be released in the form of leachates and influence the vegetation as well as soil. Such bio-chemicals are called as allelochemicals, consisted of greater amount of phenolic substances and may cause blockage of chlorophyll pathways and ultimately the process of photosynthesis. (Rice, 1984). These leachates consisting of phenols altered the pH of water and create noxious effects to the plants growing in that medium. A lot of researchers worked on leachates of plants *i.e.,* leaf leachates of *Gmelina arborea* contain many allelochemicals which are responsible for drastic inhibition of seedling growth and moisture content of different pulse crops (Shankar et al., 2014).

Osmotic stress is another influential factor that provides a great loss to the agriculture sector. Polyethylene glycol (PEG) 6000 is basically used to introduce water scarcity in plants artificially. It bears a higher molecular weight and unable to pass through apoplast, moreover, it is a sugary compound. Radhouane (2007) worked on Osmotic stress by using PEG 6000 and evaluated that the seedlings of pearl millet were affected by osmotic solution. It is typically assumed that allelopathy is not the actual criteria for inhibition as explained by Bell (1974) and Wardle et al., (1992). They suggested that reduction in plants growth cannot always be a factor of allelopathic effect, it might be due to osmotic tension in cell sap. The concept is fairly acceptable, therefore, it is important to distinguish the inhibitory effect and the real cause of inhibition in the crop, that's how the crop conservation strategy could be maintained.

The aim of this study is to examine the causal agent of inhibition in the growth of two crop plants and explored whether osmotic pressure or allelopathy. Therefore, experiment was conducted using PEG 6000 and root leachate of *E. hirta* on germination and seedling emergence of *Cicer arietinum* and *Phaseolus vulgaris* which are economically important crops and fulfil the nutritive requirement of the country.

2. MATERIALS AND METHODS

The experiment was carried out in the pattern of control randomized block design that has conducted in two phases i.e., germination phase and seedling stage phase. In both phases, there were two sets of petriplates (i) For the analysis of root exudates effects on seed germination and growth (ii) For evaluation of PEG 6000 impacts on seed germination and growth. For this purpose, fresh seeds of *C. arietinum* and *P. vulgaris* were drawn from local market and were surface sterilized. Ten seeds of each test species were placed in petridish containing Whattman filter paper 1 along with five replicates.

For leachates isolation, 30 newly borne seedlings of *E. hirta* were collected, transferred to the pots and kept in the greenhouse located in Department of Botany, Federal Urdu University. The climatic conditions comprised of mild winter having ~26^0 C of average temperature in the month of February 2017. After two weeks of transplantation, leachates were collected by following method of Wardle et al., (1992) The obtained leachates were then diluted to prepare 50, 75 and 100 % concentrations. Another treatment of non-diluted sample with five replicates was used to analyze the effects, denoted as 0% in the results.

For identification of osmotic effect, PEG 6000(s) was chosen to create osmotic hindrance, different concentrations (8%, 16% and 40%) were prepared by dissolving PEG 6000 in 200 ml of water. Osmotic potential of exudates and PEG were determined by the help of Osmometer (Knauer Semi-Micro Osmometer K-7400S) that is listed in (Table 1). Osmotic potential of all the solutions were balanced to standardize the effect of osmoticum, this was helpful in the investigation of fundamental inhibitory agent. 3ml of leachates and PEG solution of different concentrations was applied to each petridish.

The seeds were irrigated by their respective treatments at every alternate day. Seed germination was recorded on daily basis while radical and plumule elongation of seedlings were measured at alternate days. pH of all the prepared solutions was recorded by the help of pH- meter Model No. ECPCWP 65000 (Table 2).

Table 1. Osmotic pressures of Root leachates of *E. hirta* and polyethylene glycol.

PEG 6000 g/l	O. Pressure (kpa)	Root exudates ml/ml	O. Potential (kpa)
8%	12	50%	12
16%	25	75%	25
40%	55	100%	55

Where, O. Pressure = Osmotic pressure, Kpa = kilo pascal

Table 2. pH level in root leachates of *E. hirta* and PEG 6000

Spurge weed dilutions (%)	0	50%	75%	100%
Obtained pH	7.00	7.1	7.5	7.9
PEG solutions (%)	0	8 %	16%	40%
Observed pH	7.00	7.01	7.1	7.2

2.1 Statistical Analysis

Germination % was determined by the following formula.

$$Germination\ \% = [\ n/t]\ x\ 100$$

Where n = No. of seeds germinated, t = Total no of seeds.

Germination index was calculated by following formula. (Khandakar & Bradbeer, 1983)

$$S = [N1/1 + N2/2 + N3/3 +- \ - - -]\ x\ 100$$

Where "S" is the speed of germination N1/1, N2/2…..are the ratio of number of seed germinated per day.

SPSS-2000 version was utilized for the statistical analysis of the above parameters.

3. RESULTS AND DISCUSSION

3.1 Effects on germination

Osmotic pressure and pH of root leachates and PEG 6000 solutions were shown in Table 1 and 2. Emergence of seeds of selected test crops effected by root leachates and PEG 6000 were examined and listed in (Table 3, 4; Figure 1, 2). Present study showed that all concentrations of *E. hirta* root leachates had a significant effect on the germination of chick pea. Highest concentration (55 Kpa) of root exudates have produced 72% germination in *C. arietinum* while in *P. vulgaris*, seed germination rate was 90% while there was 100% germination produced from PEG induced samples, hence the germination (%) maintained a significant ($p < 0.05$) difference between both the species and treatments. It has been reported from various studies that alteration in seed germination may occur due to a number of factors *i.e.,* changes in enzymatic activity, chromosomal sequencing and functioning of secondary metabolites (Khan et al., 2018; 2020). Allelochemicals are usually responsible to induce phytotoxic effects and alter the ability of seed germination (Friedman et al., 1977; Baskin & Baskin, 2001; Asad et al., 2020). Chaves et al., (2001) revealed that the flavonoids present in *Cistus ladanifer* influenced the cotyledons size as well as limiting the germination % and slow down the cotyledon emergence. Germination indexes of both crops were declined by the insertion of root leachates, least velocity of germination was observed in chick pea (47%) while in PEG, 99% seeds were germinated. In contrary to *P. vulgaris*, % germination of *C. arietinum* is found to be more effected by the concentration of root leachates.

Table 3. Impact of *E. hirta* root leachates and PEG 6000 on germination phase of *Cicer arietinum*

	PEG 6000		**Root exudates**	
Treatments (Kpa)	G %	GI %	G %	GI %
0	100±0.0	99	100 ± 0	99%
12	100±0.0	99	99± 1	89%
25	100±0.0	99	98 ± 2	83%
55	100±00	96	72 ± 8	47%

where, G % = Germination %, GI = Germination index

Table 4. Impact of *E. hirta* root leachates and PEG 6000 on germination phase of *Phaseolus vulgaris*

	PEG 6000		Root exudates	
Treatments (Kpa)	G%	GI %	G%	GI %
0	100±0.0	99	100 ± 0	100%
12	100±0.0	95	100± 0	96%
25	100±0.0	90	99 ± 1	91%
55	100±0.0	89	90 ± 10.1	89%

where, G % = Germination %, GI = Germination index

On the other hand, PEG 6000 with all dilutions did not produce any hindrance in germinating seeds of both test crops, however, it showed some alterations in germination rate of both selected test crops at 55 kpa (89 %) over to control (100%). Our findings were similar to (Radhoune, 2007) where, lower concentration of PEG would not be able to evoke any disturbance but at higher concentration it can cause significant inhibition.

3.2 Effect on seedling emergence

Radicle and plumule elongations were measured after completion of seeds germination and presented in Table 5, 6 and Figure 1, 2. It was observed that *E. hirta* root leachates significantly inhibited seedling emergence of both test crops lowering the growth. 100 % concentration of leachates strongly inhibited root elongation in chick pea (3.5 cm) whereas in control samples roots prolonged up to 17.33cm, showing a significant difference ($p < 0.05$) in chick pea seedlings. Similar results were obtained by Rawat et al., (2013) in which leachates of sunflower possessed certain allelochemicals which were responsible for the reduction of seedling growth of different crops. By comparing both crops, chick pea was found to be greatly influenced by leachates. Our results are in agreement with the findings of Pawar & Rawal (2014), they revealed that, petals of *Delonix regia* contained different polyphenols which altered the germination and seedling growth of chick pea, while Ahmed et al., (2004) observed that *E. camaldulensis* Dehn (aqueous extract) influenced the germination index as well as seedling growth of chick pea.

Table 5. Response of *E. hirta* root leachates and PEG 6000 on seedlings of *Cicer arietinum*

	PEG 6000		Root exudates	
Treatments (Kpa)	RL (cm)	SL (cm)	RL (cm)	SL (cm)
0	17.23 a	21.23 a	17.33 a	21.11 a
12	16.19 b	19.21 b	11.2 b	15.28 b
25	13.44 c	16.52 c	8.72 c	11.96 c
55	11.97d	11.48 d	3.5 d	8.89 d

where a = 0.1, b = 0.8, c = 1, d = 1.5 (standard error), RL = root length, SL = shoot length

Table 6. Response of *E. hirta* root leachates and PEG 6000 on seedlings of *Phaseolus vulgaris*

	PEG 6000		Root exudates	
Treatments (Kpa)	RL (cm)	SL (cm)	RL (cm)	SL (cm)
0	21.99 a	26.26 a	21.98 a	26.22 a
12	20.49 b	25.11 b	21.01 b	24.18 b
25	18.24 c	22.92 c	16.02 c	19.99 c
55	14.92 d	19.49 d	13.51 d	16.72 d

where a = 0.1, b = 0.8, c = 1, d = 1.5 (standard error), RL = root length, SL = shoot length

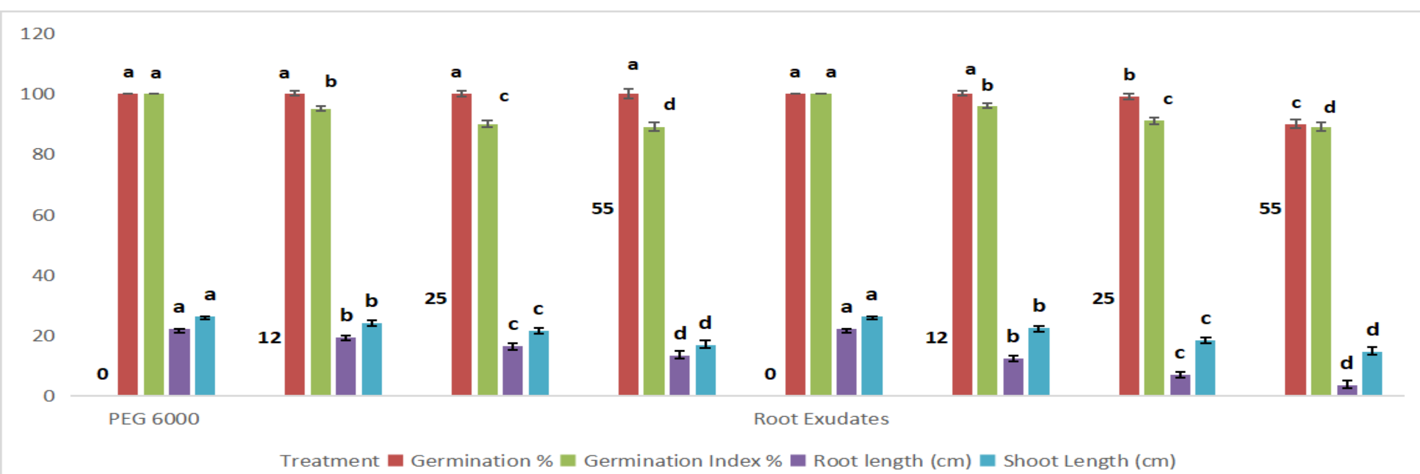

Figure 1. Estimates of Germination %, Germination index (%), root and shoot length (cm) in plants growing under influence of PEG and root leachates of *E. hirta* in *Cicer arietinum*
where a = 0.1, b = 0.8, c = 1, d = 1.5 (standard error)

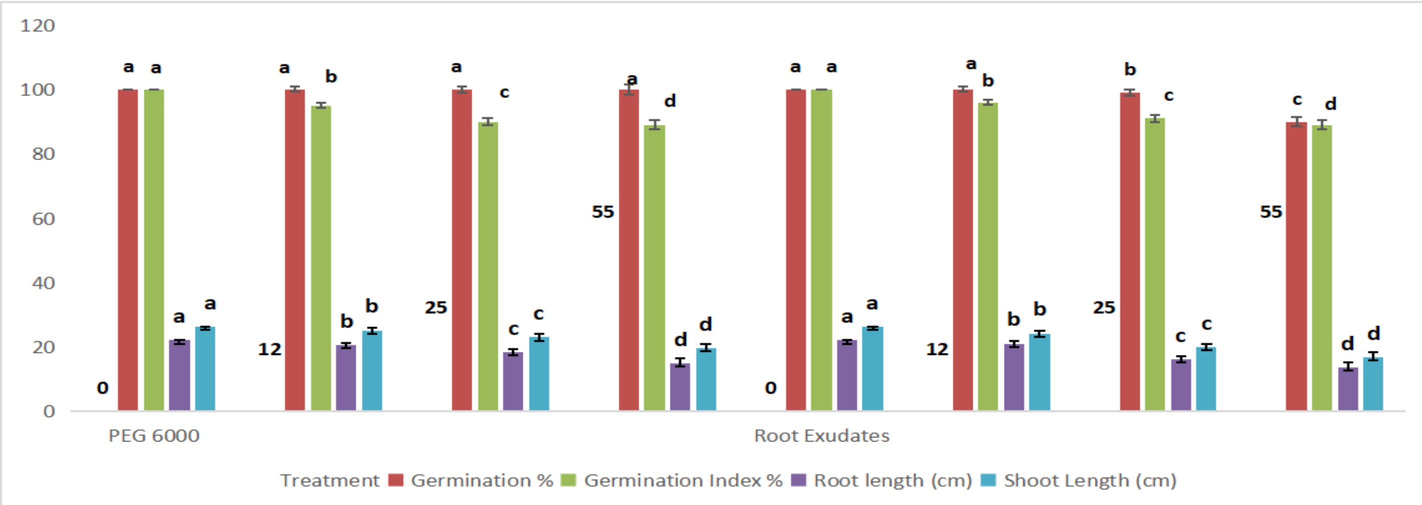

Figure 2. Estimates of Germination %, Germination index (%), root and shoot length (cm) in plants growing under influence of PEG and root leachates of *E. hirta* in *Phaseolus vulgaris.*
where a = 0.1, b = 0.8, c = 1, d = 1.5 (standard error)

However, PEG 6000 also contributed in lowering the seedling growth of both crops (Table 3, 4). Least growth of root and shoot was seen in chick pea (11.97 cm) when compared to control (17.33 cm) at 55 kpa showing significant ($p < 0.05$) difference among the samples whereas, at 12 and 24 kpa, a considerable inhibition was noticed. Our results agreed with the views of Khalid & Cai (2011), stating that Mannitol could influence the growth, mineral contents and photosynthetic pigments of lemon balm at different levels of concentrations. Moreover, Garg et al., (2019) determined that Mannitol possessed the ability to induce reduction in germination, root and shoot growth and dry matter of chilli at all concentrations.

3.3 Effect on pH

Since the pH of leachate samples was in range of 7.1-7.9 (greater than that of water i.e. pH = 7), caused inhibition in seed germination and seedling growth at relatively greater extent than those of PEG treated samples (Table 2). However, the pH range of PEG solution was somehow equivalent to the required pH. Our results supported the findings of Gentili et al., (2018) who explained that pH higher than 7 could produce drastic effects on plant height as well as inhibited flower formation.

3.4 Comparative analysis of leachates and PEG 6000 effects

Our results revealed that PEG 6000 inhibit seedling growth in both test crops, however, the causatums of root leachate of *E. hirta* was greater than that of PEG 6000. Analysis of variance (ANOVA) in a way 2-way mode contributed significant difference ($p < 0.05$) between the treatments (PEG and root exudates) in both the crops while variance among concentrations of each treatment showed weakly significant differences ($p < 0.1$) from L.S.D 0.05 (Figure 3). Additionally, PEG 6000 was failed to reduce germination factor while root leachate significantly reduced the % germination. Our results in agreement with the findings of Salam et al., (2018), stated that Mannitol showed inhibition at high concentration while *Chenopodium album* inhibited the growth of wheat and chick pea at all levels and this inhibition was entirely due to allelopathy.

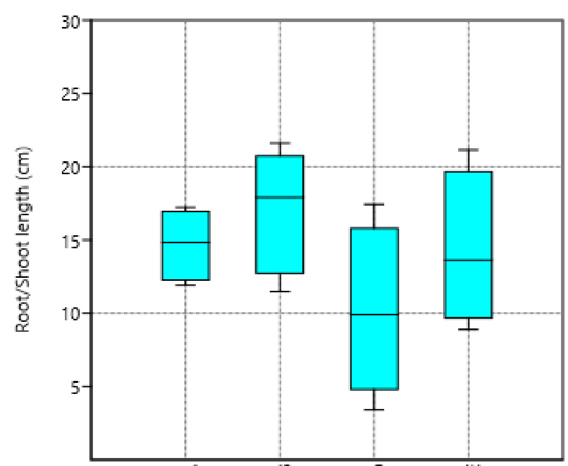

Figure 3. Difference in the root/shoot elongation between PEG 6000 and root leachates of *E. hirta* applications on treatments.

Where, A and B = Root and shoot length of PEG 6000 induced plants. D and E = Root and shoot length of *E. hirta* root leachates induced plants respectively

4. CONCLUSION

Current study showed that the inhibition in germination and seedling growth of chick pea and French beans was entirely due to the presence of allelochemicals in the root leachates of *E. hirta.* Although, PEG 6000 governs the capability of growth inhibition in different plants but in the current study there is no such evidence recorded. Nevertheless, it will remain a matter of concern to check the root cause of inhibition in plant growth rather than claiming allelopathy without including osmotic pressure trials.

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