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USE OF OSMOTIC PRESSURE IN ALLELOPATHIC TRIALS

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Abstract

This paper explains the relative suppression of crop growth as a consequence of either allelopathic expression or osmotic stress, finding the main factor of inhibition. For this study, five crops are tested namely *Zea mays* L. (Maize), *Hordeum vulgare* L. (Barley), *Cicer arietinum* L. (Chickpea), *Pisum sativum* L. (Pea) and *Phaseolus vulgaris* L. (French beans), using three weeds for allelopathic test *i.e., Portulaca oleracea* L. *Euphorbia hirta* L. and *Amaranthus viridis* L. PEG (Polyethylene glycerol) 6000 is used for seed priming against weeds to maintain osmotic balance between two types of trials. The rate of inhibition appeared too close at these predefined early stages though, but the weed stress has poorly (p<0.1) dominated over osmoticum. Seed germination has no significant difference among samples while seedling vigor index (SVI%) has the priority in the seeds treated with PEG corresponded with *P.oleracea* at 31%. Similarly, chlorophyll content at seedling stage shows highest synthesis in the same samples *i.e.,* 42 µg/mg/ml. Contrarily, the lowest SVI% was estimated as 38% from *A. viridis* samples and the lowest synthesized chlorophyll is recorded *i.e.,* 38 µg/mg/ml from the same samples. Among tested weeds, *A. viridis* has produced strong inhibition while *P. oleracea* appeared as less inhibitory or relatively influential than PEG extracts. This shows negligible or no inhibitory effects of *P. oleracea* and *E. hirta* while *A. viridis* has greater influence on crops that can be severe than osmotic stress at higher concentrations. It can be assumed that weeds that have not inhibited crop growth might have productive effects on crops in future rather than *A. viridis* might be inhibitory for the crops at later stages only at higher concentration.

Key words: Weed, Crop, Allelopathy, Osmoticum, Inhibition.

Introduction

Different plants follow different mechanisms or adaptations to compete with other plants to survive. Each plant extract has certain osmotic pressure. Plants with higher osmotic potential depress the growth of plants with lower osmotic potential. Some plants depress the growth of other plants due to faster growth or ability to grow under less favourable conditions while certain plants release toxic chemicals to suppress or enhance growth of other plants (Khan *et al.*, 2018a). This capability of plants that takes command over the processes of other plants is called allelopathy. It is important to check the impression of one plant on other, whether it is due to osmotic stress or allelopathy. Therefore in our trials we experimented to differentiate the effects of osmotic pressure, competition and allelopathy in our tested crops.

Any biological system can be greatly altered by interference. Allelopathic influence in agro-ecosystem can deteriorate or it may be beneficial for the donor plants for their growth and seed germination. In both cases, competition is the main factor that fundamentally occurs in the field which usually happens due to excessive growth of weeds side by side with the crops.

Allelopathy is regarded as a general defensive mechanism in almost all plants (Rice, 1984). This is caused by biochemicals produced in plants called allelochemicals that are naturally synthesized in plant body as a secondary bio-product (Dayan & Duke, 2014). Allelopathy is a predominant characteristic of weeds that creates a weed-crop competition in an agricultural field (Peng, 2019). Emergence of weeds among crops lowers crop growth and promotes seed dormancy due to extensive resource competition as the weeds are known to be hyperactive in extracting moisture and nutrients (Findura *et al.*, 2020). Moreover, the presence of weed is crucial for crops due to their toxins producing activity in the soil that inhibits growth and germination of other plants (Patterson, 1993). Seed germination and seedling survival are the primary and most sensitive phases in plant's life cycle (Ruan *et al.*, 2002) whereas weeds complete their life cycle very early (Noshad & Khan, 2019; Khan *et al.*, 2018) and possess an efficient competitive capability (Dayan & Duke, 2014).

Water deficit among crops act as the primary limiting factor that can cause stress severity for seed germination and viability when weed population increases in the field (Johnston *et al.*, 2002; Krupinsky *et al.*, 2006). Nutrient availability is also a hard task for crops in presence of weeds (Hans & Johnson, 2002). In such situation, weeds absorb greater amount of nitrogen, phosphorus, potassium and other ions in addition to moisture, causing nutrient deficit (Kazemeini *et al.*, 2013). Water and nutrients cumulatively develop osmotic pressure in plant cells, this additional stress can be coped by making osmotic adjustments (Zhang *et al.*, 2015). Plant's self-secreted compounds such as proline, glutamate, fructans, sucrose are known to be important in self-maintaining osmotic equilbrium (Ramanjulu & Bartels, 2002). However, external application of manitol and osmopriming of seeds with polyethylene glycerol (PEG) has also been used for osmotic adjustments to release osmotic stress (Zhang *et al.*, 2010; Chen & Arora, 2011; Yuan-Yuan *et al.*, 2010). Hur (1991) observed improvement in seed germination and seedling establishment in Italian Rye grass and Sorghum by osmopriming of seeds with PEG under drought stress. PEG compounds are water soluble neutral polymers known to decrease water potential in plants (Lawlor, 1970).

In this study, a preliminary assessment has been designed to differentiate the effect of weeds on seed germination and seedling establishment. Main objective of this study is to determine whether weed inhibition is due to their osmotic pressure or allelochemicals on early stage of crop growth.

Materials and Methods

To achieve our goal, two sets of experiments were performed. One set of experiment carried allelopathic interaction while the other set was subjected to PEG priming of seeds. Five different crops were selected on the basis of their nutritional and economic importance *i.e*, Maize (*Zea mays*), Barley (*Hordeum vulgaris*), Chickpea (*Cicer arietinum*), Pea (*Pisum sativum*) and French beans (*Phaseolus vulgaris*). Three weeds that were selected for allelopathic investigations that are frequently found in the locality and are known to possess strong allelopathic characteristics, *i.e., Portulaca oleracea, Euphorbia hirta* and *Amaranthus viridis.*

Preparation of extracts: For allelopathic investigations, 100 gm of each weed plant was uprooted from the local gardens of Department of Botany Federal Urdu University. The plants were oven dried at 70^{o}C, grounded and then the powder was dissolved in 200 ml distilled water to make extract of the weed for seed priming (Sas–Piotrowska & Piotrowski, 2012). The obtained extract was further diluted to 5%, 10% and 15% concentrations.

PEG 6000 solution was used by preparing concentrations in accordance to the osmotic pressure of weed extract concentration to standardize the osmoticum of all treatments (Lawlor, 1970). The balance of osmotic pressure in relevant concentration of weed extract and PEG elaborated in (Table 1).

Table 1. Osmotic pressures standardization chart at respective concentration of PEG (g/l) and weed extract (ml).

O. Pressure (kpa)	PEG 6000 g/l	Root extract ml
	Portulaca oleracea	
12	2%	5%
25	4%	10%
55	8%	15%
	Euphorbia hirta	
12	8%	5%
25	16%	10%
55	40%	15%
	Amaranthus viridis	
12	12%	5%
25	20%	10%
55	44%	15%

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

Seed germination: Petriplate experiment was established by placing 5 seeds per petriplate (sterilized). Each treatment had five replicates. The experiment was carried out in the laboratory of Department of Botany Federal Urdu University. Seed germination and vigor was observed on daily basis and evaluated by using following (Dias, 2001; Bradbeer, 1998; Chiapusio *et al*., 1997):

$$\text{Germination \%} = S_g/S_t \times 100$$

where, S_g = No. of seeds germinated, S_t = Total no. of seeds

Germination index: S=[N1/1 + N2/2 +N3/3 +- - - - - - - -Nn/n] x 100

where, N = No. of seeds germinated per day

Seedling establishment: After the emergence of radicle and plumule, their lengths, fresh and dry weights were recorded. SVI = (Root length + Shoot length) x Percent germination (Sun *et al*, 2012).

where SVI = Seedling viability index

WRC (%)= (FW–DW)/(TW–DW)×100 (Bars & Weatherley, 1962),

where WRC = Water retention capability, FW, DW, and TW are fresh weight, dry weight, and turgid weight, respectively.

Seedling bioassay: Chlorophyll determination in the seedlings was carried out by following Arnon (1949), following formula was used for estimation:

$$\text{Chl a (mg g}^{-1}) = \frac{12.7D663 - 2.69D645}{W} \times V$$

$$\text{Chl b (mg g}^{-1}) = \frac{22.9D645 - 4.68D663}{W} \times V$$

$$C_{X+C} = \frac{7.6D480 - 2.63D510}{W} \times V \text{ (x = xanthophylls and carotenes)}$$

Results

Inhibition increased with the increase in osmotic pressure/extract concentration. The osmotic balance was applied in the order of 12, 25 and 55 kpa (Table 1). As the concentration level of weed extract differ with that of PEG therefore a standardized level of osmoticum was maintained to figure out the weed inhibition rate. The mode of reaction by crops was similar in all five tested crops *i.e.,* germination index and vigor index both dropped at higher concentration of weeds while PEG application produced inhibition at lower rate than the weed extract samples in the same manner (Table 2). Germination index (GI) was highest in Chickpea (100% in control, 98% in 5%, 97% in 10% and 96% in 15% treatments) while lowest in French beans (99% in control, 92% in 5%, 90% in 10% and 88% in 15% samples) on treatment with *P. oleracea* weed extract (Table 2a). The adjusted osmotic pressure by PEG treated samples to balance *P.oleracea* expression produced less inhibition than weed extract samples. The highest inhibition in germination index (GI) occurred in French beans in the order of 99%, 95%, 94% and 91% in control, 4%, 8% and 24% samples (Table 2a). Seed vigor index (SVI) was highest in French beans and lowest in Maize seeds which successively decreased with an increase in concentration and osmotic pressure.

Table 2a. Mean Seed germination %, germination index (GI) and seed vigor index (SVI) of Maize, Barley, Chickpea, Pea and French beans irrigated by *Portulaca oleracea* and PEG solution.

Extract conc	Germination %	GI %	SVI %	PEG conc	Germination %	GI %	SVI %
Maize							
C	100	100	23	C	100	100	23
5%	100	98	21	4%	100	99	22
10%	100	95	19	8%	100	99	20
15%	100	90	18	24%	100	98	19
Barley							
C	100	100	28	C	100	100	28
5%	100	98	25	8%	100	99	25
10%	100	94	24	16%	100	95	26
15%	100	92	23	40%	100	93	24
Pea							
C	100	100	33	C	100	100	33
5%	100	98	30	12%	100	99	32
10%	100	95	28	20%	100	96	29
15%	100	94	25	44%	100	95	28
Chickpea							
C	100	100	37	C	100	100	37
5%	100	98	34	12%	100	99	35
10%	100	97	32	20%	100	98	34
15%	100	96	29	44%	100	98	32
French beans							
C	100	99	47	C	100	99	47
5%	100	92	46	12%	100	95	46
10%	100	90	40	20%	100	94	43
15%	100	88	36	44%	100	91	38

Conc = Concentration; PEG = Polyethylene glycerol

Table 2b. Mean Seed germination %, germination index (GI) and seed vigor index (SVI) of Maize, Barley, Chickpea, Pea and French beans irrigated by *Euphorbia hirta* and PEG solution.

Extract conc	Germination%	GI %	SVI %	PEG conc	Germination%	GI %	SVI %
Maize							
C	100	100	23	C	100	100	23
5%	100	96	19	4%	100	98	21
10%	100	92	17	8%	100	94	17
15%	100	88	13	24%	100	90	14
Barley							
C	100	100	28	C	100	100	28
5%	100	97	24	8%	100	97	25
10%	100	93	20	16%	100	95	24
15%	100	90	18	40%	100	92	19
Pea							
C	100	100	33	C	100	100	33
5%	100	97	28	12%	100	98	31
10%	100	95	27	20%	100	96	27
15%	100	93	22	44%	100	94	24
Chickpea							
C	100	100	37	C	100	100	37
5%	100	97	33	12%	100	98	35
10%	100	95	29	20%	100	97	32
15%	100	94	25	44%	100	97	27
French beans							
C	100	99	47	C	100	99	47
5%	100	94	44	12%	100	95	46
10%	100	89	39	20%	100	90	41
15%	100	86	32	44%	100	89	30

E. hirta treated seeds were more sensitive to the weed effect than *P.oleracea* extract. Maize and French beans produced lowest germination index while Barley and French beans were the crops with lowest germination index treated with PEG. SVI of French beans was highest (47%, 44%, 39% and 32% in control, 5%, 10% and 15% concentration) in *E. hirta* treated seeds while in PEG treated seeds it was again highest in French beans (Table 2b).

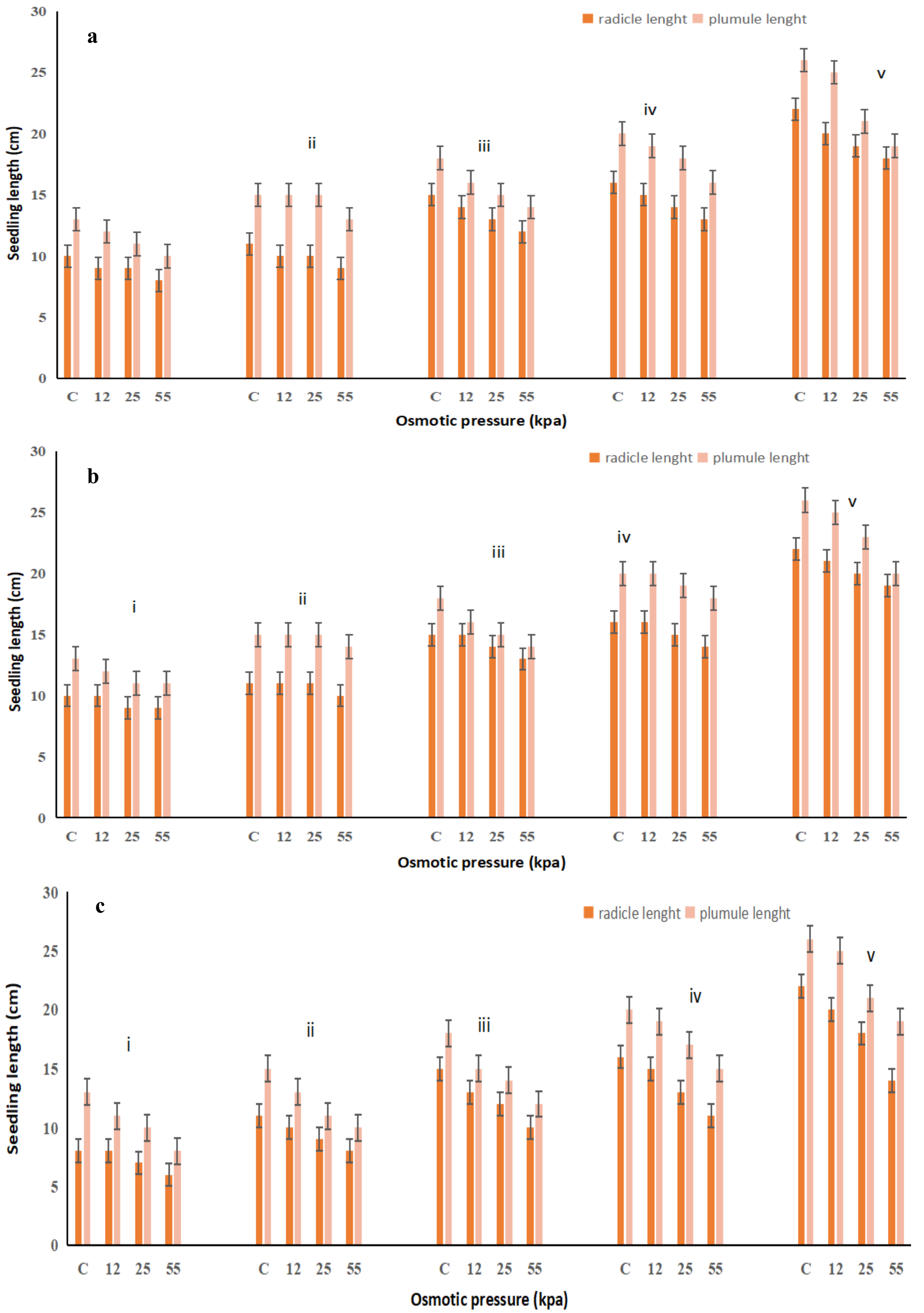
Higher concentrations of *A. viridis* produced relatively greater level of inhibition among the three weeds (Table 2c). Maize and French beans were the crops with lowest germination index while Chickpea and French beans attained highest SVI which gradually lowered with the increase in concentration/ osmoticum (Table 2c). Similar results produced in the crop seeds when treated with PEG solution standardized by the osmotic pressure of weed extract concentration.

Inhibitory effects of weed extract in contrast to osmotic pressure were slightly greater as observed in *A. viridis* treated seeds. However, PEG solution has triggered the germination index and SVI in crop seeds as shown in Table 2a, b and c, where inhibitory results were dominant in weed extract treatments.

Emergence of radical and plumule took place within 3 days of seed germination. The establishment of seedlings proceeded in all treatments at a slower rate than control plants. The highest radical and plumule elongation was observed in French beans (20 ± 2 and 25 ± 2 cm in radical and plumule respectively) in *P. oleracea* (Fig. 1a) and PEG treated seedlings *i.e.,* 21 ± 2 cm and 25 ± 2 cm in radical and plumule respectively (Fig. 1b). Similar results were observed in *E. hirta* samples with highest seedling elongation in French beans and strongest inhibition in seedling growth in Maize seedlings in both weed extract 6 ± 1 cm and 8 ± 1 cm in radical and plumule respectively) and PEG solutions *i.e.,*7 ± 1 cm and 9 ± 2 cm in radical and plumule respectively (Fig. 1d). *A. viridis* treated seedlings showed highest level of hindrance in their seedling establishment phase with the lowest seedling elongation in Maize samples *i.e.,* 5 ± 1 cm and 7 ± 1 cm (in radical and plumule respectively) in 15% weed extract samples. While highest resistance against *A. viridis* extract was exerted by French beans seedlings at 5% treatment by exhibiting 18 ± 2 cm and 23 ± 2 cm in radical and plumule length (Fig. 1e). PEG treated seedlings also showed highest elongation in French beans (19 ± 2 cm and 24 ± 2 cm in radical and plumule length respectively) at 12 kpa and lowest growth in Maize seedlings (7 ± 1 and 7 ± 1 cm in radical and plumule length respectively) at 55 kpa osmotic pressure (Fig. 1f). Hence, the seedling elongation was found to be predominantly hindered by weed extract (Fig. 1).

Table 2c. Mean seed germination %, germination index (GI) and seed vigor index (SVI) of Maize, Barley, Chickpea, Pea and French beans irrigated by *Amaranthus viridis* and PEG solution.

Extract conc	Germination %	GI %	SVI %	PEG conc	Germination %	GI %	SVI %
Maize							
C	100	100	23	C	100	100	23
5%	100	93	18	4%	100	94	19
10%	100	89	15	8%	100	92	16
15%	100	84	12	24%	100	88	14
Barley							
C	100	100	28	C	100	100	28
5%	100	95	22	8%	100	96	24
10%	100	91	17	16%	100	92	20
15%	100	90	15	40%	100	90	17
Pea							
C	100	100	33	C	100	100	33
5%	100	97	27	12%	100	97	31
10%	100	93	24	20%	100	95	25
15%	100	91	20	44%	100	94	21
Chickpea							
C	100	100	37	C	100	100	37
5%	100	97	29	12%	100	97	34
10%	100	93	27	20%	100	96	31
15%	100	92	23	44%	100	94	28
French beans							
C	100	99	47	C	100	99	47
5%	100	88	41	12%	100	90	44
10%	100	84	35	20%	100	87	39
15%	100	81	28	44%	100	85	32


a
radicle lenght
plumule lenght
Seedling length (cm)
30
25
20
15
10
5
0
ii
iii
iv
v
C 12 25 55
C 12 25 55
C 12 25 55
C 12 25 55
C 12 25 55
Osmotic pressure (kpa)
b
radicle lenght
plumule lenght
Seedling length (cm)
30
25
20
15
10
5
0
i
ii
iii
iv
v
C 12 25 55
C 12 25 55
C 12 25 55
C 12 25 55
C 12 25 55
Osmotic pressure (kpa)
c
radicle lenght
plumule lenght
Seedling length (cm)
30
25
20
15
10
5
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C 12 25 55
C 12 25 55
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C 12 25 55
C 12 25 55
Osmotic pressure (kpa)

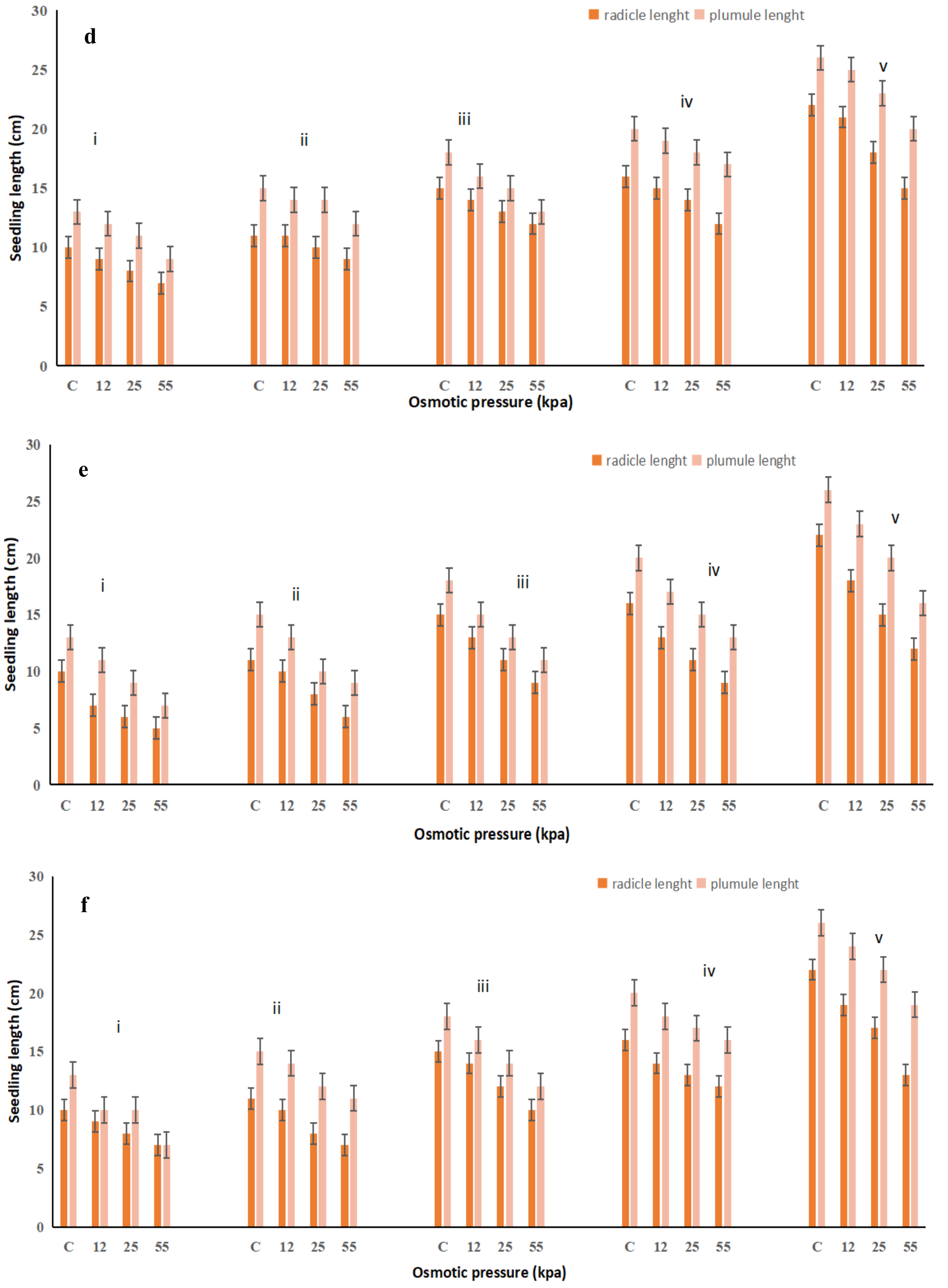
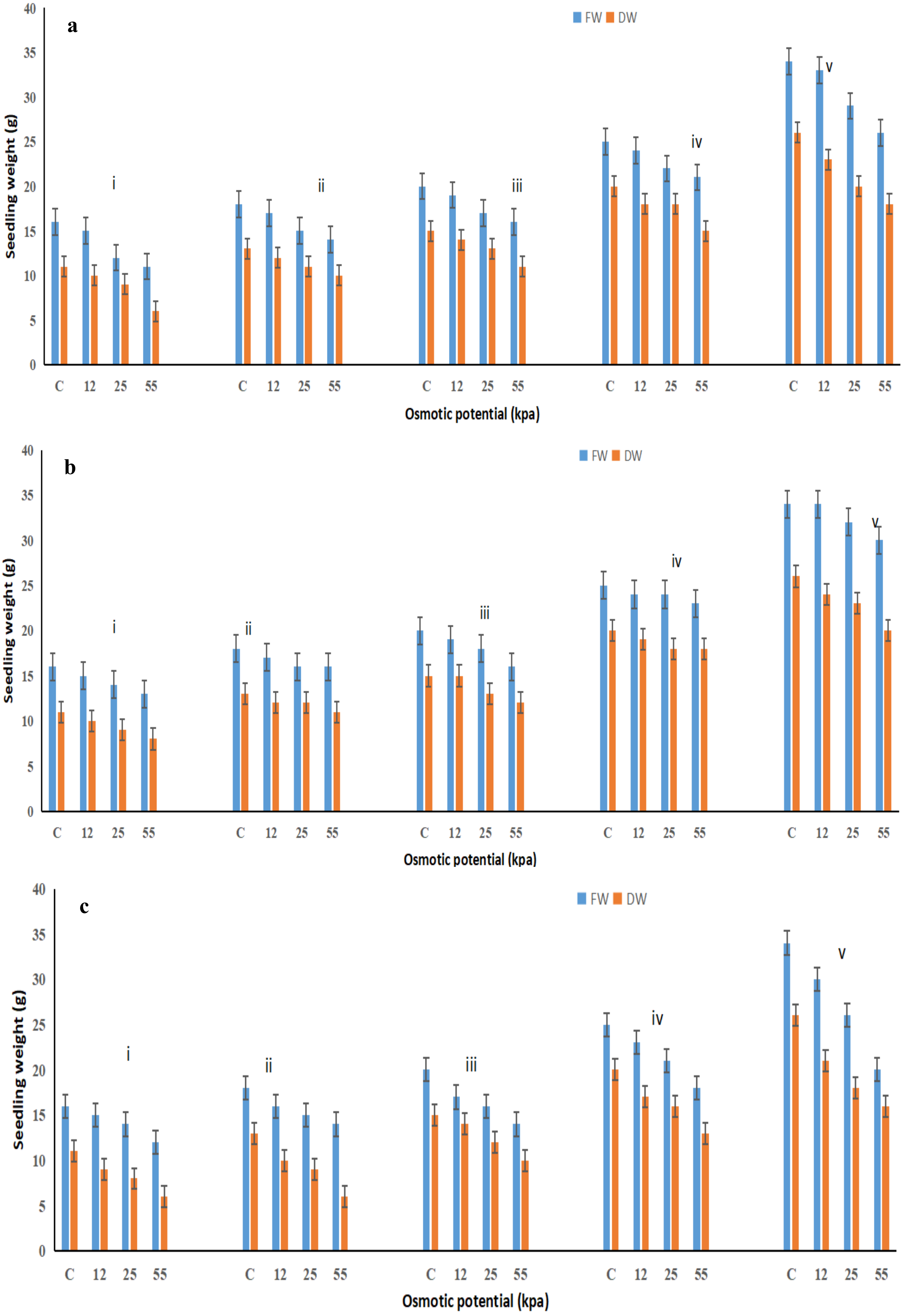


Fig. 1. Mean redical and plumule length of Maize (i), Barley (ii), Pea (iii), Chickpea (iv) and French beans (v) at different concentration of weed extract (a and b = *P. oleracea*, c and d = *E. hirta* and e and f = *A. viridis*) and PEG treated seedlings.


a
FW DW
Seedling weight (g)
0 5 10 15 20 25 30 35 40
i
ii
iii
iv
v
C 12 25 55 C 12 25 55 C 12 25 55 C 12 25 55 C 12 25 55
Osmotic potential (kpa)
b
FW DW
Seedling weight (g)
0 5 10 15 20 25 30 35 40
i
ii
iii
iv
v
C 12 25 55 C 12 25 55 C 12 25 55 C 12 25 55 C 12 25 55
Osmotic potential (kpa)
c
FW DW
Seedling weight (g)
0 5 10 15 20 25 30 35 40
i
ii
iii
iv
v
C 12 25 55 C 12 25 55 C 12 25 55 C 12 25 55 C 12 25 55
Osmotic potential (kpa)

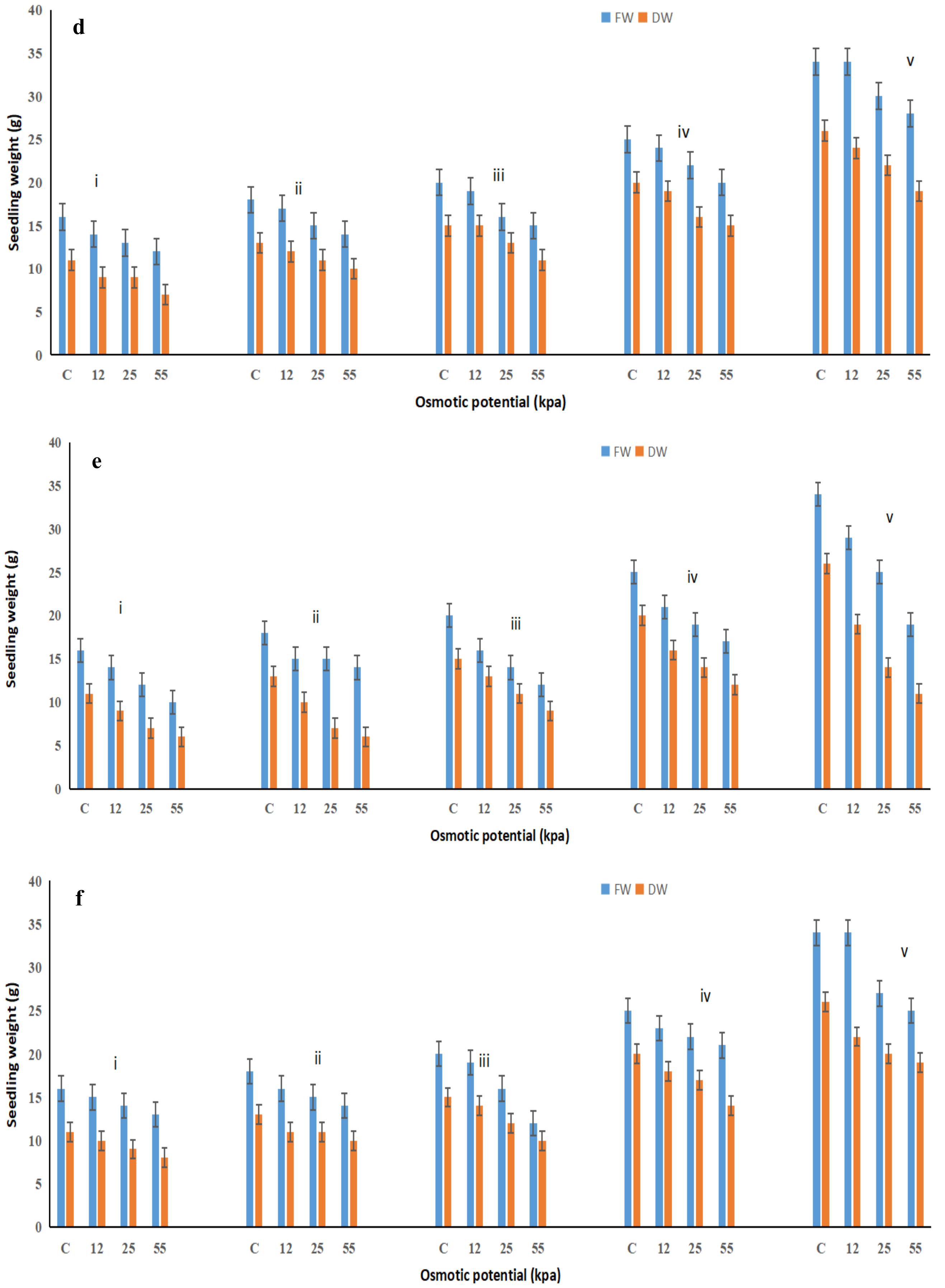


Fig. 2. Mean fresh and dry weight of Maize (i), Barley (ii), Pea (iii), Chickpea (iv) and French beans (v) at different concentration of weed extract (a and b = *P. oleracea*, c and d = *E. hirta* and e and f = *A. viridis*) and PEG treated seedlings. Fig. 2 a, c and e reveal the induction of weed extract while Fig. 2b, d and f demonstrate the induction of PEG solution to the crop under predefined conditions.

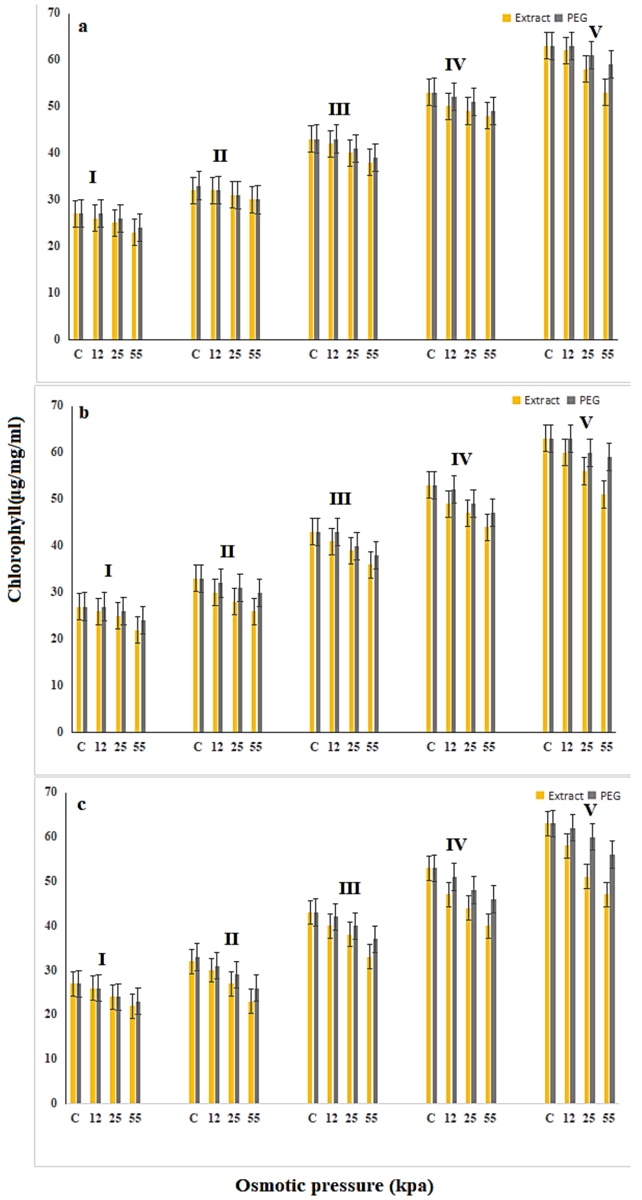


Fig. 3. Mean chlorophyll content of Maize (I), Barley (II), Pea (III), Chickpea (IV) and French beans (V) at different concentration of weed extract (a and b = *P. oleracea*, c and d = *E. hirta* and e and f = *A. viridis*) and PEG treated seedlings.

Fresh and dry weights of the seedlings of five crops were tested, showed higher inhibition in weed extracts. However, the results were less significant in comparison with PEG treatments, the seedlings mass was even lesser than control samples (Fig. 2). The highest fresh and dry weight was observed in French beans (33 ± 3.4 mg and 23 ± 3.1 mg in *P. oleracea*, 30 ± 2.9 mg and 21 ± 2.9 mg in *E. hirta* and 29 ± 2.2 mg and 19 ± 1.5 mg in *A. viridis*) from 5% weed extract treated samples. While the greater extent of inhibition was recorded in Maize seedlings (11 ± 3.4 mg and 6 ± 0.2 mg in *P. oleracea*, 12 ± 0.5 mg and 6 ± 0.2 mg in *E. hirta* and 10 ± 0.2 mg and 6 ± 0.1 mg in *A. viridis*) from 15% samples. PEG samples received the same mode of response from the crops *i.e.,* highest inhibition persisted in Maize seedlings in 55 kpa samples, while highest weight gained by French beans in the treatments where 12kpa osmotic pressure was maintained (Figs. 2b, d and f).

Chlorophyll synthesis in the seedlings of all crops was found to be in an inverse relationship with increase in concentration and osmotic potential of the treatments. Hence, greater the concentration higher will be the inhibition (Fig. 3) the phenomenon was accurately fitted in both the weed extract and PEG treatments. The lowest chlorophyll content was recorded from Maize seedlings containing 22 ± 1.9 µg/mg/ml and 22 ± 1.5 µg/mg/ml from *E. hirta* and *A. viridis* samples at 15% concentration (Figs. 3a and b) while PEG treatments also attained lowest chlorophyll content from Maize seedlings at 55 kpa concentrations *i.e.,* 23 ± 0.9 µg/mg/ml (Fig. 3c).

An overall investigative approach was developed to find the variance in the response of test crops. Two-way ANOVA produced non-significant results among within group tests while between groups there was a weakly significant (p<0.1) difference, found in both *i.e.,* seedling vigor index and chlorophyll content (Fig. 4). Highest seedling vigor was recorded from *P. oleracea* treated plants and PEG adjusted samples (30% and 31% respectively), while the lowest recorded from *A. viridis* samples (26%) as listed in Table. 3. Highest variance was evaluated in *A. viridis* and corresponding PEG *i.e.,* 84% and 85% respectively (Table. 3). This showed a greater probability of causing inhibition and fluctuations during the course of seed germination and seedling establishment regulated by the interference of *A. viridis* extract.

Chlorophyll content is an integral unit in plant physiology and its production varies from species to species hence there would be a certain difference among species chlorophyll content. However, a comparative rate of inhibition in chlorophyll content was tested. Among the crops, highest inhibition was recorded in Maize plants on treatment with *A. viridis* extract (Fig. 3). This was a lower chlorophyll synthetic rate of the crop in general as the control samples also showed lowest chlorophyll content. Lowest mean value (38%) was observed from *A. viridis* treated plants while the rest of the plants did not show any significantly contrasting values against PEG treated samples (Table 3, Fig. 4). Variance was lowest in *A. viridis* treated crops (1.5%) while the corresponding PEG contrarily gave highest variance in the seedlings of all tested crops *i.e.,* 1.8% (Table 3).

Table 3. Mean SVI and chlorophyll content of all seedlings in comparison to treated weeds.

Treatment	Mean%	STDev	Variance
SVI%			
E1	30	8.24	68
P1	31	8.1	66
E2	28	9.06	82
P2	29	9	80
E3	26	9.15	84
P3	28	9.24	85
Chlorophyll content (µg/mg/ml)			
E1	41	0.12	1.6
P1	42	0.13	1.7
E2	40	0.12	1.6
P2	42	0.13	1.73
E3	38	0.12	1.5
P3	41	0.13	1.8

Where, E1, E2 and E3 indicate *P. oleracea*, *E. hirta*, *A. viridis* respectively. P1, P2, P3 indicate PEG solutions in correspondence with *P. oleracea*, *E. hirta*, *A. viridis* respectively

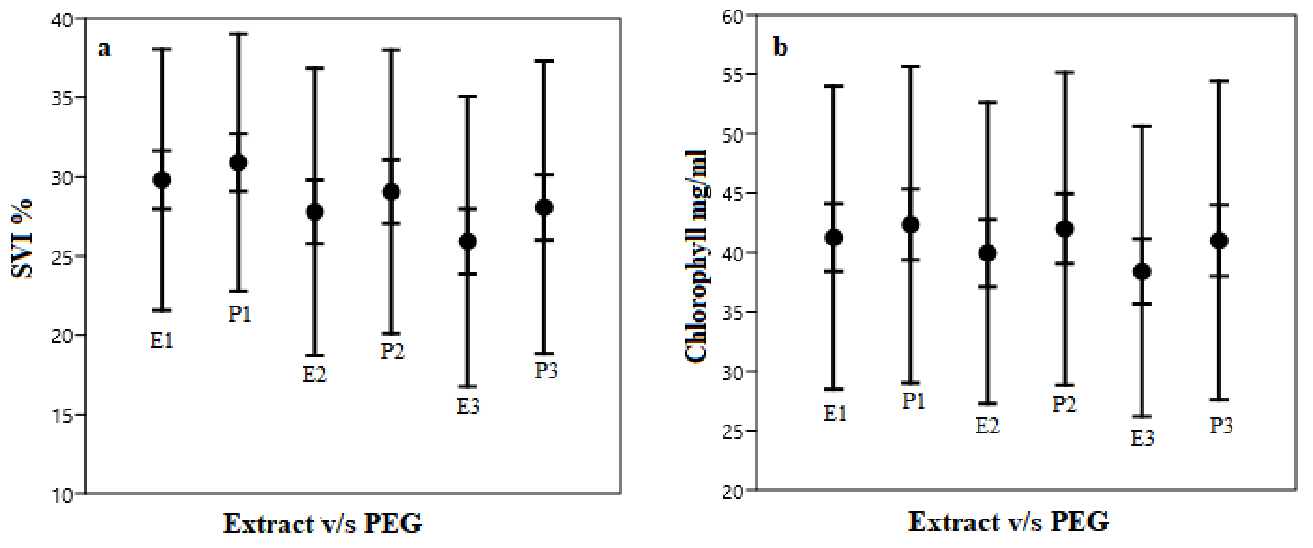


Fig. 4. A comparative whisker of seedling vigor index and chlorophyll content of all crop seedlings treated with weeds. Where, E1, E2 and E3 indicate *P. oleracea, E. hirta, A. viridis* respectively. P1, P2, P3 indicate PEG solutions in correspondence with *P. oleracea, E. hirta, A. viridis* respectively.

Discussion

Generally, allelopathy is known for causing inhibition to the plants, the actual phenomenon claims the interference of other plants that may cause either inhibition or growth promotion or sometimes no effect may be seen. There may be some other reasons of growth retardation like competition, osmotic gradient etc (Khan, 2020). Present study was elucidated to verify the actual responsible factor of inhibition involved in seed germination, growth and development, either alleopathy or osmotic disturbance. It was revealed that both factors produced poorly significant suppression in germination at their higher level however, inhibitory constraint was slightly effective in the allelopathic extracts of *Amaranthus viridis,* while *Euphorbia hirta* and *Potulaca oleracea* did not show any pronounced effects on crop growth in comparison to PEG 6000 dilutions. These observations are contradictory to the findings of Friedman, (1997); Baskin & Baskin, (2001) that secondary metabolites present in any allelopathic plant may alter the germination of seeds by affecting the activity of enzymes and sequencing of chromosomes. Furthermore, Radhoune, (2007) confirmed that PEG has no effect on germination at initial level but at higher and continuous exposure of this solution could cause retardation in genesis phenomenon of the seeds. However, it was also noticed that all the weeds exhibited no effects on total germination on test species but among them *A. viridis* was found to produce hazardous effects hence current findings are in agreement of Prinsloo & Christian, (2018) that water soluble extracts of *A. viridis* possessed inhibitory properties and could significantly reduced the germination indexes of certain vegetables as well as weeds.

Seedling establishment is one of the most crucial stage for plant growth and development (Khan *et al.,* 2020). Under allelopathic phenomenon, allelochemicals in plant involve many biochemical reactions that have resulted in various modifications (Qadir *et al.,* 2021 & 2020; Asad *et al.,* 2021 & 2020). Some of these modifications could completely affect the overall plant growth mechanism (Yu *et al*., 2003; Stein & Braga, 2010). From the results, it was obvious that seedling growth of all test crops were gradually moved towards decline as the concentration of *A. viridis* weed extract was increased, on the other hand, rest of the weeds could be productive at later stages as their activity trend did not show any inhibitory approach on crop growth. Shehata, (2014) stated that *Portulaca oleracaea* contained different phenolic compounds that play a major role in suppressing seedling growth of different crops at various concentration of extracts. However, PEG 6000 also contributed in lowering the seedling growth of all crops at all concentrations hence it cannot be concluded that the inhibition is caused by allelopathy, it could be due to osmotic imbalance in the crops. Our results correlate with the views of (Khalid & Cai, 2011) that mannitol could influence the growth, mineral contents and photosynthetic pigments of lemon balm at different levels of concentrations. Comparative studies of both factors showed that greatest suppression in seedling emergence was occupied by weeds extracts whereas, current findings claimed a on significant difference in weed extracts and PEG stimulation against inhibition except that of higher concentrations of *A. viridis* hence the other weeds cannot be considered as inhibitory for younger stages of predefined crops. However, Dafaallah *et al*., (2019) explained that *Amaranthus viridis* played a significant role in enhancing the inhibition criteria in seedling growth of various leguminious plants at all concentration.

Chlorophyll contents play a vital function in the physiology of any plant as they provide energy rich compounds in the form of ATP during photosynthesis, however, the current findings prove that all the three weeds extracts at different concentrations did not support in creating dreadful effects on total chlorophyll of all the test crops. Alamdari *et al*., (2018) confirmed that extracts of *Euphorbia hirta* possessed allelochemicals that could attack on chlorophyll contents of wheat cultivars and thus reduced the germination and seedlings elongation as well. Although, PEG 6000 also showed inhibition in total chlorophyll content of all test crops but suppression level of weeds were highest as that of PEG 6000. Our results support the findings of Salam *et al.,* (2018) that Mannitol showed inhibition at high concentration but the *Chenopodium album* inhibited the growth of wheat and chick pea at all levels and this inhibition was entirely due to allelopathy.

Within all the weeds *Amaranthus viridis* was found more potent in inducing inhibition in all genesis %, emergence growth and chlorophyll content of all the test crops in comparison to other two weeds. Sarkar & Chakarborty, (2016) found that *Amaranthus spinosus* consists of allelochemicals in large quantity and had the ability to reduced the germination factors, growth pattern, biomass, relative water content and total chlorophyll matter of rice and mustard. Therefore, it has been seen that although the osmotic potential is an strong factor in lowering germination and growth due to competition but the weed interaction can cause inhibition at a greater extent than osmotic potential. Moreover, osmotic potential is a natural phenomena that exists in all the cells and prevails due to competition while allelopathic influence is an external stress that may be toxic or inhibitory at a massive extent sometimes or may be stimulatory for growth of other plants.

Conclusion

It is concluded that allelopathic effects could not be determined without exploring the effects of osmotic pressure, both may inhibit or enhance growth of other plants. In our crops, seed germination show no significant inhibition while seedling development and chlorophyll content are inhibited by only one weed (*Amaranthus viridis*).

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