

Allelopathic Effect of Two *Cynanchum acutum* L. Populations on Emergence and Shoot Development of Barley

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ABSTRACT

Experimental study is carried out in order to assess the potential allelopathic effect of leaf, stalk and root of two *Cynanchum acutum* L. populations (Karaj and Kerman) on germination percentage, radicle and shoot length of barley (*Hordeum vulgare* L.). Polyethylene glycol solvable (PEG) is utilized to distinguish the osmotic and allelopathic effects of distillate extracted from *C. acutum*. Result showed the watery distillate of before mentioned organs in two populations had allelopathic effects on germination trait and crops development; so that by increasing the concentration rate of distillate, the germination percentage, radicle and shoot length of aforementioned crop decreased. Kerman's population showed rather osmotic potential compared to Karaj ones. *H. vulgare* germination traits respectively presented the highest sensitivity to distillate extracted from *C. acutum*. The watery distillate extracted from leaf and root had a drastic allelopathic effect compared to those that released from *C. acutum* stalks, which was dedicated the lowest allelopathic effect. Different PEG concentrations had no significant effect on germination trait. Thus, the whole inhibitor effects observed in distillates is pertained to their available allelo-chemical substance.

Nomenclature: *Cynanchum acutum* L., barley (*Hordeum vulgare* L.), Polyethylene glycol (PEG).

KEYWORDS: Allelopathic and osmotic effects, radicle length, shoot length, concentration, watery distillate.

INTRODUCTION

In arable ecosystems, plants growth and development is associated with their interaction. Plants directly affect their development through their competition to acquire the environmental nutrient elements [1] or indirectly they made this inhibition with their radicular leakage [2], obtained distillates of growing residues [3] and other soil incorporated residues [4]. Allelopathy is the biochemical reactions between two or more plants and microorganisms, which are run the natural chemical substance (allelo-chemical) release from particular specie opposed to plants physiological processes or adjacent creatures to affect those [5, 6]. The entity of allelopathic effects is verified in remained fragments and watery distillates of immense weed species, which can prevent germination and development of other species or intruded in crop growth and development processes and made yield reduction [7].

Geographical situation and regional environment situation of the plant habitat, where it growth appropriately, plays a major role in its allelopathic potential. Alternation in production of allelo-chemical compositions is considered as a functional entity of latitude and longitude of species habitat. The Plant, which grows under the higher environmental temperatures, its allelo-chemical production will increase [8, 9]. Moreover, the other associated factors of habitat like regional altitude, the soil microclimate, the intensity and duration of raining, acidity and the soil oxygen concentration can play a major role on plant allelopathic potential [8, 10, 11]. Production of plants allelo-chemical compositions attributed to their gene and environmental circumstances like temperature, light and moisture [12]. Studies showed that allelopathy is intensified in stressful and inappropriate situations. When allelopathic species, for instance, grow at the mercy of drought, salinity and drastic temperatures, as defensive mechanisms they increase production of subsidiary metabolite, since most of these metabolites categorize as venomous compositions, subsequently the plant allelopathic activities will increase regarding to the stress [12, 13, 14, 15].

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In recent years, the invasion of *Cynanchum* species and their waste conspicuously got to be ubiquitous through the farmlands, gardens (especially in perennial plants cultivation) and also in zero-tillage management system [16, 17, 18]. Numerous researches carried out, aimed to distinguish factors which contributed to higher invasive power and thriving characteristics of these species. It is revealed that some specific traits like productivity characteristic and particular growing behavior of these species known as their dominant capability; since they can generate immense number of seeds, which are armored via appropriate wing that easily make them able to commute kilometers and spread away by wind [16, 17, 19, 20]. The higher proportion of root to shoot (R/S) distinguished as a dominant factor for these species [21]. Moreover of the aforementioned elements, there are numerous researches, which attribute the production and extraction of allelo-chemicals compositions into rhizosphere as a high invasive power and thriving factor for these plants [18, 22, 23]. These allelopathic compositions can directly affect growth of adjacent plants or indirectly by altering the soil microorganisms' balance, change the growth situation in favor of *Cynanchum* spp. [21, 23, 24, 25]. A probable mechanism about alternation of microorganisms' population is indicated as well [26]. It is reported radicular spatters of *Cynanchum* Spp. have immense value of antophin compositions, which can decrease the soil fungous species [26].

Most of species from *Asclepiadaceae* family produce specific chemical compositions. Historically species of *Cynanchum* genre utilized because of their therapeutic traits. The root of these species excessively contains glycoside compositions [18]. Tri-terpenoid compositions extracted from aerial parts of plants, which have anti-tumor characteristics [27]. Other researchers extracted specific alkaloid compositions with anti-bacterial and anti-fungus characteristics from roots, fruits and leaves of these plants as well [26, 28]. In India and equatorial parts of the U.S continent, *Cynanchum arnattianum* shrubs are utilized as an appropriate pesticide and parasite repellent [29]. In china *C. atratum* utilize for its anti-fever and diuretic trait [30] and also *C. paniculatum* for its soothing effect [31]. In order to separation and identification the natural chemical compositions of *Cynanchum* spp. several investigations are carried out; and right now compositions like steroidal glycosides [32], different carbohydrates [33], alkaloids [34], phenolic [35] and tri-terpene [36] combinations are distinguished from this genus. Frequent studies, which aimed to identify and separate the produced chemical compositions of *Cynanchum acutum* L., lead to categorize these chemical materials, encompass α -amyrin, lupyl acetate, lupeol and β -sitosterol [37]; quercetin and quercetin 3-*O*- β -D-galactoside [38]; four flavonoid glycoside combinations include quercetin di-*O*-hexoside, quercetin 3-*O*-rhamnosyl glycoside, quercetin 3-*O*-galactoside and quercetin 3-*O*-xyloside [39] and ultimately two coumarin combinations like scopoletin and scoparone is distinguished [40].

Most of the accomplished phyto-chemical researches on *Cynanchum* genus revolve around their therapeutic and anti-bacterial effect, whereas few researches concerned about the allelopathic trait of their chemical compositions on adjacent plants. The initial directive research about the allelopathic effect of these plants was carried out [22]. The root watery distillate of *Cynanchum rossicum* (Klepow) Borhidi significantly reduced seeds germination of *Raphanus sativus* L. Investigation of the allelopathic potential of *C. rossicum* and *Cynanchum nigrum* (L.) Pers., non Cav showed the radicular distillate of these plants could decrease the radicle length of *Asclepias tuberosa* L., *Digitaria sanguinalis* L. Scop and *Lactuca sativa* L. respectively at the rate of 40, 20 and 25% [41]. They also cited the allelopathic potential of these species can be used as an appropriate tool for their invasion power. The allelopathic trait will be considered in the term of managing programs to control weeds emphasized by [41]. After mowing the aforementioned weed species, for example, their foliage should entirely eliminate from the farm because it can decrease crops growth and germination with its allelopathic characteristic [41].

According to high waste rates of *Cynanchum acutum* L. in Iran [42, 43]; their drastic invasion power [16, 17, 18]; allelopathic trait of nearby weed species [41, 22] and also practical evidence proved the allelopathic effects of these species on crops. Furthermore the lack of scientific information in this term provided to investigate the allelopathic effects of different organs of two *C. acutum* populations on germination and seedling growth of barely.

MATERIAL AND METHODS

To run the study, in autumn 2013 and at plant maturity stage, the foliage of *C. acutum* encompasses leaves, roots and stalks randomly aggregated from abundant shrubs approximately around five square kilometers through various fields in Karaj (Latitude: 35° 50' 24" N, Longitude: 50° 56' 20" E) and Kerman (Latitude: 30° 17' 02" N, Longitude: 57° 05' 00" E). To extract the watery distillate from aggregated organs, separately they settled at 48 °C for 48 hrs in oven in order to be dried. To provide the appropriate distillate, initially different parts of *C acutum* slaughtered to the tinier fragments and fresh grind tissues transmitted from sieves with 0.5 mm diameter. Then 100 ml sterilized water per 5 g of each plant remnant added and placed into the Stirrer machine for about 24 hrs and the whirling speed set on 200 turns per minute under the room temperature. Then the watery distillate transmitted from fourfold Watman's filter paper (number one) to refine it and for half an hour it treated in centrifuge at 3000 turns per

minute and finally the obtained distillate refined with Watman's filter paper (number one) and maintained in refrigerator to utilize in an appropriate time [44]. To provide pre-determined concentrations of 0, 25, 50, 75 and 100%, extracted distillate of different *C. acutum*'s parts aggravated to adequate amounts of sterilized water.

Experiment was conducted in a split plot factorial based on CRBD with four replications. Experimental treatments include two populations of *C. acutum* (Karaj and Kerman), different organs (leaf, stalk and root) of both populations and different distillate concentrations (0, 25, 50, 75 and 100%). Experimental units were encompassed petri dishes with dimension of 11 cm diameter and 3 cm depth. Seeds and experimental dishes sterilized in order to prevent microbes growth and activities. To do that seeds settled into solvable of hypochlorite sodium 10% (1:10) for about 10 minutes; then to eliminate the sterilized solvable remnant from seeds, they frequently rinsed with sterilized water [45]. On the next step 50 unites of seed attached on two layers of filter paper and placed in foreseen petri dish, then 10 ml of prepared distillate added to each petri dish. Petri dishes wrapped with Para-film to prevent evaporation and conveyed to germinator under the 20/30 (day/night) alternative temperature and 12 hrs of light period [42].

Under the same experimental situation and in convergence of inquiring on distillates effect, polyethylene glycol (PEG) utilizes in order to distinguish osmotic and allelopathic effects of distillates. In this point initially the watery potential of organ's distillate, concentrations and different *C. acutum* populations respectively determined by Thermocouple psychrometer machine and PEG soluble with the same potential are provided [45]. And the whole experiments carried out under the same situations.

Buds counting and ventilating accomplished daily according to International Seed Testing Association (ISTA) instruction [44]. Also the ultimate calculating of emergence percentage, radicle and shoot length separately assessed for each seed species according to ISTA instruction. The deterrent percentage of germinating is achieved with the help of beneath formula [44]:

$$IP (\%) = [(C - E) / C] \times 100$$

IP: Inhibitive percentage of germination

C: The number of germinated seeds in sterilized water (evidence treatment)

E: The number of germinated seeds in distillate treatments obtained from different *C. acutum* parts

The data of inhibitive germination percentage normalized by arc sin regulator, and the other abnormal data was regulated by square formula [46]. At least data analysis was done with utilizing the SAS ver. 9.1 Software; and graphs were draw with Excel Software.

RESULT

Germination and growth of barley's seedling

Effect of different distillate concentrations of two *C. acutum* populations on preventing seeds germination of barley is presented in Figure 1. The watery distillate of *C. acutum* could reduce the germination percentage of barley's seeds compared to sterilized water. This effect was in convergence of increasing distillate concentration; so that, by increasing concentration from 25 to 100%, the preventing value developed from 45.5 to 100% in the term of barley germination. There was no significant difference between preventing effects of distillates obtained from Karaj and Kerman population. The preventing rate in this term under the same concentrations was equal in both populations (Figure 1). Kerman's distillate with concentration of 75% entirely ceased barley germination, whereas this value for Karaj population acquired in concentration of 100%. By increasing distillates concentration from 0 to 50%, the preventing rate of barley germination sharply accelerated (from 0 to 81%). But the rather increasing of concentrations (from 50 to 100%) presented a dull increasing steep (from 81 to 100%). Increasing the PEG concentration and decreasing the water potential, resulted a linear and dull steep reduction in the preventing term of barley germinating percentage; so that, at the highest concentration level of PEG, this preventing value was just about 14% (Figure 1). Although this value was rather compared with wheat germination, it is seemed in the lowest distillate concentration of *C. acutum* (25%) the preventing rate of barley germination was rather than 45%. So conspicuously the main percentage reduction point of wheat germinating attributed to allelopathic effects of *C. acutum* (not for the sake of its osmotic effects).

The watery distillate of different organs from two *C. acutum* populations presented different preventing effects on barley germination percentage; so that the leaf's and stalk's distillates respectively dedicated the highest and lowest percentage of preventing effect on barley germination (Figure 2). The prevention percentage made by leaf, root and stalks of *C. acutum* was respectively about 79, 68 and 47% in this term. Indeed, the effect of different organs among populations was varied; hence in Kerman's population the preventing percentage of barley germination treated by root, leaf and stalk of *C. acutum* was respectively about 87, 61 and 49%. This value for Karaj ones was about 71, 75 and 45% (Figure 2). There was no significant difference between preventing effect of

obtained distillate from root and leaves ($P \leq 0.05$); also stalks distillate effect on preventing germination of barley in Karaj and Kerman population was statistically the same at the level of 5% (Figure 2).

By increasing the watery distillate concentration of two *C. acutum* populations, the barley radicle length experienced a linear and drastic steep reduction (Figure 8); so that by increasing concentration from 0 to 100%, the barley radicle length with 100% reduction got from 52.5 mm to zero point. Result showed, in the whole concentrations except the concentration of 75%, there were similar preventing effects on barley's radicle length in Karaj and Kerman population. Whereas, barley's radicle length in concentration of 75% was respectively about 8 and 0 mm in Karaj and Kerman population (Figure 3). By increasing the PEG concentration and decreasing the water potential barley's radicle length experienced a linear and dull sort of increase; so that at the highest PEG concentration, barley's radicle length got to 58 mm (Figure 3). Thus barley's radicle length reduction under the effect of watery distillate of *C. acutum* is due to its allelopathic trait.

Distillate effect of different organs from two *C. acutum* populations on barley's radicle length (Figure 4) showed the barley's radicle length when it was at the mercy of leaf, root and stalk distillates of *C. acutum*, which was respectively about 15.5, 18.5 and 40 mm. The effect of different organs between two populations was various; so that in Kerman's population this value under the aforementioned organs was respectively about 10, 23 and 39 mm and the Karaj ones assessed about 21, 14 and 41 mm (Figure 4). The highest reduction for Kerman and Karaj population in this term respectively achieved by leaves and roots distillate. The leaf distillate effect in Kerman population on barley's radicle length was in parallel with Karaj ones ($P \leq 0.05$). Also stalks distillate effects in both populations was statistically equal at the level of 5% (Figure 4).

By increasing the watery distillate of two *C. acutum* populations, barley's shoot length had a drastic and none linear reduction (Figure 5); so that by increasing the concentration from 0 to 100%, this trait with 100% reduction got from 38.5 mm to zero point. Distillate effect of Kerman and Karaj population on barley's shoot length was the same (Figure 5). By increasing the PEG concentration and decreasing the water potential, barley's shoot length linearly fell down; so that at the highest PEG concentration, barley's shoot length got to 21 mm (Figure 5). But this sort of reduction was not comparable with ones which made by *C. acutum* distillates. Therefore the main reason of this reduction in barley's shoot length treated by watery distillate of *C. acutum* can be attributed to its allelopathic effects (and it is not correlated with its osmotic effects).

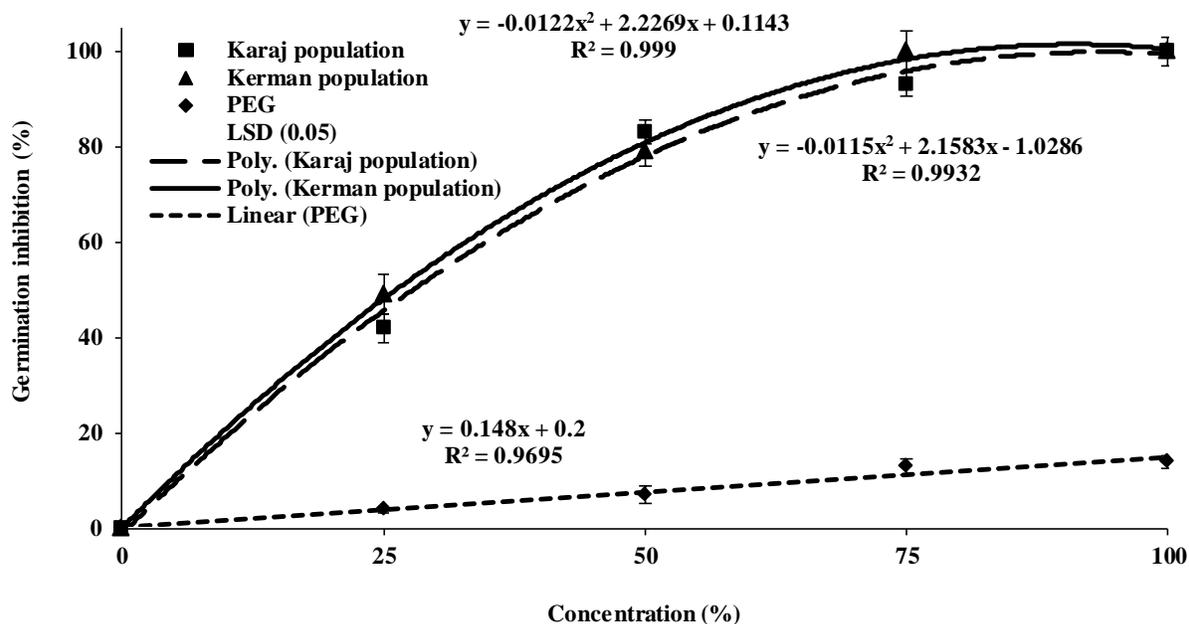


Figure 1- Watery distillate effect of two *C. acutum* populations on preventing germination percentage of barley. Means within a column followed by the same letters are not significantly difference at $\alpha=0.05$ (Duncan's multiple-range test).

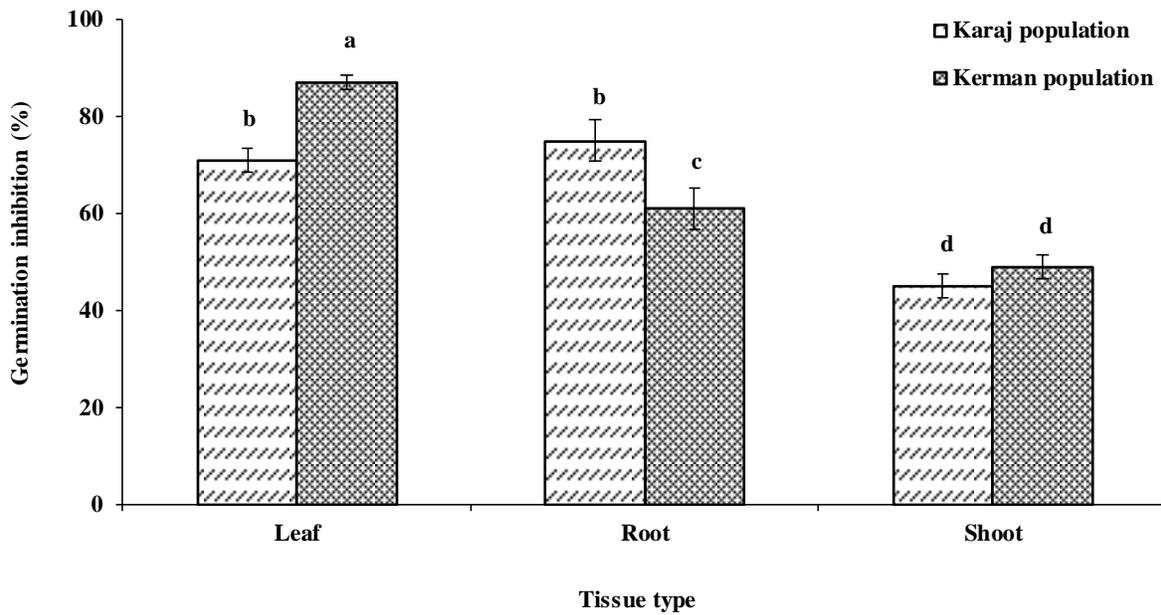


Figure 2- Watery distillate effect of different organs from two *C. acutum* on preventing germination percentage of barley.

Means within a column followed by the same letters are not significantly difference at $\alpha=0.05$ (Duncan's multiple-range test).

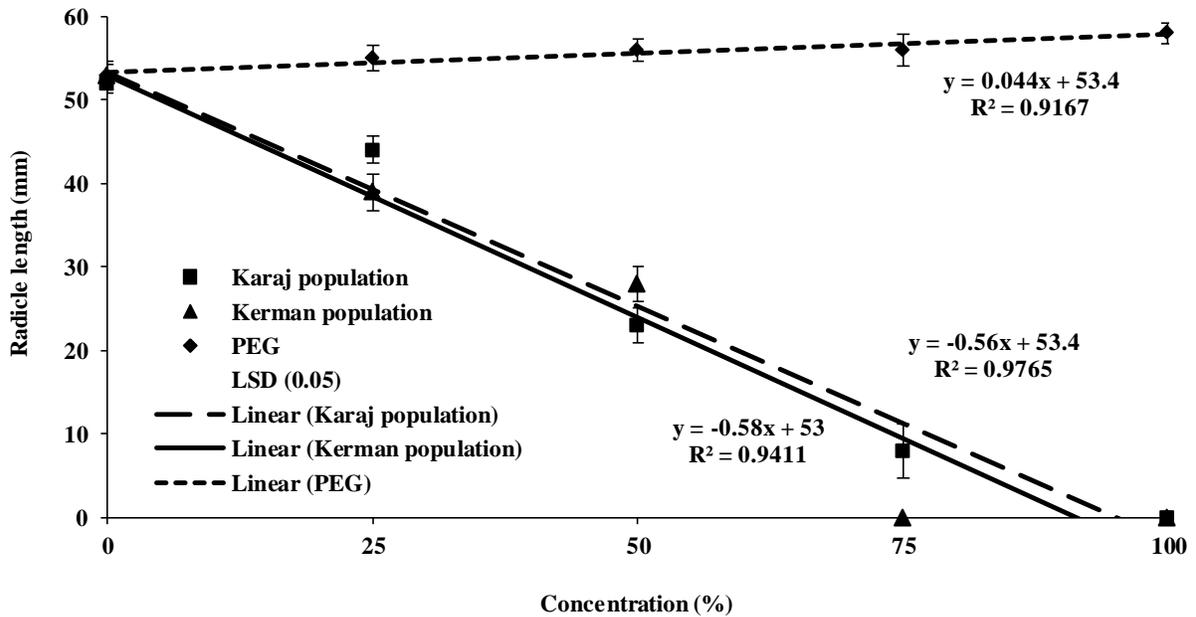


Figure 3- Watery distillate effect of two *C. acutum* populations on barley's radicle length. Means within a column followed by the same letters are not significantly difference at $\alpha=0.05$ (Duncan's multiple-range test).

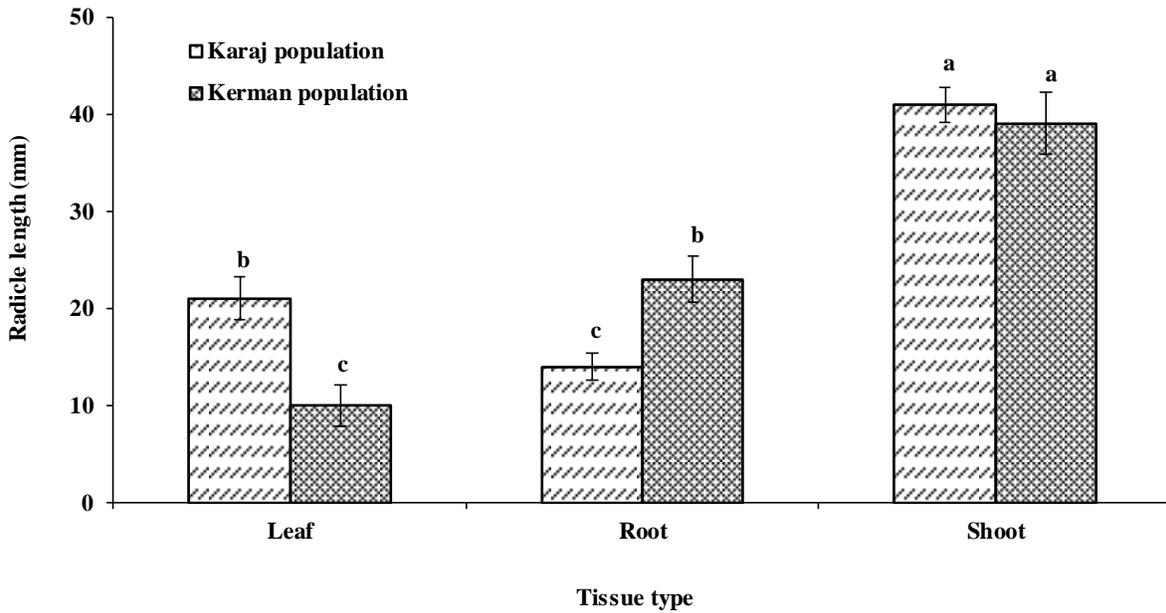


Figure 4- Watery distillate effect of different organs from two *C. acutum* on barley's radicle length. Means within a column followed by the same letters are not significantly difference at $\alpha=0.05$ (Duncan's multiple-range test).

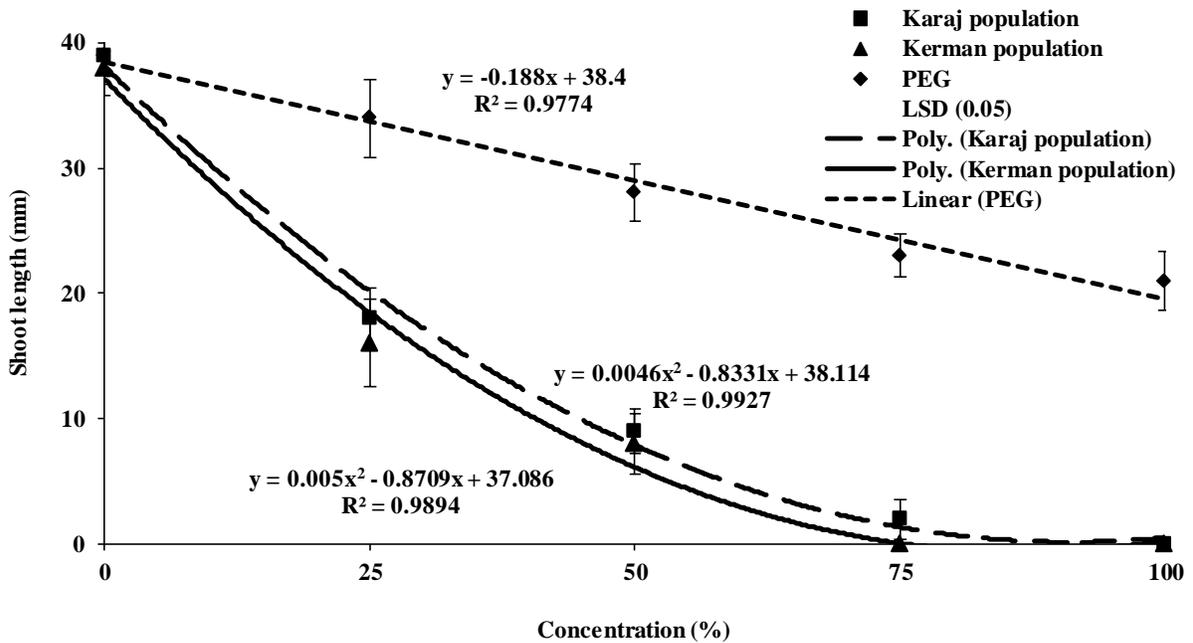


Figure 5- Watery distillate effect of two *C. acutum* populations on barley's shoot length. Means within a column followed by the same letters are not significantly difference at $\alpha=0.05$ (Duncan's multiple-range test).

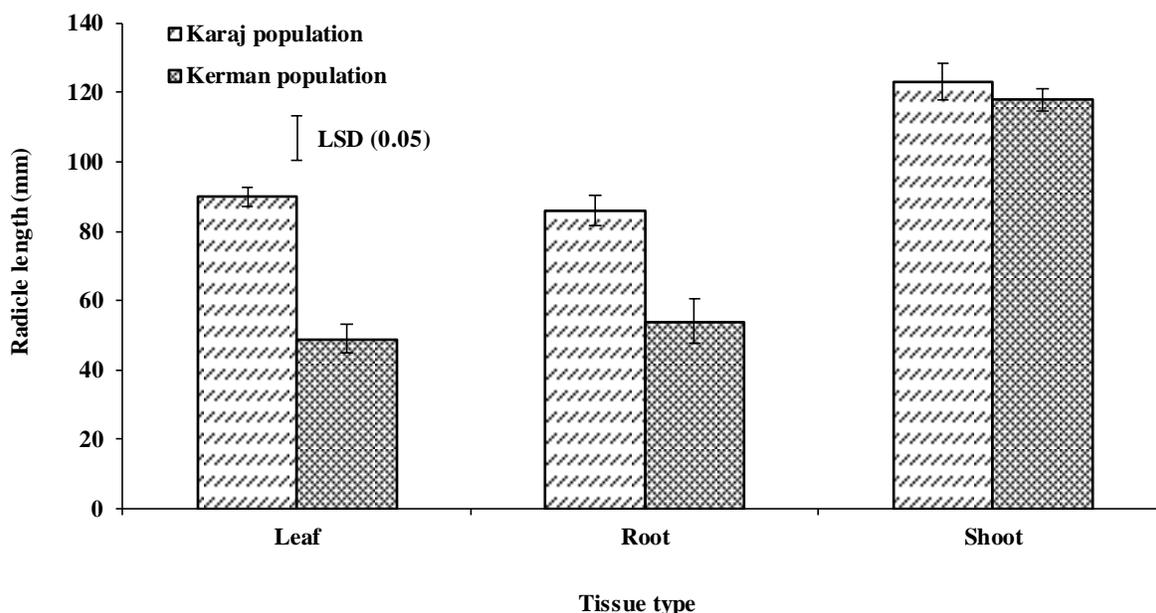


Figure 6- Watery distillate effect of different organs from two *C. acutum* populations on barley's shoot length. Means within a column followed by the same letters are not significantly difference at $\alpha=0.05$ (Duncan's multiple-range test).

DISCUSSION

By increasing the watery distillate concentration of *C. acutum*, consequently the germination percentage, radicle and also shoot length of barley decreased (Figure 1, 3 and 5). So, the germination percentage, shoot & radicle length and seedling weight of wheat can decreased with increasing concentration of *C. acutum* residue [47].

At the highest distillate concentrations, the germination percentage rate confronted with a rather sort of downfall which is attributed to the rather devastation of germinating reactions. Increasing the concentration of canola aquatic extracts caused a linear reduction in germination percentage and radicle length of *Thalaspis arvensis*, *Amaranthus retroflexus* and *Bromus tectorum* [48]. In a research aimed to investigate the allelopathic potential of *Cynanchum rossicum* and *Cynanchum nigrum* indicated by increasing the concentration of allelopathic material, the germination percentage of *Asclepias tuberosa* L., *Asclepias syriaca* L., *Digitaria sanguinalis* (L.) Scop., *Poa annua* L. and *Echinochloa crus-gallis* L. P. Beauv and also the radicle length of *Solanum lycopersicum* L. and *Digitaria sanguinalis* (L.) Scop decreased [41]. The effect of *C. acutum*'s different organs was various on germination percentage, radicle and shoot length of barley. Utterly the leaves and roots of *C. acutum* presented a rather allelopathic effect. And the lowest allelopathic value dedicated to stalk's distillate of this weed (Figure 2, 4 and 6). It is reported that leaves and roots of *C. acutum* had more allelopathic effect on germination characteristics of wheat [47].

In a research aimed to assess the root, stalk and leaf allelopathic potential of *Cynanchum rossicum* and *Cynanchum nigrum* reported the highest and lowest allelopathic value was belonged to leaves and stalks of these species [41]. Predominantly the leaves of species comprise higher concentrations of allele-chemical compositions because of their rather vulnerability to herbivores than other tissues [49, 50, 51]. Recently it is cleared that the aerial parts of plants from *Cynanchum* spp. contain sort of active biological compositions which plays a defensive role against herbivores. Not only these compositions have a defensive role, they own allelopathic trait too [52, 53]. As mustard's root owns the higher value of allelopathic materials compared to aerial organs, radicle length and also the total seedling dry weight of that will present the highest reduction if they expose under the root distillate [54]. Due to the higher allelopathic material like glucosinolatein mustard's root compared to its aerial organs, the radicle length and seedling dry weight of that affected more by root's allelopathic impacts [55].

Between two populations, according to recent study, the highest allelopathic effects on barley dedicated to Kerman ones (Figure 1 to 6). According to the study aimed to evaluate the allelopathic effect of two *C. acutum*

populations, it is reported that Ghazvin population had a more allelopathic effect on germination characteristics of wheat Compared with Moghan population. Several established theories address the impact of the environment on allelo-chemical production [47]. Studies have shown allelopathic plant species increase production of phytotoxic secondary metabolites when subjected to environmental stresses such as mineral deficiency, extreme temperatures, moisture stress, or extreme light levels [15].

Concerning to more suitable environmental circumstances of Karaj (more raining level, appropriate temperature and less salinity) compared to Kerman territory, *C. acutum* shrubs which were grown in Kerman situation tolerate drastic regional stresses; so the higher allelopathic potential in Kerman shrubs is rational. Several studies proved that under the inappropriate and stressful situation the allelopathic effect will intensify. For instance, when the allelopathic plants grow at the mercy of drought, salinity, sunshine and drastic temperatures, they increase their secondary metabolites production as defensive mechanisms; since most of these metabolites categorized in venomous compositions, so plant's allelopathic activity intensified at the mercy of environmental stress [12, 13, 14, 15]. The highest sensitivity to *C. acutum*'s allelopathic effect dedicated to barley (Figure 1 to 6). Various reactions of plant species to these allelo-chemical compositions can be attributed to different enduring mechanisms of plant and also the various rates of allelo-chemical metabolism with plant's cells between species [56].

According to result the watery distillate of *C. acutum* presented a blatant allelopathic potential on barley. Hence, *C. acutum* can immensely wreak havoc on crop yield. It is reported the root watery distillate of *Cynanchum rossicum* (Klepow) Borhidi made a sort of reduction on *Raphanus sativus* L. germination [22]. In the study aimed to investigation the allelopathic potential of *Cynanchum rossicum*, non Cav. and *Cynanchum nigrum* (L.) Pers indicated the root distillate of these species can decrease the radicle length of *Asclepias tuberosa* L. and *Digitaria sanguinalis* (L.) Scop., absolutely about 40 and 20%. Also the calculated reduction rate on germinating percentage of *Lactuca sativa* L. was about 25% [41]. It is reported the alkaloid compositions (such as 7-demethoxytylophorine, 7-demethoxytylophorine *N*-oxide, pyrroloisoquinoline) available in root, shoot and leaf of *Cynanchum rossicum* and *Cynanchum nigrum* play a major role to reduce the germination and seedling growth of sensitive plants; direct intervention of allelopathic compositions on growth of adjacent plants and also the root's leakages effect on soil's fungous population is distinguished as a thriving and dominant factor for these plants in northern America [41].

It is noted that weed species take advantage of allelopathic potential as an appropriate tool for their invasion power, they emphasized on this issue as a considerable point in the term of management programs [41]. After mowing the allelopathic species, for example, inevitably their foliage should evacuate from farmland; because remnant of these species can significantly reduce germinating and growth of arable plants due to their allelopathic trait [41]. *Cynanchum* spp. under the regular situation can immensely produce biomass [19, 57]. Since these species have a high allelopathic potential [41], therefore the allelopathic effect of their compositions will be considerable in natural ecosystems where these species immensely produce biomass. The allelopathic trait of *Cynanchum* species help them to extend appropriately in various (destroyed, poor, desirable or stable) ecosystems [18, 23]. Drastic tenacity of allelopathic intervention of these plants justifies their higher invasion power in various ecosystems; and also it is known as a main factor of their thriving behavior against plants with high competitive power [41]. Regarding to aforementioned statements and also by proving the drastic effects of these allelopathic weeds on crops, their negative impacts and damages should thoroughly be monitored to prevent probable spread of this invasive plant over the country and to facilitate depicting the appropriate managing program to control *C. acutum* in convergence of its allelopathic trait.

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