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Effect of flooding, pH and high temperature on germination behavior of two common heliotrope (*Heliotropium europaeum*) populations

Farid Golzardi[1]✉, Yavar Vaziritabar[2], Yazdan Vaziritabar[2], Mohammad Nabi Ilkaee[3], Sina Fallah Tafti[4], Shabnam Sarvaramini[5]

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Abstract

To study the response of seed germination and early growth of common heliotrope (Heliotropium europaeum) in different environmental conditions, three separate experiments were conducted. Treatments were the effect of different waterlogging levels 0, 1, 2, 4 and 8 days; acidity levels at seven levels 4, 5, 6, 7, 8, 9 and 100 and high temperature levels at nine levels 60, 80, 100, 120, 140, 160, 180, 200 and 220°C; the percentage and rate of germination, root and shoot length and fresh weight of seedlings was studied in two populations of Karaj and Ahvaz. The results showed that with increasing the duration of waterlogging in Karaj and Ahvaz all traits in both populations declined, so that the 8 days waterlogging H. europaeum growing in both populations completely stopped. With increasing pH from 4 to 7 percentage and germination rate gradually increased. The effect of high temperatures on germination levels in both populations showed an inverse relationship between increasing oven temperature and traits there. So that, by increasing the oven′s temperature the percentage rate of germination, root and shoot length and also seedlings fresh weight of two populations significantly declined.

***Keywords: Common Heliotrope, germination, waterlogging, acidity, high temperature*.**

Introduction

Heliotropium europaeum L. (*Boraginaceae*) is also known by the other common names like European heliotrope, barooga weed, caterpillar-weed, common heliotrope, European turnsole, hemp-agrimony (Clement and Foster, 1994; Holm et al., 1997; NGRP, 2014). *Heliotropium europaeum* is an erect or semi-prostrate branched annual growing with a rapid life cycle and produces a well-developed taproot (Holm et al., 1997; Parsons and Cuthbertson, 2001).

Author's Address
[1]Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
[2] Justus-Liebig-University Giessen, Institute of Crop and plant breeding I, BFS (Biomedical Research Centre Seltersberg), Schubertstr. 81, D-35392 Giessen, Germany
[3] Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran
[4] Department of Agronomy, College of Agriculture, Ahwaz branch, Islamic Azad University, Ahwaz, Iran
[5] Department of Agricultural Management and Development, University of Tehran, Karaj, Iran
E-mail: golzardi@chmail.ir

Heliotropium europaeum is reported to be native to southern and central Europe, northern Africa, and western Asia (NGRP, 2014; Parsons and Cuthbertson, 2001). Beyond this range, other species is also present in Afghanistan (GBIF, 2014), Australia (Parsons and Cuthbertson, 2001), Belgium (Verloove, 2006), China (Weber et al., 2008), India (Kaul, 1986), Pakistan (GBIF, 2014), Russia (Zhengyi et al., 2014), and South Africa (CABI, 2014). It continues growing and reproducing continuously until frost kills the plants (Parsons and Cuthbertson, 2001). Based on seed production rates of about 551 seeds per plant (Hasan and Aracil, 1991) and plant densities of 3 to 32 plants per square meter (Hunt et al., 2013; Tepe et al., 2011), this species has a high reproductive capacity.*Heliotropium europaeum* is an economically important weed because it is toxic to sheep, cattle, horses, pigs, and poultry (Burrows and Tyrl, 2001; Cavallaro et al., 2004; Parsons and

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Cuthbertson, 2001). It contains five pyrrolizidine alkaloids (Parsons and Cuthbertson, 2001) which cause liver damage and predispose animals to chronic copper poisoning and photosensitization over long periods of exposure (Holm et al., 1997; Parsons and Cuthbertson, 2001). In Australia, *H. europaeum* has directly caused losses of hundreds of millions of dollars to sheep production (Harris and Nowara, 1995; Holm et al., 1997). European heliotrope can be controlled with herbicides, control requires repeated applications (Parsons and Cuthbertson, 2001). Lastly, a congener of this species has contaminated wheat grain resulting in human illness and fatalities in central Asia (Holm et al., 1997). Moore (1956) suggested that control of *H. europaeum* could best be achieved by the establishment of competitive crop and pasture species, where annual rainfall is more than 50 cm. *H. europaeum* is an effective competitor, particularly for moisture during summer. This moisture removal can significantly reduce the yield of subsequent crops, especially in dry years (Delfosse and Cullen, 1981).During the germination process, seed as a productive unites guarantees the vitality of species. Moreover, due to the seed′s role in shrub establishment, germination is a linchpin factor in the term of novel agriculture (Akram Ghaderi et al., 2008). The germination process encompasses the rapid inception of metabolic activity, embryo development, radicle emersion and ultimately shoot emergence. Germination is the main factor to thrive weed through its establishment process; because in the ecologic niche, germination is the initial stage for weeds competition (Forcella et al., 2000). The germination process innately affects by different environmental factors like humidity, the soil salinity, nutrition elements (nitrate) and gases, light and temperature (Chauhan and Johnson, 2007; Roberts & Lockett, 1975; Chachalis & Ready, 2000).Through the germination process seeds might come across with unforeseen situations like heavy rain, flooding and so forth, which can succumbed the embryo due to the waterlogging effect, especially in the heavy soils or can be occurred in minimum or conservative tillage. In regions, where the water find in profusion, waterlogging can be acted as an appropriate solution to control the extend varieties of weed species (Wuebker et al., 2001). It is reported; flooding can reduce the *Bidens pilosa* germination (Reddy &Singh, 1992). Also there are similar reports about the flooding reduction effect on *Morrenia odorata* & *Diodia virginiana* germination (Singh & Achhireddy, 1984). The roots, stalk, plant density and seedling vigour reduction are the general effects of flooding (Huang and Wilkinson, 2000; Kozlowski, 1997; Pezeshki, 2001). During the flooding period, the gas transmission approximately interrupted into the soil, because in the watery situation the gas transmission rate fall down about 10 thousand times. In this case the soil and rhizosphere oxygen availability decreased impressively and soil confront with difficulties like the lack of oxygen (Hypoxia) or oxygen deprivation (Anoxia). Physiologically, the oxygen is the inescapable factor for seeds germination and vitality. Acidity or pH is the impressive factor on weeds germination. Chachalis & Reddy (2000) reported rather than 59% of *Campsis radicans* seeds were able to germinate at pH range of 5 to 9; whereas, at 4 to 10 pH levels their germination stopped entirely. The *Hyparrhenia hirta* seeds germination was 90 in the neuter pH level, which 65% reduced at pH levels of 4 and 10 (Chejara et al., 2008). Chachalis et al. (2008) reported the *Hibiscus trionum* seeds germination at pH ranges of 3 to 11. From the obtained result of these studies it can be inferred that the soil pH can′t act as an inhibitor factor for weeds germination and development. In the most regions of the world especially in Asia and Africa, farmers in order to wipe their lands out of weeds and previous remained cultivation ignite their farm for anticipate the forward cultivation and to facilitate the farm preparation (Roder et al., 1997). However through this blazing operation the soil surface temperature rises to 550°C for about 5 to 6 minutes (Cook, 1939). So the whole seeds and plants remained on the soil surface abolish through this operation, but this temperature decrease about 100°C per each 1 cm soil penetration depth (Sanchez, 1976). Therefore, the seeds which settled in deeper depth are safe from the lethal firing temperature and can still remain their germination vigor. The weed species have different sensitivity to these awkward temperatures. So resistance to the upper temperature can be used as effective equipment for weeds invasion power (Bhagirath et al., 2008). The ecological recognition of *P. olearcea* germination and development plays a major role to control and manage it in the long time program. Hence, this study aimed to recognize the *P. oleracea* germination behavior and its reaction to some of environmental elements.

Material and Methods

Flooding effect on *Heliotropium europaeum* germination

To investigation the flooding effect on *H. europaeum* seeds germination, an experiment was carried out based on randomized complete block design with four replications. The initial factor was *H. europaeum* population at two levels (Karaj & Ahvaz) and the second factor was the flooding periods at five levels (0, 1, 2, 4 and 8 days). To run the experiment 25 unite of seeds placed in Watman′s filter paper and then settled into the petri dishes, which had 8cm diameters. Then seeds soaked with sterilized water to the 70% of each petri dish depth and to avoid floating seeds in their bed, the three sieve paper placed on their tips. The flooding treatment carried out at periods of (0, 1, 2, 4 and 8 days). Then by passing the flooding period, each treatment irrigated by sterilized water according its humidity demand. The germinated seeds, which predominantly were emerged their radicle from the seed coat separated and ultimately the germination percentage of each petri dish is recorded (Baird and Dickens, 1991; Reddy and Singh, 1992; Singh and Achhireddy; 1984).

Acidity (pH) effect on *Heliotropium europaeum* germination

To investigation the acidity effect on *H. europaeum* seeds germination, an experiment was carried out based on randomized complete block design with four replications. The initial factor was *H. europaeum* population at two levels (Karaj & Ahvaz). The second factor was different acidities at seven levels (4, 5, 6, 7, 8, 9 &10). The seven soluble acidities provided according to Chachalis & Reddy method (Chachalis & Reddy, 2000). The seven acidity (pH 7) used as evidence treatment. To run the experiment 25 unite of seeds placed in Watman′s filter paper and then settled into the petri dishes with 8 cm wide, then 6 ml of predetermined pH soluble added to them and transferred to germinator under the 18/25°C (day/night) alternative temperature (Pahlevani et al., 2008). Ultimately in the end of the progress (fourteenth day), the number of germinated seed recorded. Seeds which have radicle length of about 2 mm or rather accepted as germinated ones.

High temperatures effect on *Heliotropium europaeum* germination

To investigate the high temperatures effect on germination percentage seeds placed in oven at 60, 80, 100, 120, 140, 160, 180, 200 and 220°C for 5 minutes. Then seeds germination percentage assessed under 18/25°C and 12/12 hrs day/night in the germinator. The seeds, which was treated in the room temperature (25°C) set as evidence treatment (Bhagirath et al., 2008). Ultimately after initial data analyzing and evaluation of their distribution process, the hypothesis of normal data distribution is investigated. The abnormal data was regulated by logarithmic formula. At least data analysis was done with utilizing the SAS ver. 9.1 Software. The comparison means is assess with Duncan's multiple-range test at level of 95% and graphs were draw with Excel Software.

Result and Disscusion

Flooding periods effect on *Heliotropium europaeum* seeds germination

By increasing the flooding period length, the percentage and germination rate of Karaj and Ahvaz population decreased; so that the flooding periods at 1, 2, 4 and 8 days reduced the germination percentage of Karaj and Ahvaz population respectively about 5, 9, 65 and 100% (in Karaj) and 6, 31, 81 and 100% (in Ahvaz) compared to evidence treatment (without flooding) (Table 1). The maximum and minimum germination percentage rate in both populations respectively observed at evidence and 8 days of flooding treatment, however there was no statistical difference between these two flooding treatments (Table 1). Also the compression means result indicated, by increasing the flooding period length, the fresh seedling weight, and radicle and shoot length decreased in Karaj and Ahvaz population. So that; at 8th day of flooding treatment, *H. europaeum* development factors (three aforementioned traits) ceased entirely in both populations. Hence, probably the aforementioned traits reduction severely associated to the oxygen reduction. In the case of oxygen deprivation aspiration processes, Krebs and electron transmission cycle interrupted and subsequently the glycolysis operation confront problematic situation, which results 2 ATP creation instead of 32 ATP produced in aerobic situation (Visser and Voesenek, 2004).Plant density, seedling vigour, radicle and stalk development reduction is the general effects of flooding reported by Huang and Wilkinson (2000); Kozlowski (1997) and Pezeshki (2001). The investigation of flooding period effects on *Diodio virginiana* and *Bidens pilosa* germination percentage showed by increasing the flooding period length, subsequently the seed germination decreased (Baird and Dickens, 1991; Reddy and Singh, 1992). However Pahlevani et al. (2005) to investigate the flooding period length effect (0, 1, 3, 7, 14 and 21 day) on *Cynanchum acutum* germination and development reported by increasing the flooding period length, no reduction observed on seed germination percentage. But the fresh weight, radicle and hypocotyl length of *C. acutum* reduced under the flooding situation compared to evidence treatment and by increasing the flooding time length this reduction rate goes up. Karaj *H. europaeum* seed population showed the higher resistance to increase the flooding period compared to Ahvaz population. Flooding made non-aerobic situation in the soil circumstances provided germination percentage reduction for *H. europaeum*. From the result it is inferred, the flooding can be acted as an appropriate solution in the term of integrated weed management if there were plants compatible with flooding when the sufficient water and suitable soil characteristic is being present. Minbashi Moeini et al. (2001) regarding to (McWhorter, 1972) accounted; the farms in the southern united states, which was treated about 2 to 4 weeks under the flooding situation as a pre-plantation treatment, had less *Sorghum halepense* population; there was no yield waste as well.

Effect of pH on *H. europaeum* germination

Increasing the acidity from 4 to 7 mount, gradually increases the percentage and germination rate of common heliotrope. So that at acidity (pH) of 7,

Table 1- Effect of waterlogging duration on the seedling emergence and growth parameters of two common heliotrope populations of Karaj (KP) and Ahvaz (AP).

Flooding period (day)	Germination (%)		Radicle length (mm)		Shoot length (mm)		Seedling fresh weight (g)		Germination Speed	
	KP	AP	KP	AP	KP	AP	KP	AP	KP	AP
0	89a	87a	16. 63a	15.54a	14.6a	14.32a	0.15a	0.14a	0.13a	0.12a
1	85a	8 a	14.86a	10.98b	12.6a	11.8ab	0.12ab	0.11b	0.11ab	0.08b
2	81a	52b	9.85b	7.12bc	11b	8.65bc	0.07c	0.07c	0.05c	0.03d
4	32c	17d	6.56bc	3.22c	6.23c	4.12cd	0.04d	0.02d	0.02d	0.01de
8	0e	0e	0d	0d	0d	0d	0e	0e	0e	0e

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

the highest germination percentage rate occurred in Karaj and Ahvaz population respectively of about 83 and 82%. Also the germination rate of these two populations was about 0.111 and 0.118%. The upper pH levels rather than 7 lead the significant germination percentage and rate reduction. Result showed at pH level of 4 and 10 these aforementioned traits entirely ceased (Figure 1 & 2). However, common heliotrope is capable to germinate in the immense range of pH levels, it prefer the acidity or neuter levels of pH. Susko and Hussein (2008) reported *Hesperis matronalis* seeds were capable to germinate of about 60% between 3 to 10 pH ranges. The appropriate pH range for its germination assessed between 5 to 8 ranges, but the germination impressively declined in upper ranges (pH 9) or in lower ranges (pH 4). *Sisymbrium* *oriental* and *Brassica tourneforti* are capable to germinate in the vast ranges of acidity (pH 4 to 10) reported by (Chauhan et al., 2006). McDonald et al. (1992) in their study aimed to investigate the effective factors on two species of *Eupatrium capillifolium* percieved the both species were able to germinate in the immense range of pH (6 to 10) the highest rate of germination occurred at pH 8. *Bidens pilosa* seeds at pH 4 to 9 germinated about 78 to 90%. Also the radicle development affected rather than germination rate in the term of higher pH ranges (Reddy and Singh, 1992). According to result of this study, acidity can not be acted as inhibitor factor for common heliotrope development.The maximum radicle length, shoot length and fresh seedling weight in Karaj and Ahvaz population achieved at pH 7 (Figure 2, 3 and 4). According to the current study, the higher and lower pH ranges of 7 acidity level had inverse effect on aforementioned traits development. The numerous researchers presented similar result in association of weeds response to pH variations; *Asclepias syriaca* L. by Evetts and Burnside (1972), *Brunnichia ovate* (Walt) Shinners by (Shaw et al., 1991), *Scoparia dulcis* L. by Jain and Singh (1991) and *Caperonia plustris* (L.) St. Hill by (Koger et al., 2004). According to figures 3, 4 and 5, the appropriate growth in aforementioned traits achieved at pH<7 in Karaj and pH>7 in Ahvaz population. Affecting the soil nutrition availability is the main pH role. In the far lower pH ranges elements like Ca, P and K are leached or become insoluble in the soil. Also in the upper pH ranges lacks of P, Fe, Mn and other micro-nutritive elements might be seen (Seeber, 1976).

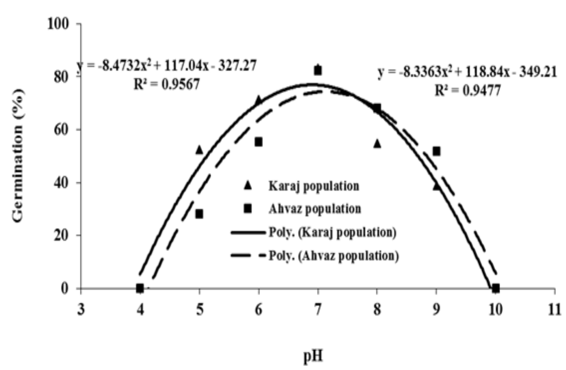

Figure 1- Effect of the acidity on Germination (%) of two common heliotrope populations (Karaj and Ahvaz).

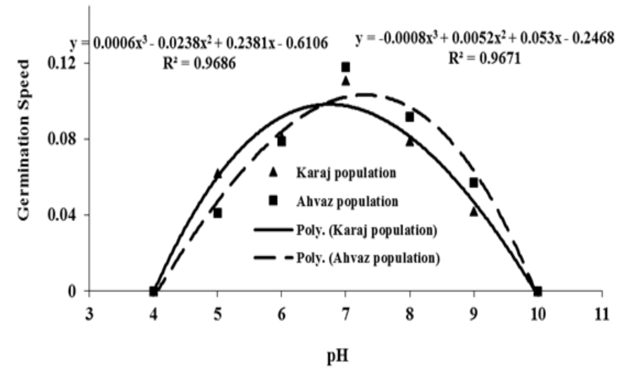

Figure 2- Effect of the acidity on Germination rate of two common heliotrope populations (Karaj and Ahvaz

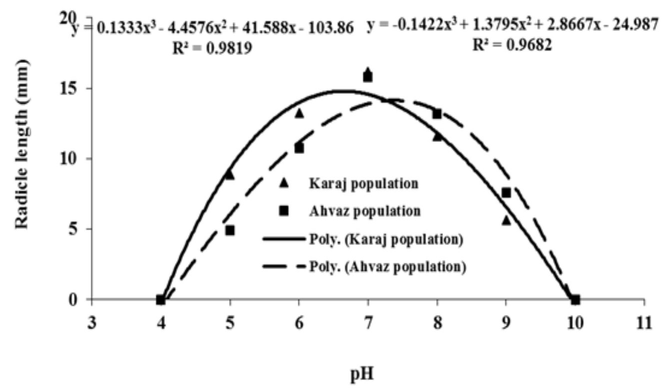

Figure 3- Effect of the acidity on Radicle length (mm) of two common heliotrope populations (Karaj and Ahvaz).

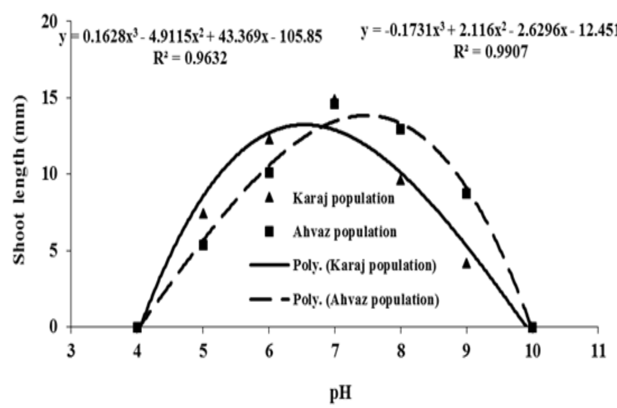

Figure 4- Effect of the acidity on Shoot length (mm) of two common heliotrope populations (Karaj and Ahvaz)

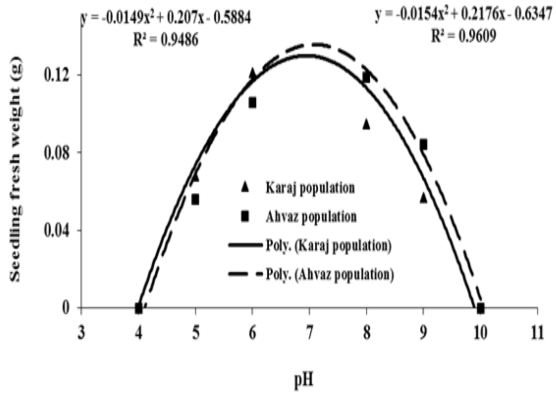

Figure 5- Effect of the acidity on Seedling fresh weight (g) of two common heliotrope populations (Karaj and Ahvaz)

High temperature effects on *Heliotropium europaeum* germination

The investigation of higher temperatures effect on *H. europaeum* germination in two populations presented a diverse correlation between increasing the oven temperature and aforementioned traits. So that, by increasing the oven temperature, the percentage and rate of the germination; fresh seedling weight and also radicle and shoot length of two populations significantly decreased. The result showed by increasing temperature from 60 to 100°C in Karaj population or 60 to 140°C in Ahvaz population, development of five aforementioned traits decreased linearly; furthermore at 100 and 140°C, the development of these traits entirely ceased in two populations (Table 2).

Table 2- Effect of high temperatures period on germination rate and percentage of two common heliotrope populations

Temperature (C°)	Germination (%)		Germination Speed	
	KP	AP	KP	AP
Control	91.5 a	93 a	0.127 ab	0.138 a
60	72 bc	81.75 b	0.101 bc	0.112 b
80	45.25 d	63.5 c	0.072 cd	0.083 c
100	10.75 e	43.5 d	0.051 de	0.059 d
120	0.0 f	22.5 e	0.0 g	0.031 e
140	0.0 f	8.25 ef	0.0 g	0.011 f
160	0.0 f	0.0 f	0.0 g	0.0 g
180	0.0 f	0.0 f	0.0 g	0.0 g
200	0.0 f	0.0 f	0.0 g	0.0 g
220	0.0 f	0.0 f	0.0 g	0.0 g

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

This issue showed the higher sensitivity of Karaj population to intensified temperatures (created by firing). The heat treatments affected plants from several ways include temperature degree, heating exposing time and consumed energy (Minbashi Moeini et al., 2001). Investigation of higher temperatures effect on *Digitaria ciliaris* (Retz) Koel and *Digitaria longiflora* (Retz) Pers presented, increasing temperature till 80°C for 5 minute could not decrease the germination percentage of both populations. Whereas, the higher temperature significantly decreased the germination percentage of those seeds; so that, *D. longiflora* seeds at 140°C and *D. ciliaris* at 180°C lost their germination vigour. Also *D. ciliaris* showed rather resistance to higher temperature compared to *D. longiflora* (Bhagirath et al., 2008). It is perceived, increasing the soil temperature via firing, for example, is the effective way to decrease the weeds seed bank. Decreasing the constant seed bank availability of the soil and eliminating the established seedling is the linchpin of weed management in the polluted farms (Altieri & Liebman, 1988).

Table 3- Effect of high temperatures period on seedling emergence and growth parameters of two common heliotrope populations (Karaj and Ahvaz)

Temperature (C°)	Radicle length (mm)		Shoot length (mm)		Seedling fresh weight (g)	
	KP	AP	KP	AP	KP	AP
Control	16.98 a	17.43 a	13.68 b	15.47 a	0.171 a	0.178 a
60	11.25 bc	13.85 b	11.22 c	13.01 b	0.115 bc	0.135 b
80	8.32 c	10.74 bc	8.45 d	10.75 c	0.073 cd	0.092 c
100	4.93 d	7.92 c	3.67 fg	6.83 e	0.049 d	0.071 cd
120	0.0 e	4.11 e	0.0 h	4.87 f	0.0 f	0.033 de
140	0.0 e	2.32 de	0.0 h	0.0 e	0.0 f	0.012 e
160	0.0 e	0.0 e	0.0 h	0.0 e	0.0 f	0.0 e
180	0.0 e	0.0 e	0.0 h	0.0 e	0.0 f	0.0 e
200	0.0 e	0.0 e	0.0 h	0.0 e	0.0 f	0.0 e
220	0.0 e	0.0 e	0.0 h	0.0 e	0.0 f	0.0 e

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

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