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Application of Different Ratios of Effluents of Ethyl Alcohol Factories on Germination of Barley

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Abstract**—**Using effluent as a sustainable water resource for agriculture not only could provide part of water needs but also would save the existing water resources, durably. Vinasse, the effluent of ethyl alcohol factories, a by-product, which is derived from sugarcane molasses, is one of the water resources that could be effectively utilized for agricultural purposes. In the present study in order to investigate the application of different ratios of water: vinasse on germination and growth of barley seedlings an experiment was designed in pots with completely randomized design with three replications and control treatment. The consequences of four irrigation levels were studied with different water: effluent ratios (100% water, 90% water & 10% effluent, 75% water & 25% effluent, 50% water & 50% effluent) on germination and growth of barley seedling components in sandy-loam soil. The results showed that, with increasing the percentage of vinasse in the irrigation admixture, the germination percentage in barley seedlings decreased, significantly, so that the decrease in germination in comparison with the control samples in the second and third treatments was 20% and 93.33%, respectively. Seed germination percentage was about 46.66. The average stem length in seedlings was 14.3 mm and the average root length was 9.37 mm. The averages of the soils Electrical Conductivity (EC) and pH which were under irrigation with different ratios of vinasse (dSm^{-1}) were 5.85 and 7.32, respectively, which showed a 76.2% increase in soil salinity.

Keywords**—**Electrical Conductivity, effluent, germination, vinasse, barley.

I. Introduction

IRAN is situated in one of the world's most arid regions. Water scarcity, limited water resources and the ever-increasing population of this country have made it impossible to satisfy the increasing water demands of the agriculture sector. Consequently, under these conditions, optimization of water use in agriculture seems to be essential [1].

Urban, agricultural and industrial effluents are one of the water resources that can satisfactorily be used in agriculture. Use of these effluents to provide the water required for urban green spaces, to grow crops that are not eaten raw, and to artificially recharge plains where groundwater levels have declined severely can be a strategic policy for reducing the pressure on conventional water resources. Use of effluents as a permanent water resource for agriculture meets some of the water needs of this sector and saves and conserves the existing water resources [2]. Vinasse is the effluent generated in the alcohol industry after alcohol distillation. It has a dark brown color and bad odor and contains various organic compounds such as acetic acid, lactic acid, glycerol, different phenols and polyphenols, melanoidins, and mineral substances including sulfate and phosphate salts, potassium, calcium, magnesium, phosphorus and nitrogen. In general, its ingredients are strongly dependent on the material from which it is extracted [3]. Generally, 8-15 liters of vinasse is generated for each liter of alcohol that is produced [4]. Vinasse is often used as a liquid fertilizer and in irrigation, mainly in sugarcane culture. However, its excessive use may have negative consequences [5]. In other words, vinasse may pollute surface soil or groundwater if its entrance into the environment is not planned [6]. One of the major problems in using vinasse is its effects on soil salinity. Most studies have reported that use of vinasse causes soil salinity and these effects increase exponentially at higher vinasse application rates [1]. Soil and irrigation water salinity are the most important limiting factors for plant growth as they cause changes in the quantities and types of metabolic substances thereby influencing growth rate [7]. When exposed to salinity stress, plants use complicated mechanisms to adapt themselves to osmotic stress and toxicity of ions. These mechanisms vary depending on plant type and its degree of sensitivity to salinity. For example, in plants resistant to salinity sodium and calcium ions are accumulated in the vacuoles and in sensitive cultivars in cell cytoplasm [8]. Germination is the stage in the plant life cycle most strongly influenced by salinity [9]. Germination can be considered as composed by two main part-processes: imbibition (water uptake) and metabolic reactions conducting the poorly defined embryo to a new plant [10]. Depending on the genetics of each plant species and the environmental conditions the seeds are exposed to, germination is a vital stage in the life cycle of plants. In arid and semi-arid regions, the frequent adverse conditions such as water salinity and scarcity disrupt water uptake by seeds during the germination process due to the negative value of matric potential [11].

The crop plant barley (*Hordeum vulgare* L.) belongs to the Gramineae family, which is the largest family of monocot crops. Compared to wheat, barley requires less water to produce a crop on an economic scale, and it is possible to grow it in regions with minimal amounts of rainfall (200-250 mm) and salinity of up to 8 dSm^{-1}. Consequently, barley is somewhat resistant to drought and salinity and is adapted to various weather conditions [12].

Therefore, the aim of this study is to investigate the effects of using different ratios of ethanol (vinasse) alcohol production

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effluents as an unconventional water source on some germination indices of barley as a relatively resistant plant to salinity. The effect of different concentrations of vinasse on EC and pH of cultivated soil has also been investigated.

II. Materials and Methods

In this research, a pot experiment was designed using a completely randomized design with three replications and a control treatment to study the effects of applying vinasse on barley seedling (Nimrooz cultivar). For this purpose, 12 identical pots were used each containing 1000 g of soil. The physical and chemical characteristics of the soil are listed in Table I. Three days before sowing the seeds, the pots were irrigated with 100 mL of specific water: effluent ratios according to the design pattern to provide the initial moisture of the soil in the pots. The water: vinasse ratios for each treatment are presented in Table II. Ten barley seeds were sown in each pot (120 seeds in total) at the soil depth of 4 cm. The required vinasse was obtained from the evaporation ponds close to the alcohol production factory in Debal Khazaee agro-industry, km 30 of Ahvaz-Abadan Road, Khuzestan Province, southwestern Iran. Fig. 1 shows a picture of these ponds, and Table III lists the chemical characteristics of the vinasse used in the experiment.

TABLE I
Texture and Chemical Properties of Soil

Soil texture		Sandy loam
Silt	%	38
Clay	%	7
Sand	%	55
pH	-	3.32
EC	dsm^{-1}	39

TABLE II
The AMOUNT of vinasse and Distilled Water Used for Each Treatment per 100 cm^3

Treatment	The amount of water for a sample (cm^3)	The amount of vinasse for a sample (cm^3)
1	100	0
2	90	10
3	75	25
4	50	50

TABLE III
Chemical Properties of Vinasse

Property	Unit	Amount
pH	-	4.8
EC	dsm^{-1}	39
K	mg/L	2035
Na	mg/L	220
P	mg/L	140
Mg	mg/L	226
Ca	mg/L	540
SO_4	mg/L	1282

Each pot received 100 mL of irrigation water every other day after sowing until the emergence of the first seedling. After that, an irrigation interval of three days was followed for 15 days. Fig. 2 shows the arrangement of the pots and emergence of the seedlings.

Fig. 1 Sewage evaporation ponds near the ethyl alcohol factory of Dabal Khazaei (Razi) agro-industry

Fig. 2 Pot arrangement and emergence of seedlings

15 days after the emergence of the first seedling in each pot, the total number of germinated seeds (the seedlings) was counted and recorded, and the data were used to determine the final germination percentage. The seedlings were thinned to two per pot to eliminate the effect of competition on their growth, and the irrigation process was continued for 20 days with the same irrigation interval. In each stage of their vegetative growth, the number of seedlings was measured and recorded (Fig. 3). At the end of the 20-day period, the pots were emptied and the lengths of the stems and roots were measured and recorded (Fig. 4).

Fig. 3 Thinning seedlings and keeping 2 ones in each pot

Fig. 4 Barley seedlings

The following parameters were evaluated to assess seed germination process: Seed germination percentage (GP) [13], seedling vigor index (SVI) [14], mean germination time (MGT) [15], and mean daily germination (MDG) [16] were determined using (1)-(4), respectively:

$$GP = (G/N)\ 10^2 \quad (1)$$

$$SVI = MSL \times MGP \quad (2)$$

$$MGT = \Sigma\ (Ti \times Ni) / \Sigma\ Ni \quad (3)$$

$$MDG = FGP / d \quad (4)$$

In these equations, G represents the number of germinated seeds during the experiment, N the total number of seeds, Ni the number of newly germinated seeds at time Ti, FGP Final germination percentage, MSL mean seedling length, MGP mean GP and d the number of days. Stem and root fresh and dry weights were measured with a digital balance and recorded to calculate barley seedling fresh weight and dry weight (FW and DW). The seedlings were then put in an oven at 80 °C for 72 hours and then weighed to calculate seedling dry weight. Length of the aerial organs (SL) and root length (RL) were measured in cm.

Given the high EC value of vinasse, the extent of its effect on soil EC and pH should be assessed. For this purpose, after applying the treatments, removing the seedlings from the pots and preparing 1:2 volume extract, the EC and pH values of the soil in the pots were measured using a STARTER 3000C EC meter and pH Meter F-11, respectively. SPSS 20 was used for statistical analysis of the data and the diagrams were drawn in Excel.

III. Results and Discussion

Table IV lists the effects of the various vinasse (obtained from the alcohol production factory) to water ratios on barley seed GP and germination indices. The mean number of germination in the 120 seeds was about 56 and the mean GP was 46.66%. The values for mean stem and root length and for the other characteristics of the seedlings, and also those for the mean soil EC and pH after removing the seedlings from the pots, are presented in Table V. Accordingly, the soil EC increased from an average of 3.32 to 5.85 which indicates the effect of vinasse on increasing EC by 76.2%. On the other hand, the average of the soil pH decreased from 7.6 to 7.3 and the soil became more acidic by 0.02% under the influence of vinasse.

A decreasing trend of mean root length and stem length was observed in treatments 1 to 4 so that this trend in terms of millimeter for root length of seedlings was equal to T1 (18.33) > T2 (16.43) > T3 (2.25) > T4 (0) and for seedlings stems length were T1 (32.21) > T2 (24.01) > T3 (0.96) > T4 (0), respectively.

TABLE IV
Germination Indices

	GP	MDG	SVI	MGT (day)	G
Average	46.66	0.46	0.11	12.55	4.66
Sd	46.77	0.41	0.13	6.21	4.67
Variance	2187.87	0.172	0.017	38.62	21.87
Sample	12	12	12	12	12

Sd = Standard deviation

TABLE V
Seedlings Growth Indices and Soil EC and pH

	SL (mm)	RL (mm)	FW (g)	DW (g)	pH	EC (dSm^{-1})
Average	19.7	14	0.93	0.12	7.32	5.85
Sd	14.89	9.09	0.001	0.13	0.19	3.67
Variance	221.91	82.80	1.003	0.018	0.039	13.48
Sample	12	12	12	12	12	12

Sd = Standard deviation

The results suggested that there were significant differences between the four treatments in daily germination at the $P < 0.01$ level. Fig. 5 shows that the highest daily germination (0.9 on average) belonged to the first treatment (pure irrigation water) and the lowest (0.68 on average) to the third treatment followed by the fourth treatment (0% germination rate). As the amount of effluent in the irrigation water increased, the MDG decreased significantly. The reason for this decrease in germination was that higher vinasse percentages led to increases in EC and entrance of vinasse into seed cells. Consequently, cellular activities were severely disrupted and germination was stopped because osmotic stress under saline conditions led to water intake by plant tissues, which is called physiological drought. In addition, ionic toxicity caused by accumulation of special ions, especially sodium, disrupted plant metabolic reactions [17]. Salinity prevents plant growth and development via complicated features such as osmotic pressure, ionic toxicity, mineral deficiency and physiological and biochemical defects [18].

The four treatments also differed, significantly, at the $P < 0.01$ level in the number of days to maximum germination. Fig. 6 indicates that the highest number of days to maximum germination (with the average of 15 days) was recorded in the third treatment. It must be mentioned that no germination was observed in the fourth treatment by the end of the experiment. Fig. 7 reveals that the highest GP was recorded in the first treatment. The mean GP of barley seeds in the second treatment was 80% and the GP with an average of 20% is related to the third treatment. In the fourth treatment the seeds germination rate was zero. It reveals that by increasing in vinasse utilization in irrigation mixture, GP decreased, accordingly. The four treatments differed, significantly, in this index at the $P < 0.005$ level.

As for the mean number of germinated seeds, there were significant differences between the four treatments at the $P < 0.01$ level. Fig. 8 demonstrates that the highest Number of germinated seeds (10 seedlings on average) was that of the first treatment and the lowest (zero on average) that of the fourth treatment. Bybordi and Tabatabai reported that reduced germination rate and percentage were related to lower turgor and to decreased water absorption by the seeds in the water uptake stage [19]. In addition, under saline conditions, osmotic and ionic stresses lead to production of reactive oxygen species (ROS) in the chloroplasts, mitochondria and apoplastic space. This oxidative stress causes peroxidation in membranes, ion leakage, and damage to nucleic acids, cell membrane and cellular structure [15], and eventually decreases crop quality and yield. In addition, it can negatively influence water quality and soil structure [20].

IV. Conclusion

Based on the results in the present study, consumption of vinasse with irrigation water due to salinity stress, reduces barley seeds germination, significantly, so that no seedlings appeared in high concentrations of vinasse. It seems that despite the high amounts of elements such as phosphorus, magnesium, calcium and especially potassium in vinasse, using this substance as a fertilizer with irrigation water may cause damage to the soil and seeds. Therefore, to use the vinasse might require major modifications to reduce the EC. Consequently, further field experiments are required to use vinasse with a composition content of less than 25% in low salinity soils.

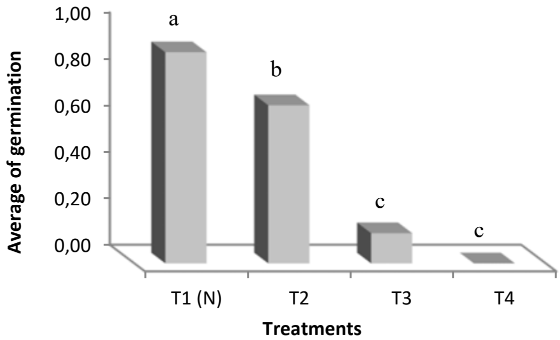

Fig. 5 Average of daily germination

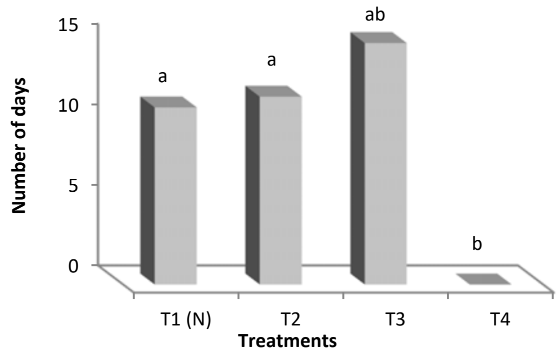

Fig. 6 Average of days to reach maximum germination

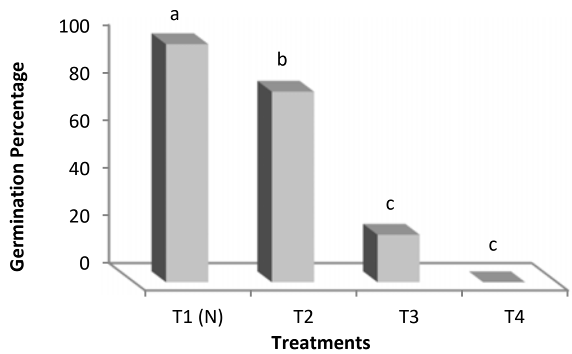

Fig. 7 Average percentage of germination

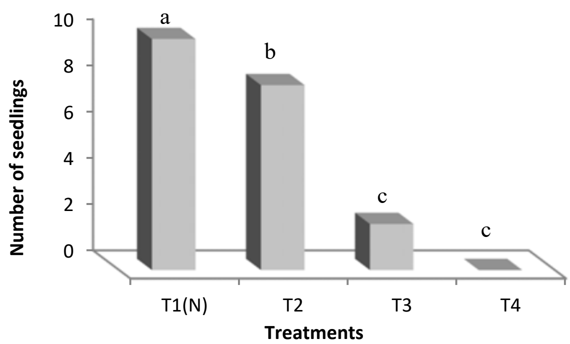

Fig. 8 Average number of seedlings

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