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Effect of Distillery Effluent on Seed Germination in Some Crops

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Abstract | **Original Research Article**

The present study has been undertaken to evaluate the effect of distillery effluent (stillage) on seed germination in *Zea mays*, *Phaseolus vulgaris* and *Arachis hypogaea*. The effect of different concentrations T0 (tap water), 10% (T1), 20% (T2), 50% (T3), 75% and T5 (100% of distillery effluent) on seed germination percentage, vigor index, tolerance index, phytotoxicity percentage, number of seed germination, the length of the radicle, seedling length, root length and radicle length were recorded for each plant species after 15 days. For *Zea mays*, T2 has the highest germination percentage; followed by T3 for *Phaseolus vulgaris* and by T0 for *Arachis hypogea*. The phytotoxicity percentage is high in T5 for *Phaseolus vulgaris*. The diluted effluent had statistically significant effects on the measured parameters. The high concentrations of stillage inhibited the root and radicle length of seedlings in *Arachis hypogaea.* Based on the tolerance to distillery effluent, the crops studied have been arranged in the following order *Zea mays* > *Phaseolus vulgaris* > *Arachis hypogaea*. The seed germination and growth parameters increased in lower (10% et 20%) concentrations of distillery effluent and the morphological parameters gradually decreased with increasing effluent concentration. We conclude that the effect of the distillery effluent is crop-specific and due care should be taken before using the distillery effluent for irrigation purposes.

Keywords: Distillery effluent; seed germination; phytotoxicity percentage; dilution.

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1. INTRODUCTION

Alcohol distilleries generate effluents known as stillage or vinasse (Arias-Martínez *et al*., 2025; Shetty *et al*., 2023). Stillage is a significant by-product of the fermentation industry. In Cameroon, stillage is produced by two distilleries. While in India and Brazil there are more than 300 alcohol distilleries (Hans *et al*., 2023). However, the impacts of these effluents are identical to those observed in several countries in the world (Tiwari and Prakash, 2021). According to the work of (Noukeu *et al*., 2016), the volumes of stillage produced per day by these distilleries in Cameroon are real sources of organic pollution for the receiving environments. In recent years, stillage has gained attention due to its high content of organic material and natural mineral nutrients. Its utilization not only enhances agricultural production but also addresses the issue of wastewater disposal (Carpanez *et al*.; 2022). Additionally, stillage enhances the availability of essential macro and micronutrients and organic matter in the soil, ultimately improving plant growth and production. In India, the use of distillery wastewater in agriculture is popular (Ratna *et al*., 2021). Distillery effluents often contain an excess of various forms of cations and anions, these constituents should be reduced to beneficial levels by diluting the effluent so that it could then be used as a substitute for chemical fertilizers (Hassan *et al*.; 2021). Moreover, stillage promotes growth and photosynthetic rates without detrimental effects. Substituting mineral fertilizers with stillage contributes to resource recycling (Stephen *et al*., 2024; Kumari *et al*., 2016). The stillage could be used as a complement to mineral fertilizers for sugarcane culture (Mikucka and Zielińska, 2020). The effluent contains N, P, K, Ca, Mg and S and thus valued as a fertilizer when applied to soil through irrigation (Mahmoud *et al*., 2019). The application of diluted stillage was found to increase the uptake of zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) in maize and wheat as compared to the control test and the highest total uptake of these minerals were found at lower dilution levels than at higher dilution levels (Moran-Salazar *et al*., 2016). Diluted stillage increases the uptake of nutrients, height, growth and yield of leafy vegetables, cabbage and mint leaf (Vaithiyanathan and Sundaramoorthy, 2017). Increase in nutrients uptake was equally observed in vegetables, pulses, condiments and root vegetables (Da Silva *et al*., 2020; Bastos *et al*., 2021). Due to the main problem of dealing with excessive quantities of stillage, alternatives have been required. For (Pires *et al*., 2016), one of the viable and economical alternatives to dispose of this

waste is its use in biofertilization. In Cameroon no information is available on the impact of distillery effluent on crops. Therefore, in this context, in order to obtain more information about the possible effects of distillery effluent on plants, the present study has been undertaken to evaluate the effect of distillery effluent on seed germination in some crops.

2. MATERIALS AND METHODS

2.1 Analysis of distillery effluent

The effluent sample was collected in pre-cleaned, sterilized plastic containers from the outlet of the sugar industry Nodiscam (Nouvelle distillerie du Cameroun) from March 2024 to June 2024. The samples were transported to the Biotechnology and Environment laboratory of the University of Yaoundé 1 for physicochemical analyses. Physicochemical parameters, such as color (Pt/co), pH, temperature, electrical conductivity (EC), total dissolved salts (TDS), chemical oxygen demand (COD), biochemical oxygen demand for 5 days (BOD_5), alkalinity, turbidity; oxidability, CO_2, dissolved oxygen, suspended solids (SS), salinity (US), nitrates (NO_3^-), nitrites (NO_2^-), ammonium ions (NH_4^+), Kjeldahl nitrogen (KKN), orthophosphate (PO_4^{3-}), calcium, magnesium, sodium and potassium (Ca^{2+}, Mg^{2+}, Na^+, K^+), cadmium and lead (Cd^{2+}, Pb^{2+}) were measured using standard methods (APHA, 2005; Rodier *et al*., 2009).

2.2 Effects of different vinasse concentrations on the germination of some seeds

The vinasse sample collected from the outlet of distillery industry was treated as 100% raw effluent identified as treatment T5 in this experimentation. For bioassays, the effluent was diluted with distilled water. The different treatments were:

T0 control: 100% tap water;
T1: 10% vinasse (10 mL of effluent + 90 mL distilled water);
T2: 20% vinasse (20 mL of effluent + 80 mL distilled water);
T3: 50% stillage (50 mL of effluent + 50 mL distilled water);
T4: 75% vinasse (75 mL of effluent + 25 mL distilled water);
T5: 100% vinasse (100 mL of effluent).

2.3 Germination Studies

Healthy *Zea mays*, *Phaseolus vulgaris* and *Arachis hypogaea* seeds were selected. Twenty healthy seeds of each plant species were incorporated in plastic jars filled with 100 g sterilized soil with 85.05% sand; 6.22% clay and 9.65% silt. The seeds were introduced into 180 jars at the rate of 60 jars per plant species with respectively 10 replications for each treatment applied. The seeds were irrigated with equal quantity of different concentrations of distillery effluent and the seeds irrigated with tap water were treated as control (T0). After seed germination, seedlings were thinned to ten healthy uniform young plants per jar and per treatment. The criterion of germination which was considered was the visible protrusion of radical from the seed coat and it was expressed in percentage (Ramana *et al*., 2002). The number of seed germination, the length of the radicle, seedling length, root length and shoot length were recorded for each plant species after 15 days of sowing. Ten seedlings were taken from each treatment and their shoot, radicle and root length were determined by using a cm scale and these values were recorded. Seed germination percentage, vigor index, tolerance index and phytotoxicity percentage of maize, beans and peanut under different concentrations of stillage were calculated using the following formulae.

$$\textbf{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total numbers of seeds sown}} x100$$

Vigor index of the seedling were calculated using the formula proposed by (Padma *et al*., 2015)

Vigor index = Germination percentage × Length of seedling.

The tolerance index was calculated by the formula of (Turner and Marshal, 1972).

$$\text{Indice de tolérance} = \frac{\text{avearge root length of the treatment with longest root}}{\text{average length of the longest rooth of the control}} x100$$

The percentage of phytotoxicity (Pp) was calculated using the formula proposed by (Chou *et al*., 1978).

$$\text{Percentage of phytotoxicity} = \frac{\text{Lenth of radicle of the control exeriment} - \text{length of radicle of treatment}}{\text{length of radical of the control experiment}} x100$$

The results and figures obtained were recorded using Excel 2016 software and XLSTAT (Addinsoft, 2025). Comparison of k samples was done using the Kruskal-Wallis and Dunn's procedure test. The Kruskal-Wallis test is often used as an alternative to the ANOVA where the assumption of normality is not acceptable. For the Kruskal-Wallis test, three multiple comparison methods are available. The method based on the comparison of the mean of the ranks of each treatment was used, the ranks being those used for the calculation of Ƙ. The normal distribution is used as the asymptotic distribution of the standardized difference of the mean of the ranks.

3. RESULTS AND DISCUSSION

Physicochemical characteristics of the distillery effluent

After the chemical characterization of the distillery effluents of alcohol production (stillage) from "Nodiscam" distillery, high concentrations of organic

matter, calcium, magnesium and potassium are presented, giving an inestimable value as fertilizer. (Table 1). Dilution of the stillage to obtain treatments T1, T2, T3 and T4 allowed a decrease in the concentration of all parameters. But the values obtained are higher than the WHO discharge standards. The pH obtained in the raw effluent T5 is acidic (4.41). Low electrical conductivity values are observed for treatments T1, T2 and T3. Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} ions show high concentrations for treatments T3 and T4. But the potassium values are very high. Four forms of nitrogen were measured in the vinasse effluents, namely: ammoniacal nitrogen (NH_4^{+}); nitrites (NO_2^{-}); nitrates (NO_3^{-}) and nitrogen of Kjeldahl (TKN).

The values of assimilable phosphorus (PO_4^{3-}) are also high in treatments T5 (9 g/L) and T4 (8.2 g/L). The high values of COD and BOD_5 show the richness of the vinasse in organic matter. The work carried out by (Arivoli *et al*., 2021) show concentrations of 29 mg/L of phosphorus and 19.6 mg/L of total nitrogen in the treatment with 60% stillage.

Table 1: Physicochemical characteristics of distillery effluent at different concentrations

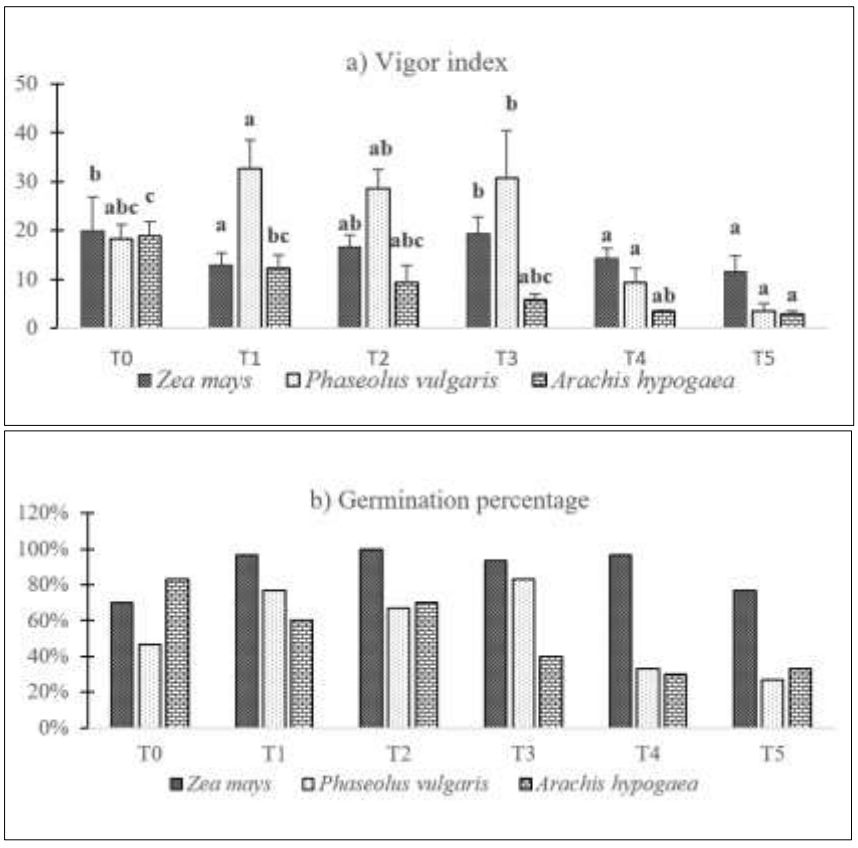
Parameter	T1	T2	T3	T4	T5 (Raw effluent)
pH	4.09	4.13	4.25	4.39	4.51
EC (µs/cm)	150.01	375.02	750.04	1350.08	1500.08
T °C	7.83	19.58	39.17	70.50	78.34
Turbidity (NTU)	21.8	54.4	108.8	195.9	217.7
Oxidation (mg/L)	57	144	287	517	574
CO_2 (mg/L)	156	391	781	1406	1562
Dissolved oxygen	0.54	1.36	2.71	4.88	5.42
MES (mg/L)	17.65	44.13	88.27	158.88	176.53
Color (Pt/co)	249.53	623.83	1247.67	2245.80	2495.33
Salinity (US)	0.98	2.45	4.9	8.82	9.8
Alkalinity (mg/L)	62	106	211	380	422
COD (g O_2/L)	20.86	47.15	54.3	97.74	108.6
BOD5 (g O_2/L)	16.75	41.87	83.74	150.74	167.49
NH4+ (mg/l)	2.4	6	12	21.6	24
Nitrites NO_2^{-} (g/L)	0.166	0.415	0.83	1,494	1.66
Nitrates NO_3^{-} (g/L)	0.8	2.8	4	7.2	8
PO_4^{3-} (g/L)	1	3.85	5	8.2	9
Mg^{2+} (mg/L)	8	19	38	69	77
Ca^{2+} (mg/L)	30	75	150	271	301
K^{+} (g/L)	93.31	158.3	316.5	569.7	633
Na^{+} (mg/L)	2.02	5.05	10.10	18.17	20.19
Cd^{2+} (mg/L)	0.01	0.02	0.04	0.07	0.07
Pb^{2+} (mg/L)	0.02	0.05	0.11	0.20	0.22
NTK (mg/L)	1.18	2.94	5.88	10.58	11.75
STD (mg/L)	102	125	231.15	416.08	462.31

Germination characteristics such as percentage of germination, germination index, vigor index and phytotoxicity percentage of maize, beans and peanut under different concentrations of stillage effluents are shown in Table 2. The germination of seeds of *Zea mays* showed a vigor index which has no significant difference between treatments T1, T2, T4 and T5; no difference between T0 and T3 but differences are observed between T0-T1; T0-T4; T0 –T5; T1-T3 and T3-T5. The tolerance index and phytotoxicity percentage are high for treatments T3, T2 and T5. However, no significant difference was observed between treatments. The germination of *Phaseolus vulgaris* seeds showed significant differences between treatments for the parameters vigor index, tolerance index and percentage of phytotoxicity (Figure 1 a, 1 c and 1 d). The germination of seeds of *Arachis hypogaea* shows significant differences between treatments for vigor index.

Table 2: Seed germination percentage (GP), vigor index (IV), tolerance index (TI) and phytotoxicity percentage (Pp) of maize, beans and groundnut under different concentrations of stillage

	Zea mays			
Treatment	GP	VI	TI	Pp
T0	70%	20±6.84b		
T1	96.66%	13.05±2.32a	40.19±19.04a	40.13±24.41a
T2	100%	16.68±2.49ab	60.93±35.29a	28.71±12.64a
T3	93.33%	19.43±3.31b	70.41±35.66a	34.42±15.77a
T4	96.66%	14.40±2.03a	54.79±29.07a	31.69±16.45a
T5	76.66%	11.68±3.16a	60.76±28.63a	57.79±20.30a
	Phaseolus vulgaris			
T0	46.66%	18.40±2.91abc		
T1	76.66%	32.77±5.72c	94.13±49.05b	19.44±12.20ab
T2	67%	28.55±3.91bc	93.90±80.82ab	10.63±8.03a
T3	83.33%	30.67±9.71c	40.52±20.36a	16.41±12.23ab
T4	33.33%	9.41±2.96cb	83.25±32.94b	22.65±19.71ab
T5	26.66%	3.50±1.59a	47.27±25.05ab	91.67±30.59b
	Arachis hypogaea			
T0	83.30%	18.95±2.91c		
T1	60%	12.23±2.86bc	97.20±10.37a	26.94±25.63a
T2	70%	9.36±3.61abc	82.88±45.44a	52.78±20.15a
T3	40%	5.8±1.25abc	51.42±22.90a	54.37±22.32a
T4	30%	3.45±0.3ab	88.48±18.44a	57.77±2.22a
T5	33.33%	2.91±0.63a	64.74±22.93a	63.33±4.72a

Figure 1.b shows that the three plants do not have the same germination percentage depending on the different treatments. However, for *Zea mays*, T2 has the highest germination percentage; followed by T3 for *Phaseolus vulgaris* and T0 for *Arachis hypogaea*. The percentage of phytotoxicity is high in T5 for *Phaseolus vulgaris*.



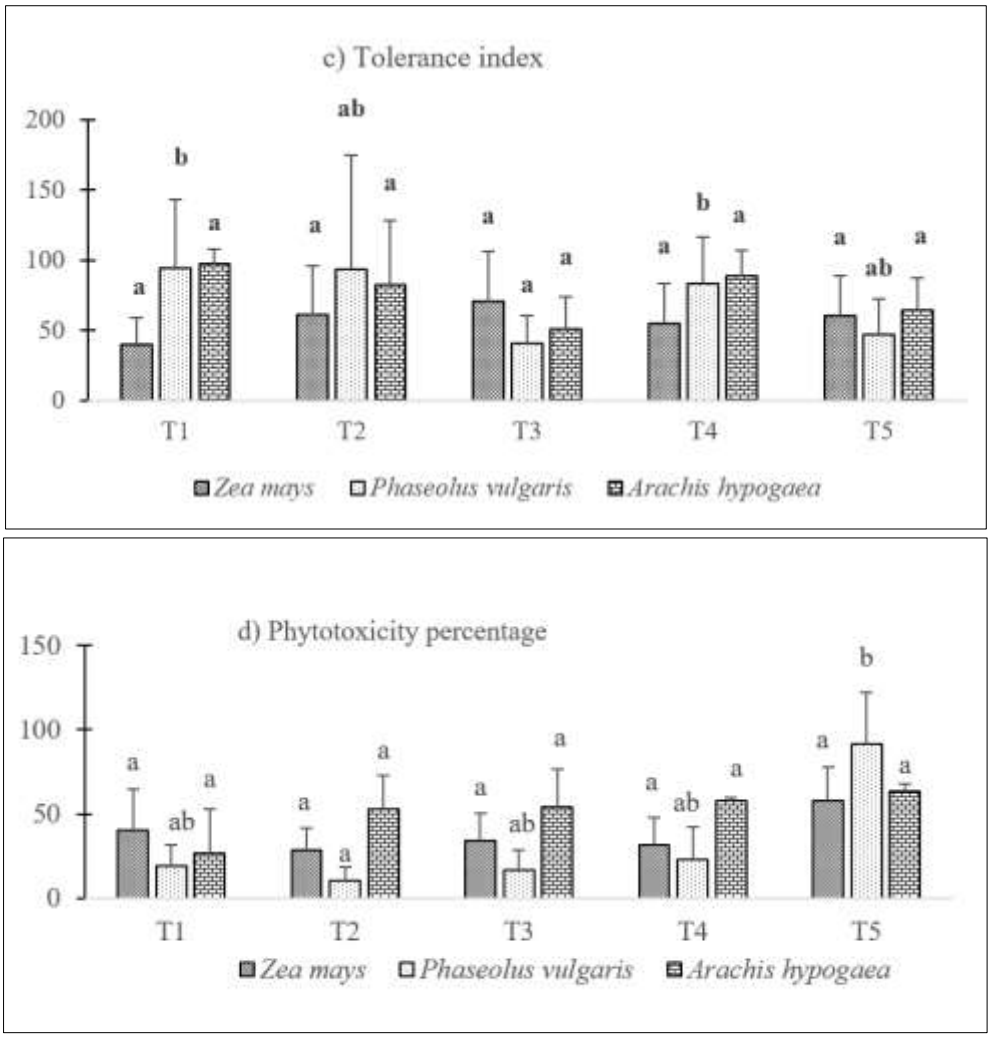


Fig.1: Impact of vinasse effluent at different concentrations on a) Vigor index,b) germination percentage, c) tolerance index and d) phytotoxicity percentage of *Zea mays*, *Phaseolus vulgaris* and *Arachis hypogaea*

The results presented in Table 3 show the effect of different stillage doses on the growth of *Zea mays*, *Phaseolus vulgaris* and *Arachis hypogaea* seeds. At the end of the germination process, it was determined that the diluted stillage had statistically significant effects on the measured parameters. The values obtained from the root measurement are high for the control test, for T3 and T4 in *Zea mays.* In *Phaseolus vulgaris* and *Arachis hypogaea*, it is the T0 treatments and the control tests which present root lengths greater than those in the treatments.

Significant differences are observed in *Zea mays* and *Phaseolus vulgaris* between the different treatments for root length. No difference observed between treatments in *Arachis hypoga*. The values obtained for the radicle length of *Zea mays*, *Phaseolus vulgaris* and *Arachis hypogaea* show significant differences between the treatments.

Radicle length is high for treatments T3, T4 and control in all three plants. For *Zea* mays, no significant difference was observed between the treatments. The radicle length is well developed in each plant. The control tests and treatments T0, T1, T2 and T3 show high values. Significant differences are observed for this parameter. Figure 2 shows the evolution of the different germination parameters measured on *Zea mays*, *Phaseolus vulgaris* and *Arachis hypogaea*. Figures 2.1.a; 2.2.a and 2.3.a; represent the radicle lengths respectively. The roots are well developed for treatments T0, T1 and T2 according to figures 2.1.b; 2.2.b and 2.3.b. Figures 2.1.c; 2.2.c and 2.3.c show the radicle length values which are high in treatments T0, T1, T2 and T3. The observation of figures 2.1.d; 2.2.d and 2.3.d show good plant growth for all treatments and for the radicle length parameter.

These observations are identical to the works of (Arivoli *et al*., 2021; Kumar, 2014) who showed that the high concentrations of sugar mill effluents inhibited the root and radicle length of seedlings. Figure 2 shows overall that treatments T1 and T2 increase the germination percentage and this may encourage the use of treated stillage as reported by (Da Silva *et al*., 2016). According to the physicochemical characteristics of the

stillage presented in Table 1 where T5 represents the raw effluent, it could be said that the high concentrations observed inhibited the germination of *Arachis hypogaea* given that it went from 10 seeds initially sown to only 4 plants that were able to survive until 15 days. Large amount of organic and inorganic substances presented in higher concentration of stillage affects the seed germination. Higher salt content can cause changes in osmotic pressure outside of the seedling (Hassan *et al*., 2021). The crop species differed widely in response to different concentrations of distillery effluent with respect to germination percentage, vigor index, tolerance index and phytotoxicity percentage. At lower concentrations, the distillery effluent did not inhibit seed germination in all the crops except *Phaseolus vulgaris* (Figure.1). In general, the germination (%) decreased with increase in concentration of the effluent but the germination was not inhibited in all the crops studied with concentration of 100% raw effluent. These results are in agreement with those observed by (Bastos *et al*., 2021; Ramana *et al*., 2002; Bustamante *et al*., 2005). The reason for the reduction in vigor index at higher concentration (T4 and T5) in all the crops and, even at lower concentrations (T0 and T1) could therefore be attributed to excessive quantities of inorganic salts and its higher electrical conductivity (EC), (Table.1). Distillery effluents concentration-dependent decrease in germination of maize (*Zea mays*), have also been reported (Padma *et al*., 2015). The work of (Ramana *et al*., 2002) showed that the effect of distillery effluent on seed germination is governed by its concentration and is crop-specificity. Similarly in this study it was observed that each plant reacted differently to each treatment. The composition of raw effluent (T5) can inhibit seed growth (Karaer, 2025; Tejeda *et al*., 2022). According to (Ungar, 2017) the germinating ability of different crops at high osmotic pressure differs with variety and species. The osmotic pressure of the effluent is higher at high concentrations which retarded germination (Akhtar *et al*., 2018). The diluted distillery effluents are beneficial for different crops as reported by (Baskar *et al*., 2003). The decline in seedling vigour index was similar to the earlier findings of (Nupur, 2025) in *Vigna radiata*.

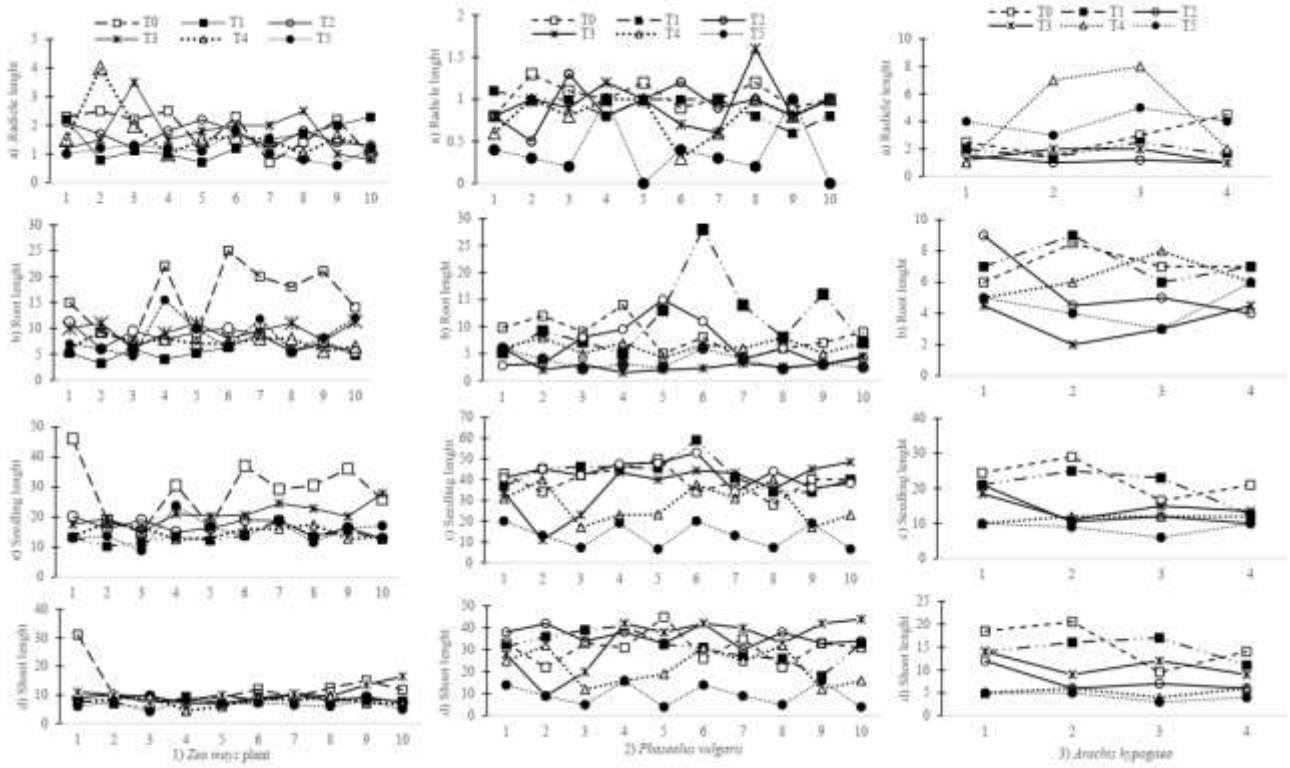


Fig.2: Effect of different concentrations of vinasse effluent on seed germination of 1) *Zea mays,* 2) *Phaseolus vulgaris*, and 3) *Arachis hypogaea*. a :Radicle lenght ; b : Root lenth ; c : Seedling lenght ; d : Shoot lenght

4. CONCLUSION

The results of the present study demonstrate that the effect of distillery effluent on seed germination is governed by its concentration and the specificity of the plant. Stillage can be used for crop irrigation, provided that it is applied in low concentrations with high potential for the sustainable use of this by-product as fertilizer complement in sugarcane culture. In this study, the concentration that presented the best performance among the treatment was 10% and 20%, with high potential for fertigation.

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Data availability: All data generated or analysed during this study are included in this published article.

DECLARATIONS

Ethics approval and consent to participate an ethics statement is not applicable in this study because the study does not involve human subjects and/or animals and/or any experiment which is under ethical consideration.

Consent for publication: Not applicable.

Plant material: The collection of plant material, complied with relevant institutional, national, and international guidelines and legislation.

Competing interests: The authors declare no competing interests.

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