

Original Research Article

Effect of Vegetable Oil Mill effluents on Physiological properties of *Brassica campestris* L. Seeds

Abstract

Comment [H1]: Revisit the abstract

This investigation aimed to test the Toxicity of soybean and mustard oil mill effluents and their impact on the Vitality Index, Seed Vigour Index, and Tolerance Index of seeds of Brassica campestris L. Certified seeds of variety Pusa Bold were obtained from the Department of Agriculture, Bundi district, Rajasthan, India. Healthy seeds with uniform size and shape were selected and sterilized with 0.1% HgCl₂ for 2 minutes and washed thoroughly twice with tap water, and soaked in distilled water for 2-3 days. Treated effluent samples were collected in plastic containers from outlets of mustard and soyabean oil mills of Bundi, Rajasthan and stored at 5°C then diluted from 0% oil mill effluent (water, control) to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% OME (not diluted). Two sets of experiments were set, one for mustard OME and another for soybean OME. Ten seeds were placed at equal distances in pre-labelled petri dishes containing filter paper soaked in water for each set of experiments. Three replicates of all the experiments were prepared and maintained. Data was collected on toxicity, vitality index on 15th day, seed vigor index on 15th day and tolerance index on 15th day. Results showed that at lower concentration of soybean and mustard oil mill effluents, all the parameters vitality index, seed vigour index, and tolerance index were favourable. Higher concentration of both influents, toxicity increases caused retardation of seed germination; the increase in toxicity was comparatively higher for soybean effluent than mustard effluent. Higher concentrations of vegetable oil mill effluent negatively affect seed vitality, vigour and this may be due to reduced tolerance and increased toxicity with higher concentrations of OME.

Key Words: Effluent, Vitality, Vigour, Seedling

Introduction

Industrialization brings economic prosperity to a nation leading to an increase in wealth, production of goods, standard of living, better transportation, better job opportunities, etc., none the less it brings the threat of pollution. Various industries are known to cause almost all possible types of pollution. In the wake of recent industrialization and fast urbanization, the quality of ground water in industrial areas has become an increasing concern due to contamination by various toxic chemicals (Meena *et al.*, 2010; Abskharan *et al.*, 2009).

Industries not only consume a lot of fresh water but also generate a huge amount of wastewater or industrial effluent that contains many inorganic-organic wastes as well as toxic elements. This production of wastewater is an inevitable outcome of industrial processes and is hazardous to the environment as in most cases, industries discharge their wastes into nearby fields, water bodies, and streams without proper treatment; which causes the introduction of many contaminants or potential

pollutants such as heavy metals into soil and groundwater which affect these precious resources (Quazilbash *et al.*, 2006; Azumi and Bichi, 2010). In the race for rapid industrialization and urbanization, groundwater quality in industrial areas is highly compromised due to contamination by industrial effluents, wastewater, and emissions, which are discharged without any treatment in these resources (Davihar, 2019; Abskharan *et al.*, 2009). It can result in deterioration of water quality and change in physico-chemical properties; by altering pH, temperature, chemical oxygen demand (COD), biological oxygen demand (BOD), heavy metal, and toxic elements.

India is an agriculture-based country and thus is a major consumer of water resources for irrigation (Singh *et al.*, 2005). The agricultural sector accounts for about 70% of total water usage, which makes it the largest consumer of freshwater worldwide (Elgallal *et al.*, 2016). The agricultural sector alone demands more water than utilizable renewable water resources (Kumar *et al.*, 2008). In water-scarce arid and semi-arid regions of the country, where the natural endowment of water is poor (Amarasinghe *et al.*, 2005), there is very high per capita water use in irrigation (Kumar *et al.*, 2008). In India poor irrigation systems and fast-track groundwater depletion result in 90% of water use in irrigation (Dhawan, 2017). Conventional water resources are more prone to contamination by industrial wastewater (Morrison *et al.*, 2001). The major risk associated with polluted water sources is that when this water is used for irrigation, it can potentially cause infectious diseases in grazing animals that feed on pastures irrigated with this water, and to people who feed on such crops (Bouwer, 2000).

Increasing population and agricultural activities cause great demand for water for irrigation while gallons of industrial effluents are disposed of untreated in nearby water bodies or fields. The rising demand for water in the agriculture sector and diminishing supply have made treated industrial effluents and domestic wastewater an attractive alternative to conventional irrigation. Application of industrial and domestic wastewater in irrigation may prove beneficial to plant growth as it provides nitrogen, phosphorus, and other nutrients to the soil, besides helping in water conservation (Rathore *et al.*, 2000). Thus, wastewater irrigation helps in nutrient recycling, reduces direct fertilizer inputs in agricultural fields, and minimizes pollution loads of the water bodies receiving wastewater (Hylander *et al.*, 2006; Thapliyal *et al.*, 2009). It was reported that most wastewater irrigation leads to an increase in the yield of most of the crops along with decreasing the demand for chemical fertilizers thus saving the total costs of farmers (Ezhilvannan *et al.*, 2011).

Effluent irrigated soil also shows change in physio-chemical properties. Untreated effluents affect soil parameters and ultimately soil fertility (Kisku *et al.*, 2000). Improper management of industrial waste products ultimately results in low productivity of soil by disposing of it in soil and agriculture fields

which leads to lower biological and chemical soil quality index and affects the overall soil quality index (Pushpanjali, 2017). Thus, industrial effluents eventually affect agriculture production and food security (Haferburg and Konthe, 2007). This paper aims to test the Toxicity of soybean and mustard oil mill effluents and their impact on the Vitality Index, Seed Vigour Index, and Tolerance Index of seeds of *Brassica campestris* L.

Material and Methods:

Certified seeds of *Brassica campestris* L variety Pusa Bold were obtained from the Department of Agriculture, Bundi district, Rajasthan, India. Healthy seeds with uniform size and shape were selected and sterilized with 0.1% HgCl₂ for 2 minutes, then washed thoroughly twice with tap water to remove traces of HgCl₂. Sterilization of seeds was followed by soaking in distilled water for 2-3 days.

Both the treated effluent samples were collected in plastic containers from outlets of mustard and soyabean oil mills of Bundi, Rajasthan, and stored at 5°C to preserve the physicochemical properties of the effluent. Effluent was then diluted from 0% oil mill effluent (water, control) to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% OME (not diluted). Two sets of experiments were settled, one for mustard OME and another for soybean OME. 10 seeds of *Brassica* were then placed at equal distances in pre-labelled petri dishes containing filter paper soaked in water for each set of experiments. Later on, 5ml of each prepared concentration of both mustard and soybean OME was used to irrigate both separate sets of treatment and then left in the dark for 48 hours for germination. Three replicates of all the experiments were prepared and maintained.

Seed vitality index was calculated using the following formula (Association of Official Seed Analysis, 1983).

$$\text{Vitality index (VI)} = S \times \text{GI}$$

Where GI is the Germination index and S is the length of seedlings.

SVI is calculated by multiplying germination percentage (GP) and seedling length (mm).

Seed vigour index = Germination percentage × length of seedling

The tolerance index of the seedling was calculated using the formula given by Turner and Marshal (1972):

$$\text{Tolerance index} = \frac{\text{Mean length of longest root in treatment}}{\text{Mean length of longest root in control}} \times 100$$

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

$$\text{Phytotoxicity \%} = \frac{\text{Radicule length in control} - \text{Radicule length in test}}{\text{Radicule length in control}} \times 100$$

Data was collected on toxicity, vitality index on 15th day, seed vigor index on 15th day and tolerance index on 15th day.

Observations and Results:

Vitality index (VI) decreases to almost 1/5 with increasing OME concentration (Table 1, Figure 1) as compared to control (without OME). After 20% OME, vitality index showed a more decreasing trend with soybean OME but after 50% OME concentration vitality index showed the same decreasing trend in both OME. The seed vigour index (SVI) also showed a marked decrease with increasing OME concentration (Table 1, Figure 1). Up to 10% OME concentration in both cases there is almost no negative impact, but after that soybean OME concentration shows more negative effect. SVI with both the OME follows the same pattern after 60% of both OME have almost the same negative effect on SVI. Tolerance index (TI) reduced to almost 50% with increasing OME concentration from 0% (water) to 100% (Effluent) (Table 1, Figure 1). Toxicity (Table 1, Figure 1) is more pronounced with soybean OME during the early germination period but later it shows that toxicity increases with increasing OME concentration with both the OME.

Table 1: Parameters related to the growth of *Brassica campestris* L. seedling with soybean OME (S OME) and mustard OME (M OME).

OME Conc. (%)	Toxicity (%)		VI on 15 th day		SVI on 15 th day		Tolerance index on 15 th day	
	S OME	M OME	S OME	M OME	S OME	M OME	S OME	M OME
0	0	0	56.88±2.	56.88±2.	650±4.	650 ±2.5	100	100

10	0	7.69±1.0 2	55.98±1. 69	55.12±1. 06	650±3. 69	640±5.6 1	100	98.46 ±2. 52
20	23±0.23	7.69±0.6 4	32.02±2. 33	35.52±1. 08	440±5. 21	488±5.2 2	85±2.6 1	93.85±3. 61
30	23±0.65	8.45±0.6 8	29.53±2. 01	35.21±2. 30	416±3. 25	496±3.0 5	80±5.1 1	95.38±3. 21
40	31±0.54	15±0.96	29.40±1. 23	34.80±1. 05	441±3. 55	522±3.1 4	75.38± 4.12	89.23±3. 33
50	30±1.69	30.77±0. 58	21.51±0. 63	22.83±0. 69	343±2. 31	312±2.1 5	75.38± 3.21	80±4.12
60	38±0.96	30.77±1. 06	25.93±0. 96	24.62±0. 94	336±3. 20	300±3.6 0	74±2.5 4	76.92±2. 14
70	62±2.33	46.15±1. 90	14.85±1. 05	15.59±0. 33	200±1. 96	210±1.9 8	62±3.2 5	64.62±6. 01
80	62±1.23	46±2.33	13.94±1. 62	15.07±2. 01	185±4. 21	200±2.1 1	57±2.5 5	61.54±2. 58
90	62±1.89	54±0.63	10.04±0. 68	11.96±1. 05	144±2. 14	156±3.0 5	55±2.1 1	60±2.91
100	62±0.98	53.85±2. 16	10.01±0. 56	11.9±0.6 8	180±3.2 5	180±1.58	55.38± 2.68	55.38±2. 55

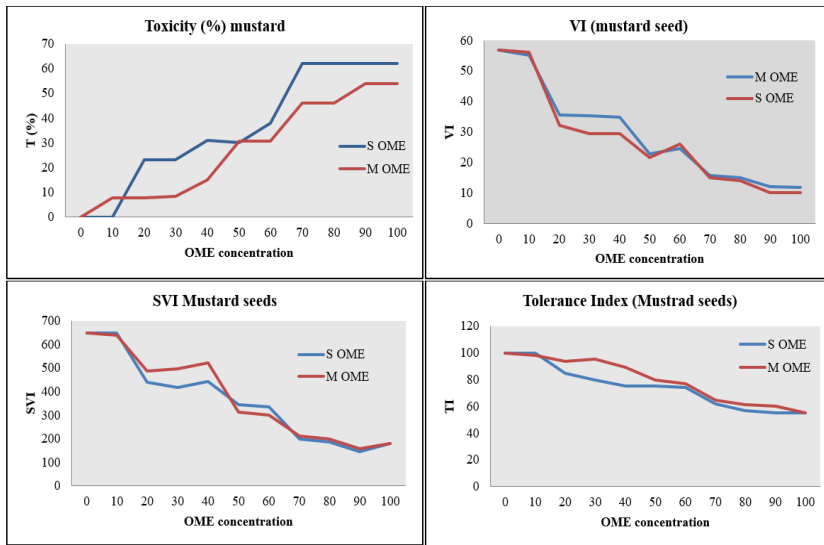


Figure 1: Effect of Soybean and Mustard OME on Toxicity (%), Vitality Index (VI), Seed Vigour Index (SVI), Tolerance Index (TI) of *Brassica campestris* L. seeds.

Discussion

Results of the present study prove that at lower concentration of soybean and mustard oil mill effluents, all the parameters studied viz. vitality index, seed vigour index, and tolerance index gives favourable results for *Brassica campestris* seeds. At the higher concentration of both influents, toxicity increases which causes retardation of seed germination; the increase in toxicity is comparatively higher for soybean effluent than mustard effluent. These findings found higher effluent concentrations to negatively affect seed germination which ultimately affect parameters under study.

Industrial effluents are usually considered as a serious problem but they can also be a solution for issues like chemical fertilizers and water scarcity when used in irrigation after proper treatment. Discharged effluent differs in quality based on the type of industrial raw material used. Therefore, physio-chemical examination of effluent is a must before its application in irrigation. Recent studies have shown the effect of the physicochemical properties of soil after the application of effluent as an irrigant (Regar and Jaiswal, 2020) and also proved that the effluent's pH, total suspended solids, and Chemical Oxygen demand can be brought to the permissible limit only after treatment (Regar and Jaiswal, 2020a). Usually, raw or untreated effluents are more toxic than treated effluents and are efficient in causing plant growth inhibition, thus the degree of treatment is a key determinant of wastewater quality (Ram Chandra *et al.*, 2004).

Many effluents may have high concentrations of heavy metals, some of which may have a major role in the growth and development of plants but are toxic beyond a certain level (**Edday *et al.*, 2006**). These metals along with many non-metal pollutants can easily enter the food chain and can disrupt mineral nutrient uptake by plants, inhibit photosynthesis, reduce enzyme activity, interfere with physiological processes, damage cell membranes, cease metabolite biosynthesis, and consequently reduce plant growth and yield (**Rengel *et al.*, 2016**; **Cole *et al.*, 2016**).

Seed vitality is one of the primary determinants of high yield that directly determines the performance of seedling growth and plant growth. It is usually defined as the ability of any seed to germinate and produce natural seedlings during provided growth conditions. The present study witnessed a reduction in seed vitality with increasing OME concentration, and seed vitality is directly dependent on seed germination and seedling growth. There are many possible reasons for delay in the germination of seeds and lower seedling growth. Some of these include salt concentration outside the seeds during germination (**Adriano *et al.*, 1973**); excess ammonia (**Kirkby, 1968**); higher solid concentration in effluent (**Saxena *et al.*, 1986**); and low oxygen supply (**Hadas, 1976**).

The seed vigour test is a key quality parameter, to test the performance of the seed lot in the field or storage, thus its assessment is important to supplement germination and viability tests. The seeds with a higher vigour index are considered to be more vigorous and are highly active during seedling emergence (**Abdul-Baki and Anderson, 1973**). Results of the present study state that seeds sown with a higher concentration of OME have low SVI and thus will be less active during seed germination and seedling emergence. This is probably due to the stressful seedbed environment of the field, as seeds are extremely sensitive to the physical stresses imposed by soil during germination and seedling expansion (**Whalley and Finch-Savage, 2006**).

Tolerance index of any seed is its ability to tolerate stress during seed germination and early development i.e., seedling development. Results of the present study state a reduction in tolerance index with increasing OME concentration i.e., seeds become less tolerant to higher OME concentration, which is probably due to an increase in stressful conditions with concentration. Seeds can withstand only a certain level of toxicity in a seedbed environment and their performance reduces after that. Soybean seeds are found to be more tolerant than mustard seeds for both types of OME.

Toxicity increases with increasing OME concentration. It may be due to the higher concentration of heavy metals, non-metals, and other contaminants of the OME, which not only affect seed germination but the overall growth and development of seedlings. The impact of toxicity of these pollutants or toxins

can also be seen in later stages of plant life as visible symptoms like chlorosis, yellowing and immature fall of leaves, poor growth, and retarded flowers, fruits, and seed yields. Therefore, it is necessary to screen crops for their sensitivity or tolerance variance to different effluents before using them in agriculture (Taghavi and Vora, 1994). Jothimani *et al.*, (2002) also focused on the need to assess the quality of treated wastewater and its effects on plant species before using it for crop production. Risk assessment and its management are also necessary before irrigation with wastewater (Salgota *et. al.*, 2006). Results of the work done by Regar *et. al.*, (2023) and Regar & Jaiswal (2020b) also show that at higher concentration of OME, seed germination and early seedling growth is negatively affected.

Conclusion

It is clear that irrigation with suitable effluent or wastewater may be beneficial up to a certain level of concentration, and the effect of different effluents can differ for the same seed. Higher concentrations of vegetable oil mill effluent negatively affect seed vitality, vigour and this may be due to reduced tolerance and increased toxicity with higher concentrations of OME.

References

1. Abdul-Baki A A, Anderson J O., (1973). Vigor determination in soybean application of dairy manure on germination and emergence of some selected crops. *J Environ Qual.* 3:396–399
2. Abskharan L.T., Ganesh, S., (2009). Chromium induced cytotoxicity in black gram (*Vigna mungo*). *Iranian Journal of Environmental health and science and Engineering.* 6(1): 17-22
3. Adriano, D. C., Chang, A. C., Pratt, P. F., & Sharpless, R. (1973). *Effect of soil application of dairy manure on germination and emergence of some selected crops* (Vol. 2, No. 3, pp. 396-399). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.
4. Amarasinghe, U. A., Sharma, B.R., Aloysius, N., Scott, C.; Smakhtin, V., de Fraiture, C., (2005). Spatial variation in water supply and demand across river basins of India. *IWMI Research Report 83. Colombo, Sri Lanka: International Water Management Institute.*
5. Association of Official Seed Analysts [AOSA], (1983). Seed Vigor Testing Handbook. AOSA, Ithaca, NY, USA. (*Contribution to the Handbook on Seed Testing, 32*).
6. Azumi S. Dan', M.H. Bichi, (2010). Industrial pollution and heavy metals profile of Challawa river in Kano, Nigeria. *J. Appl. Sci. Environ. Sanit.*, (5): 23-29.
7. Bouwer, H., (2000a). Integrated Water Management: Emerging Issues and Challenges. *Agricultural Water Management*, 45(3): 217-228.

8. Chou, C. H., Chiang, Y. C., & Kao, C. I., (1978). Impacts of water pollution on crop growth in Taiwan. II. Phytotoxic natures of six rivers and twenty-seven industrial wastewaters in Kaohsiung area, Taiwan. *Bot. Bull. Acad. Sinica* 19:107-124.
9. Cole J. C., Smith, M. W., Penn, C. J., Cheery, B. S., Conaghan, K. J., (2016). Nitrogen, phosphorus, calcium, and magnesium applied individually or as a slow-release or controlled-release fertilizer increase growth and yield and affect macronutrient and micronutrient concentration and content of field-grown tomato plants. *Sci. Hortic.* 21(1): 420–430.
10. Davihar R, Arvind PS, Sangeetha S P, Mohanakavitha T., (2019). Impact of Industrial Wastewater Disposal on Surface Water Bodies in Kalingarayan Canal, Erode district, India, *Indian Journal of Ecology.* 46(4): 823-827.
11. Dhawan V., (2017). Water and Agriculture in India, Background paper for the South Asia expert panel during the Global Forum for Food and Agriculture (GFFA)2(5). https://www.oav.de/fileadmin/user_upload/5_Publikationen/5_Studien/170118_Study_Water_Agriculture_India.pdf (Accessed on 20/10/2022)
12. Edday, N. O., Odoemelan, S. A, and Mbaba, A., (2006). Elemental composition of soils in some dumpsites, Electronically, *Journal of Environmental Agriculture and Food Chemistry*, 5(4): 1399-1465.
13. Elgallal M., Fletcher L., Evans B., (2016), Assessment of potential risks associated with chemicals in wastewater used for irrigation in arid and semiarid zones: a review. *Agric. Water Manag.*, 177: 419-431.
14. Ezhilvannan, D., P. S. Sharavanan, and M. Vijayaragavan, (2011). Effect of sugar mill effluent on changes of growth and amino acid and protein contents of maize (*Zea mays* L.) plants, *Journal of Ecobiotechnology*, 3(7): 26–29.
15. Hadas, A. (1976). Water uptake and germination of leguminous seeds under changing external water potential in osmotic solutions. *Journal of Experimental Botany*, 27(3), 480-489.
16. Haferburg, G and E. Konthe, (2007). Microbes and metals: Interactions in the environment. *Brazi. J. Microbiol.*, 39: 780-786.
17. Hylander, L.D., A. Kietliriska, G. Renman and G. Simon, (2006). Phosphorus retention in filter materials for wastewater treatment and its subsequent suitability for plant production. *Bioresource Technology*, 9(7): 914-921.
18. Jothimani, P., Prabakaran, J. and Bhaskaran, A., (2002). Characterization and impact of dyeing factory effluent on germination and growth of Maize (Co1) and Cowpea (CO-4). *Madras Agricultural Journal*, 89(10/12): 568-571.

19. Kirkby E. A. (1968). Influence of ammonium and nitrate nutrition on the cation balance and nitrogen and carbohydrate metabolism of white mustard plant grown in dilute nutrient solution. *Soil Sci.* 105-141.
20. Kisku G. C., Barman S., Bhargava K., (2000). Contamination of Soil and Plants with Potentially Toxic Elements Irrigated with Mixed Industrial Effluent and its Impact on the Environment, *Water Air and Soil Pollution.* 120(1): 121-137.
21. Kumar M. D., Sivamohan M.V.K., Narayanamoorthy A., (2008). Irrigation water management for food security and sustainable agriculture: The forgotten realities. Paper presented at the International Conference on Food Security, *Greenpeace, New Delhi.*
22. Meena, M. K., Dutta, S., & Pradhan, R. (2010). Environmental impacts of wastewater irrigation on groundwater quality—a case study of Ajmer city, Rajasthan (INDIA). *Elect J Environ Agric Food Chem*, 9, 760-766.
23. Morrison G., O. S. Fatoki, L. Persson, and A. Ekberg, (2001). Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River-pH, electrical conductivity, oxygen- demanding substance (COD) and nutrients,” *Water SA*, 27(4): 475–480.
24. Pushpanjali, Tejaswi, P.B., Sharma K.L., Mandal, U.K, Solomon Raju, A.J., Venkanna, K., Masanne, R., Samuel J., and Karthikeyan, K., (2017). Soil quality and heavy metal contamination of soils in Mindi industrial area, Visakhapatnam, Andhra Pradesh, India, *Indian Journal of Ecology*, 44(4): 774-777.
25. Quazilbash A. A., Farayal R., Naqui K. B., (2006). Efficacy of indigenous *Bacillus* species in the removal of chromium from industrial effluent. *Biotechnology*, 5(1): 12-20.
26. Ram Chandra, Kishan Kumar and Jaswant Singh, (2004). Impact of anaerobically treated and untreated (raw) distillery effluent irrigation on soil microflora, growth, total chlorophyll and protein contents of *Phaseolus aureus* L. *J. Environ. Biol.*, 2(5): 381-385.
27. Rathore, N. P., S. A. Iqbal and K. S. Panwar, (2000). Role of sugar industry in Agriculture. *Indian J. Appl. Biol.*, 15: 91-94.
28. Regar DL and P Jaiswal (2020a) Impact of vegetable oil mill effluents on soil characteristic in Bundi district, Rajasthan, India, *International Journal of Ecology and Environmental Sciences.* 2(4):25-29
29. Regar DL and P Jaiswal (2020b) Effect of vegetable oil mill effluents on seed germination and seedling growth of *Glycine max* (L.). *Eco. Env. & Cons.* 26 (2): 606-611
30. Regar DL, Dadhich P and P Jaiswal (2023) Assay on the Impact of Vegetable Oil Mill Effluent on Seed Germination and Seedling Growth of *Brassica campestris* L. and *Oryza sativa* L.; *J. Exp. Agric. Int.*, 45(2):1-9

Comment [H2]: Check if it is cited

31. Rengel Z., Bose J., Chen Q., Tripathi B. (2016). Magnesium alleviates plant toxicity of aluminium and heavy metals. *Crop Pasture Sci.*, 66: 1298–1307.
32. Salgota M., E. Huertasa, S. Weberb, W. Dottb, J. Hollenderb., (2006). Wastewater reuse and risk: Definition of key objectives, *Desalination*, 18(7): 29–40.
33. Saxena R. M., Kewal P. F., Yadav R. S., Bhatnagar A. K. (1986) Impact of tannery effluents on some pulse crops, *Indian J. Environ. Health.* 28(4). 345-348
34. Singh D. K., D. Kumar and V. P. Singh, (2005). Phytoremediation of contaminated soil. *Journal of Environmental Biology*, 5: 15-91.
35. Taghavi S. M. and Vora A. B., (1994). Effect of industrial effluent on germination and growth development of guar seed (var.PNB). *J. Environ Biol.*, 15(3): 209-212.
36. Thapliyal A., P. Vasudevan and M. G. Dastidar, (2009). Domestic wastewater for fertigation: A solution for water recycling and irrigation. *Proceedings of Micropollutant Ecohazard. 6th IWA/GRA Specialized conference on assessment and control of micro pollutants/ hazardous substances in water.* 8-10th June, San Francisco, USA, 317.
37. Turner R. G., Marshal C., (1972). The accumulation of zinc by subcellular fractions of root of *Agrostis tannis* sibth in relation of zinc tolerance. *New Phytol.* 71(4):671–676.
38. Whalley WR, Finch-Savage WE. 2006. Seedbed environment, In: Black M, Bewley JD, Halmer P, eds. *The encyclopedia of seeds: science, technology and uses.* Wallingford, UK: CAB International, 599–602