

Assay on the Impact of Vegetable Oil Mill Effluent on Seed Germination and Seedling Growth of *Brassica campestris* L. and *Oryza sativa* L.

Abstract:

Industries of any country are one of the key determinants of its economic wealth. Industries not only utilize a huge amount of water but also cause soil and water pollution by disposing of untreated industrial effluents in water bodies and agricultural land. This study aims to test the impact of mustard oil effluent on seed germination and seedling growth of *Brassica campestris* L. and *Oryza sativa* L. and to evaluate the scope of industrial effluent as irrigation water. Different parameters like germination percentage, germination index, germination rate, and mean germination time were evaluated with different concentrations of effluent to analyze the impact effluent had on plant growth. The results of the study show that industrial effluents can be used for irrigation only after appropriate dilution, increasing the concentration of effluent in irrigation water can lead to a reduction in macro and micronutrients concentration as well as heavy metal accumulation which results in the inhibition of seed germination. Thus, careful monitoring of the effects of any industrial effluent on any particular crop is a must before its application as the response of crops may vary with single effluent also.

Key Words: Industrial effluents, seed germination, seedling growth. **Introduction.**

Introduction:

Industrialization is an important indicator of the economic wealth of any country. But on other hand it gives rise to environmental pollution resulting in deteriorated soil and water quality, global warming, climate change, etc. Most industries utilize a huge amount of water and in turn, produce a considerable amount of effluents containing different organic and inorganic materials with toxic trace elements. An assessment of disposal of these effluents shows that only 60% of the total effluents disposed of are treated before disposal (Kaur, 2012), and the rest is discharged directly into the nearby water body or the soil itself.

Heavy metals and other toxins present in these effluents are a serious threat to the ecosystems (Amathussalam *et al.*, 2002). Discharge of industrial effluents without proper treatment can alter the physico-chemical properties of the receiving body by increasing or decreasing water pH, increase in temperature, change in Biological Oxygen Demand (BOD), Chemical

Comment [E1]: This study aims

Please use the past tense.

Comment [E2]: Key words:

Comment [E3]: Why...Introduction....as a key words?

Comment [E4]: (Kaur et al., 2012)

In fact, please respect instructions for authors for every citation in text:

Reference style

References must be listed at the end of the manuscript and numbered in the order that they appear in the text. Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated by the reference number in brackets [1].

Comment [E5]: *et al.*,
Not italic

Oxygen Demand (COD), heavy metals, and toxic chemical contamination. Such polluted water affects aquatic life and causes severe diseases in human beings when consumed (Morrison *et al.*, 2001), and when this water is used for irrigation causes infectious diseases in animals and humans who consume these effluent irrigated crops (Bouwer, 2000).

In agriculture-based countries like India, agro-based industries are major pillars of rural and socio-economic development and are considered to cause low pollution. In arid and semi-arid parts of the world, the use of rural and urban wastewater in agriculture is a century-old practice, but for quite some time due to scarcity of clean water, some farmers started to use industrial wastewater for irrigation (Rathore *et al.*, 2000). Around 10% of the total irrigated land utilizes untreated or partially treated wastewater globally (Winpenny *et al.*, 2013), and at least 10% of the population believes to consume wastewater-irrigated foods (WHO, 2006a & b; Hamilton *et al.*, 2007).

Using wastewater for irrigation can be beneficial or detrimental depending on the geographic region and type of wastewater used and their effect on various crops including vegetables (Ramana *et al.*, 2002). There are many reports suggesting effluent irrigation as a potential alternative to freshwater irrigation as it leads to the development of root system, increase the biological activity of soil (Hassanoghli *et al.*, 2005), increase soil fertility and permeability (Abedi Koupai *et al.*, 2006), increase soil nutrients and enhance soil properties (Rezapour *et al.*, 2012) and increase in the water-holding capacity (Saber, 1986). But on the other hand, the application of effluent as irrigation water can be detrimental as well as it contains toxic substances which may lead to soil pollution. Heavy metals like Cd, Ni, Pb, and Cr are present in high concentrations in many untreated or contaminated sewage and industrial effluents (Arora *et al.*, 1985; Narwal *et al.*, 1992). Many heavy metals like iron, molybdenum, manganese, zinc, copper, magnesium, copper, selenium, and nickel have a major role in the growth and development of plants but beyond a certain level, these are also toxic (Edday *et al.*, 2006). If soil contaminated with these heavy metals is used for the production of crops, these can easily enter the food chain (Principi *et al.*, 2006). These accumulated heavy metals and macronutrients, micronutrients, from using untreated wastewater in agriculture can disrupt the uptake of mineral nutrients by plants, inhibit photosynthesis, reduce enzyme activity, interfere with physiological processes, damage cell membranes, limit biosynthesis of metabolites, and consequently decrease plant growth and yield (Kochian *et al.*, 2004; Cole *et al.*, 2016).

Comment [E6]: Heavy metals like Cd, Ni, Pb, and Cr are present
Please explain.....like cadmium (Cd),.....and so on

The present study was conducted to study the effect of vegetable oil mill effluent on germination and seedling growth of 2 plant species; *Brassica campestris* L. and *Oryza sativa* L. and to evaluate the scope of industrial effluent as irrigation water. The study was conducted in the Bundi district in India where two vegetable oil mills (Bunge India Private Limited and Adani Wilmer Limited) are working for the last 20-25 years. Mustard and Paddy are the two crops that are main crops in the study area.

Comment [E7]: ...plant species (*Brassica campestris* L. and *Oryza sativa* L.) and...

Methodology:

The effluent was collected from oil mills (Bundi, Rajasthan) and stored at 4°C to preserve the physico-chemical properties of the effluent. Certified seeds of *Brassica campestris* L. variety Pusa Bold and *Oryza sativa* L. variety Kaveri 888 were collected from the Department of Agriculture, Bundi District, Rajasthan, India. Seeds that were healthy and uniform in shape and size were selected, sterilized with HgCl_2 , and then washed thoroughly twice to remove traces of HgCl_2 . These seeds were then soaked in distilled water for 2-3 hours.

Comment [E8]: Methodology:

I recommend:

Material and Methods

The section needs to be organised.

Please clearly present,
The experimental site

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The biological material

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The experimentally variants

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The parameters determined

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Statistical analysis

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Two sets of experiments were then settled one for mustard seeds and the other for rice seeds. Effluent is then diluted to 0% oil mill effluent (OME, control), 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% (not diluted). 10-10 seeds of mustard and rice were then placed equidistantly in pre-labeled Petri dishes containing filter paper soaked in water. After this 5ml of each prepared concentration of OME was added to both separate sets of treatment and then left in dark for 48 hours for germination. Three replicates of all the treatments including the control were prepared and maintained.

Comment [E9]: ...mercuric chloride (.....)

Comment [E10]: Effluent was then

Comment [E11]: ...this 5mL.....

Please use every time....mL.
Not....ml.

Since, the first physiological process of plant growth is seed germination Growth parameters like seed germination, germination percentage, and seedling growth are considered as criteria to assess the degree of impact on plant growth caused by pollution. The germination percentage (Association of Official Seed Analysis, 1983) was recorded on the 10th day (Sundaramoorthy *et al.* 2006), and seedling length was taken and recorded on the 15th day of seedlings growth (10th day of seed germination was taken as 0 days for seedling growth). The values of germination percentage, germination index, germination rate, and mean germination time (Ellis and Roberts, 1981) were observed for each set of treatments.

Comment [E12]:seed germination, growth

Comment [E13]: I recommend to useThe final germination percentage (FGP) (%)....

Comment [E14]: ...[] as I said before.....for every citation in text, please.

a). Germination percentage = $\frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$

Comment [E15]: I recommend: Final germination percentage (FGP)(%)=

b). Germination index = $\frac{\text{No. of germinated seedlings}}{\text{Days of first count}} + \frac{\text{No. of germinated seedling}}{\text{Days of final count}}$

Comment [E16]: Germination index (GI)=

Please, No. of germinated seeds.....NOT germinated seedlings!

c). Germination rate = $\frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \dots + \frac{X_n - X_{n-1}}{Y_n}$

Comment [E17]: Germination rate (GR)(the expression unit....)=

Where X_n = number of germinations on the nth day

Y_n = number of days from the first-day experiment

d). Mean germination time = $\frac{\sum Dn}{\sum n}$

Comment [E18]: Mean germination time (MGT)(the expression unit....)=

Where, n = no. of seeds that have germinated on day D

D = no. of days counted from the beginning of the experiment

Data for seedlings growth were is collected 15 days after 10 days of germination (the 10th day of germination was is considered 0 days for seedling growth) for both *Brassica campestris* L. and *Oryza sativa* L.

Observations and Results:

Comment [E19]: Results -bold

Different concentrations of OME have a significant effect on germination percentage (GP), germination index (GI), germination rate (GR), and mean germination time (MGT). In the case of treatment 1 (Mustard seeds), maximum germination was found at 0% (control) and 10% OME which gradually decrease towards 100% OME. The same trend is observed in the case of GI and GR. But in the case of MGT a reverse trend is observed i.e., it increases with increasing concentration of OME.

Comment [E20]: Resultsmean germination time (MGT)(Table 1).

In the case of treatment 2 (Paddy seeds); data obtained is quite a similar trend i.e., GP, GI, and GR are maximum at 0% OME or control while on the other hand, MGT increase with increasing concentration of OME.

Table 1. Parameters related to the germination of *Brassica campestris* L. and *Oryza sativa* L. seeds with mustard oil mills effluent (OME).

Comment [E21]: Data shown mean ± SE(or SD)? N=3?

OME Conc. (%)	GP		GI		GR		MGT on the 10 th day	
	Paddy	Mustard	Paddy	Mustard	Paddy	Mustard	Paddy	Mustard
0	100	100	6.39±0.63	8.75±0.62	1.75±0.01	2.18±0.63	1	1

Comment [E22]: Please explain also Final germination percentage (FGP) Germination index (GI)...and so on...

Statistical analysis is kindly recommended.

Comment [E23]: MGT

10	90±2.98	100	4.54±1.44	8.61±0.02	1.40±0.25	2.15±0.12	1.11±0.05	1
20	60±3.66	80±1.09	3.59±0.96	5.82±0.31	1.02±0.06	1.48±0.06	1.67±0.12	1.25±
30	80±2.85	80±2.34	3.80±0.56	5.68±1.01	1.17±0.04	1.46±0.11	1.25±0.13	1.25
40	80±3.74	90±0.22	3.80±0.22	6±0.61	1.17±0.61	1.58±0.67	1.25±0.04	1±0.02
50	70±1.69	60±0.69	2.79±1.20	4.39±0.09	0.94±0.02	1.1±0.22	1.43±0.05	1.67±0.04
60	60±2.68	60±1.50	2.25±1.32	4.92±0.12	0.79±0.31	1.18±0.26	1.67±0.15	1.67±0.01
70	70±5.12	50±0.56	2.66±0.98	3.71±0.62	0.93±0.01	0.93±0.06	1.43±0.21	2±0.11
80	70±3.99	50±0.68	2.90±0.87	3.77±0.33	0.96±0.08	0.94±0.02	1.43±0.06	2±0.02
90	30±2.01	40±1.62	1.77±0.77	3.07±0.69	0.48±0.03	0.76±0.13	3.33±0.11	2.5±0.03
100	40±0.89	50±1.01	2.11±1.05	3.36±0.51	0.60±0.03	0.78±0.60	2.5±0.15	2.5±0.05

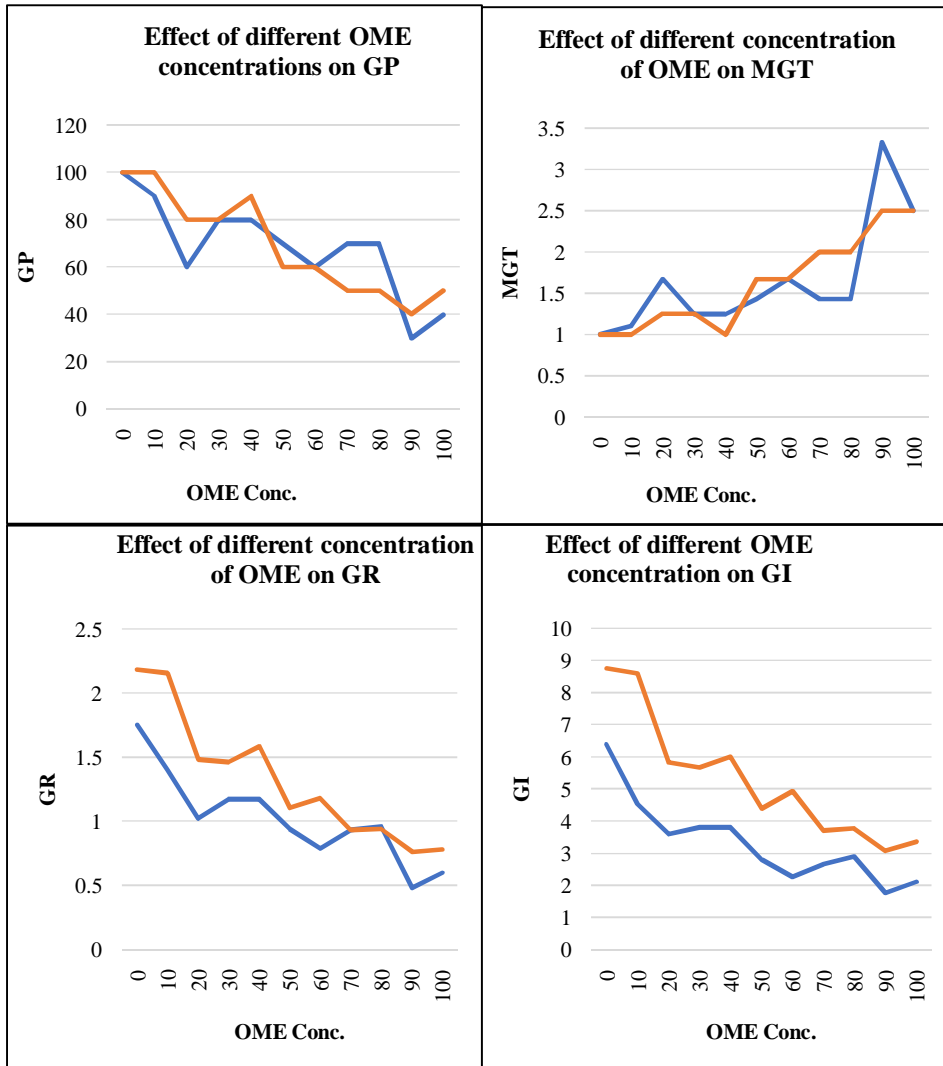
Table 2. Seedling length of *Brassicacompestris* L. and *Oryzasativa* L. after 15, 30, 45, and 60 days with mustard oil mills effluent (OME) treatment.

Comment [E25]: Table 2. Seedling length (cm) of.....

Comment [E26]: Data shown mean ± SE(or SD?). N=3?

Statistical analysis is kindly recommended.

Effluent conc. %	15 Days Seedling		30 Days Seedling		45 Days Seedling		60 Days Seedling	
	<i>Oryzasativa</i> L.	<i>Brassicacomp</i> <i>estris</i> L.	<i>Oryzasativa</i> L.	<i>Brassicacomp</i> <i>estris</i> L.	<i>Oryzasativa</i> L.	<i>Brassicacomp</i> <i>estris</i> L.	<i>Oryzasativa</i> L.	<i>Brassicacomp</i> <i>estris</i> L.
0	18.4±3.08	6.5±1.05	29.4±3.22	10.8±2.37	56±2.98	57±3.21	67.8±3.64	61.8±3.33
10	18.2±3.69	6.5±0.59	29.1±2.69	10.7±1.59	56±3.68	57±3.11	67.2±5.37	61.2±2.15
20	18.1±2.99	5.5±1.32	28.4±1.54	10.4±2.54	56±2.69	55.7±1.89	66.2±3.99	60.4±1.25
30	17.9±0.97	5.2±0.99	27.5±1.62	9.9±2.33	49.2±3.54	54.2±2.14	64.8±4.67	59.9±1.98
40	17.8±1.58	4.9±1.01	27±1.28	9.2±0.62	48.3±1.09	53±0.88	63.1±2.11	58.8±0.59
50	15.2±2.55	4.9±2.05	26.5±3.66	8.7±0.54	48±2.67	52.4±0.69	61.2±6.21	58.2±1.05
60	14.7±3.11	4.8±0.06	26.1±1.89	8.5±1.08	46.6±2.14	50.8±2.10	58.3±3.27	56.7±1.66
70	14.5±2.14	4.0±0.59	24.8±2.05	8.7±0.78	45.4±1.68	50.1±0.99	56.7±2.17	55±1.87
80	14±2.58	3.7±0.32	24.5±1.57	8.2±1.12	45.4±3.68	49.4±1.57	55.4±2.91	54.3±2.36
90	12.9±2.09	3.6±0.56	24.1±1.69	7.7±0.67	41.3±2.18	48.2±1.33	52.5±3.64	54.1±2.55
100	12.5±0.08	3.6±0.24	24±3.46	5.7±0.64	40.1±2.10	48.2±2.11	50.5±5.77	52.5±3.11



— Rice — Mustard

Figure 1: Effect of OME concentrations on different germination parameters.

Results on seedling growth show that in both cases i.e. *Brassica* and *Oryza* early seedling growth (15 days or 30 days) was more negatively affected by OME; afterward, seedlings develop tolerance to OME.

Comment [E27]: Figure 1: ?
 If in the Figure 1 there are presented the same data as inside the Tables, please delete the Figure 1.
 Data will be presented only as a one format.

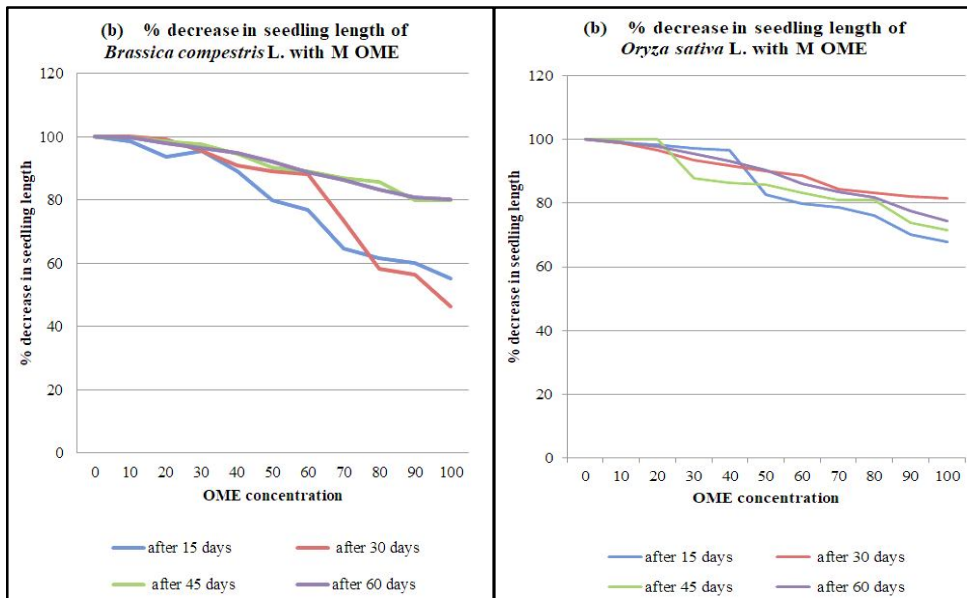


Figure 2: Percentage decrease in Seedling length of *Brassicacompestris* L. and *Oryzasativa* L. after 15, 30, 45 and 60 days with mustard OME.

Comment [E28]: First of all....the indication for the Figure 2, inside the text.

Figure 2. Percentage decrease in seedling length (cm) of
Why bold....Percentage decrease in?

Discussion:

Industrialization is an inevitable cause of pollution, by dumping untreated effluents affects both water and soil quality (Quazilbash *et al.*, 2006). In past, some poor countries used land disposals of municipal, agricultural, and industrial wastes as irrigation water as it is a source of nutrients and organic matter, due to the high cost of chemical fertilizers (Younas & Shahzad, 1998). Irrigation with wastewater has multifold benefits as it reduces the cost of irrigation, lowers the application of chemical fertilizers, conserves water, and minimizes pollution loads of the water bodies where these effluents have to be disposed of (Hylander *et al.*, 2006; Thapliyal *et al.*, 2009). Most of the crops give higher yields when irrigated with wastewater (Ezhilvannan *et al.*, 2011).

The edible seed oil industry is known to produce resources, water, energy, and wastes in large amounts; and it has been observed in many cases that the wastes and by-products are not utilized to their full potential (Welz *et al.* 2017). Mostly biodegradable organic substances are present in wastewater from oil refining units (Aslan *et al.*, 2009). Being a by-product of agricultural produce, waste from the edible oil industry has great potential for further valorization and water reuse.

The present study found an increase in mean germination time with increasing effluent concentration. There are many possible explanations for this higher salt concentration in polluted water which delays germination (Khan and Sheikh, 1976); the excess amount of ammonia causes depletion of the tricarboxylic acid cycle, reducing the respiration rate of seeds which ultimately leads to low seed germination (Kirkby, 1968). Higher concentration of solids in the effluent can also reduce energy supply by anaerobic respiration which in turn restricts the growth and development of the seedling (Saxena *et al.*, 1986); restriction of energy supply due to low oxygen to the seeds which retards the seedling growth and development (Hadas, 1976).

Seed germination percentage also reduces with increasing effluent concentration. It was reported in a study that the lowest concentration of heavy metals in industrial effluent has the least harmful effect on germination whereas at higher concentration strength of germination is significantly reduced (Peralta *et al.*, 2004). Other than this; high TDS and TSS can also change the osmotic relation between seed and water, which causes retardation of seed germination by increased salinity. The rate of germination as well as the seed germination index shows a decreasing trend with increasing OME concentration.

It is reported in the present study that the length of both radicle and plumule shows a decreasing trend with increasing OME concentration. Similar results were obtained by Bazai and Achakazai, 2006 i.e., a decrease in seedling growth with increasing OME concentration. Many authors reported that metal interference with cell division and cell elongation is the key cause of heavy metal-mediated root inhibition (Kidd *et al.*, 2004; Rout *et al.* 1997).

At lower concentrations of OME, seed germination, as well as seedling growth, are not affected, but a higher concentration of OME is known to inhibit both. Higher electrical conductivity and excessive inorganic salts are possibly the cause of this. However, effluent quality obtained from the same industry can also vary based on physical, chemical, and biological methods used for oil extraction and wastewater treatment, but untreated effluent is always more toxic than treated effluent. Results of the present study prove that at lower concentrations of mustard oil mill effluent, all parameters taken under study (GP, GR, GI, MGT) gives favourable results. A low concentration of effluents not only favours seed germination but promotes the overall growth of the plant. These findings suggest that higher effluent concentrations were found to negatively affect plant growth, but diluted effluent favoured seedling growth (Ayyasamy *et al.*, 2008).

Comment [E29]:high TDS and TSS ...
Please also explain first the abbreviations.

Comment [E30]: In fact.....the seedling length
was determined, not separately for radicle and
plumule.

The increasing demand for water for the agriculture sector and diminishing availability have made use of industrial effluents after proper treatment, an attractive alternative for irrigation. The use of treated effluent not only sorts the problem of water shortage but can prove beneficial to crop plants because of the nutrients present in it (Sahai *et al.*, 1985; Mishra and Behera, 1991).

Conclusion:

Results of the present study revealed that the response of *Oryza sativa* L. to mustard OME is comparatively more inhibitory way than *Brassica campestris* L. This concludes that different crop species have varying tolerance towards industrial OME, and that vegetable oil effluent can be used as potential irrigation water only after careful monitoring of crop species' tolerance towards that particular effluent. Results have also shown that OME can be used for irrigation only after appropriate dilution; increasing the concentration of OME in irrigation water can lead to a reduction in macro and micronutrient concentration as well as heavy metal accumulation which results in the inhibition of seed germination. Thus, proper treatment of industrial effluent as well as proper assessment of its effects on particular crop plants is a must before its application as an irrigation alternative.

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Comment [E31]: <https://journaljeai.com/index.php/JEAI/about/submissions>

Reference style

References must be listed at the end of the manuscript and numbered in the order that they appear in the text.

Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated by the reference number in brackets [1].

So, the first one will be:

1. Kaur

Example:

Reference to a journal:

For Published paper:

1. Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. *Clin Exp Allergy*. 2002;32(4):489-98.

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