# ECO-PHYSIOLOGICAL EFFECTS OF DAIRY EFFLUENT ON SEED GERMINATION AND SEEDLING GROWTH OF *BRASSICA JUNCEA* (L.) CZERN. & COSS.

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**Abstract:** Samples of dairy effluent were collected from Parag Milk Plant, Meerut. The different concentrations (25, 50 and 100%) of treated and untreated effluent were used in this experiment. Tap water served as control. Effects of dairy effluent were studies on *Brassica juncea* L., which is grown as oil crop in India. It was observed that 25% conc. of dairy effluent increased the germination percentage, seedling growth, dry weight of root and shoot, chlorophyll content and seed yield as compared to control.

**Keywords:** *Brassica juncea*, dairy effluent, germination, Meerut, pH, seedling growth

## INTRODUCTION

Industrial revolution and modern agriculture have resulted high standard of living and socio-economic per capita status of man, however, repudiated pollution (air, soil, water and sound) create problem in developed and developing countries. Infect, pollution is the result of urban-industrial technological revolution and speedy exploitation of every bit of natural resources, which have created adverse effects on flora, fauna and non-living components of ecosystems.

Rapid industrialization has left with us polluted air, green house gases, choked rivers, depleted ground water and ozone layer. Gangol Sehkari Dugdh Utpadak Sangh Limited, Partapur, Meerut is one of the 10 modern dairy industries (Pasteurization plant) of western U.P. and located geographically 29° north & 77° 40' east and situated 60 km north east of Delhi. Parag dairy generates 2000 to 3000 liters waste water after pasteurization of milk, cream, butter, milk cake, cheese powder etc. from milk.

The dairy waste water contains caustic soda, amyl alcohol, nitric acid and sulphuric acid with high B.O.D. and acidic pH (6.5) but contains fat 20%, proteins 0.15% and 109 mg/l lactose (Rodionova *et al*., 1989 and Fang, 1990). Therefore keeping above facts in view, present study was designed to evaluate the risk assessment of effluent and environmental management at Simbhaoli and its effects on *Brassica juncea* L. which is oil food crop and grown in agricultural fields.

## MATERIAL AND METHOD

The present investigation has been conducted to observe the physico-chemical characteristics of dairy effluent of Gangol Sehkari Dugdh Utpadak Sangh (Parag) Limited, Partapur, Meerut. The dairy effluent was collected at 10 a.m. for physico-chemical examination. The untreated samples were collected at the main drain of industry before entering the (E.T.P.). The treated samples were collected from outlet of the aeration tank, where biological treatment was given.

The present investigation has been aimed to obtaining the basic information of the effect of dairy effluent on *Brassica juncea* L. Czern & Coss. at Environmental Science Laboratory, Post Graduate Department of Botany, Kisan (P.G.) College, Simbhaoli. The material consisted of three cultivars of *Brassica juncea* viz., Pusa Bold, Pro-Agro 4001 and T-59.

Observations of seed germination and seedling growth were recorded. Chlorophyll content was estimated following the method of Smith and Benitez (1955). Data were analyzed statistically and critical difference (CD) was calculated at 5% level of significance. Stomatal index was observed by following formula:

$$\text{Stomatal index (S.I.)} = \frac{S}{S+E} \times 100$$

Where, S = No. of stomata per unit area and E = No. of epidermal cells per unit area

Phytotoxicity percentage (P.P.) was determined by following Chou and Muller (1972)

$$\text{Phytotoxicity percentage (P.P.)} = \frac{\text{Root length of control} - \text{Root lentgh of treatment}}{\text{Root length of control}} \times 100$$

The following yield parameters were also recorded:

1- Days to 50% flowering
2- Harvest index

$$\text{Harvest index (\%)} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

### Short term exposure experiments

Uniform, healthy and viable seeds were selected for germination. The seeds of test crop were surface sterilized with 0.1% mercuric chloride solution for 5.0 minutes and afterwards washed 5-6 times with distilled water to remove its traces. 10 seeds were arranged equidistantly in sterilized petri dishes (9 cm dia) lined with Whatman No. 1 filter paper. 10 ml of dairy effluent with 25, 50 and 100% conc. were gently poured to petri dishes and observed seed germination percentage, seedling growth and phytotoxicity percentage.

**Long term exposure experiments**

For long term exposure experiments, seeds were sown in polythene bags with a diameter of 25 cm. Each bag contained 3 kg of sandy loam soil which was well pulverized and homogenized. Ten seeds of test crop were sown (kept on soil surface) per pot. Immediately, seeds were covered with 500 g dry sieved soil to prevent crust formation over the germinating seeds. After the completion of germination, the polythene bags were irrigated with tap water as per requirement.

## RESULT

The seeds of *Brassica juncea* L. Czern & Coss. were germinated in petridishes, polythene bags as well as in field conditions also. Meteorological data were recorded for climatic factors. Physico-chemical characteristics of Parag dairy effluent have been observed. The untreated effluent revealed B.O.D. (64 mg/l), C.O.D. (160 mg/l) and treated effluent with B.O.D. (34 mg/l), C.O.D. (96 mg/l), whereas permissible limit of B.O.D. for fish is 30 mg/l and 250 mg/l for soil.

Germination of all the three cultivars has been studied with three concs. of untreated and treated effluent. It was observed that seed coat burst just after two hours in 25% conc. (breaking the seed dormancy) and seeds showed the beginning of germination on second day. Significantly higher germination percentage has been recorded in 25% conc. over 50% as well as 100% concentrations (Table 1). However, treated effluent showed higher seed germination percentage than untreated effluent in all the three concentrations. Nevertheless, 100% waste water revealed significant reduction in germination percentage over the control. It may be due to more osmotically active substances present in effluent, which creates unfavourable environment for the growth.

Effect on radical length has been observed and data revealed that its length ranges higher in 25% conc. over the control, while in 50% and 100% it reduced significantly in cv Pusa Bold and similar trends were also observed in cvs P.A. 4001 & T. 59 of *B. juncea*.

The findings on the effect of the untreated and treated effluent on average plumule length in *B. juncea* cv Pusa Bold revealed that plumule appeared on the fourth day of experiment. 50% and 100% concentrations were found inhibitory to the plumule length. On account of an intermediate acidity, nutrient like nitrogen, phosphorus, potassium are not available or fortified to seeds at the time of germination due to higher B.O.D. and C.O.D. except in 25% conc., whereas showed significant result in cvs P.A. 4001 & T- 59 follow the same pattern.

Total seedling phytomass of treated plant increased gradually up to 90 days and afterwards decreased in all the cvs of Indian mustard. Phytotoxicity percentage in root and shoot showed the order- 25 > 50 > 100 at 30 days of plant in both untreated and treated effluent due to ecotoxicity of salts and acidic pH.

Appearance of 50% flowering have been observed at 63 days (25%) 66 days (50%) and 68 days (100%) and 62 days (25%) 66 days (50%) and 67 days (100%) conc in untreated and treated effluent respectively, over the control (65 days) in all three cultivars. Thus initiation is slightly advanced under the stress of acidic nature but the data are not statistically significant. Besides, it was significant in cv T- 59 at 25% conc., where enhances formation of florigin hormone which depicts early flowering in effluent treated soil. Average values of no. of leaves and branches have been studied. No. of leaves increases up to 90 days and thereafter decreases because senescence starts in leaves (source and sink relationship) to fall down. The effect on no. of siliqua$^{-1}$ and harvest index revealed significant results of all three cvs recorded in treated as well as untreated effluent at 25% conc., while significant reduction at 100% conc in untreated and treated effluent. The same pattern occurs in all the particulars.

The relative sensitivity of three cvs of dairy effluent has been evaluated as follows: *Brassica juncea* cv Pusa Bold > P.A. 4001 > T- 59 for all attributes. Therefore, Pusa Bold is more stable, productive and tolerant hence, superior than the rest two and recommended to grow so that oil seed production may be enhanced for “Yellow Revolution” and sustainable agriculture.

**Table 1.** Effect of Parag dairy effluent on seed germination and other parameters of *Brassica juncea* cultivars.

| S. No. | Particulars | DAS** | Cultivars | Control | Untreated Effluent | | | Treated Effluent | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 25% | 50% | 100% | 25% | 50% | 100% |
| 1 | **Germination (%)** | 5 | Pusa Bold | 84.22 | 86.60 | 80.66 | 56.66 | 93.33 | 83.33 | 60.00 |
| | | | Pro Agro 4001 | 83.33 | 85.33 | 79.33 | 54.66 | 92.33 | 82.66 | 59.33 |
| | | | T-59 | 83.10 | 84.33 | 78.66 | 53.33 | 90.66 | 81.33 | 58.70 |
| 2 | **Radical length (cm)** | 10 | Pusa Bold | 9.70 | 11.33 | 9.63 | 7.03 | 12.66 | 10.63 | 8.13 |
| | | | Pro Agro 4001 | 8.63 | 10.30 | 9.13 | 7.01 | 12.30 | 10.33 | 8.10 |
| | | | T-59 | 8.61 | 9.60 | 9.00 | 7.00 | 12.10 | 10.30 | 8.05 |
| 3 | **Plumule length (cm)** | 10 | Pusa Bold | 10.63 | 12.10 | 10.30 | 8.13 | 12.90 | 10.90 | 8.13 |
| | | | Pro Agro 4001 | 10.03 | 11.90 | 10.20 | 8.03 | 12.60 | 10.30 | 8.10 |
| | | | T-59 | 9.43 | 11.30 | 10.05 | 8.01 | 12.03 | 10.03 | 8.05 |
| 4 | **Seedling phytomass (mg)** | 15 | Pusa Bold | 80.33 | 82.66* | 70.33* | 50.54* | 95.33* | 78.66* | 60.66* |
| | | | Pro Agro 4001 | 75.66 | 77.33 | 65.66 | 48.33* | 90.66* | 73.33* | 59.34* |
| | | | T-59 | 71.33 | 73.66 | 61.34 | 44.66* | 84.34* | 69.66 | 53.66* |
| 5 | **Growth index (G.I.)** | 15 | Pusa Bold | - | 1.02 | 0.96 | 0.88* | 1.03 | 0.97 | 0.90 |
| | | | Pro Agro 4001 | - | 0.96 | 0.95* | 0.88* | 1.03 | 0.97 | 0.89* |
| | | | T-59 | - | 1.01 | 0.96* | 0.89* | 1.03 | 0.97 | 0.89* |
| 6 | **Phytotoxicity percentage (P.P.)** | 30 | Pusa Bold | - | -6.50* | 5.30* | 41.40* | -10.34* | -2.69* | 36.95* |
| | | | Pro Agro 4001 | - | -8.18* | 7.42* | 44.02* | -11.02* | -1.39* | 38.10* |
| | | | T-59 | - | -10.98* | 5.03* | 42.22* | -12.56* | 0.34* | 37.64* |
| 7 | **No. of Siliquae per plant** | 75 | Pusa Bold | 106.4 | 110.2 | 98.3* | 56.4* | 130.1* | 99.4* | 70.2* |
| | | | Pro Agro 4001 | 96.2 | 100.4* | 94.2* | 50.1* | 126.6* | 99.3* | 68.3* |
| | | | T-59 | 95.3 | 97.0* | 90.0* | 44.4* | 124.1* | 94.4* | 64.5* |
| 8 | **Stomatal index** | 75 | Pusa Bold | 14.6 | 8.9 | 10.4 | 12.4 | 7.7 | 8.7 | 8.7 |
| | | | Pro Agro 4001 | 13.23 | 7.8 | 9.3 | 11.3 | 6.3 | 9.6 | 9.2 |
| | | | T-59 | 10.11 | 7.6 | 8.6 | 10.2 | 5.9 | 6.7 | 9.4 |
| 9 | **Days to 50% flowering** | 75 | Pusa Bold | 65.0 | 63.0 | 66.0* | 68.0 | 62.0 | 66.0 | 67.0 |
| | | | Pro Agro 4001 | 66.0 | 64.0 | 67.0 | 68.0 | 63.0 | 66.0 | 68.0 |
| | | | T-59 | 69.0 | 65.0* | 70.0 | 71.0 | 64.0* | 69.0 | 70.0 |
| 10 | **Harvest index** | - | Pusa Bold | 65.10 | 69.10* | 61.10* | 52.30* | 71.28 | 62.28* | 53.18* |
| | | | Pro Agro 4001 | 64.90 | 67.28 | 59.27* | 51.60* | 69.83* | 60.18* | 52.40* |
| | | | T-59 | 63.60 | 66.35* | 57.80* | 49.15 | 68.25* | 69.58* | 50.88* |

*Significant at 5% level **Days after sowing

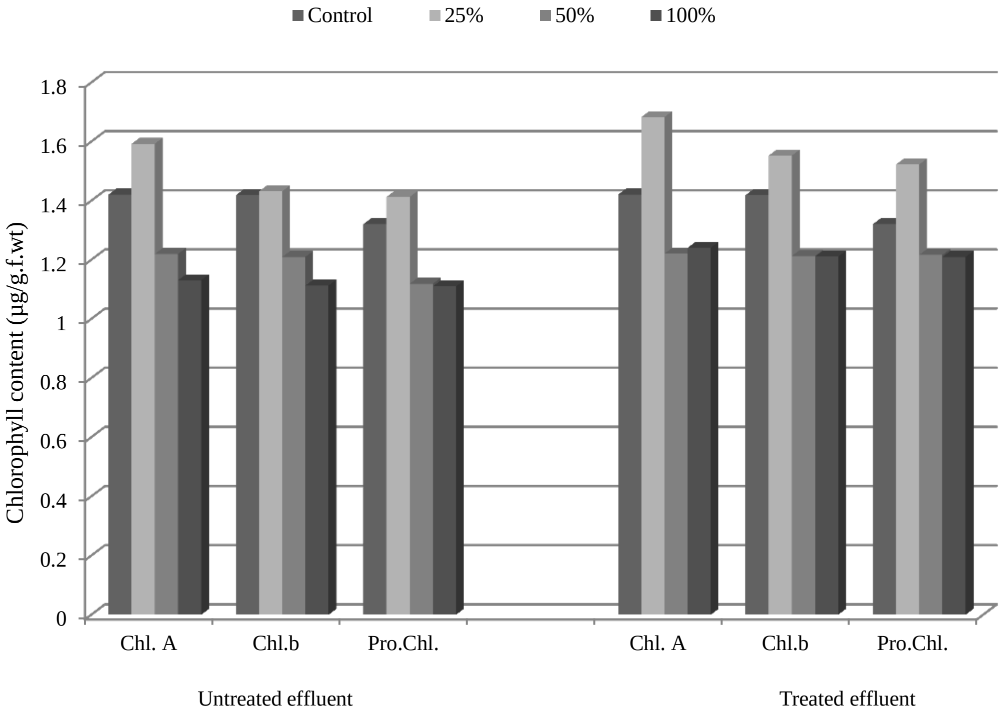


**Fig. 1**. Effect of untreated and treated dairy effluent on chlorophyll content in cotyledonary leaves of *Brassica juncea* cv Pusa Bold.

## DISCUSSION

Climate change, eutrophication and acidification (acid rain) are the process by which soil and surface water are depleted alkalies and consequently suffer an increase in acidity, results in damage to terrestrial and aquatic ecosystems. Much surface water is entirely devoid of fish, amphibians and vegetations. There has been significant damage to forest in the world.

Observations revealed that the effect of temp of effluent on plants is negligible. Thus factor is not injurious to crops which are irrigated with dairy waste water. Nevertheless, high temp of distillery effluent adversely affects the plants by alternation in salt absorption, cellular enzymes, membrane lipids and denaturation of proteins as well as brings about an imbalance between respiration and photosynthesis. It can cause chlorosis, injure and kill the protoplast (Edwards, 1932).

It was observed that the pH of the waste water used for irrigating the mustard crop in present investigation is acidic (6.23) but no noticeable effect was observed in the soil pH of all the studied plots. It may be due to the buffering capacity of the soil and leaching of salts.

Furthermore, it is also worth mentioning that pH of the waste water is under the limits of ISI (Indian Standard Institution) ranging from 5.5 to 9.9 (Naik *et al*., 1995). However, Srikantha and Srinivasamurthy (1999) have discussed 6.75 to 8.88 and 7.50 to 9.90 for treated and untreated dairy effluent, respectively due to the presence of sodium which comes from cleaning agent. The analysis of dairy effluent indicates excessive quantities of inorganic salts, organic matters and total solids applied for irrigation the crops in comparison to suggest by ISI. Misra (1965) concluded that effluent containing total solids over 2100 mg/l adversely affected crops. Observations noted that the germination of all cultivars of *B. juncea* is similar to earlier reports of dairy effluent and maximum effects have been observed with the concentrated dairy effluent followed by Naik *et al.*, 1995 and Srikantha *et al.*, 1997. The 25% conc of the effluent showed stimulatory effect whereas, higher concs (50 and 100%) significantly reduced the germination. Inhibition of seed germination at higher concs may be due to high levels of dissolved solids (D.S.).

Goel and Mandavakar (1983) concluded that 10% diluted distillery effluent can be effectively used for *Cyamopsis tetragonaloba*. Choudhary *et al*. (1987) has been found that in *Zea mays*, the germination was promotive up to 25% conc of paper mill effluent. Ali, G. (2002) have suggested the neutralization of pH by lime to dairy effluent before irrigating the effluent into mustard crop and recommended 25% conc for agricultural purpose. Therefore, industrial waste should be given desirable pre-treatment to reduce the necessity of higher dilution (need of water) for use in agriculture.

It was observed that the dairy effluent influenced the growth of the radical as well as plumule significantly. It was increased at 25% conc and reduced significantly at 50% and 100% concentrations of dairy effluent. Our results further support strongly to the findings of Naik *et al*. (1995) and Srikantha *et al*. (1998). They found that 25% conc of effluent significantly increased the plumule length whereas higher conc (100%) decreased significantly.

It was observed that lower conc (25%) enhanced formation of florigin hormone which depicts 50% early flowering in effluent treated soil. Higher conc create disturbance in hormone. Similar findings have been reported in mustard (Dongal *et al*., 1990). They opined that higher level of fertilizer delayed the flowering in comparison to the lower levels.

A decrease in harvest index and no. of siliquae were also recorded in the present study. Similar results reported by Evans and Sorger (1989). It has been studied that the higher conc of dairy effluent decreased the amount of chlorophyll a, chlorophyll b and proto chlorophyll in all cvs of *B. juncea* and maximum chlorophyll occurred in 25% dairy effluent treatment (Fig 1, 2 and 3). The reduction in chlorophyll is due to the low uptake of nutrient from soil due to lower dissolved oxygen and salt uptake which involved in the structure and synthesis of chlorophyll molecule (Treshow, 1970). Further, chlorophyll reduction may be attributed to the reduction in $Mg^{+2}$ ions which are present in chlorophyll molecule. Therefore, deficiency of $Mg^{+2}$ ions will lead to less chlorophyll synthesis. Potassium deficiency also decreased chlorophyll synthesis (Treshow, 1970). After entering the leaves, dairy effluent dissociated into $H^+$, $HSo_3^-$, $No_3^-$ and cause degradation of chlorophyll molecule by displacing $Mg^{+2}$ ions by $H^+$ ions from the tetrapyrrole ring of chlorophyll molecule resulting in the formation of photosynthetically inactive brown pigment phaeophyrin (Malhotra, 1977 and Shimazaki and Kiyashi, 1980). Accumulation of $So_4^{-2}$ and $No_3^{-1}$ may lead to the breakdown of chlorophyll and interaction between these acidic ions results into the inhibition of metabolic activity of the chlorophyll.

Hindawi *et al*. (1980) observed loss of chloroplast integrity in the injured leaves of *Phaseolus vulgaris* as a result of acid rain. Higher conc of effluent showed decreasing trend in total chlorophyll content over control. An increasing trend has been marked with the passage of time in particular conc of effluent. The chlorophyll content of fresh leaves decreases at 50% and 100% concs of effluent but increases at 25%. It may be due to higher T.D.S., T.S.S., B.O.D. and C.O.D. present in the effluent (Sahai and Neelam, 1987). Zinc present effluent also reduced the chlorophyll content (Khandelwal and Aery, 1993).

## Recommendation

Research findings recommended that effluent should be properly treated before its disposal into available land then it could be used as liquid manure, which is one the best practical method for the disposal of dairy effluents to illuminate pollution problems for "evergreen revolution". Therefore, effluent treatment plants should be installed in each pasteurization plant like distilleries to check water pollution so that water bodies like Holy Ganga can be saved from deterioration until and unless the sewage and industrial effluent are not treated properly and their mouth should be diverted towards agricultural crop without which Ganga and Yamuna action plans result will not be exciting for the management of water budget and conservation of water resources in the country.

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