MATERIALS AND METHODS

In the present investigation for quick decomposition of sugar mills and distillery waste (PMC, flyash and spent wash) collected from the Simbhaoli Sugar Mills (SSM), Simbhaoli, Ghaziabad and its distillery division. Compost of agro-industrial wastes were analyzed for physicochemical properties by standard procedure (Table-1&2) with water hyacinth (*Eichhornia crassipes*) from pond and decomposer bioinoculant *Trichoderma viride* in powder form. Envirophysiological effects of compost on seed germination percentage, growth, phytomass, chlorophyll content and yield of *Coriandrum sativum* (L.) cv. Kalmi (spice crop) and *Phaseolus aureus* Roxb. cv. K-851 [*Vigna radiata* (L.) Wilczek] (pulse crop) plant⁻¹ and test weight of 1000 seeds were studied. Sustainable impacts on physicochemical properties of the soil were also investigated.

I. EXPERIMENTAL PLANT

*Coriandrum sativum* L. cv. Kalmi, a spice crop and *Phaseolus aureus* Roxb. cv. K-851 [*Vigna radiata* (L.) Wilczek] (pulse crop) were selected for experiment.

II. GERMINATION STUDIES

The experiment was conducted in petriplates 9 cm. diameter having a depth 1 cm. The conc. of the distillery effluent were diluted with tap water as follows:

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 %</td>
<td>5 % PTDE + 95 % Tap Water</td>
</tr>
<tr>
<td>25 %</td>
<td>25 % PTDE + 75 % Tap Water</td>
</tr>
<tr>
<td>50 %</td>
<td>50 % PTDE + 50 % Tap Water</td>
</tr>
<tr>
<td>100 %</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Control</td>
<td>Tap water</td>
</tr>
</tbody>
</table>

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(a) **EFFECT OF DISTILLERY EFFLUENT ON SEED GERMINATION**

The 10 seeds were pre-treated with 0.1 % mercuric chlorides solution for five minutes to sterilize than followed by a thorough washing with water and air drying before keeping them in 9 cm. petriplates (15) for germination in triplicates concentrations (5 %, 25 %, 50 % and 100 %) with control (Tap water). The petriplates were supplied with 10 ml of respective conc. of the first day and 5 ml subsequently on alternate days at room temperature. Observation were recorded daily for a period of 10 days for parameters like seed germination length and dry weight of radicle and plumule.

**III. DECOMPOSITION PROCESS**

(a) **EXPERIMENTAL SETUP**

The organic substrates for decomposition were studied in accordance to container (Theopate et al., 1991). The organic residues viz. PMC (Press Mud Cake)-6 kg, FA (Flyash)-100 gm, DSW (Distillery Spent Wash)-6 litter (2+2+2 in 8th, 16th and 24th days), Eichho (*Eichhornia crassipes*)-350 gms and Tricho (*Trichoderma viride*)-150 gms were mixed in following treatments:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No Effluent</td>
</tr>
<tr>
<td>PTDE</td>
<td>PMC + FA + PTDE + Eichho + Tricho</td>
</tr>
<tr>
<td>T₁</td>
<td>PMC + FA + DSW</td>
</tr>
<tr>
<td>T₂</td>
<td>PMC + FA + DSW + Tricho</td>
</tr>
<tr>
<td>T₃</td>
<td>PMC + FA + DSW + Eichho</td>
</tr>
<tr>
<td>T₄</td>
<td>PMC + FA + DSW + Eichho + Tricho</td>
</tr>
</tbody>
</table>
For all treatments, spent wash and PMC was uniformly added at 2:2 (W/V) PMC : DSW. After mixing the above materials placed in the plastic container (trays). These were covered by a black polythene sheet to maintain temperature and humidity. Turning of ingredient was done to supply \( \text{O}_2 \) (Aerobic) for the decomposition after 3\(^{rd}\) days upto 32\(^{nd}\) days.

(b) **MONITORING OF COMPOST**

During the composting process, temperature, pH, EC, moisture content, organic carbon and major nutrients (N.P.K.) were recorded on 0, 8\(^{th}\), 16\(^{th}\), 24\(^{th}\) and 32\(^{nd}\) days to composting separately.

(i) **Temperature \((^\circ\text{C})\)**: The temperature was observed everyday upto 32\(^{nd}\) day in degree centigrade \((^\circ\text{C})\) unit by inserting a thermometer in sub-surface layer of bio-resources heap which revealed variation in phases (Psycrophillc, Mesophillic, Thermophillic, Stabilization and Poikiloothermic).

(ii) **pH value**: The pH of the composting mixture were determined by glass electrode method (1:2) Soil : Water (Jackson, 1967) under different phases.

(iii) **Electrical conductivity (EC)**: The EC of the compost material were determined by Salt method (1:2) Soil : Water (Jackson, 1967).

(iv) **Organic Carbon (OC) \%**: Organic carbon of compost samples was determined by chronic acid titration wet digestion method as outlined by Walkey and Black (1934).
(v) **Nitrogen (N) %**: For nitrogen determination modified Kjeldahl microanalysis was adopted (Piper, 1966).

(vi) **Phosphorus (P) %**: The total phosphorus was analysed of soil, plant and fertilizers method and outlined by Bhargwas and Raghupati (1993).

(vii) **Potassium (K) %**: The potash was estimated with the help of flam-photometer (Jackson, 1967).

### IV. MATURITY EVALUATION OF COMPOST

There are different indicators that predict the compost maturity tested through odour, appearance, moisture content, C/N, C/P and C/K ratio (Chauhan et al., 2007).

(a) **C/N ratio**: Carbon : Nitrogen (C/N) ratio was computed by dividing the OC % by N value.

(b) **C/P ratio**: Carbon : Phosphorus (C/P) ratio was computed by dividing the OC % by P value.

(c) **C/K ratio**: Carbon : Potassium (C/K) ratio was computed by dividing the OC % by K value.

### V. PLANT BIOASSAY

Plant bioassay test for maturity of compost was investigated by seed germination index \[G.I. = \text{Germination(\%)} \times \text{root growth}\] test using *Coriandrum sativum* L. cv. Kalmi and *Phaseolus aureus* Roxb. cv. K-851. Compost extracts were prepared by shaking compost samples with deionized water at a solid:water=1:2 (W/V) ratio at 180 rpm for 1 hour followed by centrifugation at 3000 rpm for 20 min and then filtered through a 0.5 \(\mu\)m membrane filter. Ten seeds
were placed in a 100x15 mm petridish containing 4.0 ml compost extracts of PTDE, T₁, T₂, T₃ and T₄ treatments. Deionised water was used as the control treatment. The number of germinated seeds were counted after 6, 12, 18, 24 and 48 hours on 32nd days of composting at 25°C to 27°C in the dark (Lau and Wong, 2001).

VI. EXPERIMENTAL SITE AND WEATHER CONDITIONS

Interplay of Physicochemical-Envirophysiological factors of compost and soil were analyzed at Environmental Science Laboratory, P.G. Department of Botany, Kisan (P.G.) College, Simbhaoli, Ghaziabad and CPRI (Central Potato Research Institute), Modipuram, Meerut following the standard procedures.

Keeping in the view weather factors, meteorological data [Temperature (max & min), Rainfall and Humidity] were also recorded (Figs.-1, 2 & 3).

VII. ENVIROPHYSIOLOGICAL STUDY

In present envirophysiological study of the effect of mature compost on Coriandrum sativum (L.) cv. Kalmi (Dhania) and Phaseolus aureus Roxb. cv. K-851 (Moong Bean) has been carried out during the year 2004, 2005 and 2006. The compost effect on seed germination, growth and yield were observed in petriplates, poly bags and field conditions. The seeds of Coriandrum sativum (L.) cv. Kalmi and Phaseolus aureus Roxb. cv. K-851 were procured from recognised centers of Seed Store Meerut and Hapur.

For germination test of 10 seeds of Coriandrum sativum (L.) cv. Kalmi and Phaseolus aureus Roxb. cv. K-851 were
taken to study in petriplates with 5 %, 25 %, 50 % and 100 % concentration and control with tap water and petriplates and polythene bags with compost as @ 5 qt./acres.

VIII(a) GERMINATION STUDY IN COMPOST (PETRIPLATES)

Surface of seeds was sterilized with 0.1 % HgCl₂ solution for 20 minutes and washed thrice. Twenty seeds of *Coriandrum sativum* (L.) cv. Kalmi and *Phaseolus aureus* Roxb. cv. K-851 than transferred to 9 cm. petriplates (18) with compost (0.757 mg compost + 150 gm soil) treatments (PTDE, T₁, T₂, T₃ and T₄) and control for germination in triplicates treatments. The petriplates were supplied with 10 ml. tap water subsequently on alternate days at room temperature (min 12°C and max 20°C or min 22°C and max 32°C). The seedling was dissected into cotyledons radicals and plumule and subjected to fresh and dry weight measurements, seedling were kept in over at 48°C to 55°C for dry weight. The data on germination %, vigour index and seedling growth were studied on 3rd, 5th, 7th and 10th DAS.

VIII(b) POLY BAG CULTURE

Seeds of *Coriandrum sativum* L. cv. Kalmi and *Phaseolus aureus* Roxb. cv. K-851 were sown in month of October and July in poly bags with a diameter of 25 cm. in triplicate treatment. Each bag contains 1.59 gm compost + 3 to 5 kg sand loam soil for Dhania and Moong Bean, respectively, which was well pulverised and homogenised. Twenty seeds were sown in each bag after emergence of seedlings, thinning was done. Only 5 plants were left per bag. During the course of experiment, plants were irrigated as required by tap water.
VIII(c) FIELD TRIAL

The seeds of *Coriandrum sativum* L. cv. Kalmi and *Phaseolus aureus* Roxb. cv. K-851 were sown in month of October and July in field trial and plot spacing from 1 m$^2$ with 0.123 kg (123 gm) (@ 5qt./acres or @ 1250 kg/hectare) compost in triplicate treatments. Twenty seeds were sown in each plot after emergence of seedling thinning was done only 5 plants were left. During the course of experiment plants were irrigated as required by tap water.

VIII(d) RECORDING OF DATA

1. **Sampling of cultivars**: Seed germination studies were conducted in petriplates, polybag culture and field trial with 20 seeds each treatments and every replicates. The sampling had been selected after 30$^{th}$, 45$^{th}$, 60$^{th}$, 75$^{th}$, 90$^{th}$ and 105$^{th}$ DAS in poly bags and field conditions.

2. **Analysis of cultivar samples**: Five samples were taken for only weight of seedling from polythene bag. Treated and control samples were collected at different intervals for root, stem, leaf and fruit. The samples were dried nearly 8 hours at 75$^0$C in an oven for phytomass.


   (i) Seed germination (%) and germination relative index (GRI)

   (ii) Length of radicle and plumule (cm plant$^{-1}$)

   (iii) Length of root and shoot

   (iv) Leaf area (cm$^2$ plant$^{-1}$)
(v) Dry weight of seedlings (mg plant\(^{-1}\))
(vi) Vigour index (V.I.)
(vii) Shoot : Root Ratio (SRR)
(viii) Chlorophyll estimation-proto chl., chl. 'a' and chl. 'b'
(ix) Plant height (cm plant\(^{-1}\))
(x) Phytomass (mg plant\(^{-1}\))
(xi) Days to first flower inflorescence/bud initiation
(xii) Days to 50 % flowering (plant\(^{-1}\))
(xiii) No. of umbel/pod (plant\(^{-1}\))
(xiv) No. of seeds (plant\(^{-1}\))
(xv) Days to maturity
(xvi) 1000 seeds weight (gm)
(xvii) Seed yield gm plant\(^{-1}\)
(xviii) Biological yield plant\(^{-1}\)
(xix) Net primary productivity (N.P.P.)
(xx) Harvest Index (H.I.) % plant\(^{-1}\)

(i) Seed germination (%) : Seed germination was calculated by following formula:

\[
\text{Germination} \% = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds planted}} \times 100
\]

(ii) Germination Relative Index (G.R.I.) : G.R.I. has been calculated as per the following equation:

\[
\text{G.R.I.} = (S) \times n (Kn-n)
\]

where,
Xn = no. of germinated seeds on \( n^{th} \) day

K = total no. of seeds

n = number of days

(iii) **Length and dry weight of radicle and plumule** : The length and dry weight of radicle and plumule were recorded in cm. and mg. With the help of thread, scale and fractional weight box as well as chemical balance respectively.

(iv) **Shoot Root Ratio (S.R.R.)** : The shoot root ratio was observed by the following formula:

\[
\text{Shoot-root ratio (SRR)} = \frac{\text{Dry weight of shoot}}{\text{Dry weight of root}}
\]

(v) **Seedling vigour index (S.V.I.)** : It was recorded as per following formula of Abdulbaki and Anderson (1973):

\[
\text{S.V.I.} = (\text{Root} + \text{Shoot Length}) \times \text{Germination %}
\]

(vi) **Leaf area (cm}^2\) : Leaf area cm\(^2\) plant\(^{-1}\) at different age of plant was estimated by the following formula:

\[
\text{Leaf area} = \text{length (cm)} \times \text{width (cm)} \times 0.678
\]

(vii) **Plant height** : Length of plant in cm plant\(^{-1}\) was recorded from ground level to the top of the longest branch on 30\(^{th}\), 45\(^{th}\), 60\(^{th}\), 75\(^{th}\), 90\(^{th}\), and 105\(^{th}\) DAS.

(viii) **Phytomass** : Phytomass (gm plant\(^{-1}\)) was studied with the help of chemical balance at different age of plants. Samples of (root and shoot) whole plant weight were kept in a perforated paper bags and dried at 80°C for 8 hours. The drying process continued till the dry weight
biomass become constant. The dried samples were weighed at room temperature and final dry weight was recorded. The samples were collected on 30\textsuperscript{th}, 45\textsuperscript{th}, 60\textsuperscript{th}, 75\textsuperscript{th}, 90\textsuperscript{th} and 105\textsuperscript{th} days after sowing (DAS).

(ix) **Days to initiation of inflorescence, first flower bud initiation and 50 % flowering**: The number of days from the date of sowing to the initiation of inflorescence, first flower bud and the appearance of flowers in 50 % plants was recorded.

(x) **Number of seeds umbel\textsuperscript{-1}/pod\textsuperscript{-1}**: These attributes were also simply counted.

(xi) **1000 seeds weight**: Weight of 1000 seeds were weighed by chemical balance.

(xii) **Seed yield plant\textsuperscript{-1} (gm)**: The seeds were weighed by chemical balance.

(xiii) **Biological yield (gm plant\textsuperscript{-1})**: The total plant dry weight excluding roots at harvest was pooled and weight recorded from each bags. Biological yield was calculated by following formula:

\[
\text{Biological yield} = \text{seed yield} + \text{phytomass}
\]

(xiv) **Net primary productivity plant\textsuperscript{-1}**: Net primary productivity N.P.P. was calculated as per following formula:

\[
\text{N.P.P.} = \frac{\text{Dry weight of whole plant}}{\text{Plant age}}
\]
(xv) **Harvest Index (H.I.) plant\(^{-1}\):** Harvest Index (%) was calculated by following formula-

\[
\text{H.I.} = \frac{\text{Seed yield plant}\(^{-1}\)}{\text{Biological yield plant}\(^{-1}\)} \times 100
\]

(xvi) **Chlorophyll estimation**

To determine chlorophyll content of leaves, seedling 0.250 gm of plant material was homogenised in a mortar and pestle with calcium. 10 ml of 80% acetone by adding a pinch of CaCO\(_3\) while grinding. The homogenate was centrifuged at 3000 RPM for 3-5 minutes and the clear supernatant was transferred to a separating funnel to which an equal volume of solvent ether was added and shaken thoroughly. The upper green layer of chlorophyll was washed thrice with distilled water added from the inner side of the funnel. The upper layer of chlorophyll in solvent either was transferred to a test tube containing a pinch of sodium sulphite. Then it was made up to a known volume with solvent ether. The absorbent of the abstract was calculated at 625 nm, 644 nm and 662 nm using red filter in a Bausch and Lamp spectrophotometer (Spectronic-20). The amount of different chlorophyll estimated following the method of Smith and Benitez (1955). The pigment’s content was expressed in fresh weight units as under:
4. **Soil analysis**

   (i) **Soil pH and EC**: 1 gm of soil sieved through the 2 mm sieve was put into conical flask. Thereafter, 5.0 ml of D.W. was poured into the flask to obtain soil : water (W/W) of 1:5.0 ml. The flask were kept on shaker for 30 min. The pH of the soil suspension was measured with the help of pH meter. EC of soil was determine with a conductivity meter known as “Salt Bridge” (Richard et al., 1968).

   (ii) **Estimation of N.P.K. and S.**: The estimation of N.P.K. and S content in soil was done before control and after treatment of compost and harvesting crop. Nitrogen was estimated by Snell and Snell (1967) method. The estimation of phosphorus was carried out by Olsen’s (1954) method. The potash was estimated with the help of flam-photometer (Jackson, 1967) and the sulphur was analyzed by the method of Chesnain and Yein (1951).

5. **Statistical Analysis**

   The data obtained for various parameters in treatment and control were subjected to statistical analysis. Sample size for the mathematical average and further statistical analysis were 10. The f value obtained from ANOVA, was compared with the tabulated value of fat at 5 % levels of significance.
Under the above comparison if the calculated value of 7 f value (tab) at 5 % level, then there is no evidence against null hypothesis and the hypothesis was accepted at 5 % level.

If the hypothesis was accepted it means that there is no significant difference among different treatment meant at respective level of significance and we do not require any further analysis.

From the analysis of variance table, one can only know that there is significant difference among different treatment but we do not know which of the treatment is showing effective difference. For this LSD (Least Significant difference) test was used. The C.D. was calculated by the following formula:

$$CD\ 5\% = S.E.\ (\text{Standard Error}) \times t_{\%\ alpha}\ (\text{error} e.f.)$$ Where, $\alpha\ % = 5\ %$ level of significance.

$$T_{\%\ alpha\ (\text{error} d.f)} = \text{tabulated value of t at } \alpha\ %.$$  

Standard error of difference was calculated by the following formula:

$$S.E.\ of\ difference = \sqrt{\frac{2\ M_{sc}}{r}}$$

Where, $M_{sc} = \text{Mean sum of square for error.}$

$r = \text{Total no. of replicates.}$

The difference between control and treatment mean, found equal or more than C.D. at 5 % level was shown superscripted with single star (*) and CD at 1 % level with double star (**). Critical difference (C.D.) value can also be used for any two treatments to know whether there is significant difference between them or not.

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