MATERIALS AND METHODS

The experiments were carried out in four consecutive seasons (2009-2013). Harvest fresh seeds of sesamum (*Sesamum indicum* L.) cv. “Rama and B-67” were used in this study which were collected from the Calcutta University Agricultural Experimental Farm at Baruipur, 24 Parganas (South), West Bengal.

After collection, seeds were cleaned and dried properly in a drying cabinet for 4 days at 35 ± 1°C to a moisture content of about 8.9% and stored in the rubber stoppered glass bottles (2.5 litres) under ambient conditions.

At first the whole quantity of seeds were divided into three lots for pre-storage, mid-storage and post-storage treatments. Then pre-storage seed invigoration treatments were given to 1-month-old seeds (harvest fresh) and stored in 100 ml capacity rubber stoppered glass bottles. Similarly, mid-storage and post-storage (pre-sowing) treatments were given to 5-months and 9-months old seeds respectively.

I. Determination on the pattern of decline in vigour and viability of sesamum (cv. Rama and B-67) seeds stored in different containers under ambient conditions

II. Studies on the effect of pre-storage seed invigoration treatments for the maintenance of storability of sesamum

III. Efficacy of mid-storage dry and wet seed invigoration treatments for the maintenance of storability of sesamum

IV. Studies on the effect of post-storage (pre-sowing) seed invigoration treatments for the maintenance of storability of sesamum

V. Efficacy of pre-storage, mid-storage and post-storage dry and wet seed treatments for improved field performance and productivity of stored sesamum

VI. Seed vigour bioassay of invigorated and non-invigorated sesamum seed

VII. Physiological and Biochemical studies on dry and wet seed invigoration treatments in relation to seed deterioration of sesamum

A. Membrane permeability

   (i) Studies on the electrical conductance of seed leachate

   (ii) Estimation of leaching of sugar
(iii) Determination of the leaching of amino acid

B. Assay of dehydrogenase enzyme activity

C. Estimation of lipid peroxidation

D. Chemical assay of volatile aldehyde production

I. Determination on the pattern of decline in vigour and viability of sesamum (cv. Rama and B-67) seeds stored in different containers under ambient condition

After harvest, sesamum seeds were cleaned and thoroughly dried in the sun to a moisture content of 8.9% and then stored (500 g seeds) in different containers namely, paper packet, cloth bag, gunny bag, polythene packet, metal tin and glass bottle under ambient conditions in the laboratory at Kolkata. Germination tests were carried out by taking seed samples from different container at monthly interval. Over 400 seeds from each treatment were placed for germination following the ISTA rules (1996). Data on germination percentage and seedling length were recorded after germination for 5 days at 20 ± 1°C temperature.

(a) Determination of moisture content

Moisture content was measured following the ISTA method (ISTA, 1996). Five grammes weighed of seed sample from each storage containers were taken in the previously weighed containers (glass vial) and placed rapidly in an electrically heated air oven, with adequate ventilation and thermostatic control. The temperature of the oven was maintained at 103 ± 2°C and drying was done for 17 ± 1 hours at that temperature. Each empty container is weighed with its lid. After weighing, the container with the seed (lid kept separately) is placed in the oven which has already been heated to the drying temperature. The oven drops in temperature when the sample is placed in it and drying period is counted from the moment of the oven was regained the required temperature. At the end of the drying period, glass vial were corked in the oven and allowed to cool for 30-35 minutes in a desiccator and then final weight was taken in a digital balance. The moisture content (M) was calculated (in percentage) on the wet weight basis by using this formulae

\[
M = M_2 - M_3 \times \frac{100}{M_2 - M_1}
\]
Where, \( M_1 \) = weight of empty containers with lid

\( M_2 \) = weight of container with lid and seed before drying

\( M_3 \) = weight of container with lid and seed after drying and cooling.

(b) Germination test

Germination test were carried out by taking seed sample from each storage containers (paper packet, cloth bag, gunny bag, polythene packet, metal tin and rubber stoppered glass bottles) at monthly intervals following the method of Punjabi and Basu (1982) with minor modification (Fig. 1). The ordinary glass plates (2 mm thick and 15 cm × 15 cm in size) and previously washed blotting paper sheets (15 cm × 12 cm in size) in running tap water followed by distilled water was used for germination test. Prior to placement of seeds for germination, seeds were slurry dressed with Dithane M-45 for 5 minutes. The blotting paper was placed on the glass plate leaving a margin of 3 cm of the plate uncovered and then seeds were placed embryo side down in a straight line on the upper end and the blotter sheet leaving a margin of 1 cm. After that, seeds were covered with 3 cm × 15 cm blotting paper strip. To keep them in position, 2 cm long (0.75 cm diameter) polythene tubings longitudinally slit on one side, were fixed on the two sides of the glass plate securedly holding the blotting paper strip. A rubber band was then fixed firmly across the two longitudinally slit polythene tubings, which were placed 4 cm above the bottom end of the plate to maintain a space between the plate and polythene bag. The plate was placed in a polythene packet (22 cm × 18 cm) containing 20 ml of distilled water and kept on a plastic holder at an angle of 66° for germination at 23 ± 1.5°C for 6 days. Over 400 seeds from each container were tested for germination as per ISTA rules (1996).

The advantages of the above mentioned method over the conventional petridish methods of seed germination are as follows (i) seedling growth was uniform, (ii) the seeds on the plate received equal amount of water, (iii) there was no chance of water logging of the seed as water travelled up by capillary movement, (iv) addition of 20 ml water was sufficient during the period of germination and further addition of water was not necessary, (v) it was possible to observe the progress of germination specially shoot and root growth from outside, (vi) data on root and shoot length could be taken easily than the petridish method, (vii) there was little entangling of the root
Fig. 1: Arrangement for sesamum seed germination on moist blotters spread on 15 cm x 15 cm glass plates enclosed in polythene envelope containing distilled water (upper figure) and placing the plates on plastic rack at an angle of 60° (lower figure); the diagram shows the seedlings before recording the final germination percentage and length of root and shoot.
system as noted in the petridish method and the roots grow vertically down (at 66° angle) without curving or coiling on the paper, (viii) fungal contamination was less and (ix) the set up was less expensive than the petridish method.

In the present study, the newly standardized glass plate blotter method was employed for all germination tests.

Root and shoot length of normal seedlings were measured to the nearest millimeter. The mean root and shoot length as given in the tables are based on normal seedlings only. The term vigour has been rather broadly used in this thesis and is based on the root and shoot length per seedling produced by the treated and untreated seeds.

II. Studies on the effect of pre-storage seed invigoration treatments for the maintenance of storability of sesamum

Method of Seed Treatment

Pre-storage seed invigoration treatments were given to 1-month-old sesamum seeds employing dry and wet treatments. The methods are given below:

(a) Dry dressing treatments

Pre-storage dry dressing treatments were given to high-vigour (1-month old) sesamum (cv. Rama and B-67) seeds with finely powdered chemicals, pharmaceutical formulations and crude plant materials following the method described earlier by Mandal and Basu (1986) with minor modifications. At first the crude plant materials were thoroughly air-dried and then chemicals, pharmaceutical products and plant materials were finely powdered and kept in the glass bottles (25 ml capacity) for treatment.

Harvest fresh seeds of sesamum (high-vigour) with a seed moisture content of about 8.9% were dry dressed with finely powdered pharmaceutical products, chemicals and crude plant materials at various doses in the previously washed and dried glass bottles at room temperature (28 ± 1°C). After treatment, bottles were tightly closed with rubber cork and shaken thoroughly to mix the chemicals, pharmaceutical products and crude plant materials with the seeds and then kept in the laboratory under ambient conditions. The glass bottles were shaken at least once in a
day upto 7 days for thorough mixing of chemicals, pharmaceutical products and crude plant materials with the seed. The untreated control seeds were not dressed but kept along with the treated seeds in the laboratory under ambient conditions till further use.

**The following treatments along with doses are given below:**

Seeds were dry dressed with finely powdered chemicals *viz*., bleaching powder @ 2g/kg of seed and *para*-amino-benzoic acid @ 500mg/kg of seed, pharmaceuticals *viz*., aspirin @ 50 mg/kg of seed and ascorbic acid @ 500 mg/kg of seed and crude plant materials *viz*., red chilli powder @ 1g/kg of seed, lemon leaf powder @ 2g/kg of seed and spinach leaf powder @ 2g/kg of seed in rubber stoppered glass bottles at room temperature (29 ± 1°C). After treatment, glass bottles were thoroughly shaken once in a day up to 7 days for mixing of finely powdered chemicals, pharmaceutical products and crude plant materials with the seeds and then bottles were kept in the laboratory under ambient conditions. The treatment schedule (Table 1a) and method of treatment were same in both the cultivars of sesamum (cv. Rama and B-67).

(b) **Wet seed treatments**

**Soaking-drying (S-D)**

Sesamum seeds were soaked in double volume of water for 2 hours at room temperature (29 ± 1°C) with occasional stirring. After 2 hours, the excess amount of water was decanted off and the seeds were surface dried by blotting paper and finally dried back to its original weight in a drying cabinet over a current of dehumidified air at 35 ± 1°C for 3-4 days (Mandal and Basu, 1983). Control seeds were not soaked but dried with treated seed. After treatment, treated and untreated seeds were then transferred to a desiccator containing fused calcium chloride for 7 days for stabilizing the moisture content of the seeds to a uniform level. After stabilization, seeds were restored in the rubber stoppered glass bottle under ambient conditions for laboratory as well as field studies.

(c) **Ageing conditions**

**Natural ageing**: To evaluate the treatment effects on germinability, both treated and untreated seeds were subjected to natural ageing under ambient conditions for 95 days. At first, equal amount of seeds were kept in a perforated paper packets for each
Treatment combination: $T_0 - T_8$

Time of Seed treatment: Pre-storage, Mid-storage and Post-Storage

Cultivar: Rama and B-67

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Pre-Storage</th>
<th>Mid-Storage</th>
<th>Post-Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_0$</td>
<td>Control (Untreated).</td>
<td>Control (Untreated).</td>
<td>Control (Untreated).</td>
</tr>
<tr>
<td>$T_1$</td>
<td>Bleaching powder @ 2g/kg of seed.</td>
<td>Bleaching powder @ 2g/kg of seed.</td>
<td>Bleaching powder @ 2g/kg of seed.</td>
</tr>
<tr>
<td>$T_2$</td>
<td><em>Para</em>-amino-benzoic acid (PABA) @ 500mg/kg of seed.</td>
<td><em>Para</em>-amino-benzoic acid (PABA) @ 500mg/kg of seed.</td>
<td><em>Para</em>-amino-benzoic acid (PABA) @ 500mg/kg of seed.</td>
</tr>
<tr>
<td>$T_3$</td>
<td>Aspirin @ 50 mg/kg of seed.</td>
<td>Aspirin @ 50 mg/kg of seed.</td>
<td>Aspirin @ 50 mg/kg of seed.</td>
</tr>
<tr>
<td>$T_4$</td>
<td>Ascorbic acid @ 500 mg/kg of seed.</td>
<td>Ascorbic acid @ 500 mg/kg of seed.</td>
<td>Ascorbic acid @ 500 mg/kg of seed.</td>
</tr>
<tr>
<td>$T_5$</td>
<td>Red chilli powder @ 1g/kg of seed.</td>
<td>Red chilli powder @ 1g/kg of seed.</td>
<td>Red chilli powder @ 1g/kg of seed.</td>
</tr>
<tr>
<td>$T_6$</td>
<td>Lemon leaf powder @ 2g/kg of seed.</td>
<td>Lemon leaf powder @ 2g/kg of seed.</td>
<td>Lemon leaf powder @ 2g/kg of seed.</td>
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<tr>
<td>$T_7$</td>
<td>Spinach leaf powder @ 2g/kg of seed.</td>
<td>Spinach leaf powder @ 2g/kg of seed.</td>
<td>Spinach leaf powder @ 2g/kg of seed.</td>
</tr>
<tr>
<td>$T_8$</td>
<td>Soaking in water for 2 hours followed by drying (S-D).</td>
<td>Soaking in water for 2 hours followed by drying (S-D).</td>
<td>Soaking in water for 2 hours followed by drying (S-D).</td>
</tr>
</tbody>
</table>

Table 1a: Treatment schedule for Sesamum (cv. Rama and B-67)
treatment (containing same amount of seeds with equal number of holes) and then all the packets were placed in a cloth bag. The cloth bag containing paper packets were kept in the shelf of the laboratory under ambient conditions for various durations. The packets were shuffled at regular intervals (2 days) for uniform ageing.

Accelerated ageing: Treated and untreated sesamum (cv. Rama and B-67) seeds were subjected to accelerated ageing under artificially controlled regimes of different relative humidities and temperature to evaluate the treatment effects on vigour and viability. Treated and untreated seeds were kept in perforated (equal number of holes in each packet) paper packets (each packet contains equal amount of seed) and then subjected to accelerated ageing at 98% relative humidity and 40 ± 1°C temperature and 93% relative humidity and 40 ± 1°C temperature for varying periods. For this purpose, the relative humidity was maintained at 98% and 93% by storing the seeds in two separate desiccator with air tight glass lid containing 380 ml of water with 20 ml of concentrated H$_2$SO$_4$ (98% RH) and 340 ml water with 60 ml of concentrated H$_2$SO$_4$ (93% RH) and keeping it in a B.O.D. incubator at 40°C temperature. Treated and untreated seeds also placed in another desiccator which contain a saturated solution of calcium chloride (maintaining 36 % RH) for quite slow ageing at 40°C temperature. During the period of accelerated ageing, perforated paper packets containing seeds were shuffled frequently to ensure uniform exposure to the relative humidity within the desiccator. The perforated paper packets containing treated and untreated seeds were kept on a porcelain plate inside the glass dessicator and the packets were shaken every day for uniform ageing. After ageing, germination tests were carried out following the inclined glass plate blotter method of Punjabi and Basu (1982) with minor modification.

(d) Germination test

Germination test of the treated and untreated seeds (minimum 400 seeds for each treatment as specified by ISTA, 1996) were done after accelerated ageing and natural ageing following the inclined glass plate blotter technique of Punjabi and Basu (1982) with minor modifications. Data on germination percentage, root length and shoot length were recorded after 5 days of germination at 29 ± 1°C temperatures.
III. Efficacy of mid-storage dry and wet seed invigoration treatments for the maintenance of storability of sesamum seed

Mid-storage dry and wet treatments were given to medium vigour (5-month-old) sesamum (cv. Rama and B-67) seed. The method of dry and wet seed treatments along with doses of chemicals, pharmaceutical products and crude plant material were same as described earlier in the previous experiment (Experiment II). The ageing conditions and germination test were also described earlier in details (vide Experiment II). The treatment schedules were same as given in the pre-storage seed invigoration treatments (earlier Experiment II).

IV. Studies on the effect of post-storage (pre-sowing) seed invigoration treatments for the maintenance of storability of sesamum

Post-storage (pre-sowing) dry and wet treatments were given to 8-month-old sesamum seeds (cv. Rama and B-67) which were stored in the rubber stoppered glass bottles under ambient conditions. Immediately after dry and wet (only surface dried seed) treatment, germination test was carried out following the method of Punjabi and Basu, (1982) with minor modifications. The method of seed treatment and treatment schedules were same as in both pre-storage and mid-storage seed invigoration treatment which are described earlier in the previous experiment (vide Experiment II and III). In soaking treatment, seeds were soaked in double volume of water for 6 h and then surface dried prior to sowing in the field to facilitate sowing.

V. Efficacy of pre-storage, mid-storage and post-storage dry and wet seed treatments for improved field performance and productivity of stored sesamum

The methodology of pre-storage (1-month-old seeds), mid-storage (5-months-old seeds) and post-storage (9-months-old seeds) dry and wet treatments for improved field performance and productivity of stored sesamum (cv. Rama and B-67) are given below:

Field Experiment

The field experiment was conducted at the Agricultural Experimental Farm of the University of Calcutta, Baruipur, 24-Parganas (South), West Bengal (latitude 22°N
and 88°-28'E longitude), during the pre-kharif (February-May) season in four consecutive years (2009–2010, 2010–2011, 2011-2012 and 2012-2013), using randomized block design (RBD) with 3 replications for each treatment (Fig. 3).

First, the land was prepared by one deep ploughing followed by two harrowings and left unused at least for 10 days to destroy the weeds and soil nematodes. After final land preparation, field was divided into 18 blocks (3 blocks each for pre-storage, mid-storage and post-storage treatment) along with two cultivars, each consists of 9 subplots measuring 6 m² (3 m x 2 m) each. A fertilizer dose of N: P₂O₅: K₂O at the rate of 80:40:40 kg per hectare was given to the field. During land preparation, 50% of the total nitrogen and the whole amount of phosphate and potassium were added. The rest of the nitrogen was supplied in two split doses, one at the time of thinning and another before flowering. Both treated and untreated seeds of sesamum (cv. Rama and B-67) were sown @ 7kg/hectare giving a spacing of 30 cm between the rows and 15 cm between the plants and sowing was done on 2nd week of February. Apart from the post sowing irrigation, the crop received 3 more irrigations and usual culture practices were followed throughout the cropping period.

Data on plant population (field emergence) were recorded after 15 days of sowing and thinning of plants were made by considering a spacing of 30 cm between rows and 15 cm between the plants. Data on plant height, number of branches, number of capsule per plant, number of seed per capsule, seed yield per unit area and 1000- seed weight, were taken after harvesting.

VI. Seed vigour bioassay of invigorated and non-invigorated sesamum seed

Seed vigour bioassay is a quantitative attribute of seed which is manifested in the rapidity of germination and seedling growth, uniformity of stand, increased tolerance to environmental stress, greater membrane integrity and higher metabolic potential (Heydecker, 1972).

Bioassay of seed vigour was done following the method of Sur and Basu (1990a) with minor modifications. In the bioassay method, germinating high vigour jute (Corchorus olitorius L., cv. JRO 524) seeds were exposed for 48 h to the gaseous emanations of stock seeds.
Fig. 3: Layout of field experiment on Sesamum (cv. Rama and B-67) showing the distribution of various treatments
For this purpose, air-tight wide mouth plastic containers (outside diameter 8 cm, height 6 cm) were used (Fig. 4). At first, sesamum seeds (cv. Rama and B-67) were slurry dressed with Dithane M-45 for 5 minutes and the jute seeds were also kept in distilled water in a petridish in submerged condition for 10 minutes. Then, sesamum seeds were placed on a disc shaped moist blotter (diameter 6 cm) in 2 rows. A thin layer of absorbent cotton was placed under the moist blotter. Five milliliter of distilled water was then poured on the blotter so that the blotter will remain moist and will provide constant water supply to the germinating seeds.

High vigour jute seeds (*Corchorus olitorius* L., cv. JRO 524) were used as bioassay material. Preliminary studies employing several bioassay materials have shown the superiority of jute over others. The seed germinate very quickly and it is possible to record seedling growth data after 48 h. The small seeds (100 seed weight 180 mg) are characterized by their uniformity in germination and high sensitivity to the gaseous emanations from stock seeds. The jute seeds were collected immediately after harvest and dried to moisture content of 7% and stored in small sealed glass bottle in a refrigerator at -10°C prior to use in the bioassay. Twenty-five jute seeds were placed in a line on a folded strip of blotter (size 22 cm × 7 cm). The blotting paper with the jute seeds were then introduced carefully inside the plastic container (stock material) in such a way that the inner wall of the plastic container was lined by the blotting paper. Then the cap of the plastic container was tightly closed (Fig. 4). The containers for all the treated and untreated seeds (sesamum seeds) and a blank (containing bioassay material jute seeds but no stock material) were kept at 20 ± 1°C. The germination percentage and seedling length of stock material (sesamum seed) and seedling length of bioassay material (jute seed) were taken after 5 days and 48 h respectively.

**VII. Physiological and Biochemical studies on dry and wet seed invigoration treatments in relation to seed deterioration of sesamum**

(A) **Membrane permeability**

Membrane functions can be estimated by membrane permeability as determined by leaching of electrolytes, sugar and amino acid from seeds.
Fig. 4: Set-up for bioassay of seed vigour
**Electrical conductivity of seed leachate (dsm⁻¹):** Electrical conductance of treated and untreated seeds were measured immediate after treatment (i.e., before ageing) and after natural ageing under ambient conditions following the method of Anderson *et al.*, (1964). To study the electrical conductance, twenty five uniform sesamum seeds of each treatment were soaked in 30 ml of distilled water for 1h at room temperature (29 ± 1°C). The seed steeped water was poured off to a 2.5 cm diameter glass test tube and the electrical conductance of seed leachate was recorded in a Systronic Electrical Conductivity Bridge (cell constant 0.756).

**Leaching of sugar (µg/ml):** The amount of sugar leached out from the seed was determined immediately after treatment (before ageing) and after natural ageing under ambient conditions following the method of McCready *et al.*, (1950) with minor modifications. Twenty five uniform sesamum seeds were soaked in 30 ml of distilled water for 1 h at room temperature (29 ± 1°C). Two millilitre of leachate was taken in a hard glass test tube and then 4 ml pre-cooled freshly prepared anthrone reagent (0.2% anthrone in 98% sulphuric acid) was added and kept in cold (5°C) for 30 minutes until a bluish green colour developed. The percent transmission of bluish green colour solution was measured on a Systronic Photoelectric Colorimeter at 580 nm. The leaching of sugar was expressed as µg glucose equivalent per ml of leachate.

**Leaching of amino acid (µg/ml):** Estimation of amino acid in the seed steeped water was done just after treatment (i.e., before ageing) and after natural ageing under ambient conditions following the method of Moore and Stein (1948) with minor modifications. Twenty five seeds of sesamum were soaked in 30 ml of distilled water for 8 h in case of before ageing and 4 h in case of after natural ageing at 30 ± 1°C temperature. After the stipulated period, 4 ml of the seed steeped water, 0.5 ml of 0.1 m acetic acid–sodium acetate buffer (pH 5.3) and 1 ml of 1% ninhydrin solution in dioxane were added in the hard glass test tube. The reaction mixture was heated for 15 minutes in a water bath at 100°C for the colour development. The colour intensity was measured in Systronic Photoelectric Colorimeter at 610 nm.

**Assay of dehydrogenase activity**

The dehydrogenase enzyme activity of treated and untreated seeds was determined following the method of Kittock and Law (1968). Thirty sesamum seeds per treatment
were placed for germination on the petridish at 20 ± 1°C. After 24 h of germination, twenty uniformly sprouted embryo for each treatment was taken in a 10 ml capacity glass vial and then incubated with 2 ml of 0.2% solution of tetrazolium chloride for 3 h at 30°C in dark. After incubation, the tetrazolium chloride solution was decanted off and the embryos were thoroughly washed with distilled water. Five milliliter of methyl cellosolve (2-methoxy ethanol) were added on the vial containing embryos and kept for 8 h at room temperature (28 ± 1°C) for red colour development (fomazan). The colour intensity was measured in the Systronic Photoelectric Colorimeter at 470 nm.

(C) Estimation of lipid peroxide formation

Lipid peroxide formation by the treated and untreated seeds were studied using thiobarbituric acid (TBA) colour reaction as outlined by Bernheim et al., (1948) with minor modifications. Five milliliter of 1% TBA solutions were added to 50 mg of dry sesamum powder (seed thoroughly crushed and made a fine powder) in a hard glass test tube and then 2 ml of 1(N) H₂SO₄ were added. The mixture was heated for 15 minutes in a water bath. After cooling, 5 ml of methyl cellosolve (2-methoxy ethanol) were added and the mixture was thoroughly shaken. It was then centrifuged for 10 minutes at 5000 rpm. The absorbance of the clear supernatant was read on a Systronic Photoelectric Colorimeter at 520 nm using boiled TBA reagent as blank.

(D) Estimation of volatile aldehydes

For the estimation of volatile aldehydes emanating from germinating treated and untreated gram seeds, the methods of Harman et al., (1982) and Wilson and McDonald (1986a) with a modified aldehyde trapping device by Sur and Basu (1990b) were followed with minor modifications using twenty sesamum seeds. In this study, plastic containers of suitable size (8.5 cm diameter) were taken in which disc-shaped blotter was placed at the bottom. A thin layer of moist absorbent cotton was placed beneath the blotter. Distilled water was applied to make both the cotton and the blotter evenly moist and the excess water was cleared off. Twenty seeds of sesamum were taken from each of the treated stock as well as from the untreated stock for the purpose. The seeds were placed in the moist blotter leaving a central circular portion of the blotter blank. Now an open fifteen milliliter capacity glass vial is placed in that central portion in which 10 ml of 0.2% (w/v) 3 methyl-2-benzo-thiazolinone
hydrazone (MBTH) solution was kept which absorbs the volatile aldehydes present in the gaseous emanations of treated and untreated sesamum stock seeds. In one glass vial only MBTH solution (0.2%) was present which was taken as the control (Blank). The whole set was kept in a temperature at 23 ± 1.5°C for 48 h (Fig. 5). During germination, evolved aldehyde from seeds were trapped of MBTH solution. After 48 h of incubation, 3 ml aliquots of the aldehyde trapping solution (MBTH) from each glass vial of different treatment set including control was taken into test tube, in which 2.5 ml of 0.23% (w/v) ferric chloride solutions were added and the mixture was incubated for 10 minutes at room temperature. To each tube, 2.5 ml of acetone were added and tightly corked. After 30 minutes, the absorbance of the solution was measured on a Systronic Photoelectric Colorimeter at 635 nm.

**Statistical Analysis**

Data obtained from the laboratory germination test, field experiment and biochemical studies were analyzed statistically to evaluate the treatment effects on the germinability and field performance and productivity following the method of analysis of variance (Fisher, 1948). Before analysis, germination percentage data were transformed to respective angles (Arc-sin value) and root and shoot length data were analyzed as such. Vigour index was calculated as the germination percentage multiplied by their respective seedling length.
Fig. 5: Set-up for chemical assay of volatile aldehyde trapping in 3-methyl-2-benzo-thiazolinone hydrazone (MBTH) solutions

Diagram shows, air-tight wide mouth plastic containers (diameter 8.5 cm and height 6 cm) with a moist blotting paper inside the bottom of the container. A glass vial (diameter 15 mm and height 20 mm) containing 10 ml of 0.2% MBTH solution were kept on the bottom of the plastic container and then seeds (sesamum) were placed on the moist blotting paper for germination at 23 ± 1.5°C. After 48 hours of germination, chemical assay of volatile aldehyde was done by taking MBTH solutions.