Chapter-III

MATERIALS AND METHODS

1. Location and climate

The study on the effects of soil solarization and its integration with fungicides and BCA's on physico-chemical and microbiological properties of soil and seedling diseases of some horticultural corps was carried out at field research centre of T. D. P.G. College, Jaunpur and department of plant pathology T.D.P.G. College, Jaunpur.

2. Experimental Design and Solarization Technique

Field trials were conducted in plots (raised beds), heavily infested with pathogens like *Pythium* sp., *Fusarium* sp. *R. solani,* S. *rolfsii* etc., as the seedlings of different vegetables have been raised uninterruptedly for the last several years. Entire details (design, replications, treatments) of the field experiments are given in Fig. 3 and 4.

Time given for solarization was 30 days. Before mulching the soil, irrigation was given to ensure enough moisture during solarization. Airtight condition and prevention of any leakage of heated air, gases, moisture etc. from the mulched area was ensured. The crops and varieties of the nursery crops raised were cauliflower (Pant Gobhi-4), cabbage (Pride of India), tomato (Pant T-3) and onion (N-53/Nashik Red). After covering the plots with polyethylene sheeting, the edges of polyethylene were tightly buried in

soil (Fig. 5). Precautions were also taken to avoid damage to the polyethylene mulch. The damage if any, was repaired immediately.

3. Determination of Soil Moisture

Moisture status of the soil was determined before and after solarization by volumetric method. Soil samples drawn from experimental plots, were weighed and dried at 110^0C in oven till constant weight was recorded. Thereafter, percent moisture content was calculated using the following formula:

$$\text{Per cent Soil moisture} = \frac{\text{(Wet soil wt.) - (oven dried soil wt.)}}{\text{Oven dried soil wt.}} \times 100$$

4. Environmental Factors

Environmental parameters like temperature, RH, rainfall and duration of sunshine were recorded daily during the period of solarization from the Airport Agrometeorological observatory of Babatpur, Varanasi.

5. Determination of Soil Properties

5.1. Soil Temperature

Daily increase and decrease in soil temperature during the period of solarization was recorded by placing soil-thermometers beneath the polyethylene film at a depth upto 10 cm. The temperatures were recorded twice daily at 7.00 A.M. and at 2.30 P.M. Finally weekly average, minimum and maximum temperatures were computed.

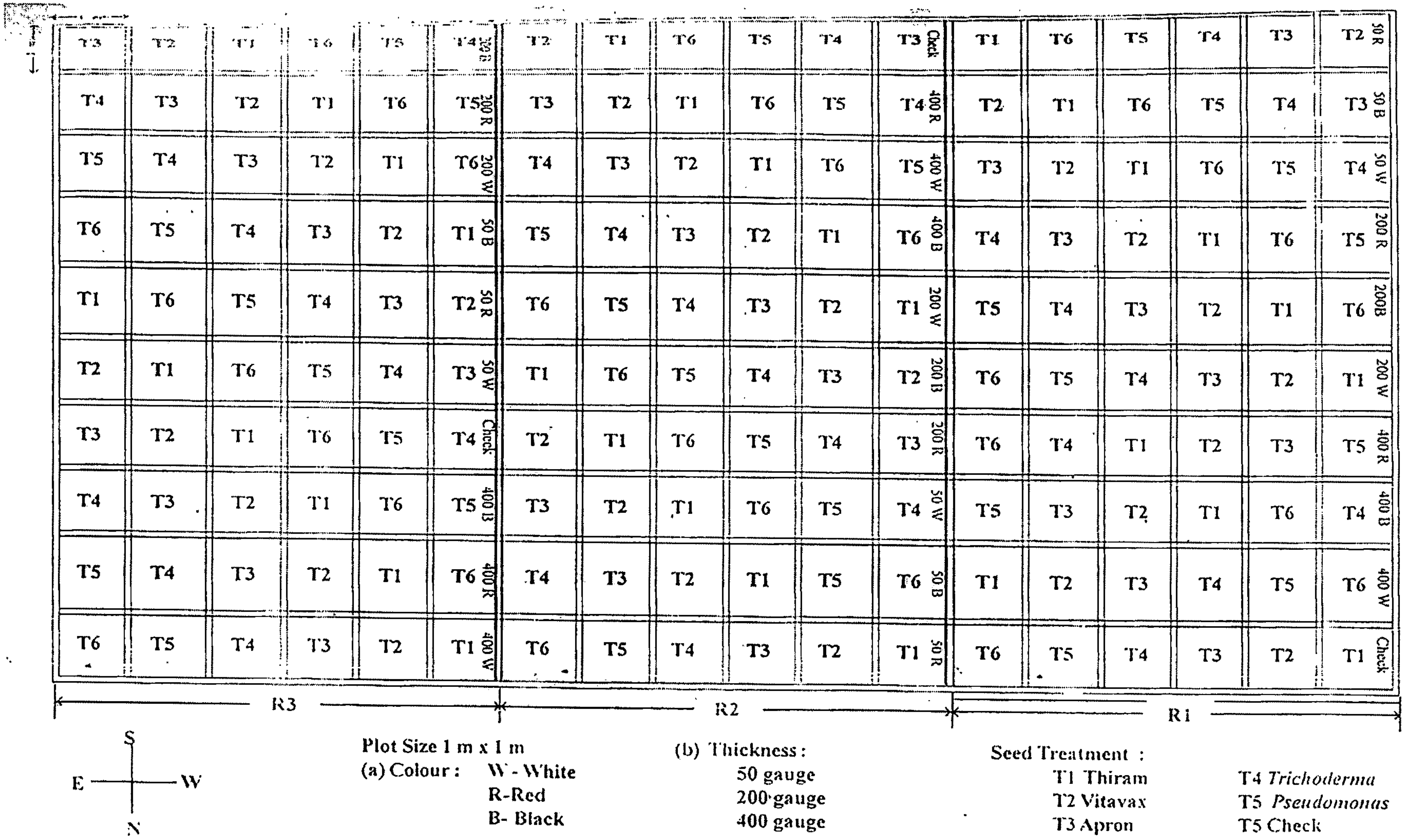

Fig. 3 : Layout of the experiment at research station T.D.P.G. College, Jaunpur.

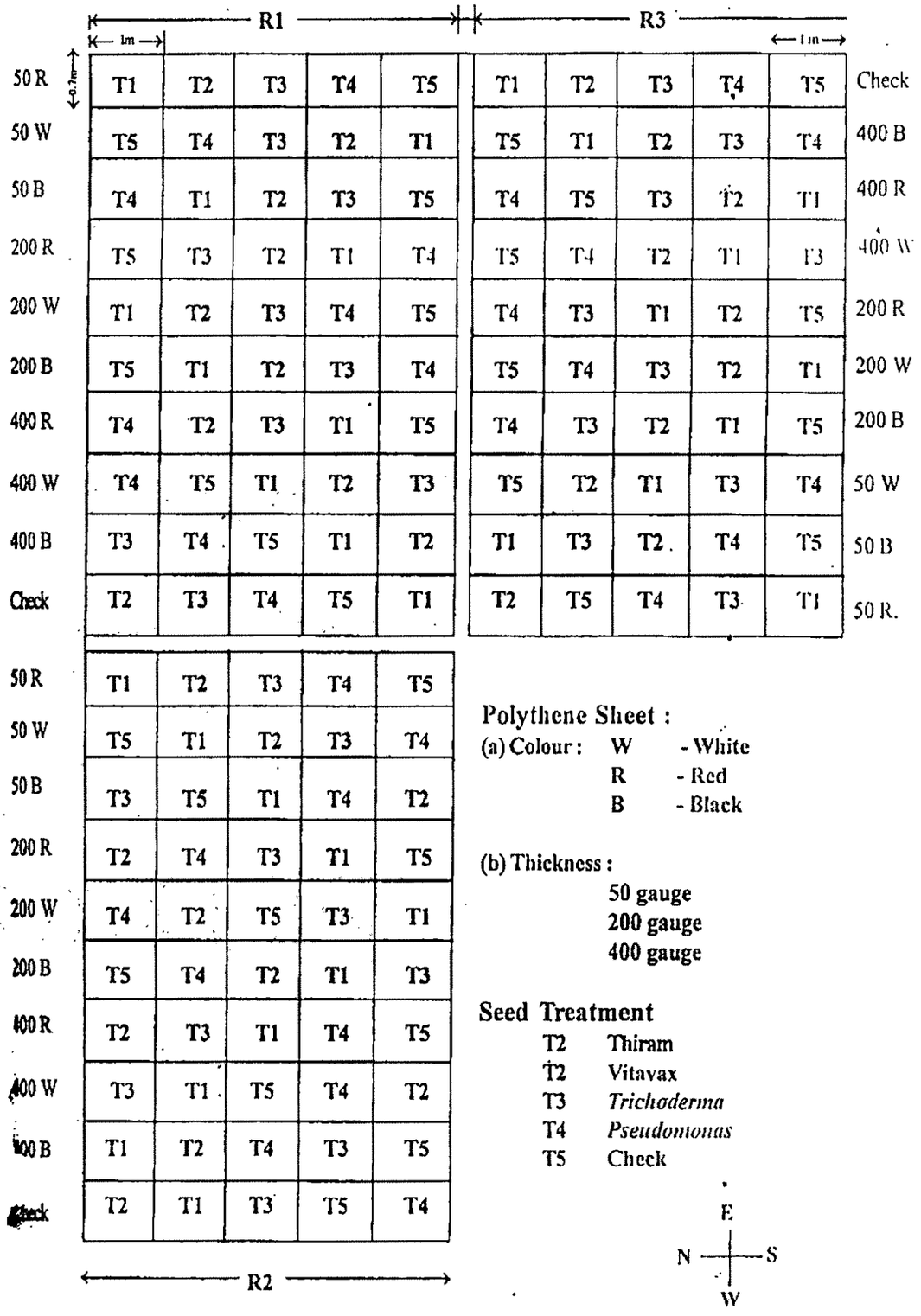

Fig. 4 : Layout of the experiment at research farm T.D.P.G. College, Jaunpur.

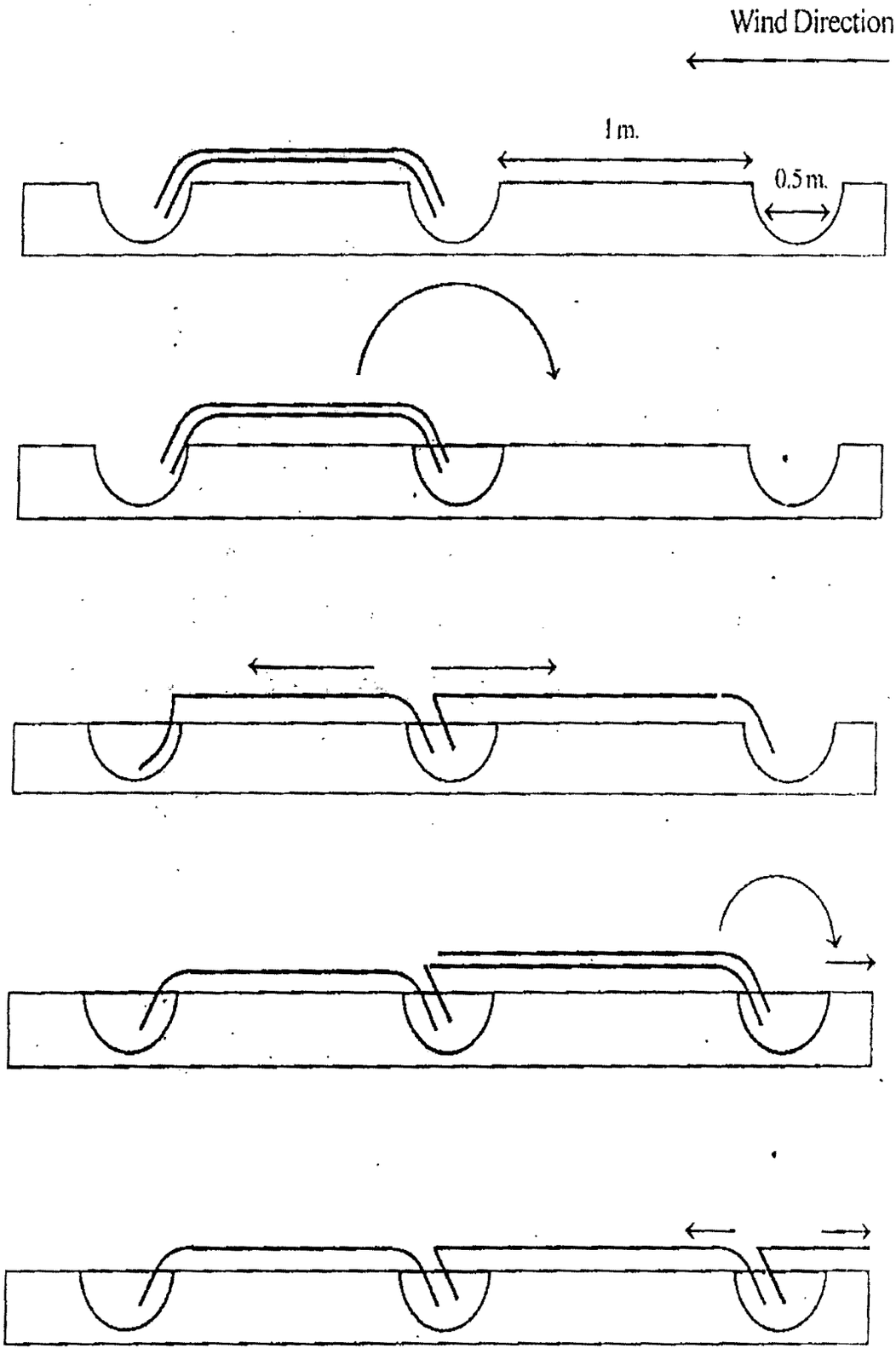

Fig. 5 : Method of placing polyethylene sheet on nursery bed for soil solarization

5.2. Soil pH

Soil pH was determined in 1 : 2 soil water suspension as described by **Jackson (1956).** Ten-gram soil was taken in a glass beaker of 50 ml capacity and 20 ml distilled water was added to it. The suspension was continuously stirred for 10 minutes and later intermittently. Observations were recorded by dipping the electrode of the digital pH meter into the soil suspension.

6. Disease-Incidence

Evaluate the effect of solarization on the incidence of pre, post-emergence and total damping-off, number of seeds expected or likely to germinate (X), number of seeds actually germinated after 7 days of sowing (A) and number of final seedling stand (B) in a row, were counted using telecounter. Damping-off incidences were computed by the following mathematical formulae:

$$\text{(i) Pre-emergence damping-off (\%)} = \frac{X - A}{A} \times 100$$

$$\text{(ii) Post-emergence damping-off (\%)} = \frac{X - B}{X} \times 100$$

$$\text{(iii) Total damping-off (\%)} = \frac{X - A}{A} \times 100$$

In order to identify pathogen-causing mortality and also to confirm the associations of pathogens with seedling

mortality, isolations were made regularly on Potato Dextrose Agar medium. The cultures, thus obtained were purified, identified and pathogenicity was confirmed.

7. Increased Growth Response

Plant growth parameters used to study IGR were plant height, fresh and dry shoots and root wt. of 30 days old seedlings. For measuring plant height, 20 plants were selected randomly from each It and measured with the help of scale. After recording plant height, same plants samples were used to record root and shoot weight.

8. Weed Population

To study the effect of solarization on weed populations, the weeds within one square feet from each plot in solarized and non-beds, solarized beds, were uprooted, identified and individual weed populations were recorded. Weed populations per square feet were also recorded before preparation of beds.

9. Effect on soil Microbiology

Soil solarization is expected to bring about certain changes in the microbial equilibrium existing in the soil. In order to evaluate and record such changes at different stages, major groups of soil microflora including fungal pathogens and some antagonists, were estimate.

Isolations from soil to assess population dynamics, were done using dilution plate technique **(Walksman and**

Starkey, 1923). In most cases selective/semi-selective media were used. Soil dilution of 1 : 10^3 was prepared from 1g air dried soil in sterilized water by employing serial dilution technique. The technique of preparing soil dilution was same for all the microorganisms, except the dilution strength varied accordingly.

One ml of desired dilution was poured in each petriplate and then 20 ml medium was poured, the plates were swirled immediately after pouring until the medium gets solidified, to get uniform distribution of soil suspension in the medium. Plates were incubated at desired temperatures and colony-forming units (c.f.u.) were recorded.

9.1 Total fungi

Dextrose Rose Bengal Agar medium **(Martin, 1950),** was used to isolate total fungi. The constituents of the medium were

KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Peptone	5.0 g
Dextrose	10.0 g
Dicrysticine	1.0 g
Rose Bengal	50 ppm
Agar-Agar	20.0 g
Distilled water	1000 ml

Rose Bengal and dicrysticine were suspended in sterilized water added to the autoclaved and cooled medium

before planting. Plates were incubated at 27 ± 2^{0}C.

9.2 *Pythium spp*

Isolations were done on selective medium developed initially by, **Singh and Mitchell (1971)** with certain modifications as suggested by **Peethamberan and Singh (1978)**. Plates were incubated at 27 ± 2^{0}C temperature for 4 days. Colonies, that developed, were counted. Each colony represented one colony-forming unit (c.f.u.). Constituents of selective medium were :

KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Peptone	5.0 g
Dextrose	10.0 g
Dicrysticine	500 ppm
Rose Bengal	50 ppm
Benlate	20 ppm
Mycostatin	1000 ppm
Metalaxyl	500 ppm
Agar-Agar	20.0 g
Distilled water	1 liter

Antibiotics, fungicides and Rose Bengal were added after autoclaving and cooling of the medium.

9.3 *Trichoderma* spp.

To estimate populations of *Trichoderma* spp., selective medium described by **Shreshta (1992)** was used with some modifications. Soil dilution of 10^{-3} strength was used.

Constituents of the medium were :

$MgSO_4.7H_2O$	0.2 g
KH_2PO_4	0.9 g
KCl	0.15g
NH_4NO_3	1.0 g
Dextrose	3.0 g
Agar-Agar	20.0g
Water to make up	1 liter
Dicrysticine	0.05 g
Apron 35 SD	0.05 g
Captan	0.05 g
Vitavax	0.02 g
Rose Bengal	50 ppm

Antibiotic, fungicides and Rose Bengal were added after autoclaving and cooling of the medium. Plates were incubated at 30 ± 10C for 4 days

9.4. Total Bacteria

Total bacteria were isolated on soil extract agar medium taking 10^{-6} dilution of soil water suspension **(Lochhead, 1950).** Composition of medium was as follows :

KH_2PO_4	0.5 g
Soil extract	100 ml
Tap water	900 ml
Glucose	1.0 g
Agar	20 g
pH (adjusted to)	7.0

Soil extract was prepared by 1000 g of garden soil with 1000 ml of water in autoclave for 30 minutes at 15 lb pressure. The suspension was filtered though double layer of filter paper.

9.5 *Bacillus* spp.

To isolate *Bacillus* spp. soil dilutions of 10^{-6} strength were prepared and heated at 800C for 30 minutes prior to plating, to kill all the vegetative cells and get only spore counts. Starch nutrient agar medium **(Ramakrishnan, 1989)** was used for isolation. Composition of medium was :

NaCl	1.0 g
Peptone	5.0 g
Beef Extract	5.0 g
Starch soluble	10.0 g
Agar-Agar	15.0 g
Water to make up	1 liter
pH (adjusted to)	7.0

9.6 Fluorescent pseudomonads

To isolate fluorescent pseudomonads, specific King's B. medium **(King *et al.*, 1954)** was taken. Constituents of the medium were :

Protease peptone	20 g
KH_2PO_4	2.5 g
Glycerol	15 ml
$MgSO_4.7H_2O$	6 g
Agar-Agar	15 g
Water to make up	1 liter

10. Seed-treatment

Three different fungicides and two-biocontrol agents were taken for seed-treatment to supplement the positive effects of soil solarization against damping-off disease. The seeds were treated by slurry method using CMC. In case of BCA's number of propagules/spores per ml were adjusted :

Fungicides/bioagent	**Chemical name**	**Rate of application (g/kg seed)**
Thiram	Dithiocarbamate	2.5 (0.25%)
Vitavax	Carboxin	2.0 (0.20%)
Apron 35 SD	Metalaxyl	4.0 (0.4%)
T. harzianum	Commercial formulation of 1.5×10^9 c.f.u.	4.0 (0.4%)
P. fluorescens	Commercial formulation of 1.5×10^9 c.f.u.	4.0 (0.4%)