3. MATERIALS AND METHODS

The present investigation was carried out in the Department of Plant Pathology. The details of material used and methodology followed during the course of study are given hereunder:

3.1 Occurrence and distribution of diseases of bell pepper

Survey of different polyhouses in Himachal Pradesh was conducted to assess the occurrence and distribution of bell pepper diseases. Six districts namely Kangra, Bilaspur, Hamirpur, Mandi, Una and Kullu were selected. Data on disease incidence and disease severity of various diseases viz. bacterial wilt (*Ralstonia solanacearum*), powdery mildew (*Leveillula taurica*), anthracnose or fruit rot (*Colletotrichum capsici*), Phytophthora blight (*Phytophthora nicotianae*), wilt (*Fusarium oxysporum*), foot rot (*F. solani*), root rot (*Rhizoctonia solani*), collar rot (*Sclerotium rolfsii*) and white rot (*Sclerotinia sclerotiorum*) were recorded and calculated by using the following formula:

\[
\text{Disease Incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants assessed}} \times 100
\]

\[
\text{Disease Severity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease scale}} \times 100
\]

3.2 Collection of diseased samples

Different capsicum growing polyhouses were surveyed during the crop season and plant parts infected with different diseases were collected, placed in paper bags and brought to the laboratory for further analysis. The samples were bench pressed between sheets of blotting paper/newspaper. Blotting paper was changed regularly till samples were dry. Dried samples were stored in dry place in paper bags for further studies.
3.3 Sterilization of glassware / plasticware

Borosil and Merck brand glassware such as Petriplates, test tubes, conical flasks, beakers, pipettes and measuring cylinders, etc. were used in different experiments conducted during the course of this study. The glasswares were dipped in cleaning solution (100 g of potassium dichromate and 500 ml of conc. sulfuric acid in 1 litre water) overnight, rinsed thoroughly under running tap water and sterilized in oven at 180°C for one hour. Working table tops and hands were surface sterilized with rectified spirit.

3.4 Preparation of media

Different types of culture media viz. potato dextrose agar (PDA), yeastal PDA and nutrient agar medium (Appendix-I) were employed for the isolation of fungal and bacterial pathogens from diseased samples. The media were sterilized in autoclave at 1.05 kg/cm³ pressure and 121.6°C temperature for 15 minutes.

3.5 Isolation, purification and maintenance of fungal/bacterial pathogens

Fungal pathogens were isolated from diseased samples of bell pepper using standard methodology on PDA medium. Small bits of infected tissues were surface sterilized by dipping in a solution of 0.1 per cent mercuric chloride for 10-15 seconds and washed thrice in sterilized water under laminar air flow. The bits were dried under two folds of sterilized filter papers and transferred to PDA slants. The tubes were incubated at 25 ± 1°C for 7-8 days and purified by single spore/hyphal tip method. Pure cultures were maintained on PDA slants.

Bacterial wilt infested plants were cut into small pieces with sterilized blade and dipped in test tube containing sterilized distilled water so that ooze from stem is mixed with water. Then, a loopful of bacterial suspension was streaked on Kelman's (1953) 2,3,5-triphenyltetrazolium chloride (TZC medium) (Appendix-I) under aseptic conditions and plates were incubated at 28 ± 1°C. After 48 hours of incubation, single colonies were picked up carefully and streaked on another TZC plate and incubated for 48 hours. After 48 hours, again
single colony was picked up and streaked on new TZC plates. This process was repeated five times to get pure culture of *R. solanacearum*. The culture was maintained on TZC slants. The typical virulent colonies of bacterium, on TZC medium were mucoid, irregular, shining and convex with production of lot of extracellular polysaccharide (EPS) around pinkish centres.

### 3.6 Evaluation of organic inputs against *Ralstonia solanacearum*

#### 3.6.1 In vitro evaluation of organic inputs

Organic inputs like matka khad, panchgavya, biosol, vermiwash, cow urine, fermented butter milk, biodynamic compost tea, NADEP compost, vermicompost and cow pat pit (Appendix-II) were evaluated under *in vitro* conditions on TZC medium. TZC medium was amended with various inputs before and after autoclaving at 0.5, 1.0, 2.0, 3.0 and 4.0 per cent concentrations and poured into sterilized plates after solidification. The culture of *R. solanacearum* was streaked on all the plates along with control plate which contained TZC medium alone without any organic input. All the plates were incubated at 28 ± 1°C for 72 hours. Per cent inhibition was calculated by using the following formula:

\[
\text{Per cent inhibition} = \frac{\text{No. of discoloured streaks}}{\text{Total number of streaks}} \times 100
\]

#### 3.6.2 In vivo evaluation of organic inputs against *R. solanacearum*

#### 3.6.2.1 Seedling infection test

Based on *in vitro* performance, seven organic formulations (matka khad, panchgavya, vermiwash, biosol, biodynamic compost tea, cow urine, fermented butter milk and homa ash) were selected to determine their *in vivo* efficacy at 10 per cent and 5 per cent by following method of Kishun and Chand (1998). Apparently healthy capsicum (California Wonder) nursery was raised in sterilized soil in iron trays (30 x 21 cm). Twenty five days old seedlings were uprooted gently and washed in running tap water to remove adhering soil and root tips
were clipped with sterilized blade. These were then washed with sterilized distilled water and dipped in organic formulations alone and in different combinations in 250 ml beakers for 10 min, 15 min, 30 min and 1 hour duration. Likewise, the capsicum seedlings dipped in sterilized distilled water for same durations served as control. One seedling representing a single replication was suspended with the help of cotton plugs in a test tube (15 x 150 mm) containing 15 ml of Hoagland’s solution (Appendix-I) in such a way that whole root system and 1/3rd of its stem remained dipped in the nutrient solution. Ten replications per treatment were used for this study.

The seedlings were allowed to stabilize in the Hoagland’s solution overnight at room temperature. The next day, each seedling was inoculated by adding 1 ml of freshly prepared bacterial cell suspension (10^8 CFU/ml) in each test tube. Seedlings inoculated with sterilized distilled water served as replicated control. The inoculated seedlings were placed on test tube stands near the window allowing maximum sunlight and incubated at room temperature. The inoculated seedlings were observed daily for the appearance of wilt symptoms regularly for 15 days.

3.6.2.2 Pot experiments under polyhouse conditions

Pot experiments were conducted inside polyhouse to test the efficacy of organic inputs evaluated under in vitro conditions. Twenty five days old seedlings were uprooted gently and washed in running tap water to remove adhering soil and root tips were clipped with sterilized blade. These were then washed with sterilized distilled water. The seedlings were dipped in organic formulations alone and in combinations in 250 ml beakers for 10 min, 15 min, 30 min and 1 hour duration. Likewise, the capsicum seedlings dipped in sterilized distilled water for same durations served as control. Five seedlings per pot were transplanted in sterilized soil. Each treatment comprised of three replications. After three days, seedlings were inoculated with the test bacterium by drenching 20 ml of inoculum (10^8 CFU/ml) per plant. The inoculated plants were observed daily for the development of symptoms regularly for 28 days.
The presence of the bacterium in wilted seedlings was confirmed by conducting the ooze test. The effect of each treatment along with control on the survivability of capsicum seedlings in different treatments was determined with reference to maximum survivability of seedlings for 15 or 30 days in the uninoculated control.

Combinations of various organic inputs were as follows:

1 - Biosol
2 - Biodynamic compost tea
3 - Fermented butter milk
4 - Vermiwash
5 - Cow urine
6 - Biosol + vermiwash + homa ash
7 - Cow urine + fermented butter milk
8 - Cow urine + fermented butter milk + vermiwash + homa ash
9 - Biosol + cow urine + fermented butter milk + homa ash
10 - Biosol + cow urine + fermented butter milk + biodynamic compost tea + vermiwash + homa ash
11 - Control

3.7 Evaluation of organic inputs against fungal pathogens

Organic inputs namely 15 days old cow urine, butter milk, biosol, vermiwash, panchgavya and matka khad (Appendix-II) were evaluated for their antifungal potential against test pathogens viz. Sclerotium rolfsii, Fusarium solani, F. oxysporum f.sp. capsici, Sclerotinia sclerotiorum, Colletotrichum capsici, Phytophthora nicotianae and Rhizoctonia solani. All these organic inputs were procured from Department of Organic Agriculture.

Organic inputs were tested by poisoned food technique (Falck 1907) with and without autoclaving. Desired concentrations of organic inputs (2.0, 4.0, 6.0, 8.0 and 10.0 %) were mixed with equal quantity of double strength sterilized PDA and poured aseptically in sterilized Petri plates. Medium mixed with equal
quantities of distilled sterilized water without any treatment served as control. Seven days old mycelial bits (5 mm) of test pathogens were placed in centre of plates and incubated at 25 ± 1°C. Data on mycelial growth were recorded when check plates were fully covered with test pathogen’s growth and per cent inhibition in mycelial growth was determined following McKinney (1923)

\[ I = \frac{C - T}{C} \times 100 \]

where,  
\( I \) = % inhibition  
\( C \) = mycelial growth in check  
\( T \) = mycelial growth in treatment

3.8 Evaluation of microbial isolates

3.8.1 Isolation of microbes from organic inputs

Predominant microbes were isolated from different organic inputs such as vermiwash, biosol, cow urine, fermented butter milk, panchgavya, matka khad, cow pat pit (CPP) and biodynamic compost (Appendix-II). Dilutions of various organic inputs ranging from \( 10^{-1} \) to \( 10^{-5} \) were prepared with sterilized distilled water and plated on potato dextrose agar, nutrient agar, actinomycetes isolation agar and Pikovskaya’s agar medium and plates were incubated at 25 ± 1°C for 48 hours in BOD incubator to obtain the bacterial / fungal isolates. The plates were observed on alternate days to record the appearance of microbes. The morphologically different microbes were isolated, purified and maintained on nutrient agar, as described earlier.

3.8.2 Screening of bacterial isolates for antifungal potential

All isolated cultures were subjected to their antifungal activity against the test pathogens. The microbes which possessed strong antifungal property were taken up for further studies.
3.8.2.1 **In vitro evaluation**

72 h old bacterial cultures grown in nutrient broth were serially diluted by transferring 1 ml of broth culture to a test tube containing 9 ml sterilized distilled water. After thorough mixing 1 ml from this dilution was transferred to next test tube containing 9 ml sterilized distilled water. The procedure was repeated in the same fashion till $10^{-6}$ dilution was obtained. 1 ml serially diluted culture from each dilution was mixed in molten medium at about 45°C and poured in sterilized plates aseptically. The plates were swirled to ensure proper mixing and left to solidify (Harley and Prescott, 1999). After solidification, seven days old mycelial bits were placed in centre of plates under laminar air flow and incubated at 25 ± 1°C. The plates without bacterial suspension served as control. Plates were monitored regularly and data were recorded when culture plates were fully covered with mycelium of pathogen and percent inhibition was calculated (McKinney, 1923).

3.8.2.2 **Determination of CFU/ml**

Pour plate method was used for determination of CFU/ml of bacterial suspension. Number of CFU/ml was calculated as under:

$$\text{CFU/ml} = \frac{\text{No. of bacterial colonies}}{\text{Dilution}}$$

3.8.2.2.1 **Pour plate method**

Nutrient agar medium seeded with different concentrations of individual microbes was poured in Petriplates and incubated at 25 ± 1°C for 48 h. After incubation, the number of colonies formed in each plate were counted with the help of a ‘STUART’ colony counter.

3.9 **Testing the antagonistic activity of bioagents**

Antagonistic activity of locally available bioagents viz. *Trichoderma koningii* (DMA-8 and JMA-11), *T. harzianum* (JMA-4 and SMA-5) and *T. viride* (Hyderabad isolate H3) were tested against test pathogens on PDA using dual culture technique (Huang and Hoes 1976). 5 mm diameter disc of actively
growing culture of pathogen was taken from the culture plates and placed at one end of Petriplate having solidified PDA medium. Similarly, mycelial disc of antagonist was placed at the opposite end of Petriplate in such a way that the distance between the pathogen and bioagent was about 5 cm. The plates containing PDA medium inoculated with pathogen alone served as control. Three replications were maintained in each treatment. The plates were incubated at 25 ± 1°C. The radial growth of the bioagents and the pathogens from the centre of disc towards the centre of the plate was recorded after the control plates were completely covered by pathogens. Mycelial inhibition percentage of the pathogen was determined as described earlier.

3.10 Study of probiotic features of organic inputs

3.10.1 Isolation of phosphate solubilizing bacteria

Phosphate solubilizing bacteria were isolated by dilution plate technique on Pikovskaya’s medium (Sundara Rao and Sinha 1963) containing tricalcium phosphate (TCP) as insoluble source of phosphate. The colonies forming halo zone of clearance around them were counted as phosphate solubilizers. The phosphate solubilizing bacterial colonies were selected, purified and maintained on nutrient agar slants for further studies.

3.10.1.1 Qualitative assay of phosphate solubilization

For screening of phosphate solubilizing activity of microorganisms, the pure cultures of bacteria were point inoculated on Pikovskaya’s agar plate and plates were incubated at 28 ± 1°C for 12 days and the clear zone of solubilization formed around colonies was recorded on alternate day.

Per cent solubilizing efficiency of bacterial isolates was calculated by using following formula:

\[
\text{Per cent solubilizing efficiency (\%SE)} = \frac{Z - C}{C}
\]

where,

\[ Z = \text{Halo zone diameter (mm)} \]
\[ C = \text{Colony diameter (mm)} \]
3.10.1.2 Quantitative assay of phosphate solubilization

The quantitative estimation of phosphate solubilizing activity was done by the vanadomolybdophosphoric yellow colour method in nitric acid system (Subba Rao 1988) in NBRIP (National Botanical Research Institute’s Phosphate Growth Medium) broth (Nautiyal 1999, Mehta and Nautiyal 2001) containing 1000 µg/ml tricalcium phosphate (TCP).

NBRIP broth containing 1000 µg/ml tricalcium phosphate was inoculated aseptically with 1 ml of 24 hours old culture broth and incubated at 28 ± 1°C upto 12 days. Five ml of growth medium from each flask was taken out on 3rd, 5th, 7th, 9th and 11th day of incubation. Filtered through Whatman No. 1 filter paper and centrifuged (R-24 REMI) at 10000 rpm for 20 minutes. 1 ml of supernatant was mixed in 2.5 ml of Barton’s reagent (Appendix-I) and final volume was made upto 50 ml with double distilled water. The intensity of yellow colour was measured with the help of spectrophotometer (Bosch and Lomb) at 430 nm after 10 minutes and the amount of solubilized phosphorus was extrapolated from standard curve.

Potassium dihydrogen orthophosphate AR grade (0.2195 g) was dissolved in one litre of double distilled water. For preparation of working solution, 150 ml of stock solution was taken in volumetric flask and volume made to 250 ml. Standard curve was prepared by taking 0, 2, 5, 8, 10, 20 and 25 ml of the working solution in 50 ml volumetric flasks to which 2.5 ml of Barton’s reagent was added and the volume was made to 50 ml by adding distilled water. Intensity of yellow colour was determined after 10 min at 430 nm spectrophotometrically. Standard curve was prepared by plotting absorbance at 430 nm vs. concentration of phosphorus (Fig. 3.1).

3.10.2 Detection and estimation of IAA production

For screening of indole acetic acid producing isolates, bacterial cultures were inoculated in Luria Bertani Broth with tryptophan concentration of 0, 2 and 5 mg/ml and incubated at 28 ± 1°C for 7 days. Cultures were centrifuged at 3000 rpm for 30 minutes. 2 ml of supernatant was mixed with 2 drops of
orthophosphoric acid and 4 ml of Solawaski’s reagent (50 ml; 35% perchloric acid, 1 ml 0.5 M FeCl₃). Development of pink colour indicated the production of IAA. Optical density was read at 530 nm and the level of IAA produced was estimated from standard curve of IAA (Loper and Schroth 1986).

Standard curve was prepared by taking standard IAA solution in 0-100 µg/ml concentration in different test tubes. The volume was made upto 2 ml with distilled water and then 4 ml Solawaski’s reagent was added and tubes were incubated for 25 minutes at room temperature. Standard curve was prepared by plotting absorbance at 530 nm vs. concentration of IAA (Fig. 3.2).

3.10.3 Detection of siderophore production

The CAS Assay (Schwyn and Neilands 1987) (Appendix-I) is the universal chemical assay for siderophore detection and is based on a siderophore’s high affinity for ferric iron. When siderophore is present, the following reaction occurs, which releases the free dye, which is orange in colour.

\[
\text{Fe}^{3+} \text{- dye (blue) + siderophore} \rightarrow \text{Fe}^{3+} \text{- siderophore production}
\]

3.10.3.1 Qualitative estimation of siderophore production

Qualitative estimation of siderophore production was done by chrome azurol sulfonate (CAS) assay with slight modification (Schwyn and Neilands 1987). All the glasswares were washed in 6 M HCl to remove traces of contaminating iron and then rinsed thoroughly with double distilled water. CAS agar plates were prepared and incubated for 24 h at 28°C for checking any contamination. Efficient phosphate solubilizing microorganisms were spot inoculated on these plates and incubated at 28 ± 2°C for 3 days. The colonies producing colour change of the medium from green to orange around the colonies were considered as siderophore producers.
**Fig. 3.1: Standard curve for Phosphorus**

\[
y = 0.001x - 0.058 \\
R^2 = 0.959
\]

**Fig. 3.2: Standard curve for IAA**

\[
y = 0.019x - 0.104 \\
R^2 = 0.995
\]
3.10.4 Lytic enzyme production by bacterial isolates

3.10.4.1 Proteolytic activity

All the bacterial isolates were screened for proteolytic activity on skim milk agar plates (Kaur et al. 1988). Separately sterilized 1 per cent skim milk was added to nutrient agar before pouring the plates. Bacterial isolates were pin point inoculated on skim milk agar plates and plates were incubated for 3 days at 28 ± 2°C and observed for proteolysis i.e. clear zone produced around the bacterial colony.

3.10.4.2 Cellulolytic activity

Cellulolytic bacterial isolates were identified by the method of Teather and Wood (1982). Bacterial isolates were grown on solid medium containing 0.1 per cent carboxymethyl cellulose (CMC). After growth, plates were flooded with 0.15 per cent congo red and washed with 1 M NaCl. A decolourised halo zone around the colony indicated CMC degrading activity.

3.10.5 HCN production

All the isolates were screened for the production of hydrogen cyanide by adopting the method of Lorck (1948). Nutrient broth was amended with 4.4 g glycine per litre and bacteria were streaked on modified agar plate. A Whatman No. 1 filter paper soaked in 2 per cent sodium carbonate in 0.5 per cent picric acid solution was placed in the top of the plate. Plates were sealed with parafilm and incubated at 28 ± 2°C for 4 days. Development of orange to red colour indicated HCN production.

3.10.6 Nitrogenase activity

Nitrogenase enzyme activity was determined in bacterial isolates by acetylene (C_2H_2) reduction technique (Steward et al. 1967, Hardy et al. 1968). Loopful culture of all bacterial isolates was streaked on nitrogen free medium slants (Jensen’s medium) and incubated at 28°C for 2-7 days. After 7 days, cotton plugs were replaced with air tight serum stoppers and 10 per cent of
atmospheric air was removed from every tube and equal volume of acetylene gas was injected using a syringe. Tubes were again incubated at 28°C for 19 hours. Then 1 ml of gas sample was drawn from the tubes and injected into gas chromatograph for ethylene estimation and similarly values were recorded for 1 ml standard ethylene gas.

Total protein of each sample tube was also determined by Lowry’s method (Lowry et al. 1951). Gas chromatogram was obtained from where Ethylene \((C_2H_4)\) produced by bacterial isolates was calculated by using the formula

\[
n \text{mole of } C_2H_4 \text{ produced hr}^{-1} \text{mg}^{-1} \text{ protein} = \frac{C \times P_s \times A_s \times V}{P_{std} \times A_{std} \times T \times P}
\]

where,

- \(C\) = Concentration of ethylene in the sample in n moles
- \(P_s\) = Peak height of sample
- \(A_s\) = Attenuation used for sample
- \(P_{std}\) = Peak height of standard
- \(A_{std}\) = Attenuation used for standard
- \(T\) = Time of incubation in hours
- \(P\) = Protein content of bacterial growth on slant in mg
- \(V\) = Volume of air space in assay vial (ml)

3.10.7 Qualitative assay for ammonia production

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were incubated in 10 ml peptone water in each tube and incubated for 48-72 hour at 28 ± 1°C. Nessler’s reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman 1992).
3.10.8 Characterization and identification of selected bacterial isolates

Bacterial isolates were characterized on the basis of morphological (type, shape, staining, colony characteristics) and biochemical characteristics (oxidase, catalase, utilization of sugar, indole, MR-VP, denitrification citrate, urease, etc.) following Harley and Prescott (1999). The bacteria were identified using standard methods described in Bergey’s Manual of Determinative Bacteriology (Holt et al. 2000).

3.11 Evaluation of botanicals

3.11.1 Collection and preservation of botanicals

Five botanicals namely *Ranunculus muricatus*, *Vitex negundo*, *Murraya koenigii*, *Melia azedarach* and *Eupatorium* species were used in the present study. Leaves of all the botanicals were collected from the surroundings of Palampur.

The leaves were oven dried by spreading them on the shelves of hot air oven over two to three layered blotting sheets at 50°C for 5 to 6 hours for two to three days. After drying, the respective plant material was ground in a blender to obtain fine dry powder. Sufficient powdery biomass of all the botanicals was stored in paper bags (Tassel bags) at room temperature for further use.

3.11.2 Preparation of extracts

Aqueous and organic plant extracts of botanicals used in the present study were prepared as given below:

3.11.2.1 Preparation of aqueous plant extracts

Fifty gram fine powder of each botanical was soaked overnight in 100 ml of sterilized distilled water (1:2 w/v) in 500 ml conical flask. Next day, the extract obtained was filtered through a double layer of muslin cloth and twice through Whatman No. 1 filter paper to get clear filtrate. This was considered as standard aqueous extract.
3.11.2.2 Preparation of organic plant extracts

Fifty gram fine powder of each botanical was soaked overnight in 100 ml of organic solvents viz., methanol, ethanol and petroleum ether in 500 ml conical flasks. The flasks were covered with aluminium foil to avoid evaporation of the solvent. The extract obtained was filtered through double layer of muslin cloth and twice through Whatman No. 1 filter paper to get the clear filtrate. The organic extracts obtained were heated on water bath at 40-50°C for 10-15 min to evaporate the solvents. The crude extracts thus obtained after evaporation were collected in flasks. The organic extracts with 100 per cent concentration were further diluted to 50 per cent by adding dimethyle sulphoxide (DMSO). This solution thus obtained regarded as standard organic extract. All organic and aqueous plant extracts were filtered with filter syringes (0.22 µ pore size) under aseptic conditions and stored in sterilized flasks (250 ml) in refrigerator for further use.

3.11.3 Preparation and evaluation of cow urine distillates of botanicals

Fresh cow urine was collected from desi cows maintained at Organic Farm, CSK HPKV, Palampur. Cow urine was stored in earthen pots. 15 days old cow urine was used for further studies.

Cow urine was distilled at 100°C using temperature controlled distillation apparatus. The distillate was stored in sterile glass flask at 4°C in refrigerator (Sathasivam et al. 2010) for further studies.

Powder of all five botanicals was soaked in cow urine over night and distilled at 50-60°C using distillation apparatus. The distillate thus obtained was collected in conical flasks and stored at 4°C.

3.11.3.1 Evaluation of antifungal potential

Distillates of cow urine alone and cow urine + botanicals were evaluated for their antifungal activity against the test pathogens (S. rolfsii, F. solani, F. oxysporum f.sp. capsici, S. sclerotiorum, C. capsici, P. nicotianae and R. solani.) by poisoned food technique (Falck 1907) with and without autoclaving. Double strength PDA medium was amended with equal quantity of distillates of cow
urine + botanicals and cow urine alone at different concentrations ranging from 0.5, 2.0, 4.0, 6.0, 8.0 and 10.0 per cent and aseptically poured in sterilized plates. Medium amended with cow urine distillate and sterilized distilled water served as control. Seven days old mycelial bits (5 mm) were placed in the centre of plates and plates were incubated at 25 ± 1°C. When control plates were covered with mycelial growth of pathogens, per cent inhibition was recorded (Vincent 1927).

3.12 Disease management under protected cultivation

Four experiments with respect to management of diseases under polyhouse conditions were conducted at four locations where capsicum was grown. The details of the selected polyhouses and predominant diseases are given in Table 3.1.

Table 3.1 Details of polyhouse locations

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Location</th>
<th>Owner's name</th>
<th>Size of polyhouse (m²)</th>
<th>Name of varieties</th>
<th>Date of transplanting</th>
<th>Predominant diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>University Vegetable Farm</td>
<td>University</td>
<td>105</td>
<td>Mekong, Peppera Pepper</td>
<td>05.04.2010</td>
<td>Bacterial wilt, anthracnose, Phytophthora blight and powdery mildew</td>
</tr>
<tr>
<td>2</td>
<td>Sungal</td>
<td>Krishan Butail</td>
<td>250</td>
<td>US-181, Peppera Pepper</td>
<td>08.04.2010</td>
<td>Bacterial wilt</td>
</tr>
<tr>
<td>3</td>
<td>Rajal</td>
<td>Rajesh Kumar</td>
<td>500</td>
<td>Bharat, Indra, Rv-1049-09, Rv-824-09</td>
<td>16.05.2010</td>
<td>Bacterial wilt, anthracnose, Phytophthora blight and powdery mildew</td>
</tr>
<tr>
<td>4</td>
<td>Chauntra</td>
<td>Lalman Thakur</td>
<td>1000</td>
<td>Bharat, Indra, Tanvi</td>
<td>20.05.2010</td>
<td>Powdery mildew</td>
</tr>
</tbody>
</table>
The details of the experiments for the management of diseases are as follows:

**Experiment – 1:** This experiment was conducted at Sungal for the management of bacterial wilt.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>Drench of Orguard (1:10) before transplanting</td>
</tr>
<tr>
<td>T₂</td>
<td>Seedling dip in biosol for 10 minutes</td>
</tr>
<tr>
<td>T₃</td>
<td>T₂ + Vermicompost (VC) @ 20t/ha</td>
</tr>
<tr>
<td>T₄</td>
<td>T₂ + Biodynamic compost (BD) @ 2t/ha</td>
</tr>
<tr>
<td>T₅</td>
<td>T₂ + Farm yard manure (FYM) @ 25 t/ha</td>
</tr>
<tr>
<td>T₆</td>
<td>T₂ + VC @ 10t/ha + BD @ 2t/ha + FYM @ 10 t/ha (5:1:5)</td>
</tr>
<tr>
<td>T₇</td>
<td>T₁ + T₂ + Drench of Orguard (1:10) at 15 days interval</td>
</tr>
<tr>
<td>T₈</td>
<td>T₁ + T₃ + Drench of Orguard (1:10) at 15 days interval</td>
</tr>
<tr>
<td>T₉</td>
<td>T₁ + T₄ + Drench of Orguard (1:10) at 15 days interval</td>
</tr>
<tr>
<td>T₁₀</td>
<td>T₁ + T₅ + Drench of Orguard (1:10) at 15 days interval</td>
</tr>
<tr>
<td>T₁₁</td>
<td>T₁ + T₆ + Drench of Orguard (1:10) at 15 days interval</td>
</tr>
<tr>
<td>T₁₂</td>
<td>Control</td>
</tr>
</tbody>
</table>

**Experiment – 2:** This experiment was conducted at Rajol for the integrated management of diseases of bell pepper.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>Drench of Orguard (1:10) before transplanting</td>
</tr>
<tr>
<td>T₂</td>
<td>Seedling dip in Panchgavya for 10 minutes</td>
</tr>
<tr>
<td>T₃</td>
<td>Spray of Vermiwash (VW) (1:10)</td>
</tr>
<tr>
<td>T₄</td>
<td>Spray of Biosol (1:10)</td>
</tr>
<tr>
<td>T₅</td>
<td>Spray of Panchgavya (1:10)</td>
</tr>
<tr>
<td>T₆</td>
<td>T₁ + T₂</td>
</tr>
<tr>
<td>T₇</td>
<td>T₁ + T₂ + Drench of Orguard (1:10) at 15 days interval</td>
</tr>
<tr>
<td>T₈</td>
<td>T₃ + T₄ + T₇</td>
</tr>
<tr>
<td>T₉</td>
<td>T₃ + T₅ + T₇</td>
</tr>
<tr>
<td>T₁₀</td>
<td>T₄ + T₅ + T₇</td>
</tr>
<tr>
<td>T₁₁</td>
<td>T₃ + T₄ + T₅ + T₇</td>
</tr>
<tr>
<td>T₁₂</td>
<td>Control</td>
</tr>
</tbody>
</table>
**Experiment – 3:** This experiment was conducted at University Vegetable Farm for the integrated management of diseases of bell pepper as follows:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>Drench of Orguard 1:10) before transplanting</td>
</tr>
<tr>
<td>T₂</td>
<td>Seedling dip in VW for 10 minutes</td>
</tr>
<tr>
<td>T₃</td>
<td>Spray of Biosol (1:10)</td>
</tr>
<tr>
<td>T₄</td>
<td>Spray of Panchgavya (1:10)</td>
</tr>
<tr>
<td>T₅</td>
<td>Spray of VW (1:10)</td>
</tr>
<tr>
<td>T₆</td>
<td>T₁ + T₂</td>
</tr>
<tr>
<td>T₇</td>
<td>T₁ + T₂ + Drench of Orguard (1:10) at 15 days interval</td>
</tr>
<tr>
<td>T₈</td>
<td>T₃ - T₄ - T₇</td>
</tr>
<tr>
<td>T₉</td>
<td>T₃ - T₅ - T₇</td>
</tr>
<tr>
<td>T₁₀</td>
<td>T₄ - T₅ - T₇</td>
</tr>
<tr>
<td>T₁₁</td>
<td>T₃ - T₄ - T₅ - T₇</td>
</tr>
<tr>
<td>T₁₂</td>
<td>Control</td>
</tr>
</tbody>
</table>

**Experiment – 4:** The experiment for management of powdery mildew was conducted at three locations namely Rajol, Chauntra and University Vegetable Farm with the following treatments:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>Milk (10%)</td>
</tr>
<tr>
<td>T₂</td>
<td>Curd (10%)</td>
</tr>
<tr>
<td>T₃</td>
<td>Butter milk (10%)</td>
</tr>
<tr>
<td>T₄</td>
<td>T₁ + T₃</td>
</tr>
<tr>
<td>T₅</td>
<td>T₂ + T₃</td>
</tr>
<tr>
<td>T₆</td>
<td>Neem oil</td>
</tr>
<tr>
<td>T₇</td>
<td>T₃ + T₆</td>
</tr>
<tr>
<td>T₈</td>
<td>T₁ + Asafoetida</td>
</tr>
<tr>
<td>T₉</td>
<td>T₁ + Sulfur</td>
</tr>
<tr>
<td>T₁₀</td>
<td>Bordeaux mixture</td>
</tr>
<tr>
<td>T₁₁</td>
<td>Control</td>
</tr>
</tbody>
</table>
3.12.1 Recording of data

Data on disease severity were recorded at weekly intervals. The disease was scored on 0-9 point scale described by Mayee and Dattar (1986) in case of powdery mildew (*Leveillula taurica*) and anthracnose (*Colletotrichum capsici*) as under:

1. Powdery mildew

<table>
<thead>
<tr>
<th>Grade</th>
<th>Disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>3</td>
<td>1-10</td>
</tr>
<tr>
<td>5</td>
<td>11-25</td>
</tr>
<tr>
<td>7</td>
<td>26-50</td>
</tr>
<tr>
<td>9</td>
<td>&gt; 50</td>
</tr>
</tbody>
</table>

2. Anthracnose

<table>
<thead>
<tr>
<th>Grade</th>
<th>Disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>3</td>
<td>1-10</td>
</tr>
<tr>
<td>5</td>
<td>11-25</td>
</tr>
<tr>
<td>7</td>
<td>25-50</td>
</tr>
<tr>
<td>9</td>
<td>&gt; 50</td>
</tr>
</tbody>
</table>

In case of bacterial wilt (*R. solanacearum*), data were recorded throughout the cropping period at weekly intervals for estimating wilt incidence. The disease severity and disease incidence was recorded and data were pooled at the end of the experiment to ascertain the effectiveness of each treatment against disease. Per cent disease severity and disease incidence was determined by using McKinney (1923) formula:

\[
\text{Disease Severity} = \frac{\text{Sum of all disease ratings}}{\text{Total number of disease ratings}} \times \frac{\text{Maximum disease grade}}{100}
\]

\[
\text{Disease Incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plant assessed}} \times 100
\]
3.12.2 Role of environmental factors on disease development

Disease progress as a function of time was recorded at weekly intervals inside polyhouse. Daily maximum and minimum temperature and relative humidity inside the polyhouse were recorded during the cropping season. The disease was correlated with meteorological factors to determine the role of weather variables, if any, on the development of disease. The role of environmental factors in the development of disease was further established by multiple regression analysis.

3.13 Statistical analysis

Data collected during the course of these investigations were subjected to appropriate statistical analysis, wherever necessary using standard procedure (Gomez and Gomez 1984). The significance of difference was tested at 1 and 5 per cent level of probability. Simple correlation and multiple regression analysis were performed using computer facility, between disease and three independent variables viz. maximum temperature \((X_1)\), minimum temperature \((X_2)\) and relative humidity \((X_3)\). The multiple linear model: \(Y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + e\) was used to describe the function relationship, where.

\[
\begin{align*}
Y &= \text{Predicted mean severity} \\
a &= \text{Intercept} \\
b_i &= \text{Regression coefficient for } X_i \ (i=1\ldots n) \\
X_i &= \text{Independent variables } (i=1\ldots n) \\
e &= \text{Random error}
\end{align*}
\]