Principles of Plant Disease Management

PRACTICAL MANUAL

Course Code: PPA 507; Credit Hours 3(2+1)

For Post Graduate Students

2024

Dr. Siddharth Singh

Dr. Manoj Kumar Chitara

Department of Plant Pathology
College of Agriculture
Chandra Shekhar Azad University of Agriculture & Technology
Kanpur - 208002

Syllabus

Practical: Methods of in-vitro evaluation of chemicals, antibiotics, bio agents against plant pathogens Field evaluation of chemicals, antibiotics, bio agents against plant pathogens. Soil solarisation, methods of soil fumigation under protected cultivation. Methods of application of chemicals and bio control agents. ED and MIC values, study of structural details of sprayers and dusters. Artificial epiphytotic and screening of resistance.

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Published: 2024

No. of copies: ……..

Price: Rs.

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Date: Course Teacher

Content

Practical No. 1

Objective: To study about Phytopathometry of plant pathogens.

Material required:.....

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Phytopathometry:- :.....

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Methods of plant disease measurement:

1) Disease incidence: :.....

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Formula:

2) Disease Severity/ Percent disease index (PDI): :.....

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Formula:

Visual methods of plant disease assessment:

i) Standard area diagrams:	ii) Descriptive scales:

Problem No. 1: Record the data of Bacterial wilt of Brinjal or Fungal wilt of Tomato and calculate disease incidence.

Observation:

Total No. of plants	No. of infected plants	Disease incidence

Formula:

$$\text{Disease incidence } (\%) = \frac{\text{Number of infected plants or leaves of plant}}{\text{Total number of plant or leaves of plant}} \text{X } 100$$

Calculation:

Problem No. 1: Record the data of Alternaria leaf spot of mustard and calculate disease severity/PDI.

Observation:

Disease grade	Total rating	No. of ratings
	Total rating =	Sum of all numerical ratings =

Formula:

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves x maximum score}} \text{X } 100$$

Calculation:

Practical No. 2

Objective: Methods of *in-vitro* evaluation of chemicals and antibiotics against fungi and bacteria

Material required:.....

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***In-vitro* evaluation of chemicals fungicides against fungal plant pathogens.**

Procedure:.....

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***In-vitro* evaluation of chemicals antibiotics against bacterial plant pathogens.**

Procedure:.....

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Formula for calculating Percent inhibition of radial growth:

$$\text{Percent inhibition of radial growth} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

Problem No. 1: **Evaluate the *in-vitro* efficacy of the given fungicides against the fungi (*Colletotrichm* sp.).**

Observations:

Name of the given fungicide	Radial growth of fungal plant pathogen at different fungicide concentration (ppm)					
	0.0	100	200	300	400	500
Copper oxychloride						
Karathane						
Bordeaux mixture						
Azoxystrobin						
Carbendazim						
Mancozeb						
Control						

Calculation:

Problem No. 2: **Evaluate the in-vitro efficacy of the given antibiotics against the *Xanthomonas axonopodis* pv *citri*.**

Observations:

Name of the given antibiotic	Colony growth of bacteria at different antibiotic concentration (ppm)					
	0.0	100	200	300	400	500
Streptocycline						
Agrimycine						
Tetracycline						
Peniciline						
Streptomycin						
Control						

Calculation:

Practical No. 3

Objective: Methods of *in-vitro* evaluation of bio-agents against plant pathogens

Material required:.....

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Procedure:.....

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Formula for calculating Percent inhibition of radial growth:

$$\text{Percent inhibition of radial growth} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

Observations:

Name of the given fungal and bacterial bio agent	Radial growth of plant pathogen (mm)					
	2 days	4 days	6 days	8 days	10 days	12 days
Trichoderma harzianum						
Trichoderma viride						
Psudomonas fluorescens						
Bacillus subtilis						
Control						

Calculations:

Practical No. 4

Objective: To estimate the ED and MIC values of the given agrochemicals

Material required:.....

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Procedure:.....

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Observation: .....

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Practical No. 5

Objective: Field evaluation of chemicals and antibiotics against plant pathogens

Material required:.....

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Procedure:.....

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Observation: .....

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Problem: *In-vivo* evaluate the efficacy of fungicides and antibiotics used in the Practical No.- 2 against late blight of potato and wilt of tomato, respectively.

Practical No. 6

Objective: Field evaluation of bio agents against fungal/bacterial plant pathogens

Material required:.....

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Procedure:.....

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Observation: .....

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Problem: *In-vivo* evaluate the efficacy of bio agents used in the Practicale No.- 3 against Alternaria leaf spot of mustard.

Practical No. 7

Objective: To study about physical methods of plant disease management

Material Required: .....

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Soil solarization:.....

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Soil sterilization:.....

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Hot water or Hot air treatment:.....

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Hot water treatment for managing loose smut of wheat

Procedure:.....

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Practical No. 8

Objective: To study effect of soil solarisation on soil borne plant pathogens

Material Required: .....

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Procedure:.....

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Practical No. 9

Objective: Methods of soil fumigation under protected cultivation

Materials required: .....

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Procedure:.....

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Practical No. 10

Objective: To study about fungicides and its classification

Practical No. 11

Objective: To study about different types of Fungicide formulations

Practical No. 12

Objective: Calculation of fungicide sprays concentrations

Formula:

$$\text{Kg of WG/WP/dust} = \frac{\text{recommended dose} \times \text{spray area}}{\text{a.i. \% of WG/WP/dust}} \times 100$$

Problem No. 1: Calculate the quantity of Captan 50% WG to apply in the one-hectare area if the rate of application is 0.5kg (500 gm) ai/ha.

Problem No. 2: Calculate the amount of Isoprothiolan 40% EC to be sprayed in Paddy at the rate of 0.25 kg a.i./ha for 2 ha.

Problem No. 3: Calculate the amount of Copper Oxychloride 50% WP to be sprayed in Potato at the rate of 0.25 kg a.i./ha for 2 ha.

Problem No. 4: Calculate the amount of Dithianon 10% EC to be sprayed in Apple at the rate of 0.25 kg a.i./ha for 1.5 ha.

Practical No. 13

Objective: To study about different tools used for Pesticide applications in field

Practical No. 14

Objective: Methods of chemicals and bio-control agents application.

Practical No. 15

Objective: Mass multiplication and bio-formulation of bio-control agents

Practical No. 16

Objective: Artificial epiphytotic and screening of resistance

Practical No. 17

Objective: To study about different types of sprayers

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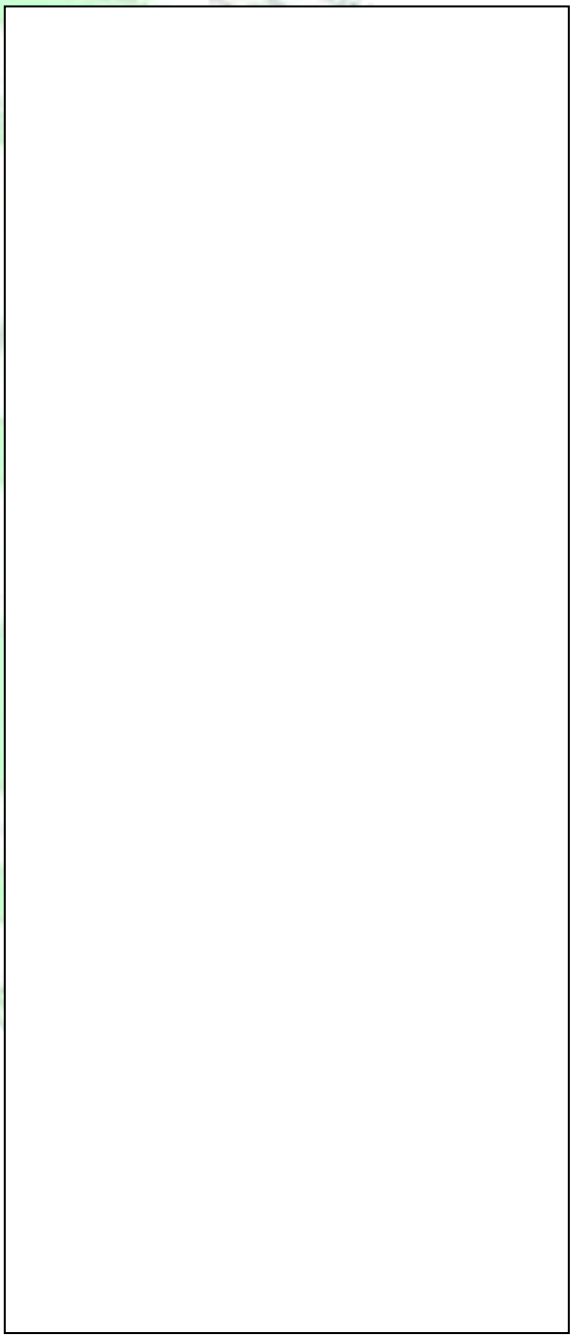
Draw a well labelled diagram of given sprayer.....



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Practical No. 18

Objective: To study about different types of dusters

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Draw a well labelled diagram of given duster .....

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Appendix

PHYTOPATHOMETRY OF PLANT PATHOGENS

Phytopathometry: Also Known as **Plant Pathometry.**

The two synonymous terms "**plant pathometry**" or "**phytopathometry**" were first coined by **Ernest Charles Large** (1953 & 1966). Phytopathometry can be defined as the branch of plant pathology (phytopathology) that is concerned with estimation or measurement of the amount of plant disease expressed by symptoms of disease or signs of a pathogen on a single or

group of specimens. Phytopathometry as defined by **Nutter *et al*. (1991)** equates with "disease assessment" and is the branch of the discipline of phytopathology that deals with estimation or measurement of the amount of plant disease (broadly encompassing detection, identification, and quantification). The measurement of disease intensity is one of the most important and often most difficult tasks in plant disease epidemiology.

Quantification of disease is essential for:

- To know the prevalence and extent of damage caused by a disease (crop loss assessment)
- To know the Pathogen population dynamics
- To develop effective management strategies
- To evaluating host resistant/pathogen virulence
- To evaluating control strategies

The three categories of disease damage:

- The whole plant is killed/ damaged
- Localized part of plant or the field is affected.
- The effect of disease outbreak persists over several seasons.

Disease is measured in term of intensity: Disease intensity can be expressed either as-

1. **Disease incidence.**
2. **Disease severity.**

The choice between evaluation of disease according to its incidence or severity depends largely on the type of disease and on the objectives.

1) Disease incidence: It is the percentage of diseased plants or parts in the sample or population of plants. It can be the proportion or percentage of diseased leaves in a plant, diseased stalks or a tiller or diseased seedlings in a field.

$$\textbf{Disease incidence}\ (\%) = \frac{\textbf{Number of infected plants or leaves of plant}}{\textbf{Total number of plant or leaves of plant}} \textbf{X 100}$$

Disease evaluation according to the incidence is suitable for:

- Most diseases in the early stages of their epidemic and
- It applies mainly to diseases which affects whole plants such as systemic virus diseases, wilts, smuts, fruits rots etc.
- Disease incidence generally tells about the prevalence of the disease in a given areas or host population.

2) Disease Severity/ Percent disease index (PDI): Disease severity is the percentage of relevant host tissues or organ covered by symptom or lesion or damaged by the disease. Severity results from the number and size of the lesions. It is more appropriate in diseases like rusts, downy and powdery mildews, leaf spots and other similar disease. It tells about the extent of damage caused by the disease.

$$\textbf{Percent disease index (PDI)} = \frac{\textbf{Sum of all numerical ratings}}{\textbf{Total number of leaves x maximum score}} \textbf{X 100}$$

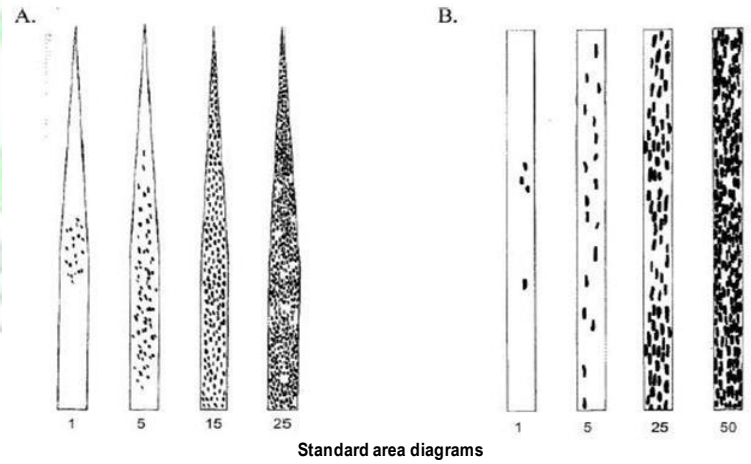
Visual assessment methods

i. **Standard area diagrams**
ii. **Descriptive scales**

i) Standard area diagrams: It allow estimation of intermediate levels of disease severity by comparing a diseased plant with diagrams showing both more and less disease.

Standard area diagrams e g. leaf rust of wheat provide 1, 5, 10, 20, 50 % leaf area infected.

Visually estimated plant disease severity



Standard area diagrams

ii) Descriptive scales: For assessing the disease severity the descriptive scales/keys have also been standardized for various diseases. These are widely used and are of many types ranging from disease rating on numerical scale to subjective estimates as severe, moderate etc.

Severity scale	
Rating	**Area covered by the disease**
0	No disease on leaf and pods
1	Small brown spot covering <1% leaf area (pin point spots on pod)
3	Brown sunken spots 1-10% leaf area (< 1% pod area)

5	Brown spots 11-25% leaf area (1-10% pod areas)
7	Circular brown sunken spots 26-50% leaf area(11-25% pod area)
9	Circular to irregular >51% leaf area (>26% pod area)

0	1	3	5	7	9

M. I. Huq (2011). Studies on the Epidemiology of Leaf Rot and Leaf Spot Diseases of Betel Vine (*Piper Betle* L.). Bangladesh J. Sci. Ind. Res. 46(4): 519-522.

Rating		Area covered by the disease/Infected plants or plant parts		
0	=	Healthy leaf, no infection	or	No or a few lesion on leaf.
1	=	Up to 5% leaf area covered	or	Up to 10% leaf area affected.
2	=	6-15% leaf area covered.	or	11-25% leaf area affected.
3	=	16-30% leaf area covered.	or	26-50% leaf area affected.
4	=	31-50 % leaf area covered.	or	51-75% leaf area affected.
5	=	above 50% leaf area covered.	or	above75 % leaf area affected.

Example:-

Disease grade	Total rating	No. of ratings
0	5	0
1	5	5
3	8	24
5	4	20
7	8	56
9	4	36
	Total rating = 34	Sum of all numerical ratings = 186

Sum of all ratings = 186
Total ratings = 34
Max. Disease grade = 9
Disease Severity/PDI = {186/ 34 x 9} X 100 = 60%

IN-VITRO EVALUATION OF CHEMICALS AGAINST FUNGAL PLANT PATHOGENS

Material required: Five-day-old culture of phyto-pathogenic fungi growing on potato dextrose agar, Petri dishes (n=3) containing approximately 20ml of potato dextrose agar amended with given fungicides at concentrations of 0, 100,200,300,400 and 500 ppm, Disposable nitrile gloves, cork borer (0.5 mm), parafilm tape, ruler, pencil, *etc*.

Procedure:

- Amend potato dextrose agar with technical/commercial grade of given fungicides to achieve final concentrations of 0, 100,200,300,400 and 500 ppm of medium.
- The fungicide should be dissolved in acetone (0.05% vol./vol.) before mixing with agar that has been cooled to 60°C.
- Non-amended media will serve as the control.
- Label each Petri dish with the appropriate concentration.
- Surface sterilize a cork borer and obtain hyphal plugs of 1-cm-diameter from the edge of actively growing *S. rolfsii* colonies.
- Inoculate Petri dishes by placing the plug hyphal side down on the center of each of the six different concentrations.
- Three replicates should be used for each concentration.
- Wrap Petri dishes with Parafilm, incubate at 27oC temperature in the dark for 3 days.
- Measure mycelial growth (in mm) from the edge of the inoculum plug at two locations perpendicular to one another, record your data, and calculate the mean mycelial growth for each concentration.
- Using logarithm (base 10) graphing paper, plot the means of growth on each of the concentrations.
- Determine the effective concentration to inhibit growth by 50% (EC50 value).

- This can be done by regressing the percent inhibition (100 – [colony diameter on amended medium / colony diameter on the control × 100]) against the log (base 10) of the fungicide concentration.
- Count the number of sclerotia produced by the fungus on each of the fungicide-amended media after 10–14 days.

Observations:

Name of given fungicide	Radial growth of fungal plant pathogen at different fungicide concentration (ppm)					
	0.0	100	200	300	400	500
Copper oxychloride						
Karathane						
Bordeaux mixture						
Azoxystrobin						
Carbendazim						
Mancozeb						
Control						

Calculations:

$$\text{Percent inhibition of radial growth} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

IN-VITRO EVALUATION OF ANTIBIOTICS AGAINST BACTERIAL PLANT PATHOGEN

Material required: Incubator • Two- to three-day-old cultures of *Xanthomonas axonopodis* pv. *citri* growing on nutrient agar supplemented with 0.1% w/ v d-glucose (NGA) or YPGA(0.5% peptone, 0.5% yeast extract, 1% glucose, 1.5% agar), Petri dishes (n=3) containing approximately 20 mL of nutrient agar amended with streptocycline at concentrations of 0, 100,200,300,400 and 500 ppm, Disposable nitrile gloves, filter paper disc (Whatman No. 42), parafilm, ruler, pencil.

Procedure:

- The bacterium *Xanthomonas axonopodis* pv. *citri* was multiplied by inoculating the loopful culture in 150 ml conical flask containing 50 ml of nutrient broth medium.
- The inoculated flasks were incubated at 27±20C for 72 h
- Amend nutrient agar with available antibiotic to achieve final concentrations 0, 100,200,300,400 and 500 ppm of medium
- Non-amended media will serve as the control
- Label each Petri dish with the appropriate concentration
- The filter paper disc (Whatman No. 42) measuring 5 mm in diameter are prepared and sterilized before use.
- The sterilized filter paper discs are soaked in the bacterial culture for five minutes and transferred onto the surface at the center of each of the six different concentrations amended medium in Petri-plates.
- Three replicates should be used for each concentration
- Wrap Petri dishes with Parafilm, place them
- Measure bacterial growth (in mm) from the edge of the inoculum disc at two locations perpendicular to one another, record your data, and calculate the mean colony growth for each concentration
- Using logarithm (base 10) graphing paper, plot the means of growth on each of the concentrations
- Determine the effective concentration to inhibit growth by 50% (EC 50 value)
- This can be done by regressing the percent inhibition (100 – [colony diameter on amended medium / colony diameter on the control × 100]) against the log (base 10) of the fungicide concentration

Observations:

Name of given antibiotic	Colony growth of bacterial plant pathogen at different antibiotic concentration (ppm)					
	0.0	100	200	300	400	500
Streptocycline						
Agrimycine						
Tetracycline						
Peniciline						
Streptomycin						
Control						

IN-VITRO EVALUATION OF BIO AGENTS AGAINST PLANT PATHOGENS

Material required: Incubator, Cultures of *P. fluorescens* isolate and *Alternaria brassicae*, Bunsen burner, Inoculation loop, Forceps, Ethanol solution, Potato dextrose agar (PDA), Petri plates.

Procedure:

I. In vitro evaluation of bacterial bio agents

- Five to seven days prior to the experiment, start growing cultures of *Alternaria brassicae* in separate PDA plates and incubate them at 28°C.
- Three to five days prior to the experiment, start growing fluorescent Pseudomonas and incubate them at 28°C.
- One day before the experiment, start the "antagonism" plates. With a sterile inoculation loop, transfer Pseudomonas to new PDA plates as a ~1 cm diameter circle close to the edge of the plates Incubate bacterial plates for 24 h at 28°C.
- On the day of the experiment, using a sterile pipette tip (hold from the tip end upside down), and cut an agar plug from the actively growing mycelium of *Alternaria brassicae*. With the help of a sterile loop, transfer the agar plug to the center of plates.
- One of the plates will be used as a "control" for *Alternaria brassicae* growth, and another one will be used to test "antagonistic" activity of fluorescent Pseudomonas isolates.
- Seal each Petri plate with Parafilm to prevent moisture loss and maintain sterility.
- Incubate plates at 28°C for 4–5 days.
- Observe plates daily, and once the mycelia of *Alternaria brassicae* fill the whole surface of the control plates, measure inhibition zone for each isolate.
- Use a ruler to measure and record the distance between the edge of the bacterial colony facing the fungus (Rs) or oomycete (Pu), and the edge of the mycelial growth of Rs or Pu. If both edges touch each other, record as "0," and consider these isolates to be non-antagonistic to the plant pathogen
- Observe if, in the inhibition halo, you can detect any colour diffusing in the agar. Orange may be indicative of antibiotics such as phenazine, whereas fluorescent yellow may be indicative of siderophore production (visible under UV light at 360 nm wavelength)

II. In vitro evaluation of fungal bio agents

- Five mm discs were cut from the periphery of actively growing ten days old culture of the test fungus with the help of sterilized cork-borer, similarly Trichoderma discs were cut with borer
- Place the discs in such a manner that both the discs lie opposite to each other (approximately 4 cm apart from each other) in petri plates (9 cm diameter) seeded with PDA (approx. 20 ml/ plate).
- Three replications were used for each treatment. All the plates were incubated at 27±10C. Petri plates without Trichoderma served as control.

Observations:

Name of the given fungal bio agent	Radial growth of plant pathogen (mm)					
	2 days	4 days	6 days	8 days	10 days	12 days
Trichoderma harzianum						
Trichoderma viride						
Pseudomonas fluorescens						
Bacillus subtilis						
Control						

Calculations:

$$\text{Percent inhibition of radial growth} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

ESTIMATION OFCONCENTRATION VALUES OF AGROCHEMICALS

In vitro poisoned food method is used to find out the ED50, ED90 (agrochemicals concentration at which 50 and 90 % population of the fungus is restricted) and MIC (minimum inhibitory concentration) values of different fungicides are derived by using dose response curve.

Median effective concentration (EC50): Statistically derived median concentration of a substance in an environmental medium expected to produce a certain effect in 50 % of test organisms in a given population under a defined set of conditions.
Note: ECn refers to the median concentration that is effective in n % of the test population

Median effective dose (ED50): Statistically derived median dose of a chemical or physical agent (radiation) expected to produce a certain effect in 50 % of test organisms in a given population or to produce a half-maximal effect in a biological system under a defined set of conditions.
Note: EDn refers to the median dose that is effective in n % of the test population

Median lethal concentration (LC50): Statistically derived median concentration of a substance in an environmental medium expected to kill 50 % of organisms in a given population under a defined set of conditions

Median lethal dose (LD50): Statistically derived median dose of a chemical or physical agent (radiation) expected to kill 50 % of organisms in a given population under a defined set of conditions

Procedure:

- Stock solutions of the chemical was prepared and from the stock solution different concentrations such as 10, 20, 30, 40, 50,100, 200,

300, 400 and 500 ppm were prepared in sterile distilled water.

- This was incorporated into 100 mL of sterilized carrot agar medium so as to get the required concentration and poured into petri dishes.
- Pathogen grows on Carrot Agar (CA) for 72 h at 24±1o C. Agar plugs of 5 mm are to be taken from the edges of the actively growing culture of the pathogen and place in the center of the sterile CA plates amended with the test fungicide.
- The required concentrations of the chemicals incorporated on to sterilized carrot agar medium and poured into sterile plates and inoculated
- Control plates contained only CA inoculated with the pathogen alone
- All the treatments should be replicated thrice
- The plates were incubated for 96 h and radial growth of mycelium is measured and percent inhibition calculated as per given formula

Calculation:

$$\mathbf{I = C\text{-}T/C \times 100}$$

Where, I= percent inhibition, C= radial growth in control and T= radial growth in treatment

Effect on pathogen sporulation:

- The stock solution prepared as above was used for studying the sporulation of pathogen.
- Different concentrations of the chemical were prepared in sterile distilled water
- Inoculum plugs of 5 mm size are cut from the margin of 72 h old culture of *P. capsici* and incubated in different concentrations of the chemicals under continuous fluorescent light for 72 h at 24 ± 1o C
- There should be three replications/treatment with 5 discs/replication
- Inoculums plugs in sterile distilled water served as control
- Observations for sporulation were taken under the microscope
- Three microscopic fields were counted/replication and the average number of sporangia produced was estimated and the reduction in sporulation compared to control and ED50 and ED90 values are calculated.

Observation:

FIELD EVALUATION OF CHEMICALS AGAINST PLANT PATHOGENS

Material required: Potato tuber/Tomato seeds, Copper oxychloride, Karathane, Bordeaux mixture, Azoxystrobin, Carbendazim, Mancozeb, etc. sprayed at recommended doses., Knapsack sprayer, weighing balance, measuring cylinder, fertilizers, measuring tape, field tags.

Procedure:

- One month old tomato seedlings raised in nursery were transplanted to in a plot size of 3 x 2 m
- Plant to plant spacing were kept 45 cm and row spacing was maintained of 60 cm the experiment was laid out in randomized block design with three replications
- All recommended agronomic practices of the zone should be adapted
- Fungicides are Copper oxychloride, Karathane, Bordeaux mixture, Azoxystrobin, Carbendazim, Mancozeb, etc. sprayed at recommended doses.
- Untreated plot will serve as control for comparing the efficacy.
- Fungicidal application should be done by Knapsack sprayer
- Three sprays of fungicides were applied at regular intervals 15 days, 30 days and 45 days of the initiation of the disease
- Data on the disease severity recorded after every fifteen days intervals of each spray
- Five plants were selected randomly in each plot and observations on severity of the disease on the foliage was recorded using 0-5 scale of Horsefall and Barette, 1945.

Disease rating scale for the assessment of early blight of tomato

Scale	Description of the symptom
0	Leaves free from infection
1	Small irregular spots covering <5% leaf area
2	Small irregular brown spots with concentric rings covering 5.1-10% leaf area
3	Lesions enlarging, irregular brown with concentric rings covering 10.1-25% leaf area
4	Lesions coalease to form irregular and appears as a typical blight symptom covering 25.1-50% leaf area

- Percent disease index (PDI) was calculated using formula of Wheeler (1969) as given below:

$$PDI = \frac{\textbf{Sum of all numerical rating}}{\textbf{Total number of leaves observed} \times \textbf{Maximum rating}} \times 100$$

- In the field experiments well mature and ripen tomato fruits are to be harvested regularly.
- Record the fruit yield per plot and extrapolated to give the value of fruit yield in tons per hectare.

FIELD EVALUATION OF ANTIBIOTICS AGAINST BACTERIAL PLANT PATHOGENS

Material required: Streptocycline, Bacterinol, sprayer, measuring cylinder, weighing balance, measuring tape.

Procedure: The antibiotics Streptocycline and Bacterinol, are to be evaluated at concentrations of 250 and 500 ppm in vivo against *X. axonopodis* pv. *punicae*.

Design: RBD; **Replications:** Three

Total two sprays of all the treatments will be undertaken at an interval of 15 days, starting first spraying at first incidence of disease. One replication was maintained as unsprayed control without receiving any chemicals. Observation on leaf bacterial blight disease will be recorded and after each spraying and last observation on leaf bacterial blight to be recorded at 15 days after last spraying. Five trees per treatment per replication are to be selected randomly and tagged. Trifoliate leaves (bottom, middle and top) from main branch on each observation and per cent leaf bacterial blight disease recorded as per the scale mentioned in sampling methodology.

Based on numerical rating / scale observed, per cent disease index / intensity were worked out applying the formula given by Mckinney (1923).

$$PDI = \frac{\textbf{Sum of all numerical rating}}{\textbf{Total number of leaves observed} \times \textbf{Maximum rating}} \times 100$$

Further per cent disease control (PDC) was worked out by formula:

$$PDC = \frac{\textbf{PDI in control plot} - \textbf{PDI in treatment plot}}{\textbf{PDI in control plot}} \times 100$$

Observation:

No.	Treatments	Conc. (ppm)	PDI* before spraying	PDI* after spraying		Mean PDI	PDC* after spraying		Mean PDC
				First	Second		First	Second	
1	Streptocycline	250							
2	Streptocycline	500							
3	Bacterinol	250							
4	Bacterinol	500							
5	Control	-							

FIELD EVALUATION OF BIO AGENTS AGAINST FUNGAL/BACTERIAL PLANT PATHOGENS

Material required: *Pseudomonas fluorescens* and *Bacillus subtilis* culture, sprayer, measuring cylinder, weighing balance, measuring tape

Procedure: Two Bio-agents namely **Pseudomonas fluorescens** and **Bacillus subtilis**, will be evaluated *in vivo* against *X. axonopodis* pv. *punicae* at concentrations of 250 and 500ppm.

Design: RBD; **Replications:** Three

Total two sprays of all the treatments will be undertaken at an interval of 15 days, starting first spraying at first incidence of disease. One replication was maintained as unsprayed control without receiving any bio-agents. Observation on leaf bacterial blight disease will be recorded and after each spraying and last observation on leaf bacterial blight to be recorded at 15 days after last spraying. Five trees per treatment per replication are to be selected randomly and tagged. Trifoliate leaves (bottom, middle and top) from main branch on each observation and per cent leaf bacterial blight disease recorded as per the scale mentioned in sampling methodology.

Based on numerical rating / scale observed, per cent disease index / intensity were worked out applying the formula given by Mckinney (1923).

$$PDI = \frac{\textbf{Sum of all numerical rating}}{\textbf{Total number of leaves observed} \times \textbf{Maximum rating}} \times 100$$

Further per cent disease control (PDC) was worked out by formula:

$$PDC = \frac{\textbf{PDI in control plot} - \textbf{PDI in treatment plot}}{\textbf{PDI in control plot}} \times 100$$

Observation:

No.	Treatments	Concentration (ppm)	PDI* before spraying	PDI* after spraying		Mean PDI	PDC* after spraying		Mean PDC
				First	Second		First	Second	
1	*Pseudomonas fluorescens*	250							
2	*Pseudomonas fluorescens*	500							

3	*Bacillus subtilis*	250							
4	*Bacillus subtilis*	500							
5	Control	-							

PHYSICAL METHODS OF PLANT DISEASE MANAGEMENT

Material required: Seed, vegetative propagative material

Principles of physical methods: This method includes soil solarization and hot water treatments.

Soil solarization: Soil solarization or slow soil pasteurization is the hydro/thermal soil heating accomplished by covering moist soil with polyethylene sheets as soil mulch during summer months for 4-6 weeks. Soil solarization was developed for the first time in Israel (Egley and Katan) for the management of plant pathogenic pests, diseases and weeds.

Soil sterilization: Soil can be sterilized in green houses and sometimes in seed beds by aerated steam or hot water. At about 50 °C, nematodes, some soil borne fungi and other water molds are killed. At about 60 and 72°C, most of the plant pathogenic fungi and bacteria are killed. At about 82°C, most weeds, plant pathogenic bacteria and insects are killed. Heat tolerant weed seeds and some plant viruses, such as TMV are killed at or near the boiling point (95-100°C).

Hot water or Hot air treatment: Hot water treatment or hat air treatment will prevent the seed borne and sett borne infectious diseases. Hot water treatment of certain seeds, bulbs and nursery stock is done to kill many pathogens present in or on the seed and other propagating materials. Hot water treatment is used for controlling sett borne diseases of sugarcane [whip smut, grassy shoot and red rot of sugarcane (52°C for 30 min)] and loose smut of wheat (52°C for 10 min).

Hot water treatment for managing loose smut of wheat

Procedure:

- The wheat seed was treated in cheesecloth bags with ample room to allow for swelling of the grain.
- A 250-liter tank, used for treatment, equipped with two motor-driven propellers which kept the water thoroughly agitated and at a uniform temperature.
- Thermostatically controlled electric heaters were employed to maintain the temperature of the 10-minute bath, which was held at 54° C. in all cases with a plus or minus variation should not exceed 0.2°C.
- The wheat seed is pre-soaked for 4 to 5 hours in cold water, then dipped momentarily in water at about 49°C, and finally immersed in water at 54°C for 10 minutes.
- Immediately after treatment the seed was spread in a thin layer to cool, and in order to reduce its moisture content to about that of the untreated seed, it was left to dry for 5 days or more at room temperature.
- Soil-germination tests in the greenhouse were made by sowing 100 seeds per flat 1x2 feet.
- Uniform spacing and depth of sowing were insured by pressing into the soil 100 one-inch pegs inserted in a board equidistantly.
- The kernels were dropped into the holes so made and then covered.

EFFECT OF SOIL SOLARISATION ON SOIL BORNE PLANT PATHOGENS

Material required: Plastic sheets, ultraviolet (UV)-resistant glue, thermometer

Principle: Soil solarization is a non-pesticidal method of controlling soil-borne pests by placing plastic sheets on moist soil during periods of high ambient temperature. The plastic sheets allow the sun's radiant energy to be trapped in the soil, heating the upper levels. Solarization during the hot summer months can increase soil temperature to levels that kill many disease-causing organisms (pathogens), nematodes, and weed seed and seedlings. It leaves no toxic residues and can be easily used on a small or large scale. Soil solarization also improves soil structure and increases the availability of nitrogen (N) and other essential plant nutrients. When clear polythene film is placed over moist soil during sunny summer days, the temperature at the top 5 cm of soil may reach as high as 52^0C compared to a maximum of 37^0C in un-mulched soil. If sunny weather continues for several days or weeks, the increased soil temperature from solar heat, known as solarization inactivates (or kills) many soil-borne pathogens, viz., fungi, nematodes, and bacteria near soil surface, thereby reducing the inoculum and its potential for causing disease.

Procedure:

Soil Preparation

- Solarization is most effective when the plastic sheeting (tarp) is laid as close as possible to a smooth soil surface.
- Preparation of the soil begins by disking, rototilling, or turning the soil by hand to break up clods and then smoothing the soil surface.
- Remove any large rocks, weeds, or any other objects or debris that will raise or puncture the plastic.

Laying the Plastic: Plastic sheets may be laid by hand or machine. The open edges of the plastic sheeting should be anchored to the soil by burying the edges in a shallow trench around the treated area.

Complete coverage:

- Plastic sheeting is laid down to form a continuous surface over the entire field or area to be planted.
- The edges of the sheets may be joined with an ultraviolet (UV)-resistant glue or anchored by laying adjacent strips of plastic and burying both edges in soil.
- Anchoring the edges in the soil may be more cost effective initially than gluing the edges together but may also result in untreated soil being close to subsequently planted crops.
- The ends of the sheets should be held in place by burying them in the soil.
- If beds are formed after complete coverage, care must be taken to avoid deep tillage that could bring untreated soil to the surface.
- Complete coverage is recommended if the soil is heavily infested with pathogens, nematodes, or perennial weeds, since there is less chance of re-infestation by soil being moved to the plants through cultivation or furrow-applied irrigation water.

Strip coverage:

- Plastic is applied in strips over preformed beds. Strips should be a minimum of 30 inches (75 cm) wide; beds up to 5 feet (1.5 m) wide are preferred because several crop rows can be planted per bed.
- In some cases, strip coverage may be more practical and economical than complete coverage because less plastic is needed and it is not necessary to join the edges of the plastic sheets together.
- Strip coverage effectively kills most pests and eliminates the need for deep cultivation after solarization.
- It is especially effective against weeds, since the furrows are cultivated.
- With strip coverage, however, long term control of soil pathogens and nematodes may be lost because pests in the untreated soil in the rows between the strips can contaminate and re-infest treated areas.

Irrigation:

- Wet soil conducts heat better than dry soil and makes soil organisms more vulnerable to heat.
- The soil under the plastic sheets must be saturated to at least 70 percent of field capacity in the upper layers and moist to depths of 24 inches (60 cm) for soil solarization to be effective.
- Soil may be irrigated either before or after the plastic sheets are laid.
- If the soil is irrigated beforehand, the plastic must be applied as soon as possible to avoid water loss; if heavy machinery is used to lay the plastic, however, the soil must be dry enough to avoid compaction.
- If the soil is to be irrigated after the plastic is laid, one or more hose or pipe outlets may be installed under one end of the plastic; drip lines may be installed before the plastic is laid; or irrigation water may be run underneath the plastic in furrows or in the tracks made by tractor wheels if the plastic sheets were applied by machine.
- Fields treated by strip coverage can be irrigated by drip lines on or in the bed.
- The soil does not usually need to be irrigated again during solarization, although if the soil is very light and sandy, or if the soil moisture is less than 50 percent of field capacity, it may be necessary to irrigate a second time.
- This will cool the soil, but because of the increased moisture the final temperatures will be greater.

Duration of Treatment:

- The plastic sheets should be left in place for 4 to 6 weeks to allow the soil to heat to the greatest depth possible.
- To control the most resistant species, leave the plastic in place for 6 weeks.
- There is little or no need to take the temperature of the soil.
- The greatest concern is to solarize the soil during a period of high solar radiation with little wind or cloud cover.

Removal of the Plastic and Planting

- After solarization is complete, the plastic may be removed before planting or, the plastic may be left on the soil as a mulch for the following crop by transplanting plants through the plastic.
- Clear plastic may be painted white or silver to cool the soil and repel flying insect pests in the following crop.
- A disadvantage of leaving the plastic on the soil is that it may degrade and be difficult to clean up.
- Treated soil can be planted immediately or left fallow without the plastic until the next growing season.
- If the soil must be cultivated for planting, the cultivation must be shallow-less than 2 inches (5 cm)-to avoid moving viable weed seed to the surface.

SOIL FUMIGATION UNDER PROTECTED CULTIVATION

Principle Soil fumigation is a chemical control strategy used independently or in conjunction with cultural and physical control methods to reduce populations of soil organisms. Soil fumigants can effectively control soil-borne organisms, such as nematodes, fungi, bacteria, insects, weed seeds, and weeds. Some are pest-specific, while others are broad spectrum biocides and kill most soil organisms. Because of treatment costs, applicators use soil fumigants primarily on high value crops, such as vegetables, fruits, and ornamentals. Very careful attention must be paid to the details of how the fumigation is done in order to guarantee excellent control of the targeted organisms and complete safety for workers and other persons close to the treated area, while also limiting the impact the product could have on our environment

Types of Soil Fumigants: Soil fumigation uses pesticide formulations that volatilize from a liquid or solid into a gas state. Soil fumigants are applied to the soil as liquefied gases, volatile liquids, or granules. Due to the high volatility of these compounds, the fumigant must be incorporated into the soil during or immediately following application. At or shortly after application, these chemicals volatilize, allowing toxic molecules to move through the air pores in the soil. Soil pests are killed when they come in contact with a toxic concentration for a long enough exposure period. For all fumigants, enough concentration and contact time with target pests are required to obtain good results.

Methyl Bromide: It is a broad-spectrum fumigant that controls many weeds and soil-borne insects, nematodes, fungi, and bacteria. However, it does not adequately control all species. Methyl bromide is toxic to all stages of insect life. It is

registered for use on a variety of crops, including ornamentals, vineyards, deciduous fruit and nuts, nursery sites, greenhouse soils, peppers, tomatoes, and strawberries.

Methyl Bromide and Chloropicrin Mixtures: Proprietary materials are available that contain both methyl bromide and chloropicrin. Such combinations are more effective than either material alone in controlling weeds, insects, nematodes, and soil-borne pathogens.

Metam Sodium: It is recommended as a pre-planting treatment to control soil-borne pests that attack ornamentals and other crops. Do not apply to crops. It controls soil-borne fungal diseases (e.g., *Fusarium, Pythium, Phytophthora, Sclerotinia*, oak root fungus, Verticillium, club root of crucifers, and Rhizoctonia), nematodes, symphylids, and germinating weed seeds of annual grasses, chickweed, dandelion, ragweed, henbit, lamb's-quarters, pigweed, purslane and suppression of perennial weeds such as quack grass.

Chloropicrin: Chloropicrin is a broad-spectrum fumigant that controls some soil-borne insects, fungi, and bacteria. It provides limited control of some weed seeds and nematodes. Although chloropicrin is often added to other fumigants in low concentrations as a warning agent, it is also added at higher concentrations (up to 75%) to increase the overall spectrum of pest control. Chloropicrin is often formulated with methyl bromide, iodomethane, DMDS, and 1,3-D. It may be formulated as the sole active ingredient.

Methyl Iodide: The effectiveness of methyl iodide is similar to methyl bromide, rendering it a potential replacement. Methyl iodide offers broad-spectrum activity like methyl bromide, but there is not the concern on impacts to the ozone layer.

Dimethyl Disulfide (DMSO): It is toxic to some weeds, soil borne nematodes, bacteria, and fungi. DMDS is a widespread natural product and is labelled for use on vegetables (tomatoes, peppers, egg plants), cucurbit crops (cucumber, squash and melons), strawberries, blueberries, and field-grown ornamentals.

1,3-Dichloropropene: 1,3-dichloropropene (1,3-D) provides nematode control, but does not provide broad spectrum weed control. The "C" formulations include chloropicrin for pathogen control.

Factors Influencing Soil Fumigation: Many factors affect soil fumigation and its effectiveness for pest control. The pest and its habits will affect fumigant selection, application rate, fumigant placement, and necessary length of exposure. Soil factors also play a key role in fumigation. Soil texture, soil condition, debris, soil moisture, and soil temperature may affect the volatility, movement, and availability of the fumigant once applied. Fumigant dosage is both pest- and soil-dependent. The following section discusses some of these factors in greater detail. After fumigation, aeration is important to make sure phytotoxicity does not occur.

Soil Texture: Soil texture influences fumigant movement and availability due to its effects on the amount of soil pore space (air spaces) and the number of adsorption (binding) sites. Fine-textured soils, such as clay, have many adsorption sites per unit area and many pore spaces.

Soil Tilth: Soil tilth is the physical condition of soil. It usually relates to the suitability of soil for planting or growing a crop. Factors include clods, moisture content, degree of aeration, rate of water infiltration, and drainage. The tilth of a soil can change rapidly, depending on environmental factors such as changes in moisture.

Soil Moisture: Soil moisture impacts the movement of a fumigant through the soil and off-gassing into the air. Improper soil moisture at the time of application can lead to poor control of target pests and could result in off-gassing. Soil moisture requirements vary depending on the fumigant. Additionally, these requirements may vary depending on a variety of factors, including soil texture, application method, and application depth.

Soil Temperature: Soil temperature correlates directly with fumigant volatility and movement. Soil temperature determines the fumigant state (solid, liquid or gas). As temperatures increase, fumigant volatility and diffusion increase. **Application Rates**: Application rates depend on several factors. Higher pest densities and targeting multiple pest species may require using higher fumigant application rates noted on the label. Furthermore, certain difficult-to-control pests and those with high population densities require using the higher rates. Pest location is also important. For example, 1,3-D rates differ depending on whether the target pest is an insect, fungus, bacterium, or nematode, and even by species of organism.

Application Methods and Soil Sealing: Fumigants can be applied to soil in many different ways. The diverse chemical characteristics of soil fumigants largely determine how the products are applied. However, the application method is also determined by it formulation, the target pest, the cost and the area or site to be fumigated. (For example, fumigating a greenhouse soil versus fumigating a mound of potting soil.)

Shank or Spray Blade Application: For shank soil injection applications, knifelike blades called shanks or chisels are mounted vertically on a toolbar behind a tractor and pulled through the soil to deliver the fumigant. A tube carrying the fumigant runs down the back of each shank. Fumigant travels from the tank to the tubing through a pressurized system. Shank traces (the grooves the shanks make in the soil) are covered with soil.

Chemigation: Application Several fumigants can be applied through irrigation systems; however, some fumigants restrict their use only to drip irrigation (chloropicrin, iodomethane, DMDS, 1,3-D). To fumigate soil by chemigation, meter and inject a liquid fumigant into irrigation water. Fumigant chemigation is applied through several types of irrigation systems.

Equipment includes an injection pump and nurse tank system.

Hot Gas Application: The hot gas, no-till application method is used for methyl bromide and chloropicrin mixtures. Heat the fumigant by passing it though a heat exchanger. Then, deliver it to the soil surface through a system of tubing or piping.

FUNGICIDES

Fungicides: Chemicals or any toxic substances which directly or indirectly kill or control the parasitic fungi are called fungicides. Chemicals used for controlling the plant diseases caused by fungal pathogens are called fungicides.

Characteristics of Ideal Fungicides:-

- It should have less or low phytotoxicity
- Stability during storage.
- Should not be toxic for warm blooded animals, earthworms, beneficial soil microorganisms and non-target organisms
- Stability during dilution
- It should have high fungi toxic activity and broad spectrum in action
- Fungicide preparation should be ready for use
- Should be available easily with affordable costs.
- Should not be inflammable or explosive
- Easy to handle and apply.
- It should have high tenacity.
- It should have high compatibility with other agrochemicals
- It should be available in different formulations
- It should be easily transportable
- It should not cause environmental pollution.

Classification of fungicides: Fungicidal chemicals may be classified in different ways.

1) Mode of action against fungi 2) Genera Use 3) Chemical constitution

Classification of fungicides: Fungicidal chemicals may be classified in different ways.

Based on mode of action

Protectants: Fungicide which is effective only if applied prior to fungal infection, is called a protectant. They mainly inhibit the reproductive organ of a pathogen. Advantage of these types of fungicides is that as they are protective in nature, they are applied in very low doses and they are creating less hazard to the environment e.g., Sulphur and Dithiocarbamates.

Curative fungicides or therapeutants: Fungicide which is capable of controlling fungus after it has caused infection, and thereby curing the plant is called curative fungicide or therapeutants. It is applied during incubation and when early symptoms are visible. They predominantly act on the vegetative as well as reproductive organs of the pathogens e.g., Metalaxyl, 1,4-Oxathin derivatives and antibiotics like Aureo-fungin.

Eradicants: Eradicants are those which remove pathogenic fungi from an infection court (area of the host around a propagating unit of a fungus in which infection could possibly occur). These cure established infection at the site of application. These chemicals eradicate the dormant or active pathogen from the host e.g., Organo-mercurials, Thiram, lime-sulphur, dodine.

Based on Mobility within the plant

Systemic fungicides: These can inhibit the development of the pathogen on regions of the plant away from the site of application. Droplets spread out on and move inside leaf tissue and provide external and internal protection. Once applied on the host, these penetrate inside the system and cure the plant irrespective of the site of application and leaves produced after the application also protected for few days e.g., Phenylamides and Benzimidazoles.

Translaminar fungicides: Translaminar fungicides penetrate into the plant tissue and are moved within a plant organ such as a leaf, but do not travel to other parts of the plant. Translaminar fungicides redistribute the fungicide from the upper, sprayed leaf surface to the lower surface of a leaf e.g., Fenamidone, Dimethomorph, etc.

Non-systemic or contact fungicides: These fungicides which are surface active and do not move inside the plant systems are usually classified as non-systemic or contact fungicides. Contact fungicides (also called protectants) remain on the surface of plants e.g., Dithiocarbamates, Copper fungicides and Quinones.

Based on General Use of Fungicides: Fungicides are used in diversified way and accordingly they may be classified. But the limitation of this type of classification is that these classes also overlapping to each other and a single fungicide may come under more than one class.

Seed treatments: Compounds used for seed or seedling dip for seed borne diseases. These are also called protectants e.g., Organomercurials, Thiram, Captan, Chloranil etc.

Soil fungicides (Pre-plant): These chemicals are applied to the soil before sowing of the seeds to control soil borne disease e.g., Chloropicrin, Formaldehyde, Vapam etc.

Soil fungicides (Post plant): These chemicals are applied after sowing of the seed to control soil borne disease e.g., Captan, PCNB, Thiram etc.

Foliage and flower protectants: These chemicals are sprayed on the plant to avoid damage to leaves and flowers e.g., Copper fungicides, Dithiocarbamates, Carbendazim etc.

Fruit Protectants: These chemicals are used to cure fruit or protect them from attack of spores e.g., Captan, Maneb etc.

Localised eradicants: These are applied locally and eradicant in their action e.g., Blitox, lime-sulphur etc.

Tree wound dressing fungicide: These are used generally as a paste to dress tree wounds e.g., Brodeaux paste, Chaubattia paste etc.

Note: In this classification a single compound, Captan could be placed in one or all the other classes. So more systematic classification of the fungicides is needed.

Based on chemical constitution: On the basis of chemical composition of fungicides, they can be grouped into two major groups (Inorganic fungicides and Organic fungicides).

Group	Fungicide	Examples
Inorganic fungicides	Sulphur fungicides	Lime sulphur, Elemental sulphur
	Copper fungicides	Cuprous chloride, Copper carbonate, Copper oxychloride, Bordeaux mixture
	Mercury fungicides	Mercuric chloride
	Lead fungicide	Red Lead
Organic fungicides	Organo metallics	Triphenyl tin hydroxide, ethyl mercuric chloride
	Thiocarbamates	Ziram, Maneb, Thiram
	Organophosphorus	Edifenphos/ Hinosan
	Quinones	Chloranil, Diclone
	Thiophanates	Thiophanate methyl
	Amide Fungicides	Metalaxyl, carboxin
	Phthalimide fungicides	Captafol, captan
	Heterocyclic fungicides	Flusilazole, hexaconazole, benomyl, carbendazim
	Antibiotic fungicides	Streptomycin, azoxystrobin

Safe handling of pesticides:

- Pesticide should be stored in their original labelled containers in tightly sealed condition.
- The Storehouse should be away from domestic water storage, tanks and flames as well as away from the reach of children.
- Always wear Personal protective equipment (PPE) such as Helmets, goggles, facemask, gloves, boots and protective clothing to protect hair, eyes, nose and skin before handling any pesticide.
- Safe handling of pesticides involves proper selection and careful handling during mixing and application.
- Read the label and leaflet carefully and calculate the required quantity of pesticide.
- Avoid spillage and prepare spray fluid in well-ventilated area.
- Stand in the direction of the wind on back when mixing pesticides.
- Don't eat, drink or smoke during mixing.
- Dispose-off the containers immediately after use.
- Spray should be done in windward direction.
- Do not blow, suck or apply mouth to any spray nozzle.
- Check the spray equipment before use for any leakage.
- Empty the spray tank completely after spraying.
- Clean the spray equipment immediately after use
- Avoid the draining the contaminated solution in ponds, well or on the grass where cattle graze.
- Decontaminate protective clothing and foot wear and wash the hands thoroughly with soap water, preferably have a bath.

First aid: In case of suspected poisoning, call on the physician immediately. Before calling on a doctor, first aid treatments can be done by any person.

FUNGICIDE FORMULATIONS

Formulation is a mixture of the active and inert ingredients in the pesticide/fungicides. The active ingredients are the chemicals that affect the target pathogen. The inert ingredients are all other ingredients in the formulation. Inert ingredients are used to dilute the active ingredient or make it safer, easier to handle, and more effective. Some formulations are ready to use, others must be further diluted by air (air-blast sprayer), water, or a petroleum-based solvent. The active ingredients are the chemicals that affect the target pathogen. The inert ingredients are all other ingredients in the formulation. Inert ingredients are used to dilute the active ingredient or make it safer, easier to handle, and more effective. Some formulations are ready to use, others must be further diluted by air (air-blast sprayer), water, or a petroleum-based solvent.

Fungicide formulation: It is a mixture of active ingredients present in fungicides.

Liquid formulation

a) Emulsified concentration (EC): These are liquid formulations which can be diluted with water before application. The active ingredient is dissolved in a solvent. The fungicides and solvents will often not mix with water, so an emulsifying agent or water dispersible oil is mixed.

b) Solution: True solutions are formulations in which active ingredient is easily dissolve in solvent like water. Solutions have the advantage of requiring no agitation after formulation is added in water.

c) Suspension or slurries: These are formulation in which a dry form of the active ingredient is mixed with a liquid. Such formulations usually contain a high percentage of active ingredients similar to wettable powders. These are mostly used as seed dressers in seed processing companies.

Dry formulation

a) Wettable Powders (WP): Wettable powder is a very common formulation for most of the fungicides. The modern wettable powders are water-dispersible which have the quality to wet easily and disperse well in water. The active ingredient is incorporated, usually at the rate of 30-80%, with a finely ground inert dust (filler) such as Kaolin, a wetting agent and a suspending agent.

b) Dust: Dusts are powders that are mixed with inert ingredients to form a product with a low percent of active material. It usually contain 1-10% active ingredient for direct application (ready to use) in dry forms.

c) Granules or pellets: The active ingredient is incorporated into small granules of inert material such as clay. The granules normally contain 3-10% of the active ingredient. Granules are incorporated into the soil.

Calculation of fungicide sprays concentrations: Being highly toxic, pesticides are not sold in its pure form. They are subjected to dilute with any carrier to avoid the hazards of poisoning to applicator or human being. The pure forms or technical grades are only used in analytical and toxicological studies. Pesticides are commercially manufactured in various formulations (by adding various additives) like emulsifiable concentrates, water-dispersible powders, dusts, granules, solutions etc. The strength or active ingredient is mentioned on the label.

Active ingredient: It is the chemical in commercial products which is directly responsible for its toxic effect.

Fungicides: Carbendazim 50 SC, Carbendazim 50 WP, Copper Oxychloride 50 WP, Difenconazole 24.9 EC, Dithianon 5, 10 EC, Dithianon 5 SC, Hexaconazole 5, 10 EC, Hexaconazole 5 SC, Mancozeb 80, 75 WP, Miclobutanil 10 WP, Propiconazole 10, 25 EC, Tebuconazole 24.9 EW, Tricyclozole 75 WP etc.

Before application or purchase of pesticides it is always strike in the mind of farmers that how much amount of insecticides or herbicides or fungicides etc. would be required for application on their farm of definite size so that he could parches only the required amount . Let us see the methods for calculating the pesticide dose with some example.

If recommended as kg a.i./ha:-

For WP, WG, dust etc. (solid forms)

Formula:

$$\textbf{Kg of WG/WP/dust} = \frac{\textbf{recommended dose} \times \textbf{spray area}}{\textbf{a.i. \% of WG/WP/dust}} \times \textbf{100}$$

Example:

1) Calculate the quantity of Captan 50% WG to apply in the one-hectare area if the rate of application is 0.75kg (750g) ai/ha.

Quantity of Captan 50% WG/ha = (0.75 x 10000 (sq.m))/ (50 x100)

Therefore, we require **1.5kg** or **1500g** of Captan 50% WG to apply in the 1 ha area.

2) Calculate the amount of Isoprothiolan 40% EC to be sprayed in Paddy at the rate of 0.3 kg a.i./ha for 2 ha.

Isoprothiolan 40% EC = 0.3 x 2 x 100 / 40

Therefore, we require **1.5 L** Isoprothiolin 40% EC.

TOOLS USED FOR PESTICIDE APPLICATIONS IN FIELD

Pesticides will only be effective if they are applied appropriately and at the right time. Methods of application vary depending on the type of pesticide, formulation, pests to be controlled, location of application and water availability.

Sprayers: A sprayer is a mechanical device in which liquids are broken up into fine droplets and discharged with some force. Essential parts are **(i) Tank or container:** (a bucket, cement tanks etc.) to hold the spray liquid: **(ii) Delivery line or discharge line:** for ejecting or discharging the spray liquid. This part again consists of (a) Delivery tube (b) lance (c) nozzle to atomize the liquid into spray, **(iii) Pump:** To create or build-up pressure in the tank.

Types of Sprayers:

Hand sprayers:

i) **Automizers:** Specifically designed for kitchen gardens,
ii) **Knapsack or backpack sprayers:** A shoulder mounted sprayer for regular spraying in small size plots.

Hydraulic sprayers:-

i) **Foot sprayers:** Pedal type pump -designed for field crops and fruit crops.
ii) **Bucket sprayers:** Pump barrel with single or double good for all kinds of small scale spraying.
iii) **Rocking sprayers:** Well suited for field crops, orchards, plantations and for general spraying.
iv) **Knapsack sprayers:** Ideal for small scale or spot spraying in vegetable gardens or vine gardens etc.,
v) **Mist blowers:** Low volume sprayers, carried on the back, for the operation. The force of the air blast produced by the fan dispenses the spray in to very fine droplets of 50 to 100 microns in size, Ideal for field crops, orchards etc.

Power operated: Most of the power operated sprayers are hydraulic type in which spray mixture is developed by the direct action of the pump on the spray liquid.

i) Portable power sprayers.
ii) Tractor mounted sprayers.

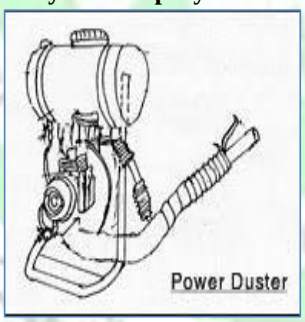
Hand sprayer

Hydraulic sprayers

Power operated sprayer

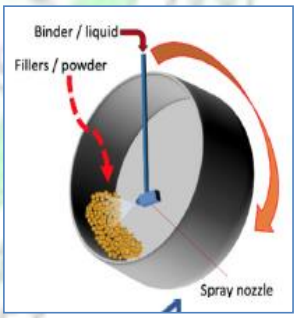
Hand Duster

Hand rotary duster



Power duster

Soil injector



Seed Dresser

Plant protection equipment

Dusters: A duster is an appliance for distributing or spreading fine particles of dry powders. The essential parts are: (i) **Container** (ii) **blower:** to create air current for injecting out dust (iii) **operating mechanism:** to work the equipment on a system of gears (iv) **agitator:** to stir the powder or dust in the container so as to make it flow uniformly (v) **Feed mechanism and level regulator:** to control the rate of flow of dust or powder (vi) **discharge line:** consisting of metal lance and a spreader nozzle.

Types of Dusters:

Hand duster: Plunger type duster consisting of a metal pump dust chamber and a discharge assembly suited for dusting small areas like kitchen gardens.
Hand rotary duster: Can be used for small to medium scale crops vegetable plots, nurseries etc.,
Power duster: excellent for quick coverage of large areas, good for all types of crops, orchards, plantation areas, the essential parts are (i) tank (ii) agitator (iii) fan and flower (iv) flow regulators and (v) distribution system. The rate of flow can be regulated

from 2 to 20 lbs per minute.
Soil injector: This is a special device for fumigating soil to a depth ranging from 12 to 18cm for the control of soil borne plant pathogens, nematodes etc.
Seed Dresser: This is a device for treating the seeds uniformly with the given chemical on the seed coat. The seed dresser is used for dry seed dressing.

CHEMICALS AND BIO CONTROL AGENTS APPLICATION

Seed or seedling treatment

Dry seed treatment: The required amount of seeds is mixed with the fungicidal powder 2hours before sowing.

Seed dip method*:* Preparing a suspension of fungicides in water in specific ratio and dipping the seed materials either dry seed or propagative part for recommended time.

Soil treatment: Applied to eliminate the inoculums or reduce the inoculum to achieve disease control target.

Methods of Soil treatment with chemicals:

Soil drenching : Fungicides are commonly use as water solvent in specific concentration for spraying and applied to the soil surface either before or after sowing / plant emerge. The fungicides suspension is applied in recommended ratio with sprayer low volume pressure so that fungicides reach a depth up to 8-12 cm.

Broadcasting: Fungicides spread over the field with help of soil or fertilizer as buffer uniformly and mixture in soil with the help of farm equipment. This method consumes excess quantity of fungicides than the other methods.

Furrow application: This method requires much less amount of fungicides per hectare than the broad cast. In this method fungicides are applied either dust or liquid form at the time of sowing.

*For the control of disease caused root and basal part of the plant.

Fumigation: Application of fumigant chemical as fungicides used specially for nematode control and some of soil is habitant fungi. In this method, usually produce gas and inserted inside the soil by narrow pipe. This method is applied in very restricted area, where valuable crop will be grown. Methyl bromide is applied by this method.

Spraying: This is most applicable general method for spraying of fungicides on crops. Water is most commonly used as solvent with wettable powder fungicides. Spraying of this method was done by two way; first high volume i.e. power machine and second hand sprayer *i.e.* low volume. These two term described the amount of liquid applied on crop.

Important terms:

Seed disinfestation: The control of spores and other forms of disease organisms on the surface of seed.

Seed disinfection: The elimination of a pathogen that has penetrated into living host and infected it. The purpose of seed disinfection is to eradicate seed-infecting pathogens from the seed coat, the embryo, or both.

Seed protection: Seed protection is the application of a chemical to protect seed from disease organisms in the soil. The purpose is to prevent seed rots and damping-off diseases caused by soil-inhabiting fungi.

MASS MULTIPLICATION AND BIO-FORMULATION OF BIO-CONTROL AGENTS

Biological control of plant diseases may be defined as any condition or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except man itself), with the result that there is a reduction in the disease caused by the pathogen.

Qualities of an effective Bio-control agent: Following are the desirable attributes of bio-control organisms or beneficial organisms to aid in assessment of their capabilities-

- Adaptable to the environmental condition/ecological capability
- Host specificity & compatibility
- Multiply faster than the host
- Reproductive potential (fecundity)
- Amenable for easy culturing in laboratory
- Should not be phytotoxic to the crop plants
- Cost effective and should give reliable control of plant diseases.
- Dissolve well in water.
- Carriers must be cheap and readily available for formulation development.
- Should be compatible with other agrochemicals.

Methods for the mass multiplication of bio-control agents:-

Mass multiplication of *Trichoderma viride*

Preparation of mother culture on Molasses yeast medium: Molasses - 30g; Yeast - 5g; Distiller water - 1000ml

The medium is prepared and dispensed into conical flasks and sterilized at 15lb pressure for 15 minutes in an autoclave. After the medium is cooled it is in inoculated with 10 days old fungal disc of *T. viride* and then incubated for 10 days for fungal growth. This serves as mother culture.

Mass multiplication: Molasses yeast medium is prepared in fermentor and sterilized as described earlier. Then after the medium is cooled, the mother culture is added to the fermentor @ 1.5 lit/ 50 lit of the medium and incubated at room temperature for 10 days. Then the incubated broth containing the fungal culture is used for commercial formulation preparation using talc powder.

Mass multiplication of *Bacillus subtilis*

Preparation of mother culture on Nutrient broth medium: Glucose - 5g; Peptone - 5g; Beef extract - 3g; Sodium chloride - 3g; Distilled water - 1000ml

The above medium is dispensed in conical flasks and autoclaved at 15lb pressure for 15 mts. A loop of *B. subtilis* is inoculated into the medium and incubated for 2 days. This serve as the mother culture.

Mass multiplication: The nutrient broth is prepared in fermentor and sterilized at 15lb pressure for 15 mts. Then the mother culture is added @ 1 lit / 100 lit of the medium and incubated at room temperature for 2 days. The medium containing the bacterial growth of *B. subtilis* is used for mixing with talc powder.

Preparation of Talc based products, Air drying of formulation and Estimation of Moisture content

Trichoderma viride: The fungal biomass collected from fermentor is mixed with talc powder at 1:2 ratio. The mixture is air dried in shade and mixed with carboxy methyl cellulose (CMC) @ 5g / kg the product. It is packed in polythene bags and should be used within 4 months.

Quality control parameters:

- Fresh product should contain hot less than 28 x 10^6cfu / g
- After 4 months of storage at room temperature, the population should be 20 x 10^6cfu / g.
- Maximum storage period in talc is 4 months.
- The talc size should be 500 microns
- The product should be packed in polythene bags
- Moisture content of final product should not be more than 20%

***Bacillus subtilis*:** The broth containing the bacteria is collected from fermentors and mixed with 250kgs of sterilized neat soil for 100 lit of broth. Then 37kgs if calcium carbonate is added thoroughly mixed, dried is shade and packed in polythene bags. This can be stored upto 6 months.

***Pseudomonas fluorescens*:** The broth containing the bacterial growth is collected from fermentor and added @ 400ml / kg of talc powder. Then CMC is added @ 5g /kg mixed well air dried to 20% moisture level and packed in polythene bags.

Quality control parameters:

- Fresh produce should contain 2.5 x 10^8cfu/g
- After 3 months of storage at room temperature the population should be 8-9 x 10^7cfu/g
- Storage period is 3-4 months
- Minimum population load should be 1.0 x10^8cfu /g
- Moisture content should not exceed 20% in the final product
- Population per ml of the broth should be 2 x 10^8cfu /g

ARTIFICIAL EPIPHYTOTIC AND SCREENING OF RESISTANCE

Pathogenic isolate of plant pathogens will be multiplied on PDA slants at 27-30°C for 15 days. The conidial suspension is prepared by harvesting conidia in sterilized distilled water by scrapping the cultures in the water and adjusted to a concentration of (4×10^4 spores/ml) for inoculation. The pathogen can be inoculated in number of ways: seed dipping, root dipping or foliar spray based on nature of the pathogen. The disease reactions will be confirmed based on disease scale.

For screening in the nursery, the seeds will be dipped in conidial suspension of pathogen for 3h and were then sown in nursery beds. However, for screening in transplanted crops, 30 days old seedlings of the test germplasm lines will be uprooted and their roots will be dipped in a freshly prepared conidial suspension for 3h before transplanting. The inoculated seedlings will then be transplanted in the field or else for foliar pathogens, the conidial suspension can be sprayed over the foliage.

Observations on disease incidence in different genotypes/varieties/ susceptible crop in the nursery beds and transplanted field will be recorded 15 and 30 days after sowing and transplanting in terms of infected plants and total number of plants and the per cent disease incidence (PDI) will be calculated. The evaluated plants or pathogens can be classified in different categories on the basis of disease scale specified for each diseases.

Activity:

1. Create an artificial epiphytotic condition for a pathogen isolated from any foliar leaf disease. And go for screening in susceptible variety to observe the virulence of the pathogen.

2. Take 5 varieties of ground nut and screen for its resistance against the Cercospora leaf spot using its disease scale. Classify in different categories on the basis of disease scale.

SPRAYERS

Sprayer is a machine used to apply liquid chemicals on plants to control pest and diseases. It can also be used to apply herbicides to control weeds and to spray micro-nutrients to enhance plant growth

The main functions of a sprayer are

- Breaking the chemical solution in to fine droplets of effective size.
- Distributing the droplets uniformly over the plants.
- Applying the chemicals with sufficient pressure for positive reaching the plants
- Regulating the amount of liquid applied on plants to avoid excessive application.

Desirable quality of a sprayer: A good sprayer should possess the following qualities-

- It should produce a steady stream of spray material in desired droplet size so that the plants to be treated may be covered uniformly.
- It should deliver the liquid at sufficient pressure so that the spray solution reaches all the foliage and spreads uniformly over the plant body.
- It should be light in weight yet sufficiently strong, easily workable and repairable.

TYPES OF SPRAYERS: Based up on the volume of liquid handled, sprayers may be classified into -

- High volume sprayer (more than 400 litres /ha)
- Low volume sprayer (5 to 400 litres/ hectare)
- Ultra low volume sprayer (ULV) spray (less than 5 litres /ha).

The selection technique depends up on type of vegetation, kind of pests and approach to the field.

Basic components of a sprayer:

(a) Pump (b) Chemical tank (c) agitator (d) Air chamber (e) pressure gauge (f) Pressure regulator (g) valves (h) Strainer (i) suction line (j) delivery line (k) nozzles

Pump: It help fluids (such as liquids or slurries, or gases) to move, from one place to another, by physical or mechanical action. Most hydraulic sprayers are equipped with positive displacement pumps capable of developing pressure. The discharge capacity of these pumps is approximately proportional to the speed.

Tank: It is a container that holds the chemical solution. It is usually made of metal sheet or synthetic rubber or plastic having good resistant quality against corrosion, erosion, and similar actions.

Agitator: It is a device which stirs the solution and keeps the contents in homogenous condition. The propeller or paddle type mechanical agitators or hydraulic agitators are very common.

Air chamber: In a reciprocating type pump, an air chamber is provided on the discharge line of the pump to level out the pulsations of the pump and thus providing a constant nozzle pressure.

Pressure gauge: It is a dial gauge which indicates the pressure at which the liquid is delivered from the pump.

Pressure regulator: The pressure regulator serves several important functions. It is the means of adjusting the pressure as required for any spray job within the pressure range of the pump.

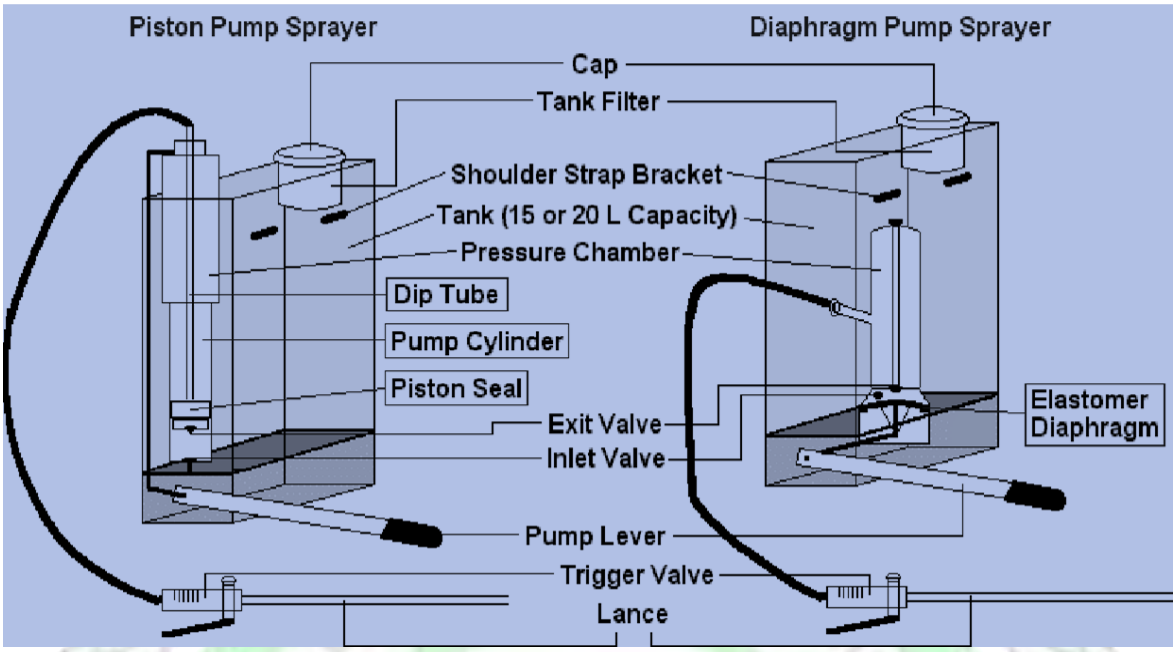
Valves: A valve is a device that regulates the flow of a fluid (gases, liquids, fluidized solids, or slurries) by opening, closing, or partially obstructing various passageways.

Cut-off valve is provided in the delivery line to control the flow from the pump.

By-pass valve is provided in the delivery line to by-pass the flow from pump to tank when flow in delivery line is reduced than the pump capacity.

Relief valve: It is an automatic device to control the pressure of fluid or gas within a range a predetermined pressure.

Strainer: It is a small circular plastic ring with nylon wire mesh to filter any dust particle coming with the chemical solution. It is included in the suction line between the chemical tank and the check valves e.g., Knapsack sprayers.



Structural components of s Knapsack Sprayer

Nozzles: It is the component which breaks the fluid in to fine droplet.

Spray gun: It is a hand held metallic or PVC pipe in which the nozzle is fitted to one end, and a flow cut off valve and a handle are fitted at the other end. The delivery hose is connected to the spray gun. It conducts the fluid from the delivery hose to the nozzle. The operator holds the gun and does the spraying job e.g., Knapsack sprayers, rocker sprayer.

Spray boom: It is a long metallic or PVC pipe to which several nozzles are fitted with. The delivery hose is connected to the spray gun. High power and high capacity sprayers are equipped with spray booms. The coverage is larger compared to spray guns e.g., Tractor operated sprayers, power tiller operated sprayers.

Over-flow pipe: It is a conduit pipe through which excess fluid from a pump is by-passed in to chemical tank by the action of a relief valve or pressure regulator.

Components of Nozzle:

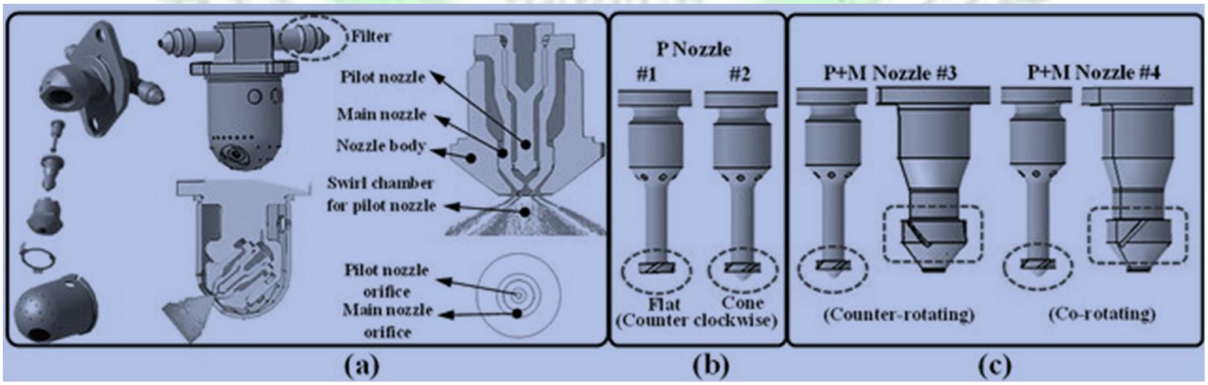
Nozzle body: It is the main component which encloses all other components of a nozzle.

Swirl plate: It is metal disc with two tangential holes which imparts a swirl or rotation to the liquid passing through it.

Nozzle disc: It is the component which breaks the fluid in to fine droplet. It is a flat disc with an orifice at the centre. When the spray solution reaches the disc from the swirl plate the disc builds up further pressure on the fluid and when the fluid passes out of the orifice, it breaks in to fine droplets. The popular nozzles are a) **Hollow cone** b) **Solid cone** c) **Fan or flat type**.

Strainer: It is a small circular plastic ring with nylon wire mesh to filter any dust particle coming with the chemical solution.

Spacer: There are two number of runner/ plastic rings placed in between nozzle plate and swirl plate and between swirl plate and strainer for effective travel of the solution.



Schematic of nozzle modification: (a) complete nozzle assembly, (b) Pilot nozzle, (c) Pilot + Main nozzle

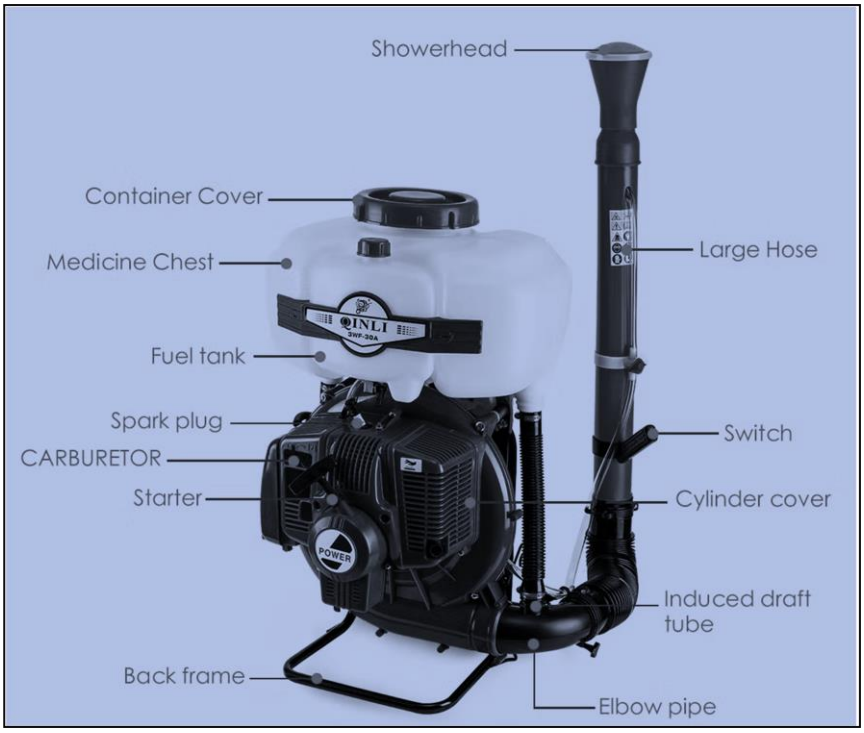
DUSTERS

Duster is a machine used to apply chemicals in dust form. Dusters make use of air stream to carry pesticides in finely divided form on the plants.

Types of dusters: Plunger type, Rotary type, Knapsack type, and Power operated duster

Plunger type - it is a simple duster with a small piston. The piston drives a current of air over the dust in the hopper. The dust is carried away through a delivery spout. Small hand pump dusters of this type are available and are suitable only where the area to be dusted is small like vegetable gardens

Knapsack type - It is a duster with the powder container carried on the back of the operator. Knapsack dusters have a hopper through which a current of air is blown to pick up the dust. The air current is produced by a lever operated leather bellows. Shoulder straps are used to carry in the field. These dusters are suitable for small areas.



Knapsack Power Mist-Duster

Rotary duster – Hand rotary dusters are useful to apply chemicals which are in powder form. It consists of a hopper, a fan, gear box, handle, delivery hose and a deflector plate. When the handle is rotates the fan rotates at high speed and draws air from outside. The chemical from hopper is fed in to the air stream in the suction side of the fan. The chemical mixes with the air, passes through the delivery line and is applied on the plants. The rate of delivery can be regulated It is used to apply powdery chemicals to vegetables, sorghum etc. crops.

Power operated duster- Power operated duster mainly consists of a power driven fan, a hopper and a delivery spout. The fan creates strong air flow which causes the dust to blow off from the hopper to a considerable distance vertically or horizontally. Direction of dust is regulated by a movable spout suitably fitted with the unit. This type of dusters is used for large areas.

Arial duster or crop duster - an aircraft is used for dusting or spraying large acreages with pesticides. Aerial spraying and dusting permit prompt coverage of large areas at the moment when application of pesticide is most effective and avoid the need for wheeled vehicles that might damage crops. The technique was greatly improved in the 1960s with the development of ultra-low-volume applicators, in which concentrated pesticides are distributed in amounts as small as 1 ounce per acre (70g/ ha).

A duster essentially consists of: (1) Hopper (2) Agitator (3) Feed control (4) Fan or blower (5) Delivery nozzle