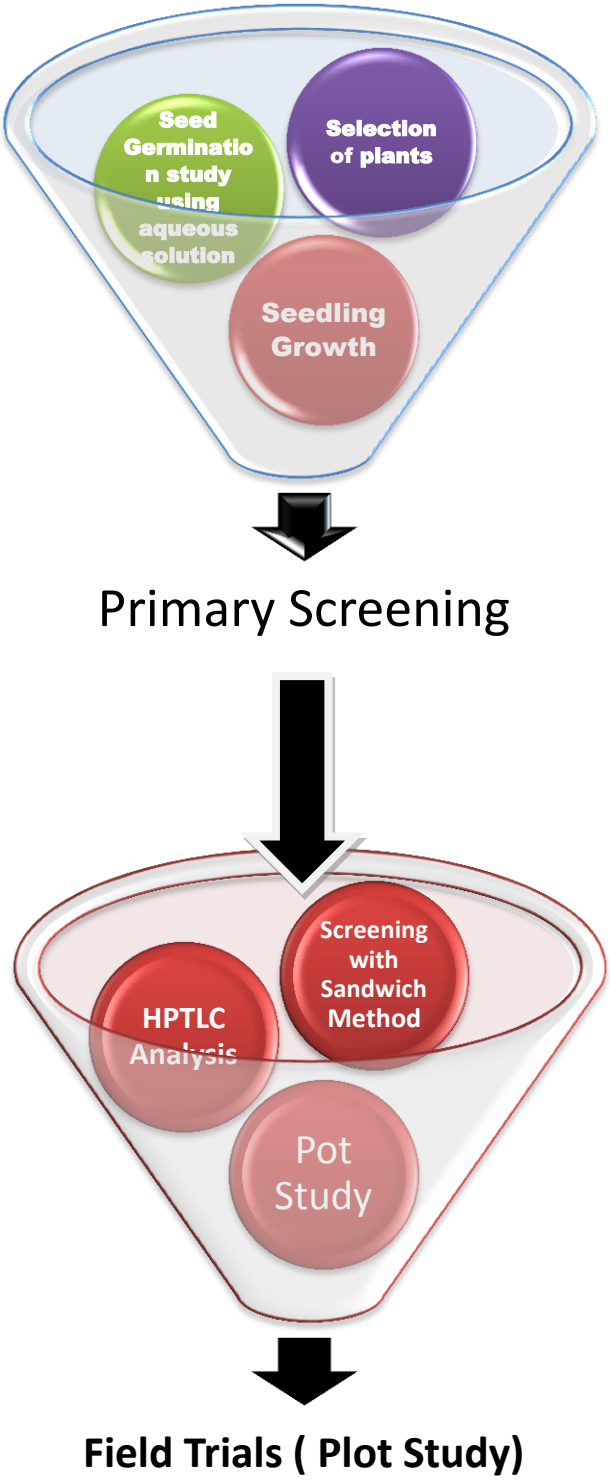
Seed Germinatio n study using aqueous solution
Selection of plants
Seedling Growth
Primary Screening
Screening with Sandwich Method
HPTLC Analysis
Pot Study
Field Trials (Plot Study)

Contents

2. Materials and Methods

To conduct the allelopathic study and analysis of parameters mentioned in objectives, below procedures and methods were followed**.**

2.1. Procurement of Plants:

Plants were categorised in three different categories i) Medicinal Plants ii) Weeds and iii) Crops. In which medicinal plants were scrutinised to check their allelopathic potential against weeds. Crops and weeds were selected to see the effect of medicinal plants on their germination/ growth.

2.1.1.Medicinal plants: Medicinal plants were collected from the nearby fields and farms. Five medicinal plants were selected for the primary screening viz. *Artemisia annua L.,Swertia chirata* Buch – Ham, *Tridax procumbens L., Tephrosia purpurea (*L.)Pers.,*Ocimum sanctum* (L.).Out of these plants, *Ocimum sanctum* L. was collected from the farms where it had been cultivated. Whereas *Tephrosia* and *Tridax* were collected from various fields near Vadodara, Anand and Navsari districts. *Artemisia* and *Swertia* were collected as dry leaf powder from IPCA Pharmaceuticals, Ratlam.Powder of *Artemisia annua* collected from the pharmaceutical company was after extraction of Artemisinine. This terpenoid is the main chemical compound used to prepare antimalarial drugs.

After collecting plants from the field, leaves were separated from twigs and kept for shade drying till 5-8% of moisture content remained.(Figure 2.1)

Figure 2.1: Shade Dried *Tridax* leaves separated from stem.

2.1.1 Weed Plants: Weed seeds were selected on the basis of their availability in the fields where legumes were growing, also some noxious weeds which were present on road sides and barren and cultivated fields around Vadodara and Anand district of Gujarat were selected. Five selected weeds were,*Parthenium hysterophorus L., Dichanthium annulatum* (Forssk.) Stapf., *Amaranthus spinosus* L.*Chloris barbata* Sw. and *Acalypha indica* L. Mature weed seeds were collected to see the impact of medicinal plants on their germination and growth.

2.1.2 Crop Plants: Leguminous plants were selected as crop plants. Selection criteria to for leguminous plants was that they are fast growing crops, two seasons are favourable for their growth and most of the legumes can find favourable conditions and are cultivated in Gujarat. Legume Seeds were collected from the Legume research institute, Vadodara.Total ten legumes were selected for primary screening with medicinal plants. *Cicer arietinum* L.(CA – chick pea), *Vigna aconitifolia* (Jacq.) marechal.(BG- Black Gram), *Dolichos lablab* L.(LB – Lablab bean), *Pisum sativum* L.(GP – Green Pea), *Vigna mungo* L.Heeper. (MB – Moth Bean), *Lens esculenta* Moench.(LE – Lentil), *Cajanus cajan* L.(PP – Pigeon Pea), *Vigna unguiculata* L.Walp.(CP – Cow Pea), *Vigna radiata* L. R.Wilczek.(GG – Green Gram), *Cyamopsis tetragonolobus* L. (FB – French Bean).

After Selection of plants, experiments were designed into four stages. First two stage experiments were conducted using Petri plates.Third stage experiments were conducted using plastic containers and in fourth stage field trials were performed as being show in flow chart.

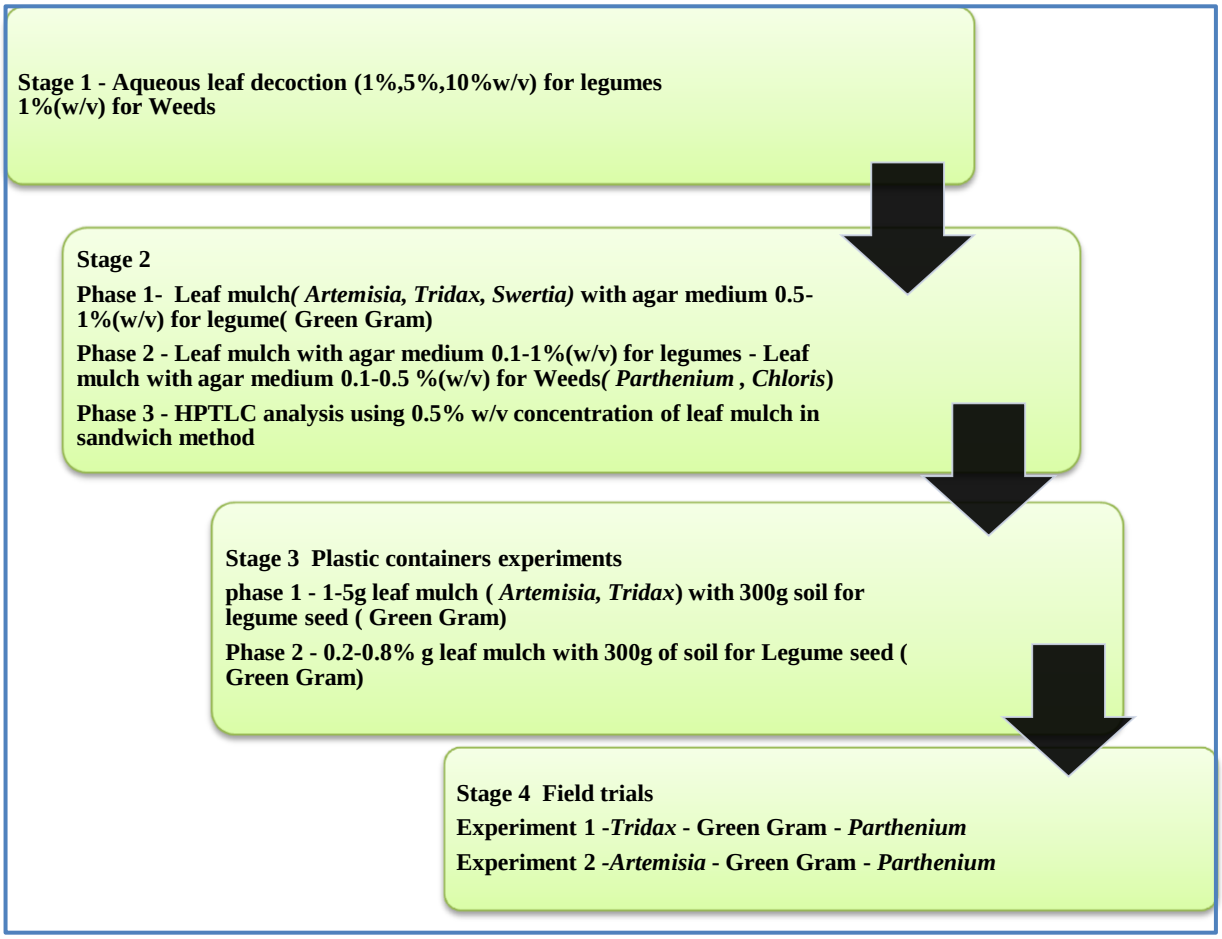

Figure 2.2: Flow Chart for Experiments conducted in the study.

2.2 Stage-1 - Primary screening: Primary screening of medicinal plants was done to test their allelopathic potential against selected legumes and weeds. Legume and weed seeds were decontaminated using Sodium hypo chlorite (1%) for ten minutes (Sauer and Burroughs,1986). Weed seeds, mainly *Parthenium* seeds went through process of scarification using 1% H_2SO_4 for five minutes. Also kept seeds in running water for 12 hours to induce germination(Usberti and Martins, 2007). Dry leaves/ Leaf Powder of

medicinal plants were soaked in distilled water for 24 hours and filtered. This Water based decoction was applied for further experiments. Initially petriplate experiment designed for legume seeds using 1,5, and 10% concentration of aqueous decoction of medicinal plants. After observing effect of these dosages, appropriate concentration (1%) was utilised to see allelopathic action of medicinal plants against weeds. Water was utilised for the primary screening as this is easily available, and economical solvent for farmers to use.

2.2.1. Legume and weed Seed germination with aqueous decoction: For primary screening. Three different concentrations of water based decoctions were utilized(1%, 5%, and 10%). As mentioned above, leaves of medicinal plant were dried in shade. 10gm of dry leaf powder was soaked in 100ml of distil water for 24 hours to make 10% total concentration. Extract was filtered with whatman filter paper 1and from this(10%) (by dilution with distilled water 5% and 1% solution was prepared. Distilled water was utilized as Control.

Glass Petri plate with 9cm diameter was kept lined with filter paper. Seeds were soaked in distilled water for three hours and ten seeds were kept in each Petri plate with three replicates for each treatment. Petri dishes were kept at room temperature, also in dark for first three days and daily observations were recorded. Every second day of observation2ml of water for control and decoction for treatment was added in each Petri plate equally if required.. A seed was considered/ counted as germinated when the radicle was protruded about 2mm (Hou and Romo, 1998).

2.2.2 Germination and Seedling growth parameters

Following parameters were assessed as per formula given by Scott (1984).

- **Germination Percentage (%)** = Total number of seeds germinated/Total number of seeds in experiment*100.

- **Mean Germination Time** was calculated by the equation,

$$\bar{t}=\sum_{i=1}^{k} n_i\, t_i / \sum_{i=1}^{k} n_i$$

Where t_i: time from the start of the experiment to the i^{th} observation; n_i: number of seeds germinated in the i^{th} time (not the total or accumulated number, but the number of particular i^{th} observation); k: last time of germination.

- **Variance of Germination Time** was calculated by the equation ,

$$s_t^2 = \sum_{i=1}^{k} n_i(t_i - \bar{t})^2 / \sum_{i=1}^{k} n_i - 1$$

Where $\bar{t}$: mean germination time; t_i : time between the start of the experiment and the i^{th} observation; n_i : number of seeds germinated in the i^{th} time (not the total or accumulated number ,but the number of particular i^{th} observation); k: last time of germination.

- **Coefficient of variation of the germination time** was calculated by the equation

$$Cv_t = \frac{S_t}{\bar{t}} * 100$$

Where S_t : standard deviation of the germination time and $\bar{t}$: mean germination time.

- **Uncertainty of the Germination process** was calculated by the equation,

$$U = -\sum_{i=1}^{k} f_i \log_2 f_i$$

Where, $f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$,

Where n_i: number of seeds germinated in the i^{th} time and k: last time of germination.

- **Synchrony of Germination** was calculated by the equation,

$$Z = \sum_{i=1}^{k} C_{n_i,2} / C_{\sum n_i,2}$$

Where $C_{n_i,2} = n_i(n_i - 1)/2$

Where $C_{ni,2}$: combination of the seeds germinated in the i^{th} time, two by two ,and n_i: number of seeds germinated in the i^{th} time

- **Seedling length** was measured for each seedling, separating root (Radicle)and shoot (Hypocotyl). parameters were assessed as below,
    a. **Root Length**
    b. **Shoot Length**
    c. **Seed Vigour Index (SVI)= seed Germination (%) X Seedling Length** (Sikha and Jha,2016)
    d. **Coefficient of Allometry = Ls/Lr**

    Where Ls= length of shoot and Lr is the length of root

2.2 Stage -2 - Germination study of selected plants using mulch in Sandwich method

The germination test was performed by sandwich method, for selected weeds and crops after primary screening. For sandwich method Agar Powder (Nacalai Tesque Kyoto, Japan) having gelling temperature of 30~ 31ºC was used and 0.5% (w/v) solution was prepared in distilled water.The experiment was carried out taking Green Gram as legume crop, *Artemisia , Tridax and Swertia* as Allelopathic plants and *Parthenium , Chloris* as weed

plant for this study.The medium and petridishes were sterilized in Autoclave. Seeds were also sterilised with Sodium Hypochlorite to avoid any contamination.

Figure 2.3: Glass Petriplate of 20cm used for sandwich method

Different weight of plant materials were taken and placed in each of the petriplates of 20cms diameter (Figure 2.3), and then agar solution was poured in each petriplate. Petriplate were kept at room temperature (25ºC) for about 30-60 min to solidify the agar solution.20 healthy seeds of green gram or weed seeds were kept in a Petri plate. Again Plate was kept in dark place for first three days. Distilled water was used as control.

All the parameters assessed in stage 1 were repeated. Addition to them GGI (Grade of Growth Inhibition)was also calculated.

- **GGI (%) =** Dry wt. of control – Dry Wt. of Treatment/Dry wt. of control X100

This study was accomplished in two phases.

2.3.1. Phase 1: For Legume Different concentrations viz. 0.5%, 1% and 5% (w/v) of selected medicinal plants were used.

2.3.2. Phase 2: For Legumes Concentration utilized were 0.1-1% (w/v), for weeds concentration selected were 0.1%, 0.25% and 0.5 %(w/v). Observation was taken every day till five days for legumes. Number of observation days was different for weeds depending on their germination time.

2.3.3.HPTLC Analysis

It was done to visualize absorption of allelochemicals by the crop seedlings. The analysis for weeds was done only for *Chloris* vs *Tridax* treatment. There was no seed germination of *Parthenium* in any treatment.

2.3.3.1. Sample collection

Green gram and *Chloris* seedlings were selected for the comparative chromatographic study. Seedlings were grown using sandwich method. Mulch concentration for all the plant was 0.5%w/v.

2.3.3.2 Preparation and extraction of plant material (sample)

- The shade dried, powdered mulch and dried Radicle and Hypocotyl material of green gram were kept in soxhlet apparatus and extraction was done by 70% Methanol, Refluxed for 3 times with equal amount of Methanol for 30 mints for each set.
  - ✓ *Artemisia*, *Tridax* and *Swertia* dry leaf mulch 2g in 150ml 95% methanol (50ml+50ml+50ml) = 1.3%w/v
  - ✓ Control Green Gram Radicle = 0.8%w/v
  - ✓ Treated legume Radicle =0.8%w/v
  - ✓ Control Hypocotyl = 1.3%w/v
  - ✓ Treated Green Gram Hypocotyl =1.3%w/v
  - ✓ Control and treated *Chloris* seedling =1.3% w/v
- Volume was reduced by 2ml keeping flask in water bath and utilized for final analysis through HPTLC.

- The methanolic seedling and leaf extracts were dissolved in HPTLC grade methanol which was used for sample application on precoated silica gel GF254 aluminium sheets.

2.3.3.3 Developing solvent system

HPTLC studies were carried out following the method of Harborne and Wagner (2004) using different solvent system and reagents initially.

Final results were obtained using best suitable solvent and reagent.

For Terpenoids , Solvent : Chloroform : Methanol (9:1).

Developing Reagent : Anisaldehyde Sulphuric Acid (AS)

For Phenolics : Solvent System : n- Butanol: Acetic acid: water-4:1:1

Developing Reagent : 0.5% Fast Blue

For Flavanoid, Solvent System: Ethyl acetate: Glacial Acetic acid: Formic acid: water-100:11:11:26

Developing Reagent: NP – PEG(Natural Products – Polyetylene glycol reagent). 1% Methanolic diphenyl boric acid β- ethylamine ester followed by 5% Ethanolic Polyetylene glycol-4000

2.3.3.4. Sample application

The samples (10µl and 15µl) were spotted in the form of bands of 6 mm width with a 100 µl sample using a Hamilton syringe on silica gel which was precoated on aluminum plate GF-254 plates (20 cm x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was manufactured by EMerck kGaA, programmed through WIN CATS software. Distance between two tracks was 10mm.Manufacturer-E.Merck KGaA, Application position on Y axis- 8 mm,1st application position -15mm.

Lamp – D2,Measurement type – Remission, Measurement mode – Absorption,Drying device in oven-at 60˚C for 5 mint.

2.3.3.5. Development of chromatogram

In silica plate of 20cm X10cm, total 18 track were plotted of 6mm band size.

Bands were applied and codes were given as per below,

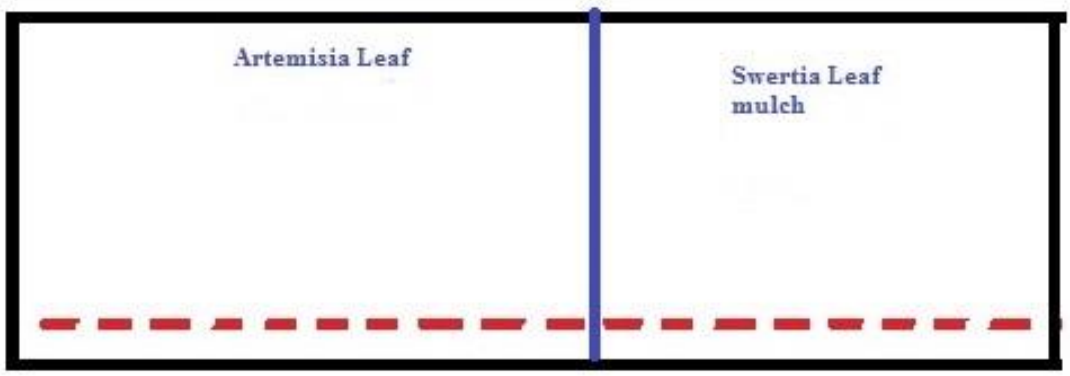

Figure 2.4: Application of extracts on TLC plate.(*Artemisia and Swertia, Tridax* leaf)

1. **Plate 1**- *Artemisia* and *Swertia* treated experiments in single plate for each run.

1. ARST – *Artemisia* leaf mulch 10µl
2. CTR – Control Radicle 5 µl
3. CTS – Control Stem 5µl
4. TAR- *Artemisia* Treated Radicle 10µl
5. TAS- *Artemisia* Treated Stem 10µl
6. ARST-*Artemisia* leaf mulch 15µl
7. CTR- Control Radicle 10 µl
8. CTS- Control Stem 10µl
9. TAR- *Artemisia* Treated Radicle 15µl
10. TAS- *Artemisia* Treated Stem 15µl
11. SWST - *Swertia* leaf mulch 10µl
12. CTR- Control Radicle 15 µl
13. CTS- Control Stem 15µl
14. TSR- *Swertia* Treated Stem 10µl
15. TSS- *Swertia* Treated Stem 10µl
16. SWST - *Swertia* leaf mulch 15µl
17. TSR- *Swertia* Treated Stem 15µl
18. TSS- *Swertia* Treated Stem 15µl

2. **Plate 2**- *Tridax* Leaf and *Tridax* Stem treated experiments in single plate for each run.

1. TLST – *Tridax* leaf mulch 10µl
2. CTR – Control Root 5 µl
3. CTS – Control Stem 5µl
4. TTLR- *Tridax* Treated Root 10µl
5. TTLS- *Tridax* Treated Stem 10µl
6. TLST-*Tridax* leaf mulch 15µl
7. CTR- Control Root 10 µl
8. CTS- Control Stem 10µl
9. TTLR- *Tridax* Treated Root 15µl
10. TTLS-*Tridax*TreatedStem 15µl
11. TSST – *Tridax* stem mulch 10µl
12. CTR- Control Root 15 µl
13. CTS- Control Stem 15µl
14. TTSR- *Tridax* stem Treated Stem 10µl
15. TTSS- *Tridax* stem Treated Stem 10µl
16. TSST - *Tridax* stem leaf mulch 15µl
17. TTSR- *Tridax* stem Treated Stem 15µl
18. TTSS-*Tridax* stem Treated Stem 15µl

For *Chloris* the codes were: CHC(*Chloris* control) CHT (*Chloris* Treated). Linear ascending development was carried out in a (20 cm x10 cm) twin through glass chamber saturated with the mobile phase.

2.4.5. Detection of spots

The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with different reagents as mentioned in section 2.4.2 as spray reagent and dried at 100ml in hot air oven for 3 min. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 254 and 366 nm, and in Light at 540nm. The Rf values and finger print data were recorded by WINCATS software.

Comparative study was done between *Artemisia*, *Tridax* and *Swertia* leaf mulch band with control and treated root shoot sample band.

2.4 Stage 3-Pot Study

Plastic containers were taken for this experiment. 15 – 20 micro holes were made at the base for aeration. This experiment was conducted to see the response of Crop to dry mulch in soil. Only Green gram and *Parthenium* were utilised for this

experiment. In each glass three seeds were sown and observations were taken till 35 days.

2.4.1. Phase 1: In each plastic container 300gms of dry soil was added and concentration of the Medicinal plants (*Artemisia* and *Tridax*) were 1gm(0.33%w/w), 3gm(1%w/w), and 5gm(1.6%w/w) per glass.

2.4.2. Phase 2: After obtaining results of Phase 1, further experiments were conducted using lower concentration of each of three medicinal plants. Concentrations were made after mixing 0.2gm, 0.4gm,0.6gm and 0.8gm of leaf mulch into 300gm of soil. Which was approx. 0.06%w/w, 0.13%w/w,0.2%w/w and 0.26%w/ w respectively.

Calculations for germination and seedling length were done as mentioned earlier.

2.4.3 Chlorophyll Analysis

During the second phase of the pot study, observations were taken till 35DAS, chlorophyll content of the leaves were analysed as below.

Chlorophyll content of Green Gram leaves was performed according to modified Arnon method described by Hiscox as below (Hiscox and Israelstam, 1979). **Methodology** : 200 mg of dry leaf tissue was taken, cut into small pieces and suspended in test tubes containing 2 mL of dimethyl sulphoxide (DMSO). Test tubes were incubated at 60° C for 20 min in a water bath. The supernatant was decanted and another 3 mL of DMSO was added to the residue and incubated at 60° C for 20 min. The supernatants were pooled and the volume was made up to 10 mL by adding DMSO. The chlorophyll extract was transferred to a cuvette and the absorbance was read in a Spectrophotometer (Genesys, UK) at 645 and 663 nm against DMSO blank

Chlorophyll a, b and total chlorophyll were calculated by the following formulae (Arnon, 1949) and results were expressed as mg/g.

1. Ch A (mg/g) = 12.7 A663 – 2.69 A645/1000X W X V
2. Ch B (mg/g) =22.9 A645 – 4.67 A663/1000x W x V
3. Total chlorophyll(mg/g) = 27.8 A652/1000XWX V

2.5Stage 4-Field Trials

After Pot experiment, Field Trials were conducted in 1X1 m^2plots. *Artemisia* and *Tridax* mulch were used to see their allelopathic effect on Green gram and *Parthenium*. Number of green gram seeds were 15gm/Plot (Approx.. 23-25 seeds per gram) and weed seeds were 3gm/ plot (Aprrox.100-107 seeds per gram)

Treatment details and layout of the experiment was as follows:

2.5.1 Experiment 1–*Tridax* mulch was utilised in two concentrations,(T-1 =40 gm/ Plot, T - 2 =80gm/plot)each treatment with two replications, Schematic diagram can be seen as below,

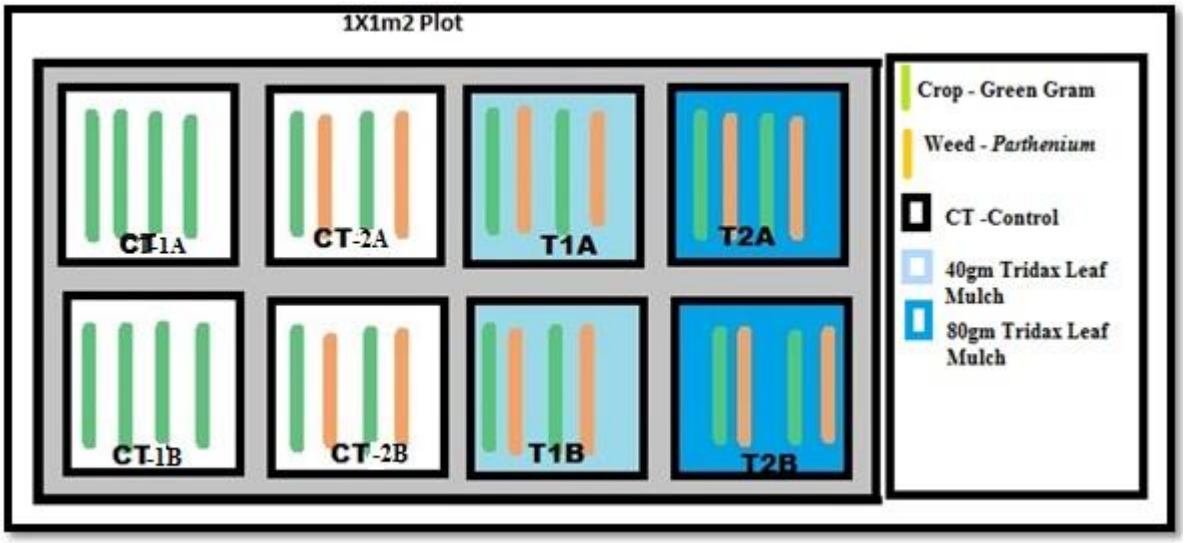

Figure 2.5: Schematic Diagram for Experiment 1 showing application in Field Trial.

Out of these eight plots, four were of control where no much was utilized. CT-1A,CT-1B and only Crop seeds were sown, Control 2, i.e CT-2A andCT-2B with only Crop and Weed seeds. In remaining four plots medicinal plant dry leaf mulch was added. T-1A and T-1B

with 40 gm of *Tridax* dry leaf mulch was sprinkled evenly over the top most layer of the soil, after mixing it with 1 kg of soil. Then as shown in the figure Crop and Weeds were sown in two lines alternately. T2A and T2B was treated with higher amount of mulch i.e. 80 gms of dry powder of *Tridax procumbens* L.(Figure 2.5).

2.5.2Experiment 2 - For this study *Artemisia* mulch was utilised in three concentrations, 10gm in T1 and 20gm for T2 and 50g for T3. Each treatment had two replicate plots, so total ten plots were utilized for one experiment.

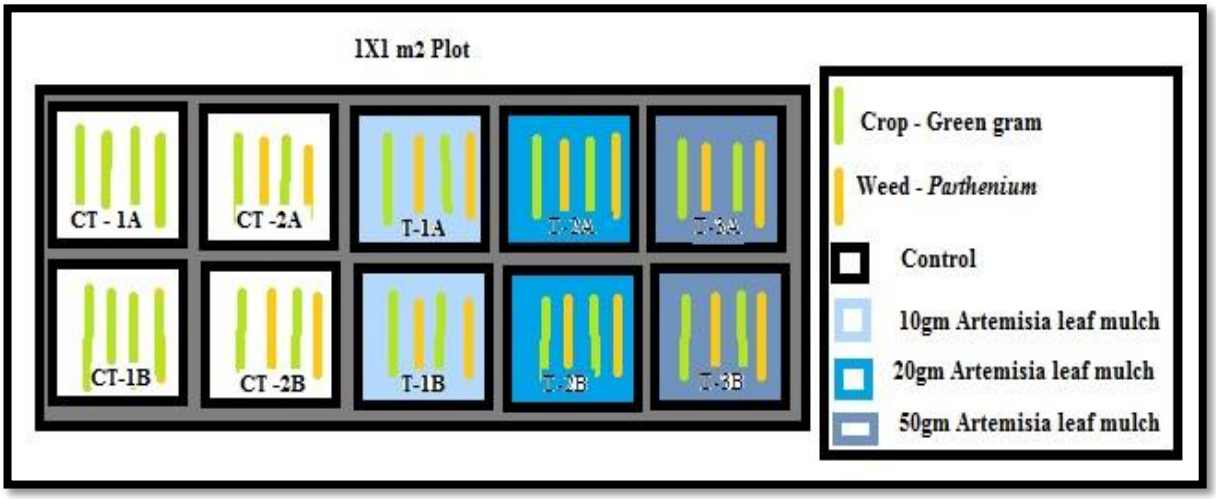

Figure 2.6: Schematic Diagram for Experiment 2 showing application in Field Trial.

Layout of this experiment was same as mentioned in Experiment 1. Only instead of *Tridax* , *Artemisia* leaf mulch was utilized. (Figure 2.6).Addition to earlier experiment two plots as T3A and T3B , were treated with 50gms of *Artemisia annua L.* mulch. As mentioned earlier Calculations were made for seedling growth.

Random collection of 25 Plants from field at four stages- 1) Vegetative stage: 20DAS*

2) Vegetative stage:40 DAS

3) Flowering stage: 60DAS

4) Seed setting stage: 80DAS

(*DAS – Days after sowing)

In addition to root and shoot growth, other Growth and yield parameters were recorded to see the effect as below,

For Crop: Number of Leaves /Plant, No. of Nodules / Plant, weight of total nodules per plant, Number of flowers or buds/ plant and biomass, Number of Pods/ plant, Biomass of Pod, Number of seeds / pod, Weight of seeds

For Weed: Number of Weeds / Plot, Number of Leaf and Chlorophyll content were analysed.

1. Leaf and leaflet counting – During the second stage – vegetative stage Number of leaf and leaflets were counted from each plot.

2. Chlorophyll content estimation was done as per above mentioned method for green gram in second stage observation, and for *Parthenium* at the last stage of the study.

3.Nodule counting– No. of nodules present on roots of the legumes were counted after 40-45th Day from the sowing as maximum nodulation can be observed after four weeks of sowing the seeds.(Adjaie *et al*, 2016).

4. Number of all flowers and Pod -were recorded at third and fourth stage of study which also included number of seeds /pod.

5. Weed Control Efficiency (%)was calculated for each plot as per formula given below

WCE (%) = Weed population in control plot –weed population in treated plot/ weed population in control plot X100

6.Quantitative Protein estimationwas done for the seeds of legumes at fourth stage followingLowrymethod (Lowry *et al,*1951). For the method 100mg of the Dry seed powder was utilized

Chemicals for protein estimation: Complex-forming reagent: Prepared immediately before use by mixing the following stock solutions in the proportion 100:1:1 (by vol), respectively:

- Solution A: 2% (w/v) Na2CO3 in distilled water.
- Solution B: 1% (w/v) CuSO4·5H2O in distilled water.
- Solution C: 2% (w/v) sodium potassium tartrate in distilled water.

Reagent: 2 N NaOH., Folin reagent (commercially available) in1 N concentration.

Method

To 0.1 mL of sample or standard,0.1 mL of 2 N NaOH was added. It was hydrolysed at100°C for 10 min in a water bath. Solution was cooled to room temperature and 1 mL of freshly mixed complex-forming reagent was added. Solution was allowed to stand at room temperature for 10 min .Then0.1 mL of Folin reagent was added using a vortex mixer, and the mixture was kept to stand at room temperature for 30–60 min (not exceeding 60 min) .

Absorbance was taken at 750nm, sample absorbance was compared with standard graph prepared with bovine serum albumin fraction, to determine the protein content.

7.Qualitative analysis of Protein using SDS gel Electrophoresis:

Qualitative analysis of control and treated legume seed protein was done by using SDS PAGE analysis to see the difference between bands obtained during the treatment.

- **SDS – PAGE- The reagents and procedure of Gel preparation was as below:**

  (a) acrylamide: bisacrylamide (1.665 ml); (b) 1.5 M Tris-HCl (1.25 ml); (c) 1% ammonium persulphate (0.5 ml); (d) 10% SDS (0.05 ml) and distilled water (1.85 ml) were taken to prepare a 5 ml separating gel mixture in SDS gel system.

  (b) 5 µl of tetra methyl ethylene diamine (TEMED) was added to the mixture, stirred well and any air trapped was removed in vacuum, just before casting the gel.

(c) 5 ml of the mixture was enough to cast a slab gel of 80x70 mm size and 1 mm in thickness. The electrophoretic tank and plates were rinsed with distilled water. The tefl on spacers were kept in position between the two plates and fixed closely to the electrophoretic tanks by tightening the screws

(d) The separating gel was cast within the sandwiched glass plates leaving sufficient space for the stacking gel. The separating gel was layered on top with distilled water to get an even surface and was kept till gel gets solidified.

(e) The surface was washed carefully with distilled water to cast the stacking gel. The surface of the separating gel was cleaned and any droplet of water trapped, was removed.

(f) The polymerization mixture was prepared with the following constitution. (a) acrylamide : bisacrylamide (0.75 ml) ; 0.5 M Tris-HCl, pH 6.8 (1.25 ml); (c) 1% ammonium persulphate (0.5 ml); (d) 10% SDS (0.05 ml) with the addition of 2.45 ml of distilled water and 5 µl of TEMED to get a gel strength of 4%.

(g) In slab gel system, after the stacking gel was poured over the separating gel, a comb made up of tefl on was inserted to form wells in to which samples were loaded. The comb was removed after 2 hr followed by cleaning the wells with electrode buffer using a syringe and a long blunt needle

(h) Procedure (SDS-PAGE) : Electrophoresis was carried out according to Laemmli (1970). Samples were prepared for electrophoresis by mixing 10µl of extracted protein, 2.5µl of 2- mercaptoethanol and 7.5µl of 0.002 % bromophenol blue in 0.0625 M tris Hcl buffer (pH 6.8), containing 10 % glycerol and 2% SDS.

(i) Samples were mixed with equal amount of 2X buffer, and boiled in water bath at 50®C for 2-3 minutes and then cooled. Gel pre – run at 5 Watt for 30 minutes.

(j) Sample applied and ran at 220V, temperature kept at 8®C.

(k) Completion of gel after 1:30 hours.

(l) The gels used were of 12% (Separating gel) and 5% (Stacking gel). Protein staining was performed using Coomassie Brilliant Blue according to Hames and Rickwood, (1990).

(m) The excess stain was removed by diffusion in destaining solution (methanol containing acetic acid).

Gel was then taken to the Gel documentation apparatus to see the band at IR and UV lights. Rf value of bands were obtained and compared with each other. In each gel control and treated seed powder was utilized.

2.6 Statistical Analysis: Statistical analysis was done for mean, standard deviation using t-test, for Two-Sample Assuming Equal Variances at P(<0.05)to analyse the significant difference between control and treated plants, especially for Seedling length at each stage of the study. The Correlation between root and shoot length for every treatment was calculated and it was matching with calculation carried for Coefficient of Allometry (Ls/Lr), hence data of correlation are not tabulated separately.