

#### 4. Discussion

Agriculture plays a main role to change the economy in developing countries such as India and adjoining neighboring countries with high population. The initial condition of institutional structure in agriculture determines to increase agricultural production for economic development. Demand of main food crops and products are increasing day by day in India. This also leads to focus on producing more yields per area and cultivation of multiple crops during one or all season. Dependency on rain to get agriculture yield in most of the region of India, is one of the concern to the country. As per FAO reports India and China are strongly heading towards the rapid economic growth. Green revolution in country helped to become it independent for the local food needs, where use of high yielding seeds, utilization of fertilizers and pesticides has benefited to get good grain yield (Gulati *et al.*, 2005). Such development also led to serious environmental issues as water and air pollution, soil erosion, loss of forest and biodiversity with increasing population and food demand. But major concern is the declining quality and availability of ground water, loss of soil fertility and suitability for cultivation day by day (FAO Report, 2016).

Good yield in agriculture is obtained by controlling diseases and pests ,specially –weeds AS per ICAR data, in India maximum (33%) loss of agriculture products occurs due to weed growth in the field as compared to all other pests. Integrated weed management can be done using allelopathic activity of other plants/crops in the field to reduce weed (Belz, 2007). In addition, Current trend of intercropping or using crop residues to control weeds are affective against use of synthetic weedicide or pesticides especially in organic producing farms. Weed management is the most difficult challenge for organic farming, as has to replace synthetic herbicides by using other cultural practices,like intercropping,

allelopathy, cover crops, crop rotation, hand weeding, mechanical weeding; weed seed predation, smoother crops, natural herbicide and competition, which involves one or other kind of chemical exchange (Wallace, 2001; Teasdale et al., 2004; Liebman and Davis, 2009; Liebman and Gallandt, 1997; Wallace, 2001; Liebman and Davis, 2009; Kalinova, 2010; Flamini, 2012). The approach to use allelochemical extracts as natural herbicides represents a future challenge. Several researchers have suggested the use of aqueous extracts, which have provided excellent results in laboratory studies, as well as under field conditions (Ercoli *et al.*, 2007). However, the identification of a plant-based chemical that shows allelopathic properties in the laboratory represents only the first step towards the development of an agronomically relevant weed control system. It is furthermore required that this chemical suppress weed growth in a farming system/field trials at large scale, and also that the chemical can be economically extracted and used as a natural herbicide (Breen and Ogasawara, 2011).

Looking at the current scenario, present study was designed to find out allelopathic potential of some medicinal plants in direction to control weeds without affecting the crops in fields. Objectives were achieved one by one by examining the allelopathic potential of Medicinal plants in different doses on weeds and crops. The study was steered to see the allelopathic potential of medicinal plants growing commonly in surrounding area as wild or cultivated for different medicinal purposes. Due to the presence of secondary metabolites in substantial quantity and quality, medicinal plants efficiently illustrate their allelopathic potential with different solvent systems (Fuji *et al*, 2003; Nasrine *et al*;2011) against other plants. Maximum effect of allelopathy can be observed through leaf leachates than root, stem or any other part of the plant (Molisch, 1937). Referring to the previous studies, current study was conducted using dry leaf mulch of medicinal plants (Fuji *et al*, 2003; Balcevic *et al*, 2015) or their aqueous

decoction (Umer *et al*, 2010) in different concentrations against noxious weeds and legumes. Practice with any expensive or harmful solvent medium was avoided to grow the seeds during the study to ease the practical approach of this work into the field and thus water-based decoction was utilized for the primary screening, as is being suggested by Ercoli *et al*, (2007).

Medicinal plants utilized for the present study were

i) *Artemisia annua* L., It is a source of an important antimalarial compound artemisinin, which has made this plant accountable for the cultivation in large area of the country, including Gujarat (Dangash *et al*, 2013). Several researches have been done stating the allelopathic activity of this plant where it inhibited growth of lettuce, *Portulaca oleracea* L. (Duke *et al*, 1987) and *Plantago ovata* L. (Mohsen *et al*, 2011). According to previous study it also inhibited the growth of *Amaranthus retroflexus* L. and *Chenopodium sp.* to a great extent but comparatively is less effective against soybean and maize (Romangi *et al* 2000). Most of the research for *Artemisia* has been conducted to see the effect of artemisinin. Leaf mulch of this plant was collected from IPCA, Ratlam (Madhya Pradesh) after artemisinin extraction and utilized for the present study. This was done to give value addition to *Artemisia* cultivation as, leaf mulch without artemisinin is a byproduct and can be further utilized as future bio-weedicide if it could prove so.

ii) *Tridax procumbens* L. is a medicinal plant available in wild. It is a common herb used in traditional medicines. It has significant medicinal properties against blood pressure, headache, wound healing etc. leaves possess insecticidal and parasitical properties. Though considered as weed at many places it has high potential as a medicinal plant (Jain and Jain, 2012). Several studies have been done to see its allelopathic potential on legumes. It showed hindering effect to the growth of black gram and Green gram at 25%, 50% and 75% concentration of fresh leaf aqueous extract (Famina *et al*, 2012). Present

study was conducted using dry leaf mulch and aqueous decoction at low concentration against ten legumes and five weeds. Here also it showed reduction in growth at 5-10% of the concentration. However, at lower concentration negligible or insignificant suppression of legume was observed while the same concentration was inhibitory for weed growth.

iii) *Tephrosia purpurea* (L.) Pers. was also available and collected as wild plant. Whole plant contains medicinal properties as anti-carcinogenic, anti-inflammatory, wound healing etc. (Barnwal et al,2014). It is also reported for its anti- microbial activity against bacteria and fungus using methanolic extract (Rayalu et al,2013). *Tephrosia* has also been reported as allelopathic plant against some arid crops ( Pearl millet, Sesame and Cluster bean) using aqueous decoction of stem, root and leaves at higher concentrations( 25% - 100%)(Sundaramoorthy S. and Sen D,1990) and against some weeds like *Acalypha indica* L. and *Amaranthus spinosus* L.( Purohit and Pandya,2013).

iv) *Swertia chirata* is a traditional medicinal plant documented in Ayurveda, siddha and Unani system of medicine. The whole plant has medicinal properties for the treatment of hepatitis, inflammation, and digestive disorders (Bhatt *et al.*, 2006). Recently, *S. chirata* extracts showed anti-hepatitis B virus (anti-HBV) activities (Zhou *et al.*, 2015). Though the plant is habituated to high altitudes because of its medicinal value its cultivation is promoted by NMPB and now it is cultivated on a large scale in central part of country also.

v) *Ocimum sanctum* L. is utilized as traditional medicine and reported for its medicinal activity in Charak samhita. It had been recommended for the treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, also has been suggested to have anti-fertility, anticancer and antidiabetic actions. (Patnayak *et. al*,2010). Allelopathic activity of the plant was also studied against legumes and weeds ( Purohit and Pandya,2013).

Crop selected were legumes, which play an important nutritional role in the diet of millions of people in India. They are also referred as the poor man's meat being vital sources of protein, calcium, iron, phosphorus and many other minerals for those who are dependent on vegetarian diet (Latham, 1997). It is also advisable to cultivate legumes as cover crop, intercrop or rotational crop with other food / cash crops as it makes soil rich in nitrogen source (ICRISAT Report, 2015).

Selected medicinal plants were utilized to study their allelopathic potential against weeds and to select dosage which would not affect the growth of legumes, if used for weed suppression in legume crop field. For this, successive experiments were conducted starting with germination studies in laboratory to plant growth studies in field. Primary aspects were studied using shade dried leaves at room temperature so leaf color and chemical composition remains unchanged to prepare aqueous decoction. Decoction was tested against legumes and weed seeds using petriplates in laboratory bioassay. It is the first step to evaluate the involvement of allelopathy (Foy, 1999; Wuh *et al*, 1998). Next step study was conducted by sandwich method using neutral agar medium. So the impact analysis of leaf mulch on seed growth could be done, without any hindrance of external chemicals or material. Second stage analysis was done using pot and then final stage of the study was done in plots as field trials. Such lined experiments to analyze allelopathic impact are also recommended in one of the recent study by Balicevic *et al.* (2015). Importance of field trials during the allelopathic study is desirable as results and response of plants can vary in laboratory and field (Breen and Ogasawara, 2011).

At the primary stage, seeds of legume and weed were germinated in petriplate with aqueous decoction of each medicinal plants using 1-10% w/v concentration. (Fuji *et al*, 2003) Different germination and seedling growth indices were analyzed to evaluate the dosage reaction. Germination study or observations are the best tools to study effect of

any substance applied exogenously (Hoagland and Williams, 2003). Key Germination parameters like germination percentage, mean germination time taken by the seeds, mean germination rate, synchronization and uncertainty in germination process are important criteria and such germination indices are recommended in order to improve the precision in seed germination bioassays (Ranal M, 2009; Maisuriya k., and Patel S., 2009; Tanveer *et al*, 2014). The practical implication of allelopathic studies with such parameters shows that allelochemicals have harmful effects on the treated plants, in the form of reduced and delayed germination and mortality of seedlings, ultimately leading to decline in growth and yield, (Anjum, T., 2005). Studying speed of germination is important as it was reported in one of the research that plants growing early remain healthy throughout the life cycle (Luis *et al*, 2008) so for most of the crop plants its seed germination and establishment are the most sensitive stages to any stress (Patade *et al* 2011). Also seedling growth was analyzed by parameters like root and shoot length, Seed vigor index (SVI) and Coefficient of allometry (CoA) (Saber *et. al*, 2013) to see the allelopathic impact after germination. Above Germination, parameters for legume seeds and weeds could help to evaluate allelopathic effect of the medicinal plants.

All observations varied with the treatments, recording germination percentage only is not sufficient to interpret any results, specially when comparison of two seed lots has to be done. Thus an integrated result incorporating various parameters becomes more authentic. (Kader, 2005). In present study green gram, cowpea and black gram were showing least effect against most of the treatment, also at higher concentration, highly affected legumes were Lentil, Lablab beans and Green pea with all five medicinal plants. Such results were obtained earlier for green gram and black gram (Sundramoorthy and Sen, 1990). In our study, Germination time varied with each treatment. Pigeon pea and green pea seed germination was inhibited but mean germination time was reduced in

treated seeds for *Artemisia* treatment. Mean Germination Time (MGT) and germination index (GI) express speed of germination and are affected by various consequences followed during germination. Impact of any exogenous material can alter biochemical and physiological process resulting in morphological expression. The response varies with species and dose (Tanveer *et al*, 2014). Our results also show similar effects.

Earlier research was done using medicinal plants like *Euphorbia guyoniana* and *Retama raetam*, aqueous extract on germination efficiency of two weeds (*Bromus tectorum* and *Melilotus indica*), where *Bromus* was more inhibited as compared to *Melilotus* at 2% - 10% concentration (Narine *et. al*, 2011). In the present study low concentration of medicinal plants was showing inhibitory effect on weeds with reduction in germination and seedling length, while only higher concentration were showing inhibitory effect on legume seed germination and seedling growth. After these results, concentration for the next level study of weed seed germination and growth was decreased to 1% w/v, as our main aim of the study was to find dosage which is not affecting legumes but only to weeds. All five seeds weeds were inhibited maximum with *Artemisia*, *Tridax* and least with *Ocimum* and *Swertia*. *Parthenium* is a noxious weed and itself shows allelopathic effect over many crops (rye, maize, and wheat) and other native weeds (Marsie and Singh, 1987). In present study Seed germination of *Parthenium* was also suppressed using *Artemisia* and *Tridax* by 60 % . Earlier similar study was conducted against *Parthenium* using *Cassia* sp. (Vitonde *et al*, 2014).

Generally allelopathic effect is not so efficient towards monocots as compared to dicots (Pudelko *et al*, 2014) But current research showed that *Dicanthium* and *Chloris* both exhibited decline in germination with *Artemisia*, *Tridax* and *Swertia* treatments. Seedling growth of *Parthenium* and *Acalypha* was reduced noticeably only in *Artemisia* and

*Tridax*. *Chloris* seeds were showing reduction in germination but not in seedling growth with any treatment .which shows reduction in germination but not to growth. The results can be justified by the fact that low germination will result in less population size which in turn will not offer much competition to the crop in field. Germination time of *Parthenium* and *Chloris* was delayed by  $2\pm 0$  days in treated seeds. Delay in germination time indicates inhibitory effect on the weed (Tanveer et al,2014).

In present study species specific inhibition was observed as *Artemisia* and *Tridax* belong to same taxonomical group, which were showing inhibition towards weed (*Parthenium*) while *Swertia* was more inhibitory to *Chloris*. . As for most of the biological interactions, allelopathy also shows species specificity. Two species of same genus are reported to show differential impact on same tested species ( Pratt and Bossdorf, 2004). Our results are also in same trend, where a single donor species extract was having varied degree of effect on different species and on other hand, one single species was affected differently by different donor spp.

Laboratory bioassay in second stage were assessed using *Artemisia*, *Tridax* and *Swertia* which showed maximum inhibition to the *Parthenium* and *Chloris* at 1% concentration. Green gram was selected for further treatment as it was most resistant towards allelopathic effect among all selected legumes. Resistance of green gram was observed in seed germination and seedling growth as well. In sandwich method, dry leaf mulch was utilized at low concentration to scrutinize effect on germination and seedling growth (Fuji *et al*, 2003). Mulch was used to check its allelopathic effect, so that it could be utilized in field trials. It has been reported that plant residues in field get decomposed and strong allelochemicals are extracted from them ,(Nekonam et al,2014). Here biomass accumulation was examined as it is a very crucial parameter to analyze the growth



inhibition (Belicevic et al,2015; Mali and Kanade ,2014). Some results were showing less seedling length in treated seeds but ultimately dry biomass was more. Reduction in seedling length might be due to suppression of cell division and cell elongation. The allelochemicals may interfere with release and synthesis of hormones like auxins and cytokinins, as has been suggested by Prasad and Shrivastava (1991). In a study conducted in our laboratory, arrestation of cells at interphase was observed, which also could be reason for suppression of seedling length (Patel, 2016). Many allelochemicals are reported to affect length or girth of the root and shoot length due to their stress effect.(Mali and Kanade,2014).) . Cotton plants in drought condition (stress condition) showed more tap root length to receive water from the depth but no change in their dry weight, coupled with increase in number and biomass of secondary roots were observed (Pace *et al*, 1999). Similarly, during the present study *Artemisia* reduced the vertical growth of the root but horizontal growth (secondary roots) was more. In study using 0.5% -5% (w/v) leaf mulch ,doses were not affecting the germination of green gram but germination speed was declined, as leaf mulch is in more concentrated form and comes in direct contact with the treated legumes, which affected more to the seedling length than germination of the legume. According to recent study root and shoot length were decreased due to allelopathy of Rice into selected weeds such as *Ipomea* sp.and *Convolvulus* sp and *Rumex* sp against Green gram, and sunflower( Afridi *et al*, in 2013). Growth inhibition was estimated on the basis of difference between dry biomass of control and treated seedlings. *Tridax* was less inhibitory to Green gram as compared to *Artemisia* at higher concentration. Grade of growth inhibition was negative even after decreasing seedling length and fresh weight in higher concentration of *Tridax*, which clearly indicates that there is no change in the dry weight of the seedlings. It was only affecting length of legume seedling but not the ultimate biomass. Concentration of the

leaf mulch was further decreased to 0.2 -0.8% w/v to see the minimum impact on green gram growth. Results were positive at concentration between 0.2-0.6% with *Tridax*, *Artemisia* and *Swertia* as compared to control. Dry Root biomass was considerably high in *Artemisia*, *Swertia* and *Tridax* at all concentrations. Inhibition was observed only in *Artemisia* 0.6 and 0.8% w/v concentration. Other than that in all other treatments growth was either equal to the control or having stimulatory effect due to presence of allelochemicals in it. Stimulatory effect of allelochemicals is quite possible and is well recorded too. It is hypothesized that the characteristic response of a recipient to the donor of allelochemical is much dependent on the concentration which may be favourable or deleterious for its growth.( An,2005).Low dose can be stimulatory due to the phenomenon described as hormesis, it has been proved also by drawing dose response curve (Duke *et. al.*,2006). Germination of weed was affected at all concentration from 0.1 -0.5%, maximum inhibition was observed at 0.5%. This helped to study next stage analysis using HPTLC and field trials. At 0.5% maximum weed seeds were inhibited whereas negligible inhibition was observed in Green gram. Above study aided to scrutinize and describe the dosage response viz.(  $\leq 0.5\%$  w/v )of *Artemisia*, *Tridax* and *Swertia* against Green gram, *Parthenium* and *Chloris* using sandwich method. Our objective to select and find allelopathic potential of medicinal plant against weed was justified, after elucidating the dose response.

After successive exploration of the above parameters, further study was conducted to see the uptake of any secondary metabolites from medicinal plants in treated plants after experiment.(Hariprasad and Ramakrishnan, 2012) Medicinal plants are the rich source of secondary metabolites, such as Alkaloids, terpenoids, Flavonoids , Phenolics and many more( Hariprasad and Ramakrishnan, 2012; Mauji ram *et al*,2011). Comparative analysis of HPTLC fingerprinting between Untreated and treated plant part was done, and leaf

mulch of particular medicinal plant was used as standard. During analysis two concentration of each sample were taken to confirm the impact. Sandwich method was applied to conduct this study to avoid any external factors affecting growth (Fuji *et. al.*, 2003). 0.5% concentration was selected for the study at which weeds (more inhibited) and legume both were affected. HPTLC Study was done for flavonoids, Terpenoids, phenolics at different wavelength (254nm, 366nm, 540nm) with green gram and *Chloris*. During this study *Chloris* and *Parthenium* growth was inhibited. *Chloris* seed germination was considerably more as compared to *Parthenium* in *Tridax* to perform the analysis. Legumes were examined separately for radicle and hypocotyl.

HPLTC analysis was done mainly for detection, separation, estimation and evaluation of diverse groups of secondary metabolites present in the plant part. Chromatographic fingerprint is considered as a rational method for more powerful and effective quality control of herbal drugs and their products (Shrivastva M, 2011). Chromatograph of the study showed various peaks at different R<sub>f</sub> values and area. Number of peak indicated the number of phytoconstituents present in the sample (Ahmad *et al*, 2015). according to one of the comparative study between HPTLC, GC, and MS, HPTLC was considered as most effective and simple analytical tool to control the quality control of raw herbal material (Mauji ram, 2010).

In recent years advance chromatographic techniques have played very important role in identifying the exact fractions of medicinal plants and adulterant in it (Liang *et. al*, 2004). HPTLC finger printing is very precise, effective and easy method to produce reliable results with digital screening. Data presentation in form of chromatogram and peak densitogram with absorbance, R<sub>f</sub> value, concentration and area makes easy process to perform quantitative analysis (Moffat, 2001). Main aim of this study was to analyse

methanolic fraction of control and treated green gram and weed against *Artemisia*, *Tridax* and *Swertia* leaf mulch. Purpose was to analyse the uptake of any secondary metabolite from these medicinal leaf part by the treated plants as compared to control. Radicle and hypocotyl analysis were conducted separately. Secondary metabolites such as flavonoid, terpenoid and phenolics which can travel to the receptor plant from donor plant during germination or seed growth procedure were analysed. Alkaloid content was negative in all the samples studied. According to one of the study conducted to see the alkaloid development at different stage of plant's life cycle, it was observed that alkaloid content was not seen till the first leaf development of the seedling. Also during the second vegetative stage alkaloid were absents.(Hashimoto *et al*,1990). Seedlings were collected after five days from the DAS and only affected one were selected for the further analysis. Methanolic fractionation was done to prepare the sample using soxhlet extraction (Sashidharan *et al*,2010). Different solvent system were tried, for Flavanoids, Terpenoids, Phenolics and Alkaloids (Wagner and Baladt, 1999). Comparative study between control and treated seedlings was done at 254nm, 366nm and 540nm wavelength, for three different groups of secondary metabolites.

Chemical uptake was observed for *Artemisia*, *Swertia* and *Tridax* for different secondary metabolite. Terpenoids and Phenolic study was showing peak similarity between chromatograph of medicinal plant leaf mulch and Treated legume seedling. Those peaks were not present in control. Many active compounds were observed in terpenoid, phenolics and flvanoid group. For *Artemisia* other than Artemisinin many other compounds were present. Majority of the allelopathic effect on other plants are recorded for Artemisinin. Presence of compounds other than artemisinine in utilized leaf mulch and their allelopathic effect on weed plant show possibility of their use as bioherbicide. In present study morphological changes due to allelopathy were observed in legumes and

weeds at higher concentration. Which shows that dry leaf mulch utilised for the study has many chemicals which can act as allelochemicals.(Widmer *et al*,2007). Allelochemicals are reported similarly for *Swertia* and *Tridax* which shown uptake in treated plant in small quantity. Even though the green gram seedling were showing little uptake of the chemicals specially terpenoid and phenolic compounds, no reduction in seedling growth was observed indicating that selected plant and dosage can safely be used for weed control in green gram field. Treatment of *Tridax* to *Chloris* showed allelopathic effect on germination, only few seeds germinated and their seedlings showed uptake of *Tridax* phytoconstituents. It may be that the selected dose (0.5%) of *Tridax* was not that detrimental as in case of *Artemisia* and *Swertia* , where total suppression of germination was observed. Growth inhibition by allelochemicals is well documented but research done on absorption of these chemicals by the receiver plants is meagre. In one of such study, it was found that there is correlation among applied dose, absorption and endogenous level of allelochemicals, ultimately resulting in suppressed growth.(Naguchi and Kujiml,2012).

Present study can reveal that chemicals from leaf mulch travels to the seedling developed using sandwich method. In future study, micro fractionation can be done to identify the chemical uptake which plays main role in inhibition of the seedling growth of weeds or crop.

Next level study was conducted with pots to examine allelopathic impact using soil as a dissipation medium and to form a base for the field trials, leaf mulch was combined with soil to see the germination and growth effect in green gram. For this study, *Tridax* and *Artemisia* were selected in different concentration separately for soil, as they were showing more inhibitory effect as compared to *Swertia*. But this time concentrations were restricted from 0.06% to 1.6%w/w. In phase 1, Green gram was indicating

reduction in seed germination and seedling growth with 1.6%w/w *Tridax*. Both medicinal plants showed stimulation in root growth and dry weight biomass at lower concentration.

Similar study was again conducted using low concentration (0.06% -0.26%w/w) from where the phase 1 was started. No significant reduction was observed for seed germination of legume. Total length was induced as concentration of *Tridax* leaf mulch was increased in soil. *Artemisia* showed no difference as compared to control. Interesting results were obtained with CoA parameter that value of CoA was considerably high in all concentrations of *Artemisia* and *Tridax* as compared to control, which point towards stimulation in shoot length: root length ratio as compared to control. Similar results were obtained for root biomass study where parameter was increasing with concentration for both the plants. Whereas shoot dry weight was with no change or reduced especially in *Tridax*. This study indicated *Tridax* inducing root biomass at low concentration and shoot biomass at higher concentration as per phase 1. *Artemisia* was not showing any significant inhibition or stimulation as compared to control. Chlorophyll study was conducted after 35DAS for all treated green gram with compare to control. Chlorophyll content decreased with *Tridax* and with 0.6g of *Artemisia*.

Field trials were conducted after analyzing *Artemisia* and *Tridax* effect on Green gram to manage weed management in a better way. Field trials are needed to examine the relative importance of allelopathy and other factors (Pratt and Bosddwarf, 2003). Two combination were studied in 1X1 m<sup>2</sup> plot. Before one month of the experiment plots were prepared and watered every 3<sup>rd</sup> day. We allowed to emerge all possible weeds. After one month weeds were removed manually before plantation was started. Second time hand weeding was performed before flowering period of the legume. Number of

nodules present on roots of the legumes was counted after 40- 45<sup>th</sup> Day from the sowing as maximum nodulation can be observed after four weeks of sowing the seeds.(Adjaie *et al*, 2006). *Parthenium* started growing for both the experiment on 45<sup>th</sup> day in control plots whereas in treated plots (T-1 and T-1) it started emerging after 55<sup>th</sup> -57<sup>th</sup> DAS. Due to delay in emergence of weeds, legume life cycle was about to complete as it was already in flowering stage. As per one recent study delay by seven days in germination of an annual weed *Agrostemma githago* (L.) in competition with barley produce 54% fewer shoots, 57% less biomass, 52% fewer flowers, 36% lighter seeds and were 23% shorter as compared to control (Theresa ruhl *et al*,2016) in such case delay in seed emergence plays a vital role in overall fitness of the plant. *Parthenium* shows allelopathic effect towards legumes like chick pea, black gram (Sikha and Jha A.,2016) food crop maize, Barley,*Brassica* sp. (Singh *et al*,2005) invasive effect to the weeds in wild such as *Chloris*, *Alysicarpus*( Msafiri *et al*,2013) showing inhibition to the seed germination, seedling length, and fresh weight of the plant(Devi *et al*,2014). In our study it was observed that,before *Parthenium* grew and could produce allelochemicals to affect legumes, legume crop was about to harvest. That will also help to reduce adverse weed effect on legumes or any other suitable crops of short duration. *Parthenium* also shows adverse effect on the nodulation of the legumes, which can ultimately reduce nitrogen content of the soil and growth of the plant (Deyama,1986). This also will be avoided by late emergence of *Parthenium*. In experiment 2 with *Artemisia* treated plot T-2 flowering stage in all legumes were induced and it was earlier by 7-8 days as compared to Control and T-1. Here legume life cycle was induced while germination of *Parthenium* was delayed. That indication of delayed germination of *Parthenium* and early flowering stage of the green gram plants in treated plots were a positive sign of the study. After emergence number of *Parthenium* was considerably increase in T-1 , but the number of

leaves and chlorophyll content of those leaves were significantly less as compared to Control and T-2. Number of weed plants grown in T-2 plots was very much less as compared to untreated plots, also number of leaves was reduced. Chlorophyll analysis was done for green gram at during 40<sup>th</sup> DAS observations. Chlorophyll result in experiment 1 showed higher content of chlorophyll b than a. Increase in chlorophyll b content or lowering of chlorophyll a content was showing allelopathic effect, similar effect was found in *Helianthus annuus* L. due to effect of *Mentha piperita* aqueous extract (Skrzypek *et al*, 2015). Total chlorophyll content was increased in treated green gram leaves during experiment 2. There are also reports where chlorophyll content of the plant was increased after treatment, such as in rice against effect of phenolics. (Yang *et al*, 2004)

Protein qualitative and quantitative study did not show any significant change. Increase or decrease in protein content or any other additional band during SDS electrophoretic analysis was not observed. This also clarifies no adverse effect of treatment on protein quality of the green gram seeds.

Significance of this study is to find out the dosage response of medicinal plants that did not affect the crops but only the weeds at higher ratio. Major research had been conducted in this subject to identify the effect of weeds – crop, crop – weeds, wild plants to cultivated plants, alien species – native plants. Here dual study was conducted to find out the best possible combinations to go for practical application. If this is applied in the farms similar effect will be helpful to the organic farmers. *Artemisia* also acts as an effective natural insecticide against (Khosravi *et al*, 2010). If grown as cover crops it will prevent entry of many animals due to its bitter character. It is also a cultivated medicinal plant so farmer can get dual benefit of growing this crop. Most of the toxic effect of



*Artemisia* are attributed to its main compound artemisinin (Movillo *et al*, 2011; Moussavi – nik *et al*, 2011) which had been already extracted from the sample used in present study. This projects a possibility that besides artemisinin other compounds present in leaf mulch also show phytotoxic effect, this gives additional value to the crop as a source of natural herbicide too.