Allelopathic Influence of Tecomastans(L.) on the Seed Germination and Biochemical Changes in Green Gram

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Abstract

The present revision was aimed to assess the germination and growing responses of green gram seeds which were exposed to various (control, 1%, 5%, 10%, and 20%) concentrations of dried leaves of Tecomastans (L.) by conducting laboratory tests. Dried leaves of Tecomastans (L.) affected negatively the radical length, reduced germination percentage, delayed germination and hindered shedding of seed coat in green gram. Bioassay readings showed that the total protein activity, amylase activity, invertase activity and protease activity were significantly reduced with increasing concentrations of the Tecomastans (L.) leaf extracts. The maximumaction of all the biochemical components of the green gram was found in control while as minimum action was found in 100% of extract concentration. The tolerance index was found to be reduced while as the phytotoxic index was found to be improved from control to 100% in day by day of the experiment. The germination index was found to be increased on a daily basis of the experiment. The possible biochemical feature responsible for such allelopathy is debated grounded on earlier reports.

Key words: *Allelopathic potential, aqueous extracts, Tecomastans, Vignaradiata, tolerance index, radical length.*

I. INTRODUCTION

Allelopathy plays animportantpart in agro-ecosystems, and affects the growth, quality and quantity of the products by the interactions among crops, weeds and trees. Generally, these interactions are deleterious to the receiver plants but may also provide a selective advantage to the donor (Rice,1984). Allelochemicals released from plant parts are largely classified as secondary plant metabolites (such as alkaloids, isoprenoids, phenolics, flavonoids, terpanoids and gluconolates etc.). These chemicals are present virtually in all plant tissues, including leaves, flowers, fruits, stems, roots, rhizomes, seeds and pollen. Among the plant parts, leaves seem to be the most consistent producers of these allelochemicals. Several chemicals can be released together and may exert toxicities in an additive or synergistic manner (Putnam and Tang,1986). Many investigators reported that large number of metabolites occur in different parts of plant and may have stimulatory or inhibitory effects on seed germination and seedling growth of other plants (Chou & Yao 1983). Systematic research approach in allelopathy was started only recently in the last two decades and allelopathic influence of multipurpose tree species on crops are being investigated under different agro-eco systems. Hence, this investigation was carried out to study the allelopathic influence of aqueous leaf extract of dried leaves of *Tecomastans*(L.) on the early stages of seed germination and biochemical changes taking place in green gram seeds.

II. CONSTITUENTS AND PROCEDURE

A. Constituents

1. Assemblage of Tecomastans(L.) leaves:

Matured leaves were collected from orchard land of Pondicherry University Experimental Farm and recognizedby using the taxonomic keys. The *Tecoma*leaves were dehydrated up for ten days at room temperature.The leaves were crushed into fine powder (Hamedet al.,2014) and prepared for aqueous extract. The dominantcompounds present in *Tecomastans*(L.) are saponins, flavonoids, tannins, phenols, anthraquinones, alkaloids and glycosides. Fig 1(A) indicates the over-all*Tecomastans*(L.)plant whereas as Fig 1(B)represents*Tecomastans* (L.)flower and leaves.

Fig 1 (A) *Tecomastans*(L.)plant

Fig 1(B)*Tecomastans* (L.)flower and leaves.

2. Controlling of (green gram) seeds:

The mung bean*Vignaradiata*(L.) correspondingly known as the moong bean, green gram, or mung is a plant species in the legume family. Well matured and verified green gramseeds were procured from the Puduva Agro Service, Govtof Puducherrycertified dealer for farm contributions. The seeds were washed well and dehydrated under daylight for 2 hr. Then the seeds were soaked with Sodium hypochlorite solution in 5min. for sterilization of the seed surface (Maharajanet al.,2007). The sterilized seeds after dry in room temperature are used for study.

3. Ingredients

Preparation of leaf powder extract (Musyimi et.,2012)

100 gram matured fresh leaves of study plant

↓

Shade dried (kept in room temperature for 10 days)

↓

Made into fine powder and larger particles are removed

↓

10 g of leaf powder dissolved in 100ml of distilled water

↓

Boiled at 60˚ C for 30 minutes

↓

Cooled and filtered in fine cloth

↓

Centrifuged at 5000rpm for 15 minutes

↓

Supernatant /Filtrate is kept as 100% concentration

↓

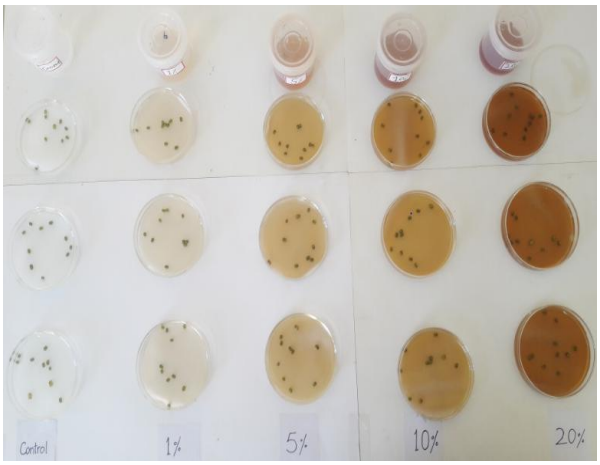
Different concentrations for experimental work are prepared with distilled water

↓

(1%, 5%, 10%, and 20% and control is distilled water)

4. Investigationalarrangement

Fig. 2 Experimental setup



Each concentration is having the number of three discs. Individually disc is single lined with whatman1 filter paper in order to provide wetness to seeds throughout the experimental phase of three days. 10 ml ofdifferent extract concentrations is added to respective discs as per the experimental setup. Total ten seeds were placed in each disc. The experimental setup is maintained at room temperature (in diffused light, which is almost dust free) for three days.

III. METHODOLOGY

Radical length was calculated by means of graph sheet and one foot scale (Ramakrishnan et al.,2014).The total protein activity in green gram seeds was measured by a modified method of Bradford (1976), amylase activity was assessed by Bernfeld method (1955), estimation of invertase activity was evaluated by a modified method of Harris and Jaffcoat(1974) and the estimation of protease activity was measured by Ladd and Butler method (1972).

IV. GERMINATION READINGS

Germination index: Germination/Emergence index (GI/EI) was calculated by following formula used by Association of Official Seed Analysis (AOSA, 1990).

VI. RESULTS

Seed fresh weight gain exposure to *Tecomastans* (L.) dried leaf extract.

$$\frac{\text{no. of days after sowing}}{\text{total no. of seeds planted}} \text{x no. of seeds germinated}$$

Tolerance Index: Tolerance index was calculated by using the formula suggested by Turner and Marshal (1972).

$$\frac{longest\ radical\ lengt\,h\ in\ treatment}{longest\ radical\ lengt\,h\ in\ control} x100$$

Percentage of phytotoxicity**:** The percentage of the phytotoxicity of the seedling is due to the presence of allelochemicals in *Tecomastans*(L.). Treatment was calculated by the formula suggested by Chiou and Muller (1972).

$$\frac{radical\ lengt\,h\ of\ control - radical\ lengt\,h\ of\ con.}{radical\ lengt\,h\ control} \text{x } 100$$

V. DATA ANALYSIS

Data on radical length, total protein activity, amylase activity, protease activity and invertase activity obtained from the study was subjected to Analysis of Variance (ANOVA-one-way-SPSS version). Means of treatment sample were compared usingleast significance difference (LSD at 0.05). Gulzar et al.,(2014).

For this bioassay10 seeds were placed on each Petri dish. Initial weight of seeds was taken and thenplaced in either water (control) or *Tecomastans* (L.) dried leaf leachate (5%) and (10%), and gain of fresh weight wasdetermined at24, 48 and 72 h of imbibition.

The inclusive result from the investigational study shows that the extract of dried leaves of *Tecomastans* (L.) is found to having high inhibitory effect on the radical length and on the chemicalconstituents of germinating seeds of green gram.Fig 9 shows the effects of *Tecomastans*(L.) leaf extract on radical growth of green gram seeds. The radical length of seedsoaked in control, 1%, 5%, 10% and 20% was found to be increased in each day of theexperimental phase and in every case it was found that radical length in control is more than treatments. The lowest mean radical length 0.4125cm was found in 24 hours of 20%concentration while as the highestmean radical length 5.1cm was found in 72 hours in control.

Similarly, Seed coat shedding process was delayed and the delay was proportional to the concentration of extract. While as the germination index was found to be improved every day. The maximum germination index was three in the last day of experiment in all the treatments. The effect of dried leaf extract on the tolerance Index and phytotoxic index on green gram seeds are represented in Fig (10). The tolerance index and phytotoxic index was found to be inversely proportional to each other, the tolerance index got decreased from control to 20% while as phytotoxic index was found to be increased from control to 20% in each day of the experimental phase.

Treatment of green gram seeds with the leaf extract of *Tecomastans* (L.) caused decline of total protein activity (Fig 11). As presented in Figure 11, total protein content was considerablyreduced in response to allelopathic effect of *Tecomastans*(L.). The maximum decline percentage in total protein content was found at the maximum leachate concentration. The total protein activity in control as well as in concentration of 10% and 20% declined with period of imbibition and in each case total protein activity was found more in control than treatments. The effect of leaf leachate *Tecomastans* (L.) on amylase content was also examined.Amylase activity in control as well as in 10% and 20% in green gram seeds weresignificantly and negatively affected by treatment with*Tecomastans* (L.) leachate (fig 12). Invertase levels were higher in control than in treated seeds (10% and 20%) as represented in (fig 13).Protease activity in control as well as in concentration of 10 % and 20% decreased with time of incubation and in each case concentration of protease was found more in control than treatments (fig 14).

VII. STATISTICAL ANALYSIS

Data were subjected to one-way ANOVA; differences between individual means were determined by the least significant difference (LSD) test at the 0.05 level of probability. Experimental design was performed as a randomized complete block design; each experiment was consisting of three treatments. Data were analyzed using SAS program.

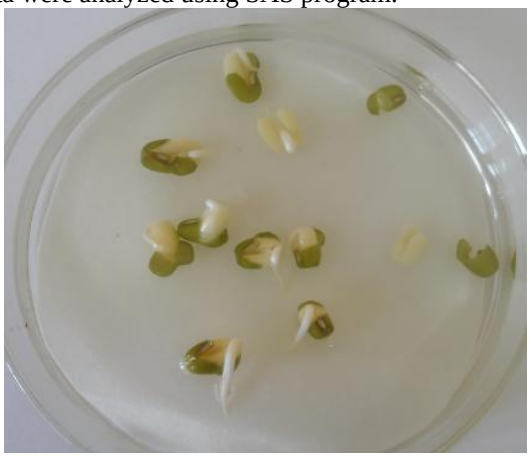


Fig 3- Seed soaked in control of dried leaf extract at 24 hours.

Fig 4- Seed soaked in 20% of dried leaf extract at 24 hours.

Fig 5- Seed soaked in control of dried leaf extract at 48 hours.

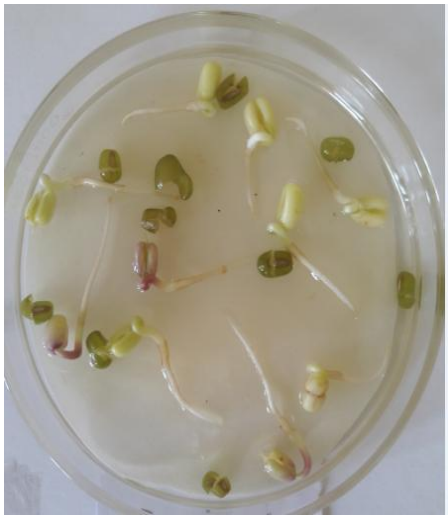


Fig 6- Seed soaked in control of dried leaf extract at 72 hours.

Fig 7- Seed soaked in 20% of dried leaf extract at 48 hours.

Fig 8- Seed soaked in 20% of dried leaf extract at 72 hours.

fig 9. Effect of aqueous leaf extract of *Tecoma Stans* on radical length of green gram

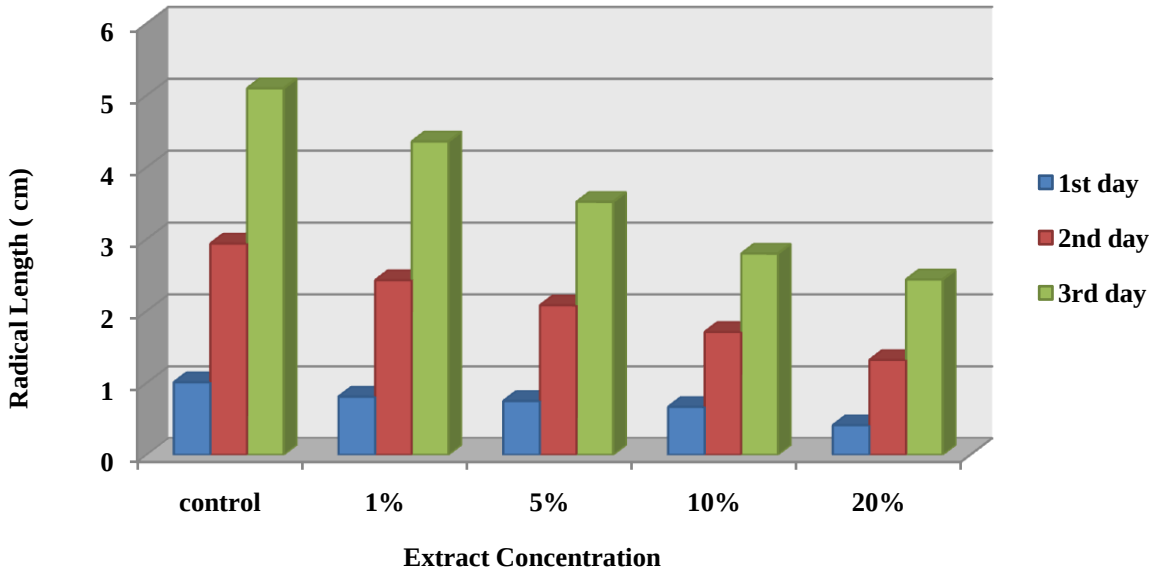
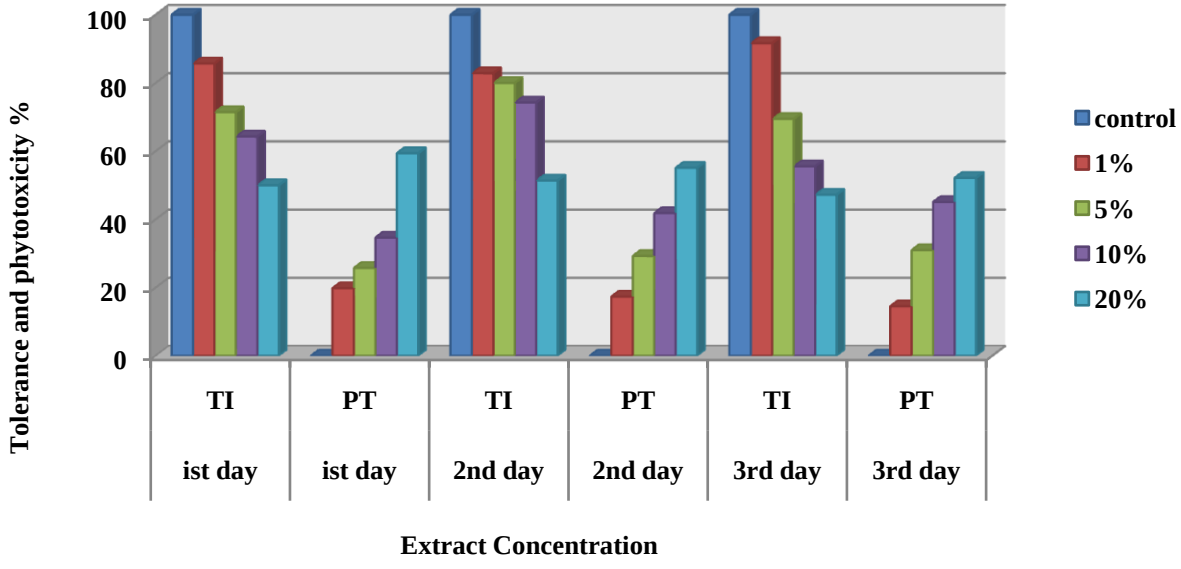


fig 10. Effect of aqueous leaf extract of *Tecoma Stans* on Tolerance and phytotoxic index of green gram



TI=Tolerance Index ; PT=Phytotoxic Index.

fig 11. Effect of aqueous leaf extract of *Tecoma Stans* on total protein content of green gram

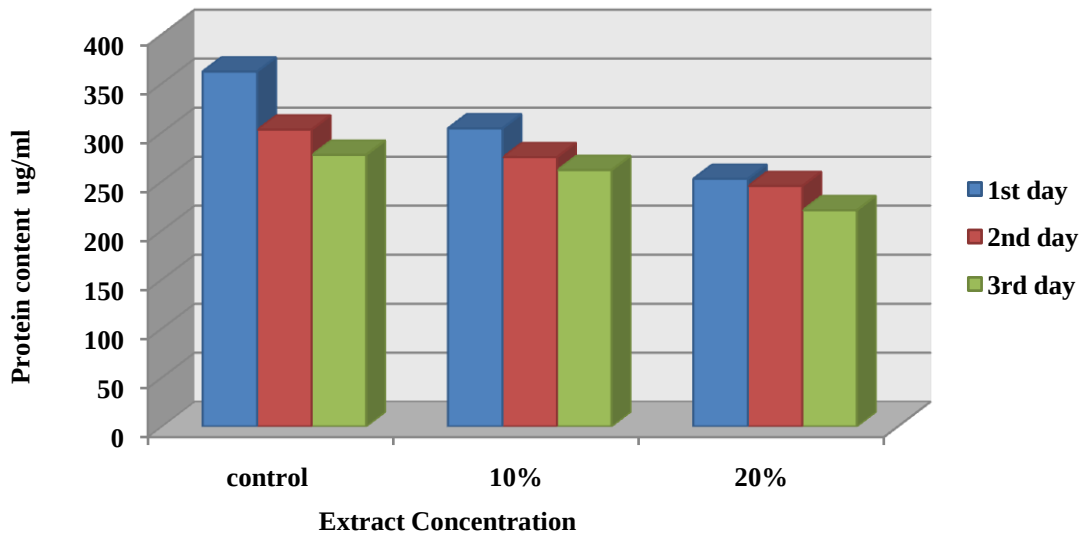
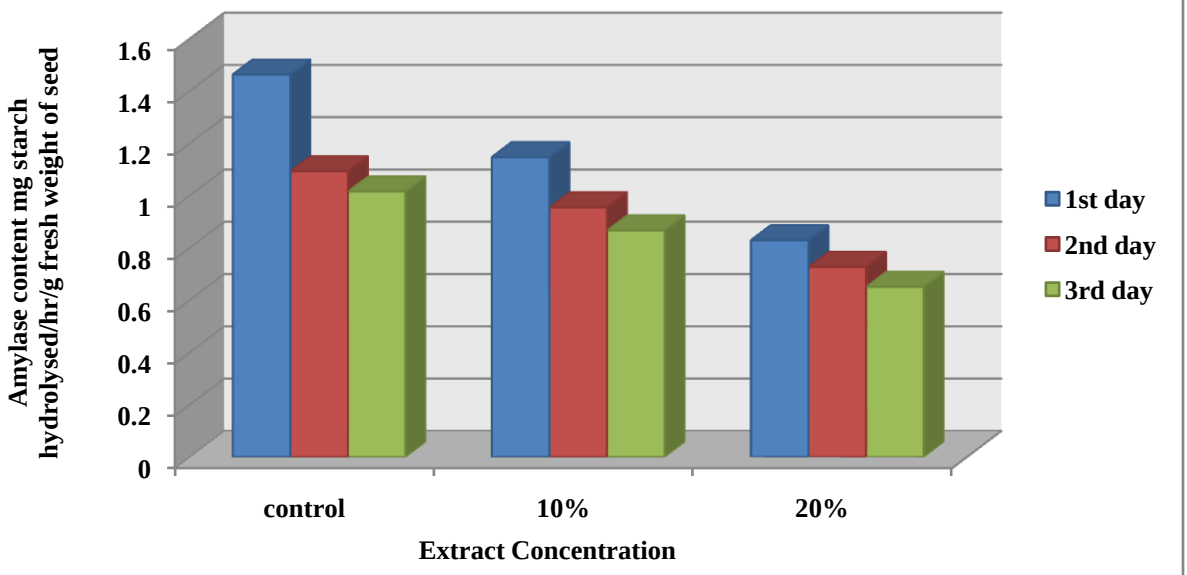
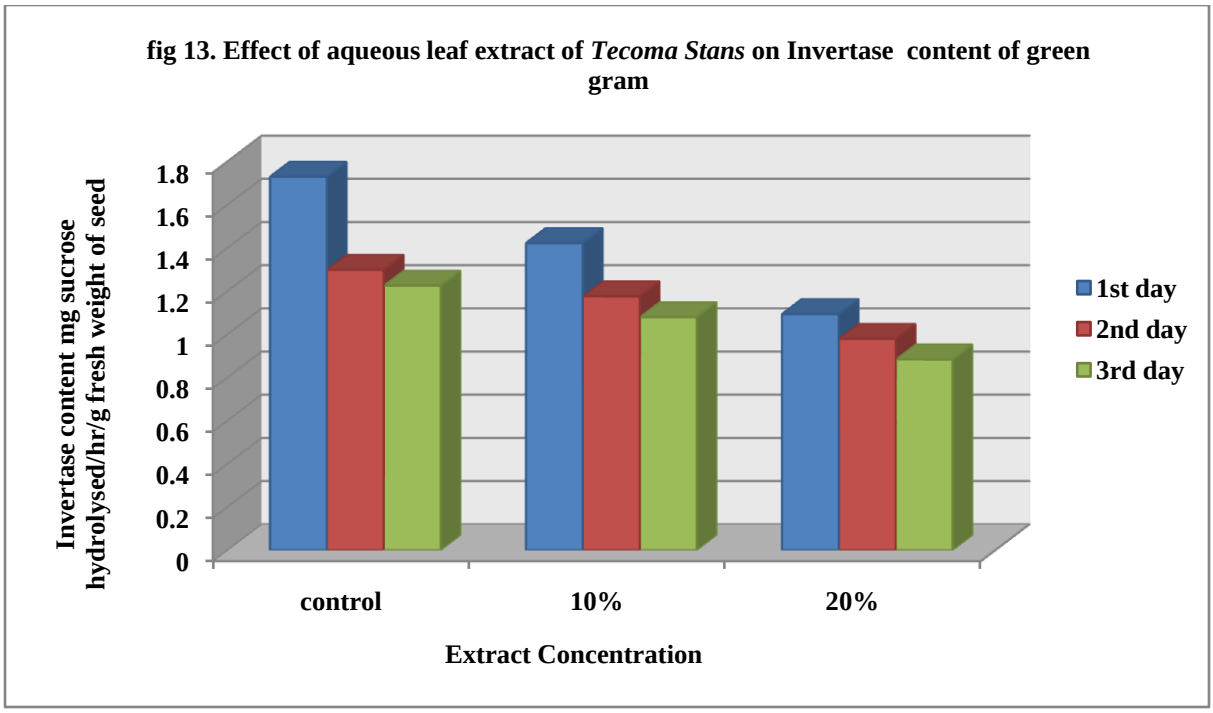


fig 12. Effect of aqueous leaf extract of *Tecoma Stans* on Amylase content of





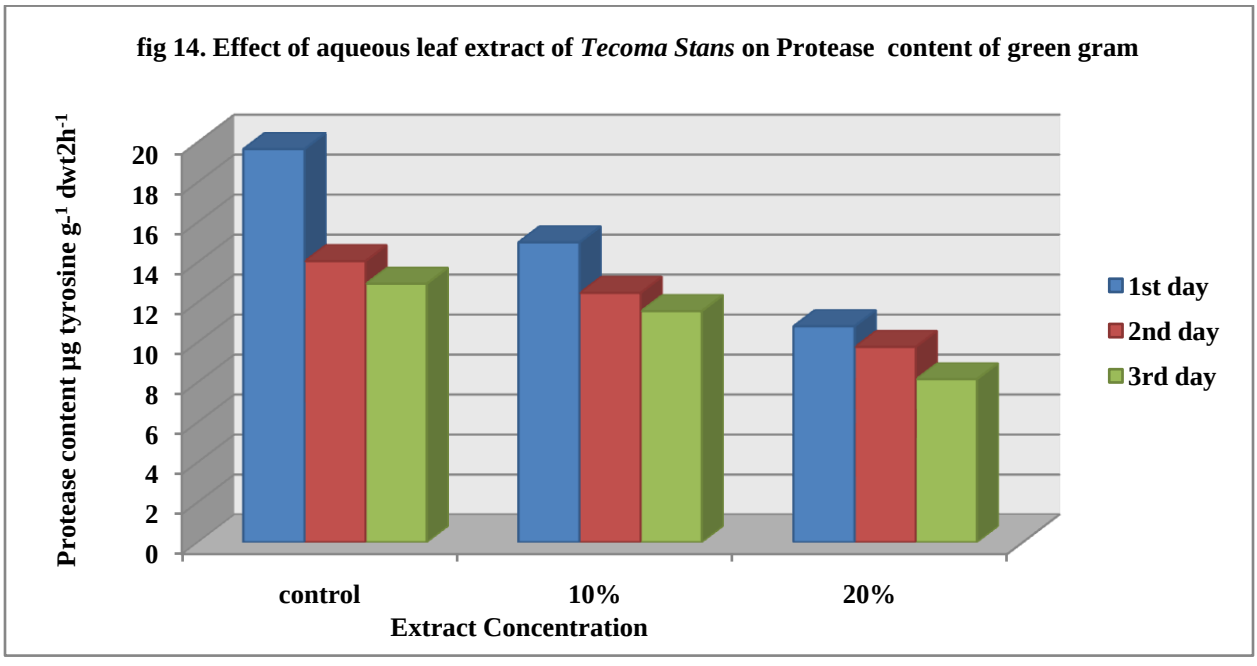


Table 1: Allelopathic effect of *Tecomastans*(L.) on the radical length of green gram seeds.

Treatments	Radical length/Mean/SD. First day	Radical length/Mean/SD. Second day	Radical length/Mean/SD. third day
Control	1.01 ±0.24698	2.94±0.31693	5.1±1.64857

1%	0.81±0.22336	2.43±0.54782	4.36±1.83436
5%	0.75±0.12693	2.08±0.49621	3.52±0.8203
10%	0.66±0.15811	1.71±0.50431	2.8±0.94281
20%	0.41±0.14577	1.32±0.48717	2.44±0.51251

Means within each row are significantly different ($p<0.05$), according to LSD test.

Table 2: Allelopathic effect of *Tecomastans*(L.) on the total protein content of green gram seeds.

Treatments	Total Protein value/ Mean/SD. First day	Total Protein value/ Mean/SD. Second day	Total Protein value/ Mean/SD. third day
Control	2.573±0.03933	2.15813±0.01685	1.97787±0.1024
10%	2.16707±0.07862	1.9612±0.03335	1.86927±0.0262
20%	1.80637±0.02438	1.75523±0.02451	1.58237±0.02662

Means within each row are significantly different ($p<0.05$), according to LSD test.

Table 3: Allelopathic effect of *Tecomastans*(L.) on the amylase content of green gram seeds.

Treatments	Amylase value/ Mean/SD. First day	Amylase value/ Mean/SD. Second day	Amylase value/ Mean/SD. third day
Control	0.325±0.00351	0.315±0.003	0.302±0.00458
10%	0.288±0.00252	0.278±0.00404	0.261±0.003
20%	0.258±0.00153	0.238±0.00351	0.224±0.2248

Means within each row are significantly different ($p<0.05$), according to LSD test.

Table 4: Allelopathic effect of *Tecomastans*(L.) on the invertase content of green gram seeds.

Treatments	Invertase value/ Mean/SD. First day	Invertase value/ Mean/SD. Second day	Invertase value/ Mean/SD. third day
Control	0.384±0.00252	0.374±0.00361	0.364±0.00404
10%	0.357±0.00306	0.342±0.00379	0.325±0.00361
20%	0.340±0.00306	0.320±0.00252	0.304±0.00416

Means within each row are significantlydifferent ($p<0.05$), according to LSD test.

Table 5: Allelopathic effect of *Tecomastans*(L.) on the protease content of green gram seeds.

Treatments	Protease value/ Mean/SD. First day	Protease value/ Mean/SD. Second day	Protease value/ Mean/SD. third day
Control	0.291±0.004	0.270±0.00709	0.256±0.00586
10%	0.251±0.00401	0.242±0.00404	0.232±0.00306
20%	0.224±0.00265	0.213±0.00351	0.187±0.004

Means within each row are significantly different ($p<0.05$), according to LSD test.

VIII. DISCUSSION

Allelopathy is the direct influence of a chemical released from one living plant on the development and growth of another (Bano et al.,2012). Plants may favorably or adversely affect other plants through allelochemicals, which may be released directly or indirectly from live, dead plants or organic residues. The keypurpose of this study was to examine the allelopathic effect of dried leaves of

Tecomastans(L.) on germinating seeds of green gramduring first three days of germination. The outcomes of this study showed that the allelopathic effect of *Tecomastans*(L.) on green gram seeds are inhibitory.

The dried leaf extract of *Tecomastans*(L.) caused a notable reduction in seedling growth of green gram seeds. The reduction of radicle length was found to be concentration dependent. This inhibition of radical length may be due to the various inhibitors which are present in dried leaves resulting in changing of macromolecules such as proteins, lipids as well as nucleic acids (Hussain and Reigosa 2011). Similar report on reduced radicle length has been made by Abu – Roman et al.,(2010) on the allelopathic effect of Spurge (*Euphorbia hierosolymitana*) on wheat (*Triticum durum*). These outcomes are also in contract to the findings of Hussain, (1985) who reported that *Azadirachtaindica* leaf extract reduced radical growth of wheat, millet, maize, lettuce and mustard. Another possibility is that the inhibition of root length may be attributed to reduction in the synthesis of carbohydrates, protein, and nucleic acids (RNA and DNA). A third possibility is that the inhibition of root length may be due to the interference of phenols in cell division, biosynthetic processes as well as mineral uptake reported to present in the leaves of *Tecomastans*(L.). The reduction on seed germination and seedling growth might be due to imbalance of metabolism and metabolite transport, regulated by various enzyme activities from seed (Padhy et al.,2000). It has been found that the influences of allelochemicals on seed germination, and the growth of root may be due to reduction in cell division (Gholami et al., 2009; Singh and Chaudhary 2011). It has been also reported that seedling root (radical) growth is sensitive to allelochemicals which inhibit cell division and elongation in the root apical meristems (Zhang and Fu 2009). Previous studies have shown that extracts from various plants tend to inhibit germination and seedling growth of a number of crop species (Sundaramoorthy and Kalra 1991). Allelochemicals might inhibit seed germination by suppressing synthesis of gibberellins and indole acetic acid (Zhang and Fu 2009) which are involved in growth of seeds or plants. It is agreed that tolerance index and phytotoxic index were inversely associated to each other.The phytotoxic index was concentration dependent that is with rise in concentration the phytotoxic index got increased while as tolerance index got decreased. Parallel report on reduced tolerance index has been made by Prabhat et al.,(2013) on the allelopathic effect of *Jatropha Curcas*and*PongamiaPinnata* edible oil yielding crop *Glycine Max*. The similar trend on increase in phytotoxicity was found by U.N. Bhale (2011) in *Sorghum vulgare* due to polluted water. Sarithaand Prasad (2008) investigated that Cd-inducesphytotoxicity in *Sorghum bicolor*(L.) during seed germination and seedling growth.

Treatment of green gram seedlings with aqueous leaf extract of *Tecomastans*(L.) resulted in decline of total protein activity. It is possible that the phenolic compounds present in the aqueous leaf extract may diminish the incorporation of certain amino acids into proteins and thus reduced the rate of protein synthesis. Prasad et al.,(1999) reported that the aerial and shoot biomass of *Rhamnusvirgatus*tree significantly reduced the protein content of *Triticumaestivum*, *Eleusinecoracana*, *Lens culinaris* and *Phaseolus mungo* as paralleled to control. Hamed et al.,(2015) stated that aqueous leaf extract of *Trichodesmaafricanum*L reduced the soluble protein, insoluble protein and total protein contents of *Portulacaoleracea*L. Padhy et al.,(2000) detected that the leachates of *Eucalyptus globulus* reduced the protein content in both the root and shoot of finger millet.

Numerous phytotoxic allelochemicals have been isolated, identified, and found toaffect a number of physiological reactions, for example, water utilization, photosystem II (PSII) efficiency, nutrient uptake, ATP synthesis, cell division, and gene expression (Blum, 1996).Cell division, production of plant hormones and their balance, membrane stability and permeability, germination of pollen, mineral uptake, movement of stomata, pigment synthesis, photosynthesis, respiration, amino acid syntheses, nitrogen fixation, specific enzyme activities and conduction tissue are known targets for allelochemicals (Rizvi et al.,1992; Wink et al.,1998).

Enzymes are the principal molecules, which are stimulated when a seed started germinating and enzymes like amylase, catalase, invertase and protease quantity varies. In the current work, reduction in enzyme activity was found with increase in concentration over a given period of time (3 days).The enzyme action was concentration dependent. Allelochemicals were reported to influence several physiological processes during seed germination such as inhibiting amylase activity. Ramakrishnan et al.,(2014) stated that leachates of *Gmelinaarborea* reduced the amylase content of green gram, red gram, black gram, and chickpea. Aurora et al.,(2009) recountedthat the aqueous leachate of *Sicyos deppei* (Cucurbitaceae) affected the amylase content in germinating tomato seeds.Both invertase and protease activity of green gram seedlings were reduced by allelochemical stress. Devi and Prasad (1992) reported that activities of amylase, invertase and

protease were suppressed by ferulic acid in tests using maze seeds and seedlings. Saritha and Prasad (2008) investigated that all hydrolyzing enzymes including amylase and total proteases exhibited a significant decrease during seed germination and seedling growth in *Sorghum bicolor* (L.) with increasing Cd concentrations.

Bhat and Yogamoorthi (2017) on comparing with the previous article findings,evaluation ofallelopathic effect of aqueous leaf extract of *Tecomastans*(L.) on seed germination and biochemical changes in *Vignaradiata*(L.)showed reduced germination percentage, hindered germination, delayed seed coat shedding, inhibited seedling growth and reduced enzyme activity and total protein content. The highest activity of biochemical constituents of green gram was found in control while as lowest activity was found in 100% of extract concentration. The tolerance index was found to be decreased while as phytotoxic index was found to be increased from control to 100% in each day of experiment. The germination was found to be increased in each day of the experiment. So from both the fresh and dried leaf extracts of *Tecomastans* (L.) It was seen that allelochemicals present in *Tecomastans*(L.) have suppressed the germination and biochemical activities of green gram.

Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone (James and Bala 2003). In the case of *Tecomastans*(L.) it is reported from the preliminary screening of this plant revealed the presence of tannins, flavonoids, alkaloids, quinones and traces of saponins and amino acids by Archana, 2013. Methanol and ethanol extracts of the leaves showed the presence of all the secondary metabolites.saponins, flavonoids, tannins, phenols, anthraquinones, alkaloids and glycosides which would be the active principles of the plant. Ethyl acetate extracts indicated the presence of saponins, tannins and phenols whereas aqueous extracts showed saponins, flavonoids phenols and alkaloids from leaves of *Tecomastans*(L.)(Minal et al.,2014). These phyto-constituents individually or synergistically interfere with various cellular metabolic activities of germinating seeds

Therefore, the allelopathic property of extracts of dried leaves of *Tecomastans* (L.) might be due to phytochemical constituents and such allelopathic characteristics is also one of the important traits in invasive plants. Concludingly, it could be stated that allelopathic weed management seems immediately advantageous as an alternative or a supplement to other weed management practices in crop production. Reduced reliance on traditional herbicides via the use of allelopathy has frequently been mentioned as environmentally favorable (Macias, 1995; Narwal et al.,1998).

In conclusion, allelochemicals present in both fresh and dried leaves of *Tecomastans* (L.)reducedgermination percentage, delayed germination, hindered seed coat sheeding, inhibited seedling growth and reduced enzyme activity and total protein content. It is therefore important that *Tecomastans* (L.) must be removed during weeding as early as they germinate in fields where green gram plants are grown. Further studies, on screening, isolation and testing of individual phytochemical constituents present in *Tecomastans* (L.) would bring out the exact bioactive compound present in the leaves of *Tecomastans* (L.) which could be potentially used in weed management.

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