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RESEARCH ARTICLE

EVALUATION OF ALLELOPATHIC EFFECT OF AQUEOUS LEAF EXTRACT OF *TECOMASTANS* (L.) ON SEED GERMINATION AND BIOCHEMICAL CHANGES IN *VIGNARADIATA* (L.)

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ABSTRACT

The effects of different concentrations (control, 10, 20, 40,80 and 100%) of aqueous leaf extract of fresh leaves of *Tecomastans* (L.) on radical length, shedding of seed coat, germination index, tolerance index, phytotoxic index and biochemical constituents of *Viginaradiata* (L.) were investigated. Aqueous leaf extract of *Tecomastans* (L.) affected negatively radical length and delayed shedding of seed coat in green gram. Significant reduction in the amount of total protein activity, amylase activity, invertase activity and protease activity were recorded in response to allelochemical stress. 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. The probable biochemical factor responsible for such allelopathy is discussed based on previous reports.

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INTRODUCTION

Allelopathy is the chemical induction or inhibition of one species by another. The degradation of allelopathic crop may produce a variety of phytotoxins in the soil causing adverse effect in other plants (Nelson, 1996; Rice, 1984). Exact information about the allelochemicals, responsible for the allelopathic effects must be sought when assessing the effects of plants with enhanced allelopathic traits. The allelochemicals are produced in above or below ground plant parts or in both to cause allelopathic effects in a wide range of plant communities. Plant parts like roots, rhizomes, stems, leaves, flowers, inflorescence, pollen, fruits and seeds are known to contain allelochemicals (Rice, 1984). Plants produce a large variety of secondary metabolites like phenols, tannins, terpenoids, alkaloids, polyacetylenes, fatty acids, steroids, which have an allelopathic effect on the growth and development of the same plant or neighboring plants. These allelochemicals when released to the soil, inhibit germination, shoot and root growth of other plants and affect nutrients uptake or naturally occurring symbiotic relationship, thereby destroying the plant's usable source of nutrients (Abu-Romman *et al*., 2010).

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Apart from the direct toxic effect on other plants, some allelochemicals are supposed to influence the availability of nutrients in the soil. Generally, phenolic acids are considered to have important influence on nutrient cycling in terrestrial ecosystems because most of the allelopathic effects are reported to be due such phenolic monomers and phenolic acids. Leela *et al*. (2014) assessed the allelopathic influence of teak *Tectonagrandis* (L.) leaves on growth responses of green gram *Vignaradiata* (L.). Kavitha *et al*. (2012) evaluated allelopathic influence of *Vitexnegundo* (L.) on germination and growth of green gram *Vignaradiata* (L.). Fara *et al*. (2014) studied the allelopathic effect from *Chromolaenaodorata* (siam weed) leaves extract on germinating seeds of *Vignaradiata*. Ramakrishnan *et al*. (2014) investigated the effect of allelochemicals from leaf leachates of *Gmelinaarborea* on inhibition of some essential seed germination enzymes in green gram. Bimal *et al*. (2016) on allelopathic effect of invasive weed (*Solanum sisymbriifolium* lamk.) on germination and seedling growth of four widely cultivated indian crops including *Vignaradiata* (L.). Manimegalai *et al*. (2012) assessed the allelopathic influence of *Tectonagrandis* leaves on the germination of black gram and green gram. Hemanth *et al*. (2015) studied the allelopathic efficacy of aqueous extracts of *Zingiberofficinale* (Rosc.) on germination, vigor, growth and yield of *Vignaradiata* (L.).Gantayet *et al*. (2014) studied the allelopathic effect of *Lantana camara* on vegetative growth and yield components of green gram. In view of these allelopathic characteristics possessed by several plants on seed germination present investigation was carried out to study the allelopathic effect of aqueous leaf extract of fresh leaves of *Tecomastans* (L.) on the early stages of seed germination and biochemical changes taking place in *Vignaradiata* (L.) (Green gram) seeds.

MATERIALS AND METHODOLOGY

MATERIALS

Collection of *Tecomastans* (L.) leaves

Matured fresh leaves were collected from garden land of Pondicherry University Experimental Farm and identified using the taxonomic keys. The plant leaves were cut into small pieces (Hamed *et al*., 2014) and made ready for aqueous extract. The major chemicals present in *Tecomastans* (L.) are saponins, flavonoids, tannins, phenols, anthraquinones, alkaloids and glycosides. Fig 1(A) represents the general *Tecomastans* (L.) plant while as Fig 1(B)shows *Tecomastans* (L.) flower and leaves.

Fig 1 (A) Tecomastans (L.) plant

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

Procurement of *Vignaradiata* (L.) (green gram) seeds

The mung bean *Vignaradiata* (L.) also known as the moong bean, green gram, or mung is a plant species in the legume family. Well matures and tested *Vignaradiata* (L.) seeds were purchased from the Puduva Agro Service, Govt of Puducherryan authorized dealer for farm inputs. The seeds were washed well and driedunder sunlight for 2 hr. Then the seeds were soaked with Sodium hypochlorite solution in 5min. for sterilization of the seed surface (Maharajan *et al*., 2007). The sterilized seeds after dry in room temperature are used for study.

METHODOLOGY

Aqueous extract of the fresh leaf preparation (Musyimi *et al*., 2012)

100 gram fresh leaves of study plant

↓

The leaf washed in running tap water for removing the surface contaminants and dust

↓

The plant materials were chopped into small pieces with cutter

↓

50 g of leaf dissolved in 500ml 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 considered as 100% concentration

↓

Different concentrations for experimental work are prepared with distilled water

↓

(10, 20%, 40%, 80% and 100% and control is distilled water)

Experimental set up

Triplicate discs for each concentration is set. Each disc is single lined with whatman1 filter paper to provide wetness to seeds throughout the experiment period (3days). 10 ml of extract (different concentration) added to each disc. 10 seeds were placed in each petri disc. Maintained at room temperature (in diffused light and dust free) for 3 days.

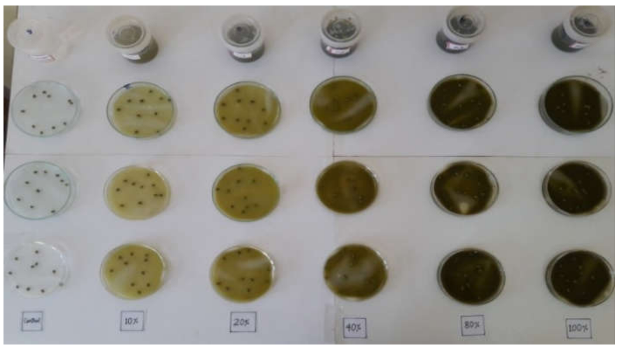


Fig. 2. Experimental setup

Measurement

Radical length was measured by using the graph sheet and one foot scale (Ramakrishnan *et al*., 2014).

The total protein activity was measured by a modified method of Bradford (1976), amylase activityin green gram seeds was estimated by Bernfeldmethod (1955), estimation of invertase activity was done by a modified method of Harris and Jaffcoat (1974) and the estimation of protease activity was measured by Ladd and Butler method (1972).

GERMINATION STUDIES

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

$$GI = \frac{\text{Number of days after sowing x Number of seed germinated}}{\text{Total Number of seed planted}}$$

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

$$\text{Tolerance Index (TI)} = \underline{\text{longest radical in treatment}} \text{ x } 100$$

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

$$\text{Phytotoxicity (\%)} = \frac{\text{Radical length of control} - \text{R length of treated sample}}{\text{Radical length of control}} \text{ x } 100$$

Data analysis

Data on radical length obtained from the study was subjected to Analysis of Variance (ANOVA-one-way-SPSS version). Means of treatment sample were compared using least significance difference (LSD at 0.05). Gulzar *et al*. (2014).

RESULTS

The overall result from the experimental study shows that the extract of fresh leaves of invasive plant *Tecomastans* (L.) is found to having high inhibitory effect on the radical length and the biochemical constituents of early germinating seeds of green gram. Fig 11 shows the effects of *Tecomastans* (L.) aqueous leaf extract on radical growth of *Vagina radiata* (L.). The length of the radical of seed soaked in control, 10%, 20%, 40%, 80% and 100% (fig 11) was found to be increased in each day of the experiment and in each case radical length was found more in control than treatments.

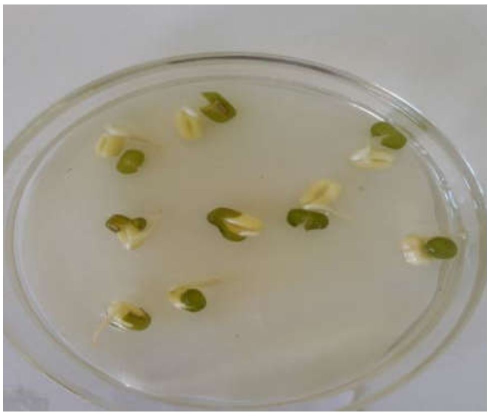


Fig.3. Seed soaked in control of aqueous leaf extract at 24 hours

Fig.4. Seed soaked in 100% of aqueous leaf extract at 24 hours

Fig.5. Seed soaked in control aqueous leaf extract at 72 hours

Fig.6. Seed soaked in 100% aqueous leaf extract at 72 hours

The minimum mean radical length 0.56cm was found in 24 hours of 100%concentration and the maximum mean radical length 6.41cm was found in 72 hours in control. There were significant differences ($p<0.05$) in the radical length during three days of germination period. The shedding of seed coat was found to be delayed with increase in concentration. The germination index was found to be increased each day.

Fig.7. Highest radical length of seed in control of aqueous leaf extract at 24 hours

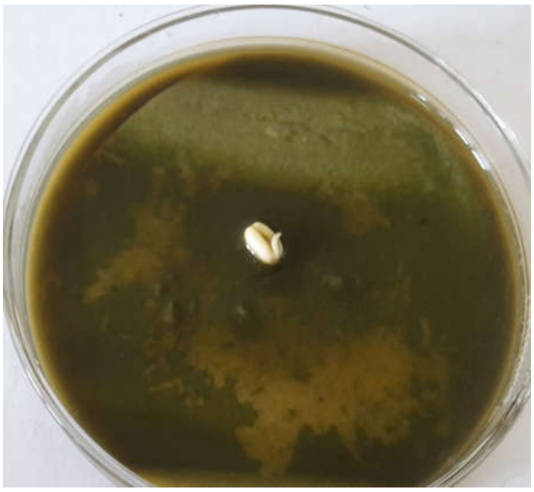


Fig.8. Highest radical length of seed in 100% of aqueous leaf extract at 24 hours

The germination index was 3 in third day in all the treatments. The effects of *Tecomastans*(L.) aqueous leaf extract on the germination index, tolerance index and phytotoxic index of *Vagina radiate* (L.) are represented in Fig (12, 13 & 14).The tolerance index was found to be decreased from control to 100% in each day of experiment, while as phytotoxic index was found to be increased from control to 100% in each day of experiment. The highest tolerance index 100 was found in control in all the three days of experiment, while as the lowest tolerance index 30.37 was found in 100% in 3rd day of experiment. Treatment of *Vaginaradiata* (L.) with the aqueous leaf extract of fresh leaves of *Tecomastans* (L.) resulted in reduction of total protein activity (Fig 15).

Total protein activity in controlas well as in concentration of 40% and 100% decreased with time of incubation. As per ANOVA analysis, there were significant differences ($p<0.05$) in the total protein activity. Amylase activity in control as well as in 40% and 100% concentration decreased with time of incubation and in each case of concentration amylase activity was found more in control than treatments (Fig 16). The minimum amylase activity 0.712mg starch hydrolysed/hr/g fresh weight of seed was found in 72 hours of seed germination in 100% and the maximum amylase activity 1.584mg starch hydrolysed/hr/g fresh weight of seedwas found in 24 hours of seed germination in control.

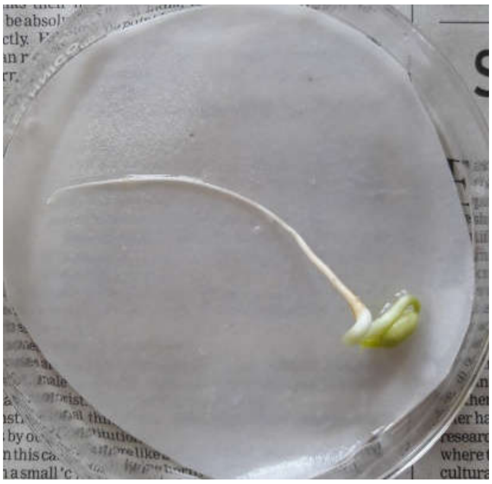
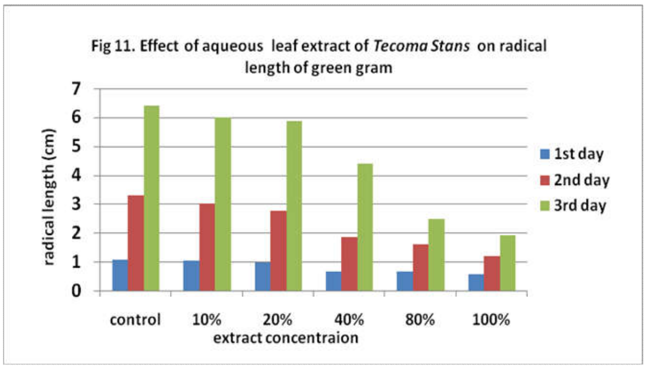
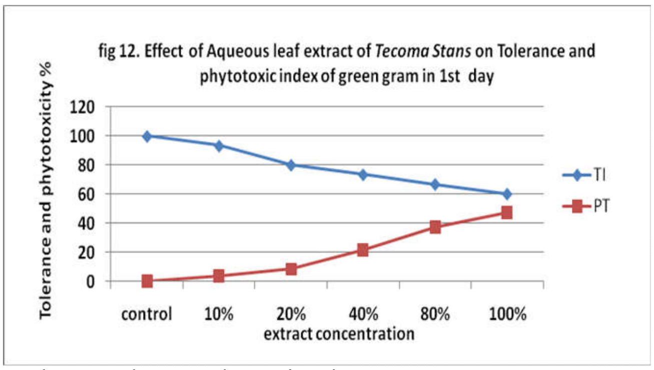


Fig.9. Highest radical length of seed in control of aqueous leaf extract at 72 hours

Fig.10. Highest radical length of seed in 100% of aqueous leaf extract at 72 hours





TI=Tolerance Index ; PT=Phytotoxic Index.

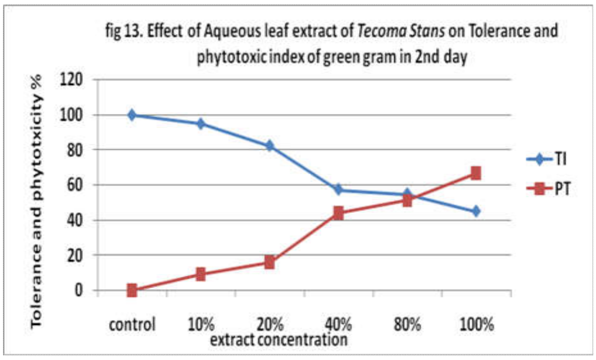


fig 13. Effect of Aqueous leaf extract of *Tecoma Stans* on Tolerance and phytotoxic index of green gram in 2nd day

TI=Tolerance Index ; PT=Phytotoxic Index.

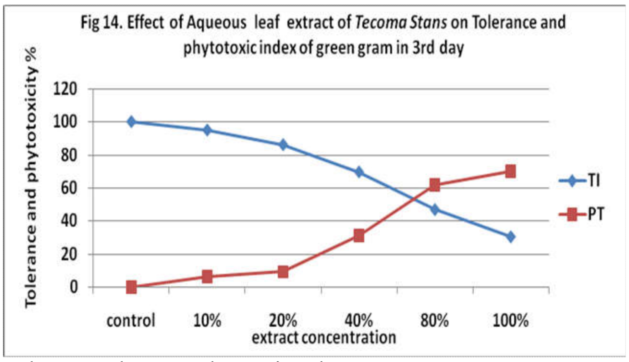


Fig 14. Effect of Aqueous leaf extract of *Tecoma Stans* on Tolerance and phytotoxic index of green gram in 3rd day

TI=Tolerance Index ; PT=Phytotoxic Index.

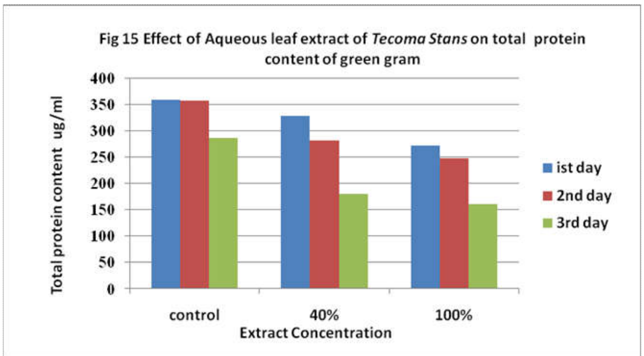


Fig 15 Effect of Aqueous leaf extract of *Tecoma Stans* on total protein content of green gram

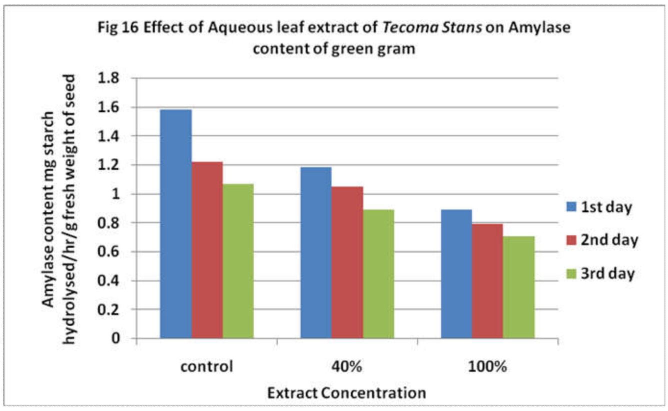


Fig 16 Effect of Aqueous leaf extract of *Tecoma Stans* on Amylase content of green gram

As per ANOVA analysis, there were significant differences ($p<0.05$) in the amylase activity. Invertase activity in control as well as in concentration of 40% and 100% decreased with time of incubation and in each case of concentration invertase activity was found more in control than treatments (fig 17). There were significant differences ($p<0.05$) in the invertaseactivity. Protease activity in control as well as in concentration of 40% and 100% decreased with time of incubation and in each case of concentration protease activity was found more in control than treatments(Fig 18).

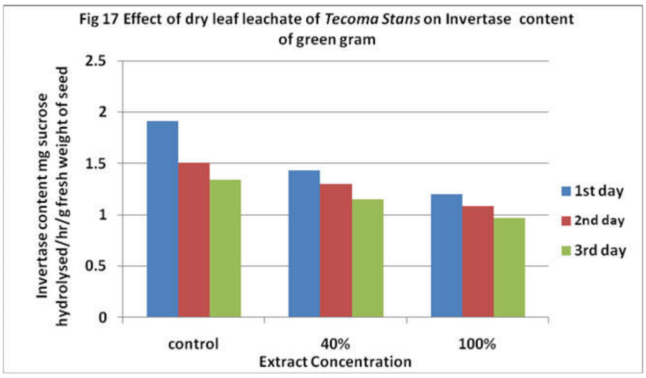


Fig 17 Effect of dry leaf leachate of *Tecoma Stans* on Invertase content of green gram

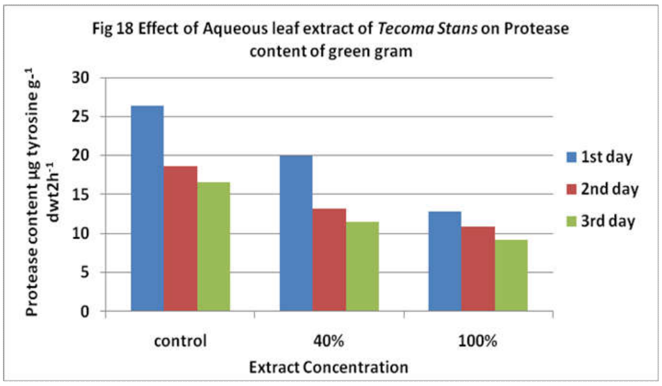


Fig 18 Effect of Aqueous leaf extract of *Tecoma Stans* on Protease content of green gram

As per ANOVA analysis, there were significant differences ($p<0.05$) in the protease activity.

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 main aim of this study was to examine the allelopathic effect of aqueous leaf extract of *Tecomastans* (L.) on germinating seeds of green gram *Vignaradiata* (L.) during first three days. The results of this study indicated that the allelopathic effects of aqueous leaf extract of *Tecomastans* (L.) on green gram are inhibitory. The aqueous leaf extract of *Tecomastans* (L.) also caused a remarkable reduction in seedling growth of *Vignaradiata* (L.).The inhibition of radicle length was found to be concentration dependent.

This inhibition may be due to the inhibitors which are present in fresh 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 results are also in agreement 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 understood that tolerance index and phytotoxic index were inversely related to each other .The phytotoxic index was concentration dependent that is with increase in concentration the phytotoxic index gets increased while as tolerance index gets decreased. Similar report on reduced tolerance index has been made by Prabhat *et al*. (2013) on the allelopathic effect of *Jatrophacurcas* and *Pongamiapinnata* edible oil yielding crop *Glycine Max*. The same trend on increase in phytotoxicity was found by U.N. Bhale (2011) in *Sorghum vulgare* due to polluted water. Saritha and Prasad (2008) investigated that Cd-induces phytotoxicity in *Sorghum bicolor* (L.) during seed germination and seedling growth.

Treatment of green gram seedlings with aqueous leaf extract of *Tecomastans* (L.) resulted in reduction of total protein activity. It is possible that the phenolic compounds present in the aqueous leaf extract may reduce 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 decreased the protein content of *Triticumaestivum, Eleusinecoracana, Lens culinaris* and *Phaseolusmungo* as compared to control. Hamed *et al*. (2015) reported that aqueous leaf extract of *Trichodesmaafricanum* L reduced the soluble protein, insoluble protein and total protein contents of *Portulacaoleracea* L. Padhy *et al*. (2000) noticed that the leachates of *Eucalyptus globulus* reduce the protein content in both the root and shoot of finger millet.

Many phytotoxic allelochemicals have been isolated, identified, and found to influence 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 tissueare known targets for allelochemicals (Rizvi *et al*., 1992; Wink *et al*., 1998). Enzymes are the chief molecules, which are stimulated when a seed started germinating and enzymes like amylase, catalase, invertase and protease quantity varies. In the present study, activity of enzymes was found to decrease with increase in concentration over a given period of time (3 days).The enzyme activity was concentration dependent. Treatment of green gram seedlings with fresh leaf extract of *Tecomastans* (L.) resulted in reduction of amylase activity. Ramakrishnan *et al*. (2014) reported that leachates of *Gmelinaarborea* reduced the amylase content of green gram, red gram, black gram, and chickpea. Aurora *et al*. (2009) reported that the aqueous leachate of *Sicyos deppei* (Cucurbitaceae) reduced the amylase content in germinating tomato seeds.

Inhibition of seed germination of crop plants might be the interfere with activities of peroxidase, alpha-amylase and acid phosphates (Alam and Islam 2002). Treatment of green gram seedlings with fresh leaf extract of *Tecomastans* (L.) resulted in reduction of both invertase and protease activity. 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. 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 studied- 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 fresh 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). 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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