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RESEARCH ARTICLE

Evaluation of bioherbicidal potential of *Nerium oleander* on growth and development of Bermuda grass (*Cynodon dactylon L.).*

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Abstract

The bioherbicidal efficacy of aqueous extracts of *N. oleander* on germination percentage, seedling growth, weed biomass and biochemical parameter of Bermuda grass (*Cynodon dactylon* L.) were tested. As per result, it was observed that aqueous extracts of *N. oleander* had a significant retardation effect on bermuda grass. At 20(g/l) concentrations, inhibitory effect was maximum than other lower extracts. Seedling length, fresh and dry weights was also reduced significantly over control. The phytotoxic effect was directly proportional to the intensity of aqueous extract concentration. At 20(g/l) extract concentration, inhibition on bermuda grass was highly notable (upto 79%) which is quite prove to use *N. oleander* as a bioherbicidal agent to control bermuda grass in paddy fields.

INTRODUCTION

Since the first implementation of synthetic herbicides in crop protection systems, weeds have been continuously developing their resistance against their counter herbicides. In agricultural system, weeds are of concern because they compete with cultivated crops for water, light, nutrients, space and perhaps under certain conditions they compete for carbon dioxide, which is essential for photosynthesis. Besides resource completion, weeds affect crop plants by releasing toxic substances, harboring insects and plant pathogens. Among the entire weeds, bermuda grass consider as one of the well known worst weed (Messiha et al., 1993; William and Hirase; 2004 and 2005). During the course of agricultural practices, different types of synthetic herbicides have used to control Bermuda grass (Ferell et al., 2004). Since one side, these herbicides played vital role to control weed but another side they become major threat for health issues of humans. In light of these characteristics of weeds and their hazards, it becomes imperative to control them. In the earlier times, since no synthetic chemicals were known, crop rotations, polyculture and other management practices were tried that were low input but sustainable.

With the discovery of synthetic herbicide in the early 1930s, there was shift in the weed management practices toward high input and target oriented ones, but the use of herbicides has proved an increasing incidence to resistance to herbicide in weeds. By these reasons, other important factor that should be considered is sustainability. New compound must be environmental friendly (Evan, 1999). But the term, sustainable technology include different point of view: agrochemical, environmental and economical. There is a need to discover safe compounds with new sites of action: however, this is not enough. Novel compounds should have a wide range of activities but be targeted at one particular problem (Macias et al., 2004). Allelopathic extracts could be used to control the growth and development of weeds (Chon and Kim, 2002 and 2004; Chon et al 2003; Singh et al 2003 and El-Rokiek et al 2006). Hence, the present investigation was made to find bioherbicidal potency of *N. oleander* to control or suppress the growth and development of Bermuda grass.

2. Materials and Methods

2.1. Experimental site condition

The experiment was conducted at the Department of Botany, Annamalai University, Annamalai Nagar from the period of June 2013 to December 2013. Annamalai University is located at 11.4° North latitude, 79.73° East longitude and about 5.248 meters altitude from the sea level. During the experimental period, the mean of minimum and maximum temperatures were 29^0 C to 38^0 C. The relative humidity ranged from 63 to 78% (Courtesy, Department of Agriculture, Annamalai University). Detailed information of experimental soil is given in table no 2.

2.2 Sample collection

Fresh leaves of *N. oleander* were collected from nearby area of Annamalainagar and Bermuda grass seeds (viable 100 seeds) are subjected to recover from post harvest paddy fields of Annamalai Nagar.

2.3. Aqueous Extract preparation

The leaves of *N. oleander* were rinsed thoroughly with tap water and then finely chopped and sterilized by 0.2% mercuric chloride ($HgCl_2$) solution for 10-15 min and then kept in oven at 80^oC for 24 hrs. Finely dried leaves were grinded with the help of ordinary grinder until a fine powder is formed. For preparing the different desirable concentrations of plant sample, weight per volume method was used i.e. for 1(g/l) extract concentration, 1g dry powder of plant sample was dissolved in 1L of water. Likewise 2.5(g/l). 5(g/l). 10(g/l), 15(g/l) and 20(g/l) extract concentrations were made (Chou and Muller (1972). Osmometer (model G-66) were used for measuring the ionic concentration of aqueous extracts. The osmolarity of the 1, 2.5, 5, 10, 15 and 20(g/l) aqueous extracts of *N. oleander* was recorded as 40, 94, 129, 142, 159, and 175 milliosmols, respectively.

2.4. Bioassay for germination percentage

2.4a. Petri dish culture

Aqueous extract treatments were used for petri dish experiment i.e. 0 (as control) 1(g/l), 2.5(g/l), 5(g/l), 10(g/l) and 20 (g/l). Viable seeds are sterilized in Sodium hypochlorite (NaClO) for 5 min and placed on filter paper in Petri dishes and watered with 5 ml of distilled water. Treatments were arranged within the growth chamber in a completely randomized design (CRD) with 5 replicates. Growth chamber conditions were 12 h light and a temperature of 25^oC. A total of 175 Petri dishes was monitored. Seed germination was recorded every day for 10 days.

2.4b Bioassay for seedling growth (physical and chemical parameter)

After 15 days, 30 days and 45 days of sowing, weed seedlings were uprooted, washed thoroughly and used as material for analysis of physio-biochemicals parameters of bermuda grass. All parameters were calculated by reference to the control plants.

For the calculation of percentage of inhibitory effect on the radicle and plumule elongation, percentage to the control was calculated as per formula,

$$I=100-(E_2\times 100/E_1)$$

Where, I is the % inhibition, E1 the Radicle and plumule elongation of control plant and E2 the Radicle and plumule elongation of treatment plant.

2.4c. Preliminary Phytochemical analysis of *N. oleander* L. (Suganya et al. 2012)

The phytochemical constituents of the Pet. ether, n-hexane, chloroform, ethyl acetate, ethanol and Methanolic extraction of *N. oleander* leaf was investigated for the presence of phytochemical constituents such as Alkaloids, Flavonoids, Saponins, Cardiac glycosides, Glycosides, Saponins glycosides terpenoid and Tannins by phytochemical screening test (Table-1). Methanolic extract showed presence of Saponins, cardiac glycosides and terpenoid. Alkaloid, cardiac glycosides and tannins were observed in Ethanolic extract. Ethyl acetate extract indicated the presence of alkaloid, terpenoid, cardiac glycosides and steroids. Chloroform extract showed presence of all the phytochemical constituents except steroids. Regarding Pet. Ether extract, cardiac glycosides, glycosides, Saponins glycosides and terpenoid were found present similar n-Hexane showed availability of flavonoid, glycosides, steroids, tannins and terpenoids.

3. Data analysis

All the experiments were performed in a completely Randomized Block Design (RBD) and repeated thrice. For each treatment were maintained with five replicates. The data of bermuda grass were subject to the Analysis of Variance (ANOVA) by using IBM SPSS Statistics version 16 and thereafter significance was tested for all the

values by the variance ration (i.e. F-value) at the 0.05% level. Tukey's Multiple Range Test (TMRT) is used for understanding the significance among treatments at significance ($P < 0.05$).

4. Result and Discussion

4.1. Effect of shade dried leaves of *N. oleander* L. on germination percentage of Bermuda grass on 10th DAS

Seed germination is a very critical stage, especially under stress condition (Allelopathic stress). During germination, biochemical changes take place which provides the basic framework for subsequent growth and development. In this regard, figure 3 showed significant reduction in weed germination when treated with aqueous extract of *N. oleander*. Compared with control, the germination rate was decreased with increasing of extract concentrations. At lower concentration, germination percentage was insignificantly recorded but at higher level, germination percentage significantly decreased over the control. The highest inhibition (70%) effect was observed in the 20(g/l) extract treatment. This indicated that the inhibitory effect was due to some water soluble phytotoxins release from the leaf residue. Similar finding was mentioned by Rezaei and Khajedolin (2008) who said that extract of the two allelopath species significantly decreased the seed germination and germination index of *O.viciaefolia* L. The germination index can reveal the speed of germination, and a higher germination index indicates healthy condition of seed. (Yan and Sun, 2000). Delay in seed germination due to allelochemicals of donor plant which cause disturbance in cell division and cell elongation.

4.2. Effect of shade dried leaves of *N. oleander* L. on seedling growth and weed biomass of Bermuda grass on 15th DAS, 30th DAS and 45th DAS.

Different levels of aqueous extract of *N. oleander* had significant effects on seedling growth and weed biomass of bermuda grass. As shown in figure 4, the effect of *N. oleander* leaf aqueous extract on seedling growth and biomass had different responses against different treatments. Phytotoxicity of treatment was unevenly increased with the increasing of percentage of extract treatment. Result has also shown that root length of target plants is more influenced under aqueous extract compared to shoot length. This finding is supported by Alam et al (1997) who mentioned that, generally root faced more allelopathic effect than shoot due to the fact that, they were in direct contact with the allelochemicals which may not have been translocated rapidly to the shoot. Our finding showed that, seedling growth and weed biomass was reduced with increasing of extract concentration. Statistically pronounced significant effect was found at 20(g/l) treatment followed by 15% and 10% treatment respectively. Fig 4 (A) represented that the phytotoxic effect on root length was observed as 53%, 67% and 42% at 15th DAS, 30th DAS and 45th DAS respectively by 20(g/l) aqueous extract. Similar finding is reported by Tsang et al (2003) who mentioned that at lower aqueous concentration of *M.tanarius* caused significant reduction (upto 70%) in seedling growth of *Bidens pilosa*. Regarding root inhibition, Alam et al. 1997 mentioned that phenolics compounds in allelochemicals represent one of the largest groups which can significant inhibit root cell division.

Figure 4 (B) showed shoot inhibition by extract treatment of *N. oleander*. At 20(g/l) extract value, inhibition was 50%, 65% and 34% over the control at 15th DAS, 30th DAS and 45th DAS respectively. Similar finding observed by Datta and Bandyopadhyay (1986) who demonstrated that leaf and inflorescence extract of *Amaranths spinosus* L., *Chrysopogon aciculatum* Trin. and *Eupatorium odoratum* seriously affected the vegetative and reproductive phases of wheat and mustard plants. Our finding is also supported by Bisio. A. et al (2010) who mentioned that seedling growth of both *Papaver* and *Avena* was reduced by treatment with the various tested concentration of *Salvia* exudates.

Figure 5 (A) and (B) showed fresh and dry weights of bermuda grass was significantly decreased when treated with aqueous extract of *N. oleander* as compared to control (table 4). The maximum inhibition on fresh and dry weight was observed at 20(g/l) extract treatment followed by 15% and 10%. At 20(g/l) aqueous extract concentration fresh weight and dry weight was observed as 60%, 71%, 64% and 65%, 77%, 70% over the control at 15th DAS, 30th DAS and 45th DAS respectively. Similar finding was observed by Blaise and Tyagi (1996) reported that the fresh leaves and roots of *Eucalyptus* adversely affected the growth of wheat and maize than cow pea. Rajangam (1997) also reported that an aqueous extract of fresh leaves, shoots, and roots of *Heliotropium indicum* inhibited the dry matter of rice seedling.

4.3. Effect of shade dried leaves of *N. oleander* L. on chlorophyll and sugar content of Bermuda grass on 15th DAS, 30th DAS and 45th DAS.

Figure 6 shown the value of chlorophyll and sugar content of bermuda grass when treated with *N. oleander* with various concentrations at different time interval. All the data revealed the significant inhibition on chlorophyll and sugar content over the control. Fig 6.A showed that at 20(g/l) extract concentration, chlorophyll showed 47%, 66% and 52% inhibition over the control at 15th DAS, 30th DAS and 45th DAS respectively. Similar result was observed by Parvez et al (2003) who concluded that aqueous extract of *Tamarindus indica* has strong inhibitory effect on chlorophyll content of bermuda grass. Our result also expressed that, inhibition of chlorophyll content was extract concentration dependent. Our finding is also in collaboration of Sahid and Sugan (1993) who found that aqueous extract of *Lantana camara* has very strong ability to suppress the cotent of chlorophyll. Wubert et al. (1996) also reported that compounds extracted with methanol from the bulbs of *Gladiolus spp*. reduced the chlorophyll in the seedling of *Lapidium sativum*.

Fig 6.B shown the sugar value of bermuda grass treated with *N. oleander*. At 20(g/l) extract concentration, sugar content was drastically reduced followed by 15% and 10%. Since our result stated strong inhibition on chlorophyll content therefore reduction in sugar content was also so obvious. At 20(g/l) extract concentration, sugar content was recorded as 67%, 76% and 64% reduction over the control at 15th DAS, 30th DAS and 45th DAS respectively. Our finding is supported by Riti (2011) who found the dry leaf residue of *Hyptis suaveolens* L. significantly reduced the amount of the total sugar observed in leaf and stem of *Parthenium* over by various treatment. The reduction in sucrose in might be due to inhibition of sucrose synthesis from fructose and glucose in a reaction catalyzed by sucrose synthetase. Our result is coincide by Prasad et al (1999) who stated that application of Aerial and root biomass of *Rhamus virgattus* trees significantly decreased the sugar and starch content in all test crops as compared to control.

4.4. Effect of shade dried leaves of *N. oleander* L. on amino acid and protein content of Bermuda grass on 15th DAS, 30th DAS and 45th DAS.

Figure 7 showed the effect of *N. oleander* on amino acid and protein content of bermuda grass. Both the biochemical parameter i.e. amino acid and protein showed significant inhibitory effect over the control when treated with aqueous extract of *N. oleander* L. they also showed significant inhibition on higher concentration of aqueous extract. At 20(g/l) extract concentration, inhibition on amino acid and protein content was 54%, 59%, 46% and 42%, 63%, 59% over the control at 15th DAS, 30th DAS and 45th DAS respectively. Similar findings was observed by Bhagvathy and Xavier (2007) who mentioned that the sorghum plants treated with high dose application of *Eucalyptus* leaf extracts decreased the amino acid protein content of sorghum. Similar finding was observed by Prasad et al., (1999) who reported that the aerial and shoot biomass of *Rhamnus virgatus* tree has strong ability to suppress the protein content in all the test crops (*Triticum aestivum, Eleusine coracana* and *Leans culinaris*).

Table 1. Preliminary Phytochemical analysis of Exoecaria agallocha L. (Suganya et al., 2012)

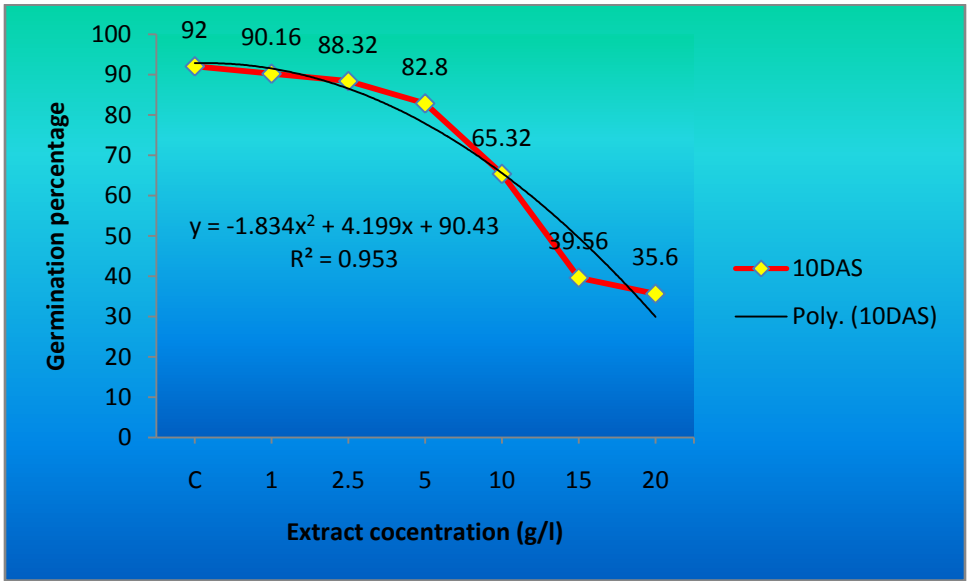
Phytochemical	Organic solvent					
	Pet. Ether	n-Hexane	Ethanol	Ethyl acetate	Chloroform	Methanol
Alkaloid	-	-	+	+	+	-
Cardic glycosides	+	-	+	+	+	+
Flavonoid	-	+	-	-	+	-
Glycosides	+	+	+	+	-	-
Saponins	-	-	-	-	+	-
Saponins glycosides	+	-	+	-	+	+
Steroids	-	+	-	+	-	+
Tannins	+	+	+	-	+	+
Volatile oil	-	+	-	+	-	+

(+) symbol represent availability of Phytochemical and (-) symbol represents absence of Phytochemical

Table 2. Physiological properties of soil of Experimental pots (pre- treatment and post – treatment)

Parameters	Mature leave extract treatment on 30 DAS	
	Bermuda grass	
	Pre-treatment	Post- treatment
Texture	Light clay	Light clay
Sand (%)	64.7	56.9
Silt (%)	31.6	28.3
Clay (%)	23.8	24.7
pH	7.14	6.99
EC(ds/m)	1.25	.52
Organic carbon (%)	.25	.10
Total nitrogen (%)	.89	.48
Available P (ppm)	.20	.10

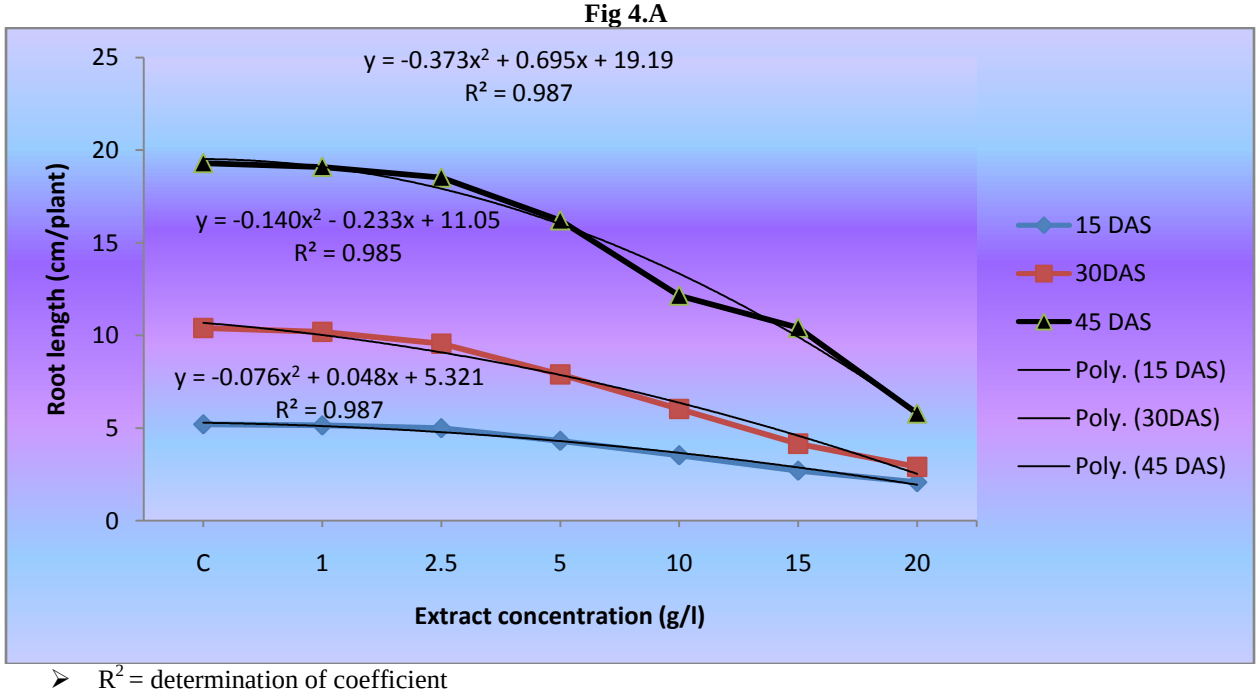
Fig.3. Effect of aqueous extract of *N. oleander* L. leaves on germination percentage of Bermuda grass on 10 DAS.



- R^2 = determination of coefficient
- y= value of germination percentage with concern extract treatment.

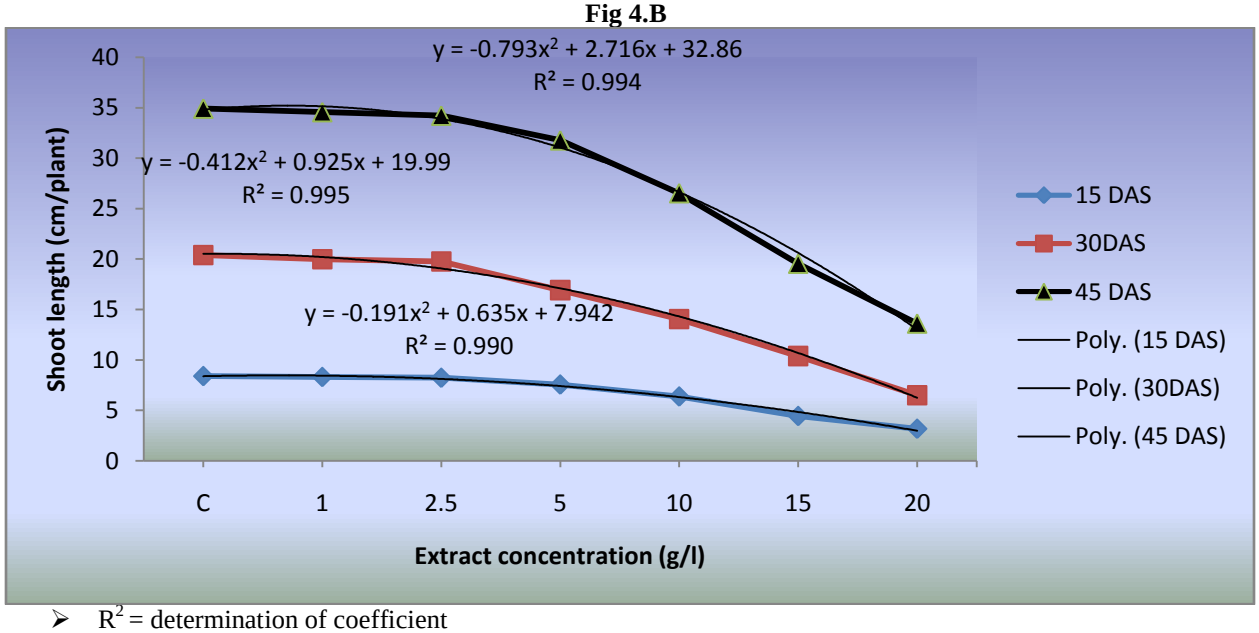
Fig 4. Effect of aqueous extract of *N. oleander* L. leaves on seedling length (A: root length(cm/plant), and B: shoot length (cm/plant) of bermuda grass on 15, 30 and 45 DAS.

Fig 4.A



- R^2 = determination of coefficient
- y= value of root length with concern extract treatment.

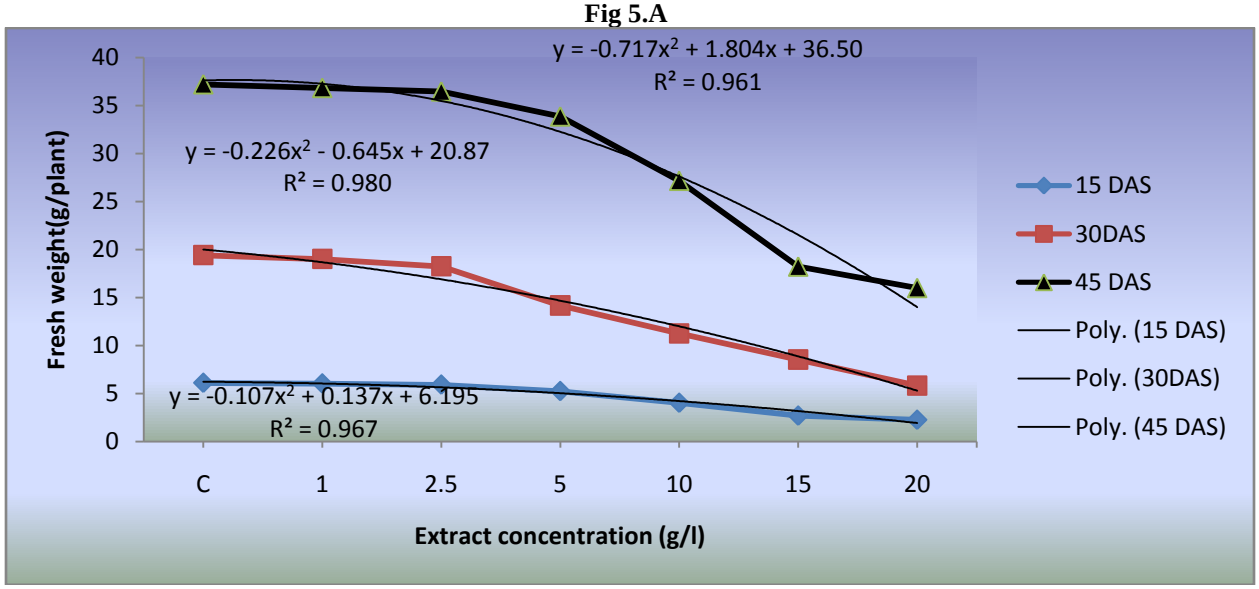
Fig 4.B



- R^2 = determination of coefficient
- **y= value of shoot length with concern extract treatment.**

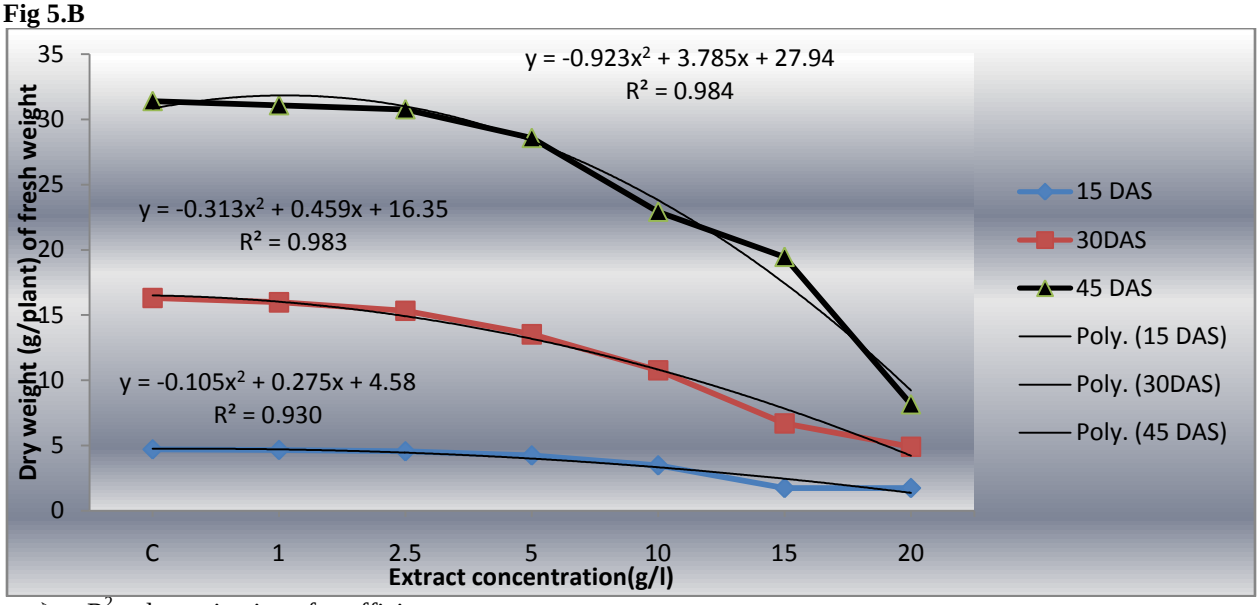
Fig 5. Effect of aqueous extract of *N. oleander* L. leaves on weed biomass A: fresh weight (g/plant) and B: dry weight (g/plant) of bermuda grass on 15, 30 and 45 DAS.

Fig 5.A



- R^2 = determination of coefficient
- y= value of fresh weight with concern extract treatment.

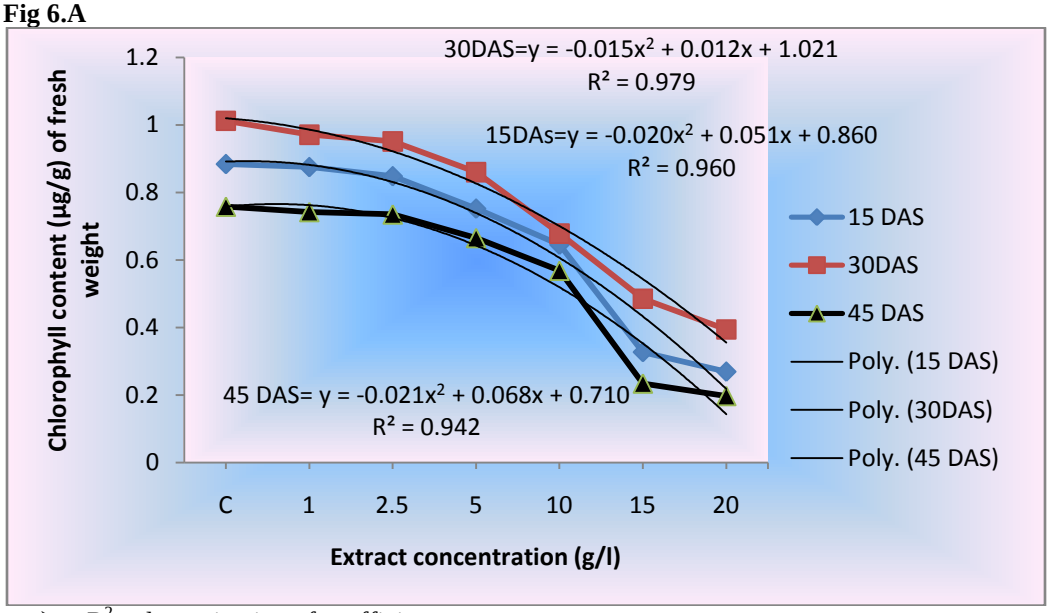
Fig 5.B



- R^2 = determination of coefficient
- y= value of dry weight with concern extract treatment.

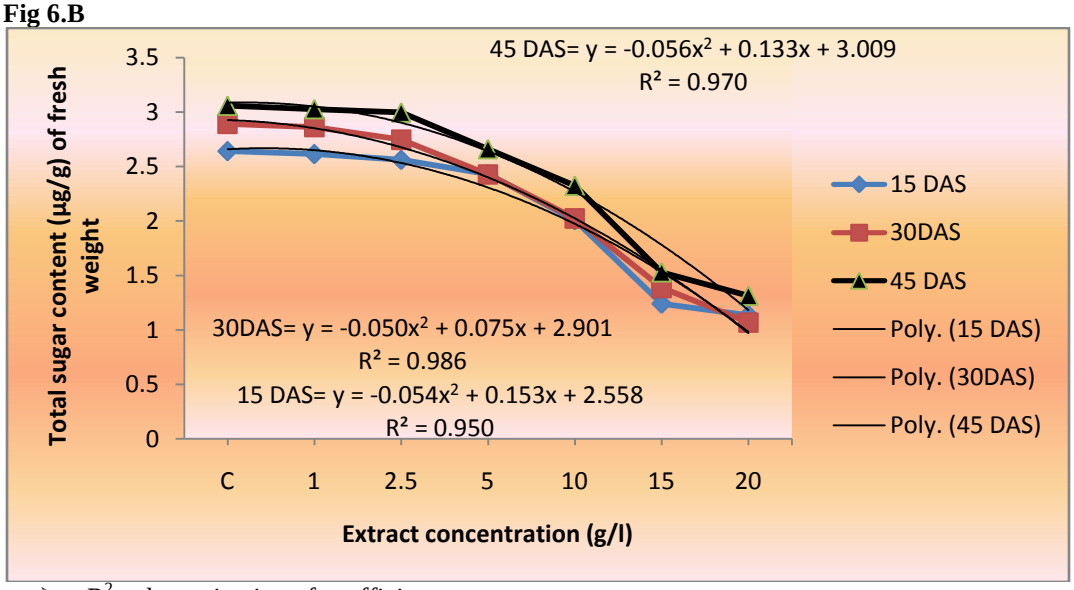
Fig 6. Effect of aqueous extract of *N. oleander* L. leaves on (A) total chlorophyll content (µg/g) and (B) total sugar content (µg/g) of bermuda grass on 15, 30 and 45 DAS.

Fig 6.A



- R^2 = determination of coefficient
- y= value of total chlorophyll content with concern extract treatment.

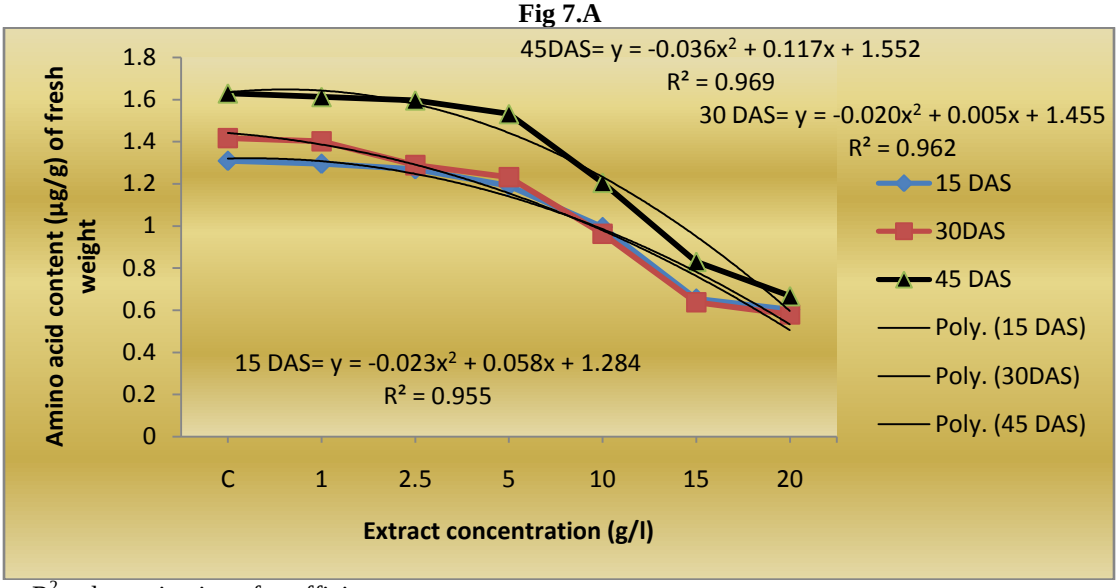
Fig 6.B



- R^2 = determination of coefficient
- y= value of total sugar content with concern extract treatment.

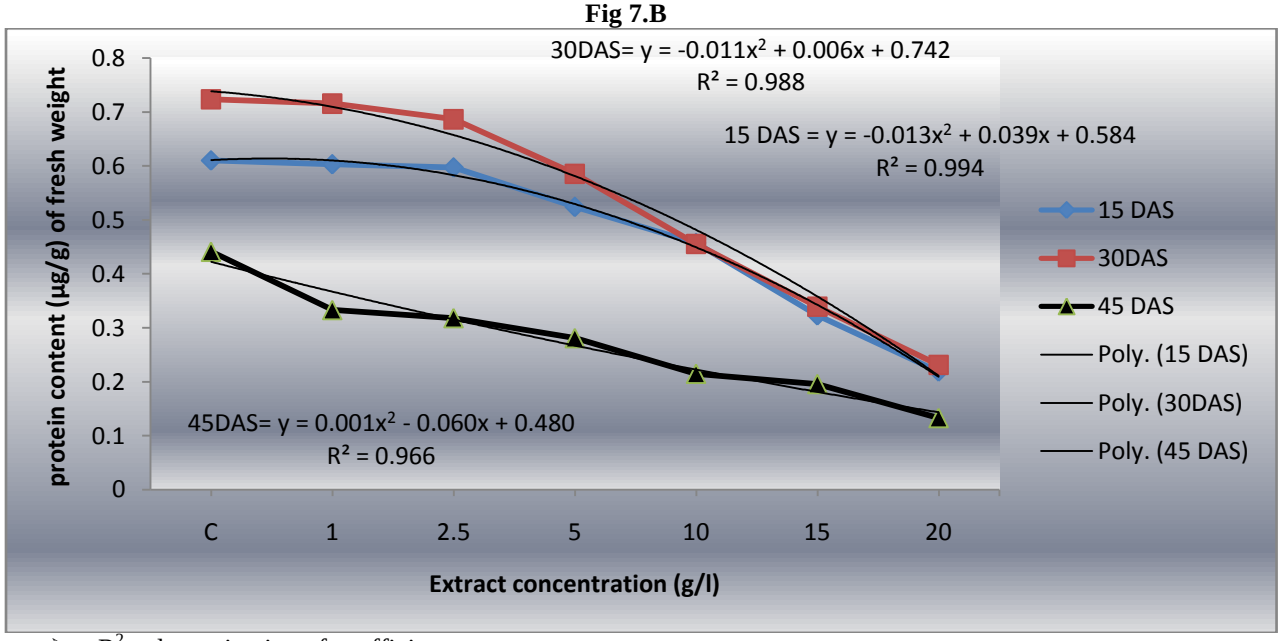
Fig 7. Effect of aqueous extract of *N. oleander* L. leaves on (A) amino acid content (µg/g) and (B) protein content (µg/g) of bermuda grass on 15, 30 and 45 DAS.

Fig 7.A



- R^2 = determination of coefficient
- y= value of amino acid content with concern extract treatment.

Fig 7.B



- R^2 = determination of coefficient
- y= value of protein content with concern extract treatment.

Conclusion

From the present study, overall it may be concluded that *N. oleander* has potential phytotoxic effect to made adverse effect on growth and development of bermuda grass. Result also revealed that at mid of treatment (30^{th} DAS), phytotoxic effect was at maximum level. Therefore it can be concluded that, *N. oleander* can be used as a source of Bioherbicide to control bermuda grass.

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