Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi
Iğdır University Journal of the Institute of Science and Technology

Allelopathic Effects of Flower Extract of Oleander (*Nerium oleander*) on the Germination of Seed and Seedling Growth of *Lolium multiflorum*

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ABSTRACT: An experiment on the allelopathic effects of *Nerium oleander* on germination and growth of Italian ryegrass (*Lolium multiflorum* Lam) was conducted at the Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Field Crops Laboratory during January 2017. The experiment was laid out in completely randomized design (CRD) with three replications. Four concentrations of oleander flower extract i.e control, 10, 20 & 40 g L^{-1} were used in this experiment. The experiment was performed in climatic cabinet having 20 °C temperature. The seeds were kept for 14 days and then different attributes were studied such as germination %, seedling length, radicle length, plumule length, seedling fresh and dry weight and seed vigor index. All the studied parameters were significantly affected by oleander flower extract concentrations. Results showed that maximum germination %, seedling length, radicle length, plumule length, seedling fresh and dry weight and highest seed vigor index was recorded in control treatment. While minimum values for all the above mentioned parameters were observed at 40 g L^{-1} of extract concentration. It indicated that *Nerium oleander* flower extract suppress growth of Italian ryegrass and thus can be used for the control of Italian ryegrass in the crops. However, all other factors need to be studied for sustainability.

Keywords: Allelopathy, Germination, *Lolium multiflorum, Nerium oleander* extract.

Zakkum (*Nerium oleander*) Çiçek Ekstraktının *Lolium multiflorum*'un Tohum Çimlenmesi ve Fide Gelişimi Üzerine Allelopatik Etkileri

ÖZET: Tek yıllık çimin (*Lolium multiflorum* Lam.) çimlenme ve gelişimi üzerine zakkum (*Nerium oleander*) çiçeklerinin allelopatik etkisini belirlemek üzere yapılan bu çalışma, Kahramanmaraş Sütçü İmam Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü Laboratuvarında 2017 Ocak ayında yürütülmüştür. Deneme tesadüf parselleri deneme desenine göre 3 tekerrürlü olarak kurulmuştur. Çalışmada 4 farklı zakkum çiçeği ekstraktı (kontrol, 10, 20 ve 40 g/lt) kullanılmıştır. Araştırma iklimlendirme kabininde 20 °C'de gerçekleştirilmiştir. Tohumlar 14 gün süre ile çimlendirmeye tabi tutulmuş daha sonra; çimlenme oranı, fide uzunluğu, radikula uzunluğu, plumula uzunluğu, fide yaş ve kuru ağırlığı ve vigor indeks gibi temel belirleyici çimlenme ve fide gelişimi unsurları ölçülmüştür. İncelenen tüm özellikler farklı konsantrasyonlardaki zakkum çiçek ekstraktlarından önemli derecede etkilenmiştir. En yüksek çimlenme oranı, fide uzunluğu, radikula uzunluğu, plumula uzunluğu, fide yaş ve kuru ağırlığı ve en yüksek vigor indeks değerleri kontrol uygulamasında elde edilmiştir. Yine aynı özellikler için en düşük değerler 40 g/lt konsantrasyonunda elde edilmiştir. Zakkum çiçek ekstraktının tek yıllık çimin gelişimini baskı altına aldığı buna bağlı olarak tarım alanlarında tek yıllık çimin kontrol altına alınmasında kullanılabileceği görülmektedir. Ayrıca sürdürülebilirlik açısından bu alanda diğer tüm faktörlerle ilgili çalışmalar da yapılmalıdır.

Anahtar Kelimeler: Allelopati, çimlenme, *Lolium multiflorum, Nerium oleander* ekstraktı.

Cilt/*Volume:* 8, Sayı/*Issue:* 1, Sayfa/*pp:* 309-317, 2018
ISSN: 2146-0574, e-ISSN: 2536-4618 DOI: 10.21597/jist.407890

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Geliş tarihi / *Received:* 04.10.2017
Kabul tarihi / *Accepted:* 14.11.2017

INTRODUCTION

The term allelopathy refers to the interaction among plant species and or with microorganisms. This interaction among plant species may be negative or positive. Rice (1974) defined that the harmful effect of plants on each other either directly or indirectly through release of chemical compounds are called allelopathy. Even though plant not always affect each other negatively but negative response can easily be observed (An et al., 1998). Dakshini et al. (1999) used the word allelochemical for the compounds that are released by the plants in the environment. The phenomenon present in the nature from very long time but significant work on allelopathy was started in the past few decades (Weston, 2005). Thus, exploring the allelopathy of different plants in all the regions of the word is necessary to explore the possible uses of plant based chemicals. Almost half of the population lives in rural area of turkey and most of them employed in agriculture. So, that's way agriculture has its importance in Turkish community and have a good impact on human life of rural people of turkey economically and socially. Agriculture contributes more than 15% of gross national products. However processed crops are not included in it (TKIB, 1997). In the early 19th century, control of pests and weeds were started through chemicals instead of using biological methods of pest control. Although integrated pest management techniques were also introduced in 1970. But still there is a large number of cultivated areas, where herbicides are used for weed control (Uludag et al., 2006). Use of herbicides for weed control create various problems such as increase resistance of weed against particular herbicide, chemical residue of the herbicide remains in the soil and it also have hazardous effects on the environment. So, to avoid all the concerns related to the use of herbicide an alternative farming system is necessary which is based on the utilization of natural substances for weed control (Waller, 2004; Khan et al., 2016). Allelopathy can be used is a tool for weed control. It may reduce the use of synthetic herbicides. It can help in reduction of soil and environmental pollution (Chon et al., 2002; Afridi and Khan, 2015). Various scientists used different plants to suppress the growth of weeds such as number of shoots and tuber formation of *Cyprus esculantus* can be reduced by the application of shoot residues of *Chenpodium album* (Alam et al., 2001). *Nerium oleander* is an evergreen shrub. It is cultivated in the subtropical region of the world. It can grow on marginal land having poor soil and has the ability to tolerate drought conditions. It is used as an ornamental plant in various landscape designs in Turkey. Therefore, it can be found in various parts of the country in abundant. It is known to have allelopathic effect on various weeds such as *Lolium multiflorum, Alopecurus myosuroides, Arundo donax* and *Lactuca sativa* (Uygur and İskenderoğlu, 1997). The present study was designed to investigate the allelopathic effects of flower extract of oleander (*Nerium oleander*) on *Lolium multiflorum* for possible use in field conditions. Such studies might be helpful for formulating plant based chemicals for control of other weeds in natural ecosystems.

MATERIAL AND METHODS

The experiment was conducted in the climatic cabinet of the Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Field Crops Laboratory during January 2017. The experiment was laid out in completely randomized design (CRD) with three replications. Two types (red and white) of fresh flowers were collected from *Nerium oleander*. These flowers were dried under room temperature. The dried flowers were then weighed 10, 20 and 40 g, respectively. While for control treatment distilled water was used. These weighed dry flowers were put in the water and the final volume was adjusted to one liter for each 10, 20, 40 g. All the solutions were kept at room temperature for 24 hours and then filtered with Whatman 1 filter paper. The resulted extracts were stored under room condition in the lab. Then 100 seeds of L. *multiflorum* were collected and sterilized with 5% bleach (NaClO) for 5 minutes before sowing. 25 seeds were allocated to each treatment including a control. Petri dishes were prepared according to the treatments contain seeds, were placed in the air conditioning cabinet at 20 ^{o}C in light environment and it was leaved to germinate. It was kept for 14 days, irrigated accordingly and then the following attributes were studied: Germination rate, radicle length, plumula length, seedling length, seedling fresh weight, and seedling dry weight and seed vigor index. Germination percentage was recorded by counting number of germinated seeds and then it was divided by total number of seeds multiply by 100. For recording seedling length, seedlings were measured

with measuring tape. Similarly, for radicle and plumula length, both were separated and then measured with measuring tape. Then both the parts were weighed to determine fresh biomass. These plant parts were then kept at 78 ºC for 24 hours and then seedling dry biomass was obtained. For vigor index, seedling length was multiply with germination percentage. The data regarding in the study were subjected to analysis of variance using the SAS 9.3 statistical program (SAS 9.3). Averages compared to Duncan's Multiple Range (DMR) test (Steel and Torrie, 1980).

RESULT AND DISCUSSION

According to the statistical analysis all the study parameters were significantly affected by various concentrations of *Nerium oleander*.

Germination %

In this study, while the highest germination rate (93.33%) were obtained in control treatment. However, with increase in concentration of flower extract, a decrease in germination percentage was noted. Minimum germination was recorded in 40 g L^{-1} extract of *Nerium,* applied to ryegrass seeds (Fig. 1). This decrease in germination may be due to the inhibitory effect of high concentration of Nerium extract. Uludag et al. (2005) used various brassica species to find out its allelopathic effects.

They concluded from their studies that it is effective against Johnson grass and can be used for reducing its population. Our results are in line with those reported by Iskenderoglu (1995) who also stated that Nerium extract can be used to reduce the germination percentage of *Lolium multiflorum*. Similarly Ashrafi et al., (2008) also concluded that shoot extract of Neem (*Azadirachta indica*) significantly reduced germination of various weed species. It can be concluded that flower extracts of *Nerium oleander* can be used against *L. multiflorum*.

This inhibitory effect might be true for other weed species as well. Therefore in light of the present studies, it can be concluded that the presence of *Nerium oleander* in area can affect the germination of surrounding plant species.

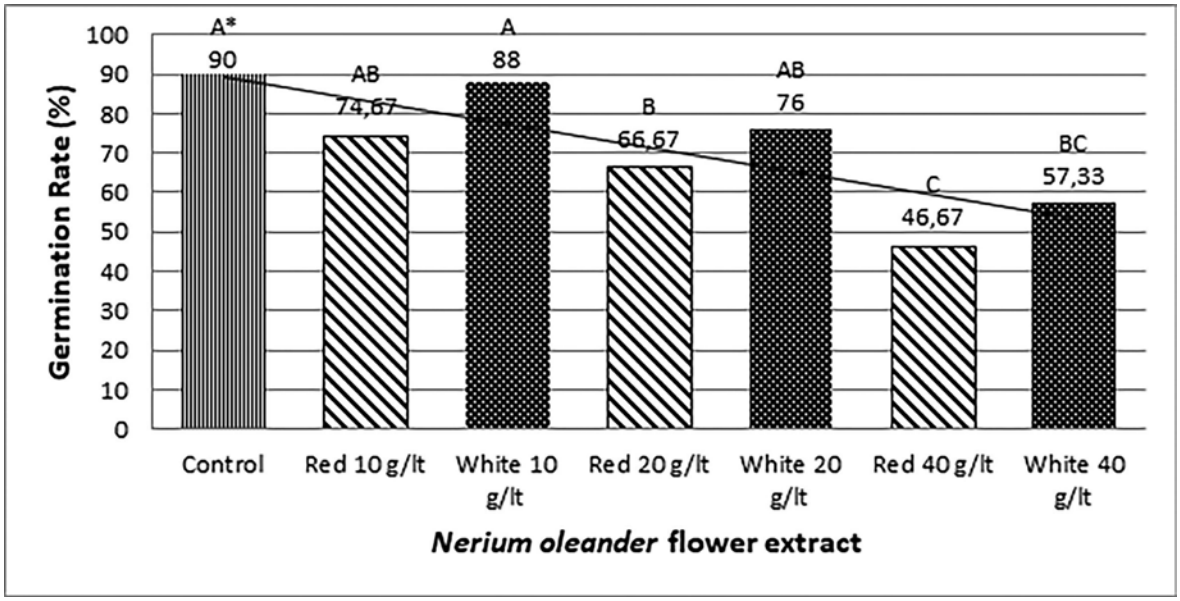

Figure 1: Allelopathic effects of flower extract of Oleander (*Nerium oleander*) on the germination % of *Lolium multiflorum*.

Radicle length (cm)

Radicle length of ryegrass was also significantly decreased with increase in Nerium extract concentration (Fig. 2). Maximum radicle length was recorded in control treatments which was decreased with increase in concentration. This decrease in radicle length might be due to inhibitory effect of flower extract of Nerium on the growth of Italian ryegrass. Karaaltin et al. (2004) reported that *Nerium oleander* extract significantly reduced radicle length. They further

stated that this decrease in the radicle growth is due to the allelopathic effect of Nerium extract. Another study suggested that essential oil extracted from the flower and leaves of various plants such as *Salvia officinalis, Artemisia vulgaris* and *Ocimum basilicum* caused phytotoxicity. These extract inversely affected germination of seed and seedling growth of *Lolium perenne* (Onen, 2003). Radicle length of *Medicago polymorpha* L. was negatively affected by by the application of various plant extract used for its allelopathic effects (Algandaby and El-Darier 2016). They also reported that radicle is more effected then plumule. Similarly Ashrafi et al., 2008 found that radicle growth is more sensitive to allelochemical application then plumule. Radicle is an important growth related variable and can significantly affect the overall growth of the plants. In the present studies, it was observed that radicle length of ryegrass was negatively affected. Therefore the *N. oleander* extract can be successfully used for the suppression of *L. multiflorum*. In addition to flower, it is suggested that all other parts of *N. oleander* may be used against all weeds for possible management. This may decrease the total herbicide use in an agro-ecosystem.

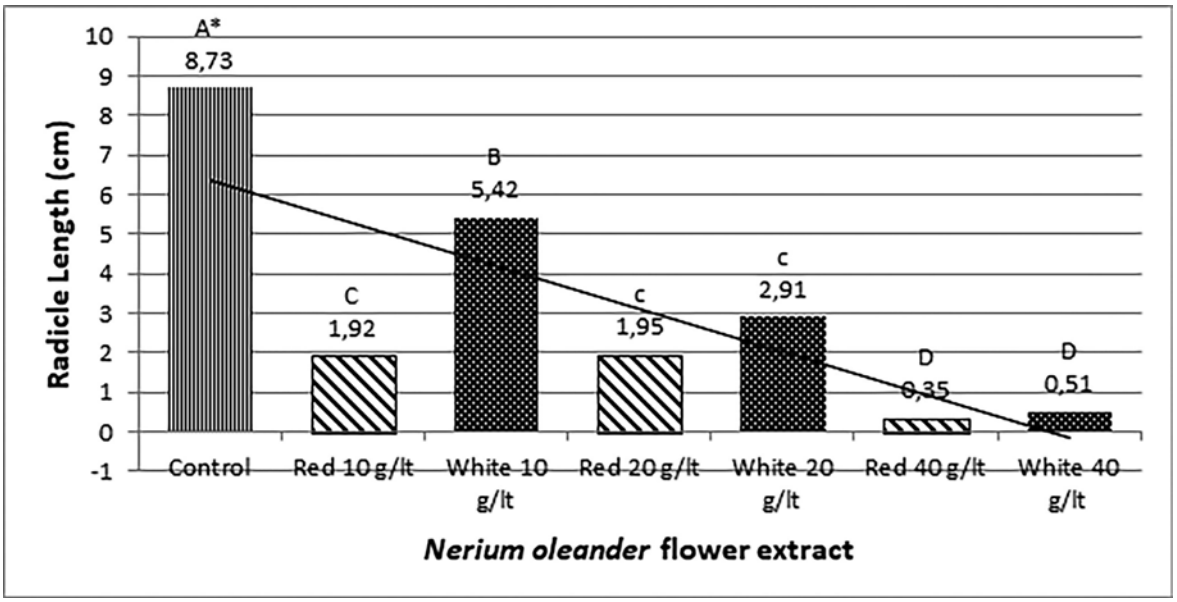

Figure 2: Allelopathic effects of flower extract of Oleander (*Nerium oleander*) on radicle length of *Lolium multiflorum*.

Plumule Length (cm)

According to the results of the experiment, plumule length also negatively affected by the application of Nerium flower extract (Fig. 3). It is clear from the data shown in figure 3 that increase in extract concentration decreased the length of plumule. This decrease in plumule length is attributed to the inhibitory effect of Nerium flower extract at higher concentration. This shows that *N. oleander* flowers contain certain phytochemicals that retard the growth of ryegrass. Therefore, bioassay techniques are suggested to identify those chemicals and isolate for practical application for weed control. In a similar studies, Karaaltin et al. (2004) tested *Nerium oleander* extracts taken from various parts of the plant such as stem, bud, root and leaf extracts for its allelopathic effects on wheat and beans. They suggested that seeds of both plant species were negatively affected by the extract of Nerium. However, the growth of plumule and radicle were more prominently affected. Ashrafi et al., (2008) also reported decrease in the plumule length in wild barley due to application of sunflower extract. Reduction in plumule length of *Medicago polymorpha* L. was found when treated with plant extracts of *Achillea santolina*, *Artemisia monosperma*, *Pituranthus tortuosus* and *Thymus capitatus* which is due to the allelopathic effects of these plant extracts on it (Algandaby and El-Darier (2016). In light of these studies, it is suggested that presence of *N. oleander* in an area can result in the reduction of growth of ryegrass and plant species. Therefore exploration of this plant for their allelopathic effects is needed to be used for the welfare of human beings.

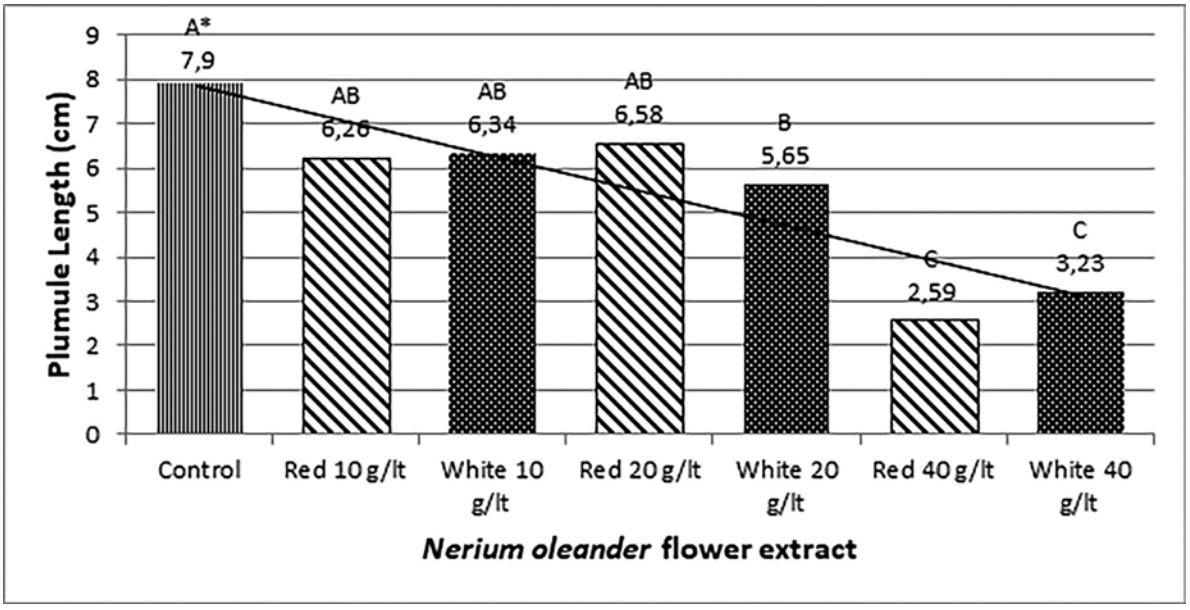

Figure 3: Allelopathic effects of flower extract of Oleander (*Nerium oleander*) on plumule length of *Lolium multiflorum*.

Seedling Length (cm)

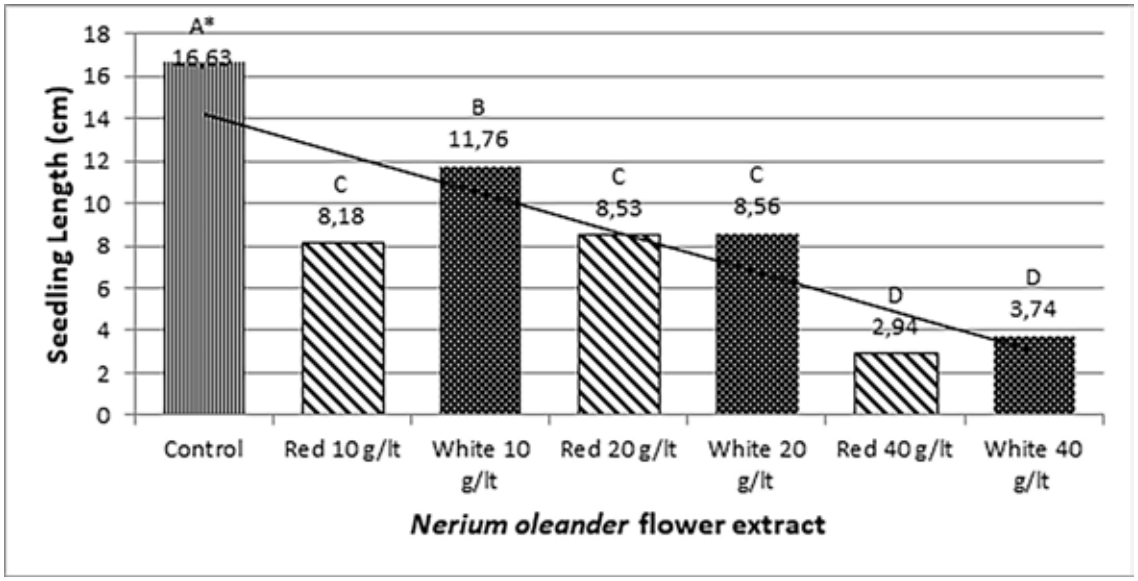

Figure 4: Allelopathic effects of flower extract of Oleander (*Nerium oleander*) on seedling length of *Lolium multiflorum*.

Seedling length of ryegrass was studied under various flower extract concentrations of *N. oleander* and was significantly decreased. Results (Fig. 4) shows that increase in the extract concentration of *N. oleander* negatively affected seedling growth of ryegrass. Study of Macias et al., (2004) suggested that allelochemicals that contain phenolic compounds stimulate seedling growth and development at low concentrations. While increase in its concentration inhibits its growth. This decrease in the seedling growth may be due to the presence of Rutin and Querecetin which play a vital role in suppressing growth of seedling and act as allelochemicals (Kuamr et al., 2011). Reduction in seedling growth of wild barley was reported by Ashrafi et al., 2008 when treated with leaf extract of sun flower. These results depicted that *N. oleander* flower extracts

can be used for suppressing ryegrass and other weeds. Therefore more studies are needed to use different concentrations of *N. oleander* against all other weeds. Such studies will be of great interest for the ecologists, agriculturists and plant scientists. Because the presence of phytochemicals can cause ecological changes in an agro-ecosystems of an area. As seedling length is an important variable for the growth of plants therefore any decrease in seedling length will greatly affect the overall production of that plant.

Seedling Fresh Biomass (g)

Fresh biomass of seedling shows the overall growth of any plant species. Seedling fresh biomass of Italian ryegrass was subjected to various concentrations of *Nerium oleander* extracts and the effect was found significant (Fig. 5). A negative correlation was noted in fresh biomass of seedling with *N. oleander* extract concentration. With increase in concentration of *N. oleander,* fresh biomass was decreased. This decrease might be due to the inhibitory effect of *Nerium* extract on the growth of Italian ryegrass.

Decreased in growth and development of plant morphological attributes affect fresh and dry biomass. Similar results were recorded by Algandaby and El-Darier (2016), who concluded that decrease in fresh biomass was found in *Medicago polymorpha* L. seedling when treated with plant extracts of *Achillea santolina*, *Artemisia monosperma*, *Pituranthus tortuosus* and *Thymus capitatus* which have allelopathic effects on it. A significant decrease in seedling weight was recorded in the cucumber seedling which was treated with high concentrations of plant extract having allelopathic effect (Terzi et al., 2003).

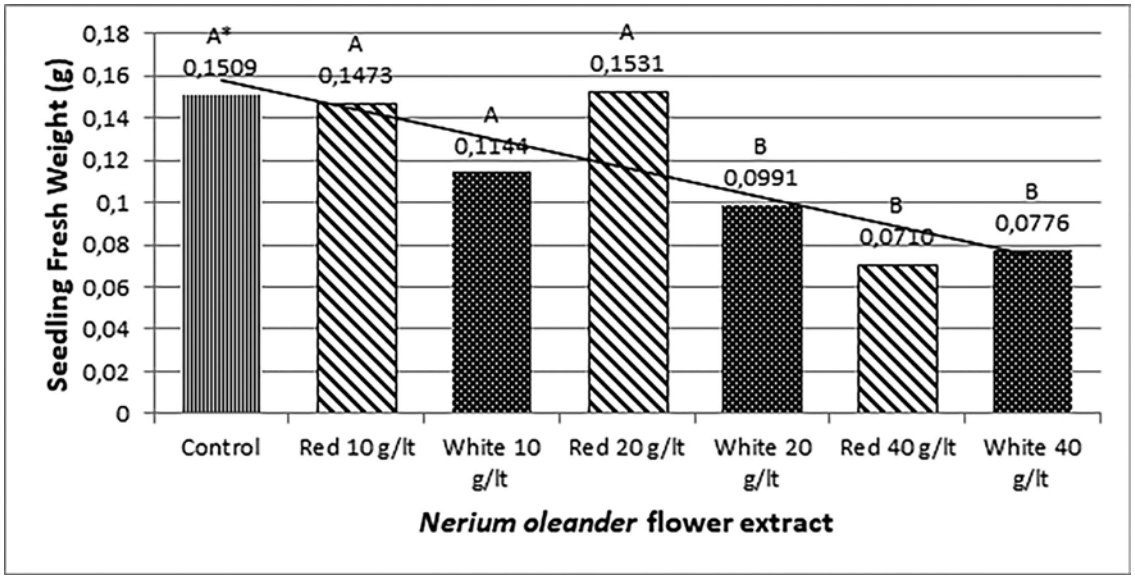

Figure 5: Allelopathic effects of flower extract of Oleander (*Nerium oleander*) on seedling fresh weight of *Lolium multiflorum*.

Seedling dry Biomass (g)

Dry biomass of any plant is an indicator of its photosynthesis and competitive ability. The plants that attain bigger vegetative growth can outcompete other plants. Figure 6 shows that dry biomass of seedling as also significantly affected by different concentrations of both type of oleander flowers used in the experiment. Decrease in dry biomass was recorded with increase in concentrations of the extract. This decrease is attributed to the inhibitory effect of oleander extract on growth parameters of Italian ray grass. Decrease in morphogenetic potential due to restricted growth may lead to reduce dry biomass accumulation. Similar results were recorded by Algandaby and El-Darier (2016). They suggested that dry biomass of *Medicago polymorpha* L. seedling was significantly decreased

when treated with plant extracts of *Achillea santolina*, *Artemisia monosperma*, *Pituranthus tortuosus* and *Thymus capitatus* which have allelopathic effects on it. In light of the present studies, it is suggested that *N. oleander* flowers contain certain chemicals that are harmful for the other plants. Therefore, the water extracts of the flowers can be used to suppress the ryegrass. However, more studies are needed to investigate the effect of various concentrations of *N. oleander* against ryegrass at various stages. Because it has been noted that behaviour of the allelochemicals is changed with the change in different environmental factors.

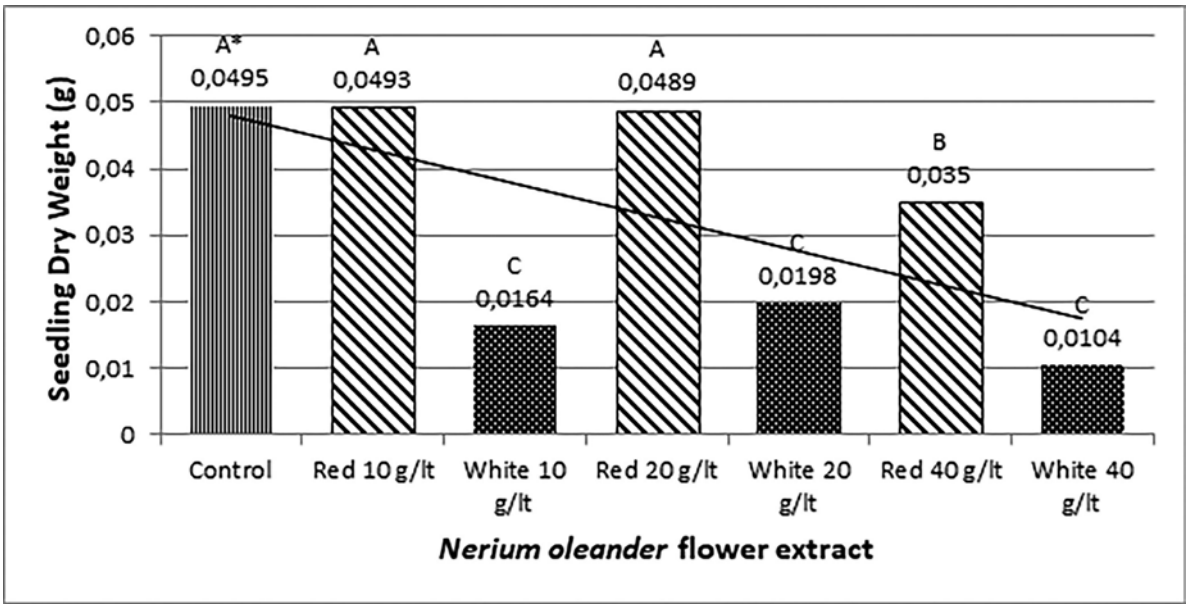

Figure 6: Allelopathic Effects of Flower Extract of Oleander (*Nerium oleander*) on seedling dry weight of *Lolium multiflorum*.

Vigor Index

Seed vigor index is an important variable that determine the competitive ability of a plant species. It was found significant for ryegrass under various concentrations of flower extracts of *N. oleander*. Seed vigor index of Italian ryegrass was reduced in extract concentration of *N. oleander* as shown in figure 7. Overall flower extract of *N. oleander* at a concentration of 40 g L^{-1} was found more effective against Italian ryegrass. In analogous studies, Ashrafi et al., 2008 observed decrease in various attributes of wild barley when treated with extract of plant having allelopathic properties. In addition, Algandaby and El-Darier (2016) also found significant reduction in the population of *Medicago polymorpha* L. when treated with various allelopathic plants. Onen, (2003) also conducted a study on the allelopathic effect of *Salvia officinalis*, *Artemisia vulgaris* and *Ocimum basilicum* on *Lolium perenne*. He observed that all the extracts significantly reduced the morphological attributes. Overall our studies showed that flower extracts of *N. oleander* has allelopathic effects against Italian ryegrass. All germination and seedling growth related variables of Italian ryegrass is negatively affected. Therefore this plant needs to be explored for further investigations to be used against other weed species.

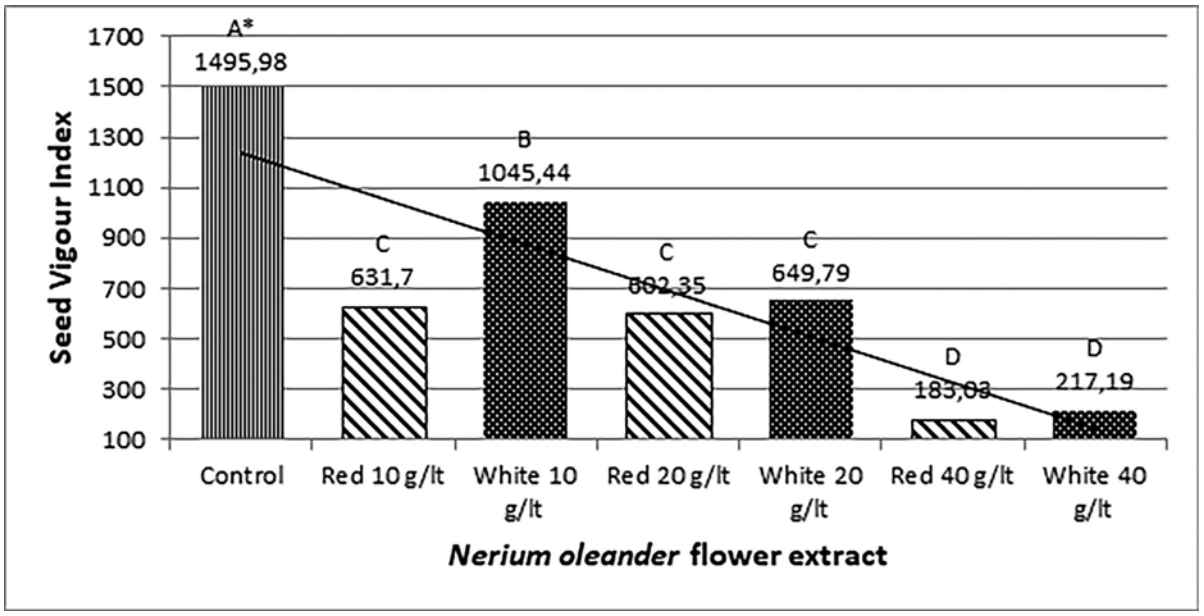

Figure 7: Allelopathic Effects of Flower Extract of Oleander (*Nerium oleander*) on the seed vigour index of *Lolium multiflorum*.

CONCLUSION

Overall application of flower extracts of *Nerium oleander* at various concentrations suppress germination and growth related parameters of Italian ryegrass. So, it can be used to reduce Italian ryegrass population in a given environment. Further research is needed to investigate its effects against various weed species.

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