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Full Length Article

Neem (*Azadirachta indica*) Oil Affects Morning Glory (*Ipomoea purpurea*) Seedling Development

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Abstract

The weed *Ipomoea purpurea* L. (Convolvulaceae), known generically as morning glory, is an important invasive plant introduced in Brazil, which not only causes significant losses in annual crops, but also makes harvesting difficult and already presents herbicide-tolerant biotypes in the country. In this context, alternative and sustainable ways of managing this plant are sought. The objective of this work was to evaluate the effect of neem oil on morning glory seeds and seedlings. The experiment was carried out in a completely randomized design, with six treatments composed of different oil dilutions, applied before and after germination, in addition to the control. Based on the experiment, it was observed that neem oil delayed seed germination by up to 1.4 days, although without significance. On the other hand, seedling fresh weight was severely affected by application after sowing, leading to a symptom of leaf blight. Morning glory seedling survival followed the same pattern, where there was a significant reduction by the application of neem oil only after sowing. It was concluded that neem oil has a phytotoxic effect on *I. purpurea* seedlings, reducing their survival and fresh weight when applied after germination, as well as influencing (delaying) seed emergence when applied to the soil. The study of the effect of lower concentrations, close to economically viable and practiced in the field, should be carried out in the future. © 2022 Friends Science Publishers

Keywords: Allelopathy; Alternative management; Phytotoxicity; Vegetable oils; Seed germination; Seedling survival

Introduction

According to the broadest definition by the FAO (2010), a pest is any species, race or biotype of plant, animal or pathogen that is harmful to plants or plant products, and thus weeds are included as important agricultural pests. Morning glory (*Ipomoea purpurea* L.) is a weed belonging to the Convolvulaceae family. It is an annual plant with a climbing habit and native to Mexico (Fang *et al.* 2013); that is, it is an exotic pest. It was introduced in Brazilian territory, where it is already widely distributed (CABI 2021). It is an important invasive species for the country, as it has asynchronous germination, remaining viable in soil seed banks for long periods (Jha *et al.* 2015) and harms mechanized harvesting, becoming entangled in plants and jamming machines (Piccinini *et al.* 2018). In addition, some biotypes in Brazil have already developed herbicide tolerance (Pazuch *et al.* 2017) and they serve as a reservoir for plant pathogens such as bacteria *Xylella fastidiosa* Wells (Wistrom and Purcell 2005) and insects (Simms and Rausher 1989). In soybeans, for example, losses of 27 to 45% have been reported (Piccinini *et al.* 2016). Such factors should be considered a warning for the future difficulty of managing this weed plant in Brazil.

According to the electronic platform for phytosanitary pesticides in Brazil (Agrofit 2021), there are approximately 258 products for the chemical control of morning glory. As mentioned, glyphosate-tolerant biotypes have already been reported in the country (Pazuch *et al.* 2017), making it necessary to search for alternative sources of pest management.

Neem (*Azadirachta indica* A. Juss) is a tree species that belongs to the Meliaceae family and has India as its center of origin (Alzohairy 2016). Neem oil, extracted from its seeds, has numerous uses and is widely used to protect plants against insects (Campos *et al.* 2016), although its use in weed management is not common. The product is natural, and it does not affect mammals or the environment (Isman *et al.* 1990), although it has several bioactive substances such as azadirachtin and meliantrol (Campos *et al.* 2016). According to Kato-Noguchi *et al.* (2014) nimbolide B and nimbic acid are the main substances with allelopathic properties present in this plant.

The influence of neem leaf extract or its residues incorporated into the soil has been studied in cultivated plants or in weeds (Xuan *et al.* 2004; Ashrafi *et al.* 2008; Ogundare *et al.* 2016), as well as the effect of neem oil on seed germination and plant development. Souza Filho *et al.* (2009) evaluated the effect of this oil on plants such as "touch-me-not" (*Mimosa pudica* Kunth.) and sicklepod (*Senna obtusifolia* L.). Some studies have also reported the phytotoxic effect of the oil (Pinheiro *et al.* 2009). In addition to this report, we have already evaluated the *in vitro* effect of neem oil on morning glory seed germination (Andrade and Marques 2021). Given the above, the aim of this study was to evaluate the allelopathic effect of neem oil on the development of *I. purpurea* seedlings.

Materials and Methods

Test location

The work was carried out in Planaltina, Brasília – DF, Brazil (15.58ºS, 47.73ºW), constituted by the Cerrado biome, during the month of July 2021. According to Köppen's classification, the municipality of Planaltina-DF has a seasonal tropical climate of megathermal savannah, with average annual precipitation of 1,400 mm, and the average temperature of the coldest month is above 18ºC. Rainfall is concentrated between the months of October and March, with a dry period from April to September, and an average minimum temperature of 15.9ºC, maximum of 26.4ºC (Cardoso *et al.* 2014).

Obtaining neem oil and morning glory seeds

The neem oil used was also purchased from a commercial supplier, being a product of the insecticide / fungicide class (Tetranortriterpenoid Chemical Group), in the formulation of Emulsifiable Concentrate (EC) and classified by the Ministry of Agriculture of Brazil in Category 5 - product unlikely to cause acute damage (Agrofit 2021). The seeds of *Ipomoea purpurea* were also purchased from a commercial supplier, already chemically treated, in order to avoid post-harvest fungi, and the batch was within the expiry date.

Experimental setup

From the original concentration of the product (85%), dilutions were made to 65 and 45%. Then, in 200 mL disposable cups containing commercial substrate, morning glory seeds were planted. Seven treatments were evaluated, T1 (control, without oil), and to another three of them were applied 1 mL of neem oil before germination (T2 to T4), immediately after sowing, while for the remaining three (T5 to T7), 1 mL of oil was applied on the seedlings that had already emerged, seven days after sowing. The seedlings were kept on a bench in full sun and were watered three times a day. The readings of the experiment were daily, where the number of seeds was noted (only from treatments T1 to T4). At the end of the experiment (after 21 days), the number of live seedlings and the fresh weight of all treatments were measured. Before weighing, the seedling roots were rinsed under running tap water.

Experimental design and statistical analysis

A Completely Randomized Design (CRD) was used with five replications, consisting of one cup and six seeds each, totaling 30 seeds in each. Based on the germination data, the average emergence time (Eq 1) and the germination percentage (Eq 2) were calculated using the following equations (Santana and Ranal 2004):

$T = (\sum(f_{i\,x}\, x_i))/(\sum f_i)$ (days) average germination time (Eq 1)

Germination index (%) = (germinated seeds)/(total number of seeds) ×100 (Eq 2)

Where f_i= number of seeds germinated on the i^{-th} day; and x_i = number of days counted from sowing to the day of reading.

The test data were submitted to analysis of variance (ANOVA) using the SISVAR 5.6 Program (Ferreira 2014). The average values of the germinability parameters were compared by the Tukey test, at 5% probability.

Results

Germinability

Based on the germinability data (Fig. 1), it was observed that the neem oil, applied before germination, did not show a significant effect on the emergence time of morning glory seeds, despite all treatments having delayed germination at 0.27 (45%), 1.4 (65%) and 0.93 days (0.85%). There was no interference in germination, with 100% germination observed.

Fresh weight of seedlings

Regarding the fresh weight of seedlings, only application after emergence significantly reduced this parameter (Fig. 2). Neem oil at concentrations of 45, 65 and 85% caused evident phytotoxicity in the seedlings, leading to a symptom of leaf blight of the morning glory's aerial parts, which did not recover; some plants escaped this effect, although with reduced weight (Fig. 3).

Seedling survival

Another parameter evaluated was the survival rate of the morning glory seedlings submitted to the application of neem oil (Fig. 4). The control and treatments where oil was applied before germination showed survival of almost 100%, that is, an average close to 6 plants per treatment/replicate. On the other hand, for the treatments in which the oil was applied to morning glory seedlings, after germination, there was a high mortality, varying between 51 and 62%, and those that survived showed reduced weight.

Discussion

The concentrations of neem oil at 45, 65 and 85% were tested *in vivo,* based on the results of previous *in vitro* germination studies (Andrade and Marques 2021). These are not usual

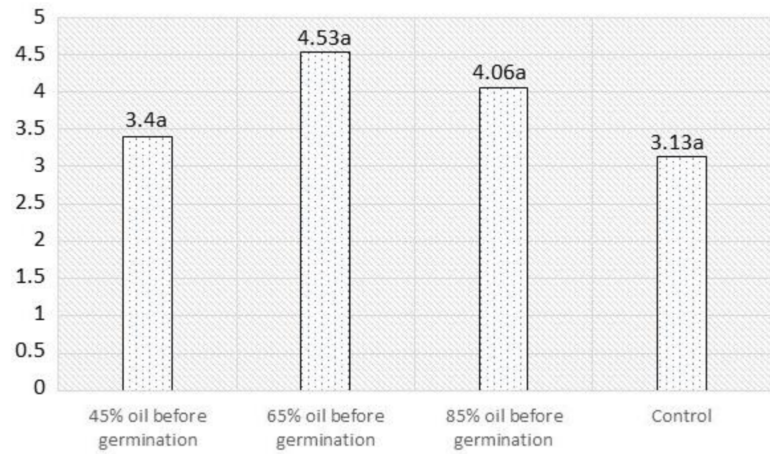

Fig. 1: Average emergence time, in days (y-axis), of morning glory (*Ipomoea purpurea*) seeds treated with different concentrations of neem oil (x-axis) before germination. Means followed by the same letter do not differ significantly by the Tukey test (P < 0.05)

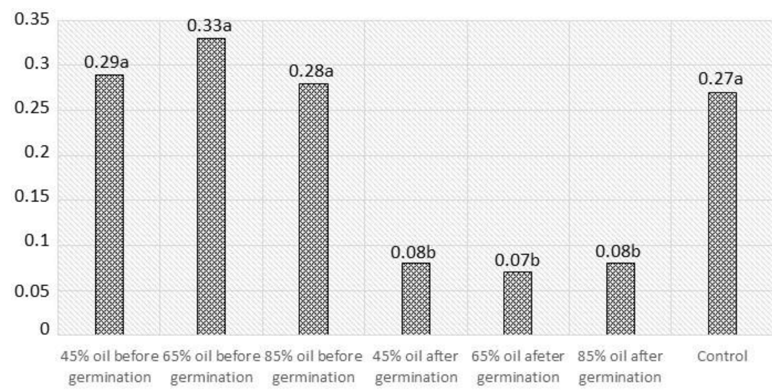

Fig. 2: Average fresh weight of seedlings on the 21st day, in grams (y-axis), of morning glory seedlings (*Ipomoea purpurea*) treated with different concentrations and times (before or after germination) of neem oil (x axis). Means followed by the same letter do not differ significantly by the Tukey test (P < 0.05)

concentrations in practice, since the commercial product is recommended for use at concentrations/doses that vary between 0.6 and 1.2%. At the concentrations used here, high phytotoxicity was observed, inducing late blight symptoms of *I. purpurea* seedlings. At these concentrations, weight and survival were reduced, and germination was delayed.

However, even at lower concentrations, phytotoxic effects of neem oil have been reported. Pinheiro *et al.* (2009) report that the 5% concentration was phytotoxic to common bean (*Phaseolus vulgaris* L.). Regarding weeds and corroborating the present work, Souza Filho *et al.* (2009) described that the oil, at a concentration of 3%, affected germination and development of “touch-me-not” (*Mimosa pudica* Kunth.) and sicklepod (*Senna obtusifolia* L.) plants, reducing the hypocotyl and roots of these plants.

Although this is not the focus of the work, the allelopathy of extracts from this plant has also been studied. Xuan *et al.* (2004) reported that neem bark and leaf extract (dried, powdered and at 5% concentration) inhibited germination and growth of cultivated plants, such as alfalfa (*Medicago sativa* L.), Adzuki bean (*Vigna angularis* (Willd.) Ohwi and H. Ohashi), carrot (*Daucus carota* L.), radish (*Raphanus sativus* L.), rice (*Oryza sativa* L.) and sesame (*Sesamum indicum* L.), as well as weeds such as Indian goosegrass (*Eleusine Indica* (L.) Gaertn (Elein)), pickerel weed (*Monochoria vaginalis* (Burm.f.) C. Presl.) and Indian jointvetch (*Aeschynomene indica* L).

Neem oil delayed the germination of morning glory seeds by up to 1.4 days, even when applied to the soil, as demonstrated *in vitro* (Andrade and Marques 2021), although without significant difference. Regarding the germination index, the present results agree with the reports by Ferreira and Áquila (2000), in which allelopathy did not exhibit an (significant) effect on germination, being more efficient in delaying it. On the other hand, studies by this same research group report that the aqueous extract of guaco (*Mikania glomerata* Spreng) (Castro *et al.* 2021), the alcoholic extract of eucalyptus-lemon (*Corymbia citriodora* (Hook.) K.D. Hill and L.A.S. Johnson) (Fonseca and Marques 2021) and castor oil (*Ricinus comunnis* L.) significantly reduced morning glory germination (Oliveira *et al.* 2021).

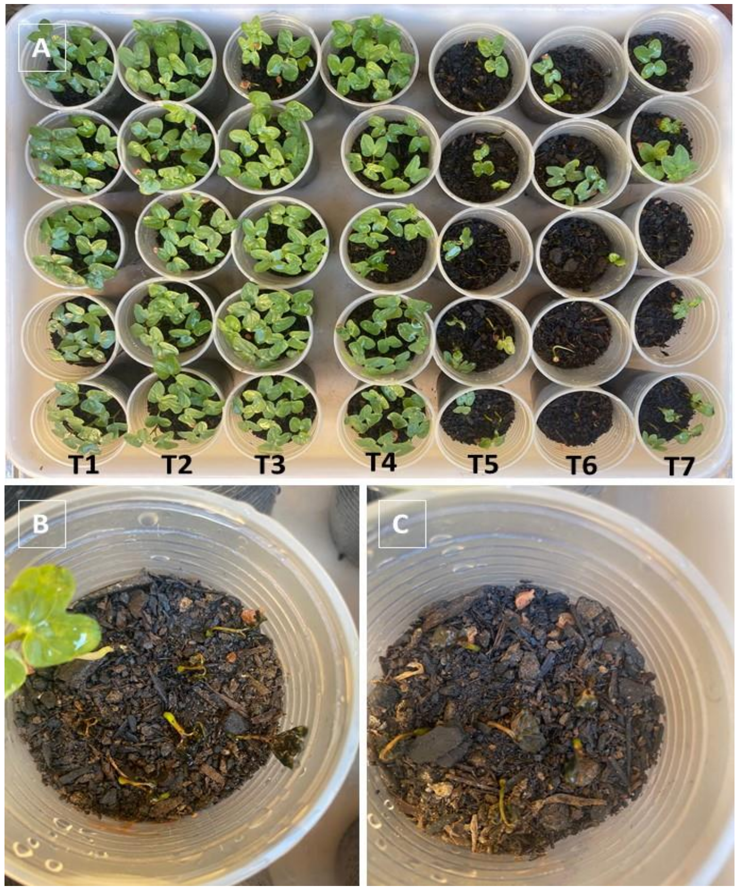

Fig. 3: Neem oil allelopathy bioassay using morning glory seed seedlings, where: A) seedlings gathered on the day of the test reading (T1 = controls, T2 to T4 = oil applied after sowing, T2 = 45% oil, T3 = 65% oil and T4 = 85% oil; T5 to T7 = oil applied after emergence, T5 = 45% oil, T6 = 65% oil and T7 = 85% oil); B) treatment with 65% neem oil applied after emergence, where an escaped seedling is observed and the others show late blight and C) treatment with 85% neem oil (pure) applied after emergence, where the complete burning of the seedlings is seen

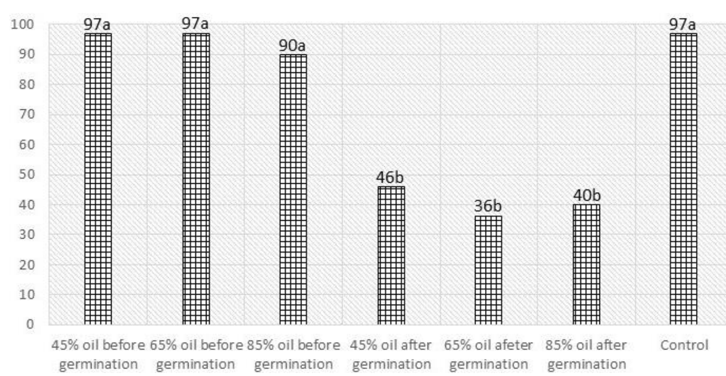

Fig. 4: Surviving seedlings (%) of morning glory (*Ipomoea purpurea*) when treated with different concentrations of neem oil and times (before or after germination) (x-axis). Means followed by the same letter do not differ significantly by the Tukey test ($P < 0.05$)

Conclusion

Neem oil has a phytotoxic effect on *I. purpurea* seedlings, reducing their survival and fresh weight when applied after germination, in addition to influencing (delaying) seed germination when applied to the soil. The effect of lower concentrations that are close to economically viable and practiced in the field should be studied in the future.

Author Contributions

ICG, MSM and EM planned the experiments and interpreted the results, ICG and EM made the write up and statistically analyzed

Conflicts of Interest

All authors declare no conflict of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable to this paper

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