Original Article: Neem Oil Influences Morning Glory Seed Germination

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Introduction

he economic impact caused by weeds is often incalculable since it depends on the species in question, the efficiency of the adopted control measures, and the infestation level in the area. In some cases, they are considered an important phytosanitary problem (Soltani et al. 2017). In Brazil, at least 54 cases of herbicide resistance have been reported, a number that grows every year (Weedscience 2021).

Morning glory (*Ipomoea purpurea* L., Convolvulaceae) is a highly harmful weed species in Brazil, mainly in annual crops, such as sugarcane, soybeans, and maize, as its branches intertwine in crops, making harvesting difficult (Piccinini et al. 2018). Their seeds germinate asynchronously. They remain in seed banks in the soil and germinate at different periods, which makes their control difficult (Jha et al. 2015). Other important factors are that the plant is a host to pathogens and mites (Moreira and Bragança 2011) and already has biotypes tolerant to glyphosate (Monquero et al. 2004, Monquero and Silva, 2007, Pazuch et al. 2017).

Given the numerous cases of pest resistance to synthetic chemicals, there is a growing need to use different strategies in integrated weed management (IWM). IWM can use cultural, preventive, mechanical, physical, chemical, and

<u>ABSTRACT</u>

In Brazil, morning glory (Ipomoea purpurea L.) is a prominent weed found in sugarcane, maize, and soybean crops, harming mechanized harvesting. Its seeds germinate asynchronously, making their management difficult, and glyphosate-tolerant biotypes are also observed in the country. Neem (Azadirachta indica L.) is a medicinal plant, and farmers have widely used its oil for alternative pest and disease management. In this context, this work aimed to evaluate the effect of neem oil, at different concentrations (85, 65, 45, 25, and 5%), on the germination of morning glory seeds. The experiment was carried out in gerboxes, in a completely randomized design, and consisted of the five treatments mentioned and a control (without oil), with four replications containing 50 seeds each. The data obtained were used to calculate the germination time and index. Based on the analysis of the data obtained, it was observed that neem oil at concentrations of 85, 65, and 45% delayed the germination of I. purpurea seeds by 1.83, 1.28, and 1.42 days, respectively. Regarding the germination index, it was observed that oil concentrations of 85 and 45% significantly reduced germination by less than 5 and 6%, respectively. The present study evidences the antagonistic potential of neem oil concerning morning glory seeds. It can be used in alternative management programs for this invasive plant, delaying and reducing germination. Further studies will be carried out to evaluate the effect on seedlings.

even biological control as tools, when possible (Korres 2018), among other alternative measures, like using plant oils (Abouziena and Haggag, 2016).

Among the alternative weed control measures is allelopathy, which is a positive or negative interaction of one plant with another through chemical compounds released by them (Mehdizadeh and Mushtaq, 2020). Plants with allopathic principles have been used in intercropping and as a source of new herbicide molecules (Dayan and Duke 2009).

Neem (*Azadirachta indica* A. Juss) is a tree that belongs to the Meliaceae family and has its origins in India. It is a plant with therapeutic and medicinal benefits found in its seeds, bark, and leaves (Alzohairy, 2016). The oil, produced through pressing its seeds, has already been used in plant protection (Campos et al. 2016). One of its main advantages is that it does not affect mammals or the environment (Isman, 1999). The oil contains a diverse group of bioactive substances with high biological effects: azadirachtin, meliantrol, and salanin (Campos et al. 2016).

Kato-Noguchi et al. (2014) highlight nimbolide B and nimbic acid as the main allelopathic substances present in the neem plant. The influence of neem extract or plant residues on crops or weeds has also been studied (Xuan et al. 2004, Ashrafi et al. 2008, Ogundare et al. 2016). However, references to neem oil allelopathy are scarce. Souza Filho et al. (2009) evaluated the effect of neem oil on germination and the seedling development of weeds, namely the touch-me-not plant (*Mimosa pudica* Kunth.) and sicklepod (*Senna obtusifolia* L.). For cultivated plants, Kume et al. (2020) evaluated the effect of neem oil on the germination speed of lettuce (*Lactuca sativa* L.) and arugula (*Eruca sativa* L.).

Given what is stated above, this study aimed to evaluate the effect of neem oil on the seed germination of *Ipomoea purpurea* L.

Materials and Methods

The study was conducted in the Federal District, in central Brazil (15.58 °S, 47.73 °W), a region dominated by the Cerrado biome, in March 2021. According to the Köppen classification, the location has a Tropical seasonal climate of megathermic savannah, with an average annual precipitation of 1,400 mm (Cardoso et al. 2014). The seed lots of *Ipomoea purpurea* were purchased from a commercial supplier, already chemically treated (to prevent fungus), and within the expiry date.

The neem oil used was also purchased from a commercial supplier, being a product considered an insecticide/fungicide (Chemical Group Tetranortriterpenoid), in the formulation of Emulsifiable Concentrate (EC) and classified by Brazil's Ministry of Agriculture in Category 5 - Product Unlikely to Cause Acute Damage (Agrofit, 2021).

From the initial concentration of the product (85%), dilutions were made with sterile distilled water to 65, 45, 25, and 5%. Then, gerboxes containing three sheets of autoclaved germitest paper were used, in which 50 seeds were deposited, and 2 ml of each dilution (treatments) of the oil were added. The gerboxes were sealed with plastic wrap to prevent drying, and then they were kept at room temperature and exposed to light. As moisture was lost, the seeds were moistened. Data were collected daily: the number of seeds germinated each day was recorded, and the germinated seeds were then discarded (Castro et al. 2021).

The design used was completely randomized (CDR) with four replications, consisting of 50 seeds in each of the five treatments (Neem oil dilutions) plus the control (seeds with the addition of sterile distilled water only). Based on the germination data, the mean germination time (Equation 1) and the germination percentage (Equation 2) were calculated using the following equations (Santana and Ranal 2004):

 $T = \frac{\sum (f_i * x_i)}{\sum f_i}$ (days) average germination time (Eq

Germination index (%) = $\frac{germinated seeds}{total number of seeds} *$ 100 (Eq 2)

Where f_i is the number of seeds germinated on the ith day; and x_i is the number of days counted from sowing to the day of reading.

The data were submitted to analysis of variance (ANOVA), followed by the Tukey test, at a

significance level of 5%, using the Sisvar 5.6 program (Ferreira, 2014).

Results and Discussion

The seeds of *Ipomoea purpurea* germinated by the 14th day, with an average germination index of 98.5% for the control treatment, which agrees with previous reports that indicate total germination between 6 and 19 days, in addition to a variation in the germination rate of the species, in different seed lots, between 84 and 98% (Castro et al. 2021). Based on the statistical analysis of the data, it was possible to observe that the gerboxes with a neem oil concentration of 85% (Figure 1) were the ones that delayed the germination of morning glory seeds (1.83 days). However, they did not differ much from those with 65% and 45% concentrations, which delayed the germination by 1.28 and 1.42 days, respectively. Those with 25% and 5% concentrations did not differ significantly from the control treatment, although they delayed the germination rate by 0.25 days and advanced it by 0.2 days, respectively.

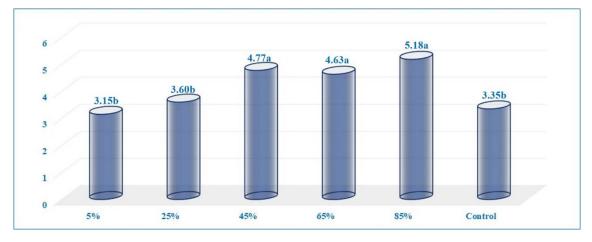


Figure 1. Average germination time in days (y-axis) of *Ipomoea purpurea* seeds treated with different concentrations of neem oil (*Azadirachta indica*) (x-axis). Means followed by the same letter do not differ statistically by the Tukey test (P < 0.05).

Regarding the germination index, it was observed that treatments with neem oil concentrations of 85 and 45% significantly reduced germination by 5 and 6%, respectively (Figure 2). The values in other treatments did not differ significantly from the control treatment, although they were 1.5% lower.

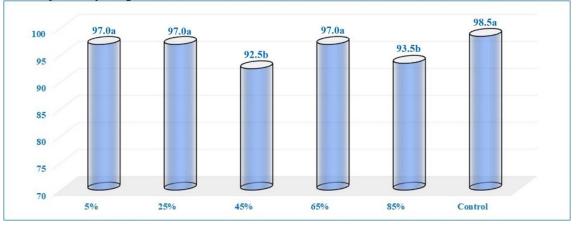


Figure 2. Germination index (y-axis) of *Ipomoea purpurea* seeds treated with different concentrations of neem oil (*Azadirachta indica*) (x-axis). Means followed by the same letter do not differ statistically by the Tukey test (P < 0.05).

Neem oil contains a diverse group of bioactive substances such as azadirachtin, meliantrol, and salanin (Campos et al. 2016). However, according to Kato-Noguchi et al. (2014), nimbolide B and nimbic acid B are the main substances with allelopathic effects present in the composition of its leaf extract, which has inhibited the growth of roots and shoots of barnyard grass (*Echinochloa crus-galli* (L.) Beauv.). Ogundare et al. (2016) observed that neem residues applied in the field in eggplant (*Solanum melongena* L.) cultivation reduced weed infestations significantly, but without mentioning which invasive plants they affected.

Xuan et al. (2004) reported that neem bark and leaves (dried, powdered, and at a concentration of 5%) strongly inhibited the germination and growth of several crops, such as alfalfa (*Medicago sativa* L.), bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi), carrot (*Daucus carota* L.), radish (*Raphanus sativus* L.), rice (*Oryza sativa* L.), and sesame (*Sesamum indicum* L.), as well as that of weeds, such as barnyard grass, pickerelweed (*Monochoria vaginalis* (Burm.f.) C.Presl.) and Indian jointvetch (*Aeschynomene indica* L.), in a bioassay and soil.

Analyzing the allelopathic effect of n-hexanesoluble, acetone-soluble, and water-soluble fractions obtained from shoot extracts of *A. indica*, Ashrafi et al. (2008) observed significant reductions in the germination and growth of the roots and hypocotyls of cockscomb (*Amaranthus rotundus* L.), Canada thistle (*Cirsium arvense* L.), crabgrass (*Digitaria sanguinalis* L.), wild mustard (*Sinapis arvensis* L.), lettuce and ryegrass (*Lolium ultiforum* Lam.), as the extract concentration increased.

The reports of Kume et al. (2020) corroborate those mentioned and the present study since they found that neem oil, at a concentration of 10% and 20%, also interfered in the germination speed of cultivated plants lettuce and arugula. Along the same lines, Souza Filho *et al.* (2009) describe that the oil, at a concentration of 3%, affected the germination and seedling development of two weeds: the touch-me-not plant (*Mimosa pudica* Kunth.) and sicklepod (*Senna obtusifolia* L.).

In the present study, the reduction in the germination of the weed seeds in question was

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significant, which can be considered an important characteristic. Aqueous extracts from several plants were also able to delay the germination of morning glory by up to 5 days. Only guaco (*Mikania glomerata* Spreng) extract interfered in the germination of *I. purpurea* in that work (Castro et al. 2021). In another work, by this same research group, the alcoholic extract of lemonscented gum (*Corymbia citriodora* (Hook.) K.D. Hill and L.A.S. Johnson) delayed and reduced the germination of *I. purpurea* (Fonseca and Marques, 2021). Inoue et al. (2010) also observed a significant decrease in the germination of *I. grandifolia* by using a hydroalcoholic extract of araticum-do-cerrado (*Annona crassiflora* Mart.).

Based on our current results, further studies will be carried out to evaluate the effect of neem oil on morning glory seedlings.

Conclusions

It was concluded that neem oil affects the germination time of *Ipomoea purpurea* seeds at 85, 45, and 65% concentrations, especially for 85%.

On the other hand, neem oil concentrations of 85 and 45% can reduce the germination rate, which indicates that neem oil can be an essential bioherbicide for the management of morning glory.

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