Article

Evaluation of propolis extract in preventing weed seed germination

Shahram Dadgostar, **Jamasb Nozari**
Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
E-mail: nozari@ut.ac.ir

Received 28 June 2020; Accepted 5 August 2020; Published 1 December 2020

Abstract
As you know, weeds can annihilate a large part of agricultural crops. Therefore, many expenses for controlling and destroying weeds are spent on the purchase of herbicide. They cause environmental pollution and have side-effects to human. Propolis is one of the most valuable bee products that have many applications including medicine, agriculture and animal husbandry. In this study, a new application has been introduced that can be so effective in agriculture field. For this reason, propolis extract was used in 4 concentrations. The combinations of propolis extracts were tested on germination of wild barley, oat and cattle cotton. For primary treatment, the seeds were grown in Peat Moss and each day 2 ml of the specified concentrations of the extract were injected. Three replicates for each treatment and three replicates without treatment were considered as controls. All treatments and control were irrigated daily. After determining the best concentration in preventing germination of seeds, the duration of starting germination after application of this extract in the ground was investigated. For this purpose, germination of wheat was studied after using of the 1:2 extract in culture tray at times 1, 8, 16 and 24 hours after treatment. The results of primary treatment showed a significant effect of propolis extract compared to the control. Also, the results of the secondary treatment showed this extract does not prevent germination of main plant's seed after soil treatment. In conclusion, this extract can be recommended as an herbicide before cultivation on farms.

Keywords propolis; herbicide; peat moss; seed germination; ethanolic extract.

Proceedings of the International Academy of Ecology and Environmental Sciences
ISSN 2220-8860
URL: http://www.iaees.org/publications/journals/piaees/online-version.asp
RSS: http://www.iaees.org/publications/journals/piaees/rss.xml
E-mail: piaees@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Propolis is a mixture of wax and resin collected by bees from plants. This honey bee production is used as a natural cement to cover the holes within the colony, to disinfect their rooms and for pasting different parts of the hive to each other. The most important role of propolis in the hives is to prevent microbial growth and activity of microorganisms. Another application of propolis is related to medicine, antibacterial and antifungal drugs (Grunberger et al., 1995; Gafar and Sacalus, 1987). Moreover, reports on the effectiveness of propolis in controlling plant and animal diseases are available (Fahmy and Omar, 1989; Zawadzki et al., 2011). Several

compounds are noted that propolis contains polyphenols, amino acids, steroids and non-organic compounds. . The quality of propolis is relevant to the geographical location of hive, plants which bees have used for years to collect propolis (Omar, 1989; Barbern et al., 1993). Propolis productions are the important part of apicultural experiment because this component has many beneficial applicants. One of the common methods used for collecting propolis is the trap on the hive which was designed based on honey bee's behavior to isolate her colony against light and coldness (Hogendoorns et al., 2013).

Along with plant pests and diseases, weeds are also the most important factors causing great damage to crops. Every year a lot of crops are destroyed due to weed growth. A great amount of herbicides are used in order to destroy weeds, which most of them are commercially-based chemical synthesis. These herbicides are sometimes harmful to human, pollinators and other non-target organisms (Zhang, 2018). Many studies about the inhibitory effect of propolis on the germination of pathogenic fungi and proliferation bacteria were carried out (Moawad et al., 2001). Behiti (2013) examined the effect of propolis on prevention of potato spoilage. In his study, fungi and bacteria that caused decay in the potato were investigated. Abeer Hashem et al. (2012) observed inhibitory effect of propolis on *Aspergillus parasiticus*. According to the study, germination and production of the fungus' conidia decreased by increasing the concentration of propolis.

Sorkun et al. (1997) in Turkey reported that propolis extracts prevented plant cells division. In another study, propolis extracts cause prevention or delay of seed germination (Sorkun et al., 1997; Sorkun and Bozcuk, 1994). This study showed that propolis could be used to prevent the growth of weeds. The best time for preventing the growth of weeds is preventing germination of seeds before they are dispersed in the environment. Consequently, based on the natural substance derive from bees, can be produced pre-emergence herbicides that we evaluated this effectiveness in this study. On the other hand, in this study, effect of non chemical product of honey bee on seed germination was evaluated.

2 Material and Methods

2.1 Seeds cultivation

In this experiment, two groups of plants, such as broadleaf and narrow-leaf weeds, were used. The seeds of wild barley (Poaceae: *Hordeum spontaneum*) and oats (Poaceae: *Avena sativa*) as narrow leaves and seeds of milk thistle (Asteraceae: *Silybum marianum*) and cattle cotton (Malvaceae: *Abutilon striatum*) as broadleaf were utilized. The seeds in trays cultivation in the context of peat moss to ensure food security at a temperature of 25 °C and photoperiod of 16: 8 were planted. There were three replicates for each solution and three replicates without treatment as control were applied.

2.2 The primary solution

To make a stock solution, the ethanolic extract of propolis was used. 10 g of laminate propolis was placed in a freezer for 5 days; Then crushed with a mortar and a ratio of one to ten (1:10) with 96% alcohol was stirred for 7 days. This combination was rattled in a shaker every day for a short time. After 7 days, insoluble material was separated by filtration and the remaining extract was used for experiments.

2.3 The secondary solution

The primary solution of distilled water at four concentrations (1:1, 1:2, 1:3 and 1:4) was diluted. The most concentrated ratio had one unit of water and one unit of ethanolic propolis extract and the most diluted ratio had 4 units of water and one unit of propolis extract. Secondary solution produced milky emulsion which with reducing the concentration its color was faded. These serial dilutions were used for seed treatment.

2.4 The primary treatment

To determine which secondary solution concentration had the highest efficiency in the prevention or postponement of weed seed germination, narrow-leaf and broadleaf were treated with each concentration in

triplicate. For each seed and concentration, three controls without treatment were considered. Since the seeds planted in the bed were treated with 2 ml of different ratios of secondary solution every day. After the treatment, all the tested and control samples were irrigated with 5 ml of distilled water to provide humidity for seed germination. Germination was evaluated every day. Index of measuring buds was exiting 2 cm of buds from the peat moss surface.

2.5 The secondary treatment

After the most effective treatments were found in different concentrations, the reconstruction test of use of herbicides in wheat fields was performed based on the times to show how seed can be germinated after treatment. For this purpose, the ratio of 1:2 (water: extract) was used. The advantage of the concentration (1:2) is the low cost; In addition, it is the first concentration after the concentration of 1:1 which had the greatest impact. Culture bed (Peat Moss in cell culture trays) with 2 ml of 1:2 solutions was treated and every eight hours (clocks 1, 8, 16, 24) seeds in the bed were cultivated. After 24, hours all the seeds were irrigated with 5 ml of distilled water to provide moisture for germination. Since the Milk thistle seeds were not totally germinated in the primary treatment, they were removed from the study. In this test were three replications for each treatment and three replicates of control in which no treatment was used. To determine the effect of the solution 1:2 on main seed germination after planting (in this experiment, wheat was used as main seed); an experiment was conducted to evaluate speed and quality of wheat and weed growth after soil treatment with the solution. Before doing this test, bed planting with a secondary solution to a ratio of 1:2 was treated and then wheat and wild barley were planted in three replications. Three replicates were used as control in this test (Fig. 1).

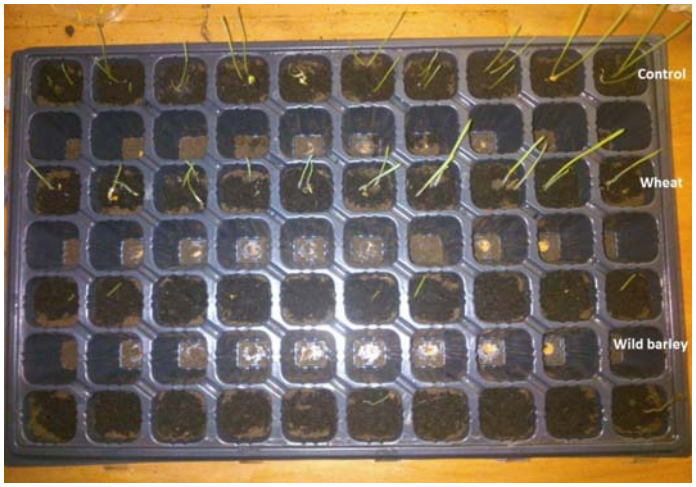

Fig. 1 Growing tray and comparison of wheat germination in different treatments and control. The first and second rows are control and wheat, respectively. The next two rows are Wild barley seeds that grow three days later than wheat.

3 Results and Discussion

The data was recorded daily and analyzed by the SPSS V. 20 software. In this study, cross-tab analysis and Pearson's Chi-square test were used.

As you can see in Table 1, based on the results of cross-tab analysis, none of the seeds were germinated at 1:1 and 1:2 concentrations. At the 1:3 concentrations, none of cattle cotton was germinated; however, in oat and wild barley, 67% lack of germination was observed. By reducing the concentration, the germination rate increased in comparison with 1: 3 in oat, but no change was observed in wild barley.

Table 1 Comparison of seeds germination percentage in the control and different concentrations of the treatment.

Seed			Results		Total
			0	1	%
Wild barley	Concentration	0	0	100	100
		1	100	0	100
		2	100	0	100
		3	67	33	100
		4	67	33	100
	Total		334	166	500
Cattle cotton	Concentration	0	33	67	100
		1	100	0	100
		2	100	0	100
		3	100	0	100
		4	67	33	100
	Total		400	100	500
Oat	Concentration	0	0	100	100
		1	100	0	100
		2	100	0	100
		3	67	33	100
		4	33	67	100
	Total		300	200	500

The untreated control seeds germinated earlier than the treated plots. After 3 days, the first buds of samples treated with a concentration 1:4 were observed; and after 4 days, seeds treated with a concentration of 1:3 initiated germination. Only one of the three replications treated with 1:2 was germinated in wild barley and oats, so some of the others did not germinate. In General, Chi-square test showed that the germination rate of the three types of seeds with the control had a significant difference ($df = 4$, $p<0.0005$).

As you can see in Fig. 2, the highest germination was in the control group and the germination of the seeds decreased with increasing concentration. At 1:1 concentration, there was no germination, and germination was reduced by decreasing the concentration of extract (1:4).

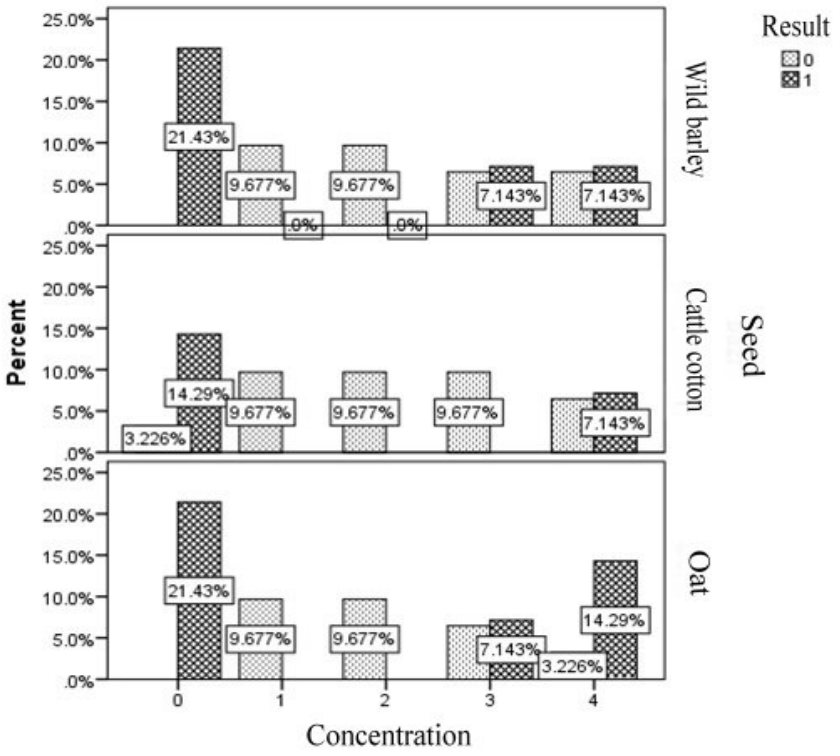

Fig. 2 Chart between the efficacies of different concentrations of extract in preventing germination as compared to control.

Result of secondary treatment show that wheat and wild barley seeds germinated more quickly. First the control seeds germinated in 3 days, and then seeds planted at clock 16 and 24 germinated after treatment.

In the soil that was treated with propolis solution, wheat germinated without loss of its quality after 4 days; however, seeds of wild barley germinated 3 days later with wheat at germinating on 7th day.

Based on the results, the concentrations produced by a ratio of 1:3 and 1:4 had little effect. On the other hand, concentration of 1:1 completely inhibits the germination of seeds of both broadleaf and narrow leaves. But due to the high cost of the concentration production, the first effective concentration after that, the 1:2 as recommended treatments to control weed seeds, have been proposed. If we want to classify this solution as an herbicide, we can say the propolis extract can be classified as before-planting herbicide. Based on residue tests of these compounds after a time, propolis extract can be used in the soil before planting and a week after the main planting like wheat is cultivated. Therefore, use of this compound, two days before planting, can prevent weed seeds from germination in soil. A pause after taking this compound in soil and plant cultivation can help prevent the residual extract in the soil (Behrooz, 2019).

4 Conclusions

Another benefit of propolis extract as a pre-planting herbicide is its bacterial and fungicidal properties. The use of this compound in soil, in addition to preventing weed seed germination, the growth of bacteria and fungi are prevented (Moawad et al., 2001). The properties of propolis extract can reduce the risk of soil-borne fungal and bacterial disease. Furthermore, synthetic chemical compounds are not used in the construction of these extracts; therefore, it has no harmful residues in nature or pollution to the environment and animals. Another advantage of the propolis extract is the low cost when compared to chemical herbicides.

Sorkun and Bozcuk (1994) reported that alcoholic extract of propolis cause prevention or delay of seed germination and in this study, it was found, depending on the concentration of propolis extract, to prevent or delay the germination of weed seeds. Moreover, results show that propolis extract can prevent broad leaf seeds germination more than narrow leaves. By changing the concentration, it can also increase the effectiveness on the narrow leaves.

Acknowledgments

We thank Lady Diona Cerelli for assistance with English editing, and her comments that greatly improved the manuscript

References

Abeer Hashem, Abdullah EF, Alwathani HA. 2012. Effect of propolis on growth, aflatoxin production and lipid methabolism in *Aspergillus parasiticus* Spear. Pakistan Journal of Botany. 44(3): 1153-1158

Bahtiti Nawal. 2013. Study of preservative effect of "Propolis" on the storage quality of mashed potatoes. Food Science and Technology 1(2): 17-20

Barberan Tomas FA, Garcia-Viguera C, Vit-Oliver P, Ferreres F, Tomas-Lorente F. 1993, Phytochemical evidence for the botanical origin of tropical propolis from Venezuela. Phytochemistry, 34: 191-196

Behrooz A. 2019. Effect of different levels of fluoride on Almond (*Prunus Amygdalus*) seedling. Proceedings of the International Academy of Ecology and Environmental Sciences, 9(3): 98-103

Fahmy FG, Omar MOM. 1989. Potential use of “propolis” to control white rot disease of onion. Assist Journal of Agricultural Sciences, 20: 265-275

Gafar M, Sacalus AM. 1978. Treatment of Common Chronic Recurring Aphthae with Propolis, A Remarkable Hive Product Propolis. 133-138, Apimondia Publishing House-Bucharest-Romenia, Romania

Grunberger D, Banerjee R, Eisinger EM, Oltz L, Efros M, Coldwell V, Nakanishi K. 1995. Preferential Cytoxicity on Tumor Cells By Caffeic Acid Phenethyl Ester Isolated From Propolis. Propolis Research, 79-86, North Yorkshire, UK

Hogendoorn EA, Sommeijer MJ, Vredenberget MJ. 2013. Alternative method for measuring beeswax content in propolis from the Netherlands. Journal of Apicultural Science, 57(2): 81-90

Moawad AA, Aly SA, Galal EA. 2001. Effect of Egyptian honeybee propolis on the growth of *Aspergillus ochraceus* and ochratoxin A production in Ras cheese. 8th Egyptian Conference for Dairy Science and Technology. 347-356, Cairo, Egypt

Omar M. 1989. Some characteristics of propolis from Upper Egypt. Proceedings of the Forth International Conference on Apiculture in Tropical Climates. 88-92, Cairo, Egypt

Sorkun K, Bozcuk S, Gomurgen AN, Tekin F. 1997. An inhibitory effect of propolis on germination and cell division in the root tips of wheat seedlings. Bee Products: Properties, Applications, and Apitherapy Symposium. 129-135, Tel Aviv, Israel

Sorkun K, Bozcuk S. 1994. Investigation of the Effect of Propolis on Seed Germination of Some Culture Plants XII. Ulusal Biyoloji Kongresi 6–8 Temmuz, Edirne-TÜRKIYE. 54-59, Turkey

Zhang WJ. 2018. Global pesticide use: profile, trend, cost/benefit and more. Proceedings of the International Academy of Ecology and Environmental Sciences, 8: 1-27

Zawadzki F, Prado IN, Marques JA, Zeoula LM, Rotta PP, Sestari BB, Valero, MV, Rivaroli DC. 2011. Sodium monensin or propolis extract in the diets of feedlot-finished bulls: effects on animal performance and carcass characteristics. Journal of Animal and Feed Sciences, 20(1): 16-25