ANTIFUNGAL AND ANTI-TERMITE ACTIVITIES, TOTAL PHENOLIC CONTENT OF *PROSOPIS FARCTA* EXTRACTS; ATTEMPTS TO DEVELOP WEED BIOCONTROL METHOD AGAINST IT

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Prosopis farcta is a widespread weed in the Near East and its an invasive plant of southwestern parts of the USA. Despite being sufficiently studied in the past, some of its activities were not published. In this research, we studied the antifungal and anti-termite activities of four extracts of the plant aerial parts: aqueous, ethanolic, ethyl acetate and hexane. In addition, since the published reports of total phenolic content (TPC) are not consistent, we tested this as well. We also tested the potential of the aqueous extract of *Carya illinoinensis* as possible weed biocontrol against *P. farcta*. The n-Hexane extract had the highest antifungal and anti-termite activities. TPC was found around 13.9 mg of gallic acid equivalent for 1 g of dry ethanolic extract (highest). The attempts to use an aqueous extract of *C. illinoinensis* for weed biocontrol of *P. farcta* achieved very limited success

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Introduction

Prosopis farcta is one of the most widespread plants over the southwestern regions of Asia, and it is n invasive plants in eastern USA.[1] It belongs to the *Fabaceae* family and the genus of Prosopis that includes 44 species.[2] Archeological findings indicate that ancient peoples of the eastern Mediterranean basin used this plant, mainly for food.[3,4] Most cultures of the Middle East have used *P. farcta* in their traditional medicines. One of the notable known uses is for the treatment of diabetes.[5] In Pakistan, it is reported that traditional societies use the plant for many purposes such as medicinal (humans and animals), animal food and fencing.[6] In the folk medicine of Jordan, *P. farcta* is used as antispasmodic and analgesic.[7] But according to published data, it is evident that Iranian traditional medicine has used this plant more than others. The uses included: blood thinner, antidiabetic, sterilizer (hands), rashes treatment, anti-atherosclerosis and menstruation pain.[8-13]

P. farcta was mentioned in several review articles, but all of them are related to the Prosopis genus in general and none of them scans the literature known about this species in particular, despite the extensive knowledge about it. Prabha et al. reviewed phenolics of *Prosopis* and their potential pharmacological uses.[14] In addition to phenolics, they indicated some other interesting compounds such as alkaloids present in *P. juliflora*, see Figure 1.

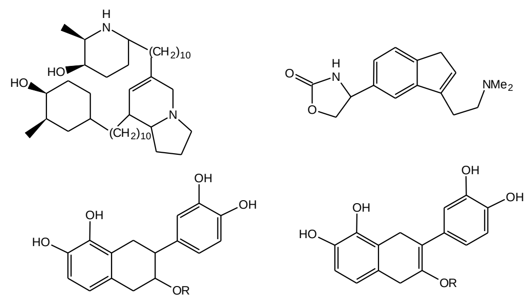


Figure 1: Pharmacologically active compounds found in P. juliflora (Ref. [14])

Persia et al. published an article about the toxicity of *Prosopis* species, but they do not indicate the source of toxicity, i.e., the toxic compounds.[15] Finally, a group of researchers from the USA published a review article of controlling Prosopis species, which are invasive plants in the New World. The interest focuses mainly of Prosopis species that grow as trees, and less of P. farcta, which is more of a low, shrubby plant type.[16]

Reviewing of chemical composition and properties

The complete, systematic chemical composition of P. farcta was never published. All published studies so far reported only partial compositions, where they mainly included groups of compounds (phenolics, fatty acid …etc.) or characterization of certain natural products, mostly previously known, such as phenolics and flavonoids.

In Table 1 we summarized the findings of these published studies.

Table 1. Reported findings of chemical composition studies of *P. farcta*

Methods and Findings
The dry powder prepared from the whole plant (excluding seeds) was extracted with various solvents and analyzed by HPLC or GCMS (for volatiles). Most isolated compounds were phenolics and there were slight differences between two locations. See Figure 2. [17,18]
Dry powder of fruits was extracted with water and ethanol. Analysis method is not indicated but compounds groups that were found are mainly phenolics. [19]
A Method was developed to increase the yield of anthocyanins in callus cultures. [20]
Methanolic extract of aerial parts was analyzed for major compounds families. Analysis methods are not indicated. [21]
Fatty acids composition of seeds oil was analyzed after preparing methyl esters. Linoleic, oleic and palmitic acids constitute more than 90 percent of the fatty acids. [22]
Quercetin content of the plant fruits was measured from several locations in Iran, after extraction with acidic hydromethanol. See Figure 2. [23]
Oil extracted from seeds was analyzed and found containing a high concentration of protein (18%), unsaturated fatty acids (UFA) and low total phenolic content (1.7 mgGAE g^{-1}). [24]
Aqueous extract of dry aerial parts was prepared and total phenolic content was measured and found 17.3 mgGAE g^{-1}. [25]
HPLC analysis of acetone and methanol (successively) extract of different parts of the plant, harvested from various locations. Many compounds were identified, none is new. [26]
Comprehensive analysis for compound groups and minerals was performed after various extractions of plant parts. [27]
Aerial parts of the plant were extracted with: methanol, then with, *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol. All extracts were analyzed mainly using GCMS. Many compounds were identified, none new. [28]

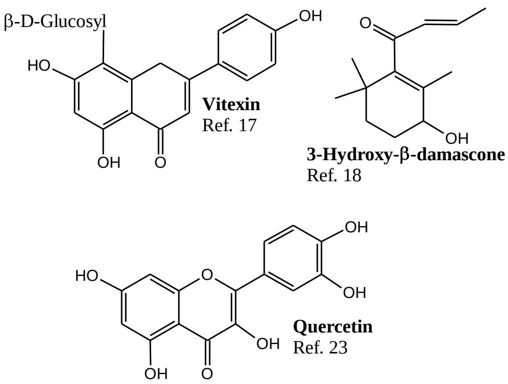


Figure 2: Selected compounds found in P. farcta

Table 2. Medicinal activities of *P. farcta* and their related properties

Tested Activities, Methods, Findings
Antibacterial The dry powder of fruits was extracted with H_2O and EtOH, and extracts were tested against antibiotic-resistant bacteria. Extracts had moderate activity.[19] MeOH extract of aerial parts was prepared and tested against antibiotics resistant bacteria and was found active.[21] Aerial parts of the plant were extracted with various solvents and tested against different bacteria. Each extract was more active than others against specific bacteria.[28] MeOH and *n*-hexane extracts of aerial parts were prepared and tested against the major pathogenic bacteria of fish, including *A. hydrophila*, *Y. ruckeri* and *S. iniae*. MeOH extract was more active.[29] Aq. ethanolic and MeOH pods extracts were tested against *S. paucimobilis*. EtOH and MeOH were more active than aq. extract.[30] EtOH extracts showed high antimicrobial activity.[31]
Anticancer Among extracts of aerial parts that were prepared with six different solvents, the EtOAc extract had the highest activity.[28] All plant aq. EtOH (80 %, v/v) extract was active against HT-29 cancer cell lines.[32]
Antidiabetic Aqueous extract of aerial parts was found active α-Glucosidase and α-Amylase inhibitor.[25] STZ-induced diabetes in male rats was treated with ethanol/water (70-80%) or methanolic various plant parts extract, or directly fed with solid extract. Clear activity was observed.[33-39,48] Diabetes was induced in cell lines with various agents and treated with plant infusion and *n*-hexane and acetone extracts. All plants products had glucose lowering effect.[40]
Antihyperlipidemic Blue-neck male ostriches were fed with seeds for 30 days and various bioactive materials were monitored in their blood. HDL cholesterol, total protein, and globulins levels increased, whereas LDL cholesterol, inorganic phosphorus, and γ-GT activity decreased.[41] Aqueous root extract reduced lipids in the blood of high cholesterol diet rabbits and had aorta protective effect.[42,43] Aqueous root extracts were active lipids lowering in rabbits livers.[44]
Antioxidant Methanolic extract of seeds was prepared and tested using three methods: TAC, DPPH, ABTS. Highly active.[24] Aqueous extract of aerial parts was tested with FRAP and ORAC methods and found moderately active.[25] Various extracts of aerial parts (including ultra-sonic assisted) were prepared and tested for antioxidant activity (DPPH): moderate.[27] *n*-Butanol extract had the highest antioxidant activity (ABTS), among six extracts that were prepared using different solvents.[28] Ethanolic extract of fruits was tested for antioxidant activity (DPPH, FRAP, ABTS) and found highly active. Total phenolic contents wad determined (62 mg GAE g^{-1}) and 27 compounds were identified. None new.[31] 80% EtOH/H_2O leaves extract had clear antioxidant (lipids) effect on STZ-induced diabetes in rats.[38] 70% Aq. EtOH fruits extract had explicit activity (FRAP)[39]
Cardioprotective Aqueous root extract had aorta protective effect.[43,45] Aqueous root extract had blood pressure lowering effect.[46]

Fertility
70% Ethanol/water fruits extract improved fertility of diabetic rats[39]

Hepatoprotective
Hydro-alcoholic extract (plant parts unknown) reduced level of malondialdehyde in liver.[38] Root aqueous extract had significantly decreased rabbits liver injuries.[44] 80% Ethanol/water extract had a protective effect against acetaminophen-induced hepatotoxicity in rats.[47] Hydroalcoholic pod extract had malondialdehyde level (in the liver) lowering in STZ-diabetic rats.[48] The methanol extract of aerial parts was active against CCl_4-induced liver toxicity in rats.[49]

Wound healing
Fruit powder and its aqueous extract found effective in healing wounds in STZ-induced diabetic rats.[50,51] Wounds made in healthy rats healed faster after treatment with a mixture of *P. farcta* and Ghee (butter, Persian).[52]

Energy production
Biomass was produced from the plant, using sodium tetraborate as most efficient catalyst.[53]

Nanoparticles synthesis
Silver nanoparticles (AgNP's) were produced from $AgNO_{3(aq)}$, using *P. farcta* aqueous extract as a reductant. AgNP's were tested for antibacterial and/or antioxidant activity.[54,55] Gold nanoparticles (AuNP's) were produced by the reduction of $HAuCl_4$ with aqueous leaves extract, and tested for anticancer activity.[56]

Reviewing medicinal activities and related properties

Most of the medicinal activities of *P. farcta* were studied and published. In addition to the classical activities such as antioxidant, antibacterial and antidiabetic, many other properties were also published, including preparation of nanoparticles. In Table 2 we summarized these published reports, mostly sorted by alphabetical order for the convenience of interested readers.

Prosopis farcta as a weed and weed control methods

The genus of *Prosopis* is described by some authors (USA) as "one of the world's worst woody invasive plant taxa".2 In Iran, *P. farcta* is considered as one of the worst weeds that grow wildly, mostly in the best and most fertile agricultural lands, and by this, it prevents the growth of many crops.[57,58]

Weed control is a worldwide issue, and 2016, the global spendings of weed control exceeded $B 40, not including manual weed unrooting.[59] All known methods used to control Prosopis species are based on chemical herbicides.[60] In Jordan, where P. farcta is a severe national problem, many synthetic herbicides were used separately and in combinations, but none of these methods proved successful.[61]

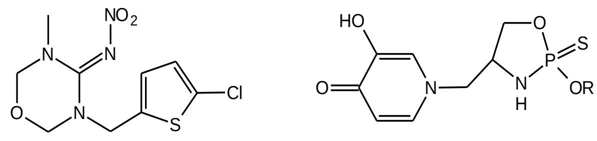
In addition to short term health concerns of chemical weed control (toxicity), it has significant adverse long term effects.[62] To avoid them, many efforts are being invested in the research of biocontrol of weeds, with a clear preference of environment-friendly methods. In Iran, studies were conducted to control *P. farcta* by using it as food for Nephopterygia austeritella (moth), with very limited success.[63] More successful were the attempts using another moth species, Stator limbatus, but also with limited success and method complexities.[64]

To the best of our knowledge, no plants material was used for biocontrol of *P. farcta* as published in the case of some other weeds.[65]

Antifungal and anti-termite activities of plant materials

In our previous publications,[66,67] we reported the antifungal activities of four plants. We also compared the use of synthetic antifungal agents with plant materials (extracts and pure natural products) that have the same property, and we showed the advantages of plant materials. Special attention was paid to reported antifungal activity against Rhizopus stolonifera (black mold), the same fungus that we tested the extracts of *P. farcta* against it.

Termites are social insects that belong to the Isoptera infraorder.[68] They cause major damages to forests,[69] buildings,[70] and many other wood-including facilities. In Israel, the most common species of termites is *Kalotermes sinaicus*.[71] Many synthetic chemicals were prepared and proved successful anti-termite agents.[72,73] Some of these compounds are shown in Figure 3.



Thiamethoxam

Mimosinol Derivatives
R = Me, Et, *n*-Pr

Figure 3. Structures of some anti-termite synthetic compounds[72,73]

But as for most synthetic insecticides, these chemicals have many adverse side effects, such as toxicity to living organisms and damages to the environment. These effects lead to the development of plant-derived insecticides.[74] Many studies were published in recent years about this subject, and the research of F. Abdullah her colleagues from Malaysia is one of the most important.[75]

Experimental Section

Gallic acid was purchased from Merck Co. (Germany). All other chemicals were purchased locally in at least analytical grade.

Prosopis farcta (aerial parts) were harvested from the wild near our laboratory in Kfar-Qari (northern Israel). The green material was washed with distilled water and air dried for 4 weeks. The dry matter was ground into a fine powder and stored at -12 °C in sealed containers.

500 g of plant powder was stirred in 1000 mL of solvent (water, ethanol, ethyl acetate, n-hexane) for 24 h at 50 °C (n-hexane, 30 °C). Suspensions were allowed to cool to room temperature and filtered (Munktell quant. Grade 393) to obtain clear solutions. These were evaporated to dryness with rotary evaporator: aqueous extracts at 60 °C, ethanol, ethyl acetate and n-hexane extracts at 50 °C. All four extracts were solids, and they were stored in screw-capped vials at -12 °C. Extraction yields are shown in Table 3.

Antifungal activity tests

The antifungal assay was performed according to the method we reported in our previous publication, with no modifications.66 Rhizopus stolonifer was grown on whole wheat bread and extracted with water. The center of each Petri dish was inoculated with 5 mm diameter disc of fungal mycelium, taken from pure culture (7 days old). Then, all inoculated dishes were incubated at 25 °C for 6 days and the radial mycelial growth was measured. The antifungal activity of each extract was calculated in terms of the inhibition percentage of mycelia growth by using the following formula:

$$\% \text{ Inhibition}=[(d_c - d_t)/d_c]\text{x}100$$

where

d_c is the average increase in mycelia growth in control

d_t is the average increase in mycelia growth in treated samples with extracts

In all experiments, the control was the extraction solvent and we performed the antifungal tests using two concentrations for each extract: 10 % and 20 % (w/w). See Table 4 and Figure 4.

Total phenolic content test

The 2 L oxidative solution (1000 ml of 0.016 M sulfuric acid solution and 1000 ml of 0.004 M solution of KMnO4) was prepared according to the new method that we reported in our previous publication,67 with no changes. The final oxidative solution was prepared by combining the acid (0.016 M) and permanganate solution (0.004 M), which was stored in 4 °C in a sealed flask. Also, we used the calibration curve of gallic acid titration that we reported in the same publication. The titration of plant extracts was done according to the same method, with no changes. In a 100 ml Erlenmeyer flask that contained a magnetic stir bar, 100 mg of dry plant extract were suspended with 10 ml of distilled water and stirred for 5 minutes. The solution/suspension was titrated with the oxidative solution, with pH monitoring. Titration speed was 2 ml min^{-1}, with continuous gentle stirring. Results are shown in Table 5 and Figure 5.

The anti-termite activity of extracts

Anti-termite activity was performed according to the method that was reported by O. K. Ndukwe and his colleagues, with slight modifications.[76] Termites, *Kalotermes sinaicus*, were collected from infested tree trunk found in a nearby forest, put in glass trays and were immediately put in the Petri pre-prepared dishes.

Strips of filter paper (1x1 cm, Munktell quant. Grade 393) were saturated with 10% extract solution (w/v), each extract in its original extracting solvent. Then, paper strips were allowed to dry for 5 h. In the center of a 10 cm diameter petri dish, an extract loaded filter paper was placed with 10 termites, of unknown sex and age. Controls of this experiment were dry strips of filter paper loaded with solvents.

Termites were kept in these dishes for 4 weeks and were observed 3 times every day (07:00, 12:00 and 17:00). Mortality rate by the end of the 4 weeks was calculated as:

$$\%\text{Dead termites} = (\text{number of dead termites})\text{x}10$$

Results are shown in Table 6 and Figure 6.

Attempts to develop biocontrol against P. farcta

500 g of fresh green peels of Carya illinoinensis were crushed (blender) to a homogeneous and soaked in 1 L of distilled water at 35 °C, for 24 h. Then the suspension was filtered and the filtrate was stored in a sealed bottle at 4 °C.

20 plants of *P. farcta*, most of the same height, were unrooted and planted in 20 identical flowerpots that each contained 2 kg of the same soil, that was brought from the same field (40 kg), and mixed before distribution into the pots. Each plant was fertilized with 5 g of potassium nitrate and irrigated with 500 ml of water. 10 of the plants were irrigated every 2 days with 20 ml of water each (control) and the other 10 were irrigated with 20 ml of *C. illinoinensis* extract every 2 days. All plants were kept in a laboratory hood under the same conditions of air flow, light and temperature. After 10 days, we tested the viability of the plants in two ways: if they are alive or dead, and if they are alive, are they partially dry or not. See Table 7 and Figures 7.

Results

Statistical analysis

Except for extractions (Table 3), that each was done in a single experiment, all data presented below, are average values of three experiments that we performed for each test.

Antifungal activity tests

Antifungal activity was measured as the inhibition percentage of mycelia growth of Rhizopus stolonifer. Two concentrations of extracts were used, 10% and 20% (w/w) in the extraction solvent and the results are shown in Table 4 and Figure 4.

Total phenolic content

Total phenolic content was determined by the method reported by us.[67]. Results are shown in Table 5 and Figure 5.

Table 3. Yields of extractions of *P. farcta* with four different solvents

Solvent	Water		Ethanol		Ethyl acetate		*n*-Hexane	
Yield	mass	%[a]	mass	%	mass	%	mass	%
	32.8	6.56	36.6	7.32	20.1	4.02	2.9[b]	0.58

Extraction yields: a - for each extraction, 500 g of dry plant powder (aerial parts) were extracted; b - since this yield is very low, we repeated this extraction three times and this is an average value.

Table 4. The antifungal activity of *P. farcta* extracts against *R. stolonifera*

Solvent	Water		Ethanol		Ethyl acetate		*n*-Hexane	
Extract Concentration (%, w/w)	10	20	10	20	10	20	10	20
Inhibition (%)[a]	18.2	22.1	26.3	27.9	26.3	27.8	32.5	36.8

a. Extraction solvent in each experiment was used as a control and resulted in 0 % inhibition.

Table 5. Total phenolic content of (TPC) extracts of *P. farcta*

Extract	Water	Ethanol	Ethyl acetate	*n*-Hexane
TPC[a]	12.4	13.9	10.9	5.1

a. mg of gallic acid equivalent in 1 g of dry extract

Table 6. The mortality rate of termites (*K. sinaicus*) as a result of feeding extracts loaded paper

Time (d)	----	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DR (%) WE	**TG**	0	0	0	0	10	10	10	20	20	20	30	30	30	30
	CG	0	0	0	0	0	0	10	10	10	10	10	10	10	20
DR (%) EE	**TG**	0	0	10	10	20	20	20	20	30	30	40	40	40	40
	CG	0	0	0	0	0	0	10	20	20	20	20	30	30	30
DR (%) EAE	**TG**	0	0	10	10	20	20	30	30	40	40	40	40	40	60
	CG	0	0	10	10	10	10	10	10	10	20	20	30	30	30
DR (%) HE	**TG**	0	10	10	20	40	50	70	80	90	100	100	100	100	100
	CG	0	0	0	0	10	10	20	20	20	20	20	20	30	30

DR, Death rate. WE, water extract; EE, ethanol extract; EAE, ethyl acetate extract, HE, n-hexane extract, TG, test group; CG, the control group

Table 7. *P. farcta* with dry branches after irrigation with aqueous extract *C. illinoinensis*

Number of Irrigations[a]	1	2	3	4	5
Plants with dry branches[b]	0	2	3	3	3

a) Every 2 days, 20 ml of C. illinoinensis aqueous extract for each plant; b) Total number of plants in test group n=10

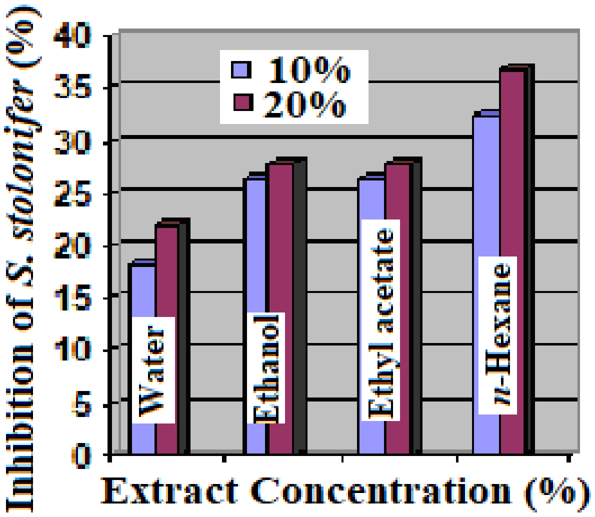


Figure 4: Inhibition (%) of R. stolonifer by extracts of *P. farcta*

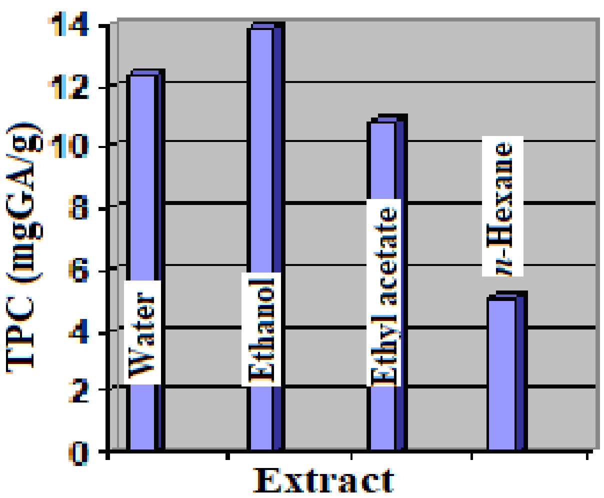


Figure 5. Total phenolic content of extracts of P. farcta (mg gallic acid in 1 g of dry extract)

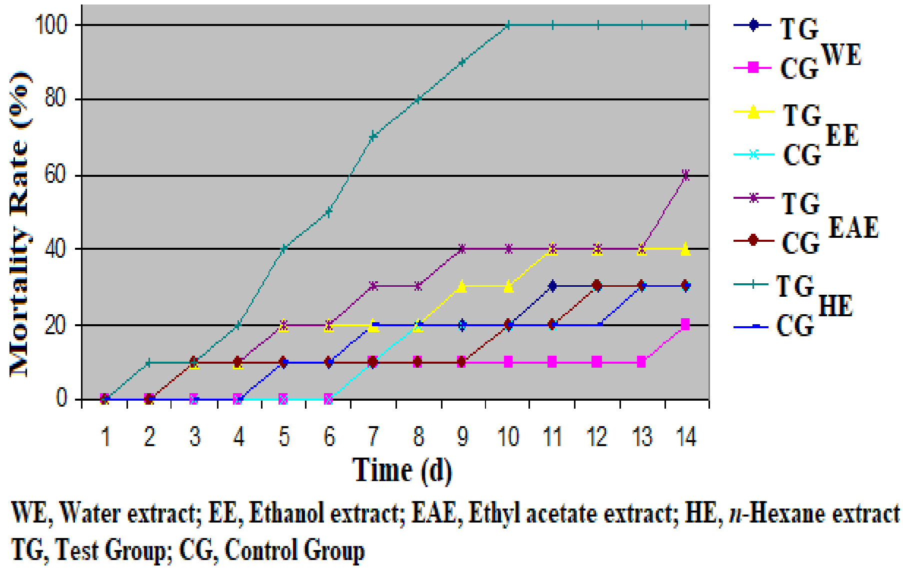


Figure 6. The mortality rate of termites (*K. sinaicus*) as a result of feeding extracts loaded paper

Anti-termite activity

Anti-termite activity was measured over 4 weeks. Results presented here are by day, where the mortality rate of termites (%) is presented in tested and control groups. Table 6 and Figure 6.

Biocontrol of *P. farcta* with *C. illinoinensis* aqueous extract

After irrigation of 10 plants of *P. farcta* with 20 ml of *C. illinoinensis* aqueous extract every 2 days (control irrigated with water), none of them died. But after the fourth day (second irrigation with extract), some of the test group plants started showing dry branches. The control group plants continued growing normally with no dry branches. Results are summarized in Table 7 and Figure 7.

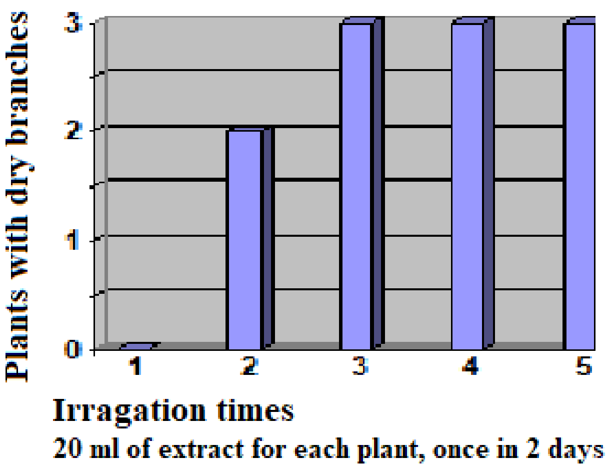


Figure 7. *P. farcta* with dry branches after irrigation with aqueous extract C. illinoinensis

Discussion

In this research, we studied some of the medicinal properties of *P. fracta*, that in addition to being a plant with high medicinal and other practical potentials, it is also a very widespread weed that harms agricultural fields. It is an invasive species not only in locations very far away of its natural habitat, but it also invaded parts of Western of Asia where it did not grow in the past.[77] So, in addition to studying some of it's not reported medicinal properties, one of the objectives of this research was to develop a biocontrol method against it. Two considerations were taken into account. First, the use of synthetic chemicals as herbicides has many adverse health and environmental effects. An excellent example of this can be glyphosate (Figure 8 A).[78]

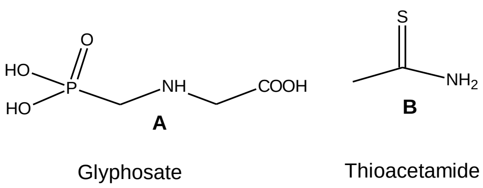


Figure 8. Structures of Glyphosate (A) and thioacetamide (B)

The second consideration is that use of plants extracts as herbicides is already known, especially of C. illinoinensis aqueous extract, that we used.[79] But success in this part was very limited: none of the tested group plants died. Some of them partially dried but they lived and a few weeks after treatment, they were all green again. This means that many more tests of this method are needed, but we also plan to use extracts of other plants, known for their toxicity.

As we have presented, medicinal and other properties of *P. farcta* are being published continuously. For example, in addition to testing its potential as an antioxidant according to classical methods (DPPH, FRAP, ABTS, etc.), some interesting studies are published. M. Zehab et al. tested the activity of seed hydroalcoholic (50 %) against oxidative stress that was orally induced in rats by thioacetamide (Figure 8 B).[80]

One of the most interesting reports that were recently published, examines the concentration of sesamin in *Cuscuta palaestina*, a parasitic plant that grows over other plants, such as *P. Farcta*.[81] Among five very common plants (near east) host plants (*P. farcta, Portulaca oleracea, Corchorus olitorius, Malva sylvestris* and *Cichorium intybus*), *C. Palaestina* that grew on *P. farcta* contained the highest concentration of sesamin: 8.45 ppm. Despite this, researchers proved that sesamin is produced by the parasitic plant and not transferred to it by the hosting plant.

Published reports of the different properties of P. farcta are mostly consistent, and in these cases, we did not examine these reports. This was not the case of the total phenolic content (TPC). A. Molan et al.,[25] reported 17.3 mg of gallic acid equivalent in 1 g of dry extract, while E. Karimi et al. reported 24.2.[26] This is not clear since both groups reported these results for methanolic extract. Our results are lower than both reports, 13.4 mg, but we tested ethanolic extract, which is expected not to be meaningfully different.

Toxicity of *P. farcta* to humans is still not clear. The only evident reported case was published in Turkey, where children (3.5-6 years old) consumed seeds with pods.[82] No other reports were published (or known) before or after this case. On the contrary, aerial parts of the plant can be used in many ways for wound healing as can be seen in the concise review article of Bahmani and Asadi-Samani.[83] But despite this, we reported here that extracts of aerial parts could be toxic to fungi and termites. For anti-termite activity, several efficient and facile methods were published and we found the method of O. Ndukwe best,[76] even though, A. Alshehry's method is also very useful.[84]

Conclusions

A- Many of the medicinal properties of P. farcta were investigated, but others were very partially studied (anticancer) or did not (antiobesity, anti-nervous system disorders).

B- The complete chemical composition of the plant is not known, and there is an urgent need for this, to promote drug discovery and other applications.

C- We reported good results of antifungal activity of the plant extracts. This research needs more studying in order to identify the active natural products responsible for this activity.

D- Our findings of anti-termite activity are very encouraging. Further research is needed.

E- n-Hexane extract proved very active. Special attention must be paid to this fact.

F- Biocontrol of P. farcta is still a very challenging subject since this plant is a weed that harms wide agricultural areas. Intensive additional research must be done.

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