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Submitted to the U.S. Department of State and U.S. Agency for International Development

Project and award number: M39-014- SIS70021GR3013

TITLE OF PROJECT: Use of brackish and recycled water to grow willow (*Salix* spp.) silage ('safsafage') as a healthy strategic feed for large and small ruminants

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Italics indicate project members who have retired or left the project. Klein is retired, but is continuing as a consultant.

**Asterisk indicates PI in Israel (Moallem, as of April 2023) and in Jordan (Awabdeh).*

Section I: Technical Progress

A) Research Objectives:

Main Objective:

The main objective of this project is the production of silage from willow ('safsafage') trees grown using non-potable water (saline or treated wastewater) as a nutritional and nutraceutical supplement for cattle, sheep and goats

Technical Project Objectives

1. Select Middle Eastern willow ecotypes for maximal seasonal and nutritional yield under irrigation with naturally-occurring brackish water or treated wastewater
2. Implement 1-3 ha plantations of willow using naturally-occurring brackish water or treated wastewater for mass production of silage in Jordan and Israel
3. Establish and disseminate best practices for making silage from willow ('safsafage')
4. Evaluate the performance of cattle, sheep and goats that are fed willow silage

Research Accomplishments July-December 2024:

Objective 1. Azaizeh received from Klein dried ground material from the May 2024 harvest of three willow genotypes grown in saline/brackish water (EC4-6, depending on Israel Water Authority at any given day) at ARDOM. His lab's analysis of the phytoactive components is as follows:

Genotype/Tree part	% Extraction yield	Phenol mg GAE/g extract	Flavonoid mg rutin \g extract	Salicylic acid mg \g extract
Americai twigs	14.2 a	163 a	145 a	50.7 a
Americai leaves	30.4	154 B	113 B	57.0 B
Red twigs	13.1 b	153 ab	124 b	60..5 ab
Red leaves	31.2	153 AB	109AB	71.1 A
White twigs	12.9 b	183 b	143 a	63.8 b
White leaves	29.8	173 A	119 A	69.5 A

Mean separation of 2 biological replicates in duplicate (n=4) by Tukey's Honestly Significant Difference test. Americai is Genotype C, Red is Genotype A and White is Genotype B (Muklada et al., 2020). Differences between twigs are separated by small letters and between leaves by capital letters. GAE= gallic acid equivalents.

There was no significant difference in % extraction yields within tissues between genotypes, except that Americai twigs had 8% higher extraction yield than the other two genotypes. Overall, Genotype B ('White' ecotype) had the greatest phytochemical content, which is in line with previous results. Trees of genotype B are usually much larger than those of the other two genotypes (Klein, Cohen and Muklada, unpublished), thus it can produce more healthy forage than the other two genotypes, both in quantity and in quality.

The silage made from this harvest has matured, and we will shortly measure its contents of healthy phytocompounds.

Objective 2. The planting at ARDOM in the southern Arava on supposedly virgin sandy soil became infested with reeds (*Phragmites australis* (Cav.) Trin. ex Steud.) over the years, despite initial efforts to weed the plot (See illustration below) By 2024 it was difficult to navigate the plots and to distinguish willow genotypes among the foreign foliage. Willow yield had plummeted. We made a final safsafage harvest in May 2024 (results detailed above and in report 7). In December 2024 Klein did a "rescue harvest" of all 9 ecotypes at ARDOM, and transported labelled cuttings to Muklada's laboratory at Gilat research center (see other illustration below). Muklada made multiple cuttings from each ecotype, and

will establish a 2000 sq m (2 dunam) plot with defined genotype and ecotype subplots on the banks of Nahalal stream at the Model Farm of ARO's Neve Yaar Research Station. The plot will be used both for purifying the water of the stream and for making safsafage for future animal feeding experiments and for project M42-038 (may its funding be assured speedily in our days) "Assessing the potential of willow trees as nectar and pollen source for honey bees under low-quality water irrigation".



(Left) Willow plot at ARDOM (looking east towards Jordan), infested with reeds. (Right) Muklada in his lab at Gilat, sorting harvested cuttings from all nine ecotypes prior to preparation for planting and maintaining the genetic range of the willow collection.

Objective 4. Evaluate the performance of cattle, sheep and goats that are fed willow silage

We had hoped to carry out a feeding experiment with safsafage for dairy calves in October 2023, but the outbreak of war prevented that. In the event, technical help has been limited because of constant call-ups to army reserve duty. We harvested another few hundred kg of willow and prepared safsafage in October, and the silage is maturing in barrels at Volcani Center. The trees used were grown with fresh water at a farm in central Israel that grows willows for ritual use during the Jewish holiday of Sukkot, but the plantings are only from one genotype (probably B). In any event, it is complicated and expensive to test all three genotypes in a calf-feeding experiment, so the safsafage from ARDOM will also be combined. We hope to conduct the experiment in April-May 2025, comparing weight gain, health (blood markers), and safsafage acceptability in calves fed from fresh- and saline-water irrigated willows, plus non-supplemented control feed.

C) Scientific Impact of Cooperation. The collaboration continues to be strong and mutually beneficial. Communication by whatsapp and email is frequent. The annual meeting was delayed by war and then finally took place in July 2024 at the International Congress on Natural Products Research meeting in Krakow, where Azaizeh delivered a talk and a poster was presented in the name of all the participants in the project.



Left to right: a) Awabdeh and Moallem at the meeting b) Klein, Moallem, Azaizeh and Awabdeh in front of the group's poster (Credit to MERC was printed at the bottom of the poster) c) Klein, Awabdeh, Moallem and Azaizeh discussing Continuation Project(s) and the continuation of M39-014.

D) Description of Project Impact: Two publications have resulted so far from research connected with the current proposal. A total of nine publications have appeared so far from the current Israeli-Jordanian consortium (previous MERC 33-018 project and the current one), in addition to two PhDs and two MSc degrees being awarded in Israel and Jordan based on MERC-sponsored willow research. A number of the same participants are involved in a third approved MERC project on honeybee use of willow (M42-038). However, despite receiving final approval by MERC, the project has not yet been implemented, due to political considerations.

E) Strengthening of Middle Eastern Institutions: Dr. Awabdeh has put NARC on the map regarding growing and using willows for small farmers of goats and sheep in Jordan. He has distributed many willow cuttings to small-holder farmers, many of whom are women. The plantings in saline- and recycled-water sites that are currently being established will strengthen the use of willows in a range of agricultural environments, and will help decrease reliance on imported nutritional supplements for goats and sheep in Jordan.

F) Future Work: Moallem and Klein still plan to feed yearling calves later in the coming year (2025) with safsafage made from willows grown with saline irrigation at Arava R&D and grown with fresh water at a commercial willow site in central Israel, as mentioned above. In Jordan, Awabdeh is continuing establishment of plantings at Ramtha and Khaldia, and has made safsafage to test in the lab and as a feed supplement for sheep. Awabdeh has worked separately with Azaizeh and with Muklada to submit two Continuation proposals to MERC, based on use of olive oil waste and on improved production/treatment of willow, respectively, both for healthy food supplements for farm animals. The fate of such projects is unknown, given the current US government's reorganization/shutdown of USAID. The present project has received a one-year extension, but there are managerial issues as detailed below.

Section II: Project Management and Cooperation

A) Managerial Issues:

1) ARDOM has finished its participation in the project, since the willow plot there is a) irremediably overgrown with reed plants and b) destined to be the site of an electrovoltaic farm.

2) In light of the effective (temporary until March, we fervently hope) closure of USAID by the Trump administration, grantees have been instructed not to be in touch with the MERC office. Klein will no longer be paid to administer the project, which in any event is frozen. On the other hand, trees and animals still have to be tended, in the hope that our project will be allowed to continue. All of us are working on the project, on a low flame....

B) Special Concerns: It used to be only the Hamas/Hezbollah war. Now it's the (temporary, we hope) closure of MERC projects, which have productively funded our team for the benefit of regional agriculture.

C) Cooperation, Travel, Training, and Publications: Awabdeh, Azaizeh, Moallem and Klein met at the International Congress on Natural Products Research in Krakow in July 2024, where Prof. Moallem finally met Prof. Azaizeh and Dr. Awabdeh. The names of all contributors to the project were listed as authors on the poster presentation, and Prof. Azaizeh also gave an oral presentation on the topic.

D) Request for Department of State and USAID Actions: Come back, please. We look forward to your return. Our collaborative work is useful and needed in many economic, scientific and political ways.