

Research project:

The chamal (*Dioon edule* Lindl.) in the state of San Luis Potosí

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Abstract

Dioon edule is a widely distributed species in the Huasteca zone, state of San Luis Potosi, Mexico, where its habitat has become deteriorated and populations reduced since crops and cattle breeding incursion. Although it is known that seeds and young leaves of chamal (*D. edule*) produce a deadly effect in cattle, for the indigenous people named *xi'oi* that inhabit this region, chamal harvest has had a major role in their diet from time immemorial, as a substitute of maize during bad times for yield. It is common to eat boiled and grounded seeds of chamal, although warnings of “fatal consequences” when they are not well cooked. This situation leads to a multidisciplinary research approach. La Palma region was selected to carry on this study, based on previous knowledge of chamal utilization by *xi'oi*. The purpose of the present study is to know what the situation of chamal habitat and natural populations is, what ethnobotany categories can be recognized, how traditional seeds cooking process is developed and how toxins could affect people that eat them. In this order, we will perform the chamal localities situation, habitat characterization and populations' structure analysis; ethnobotanical exploration with male key informants and interviews with housewives, and; neurotoxic effect experiment and neurological tests with Wistar rats in the laboratory.

Description

Mexico comprise approximately 20% of the cycad species richness distributed in the world and 80% of these are endemic. Based in the information about cycad species distributed in Mexico, at least nine species are present in the state of San Luis Potosi, Mexico: *Dioon edule*, *Zamia fischeri*, *Z. loddigesii*, *Ceratozamia hildae*, *C. kuesteriana*, *C. latifolia*, *C. mexicana*, *C. microstrobila*, and *C. zaragozae*. Of these, *Dioon edule* Lindl., is the most widely distributed, particularly in the Huasteca zone located in the Sierra Madre Oriental. Habitat deterioration of *D. edule* is evident, since many natural forests have been converted into pasture land for cattle or sugar cane fields. Cattle breeding have been devastating for many *D. edule* populations, because the species is considered extremely harmful for animals, and plants extermination is an effective option to avoid damage.

According to people, when cattle consume young leaves or cones, these produce a deadly effect starting with diarrhea, hind legs paralysis and finally decease. This could be attributed to toxic secondary metabolites produced in cycads that apparently serve as protection against predators. Although it has been suggested that these are essential for development and

maintenance of herbivorous associated to cycads, two of these compounds, the cycasin and macrozamin, could cause severe intoxication with carcinogen and neurotoxic effects in mammals. One study has reported low values of cycasin (0.1% fresh weight) and macrozamin (0.6% fresh weight) in *D. edule* seeds, but concluded that they are highly toxic for rodents.

However, one portion of the Huasteca region is inhabited by the indigenous people named *xi'oi*, whose major activity is agriculture based on maize, bean and sugar cane crops. Hunting does not represent a main source of food, but they consume gathering products, like maguey (*Agave* spp.) and chamal (*Dioon edule*) derivatives. Chamal harvest has had a major role in *xi'oi* diet from time immemorial, since this is a substitute of maize as nutritious basis for humans when maize and bean yield get lost. It is common for *xi'oi* people consuming tamales, gorditas and tortillas of chamal, obtained cooking dough made of boiled and grounded seeds. Some cook recipes alert of not well cooked chamal foodstuff that cause vomit, gastrointestinal disease or “fatal consequences” in people when consumed. Besides chamal common use as a food, it also has been used as medicine, ornament and others.

La Palma region (21° 47'N; 99° 28'W) was selected to carry on the research, based on the previous knowledge of utilization of chamal (*Dioon edule*) by *xi'oi* indigenous people that live in this region. We have stated three questions: 1) what is the situation of *D. edule* natural populations and their habitat? 2) what and how many ethnobotany categories can be recognized for *D. edule* plants? 3) how toxins present in the seeds of *D. edule* could affect *xi'oi* people that use to eat them?

To answer these questions in the first place, localities will be situated along an altitude gradient (600, 800 and 1000 m above sea level), where *D. edule* plants abundance and use frequency as food, medicine, ornamental and trade object among other uses, correspond. Planned field routes will be covered in the company of male key informants that will be selected on the basis of their knowledge, leadership and moral influence on their communities as well as their willing to collaborate with us. They will help us to select localities that reach the criterion of plant abundance and use frequency correspondence.

Selected localities will be geopositioned with a GPS, and the habitat characterized defining vegetation type, floristic composition, soil characteristics, superficial lithology, geomorphology and disturbing signals, like deforestation, fire, cattle incidence and others. *Dioon edule* population structure and spatial distribution will be characterized using 10 x 10 m plots arranged in transects.

Plants in each plot will be labeled and mapped, and on each plant height and diameter will be measured, number of leaves per plant counted and the reproductive condition noted.

With the same male key informants we will have open interviews in order to identify and give details of ethnobotanical categories related to *D. edule*. Regarding to plants commercialization, we will investigate the trade form, worth and volume as well as main markets in the region. We also will have interviews with housewives, preferably with selected male key informants', in order to know in great detail the several ways of seeds preparation as food, i.e. meal type and culinary variants, emphasizing in toxin removing procedure. Furthermore, we will record and collect additional plants that are included as secondary option in the chamal food culture, just as register this species presence or absence in the interviewed housewives plots.

Determination of neurotoxic effect of *D. edule* will be evaluated using male Wistar rats. Rats will receive human care and the experimental part will keep the Institution's guidelines and the Mexican Official Norm (NOM-062-ZOO-1999) regarding technical specifications for production, care, and use of laboratory animals. The animals will be assigned to seven groups; two groups control (-/+) and five groups of *D. edule* from different localities. Control rats will receive for two weeks deionized water daily by gavage; 2-Amino-3-(methylamino)-propionic acid (BMAA) rats will receive the dose by i.v. acutely; and, water extract of seed flour of *D. edule* rats will receive for two weeks the dose by gavage. The experimental dose will be based upon previous reports in rats which showed steady-state brain concentrations of 10 to 30 $\mu\text{g/g}$ and possibly could cause alterations in behavioral and locomotive activity.

Upon completion of the two weeks experimental period, the animals will be evaluated by two neurological tests. The first test is the Locomotor Rating Scale Basso, Beattie, Bresnahan (BBB), because this scale is a valid and predictive measure of locomotor recovery able to distinguish behavioral outcomes due to different injuries and to predict anatomical alterations at the lesion center. The second test is the evaluation of locomotion by unrestricted gait, because this method allows illustrate the spatio temporal sequence of hind limb movements during 4 to 6 step cycles. Swing (flexion, -F-, plus extension, -E1-) and stance phase (extension, -E2+E3-) duration, stride length, duration and speed will be measured.

How the project fits the interest of TCS

We consider that this research project approach must be multidisciplinary, and the proposal includes ecological, ethnobotanical and toxicological investigation. We are awareness that this is

just the beginning of a long-term research, but our expectation at the end of the first year includes benefits like ecology, ethnobotany and toxicology research promotion on an endangered species poorly known in the state of San Luis Potosi; conservation promotion of existing populations of *Dioon edule*; compilation of traditional knowledge of *xi'oi* people related to this species; and exploration of possible toxic effects of this species seeds as food.

Timetable

Activity	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
Localities selection	■	■	■									
Exploration with male key informants		■	■	■	■	■						
Housewives' informants interviews				■	■	■	■	■	■	■		
Localities habitat characterization	■	■	■	■	■	■	■	■	■	■		
Population structure and distribution characterization		■	■	■	■	■	■	■	■	■		
Neurotoxic effect experiment and neurological tests								■	■	■		
Data analysis				■	■	■	■	■	■	■	■	■
Progress report						■						■

Budget

Currently we are not able to apply for funds from the Autonomous University of San Luis Potosí [Universidad Autónoma de San Luis Potosí] Research Support Fund, but we would be eligible in November 2008 to continue developing the project. Besides, we have not received any financial support because we are going to start the proposed project.

Activity	Amount (US\$)
Expenses for localities selection	270
Expenses for field routes exploration and populations' measurements	600
Expenses for accommodation and interviews with informants	500
Garmin® GPS	380
2-Amino-3-(methylamino) propionic acid hydrochloride	380
60 male Wistar rats	300
Wistar rats maintenance and care	70
TOTAL AMOUNT REQUESTED	2500