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Research Article

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Biological Importance of wild Sunflower Seed (*helianthus annuus* l.) Weed of North Tamaulipas, Mexico

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Abstract

Helianthus annuus L. or wild sunflower is a weed that reproduces by seed, and affects sorghum and corn crops. The objective was to determine the importance of H. annuus seed and soil salinity in crops in northeastern Mexico. The seed was hydrated for 24 hours to evaluate germination and vigor, then the seeds were placed on filter paper in Petri dishes at 5° C for seven days at 25° C. The variables to be evaluated were: seedlings, percentage of dead and hard seeds, and vigor at the first count. The incidence of fungi associated with the seed was also evaluated, which were morphologically identified, as well as the incidence of insects in the heads, the number of larvae and adults were recorded. It was determined that the soils of the sampled localities have low salinity levels (CE=1.23 dS m^{-1}), with high levels of sodium and sulfates (10.6 me L^{-1}). The effect of the collection on the physiological and sanitary condition of the seed was observed. The mean was 67.2 and 5.2 % of normal and abnormal seedlings, 8.7 and 20.5 % of dead and hard seeds, with 2.0 % vigor. That is, this species has a percentage of seeds that can remain in the ground in dormancy to reproduce. Alternaria alternata, Fusarium oxysporum and the beetle Lasioderma serricone were the pathogens found in wild sunflower seed.

Keywords: Importance, Wild Sunflower, Seed, Dormancy

1. Introduction

The Asteraceae family is characterized by having pseudontic inflorescences (simulating a flower), which are called heads or capitula [1]. The wild form of the species *Helianthus annuus* L. commonly known as wild sunflower or polocote is native to northern Mexico and the western U.S. [2,3]. It is a weed of economic importance in the north of the state of Tamaulipas, mainly in the localities of San Fernando, Matamoros, Valle Hermoso, Río Bravo and Reynosa, important producers of sorghum [4,5]. According to *H. annuus* is an annual plant, with a height of up to three meters, a simple or branched erect stem, generally robust and rough [6]. The head or flowers are solitary or grouped at the end of the stems. The fruits and/or seeds are achenes oblong ovoid, somewhat compressed, 3.5 to 5.5 mm long, greyish, often mottled, pappus with two lanceolate scales, deciduous.

The seed of this species is of great agronomic and biological importance, as it ensures offspring in the next generation, and it is possible to observe changes in the response to the frequent application of herbicides [7]. Annual weeds such as wild sunflower form a population from viable seed, which has different degrees of dormancy which varies according to the species in the genus Helianthus [8]. Part of the seed remains as a reserve in the soil, which has the ability to germinate and establish populations in the following seasons [9]. *H. annuus*, in addition to competing directly with the crop, has the potential to be an alternate host for insects,

pests, and pathogens; it also has a broad capacity to adapt to environmental conditions in agroecosystems [10,11]. An important factor for its adaptation is the type of soil, since its physical and chemical properties influence the variability, distribution and prevalence of weeds in the field [12].

Cultivated sunflower is moderately tolerant to soil salinity without causing a significant reduction in yield up to 4.8 dS m^{-1} sensitivity is higher in early stages of growth at < 2.0 dS m^{-1} [13,14]. The wild sunflower has a wide distribution and covers almost the entire area of northeastern Mexico, to date there are no studies or reports of the importance of the seed of *H. annuus* in the aforementioned region. The objective of this research was to determine the biological importance of the wild sunflower seed *H. annuus*, as well as to determine the soil salinity conditions in the sampling area in northeastern Mexico.

2. Materials and Methods

2.1. Sampling area and Biological Material: Seed was collected during the month of May and June 2023, from individual plants with a similar phenological state, using a simple sampling technique [15]. In the municipalities of northeastern Mexico in the state of Tamaulipas (Table 1): San Fernando (3 localities); Matamoros (1 locality), Río Bravo (3 locality) and Valle Hermoso (3 locality). The size of each sample/locality was 100 plants, at harvest the capitula or inflorescence were integrated into a composite sample. Subsequently, the inflorescences were transported in paper bags to dry in a forced-air stove at a temperature of 34 °C, for 72 h. The dried seed was threshed and cleaned of the largest impurities of the capitulum and leaf; subsequently, the samples were cleaned manually. The samples were kept at room temperature (25 °C) in the Water, Soil and Plant laboratory of the CERIB-CIRNE, until the time of testing. In addition; from each locality, the soil was sampled to determine electrical conductivity (dSm^{-1}), Ca, Mg, Na and K minerals (me L^{-1}), salts: carbonates, chlorides and sulfates (me L^{-1}) were also sampled by analytical methods, the results were made in triplicate [16].

Locality	Crop	Sampling		Geographical Location	
		Identification	Date	N	W
San Fernando	Sorghum	L1a	23/05/2023	24,73	-97,94
San Fernando	Sorghum	L1b	23/05/2023	24,76	-98,00
San Fernando	Sorghum	L1c	23/05/2023	24,76	-97,99
Matamoros	Sorghum	L2a	05/06/2023	25,51	-97,70
Río Bravo	Corn	L3a	14/06/2023	25,82	-98,12
Río Bravo	Sorghum	L3b	14/06/2023	25,85	-98,12
Río Bravo	Sorghum	L3c	12/05/2023	25,97	-98,02
Valle Hermoso	Sorghum	L4a	12/06/2023	25,69	-97,78
Valle Hermoso	Sorghum	L4b	12/06/2023	25,69	-97,78
Valle Hermoso	Corn	L4c	12/06/2023	25,70	-97,78

Table 1: Origin of wild sunflower seed collections in northeastern Mexico

2.2. Latency, Germination and Viability: Treatment to break latency was first performed with pre-hydration. A sample of two grams of clean seed was placed in 50 ml of drinking water (0.026 dSm-1 electrical conductivity and 7.23 pH) for 24 h, at laboratory temperature (25 ºC), then the water was drained and the standard germination test was performed [17]. The seed was sown on October 29, 2023 on filter paper, 20 seeds per plastic Petri dish (8 cm), previously watered, with four replications for each collection. The boxes were kept at 5 °C, in a germination chamber, for seven days. Subsequently; they were removed and kept at laboratory temperature (25 ºC), as well; the counts were made every third day until they stopped germinating [18]. The variables were: normal seedlings (PN) with feather and radicle, when observing complete development and abnormal seedlings (PA) when there is deformation of these fundamental structures, dead seeds (SM) which did not show reaction or pigmentation staining in their vital structures, with tetrazolium; during the viability test and hard seeds (SD), those that showed viability, but do not hydrate and do not germinate after 14 days after planting; the results were expressed as a percentage [17,19]. Likewise, the vigor of the seed (V) was quantified with the technique at the first count, in this case it was three days after planting [20].

Feasibility test. It was performed with 1.0% tetrazolium (chloride of 2, 3, 5 triphenyl tetrazolium) was used to verify the viability of the seed; was diluted in 100 ml of the phosphate buffer (1.1% potassium diphosphate and 0.9% sodium monophosphate [21]. The seeds that did not germinate from each Petri dish were placed in 5 ml of water/24 h at room temperature, then cut into halves. The boxes were covered with the Tetrazolium solution for 24 h at room temperature; at the end they were drained and washed [22]. The indicator of viability was the red or purple coloration of the embryo, due to the reduction of tetrazolium due to the respiratory activity of the cells; pale colors or lack of coloration indicate poor viability or death of the embryo [23].

3. Identification of Agents Associated with Wild Sunflower Seeds

3.1. Isolation and Identification of Mycoflora: After the germination test, the Petri dishes were observed by means of a stereoscopic microscope (Zeiss Discovery V8 Stereo Plan 51, 0 X), to inspect the coat or pericarp of the seeds and identify any fungal growth. Subsequently, with a sterilized scalpel, the mycelium samples were taken, then they were transferred to Petri dishes with PDA, using the scratching technique, four replications per collection. The boxes were incubated at laboratory temperature (25 °C) for eight days, after which the colonies were identified, according to their morphological characteristics, from conidiophores, colony color, mycelium type, and conidia [24-26]. The variables that were determined were: percentage of colonized seeds (SC=number of seeds with mycelium development/total seeds x100), to calculate the percentage of infection to determine the quantitative differences between fungi, which colonize the seed, the relative abundance or incidence of the species was calculated it was calculated as follows: I= Total number of colonies of a species/ total number of colonies of all fungi [27].

3.2. Insects: From each municipality, a sample composed of 1 kg of soil was made, which was later divided into four subsamples of 250 g each, these were screened separately to quantify the presence of insects per sample; total number of adults (AI) and larvae (L). Subsequently, the individuals were preserved in 70% alcohol + 30% glycerin in entomological jars and were reviewed by Dr. José Francisco Rodríguez Rodríguez at the Entomology Laboratory of the Bajío Experimental Field, Guanajuato, Mexico of INIFAP. The taxonomic keys described by were used for their identification [28].

3.3. Statistical Analysis: The data from the results of the germination test, vigor and the sanitary test (mycoflora associated with the seed and insect incidence) were analyzed under a completely randomized design, with four replications, as a variation factor were ten collections (NC) of wild sunflower seed. An analysis of variance was performed and the differences between the means of the treatments were compared using Tukey's test (p≤0, 05). Simple Pearson correlations were made with the soil variables; The analysis was carried out with the SAS program [29]. The percentage data were transformed by arcosine of the square root of X/100 before analysis to homogenize variances; however, in the tables the results are presented with the data retransformed by sine Pérez.

4. Results and Discussion

The soils of the region under study where wild sunflower grows showed low salinity levels from 0.6 to 1.1 dS m-1; except for the sample from point L3a (Río Bravo) that had levels of 3.9 dS m^{-1}, which is considered moderate and this can be attributed to the concentration of sodium, chlorides and sulfates (31, 8.7 and 30.3 m L^{-1}) which were the highest values recorded among all the samples (Table 2). The correlations associated with soil conductivity, with the highest statistical value (p≤0.001) were the concentration of calcium (r= 0.87), sodium (r= 0.89), chlorides (r=0.84) and sulfates (r=0.86). In areas where soil sodium levels range from 5 to 200 m L^{-1}, in cultivated sunflower they suppress the rate and time of seed germination [30]. Ma report that soil salinity is the main factor limiting the vegetative growth of sunflower from sowing to sprouting, with moderate values of electrical conductivity (2.5-3.6 dS m-1) to very high levels (9.6-10.7 dS m^{-1}) [31]. A direct effect of salinity on cultivated sunflower is the reduction in leaf size and number during vegetative growth [32]. In other words, this weed would have low chances of germinating and establishing itself in soils with high levels of calcium, sodium, chlorides and sulfates. Therefore, it can be deduced that the tendency of wild sunflower would be to invade areas with greater soil fertility, since soil salinity is a condition of soil degradation; and affects the genus *Helianthus* [33].

L	EC	Ca	Mg	Na	K	Carbonates		Chlorides	Sulfates
	(dS m^{-1})					($Ca(HCO_3)2$	($CaCO_3$)	(Cl-)	(SO_4^{2}-)
		(me L^{-1})							
L1a	0.6 b	2.8 c	1.3 a	9.2 c	0.8 a	2.7 a	0.1 b	1.4 c	10.0 c
L1b	0.6 b	2.7 c	1.2 a	8.5 c	0.7 a	2.5 a	0.1 b	1.3 c	9.3 c
L1c	1.0 b	2.1 c	1.1 b	6.0 c	0.6 a	3.1 a	0.1b	1.5 c	4.5 d
L2a	1.8 b	4.5 b	2.4 a	19.0 b	0.5 a	0.5 b	0.3 b	3.9 b	21.8 b
L3a	3.9 a	6.2 a	1.5 a	31.0 a	0.7 a	0.2 b	0.2 b	8.7 a	30.3 a
L3b	1.0 b	2.0 c	2.0 a	5.3 c	0.3 a	0.4 b	1.6 a	3.0 b	5.3 d
L3c	0.6 b	3.0 b	1.3 a	12.4 b	0.1 a	0.4 b	0.2 b	5.0 b	11.2 c
L4a	1.0 b	1.8 c	2.0 a	5.7 c	0.5 a	0.4 b	1.2 a	2.0 c	6.5 d
L4b	0.7 b	1.5 c	2.0 a	2.9 d	0.5 a	0.3 b	1.6 a	2.0 c	3.0 d
L4c	1.1 b	2.1 c	1.1 b	6.0 c	0.6 a	3.2 a	0.0 b	1.5 c	4.5 d
media	1.23	2.87	1.59	10.6	0.53	1.4	0.54	3.0	10.6

L: Locality; EC: Electrical conductivity; Ca= Calcium; Mg= Magnesium; Na=Sodium; K=Potassium; $Ca(HCO_3)2$= Calcium bicarbonate and $CaCO_3$=Calcium carbonate. Columns with the same letter are not statistically different (Tukey ≤ 0.05).

Table 2: Parámetros de la calidad de suelo, asociados con el nivel de salinidad

The analysis of variance showed a significant effect of the collection on germination and vigor results. The differences in the level of latency between the different collections in this experiment (Table 3); attribute it to the effect of the agroclimatic conditions that prevail during seed development and to the rate of desiccation in the mother plant as reported in cultivated sunflower [34]. Likewise, the interactions between genetic background versus environmental conditions and their effects on the dormancy level of cultivated sunflower seeds are not well understood [35].

In the comparison of means, statistical and contrasting differences were observed between the collections (Table 3). In the collections of San Fernando, this difference is marked, in the percentage of normal plants; were statistically similar in the samples. report in cultivated sunflower, 25 °C of optimal temperature for germination and significant seedling development. The results of this evaluation prove that also for wild sunflower the temperature of 25 °C was favorable for the development of normal seedlings. report genotypes with 80.0% germination or root emergence in cultivated sunflower, and only form between 30.0% and 40.0% of normal seedlings [8,36]. In this experiment, on average, the number of abnormal seedlings was 5.2 % (Table 2), i.e., seeds that were hydrated, but in most of them, the lack of elongation of the hypocotileus and the emergence of cotyledonar leaves were observed; likewise, this species presented epigeal germination. Another important aspect of the test was the number of hard seeds. On average (20.5%) it was 3.9 times higher, compared to the number of abnormal seedlings (5.2%) and 2.4 times higher than the number of dead seeds (8.7%), and even the Matamoros collection (L2a) had 36.2% hard seeds. These results are related to the presence of dormancy in the seed of the genus *Helianthus* [33]. In many species, it is reported that water treatment favors and standardizes seed germination especially those with hard heads such as alfalfa on the other hand, indicate that pre-hydration in the seed can occur unevenly, leading to staggered germination [37-39]. During this evaluation, a differential germinative capacity of the seeds was observed, since, 72 h after the start of the germination test, statistical contrasts in vigor were recorded at the first count (Table 3).

In the L1a of San Fernando, a vigor of 6.9% was obtained, while L1b presented a value close to zero, the same occurred with the collection of Matamoros with zero percent, even, after 14 days, at the end of the test it had the highest number of hard seeds (36.2%). This response can be attributed to the structure of the pericarp, which constitutes a barrier to hydration, as occurs in cultivated sunflower, on the other hand, use scarification in seeds of wild species of the genus *Helianthus*, to improve germination; this procedure consumes time, and in small seed such as that of the polocote (4±0.16 mm), it can damage the embryo region and reduce the number of individuals [8,40].

Locality	**Collections**	**Germination and vigor (%)**				
		PN	**PA**	**SM**	**SD**	**V**
San Fernando	L1a	80,0 ab	5,0 b	5,0 c	10,0 c	6,9 a
San Fernando	L1b	58,7 c	12,5 a	20,0 a	8,8 c	0,93 bc
San Fernando	L1c	75,1 ab	8,7 ab	6,2 ab	10,0 c	1,3 bc
Matamoros	L2a	60,0 bc	1,2 c	2,5 c	36,2 a	0,0 c
Río Bravo	L3a	66,2 bc	3,7 b	2,5 c	27,5 ab	1,6 bc
Río Bravo	L3b	61,2 bc	6,2 b	11,2 ab	21,4 b	3,8 ab
Río Bravo	L3c	76,2 ab	2,5 bc	5,0 c	16,3 bc	1,3 bc
Valle Hermoso	L4a	51,2 c	5,0 b	17,5 a	26,3 b	2,2 bc
Valle Hermoso	L4b	58,7 c	3,7 b	3,7 c	33,9 ab	0,62 bc
Valle Hermoso	L4c	80,0 ab	3,7 b	3,7 c	12,6 c	0,93 bc
media		67,2	5,2	8,7	20,5	2,0
CV (%)		13,6	18,9	21,2	16,6	25,7

PN: normal seedlings and PA: abnormal seedling ; SM: dead seeds and SD: hard seeds. V: Seed vigor. Columns with the same letter are not statistically different (Tukey ≤ 0.05).

Table 3: Germination and vigor of wild sunflower seed (*H. annuus*)

In this experiment, morphological and molecular identification showed only two species in association with wild sunflower seed: *Alternaria alternata*, which is reported in sunflower cultivated in different parts of the world; as the main causative agent of leaf blight as for example in South Africa and *Fusarium oxysporum* [41]. In cultivated sunflower, several species are reported as a causative agent of wilt including *F. oxysporum* and *F. helianthi* [42]. Addrah identified five species of Alternaria spp. in sunflower seed: *Alternaria tenuissima, Alternaria alternata, Alternaria helianthiinficiens, Alternaria longipes,* and *Alternaria tamaricis*, in addition to three species of *Fusarium spp*. such as *Fusarium oxysporum, Fusarium incarnatum* and *Fusarium proliferatum*, as

well as Verticillium dahliae and Cladosporium cladosporioides. The presence of pathogens in the seed of wild species, such as *H. annuus*, is a mechanism for controlling the weed population, especially when the seed is found deeper in a seed bank in the soil [43,44].

In this trial, low diversity of species that contaminated wild sunflower seed was observed. The results showed that there was a significant effect of the collection on the incidence of fungi that colonized the seed, on average it was 11.3 % (Table 4). Within the same locality, there were statistical differences in the incidence of *Fusarium*. In the town of San Fernando there were no statistical differences between the L1b and L1c collections (25.0 and 16.3 %), while the L1a collection had low frequency (3.8 %); with respect to the three collections from the town of Río Bravo, L3a (2.5%) and L3c (11.3%) had the highest statistical contrast and of the three collections from the town of Valle Hermoso they had the highest statistical contrast between L4a (28.8%) and L4b (3.8%). The L1b collections from San Fernando (25.0 %) and L4a from Valle Hermoso (28.8 %) statistically surpassed the L2a collection from Matamoros (6.3 %) in the incidence of *Fusarium* (Table 4). With respect to the incidence of *Alternaria* among the different localities and collections was zero to low (0.0 to 1.3%), only in the locality of Valle Hermoso was the highest incidence with 8.8%, statistically surpassing the rest. In general, *F. oxysporum* was present 3.5 times higher than *A. alternata* in this evaluation.

Locality	**Collections**	**SC (%)**	**Incidence (%)**	
			FUS	**ALT**
San Fernando	L1a	3,8 c	8,8 ab	0,0 b
San Fernando	L1b	25,0 a	5,0 abc	0,0 b
San Fernando	L1c	16,3 ab	10,0 a	0,0 b
Matamoros	L2a	6,3 bc	2,5 bc	1,3 b
Río Bravo	L3a	2,5 c	1,3 c	0,0 b
Río Bravo	L3b	6,3 bc	2,5 bc	1,3 b
Río Bravo	L3c	11,3 ab	3,8 abc	0,0 b
Valle Hermoso	L4a	28,8 a	6,3 abc	1,3 b
Valle Hermoso	L4b	3,8 c	6,3 abc	8,8 a
Valle Hermoso	L4c	8,8 bc	3,8 abc	1,3 b
media		11,3	5,0	1,4
CV (%)		30,4	33,5	37,9

SC: Seeds colonized by mycelium; FUS: Colonies of *Fusarium oxysporum*, ALT: Colonies of *Alternaria alternata*. Columns with the same letter are not statistically different (Tukey ≤ 0.05).

Table 4: Fungi and insects associated with wild sunflower seed (*H. annuus*)

In seed from 12 weeds collected in Tocantins, Brazil, an incidence of *Fusarium* sp. is reported between 5.5 % and 82.0 %, while *Alternaria* sp. has a range between 0.0 % and 45.0 %, these values are a function of the weed they colonize; in addition, the frequency of the former was on average 22.0 %, while the latter was 8.0 %, in other words, *Fusarium* sp. it is 2.8 times more than *Alternaria* sp. [11].

Larvae and adults of *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Anobiidae), commonly known as the tobacco weevil, were found. Adults of *L. serricorne* are oval and hairy body, 2 to 3 mm long, and reddish-brown in color [45]. High populations of *L. serricorne* were observed in the town of San Fernando compared to the municipalities of Matamoros, Río Bravo and Valle Hermoso, respectively. No statistically significant difference (P=0.001) was observed per sampling point within each municipality; however, a statistical difference was observed between the localities of a municipality (Table 5). *L. serricorne* is a major pest in the food and tobacco industry in many parts of the world, attacking mainly during the preservation and storage stage of food products of plant and animal origin [46,47].

Locality	**Collections**	**LV (%)**	**IA (%)**
San Fernando	L1a	40,0 a	16,0 a
San Fernando	L1b	39,0 b	13,0 ab
San Fernando	L1c	29,0 ab	10,0 bc
Matamoros	L2a	17,0 bc	9,0 bc
Río Bravo	L3a	16,0 bc	7,0 bc

Río Bravo	L3b	15,0 bc	7,0 bc
Río Bravo	L3c	11,0 c	6,0 bc
Valle Hermoso	L4a	12,0 c	7,0 bc
Valle Hermoso	L4b	9,0 c	4,0 c
Valle Hermoso	L4c	11,0 c	7,0 bc
media		19,9	8,6
CV (%)		25,0	23,6

IA: Insects in the adult state and LV: Insect larvae. Rows with the same letters are not statistically different (Tukey ≤ 0.05).

Table 5: Incidence of *Lasioderma* serricone in the seed of the polocote (*H. annuus*)

Our observations indicate that the wild sunflower seed is a host of *L. serricorne*, the larvae bore into the seeds and feed on them. Later, when the adult comes out, it mobilizes to colonize other wild sunflower plants or polocote, and even migrate to grain stores where they represent a greater problem. In addition to the direct problems that wild sunflower represents as a weed in northeastern Mexico, it is important to consider the secondary damage caused by being a host of a pest of great economic importance such as *L. serricorne*.

5. Conclusions

The wild sunflower populations that were obtained from the different localities of Northeast Mexico were characterized by growing in soils with low levels of salinity and concentration of salts except for sodium and sulfates. Differences were observed; in the germinative capacity of the seed between localities; that is, in biological terms, there was a remnant of hard seed, as a result of dormancy; which can germinate and form new populations of this weed. Fusarium oxysporum and Lasioderma serricone were found in the seed of the wild sunflower Alternaria alternata as a host of pests and diseases [48-53].

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