Introduction of the neem tree in Mexico, in vitro propagation and validation of its properties against stored-product insects

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Abstract

Neem seeds from Philippines were sown in México on 25 August 1989, with a 91% emergence. In 1990, 444 small trees were distributed around México and 55 were planted in our school campus. Trees (47) from seed from Honduras, were distributed in 1992. The dissemination, up to date, includes 171 sites in 20 of the 31 States, and the Federal District of México. Seed from trees planted in Nuevo León was sown in 1993 and 178 Mexican little trees were obtained.

Work was undertaken to propagate neem trees by cuttings. Fifteen to thirty percent of the cuttings planted developed roots and survived, either in a greenhouse or in the field. Calli occurred in all petiole explants cultured, but no further development was observed. At week 20, shoots and roots were present in 75 and 100% of the foliar explants, respectively, in medium MS added with 0.4 mg/L thiamine and 100 mg/L mio-inositol.

Leaf powder (1.5 to 2.5%) in maize caused significant mortality of *Sitophilus zeamais*; grain with a 0.5 to 2.5% dose had less damage than the control 60 days after infestation. Doses 2.0 to 6.0% produced adult mortality, and reduction of total population of *Tribolium castaneum* infesting maize, 15 and 78 days after infestation, respectively. Neem leaf powder did not show any type of action against *Zabrotes subfasciatus* in stored pinto beans.

Introduction

Many subsistence farmers in México store a large part of the harvested grains in their own modest farms. The amounts stored are small and it is not economical to buy chemicals to control the damaging insect pests. Furthermore, insecticides are not easily available or they are sold only in big containers, not easily utilised by farmers. Under these conditions, it would be valuable to be able to rely on using insecticidal plants. The use of botanicals for insect control is becoming more popular in México, now that the disadvantages of synthetic insecticides, such as toxicity, contamination and resistance are better understood.

Plants with insecticidal properties are abundant in nature (Burroughs et al. 1988, Ahamed et al. 1984). A large number of plants that can be used to control insects has been reported in México (Lagunes-Tejeda and Rodríguez-Hernández 1989, Vidal-Espejo and Leos-Martínez 1993, López-Sanchez and Leos-Martínez 1993).

A few plants, distributed worldwide, have been found so efficient for insect control that many countries have imported them. The neem tree (*Azadirachta indica* A. Juss) from the Indian-Pakistani region, is now grown in many countries of the world (Ahamed and Grainge 1986). A description of the first program to legally introduce and distribute the neem tree into México follows. The objectives of this study were: 1) to distribute the neem tree to all possible regions of México; 2) to develop techniques for in vitro propagation; and 3) to evaluate the insecticidal properties of the neem tree against stored-product insects.

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Importation and Dissemination

The initial arrangements for importation of the neem tree into México were commenced in 1987 with the cooperation of Dr M. S. Swaminathan, who was director of the International Rice Research Institute (IRRI) in Manila, Philippines and Dr Ramesh C. Saxena from the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya), who at that time was based in the Department of Entomology of the IRRI as the project coordinator on botanical pest control.

The permit for neem seed importation from the Philippines was given by the Dirección General de Sanidad y Protección Agropecuaria y Forestal (General Direction of Agronomic and Forestal Health and Protection) of the Secretaría de Agricultura y Recursos Hidráulicos (Secretary of Agriculture and Hydraulic Resources) of México. Special help was given by the Dirección General de Sanidad Vegetal (General Direction of Plant Health) of the State of Nuevo León. The permit was granted with the following sanitary prerequisites: certificate of origin, international phytosanitary certificate, treatment with aluminium phosphide, and visual inspection. The Mexican exterior quarantine No. 14 against the Khapra beetle, *Trogoderma granarium* Everts, was in effect.

At 9 August, 1989 Dr Saxena sent about 200 g of freshly harvested neem seeds (700 units) collected in Manila, Philippines, from a 8-year-old tree of Indian origin. The shipment arrived on 15 August, and included a Phytosanitary Certificate (FAO International Plant Protection Convention) that guaranteed the material free of pests and diseases. The seeds had been prepared for storage and sowing. The pulp of the fruits had been removed and the kernels were dry, clean and treated with a fungicide. The seeds were large and uniform in size, around 1.5 cm long.

Seeds were sown on 25 August 1989. A mixture of 2 parts of river sand, 2 parts of garden soil, and 1 part of perlite, was prepared as the sowing bed. The mixture was placed in plastic bags of 1L capacity and seeds were sown individually 1.5 cm deep. The bags stayed inside a greenhouse with temperatures around 30°C. The soil was kept moist for 30 days with weekly waterings containing 0.1% of the fungicide Captan. Waterings and fungicide applications were then reduced. The emergence of plants started six days after sowing and reached a maximum of 91%, 28 days after sowing, on 22 September. A total of 636 plants were obtained and numbered. The seedlings were moved to bags of 5L capacity in November.

Three problems damaged and killed some seedlings. On 24 December, a heavy frost (which devastated the citrus region) affected the seedlings located close to a half-open door and killed five. When the seedlings were about 25 cm high, a general chlorosis had to be corrected with foliar fertilisations.

Several fertilisers were tried initially and 0.5% urea was selected and used subsequently. The worst problem was the root disease *Rhyzoctonia* spp., which killed some 86 plants, this was subsequently controlled with Captan.

The plants stayed inside the greenhouse until May, when they were transferred to the field under a roof made of branches to give shade.

Temperature and relative humidity inside the greenhouse and in the shady location were registered periodically with a hygrothermograph. Height was measured periodically in 25 plants (Table 1); it was very variable: the range at the last estimate (3 July 1990) was 23.0–118.5 cm, with a mean value of 69.7 cm. No dimensions were taken after this time, because the tallest plants were donated first.

The dissemination of neem began in April 1990. Some of the small trees were planted personally, but most of them were planted by someone else; in both cases there was no monetary charge. The objective was to plant at least one tree in most municipalities of Nuevo León State and a few in each of the other States of México. Preference was given to locating plants in public schools, higher education institutions, and rural research and development areas, however, many plants were given directly to farmers. Individuals who received plants, signed a letter of agreement in which they agreed to take care of the trees in the best possible way and to participate in further dissemination.

Many plants were given to the participants of the II National Symposium on Plant and Mineral Substances for Pests Control) of the Mexican Society of Entomology held at Oaxaca city in the south of México, in May 1990.

Table 2 is a record of neem plants from Philippine seeds donated to particular institutions. A number of 394 small trees were distributed in 171 different locations of 85 municipalities of 19 of the 31 states of México, and two delegations of the Federal District. The other 50 plants were given to Dr Angel Lagunes-Tejeda (Colegio de Postgraduados, Chapingo, México State) for him to keep or for further distribution. In addition, 55 small trees were planted in our institution: 20 in the gardens and 35 to make a small orchard. The 46 remaining trees of the 636 seedlings produced, were given (or taken) without filling a registration form. Around 545 small neem trees were planted in México during 1990.

Dr Simon E. Malo, director of the Escuela Agrícola Panamericana Zamorano in Honduras, Central América sent 200 g of seeds on 3 November 1990; they arrived 10 days later. The trees in Zamorano were generated from seeds from Haiti planted since 1981. The shipment included a certificate indicating that treatments and inspections rendered the seeds free of any pest or contamination. The condition of these seeds was very similar to that of those from the Philippines, but their size was smaller and more variable.

A selection of the seeds from Honduras was made; of these, 89 were eliminated because they were broken, and the rest were classified into four groups according to their size. Seed was sown on 16 November 1990. Conditions and procedures were similar to those used for the seed from the Philippines, but the soil mixture consisted of 2 parts of garden soil and 1 part of sawdust. Similar fungicide treatments were used, and the characteristics of the seeds and data on germination and plant height are given in Table 3.

Germination was very poor and slow in contrast with that of the Philippines' seeds. The effect of seed size on percentage of germination was recognisable. The highest germination values were 10.7 and 11.0% for large and medium seeds, respectively. Only 2.6% germination was obtained from tiny seeds. A total of 104 seedlings emerged, but only 73 plants survived after six months. The height of the plants one year after sowing, still exhibited the effect of seed size; nevertheless, the plants from large, medium, and small size seeds from Honduras were, on the average, taller than the plants from the Philippines seeds of the same age. Temperature was not the reason for the germination delay, since it was equal or higher than in the Philippines seed sowing. Morning mean monthly temperatures from November, 1990 to June, 1991 were: 25, 26, 24, 25, 30, 34, 32, 34°C respectively. The first and last plant emergence was 20 and 62 days after sowing, respectively.

Forty-five small trees were distributed in Nuevo León State; with only one municipality being added to the 22 already covered. Two plants were given to an institution in Guadalajara; this State is additional to the list of Table 2. In July 1992,

Table 1. Environmental conditions and height of neem plants, whose seeds from Philippines were sown in 1L bags inside a greenhouse of the Facultad de Agronomía, U.A.N.L., México, on 25 August 1989.

	Mean temperature (°C)		Mean relative humidity (%)		Height of plants (cm)	(date)
	9:00 hours	14:00 hours	9:00 hours	14:00 hours		
Greenhouse						
Sep.	27	34	62	44	8.5	(25 Sep.)
Oct.	25	36	55	42	11.9	(27 Oct.)
Nov.	22	28	54	38	15.1	(21 Nov.)
Dec.	15	23	60	46	22.1	(6 Dec.)
Jan.	19	27	57	41	22.8	(29 Jan.)
Feb.	19	29	61	36	22.9	(14 Feb.)
Mar.	19	28	62	43	26.8	(22 Mar.)
Apr.	24	34	66	38	37.9	(17 Apr.)
May	25	32	62	42	52.6	(3 May)
Shade						
May	28	35	69	47	—	—
Jun.	30	36	57	36	—	—
Jul.	26	33	68	44	69.7	(3 Jul.)
Aug.	26	33	71	49	—	—
Sep.	25	30	70	47	—	—

14 trees were added to the orchard, but a frost in February 1993 killed 12 of them. Twelve plants are missing.

Some trees (seeds from Philippines) at the school campus and nearby towns, produced a few flowers in 1992, with full bloom and fruit production occurring in 4-year-old trees in 1993. Fruits were collected and segregated into eight groups (see Table 4) according to individual tree; method of collection; and quality (maturity, colour, humidity). The pulp was removed and the seeds were sown, watered, and treated with fungicide as with the Honduran seeds. Conditions were also similar to those described for previous sowings.

The date of sowing was 26 August. Two weeks later, five plants had emerged, with the last of the plants emerging on the 47th day. Table 4 shows that the rate of germination reflected the condition of the seed. The highest value (68.9%) was for first quality seed (No. 7) from properly ripened fruits that were tumbled off the tree with a gentle agitation, and sown promptly. The fruits picked from the ground generated a reasonably high germination as long as they were first or second quality; respective values were 55.3 and 46.1%. The other groups of seed had lower germination percentages because the seeds were too old (No. 3) or because of a insufficient maturation (No. 5 and 6); these values were, nevertheless, higher than the ones for the Honduras seed. The 178 plants obtained will be planted this year.

Cuttings from neem trees were taken for plant propagation. Nine attempts were made with poor but encouraging results from 1991–1993. In each attempt, 70–100 stakes (25 cm long and 1–2 cm diameter) were planted. Their base was impregnated with a commercial root promoter, and they were inserted 1/3 of their length, either in perlite inside a greenhouse, or in regular farming soil in the open field.

Reasonably good results occurred in 1993, by using cuttings from 3–4-year-old trees. In three of the attempts, either in the greenhouse or in the field, 15–30% of the cuttings developed roots and survived. Propagation was most successful with cuttings from one-year-old wood, with a diameter of 2cm, and kept in moderate temperatures with continuous soil and air moisture.

In vitro propagation

The growth of explants from stem, petiole, and leaflet, was assessed on a basic medium MS (Murashige and Skoog, 1962) added with 0.4 mg/L thiamine and 100 mg/L mio-inositol and

Table 2. Dissemination of neem plants in México from Philippines seeds.

States	No. of municipalities	No. of sites	No. of trees	Given to institutions	Given to individuals
Aguascalientes	1	1	6	6	0
Chiapas	2	2	3	2	1
Chihuahua	2	2	17	17	0
Coahuila	1	1	15	15	0
Distrito Federal	2[a]	2	3	0	3
Durango	2	2	4	4	0
Guanajuato	2	2	9	9	0
México	3	4	17	15	2
Michoacán	1	1	2	0	2
Morelos	4	4	16	13	3
Nuevo León	22	82	170	76	95
Oaxaca	15	28	49	24	25
Querétaro	1	1	1	0	1
San Luis Potosí	8	15	25	11	14
Sinaloa	1	1	2	2	0
Sonora	2	3	10	4	6
Tabasco	1	1	10	10	0
Tamaulipas	4	4	7	4	3
Veracruz	7	12	25	24	1
Zacatecas	3	3	3	2	1
19 States and the D.F.	87	171	394	237	157

Table 3. Germination and plant height of neem, whose seed from Honduras was grouped according to size and sown in 1L bags in a greenhouse of the Facultad de Agronomía, U.A.N.L., México, on 16 November, 1990.

Seed size	Mean weight (g)	No. of seeds in group	No. of seedlings emerged	Germination (%)	No. and (%) of survivors in 6 months		Mean height in 1 year (cm)
Large	0.164	430	46	10.7	35	(76.1)	87
Medium	0.135	244	27	11.0	21	(77.8)	74
Small	0.127	245	15	6.1	9	(60.0)	76
Tiny	0.097	580	16	2.6	8	(50.0)	59

Table 4. Germination and height of neem, whose seed from Marín, Nuevo León, was grouped according to various factors and sown in 1L bags in a greenhouse of the Facultad de Agronomía, U.A.N.L., México, on 26 August, 1993.

Seed group[a]	No. of seeds in group	No. of seedlings emerged	Germination (%)	No. and (%) of survivors in 6 months		Mean height in 6 months (cm)
1	38	21	55.3	–[b]	–	—
2	39	18	46.1	–[b]	–	—
3	42	7	16.7	7	(100.0)	22.7
4	65	10	15.4	8	(80.0)	23.6
5	225	38	16.9	13	(34.2)	18.0
6	110	23	20.9	14	(60.9)	19.8
7	196	135	68.9	126	(93.3)	20.9
8	107	14	13.1	10	(71.4)	21.3

[a]Characteristics of the seed groups.
1.Tree 395; fruits from ground; 1st quality: properly ripe, yellow, juicy.
2.Tree 395; fruits from ground; 2nd quality: over-matured, brownish, dryer.
3.Tree 395; fruits from ground; 3rd quality: aged, dark-brown, parched.
4.Various trees from campus; fruits from ground; mixed quality.
5.Tree 477; fruits picked from branches; 1st quality but some unripe.
6.Tree 480; fruits picked from branches; 1st quality but some unripe.
7.Tree 494; fruits tumbled from branches to ground; 1st quality.
8.Various trees from off campus; fruits from ground; mixed quality.
[b] Seedlings were killed by accident in December 1993.

on this medium supplemented either with 0.5 mg/L 2, 4-D plus 0.05 BAP, or with 0.5 BAP plus 0.5 NAA. Ten cultures per treatment were maintained at 26 ± 1°C with 16 hours of white illumination of about 3000 lx and 8 hours of darkness per day.

Stem and petiole explants were 1 cm long; leaflet explants were 1 cm/cm^2. All explants were from young branches of a three-year-old tree, situated in the school campus. They were washed with soapy water and rinsed with running tap water. Explants were immersed for 30 seconds in 80% ethanol and then placed in a 0.6% (weight × volume) solution of sodium hypochlorite (with a drop of Tween 20 added per 100 mL) for 20 minutes. Before inoculation, they were rinsed three times with sterile water (Narayan and Jaiswal 1985).

No contamination occurred in petiole and leaflet explants, but all the stem cultures were ruined. Two weeks after media inoculation, all petiole and leaflet explants showed a slight thickening and curling. At the fourth week, all petiole explants were larger and exhibited calli; likewise, all foliar explants were bigger and curling more conspicuous, but calli were present in 100% of cases until the seventh week. No further development was observed in petiole explants in any medium, nor in leaflet explants in the basic medium supplemented with 0.5 mg/L 2, 4-D plus 0.05 BAP. By the fifteenth week, both in the basic medium and that supplemented with 0.5 BAP plus 0.5 NAA, all the leaflet explants formed independent granular protuberances, and 25% of them showed shoot buds and roots. In the last evaluation, 20 weeks after inoculation, shoots and roots were present in 75 and 100% of the foliar explants in the basic medium, and shoot and root formation of 75 and 50% in the medium supplemented with 0.5 BAP plus 0.5 NAA.

Control of Stored-product Insects with Neem

Three tests were done to evaluate the insecticidal properties of neem leaf powder. Leaves were dried in the shade, ground, and sieved through a 1 mm mesh screen to obtain a fine dust. Experimental units consisted of 1 L glass jars containing the grain (100 or 120 g), that was mixed with the neem powder and infested with one male and one female young adult/10 g. Conditions in the environmental chamber for the tests were optimal for insect development. Four replications were made for each treatment and for the control without application.

In the first test, doses 0.5–2.5% (weight × weight [w × w]) of neem leaf powder were tested to control *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in maize (López-Sanchez and Leos-Martínez 1993). Grain with the 2.5% dose, had statistically a higher mortality of introduced adults than the control 15 days after the infestation. Doses 1.5 and 2.5% at day 30, and dose 2.0% at day 60 had a higher mortality than the control. The mortality (corrected with Abbot's formula) obtained as the mean for all doses at days 6, 15, 30 and 60 was 9.6, 38.2, 46.8 and 47.7%, respectively. The percentage of damaged grains at day 60 was 14.3% in the control and 4.0% in the neem treated units (mean of all doses); any dose had significantly less damage than the control. *Chenopodium ambrosioides* L., a herbaceous plant well known in México, produced a better insect control than neem in this study; however, the insecticidal properties of neem grown in México were shown for the first time.

Two recent studies report the effect of neem leaf powder against *S. zeamais* in stored maize. Cobbinah and Appiha-Kwarteng (1989) found a dose of 0.75% (w × w) was innefective; more insects and the same damage occurred in the treated and control maize after 90 days. Although in our test the effect of a high dose was not always statistically superior to the effect of a low dose, it was discernible that higher doses performed better. It is likely that a dose of 0.75% will fail in some situations. Kossou (1989), on the other hand, used a very high dose of 5.0% (w × w) and found 40.0% mortality (corrected with the Abbot's formula) 72 hours after infestation. He also found 100 and 88% mortality (not corrected) in non-aerated and aerated conditions, respectively, after 60 days. This dose must have been very toxic to insects, if it killed them so quickly; we did not find such a rapid mortality.

In the second test, the effect of neem leaf powder upon life, oviposition, population development and damage of the

Mexican bean weevil *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) was evaluated during 42 days in stored beans (*Phaseolus vulgaris*). Five doses were tested: 0.0, 0.5. 1.0, 1.5, and 2.0% (w × w). Neem showed no action against this weevil in this test.

No references were found demonstrating effectiveness of neem leaf powder against any bruchid. Golob and Webley (1980) cited a work by Alam who found neem treatment ineffective against *Callosobruchus chinensis* (L.). Nevertheless, several works have shown a powerful action of other neem materials against bruchids, including leaf extracts. Ambika et al. (1981) stated that leaf extracts at 2.5 or 5.0% were effective (as much as Hydroprene) in suppressing progeny production of *Callosobruchus chinensis* reared in green gram Adult mortality 24 hours after exposure was considerable—15.3% with the 5.0% dose and 11.9% with the 2.5% dose. Other materials effective in controlling bruchids include kernel powder and seed oil (Jotwani and Sircar 1967; Ivbijaro 1983; Ali et al. 1983; and Saxena et al. 1988).

The third test studied the effect of leaf powder from different trees on *Tribolium castaneum* (Herbst) infesting maize. Adult mortality, 15 days after infestation, was significantly greater in grain treated with various doses (2–6%) of leaf powder than of the control. At day 78 after infestation, the number of adults in some of the treatments was statistically lower than that of the control. The total population, including larvae and pupae, was lower in any of the treatments than in the control. Mortality at day 78, was greater in any of the treatments than in the control.

These results are in agreement with those by Atwal and Sandhu (1970) who found that 2% neem leaf powder suppressed an increase in the population of *T. castaneum*.

Conclusions

The neem tree is now growing in México, but our dissemination program is far from finished. Being from only two different sources, the trees in México have a very limited variability. The fear of disease, like the one that occurred in West Africa (Price 1992), and other problems related to a narrow genetic base, restrain us from making an extensive distribution of the present material. We ask the international scientific community to participate in our efforts to establish the neem tree in México.

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