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Chapter

Importance of the Natural Incidence of the *Fusarium* Genus in Food Crops Established in Northern México

Julio César Ríos Saucedo, María Gabriela Ramírez-Valadez, Saúl Santana Espinoza, Maihualy Martínez-Fernández and Rigoberto Rosales-Serna

Abstract

The incidence of the *Fusarium* genus causing root rot is reviewed in crops showing high importance for food supply and to obtain regular income by farmers in the highlands of Northern México. Pathogen incidence was evaluated under field conditions in multiple sampling locations for common beans (*Phaseolus vulgaris* L.) and several chili peppers (*Capsicum annuum*) local cultivars (landraces and bred cultivars). Five commercial plots for registered and certified seed were also evaluated in common beans to be used in the 'seed refreshing program' implemented for the cultivar Pinto Saltillo, considered as the main variety sown in the highlands of México. High *Fusarium* genus incidence and its interactions with other fungi species, such as *Rhizoctonia solani* and *Pythium* spp., cause high losses in plant population, commercial yield and seed quality in food crops grown in Northern México. The natural incidence of plant disease caused by the *Fusarium* genus and its negative effect on crop survival and the reduction of commercial yield and seed quality is fully reviewed. Plant disease resistance, crop breeding and the influence of the environmental conditions were also considered.

Keywords: *Phaseolus vulgaris*, *Capsicum annuum*, root rot, commercial yield, seedborne pathogens

1. Introduction

The *Fusarium* genus includes several fungi species considered as the most important soil and seedborne pathogens causing plant diseases with high yield, quality and economic impact in several food and cash crops sown in the highlands of México [1]. The main effect of the *Fusarium* genus is the root and crown rot observed in vascular bundles of common bean (*Phaseolus vulgaris* L.), chili pepper (*Capsicum annuum* L.), maize (*Zea mays* L.) and other crops used as food, fodder and to obtain cash for urgent expenditures by the poor farmers in Northern México [2]. The main *Fusarium* symptoms in the plants are observed at the soil surface or

below the ground including damage in root crown and embryonic root, losses of root water-absorbing surface, leaf wilting and plant death [3, 4]. As a result, low plant densities are commonly observed in common bean and chili commercial plantings in most of the producing areas of Northern México [5, 6].

Under rainfed conditions losses in plant population are aggravated in years registering drought stress [7] after the common bean seedlings emergence. Plant losses are also observed under irrigation [8] when high temperatures and fast drying of the soil surface are registered, mainly after plant emergence (V_1) and the primary leaves unfolded (V_2) stages in common beans [9] and the second week after chili seedlings transplant in cultivated soil. Under those conditions, no post-embryonic adventitious root growth and plant death are observed due to severe damage in the embryonic root caused by the fungi complex (*Fusarium* spp., *Rhizoctonia* spp., and *Pythium* spp.) [10].

Plant losses in the early stages of growth in common bean and chili are considered one of the main causes of reducing yield and the quality of the commercial product (seed and chili pepper: fresh, dried, or processed fruits) [5, 11–13]. Decrement of the income is also observed by farmers, considering that low-input agriculture is the best option for reducing risks in crop production thus obtaining low yields. The actual measurement of the incidence and severity levels of plant pathogens causing root and crown rot, as well as diseases in the aerial plant organs is necessary to establish control strategies according to agroecological and sustainable agriculture. Identification of plant disease agents is the key to the development of effective control and management strategies [14]. Actual evaluation of disease problems related to the *Fusarium* genus in the state of Durango was included in this study regarding the importance of common bean and chili peppers as important food-producing crops.

2. Common bean

Common bean is an important cash crop used as a food, nitrogen-fixing plant and organic matter source [15], thus helping to reach sustainability in the agriculture performed under drought-prone and irrigated areas in the Mexican highlands of Northern México, including the states of Zacatecas, Durango and Chihuahua. In this area near 1.6 million of hectares are annually sown and more than 1.0 million of tons of grain are produced, at a rate of 0.69 t ha^{-1} [16]. Common bean cultivars Pinto Saltillo (improved) and Negro San Luis (landrace) are the most important varieties, according to the planted area and grain volume produced. Sowing recommendations include 35 to 50 kg ha^{-1} of seed [17] to obtain plant densities ranging from 100,000 to 120,000 plants ha^{-1}. In despite of recommendations low plant densities (<80,000 plants ha^{-1}) are very common in commercial plantings through the common bean-producing areas of Northern México, thus lower seed yield is also observed across locations and years [2].

Reduction in plant densities reached values lower than 20% of the recommended levels, and the lowest levels (12,500 plants ha^{-1}) were related to the lowest seed yield [5]. Plant densities reduction in early stages of crop growth were related to the low seeding rates, mechanical soil impedance and seedlings death caused by the fungi complex including *Fusarium solani* f. sp. *phaseoli*. Identification and predominance studies of pathogenic fungi are important to establish control strategies to reduce disease problems. Studies on fungi genetic and pathogenic diversity are also important for efficient disease control in several crop-producing areas of Northern México. Several strategies were used for fungal disease control including

crop genetic breeding, crop rotation and other modern agroecological and chemical management technologies.

2.1 *Fusarium* natural incidence

Twenty root samples were taken during the 2007 growth cycle in 15 common bean commercial plots distributed across "Los Llanos" de Durango and the Valleys of Poanas, Guadiana and Canatlán [10]. Fungi genus predominance was recorded using presence or absence in each root sample. Seventy-five fungi strains were isolated mainly belonging to three genera as follows: 54% for *Fusarium* sp., 10% *Macrophomina* and 2% *Pythium*. *Fusarium* resulted in a genus widely distributed, present in all the sampled locations and within genus three species were identified as *Fusarium oxysporum*, *F. solani* and *Fusarium graminearum* [10].

The predominance of *Fusarium* was observed in 93% of sampled locations and in 75% of the samples was the only genus of fungus found, affecting all the seed commercial classes (pinto, shiny black and flor de junio), while *Macrophomina* was associated mainly with pinto cultivars [10]. Results corroborated previous findings which demonstrated a close relationship between the *Fusarium* genus and common bean root rot in the state of Durango [1]. Systematic studies for fungi phenotypic, pathogenic, and genetic variation need to be implemented to select efficient, sustainable and agroecological disease control methods.

Another study was performed in Durango during 2020, including seven commercial plots at two important common bean producing areas (**Table 1**). The importance of *Fusarium* fungus was corroborated and variation in presence values from 50 to 100% were obtained. Other fungi genera were found at different levels of presence including *Rhizopus* (0–40%), *Pythium* (10–30%), *Erysiphe* (10–20%) and *Cercospora* (10–20%). The *Fusarium* fungus remains as the major plant disease problem in Durango, where integral control programs are necessary including crop genetic improvement, crop rotation, and other agroecological control methods.

Preventive chemical control and qualified (registered and certified) seed use are also considered. *Fusarium* control is an important issue due to some fungal species on stored common bean can also release mycotoxins (fumonisin) which causes human mycotoxicoses upon consumption, esophageal cancer and interferes with sphingolipid metabolism [18].

		Fungi genera				
Municipality	**Plot**	***Fusarium***	***Rhizopus***	***Pythium***	***Erysiphe***	***Cercospora***
Durango	1	50	40	10	20	20
	2	100	40	30	10	20
	3	80	40	20	10	20
[1]G. Victoria	[2]J. G. R.	60	30	20	10	20
	A. A.	70	30	20	20	10
	I. A.	60	0	30	10	20
	C. C.	70	0	10	10	10

[1]G. Victoria = Guadalupe Victoria.
[2]J. G. R. = José Guadalupe Rodríguez, A. A. = Antonio Amaro, I. A. = Ignacio Allende and C. C. = Calixto Contreras.

Table 1.
Isolation frequency of pathogen fungi related to common bean root rot at different municipalities of Durango, México. 2020.

2.2 Genetic improvement

Few systematic studies have been implemented in Durango for root diseases, mainly due to difficulties observed for plant root extraction, destructive sampling methods, and laboratory requirements for pathogen isolation and conservation, as well as for selecting crop-resistant germplasm. Studies concluded that low genetic diversity was observed for resistance in the plant pathogen-host represented by multiple common bean cultivars belonging to different genetic races and variable seed sizes and color. Sources of resistance against *Fusarium* wilt were identified in flor de mayo (pink) germplasm [19], mainly related to the Jalisco Race [20].

Low genetic resistance to *Fusarium* spp. has been identified in cultivated common bean populations, and the genetic resistance is quantitative [21] and hence, strongly influenced by the environment [22]. Other studies reported dominance in the control of the character with additive effects in common bean indicating that selection should be easy using efficient inoculation and selection methods [23]. In Durango, no direct selection was performed for *Fusarium* root rot in common bean, however, results indicated that resistance to FSP was more frequent in black beans [11]. In despite of the general observations, pinto seeded cultivars (Durango Race) showed capability for rapid adventitious root growth to maintain water absorption from the superficial soil layers and reduce losses in plant population.

2.3 Crop rotation

A common bean monocropping system is a common agricultural practice in most of the production areas in Northern México, thus aggravating problems and damage caused by *Fusarium* and other soilborne and seed transmitted pathogens. Crop rotations under rainfed conditions depend on the rain occurs during the May to August period. Early rains (May and early June) favor maize plantings while oats sowing is preferred when late raining periods (after middle August) are observed. Common bean is preferred to be sown when the rains are registered in late June to middle July. Under irrigation forage crops such as corn, sorghum, grasses, lucerne and oats are preferred due to pressure exerted by cattle farmers. Systematic crop rotations are required in Durango to reduce plant root and aerial pathogen problems in several crops and advances in sustainability could be also achieved by reducing water use by planting low water requirement crops.

2.4 Agroecological control methods

Trichoderma sp. showed high efficiency as a control agent for a wide range of aerial and soilborne plant pathogens and this trait makes it an excellent candidate for controlling saprophytic growth of *Fusarium* [24]. Some attempts were made to evaluate to control efficiency of this natural soil organism by reducing plant damages and yield losses caused by *Fusarium*. Results are considered ambiguous and *Trichoderma* use are not yet included in common bean crop management recommendations. Other options have been explored such as plant-based biopesticides [25] without actual use at the commercial level.

2.5 Chemical control

Disease chemical control starts with the seed treatment, but in most of the production areas in Durango the use of a fungicide is considered only in qualified seed production programs, which include several quality categories: basic, registered, certified and declared seeds [26, 27]. Most of the farmers consider chemical treatment as a

"fallacy" used only to justify the increment in seed prices without additional benefits for plant disease control under field conditions. Chemical products recommended in common bean seed treatment are: Metacaptan® (Captán + Metoxichloro), Thiram (Tebuconazole + Thiram), Terrazán [(Quintozeno-(pentacloronitrobenceno)], vitavax (Carboxín + Captán), Ridomil (Metalaxil-M) and Benlate (Benomyl).

The field visual evaluations showed some beneficial effects of the chemical seed treatment for increment the seedling emergence and plant survival at the early stages of the common bean development. Although systematic evaluation of subsequent fungicide effects on seedling and adult plant disease control and seed yield are necessary to reinforce the recommendations for its use. Several fungicides showing contact and systemic effects are recommended to reduce root rot incidence and severity, but results are not conclusive. Plant genetic resistance is preferred across the common bean production areas along México where several cultivars have been developed [28].

2.6 Qualified seed

Qualified seeds used in México for common bean commercial plantings are known as basic (foundation), registered (first generation certified), certified (second generation certified) and "enabled" seed. Standard seed treatment include fungicide (Metacaptan®, Thiram, Terrazán, Vitavax, Ridomil and Benlate), insecticide (Deltamethrin) and rhodamine as a colorant [26]. Effectivity on seed treatment needs to be evaluated due to the increase in the number of companies dedicated to seed production under several environmental conditions and variation in compliance with the regulations. In 2021, five seed lots from different sources were evaluated considering quality, which includes the genetic, physiological, physic, and sanitary traits (**Table 2**). The standard germination test is the most used probe to evaluate the physiological quality of a seed lot.

The pathogen attack on seeds is one of the factors that leads to the physiological quality loss, reducing the germination rate and the vigor of the seed lots, which end up precluding the final stand of the crop, resulting in productivity and economic losses to the farmer. Fungi are considered as the most important among pathogens due to the higher number of species and the damage caused both in yield and seed quality [29]. The mixture containing Carbendazim + Thiram in its composition are efficient in the control of pathogens regardless of the application time of products [29].

The sanitary quality of the seed used in México needs to be evaluated to reduce problems during the seed germination and seedling emergence periods, mainly under field conditions. Some chemical products were identified for their efficient control of different fungus although some phytotoxicity effects were also observed and delayed protection by fungicide controlling *Fusarium* on seeds [29]. In 2021, five

Seed Lot (origin)	Category	Year of production	Reception date	Amount (kg)
1. Chihuahua 1	Registered	*SS-2020	04/12/20	3000
2. Chihuahua 2	Registered	SS-2020	15/12/20	3000
3. Durango 1	Certified	SS-2016	15/02/2021	10,030
4. Sinaloa	Registered	AW-20	30/03/2021	3000
5. Durango 2	Registered	SS-2020	15/06/2021	9000

**SS = spring–summer growth cycle; AW = autumn-winter growth cycle.*

Table 2.
Reception data of five qualified seed lots to be used in reinforcing Pinto Saltillo common bean commercial plantings in Durango, México.

seed commercial lots (**Table 2**) were evaluated according to the physiological quality tests at the INIFAP's Valle del Guadiana Experiment Station, located in Durango, Méx. Standard germination test was performed, using a soil-based substrate, and considering the SNICS (Servicio Nacional de Inspección y Certificación de Semillas) recommendations for sample size (30 samples) in each seed lot. The seed lot number 4 from Sinaloa registered the highest emergence level (96%), reaching that value in the shortest period (8 days after planting; DAP); while, lot number 3 (from Durango) showed the lowest emergence value (65%), reached at 12 DAP (**Figure 1**).

Reductions in seed germination levels were related to the production environment and storage conditions and duration. Other negative factors were mechanical damages caused during the harvest-threshing and seed cleaning processes, as well as the seed fungi load and chemicals used for the seed treatment [29, 30]. Seedlings wilting was also observed during the germination test due to soil infestation and seed contamination by fungi. Therefore, seed studies for soil and seedborne pathogen load were performed under controlled laboratory conditions.

2.7 Seed health tests

Pinto Saltillo is an improved common bean cultivar showing high yield and disease susceptibility in aerial (Common Bacterial Blight) and root zone (*Rhizoctonia* spp., *Fusarium* spp., and *Pithyium* spp.). Most of these diseases are seedborne and the infestation/contamination of the seed may occur during harvest-threshing activities, processing and handling. The pathogen may, thus, be carried with the seeds in three ways: Admixture (pathogen are independent of seed but accompany them), External (pathogen present in seed surface as spores, oospores and chlamydospores) and internal (pathogens establish within the seed with the definite relationship with seed parts) [31]. The pathogen *Fusarium* is soil as well as seedborne in nature and the colonization percent of *F. solani* was highest as compared to other isolated fungi. Seed germination rate was also reduced (50%) in soil infested with *F. solani* where seedlings mortality reached 93.3% [32].

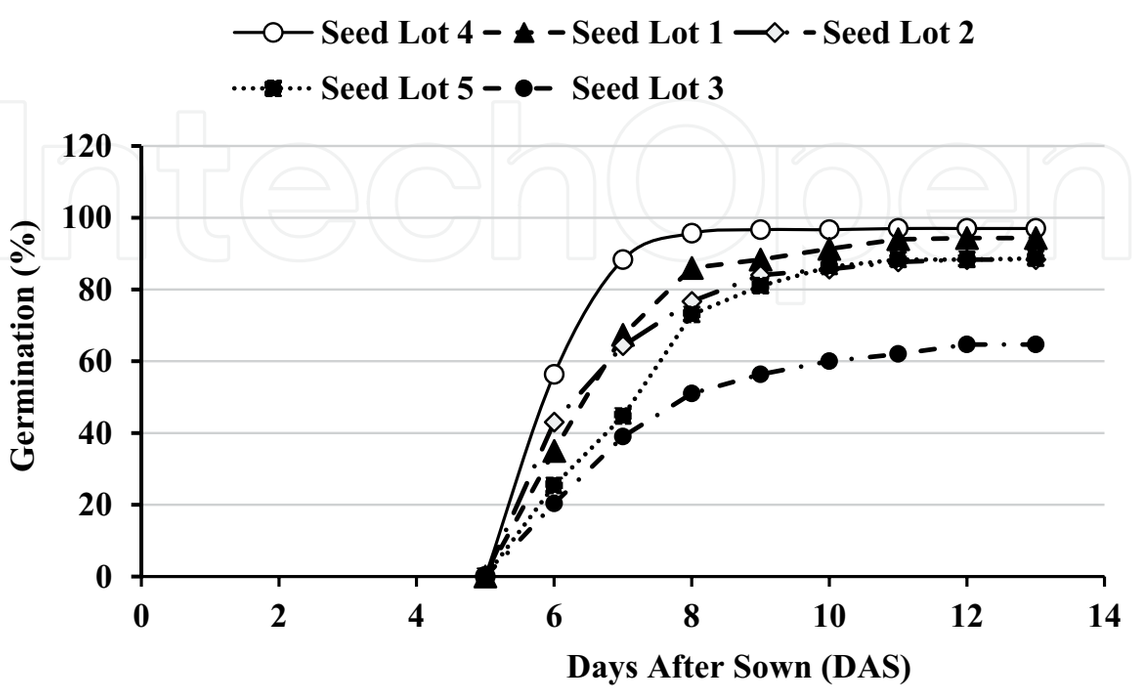


Figure 1.
Germination standard test performed in five qualified common bean seed lots obtained in México. 1) Chihuahua 1, 2) Chihuahua 2, 3) Durango 1, 4) Sinaloa y 5) Durango 2.

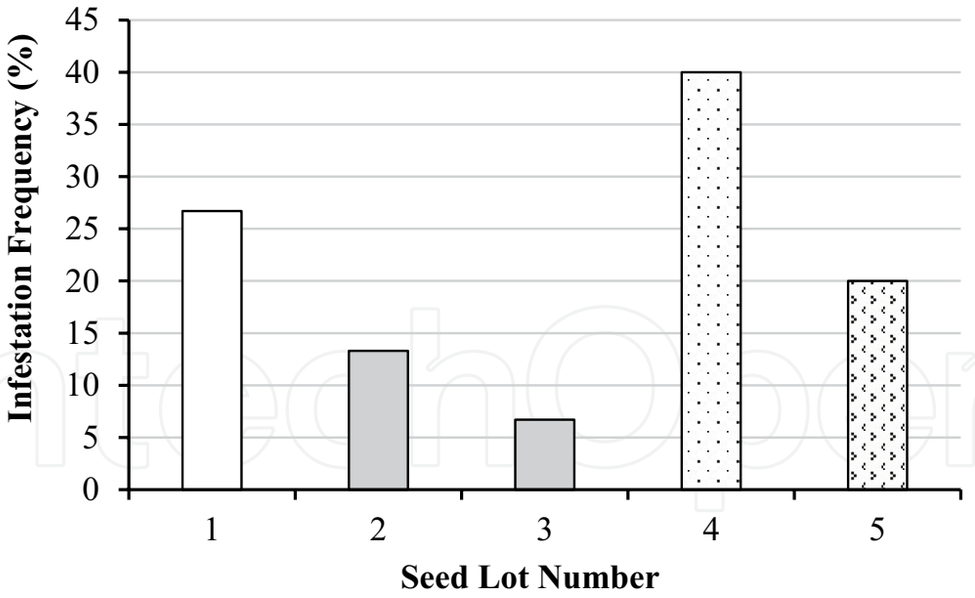


Figure 2.
Fusarium fungus infestation frequency observed in seed lots from different states to be used in Durango. 1) Chihuahua 1, 2) Chihuahua 2, 3) Durango 1, 4) Sinaloa y 5) Durango 2.

Agar plate is considered the most common method used for identification the of seedborne fungi. In 2020, *Fusarium* presence in Pinto Saltillo seed lots from different origins (**Table 1**) was determined by triplicate placing seeds onto sterile agar media (Potato Dextrose Agar: PDA) to encourage the growth of the fungus [33]. Variation of the infestation frequencies was registered among seed lots from different seed sources from México (**Figure 2**). High frequencies (40.0%) were detected in Pinto Saltillo seed lots produced in Sinaloa during the Autumn-Winter (2020–2021) growth cycle where several *Fusarium* hospedant were cultivated (chickpea, tomato, common beans and maize) [2].

Fusarium isolates from Sinaloa, Méx., showed differences in aggressiveness; and *F. falciforme* was the most aggressive compared to *F. oxysporum* [34], and isolates of both complexes triggered similar aerial symptoms of yellowing and darkening of the vascular tissues in tomato plants. But only *F. falciforme* isolate triggered necrosis in the plant crowns [34]. Seed lot 3 showed low frequencies for *Fusarium* fungus incidence (6.7%), mainly due to the longest storage period since was produced in the 2016 Spring–Summer growth period. Long storage period reduced pathogen fungus load but low seed germination and seedlings damage (injured leaves) were also observed.

3. Chili peppers

In México, generic name of chili pepper (chile; *C. annuum* L.) is used to denominate several plant cultivars mainly known with a local names such as: chile ancho, jalapeño (processed chipotle), serrano, mirasol (dried guajillo), and pimiento morrón reaching the 70 and 80% of the national production [35]. Chili pepper is one of the most important vegetable crops used in México as condiment and food flavor and its also considered as an important cash crop [36] grown in several production areas in Northern México, providing additional income to the farmers.

In the Northern highlands of México, the planted area for fresh fruit harvest of chili pepper in 2020 reached 37,440 ha in Zacatecas, 30,772 ha in Chihuahua and 4,136 ha in Durango [16]. In Durango, several chili cultivars and landraces are

planted [jalapeño, poblano-ancho, puya, mirasol-guajillo, árbol, cola de rata and tornachile (chile güerito)]; while in Chihuahua and Zacatecas, the jalapeño, serrano and habanero cultivars are preferred. The highest chili production is obtained in Chihuahua (722,937 t) where the yield rate is 24.0 t ha^{-1}. The state of Zacatecas produces 458,943 t and the yield average is 12.4 t ha^{-1}. In the state of Durango the chili production overpasses 48,035 t and the lowest yield at the North Central region of the Mexican highlands is obtained (11.6 t ha^{-1}) [16].

Chili crop management system includes seed obtention from dried fruits, sowing and nursery growth (almácigo), and transplant under field conditions, using rows 0.81 to 1.20 m apart [35]. Modern management techniques include the use of mulch and drip irrigation [37] to increment yield and water productivity. Several plant pathogens are observed in chili plantations established in Durango, causing phytosanitary problems, low yield and reduced fruit quality; as well as generalized plant or whole plot losses. Plant pathogen problems include viruses (Cucumber Mosaic: CMV, Potato Y: PYV, Alfalfa Mosaic: AMV, Tobacco Mosaic: TMV and TEV), fungi (*Phytophthora capsici*, *Rhizoctonia solani*, *Fusarium* spp., *Pythium* spp.) [12] and bacteria (*Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*) [38].

The pathogenic syndrome known as chili plant wilting 'Secadera' (CPW), is the most important disease in all the producing areas of México, causing total yield losses (100%) and the planting area was reduced by 60% in some states [39]. CPW is mainly caused by the obstruction of the vascular bundles provoked by the phytopathogen fungi infection (*Fusarium oxysporum, Rhizoctonia solani*, and *Phytophthora capsici*) in roots or root-crown [34]. The plant pathogen, mainly *Fusarium*, relative importance need to be evaluated due to its alimentary, health, economic and social implications.

3.1 *Fusarium* natural incidence

Root and crown samples were taken when 'Secadera' (Damping-off and CPW) symptoms (symptomatic plants) were observed at the main chili-producing areas in the state of Durango.

3.1.1 Study 1

Eleven municipalities were included (**Table 3**) and random sampling included 26 commercial plots established under irrigation in each of the chili pepper production areas across the state of Durango where temperate and warm climates are registered. Plots were georreferentiated for map construction (**Table 3**). Direct sampling was made in plots showing typical Damping-Off and CPW symptoms, such as: yellowing and wilting in upper leaves, wilting symptoms in all parts of the plant; leaves showing dark-green color remaining attached to the plant, root crown narrowing and plant death. Samples consisted of 10 plants in each plot, which were dissected and tissue samples were taken in the root crown and embryonic root. Samples were transported in paper bags with an identification label, including municipality, location, geographic coordinates and crop cultivar, then were sun-dried and stored at room temperature until processing.

3.1.2 Study 2

Seven chili pepper sampling sites were included at two municipalities in the state of Durango (**Table 4**), plots were mainly established under irrigation in the temperate climate regions. Plots were georreferentiated for map construction (**Table 4**). Direct sampling was made in plots showing typical Damping-Off and CPW symptoms. Samples consisted of 10 plants in each plot which were dissected,

Sample number	Municipality	Plot	Geographic localization	Cultivar local name
1	Simón Bolívar	Simón Bolívar	N 24° 45′ 42.0″; W 103° 10′ 51.5”	Ancho
2	Simón Bolívar	Flores Magón	N 24° 44′ 43″; W 103° 10′ 29.0”	Ancho
3	Lerdo	El Refugio	N 25° 25′ 59.0″; W 103° 44′ 57.3”	Guajillo
4	Lerdo	21 de marzo	N 25° 26′ 45.2″; W 103° 45′ 13.0”	Guajillo
5	Mapimí	Perimex	N 26° 32′ 36.6″; W 104° 08′ 15.8”	Jalapeño
6	Nazas	Francisco Sarabia	N 25° 18′ 12.6″; W 103° 58′ 20.5”	Guajillo
7	Nazas	Agustín Melgar	N 25° 16′ 25.3″; W 103° 58′ 20.5”	Guajillo
8	Nazas	Lázaro Cárdenas	N 25° 16′ 25.0″; W 104° 02′ 43.8”	Guajillo
9	Nombre de Dios	Francisco Munguía	N 23° 47′ 33.6″; W 104° 06′ 15.2”	Puya y Árbol
10	Peñón Blanco	J. Agustín Castro	N 24° 38′ 51.0″; W 103° 56′ 01.1”	Ancho
11	Poanas	Dago 1	N 23° 58′50.2″; W 104° 03′ 54.0”	Mirasol
12	Poanas	Pozo 12	N 23° 59′ 29.3″; W 104° 04′ 00.7”	Ancho y Puya
13	Poanas	UTP 1	N 23° 56′ 26.7″; W 104° 02′ 56.8”	Ancho y Puya
14	Poanas	UTP 2	N 23° 55′ 53.9″; W 104° 02′ 57.8”	Ancho y Árbol
15	Poanas	UTP 3	N 23° 56′ 26.7″; W 104° 02′ 56.8”	Ancho
16	Poanas	Pilares	N 23° 52′ 24.7″; W 104° 02′ 40.2”	Puya
17	Rodeo	El Parián	N 25° 09′ 45.2″; W 104° 32′ 40.2”	Ancho
18	Rodeo	Primo de Verdad	N 24° 54′ 52.7″; W 104° 28′ 5.5”	Ancho y Cola de Rata
19	Rodeo	La Cuesta	N 25° 01′ 47.7″; W 104° 28′ 40.9”	Ancho
20	S. Juan del Río	El Crucero 1	N 24° 46′ 58.4″; W 104° 30′ 56.9”	Ancho
21	S. Juan del Río	El Crucero 2	N 24° 46′ 51.3″; W 104° 31′ 14.8”	Ancho
22	S. Juan del Río	Francisco de Ibarra	N 24° 47′ 26.3″; W 104° 30′ 38.2”	Ancho y Tornachile
23	S. Juan del Río	Santa Rosalía	N 24° 53′ 44.6″; W 104° 26′ 17.4”	Ancho y Cola de Rata
24	S. Juan del Río	José María Patoni	N 24° 52′ 51.7″; W 104° 26′ 17.1”	Ancho

Sample number	Municipality	Plot	Geographic localization	Cultivar local name
25	S. Pedro del Gallo	La Laborcita	N 25° 29′ 21.3″; W 104° 24′ 03.0”	Ancho y Guajillo
26	S. Luis del Cordero	San Luis del Cordero	N 25° 24′ 42.0″; W 104° 16′ 03.0”	Árbol

Table 3.
Geographic localization of plots included in chili sampling implemented in plants showing plant wilting (CPW) symptoms at different municipalities of the state of Durango, México.

Sample number	Municipality	Plot	Geographic localization	Cultivar local name
1	Durango	CEVAG 1	N 24° 44′ 43″; W 103° 10′ 29.0”	Ancho
2	Durango	CEVAG 2	N 24° 44′ 43″; W 103° 10′ 29.0”	Ancho
3	Durango	CEVAG 3	N 24° 44′ 43″; W 103° 10′ 29.0”	Ancho
4	G. Victoria	J. G. Rodríguez	N 25° 26′ 45.2″; W 103° 44′ 57.3”	Guajillo
5	G. Victoria	A. Amaro	N 26° 32′ 36.6″; W 104° 08′ 15.8”	Jalapeño
6	G. Victoria	I. Allende	N 25° 18′ 12.6″; W 103° 58′ 20.5”	Guajillo
7	G. Victoria	C. Contreras	N 25° 16′ 25.3″; W 103° 58′ 20.5”	Guajillo

Table 4.
Geographic localization of plots included in chili sampling implemented in plants showing plant wilting (CPW) symptoms at two municipalities of the state of Durango, México. 2020.

and tissue samples were taken in the root crown and embryonic root. Samples were transported in paper bags with an identification label, including municipality, location, geographic coordinates and crop cultivar, then were sun-dried and stored at room temperature until processing.

3.2 Morphological characterization

Two classes of fungi (Anamorphic and Oomycota) were isolated including three different genera morphologically differentiated (**Table 5**). The most abundant genus was *Fusarium*, followed by *Rhizoctonia* and in a lower extent the Omicete *Pythium*. *Fusarium* were detected in 100% of the samples collected in 8 locations at municipalities of Simón Bolívar (2), Lerdo (1), Rodeo (1), San Juan del Río (3) and San Pedro del Gallo (1). This plant pathogen was present in variable proportions (18–100%) of samples at 25 locations (96%). *Rhizoctonia* were detected in 100% of the samples at five locations, such as: Simón Bolívar (2), Nombre de Dios (1), Poanas (1) and San Luis del Cordero (1). This fungus was present in 23 (88.5%) of the total samples with presence levels ranging from 18 to 100% across locations. A low incidence level was observed for *Pythium* and absence was registered at 16 locations (61.5%), with presence levels from 20 to 70% of the samples with the highest value (70%) at the location of J. Agustín Castro.

Results of the isolation frequency showed that *Fusarium* was the fungus present in most of the samples of plant material showing CPW symptoms, regardless of the sample site of origin, except for San Luis del Cordero. *Fusarium* was the widespread and frequently pathogenic genus of plant fungus, followed by *Rhizoctonia*, while in most of the sampling sites absence of oomycetes (*Pythium*) was found, mainly at the

	Municipality	Plot	Frequency (%)		
			Fusarium **sp.**	*Rhizoctonia* **sp.**	*Pythium* **sp.**
1	Simón Bolívar	Simón Bolívar	100	100	44
2	Simón Bolívar	R. Flores Magón	100	100	45
3	Lerdo	El Refugio	100	0	0
4	Lerdo	21 de Marzo	60	30	20
5	Mapimí	Perimex	60	40	60
6	Nazas	Francisco Sarabia	40	30	60
7	Nazas	Agustín Melgar	80	50	30
8	Nazas	Lázaro Cárdenas	40	70	50
9	Nombre de Dios	Francisco Munguía	40	100	0
10	Peñón Blanco	J. Agustín Castro	80	60	70
11	Poanas	Dago 1	33	67	0
12	Poanas	Pozo 12	73	27	0
13	Poanas	UTP 1	50	70	0
14	Poanas	UTP 2	89	33	0
15	Poanas	UTP 3	63	88	0
16	Poanas	Pilares	18	100	0
17	Rodeo	El Parián	63	38	38
18	Rodeo	F. Primo de Verdad	100	33	0
19	Rodeo	La Cuesta	75	58	0
20	San Juan del Río	El Crucero 1	100	0	0
21	San Juan del Río	El Crucero 2	100	18	0
22	San Juan del Río	Francisco de Ibarra	100	0	0
23	San Juan del Río	Santa Rosalía	70	60	0
24	San Juan del Río	José María Patoni	50	50	0
25	San Pedro del Gallo	La Laborcita	100	63	38
26	San Luis del Cordero	San Luis del Cordero	0	100	0

Table 5.
Isolation frequency of pathogen fungi related to chili pepper plant wilting at different municipalities of Durango, México.

municipalities of the semi-desertic region (Lerdo, San Juan del Río and Rodeo), but also in the highland valleys of Poanas and Nombre de Dios. Absence of *Pythium* was related to the phenological stage of sampled chili pepper populations since this fungus effect is mainly observed in the early stages of crop development. Soilborne pathogen *Fusarium oxysporum* and *Rhizoctonia solani* are the most common diseases causing root-rot and plant wilt in chili pepper cropping fields [36, 40]. These fungi are also observed in common bean and cereals planted in Durango for fodder and food, where the contamination with *Fusarium* species is one of the major sources of mycotoxins [41].

In the municipalities belonging to 'La Laguna' region (Mapimí and Nazas), the frequency of isolation of *Fusarium* was like that observed in other sampling sites, however, that of *Rhizoctonia* decreased and *Pythium* presence increased

considerably compared to the municipalities of the highlands region (Poanas) and that of the semi-arid sampling sites (**Table 5**). The use of mulch influenced increments for *Pythium* presence at some locations such as J. Agustín Castro.

In the second study, *Fusarium* also showed the highest presence scores (50 to 90%) at "Valle del Guadiana" and "Los Llanos" regions (**Table 6**). Other fungi species related to the common bean cropping systems such as *Uromyce*s and *Pythium*, also showed high presence levels. Other cosmopolitan fungi species *Rhizopus* (Zygomycetes) registered a high presence in plant samples, due to its omnipresent nature as an air contaminant, fast growing rate, and versatility of growth conditions (temperature and relative humidity) [42].

The low presence of *Phytophthora* fungus was observed in most of sampling sites (85.7%), due to increased novel sowing areas for chili peppers opened under irrigation at the municipalities of Durango and Guadalupe Victoria. Increments in the chili pepper area were related to recurrent crop complete losses registered in the main producing area of Poanas, Villa Unión and Nombre de Dios. Potato plantations also influenced the presence of *Phytophthora* fungus in both studied municipalities. Other fungi species (*Erysiphe* and *Cercospora*) were found, causing mildew in several crops and leaf or pod spots in common beans. Several fungi genera were detected in cultivated soils of Durango, causing severe economic losses in horticultural and agricultural crops.

3.3 Morphological identification

Different fungi and oomycetes show adaptation under different growth media, temperature, and light quality, then producing consistent characteristics that can be used for morphological identification [43]. The colony morphology of the *Fusarium* fungi species of the six most frequent isolates obtained in Durango was determined in pure culture using three different culture standard media: PDA (Potato Dextrose Agar, Difco®), Corn Flour Agar (CA) [44], and SDA (Sabouraud Dextrose Agar, Difco®) also known as Spezieller Nährstoffarmer Agar or Special Low-Nutrient Agar (SNA). Several characteristics were evaluated in fungi colonies, such as: pigmentation (color and hue) of the surface on the front and back of the colony, texture of the colony surface (cottony, resupinate, velvety, powdery, crustaceous, soaked, embedded, yeast-like, sticky, homogeneous or heterogeneous, presence or absence of elevation), margin type of the colony (smooth, regular, irregular, restricted, diffuse), pattern (radiated, flower-shaped or arachnoid), formation of resistance structures (sporodochia), mycelial type and growth rate.

Municipality	**Plot**	*Fusarium*	*Uromyces*	*Rhizopus*	*Phytophtora*	*Pythium*	*Erysiphe*	*Cercospora*
Durango	1	60	20	70	10	0	0	0
	2	70	20	0	20	0	0	0
	3	90	0	50	0	0	0	0
Victoria	*J.G.R	80	30	60	10	10	10	0
	A. A.	70	20	30	20	20	20	10
	I. A.	70	10	40	30	0	0	0
	C. C.	50	0	40	20	0	10	0

**J. G. R. = José Guadalupe Rodríguez, A. A. = Antonio Amaro, I. A. = Ignacio Allende, C. C. Calixto Contreras.*

Table 6.
Isolation frequency of pathogen fungi related to chili pepper root rot at different municipalities of Durango, México.

3.4 Colony characterization of *Fusarium* sp.

Strain EV1r. The growth of *Fusarium* sp. EV1r in PDA medium developed a fast-growing colony (4 days) with beige color (**Figure 3A**). The texture of the colonial surface was cottony presenting a smooth and irregular margin. On the other hand, the development of the strain e EV1r in corn flour agar (CA) developed a fast-growing white colony with a radial pattern and regular margin with a powdery texture (**Figure 3B**). In addition, the EV1r strain was also grown in SNA medium showing rapid growth (5 days) with a white front and back color with the presence of mycelium (**Figure 3C**).

Strain H1Zra. The growth of *Fusarium* sp. strain H1Zra in PDA medium developed a fast-growing colony (5 days) showing white color on both sides (**Figure 4A**). The texture of the colonial surface was cottony with the presence of aerial mycelium and irregular margins. On the other hand, the development of the *Fusarium* H1Zra strain on corn flour agar (CA) developed a fast-growing colony with white color, radial pattern, and regular margin. A velvety texture with the absence of aerial mycelium (**Figure 4B**) was also observed. In SNA (Synthetic Nutrient-Poor Agar) medium, the H1Zra strain showed slow growth (8 days) with an opaque colony and little mycelial development (**Figure 4C**).

Strain H1Zrb. The growth of *Fusarium* sp. H1Zrb in PDA medium developed a fast-growing colony (4 days) of white color with a slight yellow color in the periphery of the colony (**Figure 5A**). The texture of the colonial surface was velvety with the presence of mycelium with a regular margin. The development of the H1Zrb strain on corn flour agar (CA) developed a fast-growing white colony with a radial pattern and regular margin with a velvety and powdery texture with the absence

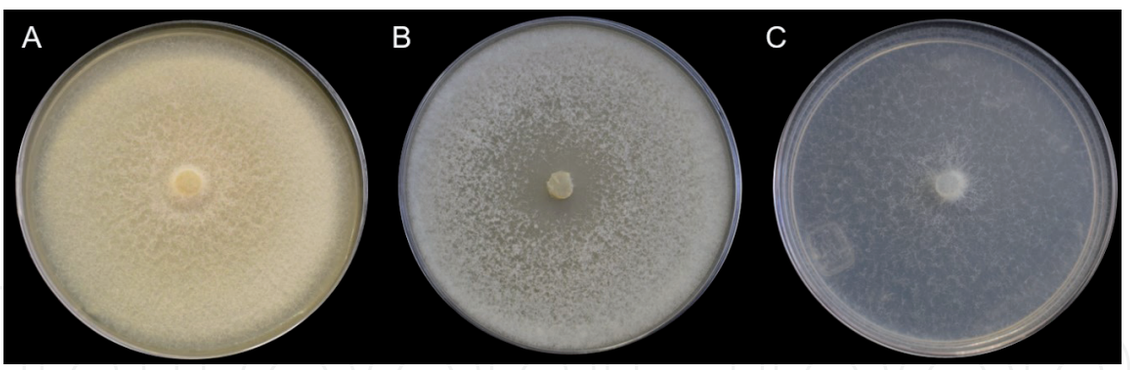


Figure 3.
Colony morphology study in Fusarium *sp. strain EV1r. Growth media: A) potato dextrose agar-PDA, B) corn flour agar-CA, and C) special low-nutrient agar-SNA.*

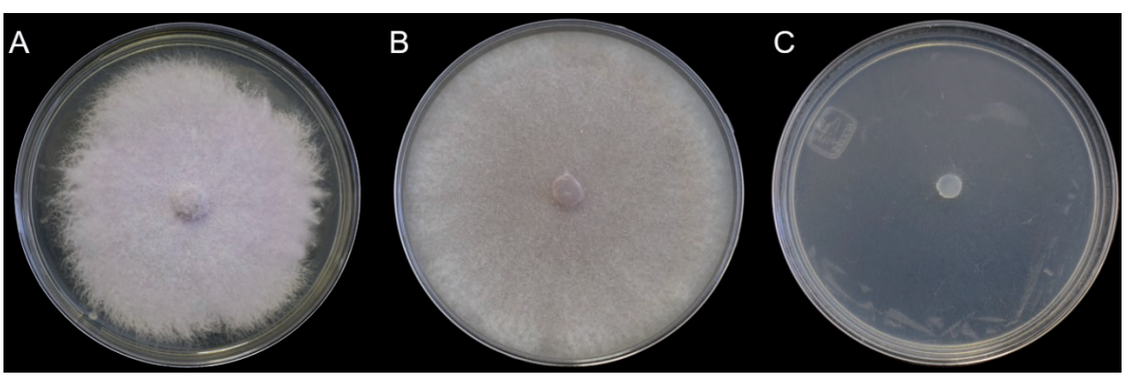


Figure 4.
Colony morphology study in Fusarium *HIZra strain. Growth media: A) PDA B) CA C) SNA.*

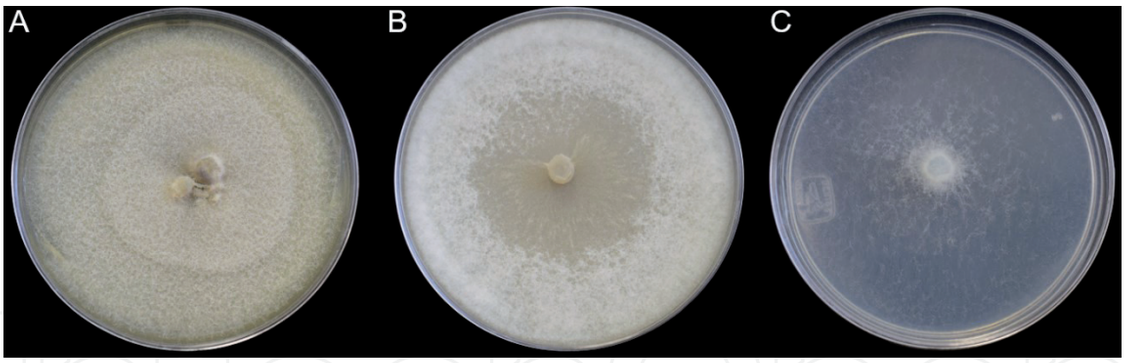


Figure 5.
Colony morphology study in Fusarium sp. strain HIZrb. Growth media: A) PDA B) CA C) SNA.

of aerial mycelium (**Figure 5B**). In SNA medium, the *Fusarium* sp. H1Zrb strain showed rapid growth with an opaque white colony with low mycelium development (**Figure 5C**).

Strain K4Zr. The growth of *Fusarium* sp. K4Zr in PDA medium developed a fast-growing white colony with the presence of growth rings (**Figure 6A**). The texture of the colonial surface is cottony with the presence of aerial mycelium with a regular margin. The development of the K4Zr strain on corn flour agar (CA) developed a rapidly growing white colony with a radial pattern and regular margin with a cottony texture with the presence of floccose mycelium (**Figure 6B**). On the other hand, in the SNA medium, the K4Zr strain showed slow-growth with an opaque white colony with a flat texture with little development of mycelium (**Figure 6C**).

Strain K5Zr. The *Fusarium* sp. strain K5Zr inoculated on PDA medium developed a slow-growth colony (8 days) showing opaque yellow color, with a floral pattern and irregular margin (**Figure 7A**). The texture of the colonial surface was creamy with the absence of aerial mycelium. The development of the K5Zr strain on corn flour agar (CA) developed a rapidly growing white colony with a radial pattern and regular margin with a cottony texture and abundant aerial mycelium (**Figure 7B**). In the SNA medium, the K5Zr strain showed restricted and diffuse growth with an opaque white colony and irregular margin (**Figure 7C**).

Strain C3WC. The growth of *Fusarium* sp. strain C3WC in PDA medium developed a slow-growing colony (4 days) of white color, with regular pattern and margins (**Figure 8A**). The texture of the colonial surface was flat and velvety with the absence of aerial mycelium. On the other hand, the development of the C3WC strain on corn flour agar (CA) developed a colony of white color with a slight brownish color of rapid growth. Radial pattern and regular margin with a cottony

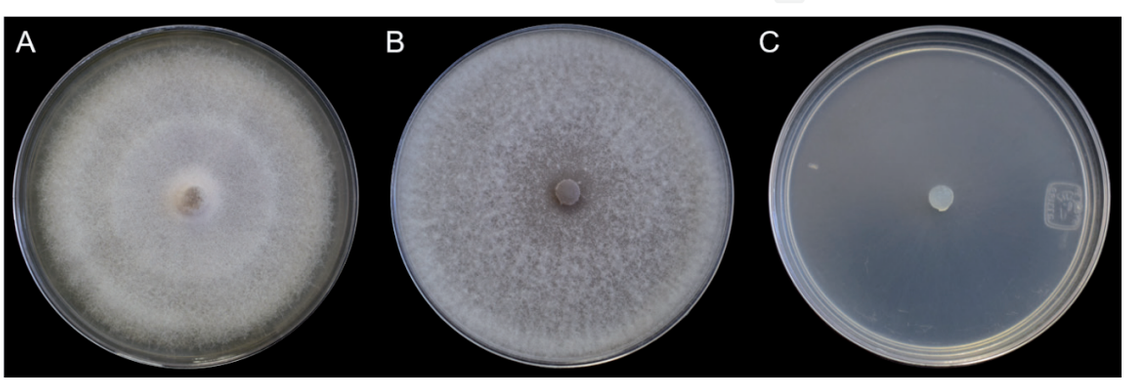


Figure 6.
Colony morphology study in Fusarium sp. strain K4Zr. Growth media: A) PDA B) CA C) SNA.

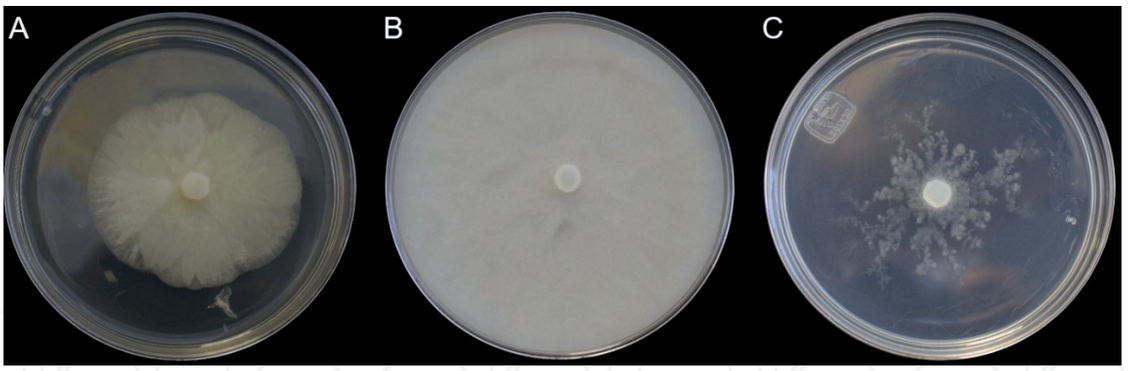


Figure 7.
Colony morphology study in Fusarium sp. strain K5Zr. Growth media: A) PDA B) CA C) SNA.



Figure 8.
Colony morphology study in Fusarium sp. strain C3WC. Growth media: A) PDA B) CA C) SNA.

texture and abundant aerial mycelium were also observed (**Figure 8B**). In the SNA medium, the C3WC strain showed a slow-growth colony with an opaque white color and irregular margins and little mycelium development (**Figure 8C**).

3.5 Genetic improvement

In México, 19 accessions of native chili pepper collected in the state of Morelos and 11 serrano chili accessions were selected considering its resistance to *Phytophthora capsici* that can be used in crop breeding [45, 46]. Twenty-six plant accessions were also identified in chili pepper with at least one individual showing resistance to *Fusarium* spp. and only two accessions from the gene bank resulted resistant to *P. capsici* and the mix including *Fusarium, Phythophtora* and *Rhizoctonia* [13]. Despite the germplasm selection for soil and seedborne fungal disease resistance no common bean and chile pepper cultivars for a specific response to soil fungi complex have been released in México.

3.6 Crop rotation

Crop rotation in the chili pepper production areas is influenced by farmer's tradition and the high economic income obtained with the fresh, dried and processed fruits, difficulting changes in the cropping systems. A similar response was observed for the common bean production under rainfed and irrigation monocropping systems. Then agronomic management recommendations need to be adjusted considering agroecological practices, including crop rotation, that contributes to improve the productivity and sustainability of local agroecosystems.

3.7 Agroecological practices

In common bean, chili pepper, maize and most of the crops, agroecological practices and integrated management systems need to be implemented and systematized. Agroecological practices are poorly used in current agriculture in North-Central México, and some components need to be validated at the commercial level including biofertilizers, organic matter incorporation into the soil and the use of natural pesticides, as well as crop choice and crop rotation. Other options include intercropping, relay intercropping, agroforestry with timber, fruit or nut trees; allelopathic plants use (sunflower), direct seeding into living cover crops or mulch, reduced tillage, drip irrigation, biological pest control, and cultivar choice [47].

In Durango, marginal advances were achieved in organic fertilizer production and use, most of the products are not often available in sufficient quantity. Industrial production has been obtained only for compost in "La Laguna" region but high prices have been observed making it unaffordable for farmers. Lombri-compost, fulvic acid and other liquid biofertilizers were also produced at low amounts and short time period effects, variable composition, and ambiguous results have been reported. Some interesting results were obtained by using Biological Nitrogen Fixing bacteria (*Rhizobium* spp. and *Azotobacter* spp.) and mycorrhizic fungi, but their production and distribution need to be reinforced. High biomass producing species with appropriate carbon to nitrogen ratio (25) has been selected (*Pennisetum* sp.) to reduce costs for direct organic matter incorporation into soils [48], stabilize pH (6.5–7.0) and naturally release soil minerals for plant nutrition.

Studies on organic pesticides need to be strengthened obtain clear results and to generate recommendations for commercial plantings of common bean, chili pepper and other food and fodder crops. Crop choices have been explored in Durango, using canola (*Brassica* spp.), chickpea (*Cicer ariethinum*), amaranth (*Amaranthus* spp.), barley (*Hordeum vulgare*), sunflower (*Helianthus annuus*), sorghum (*Sorghum bicolor*), and Oats (*Avena sativa*). Some problems need to be solved to improve adoption programs for these crops, such as mechanization from sown to harvest, efficient production storage and commercialization process.

Agroforestry with timber (Scott's Pine: *Pinus greggii*) was implemented using governmental programs, but then abandoned due to prolonged technical periods (10 to 12 years), poor technical support, and food requirement by farmer's families. Studies corroborated that the common bean is better adapted in early years of Scott's pine plantations compared to forage crops (oats and maize) [49]. Increments in Wichita pecan tree (*Carya illinoensis*) plantation area have been observed in the last 5 years in Durango, due to high prices of the pecan nuts and commercial competence to apple production, although influence on local agriculture has not yet been determined. Sunflower is an allelopathic crop which has been used in Durango to reduce problems observed with perennial grasses, mainly bermuda grass (*Cynodon dactylon*). Although, difficulties has been also observed for seed supply and during the crop harvest and seed (achene) commercialization processes.

Direct seeding into living cover crops was probed without success due to pressure exerted by beef/dairy livestock production and the preferential use of crop residues and cover crops as fodder instead as a natural amendment, then low organic matter content is commonly registered in agricultural soils. Low organic matter combined with alkaline reaction in the soils reduces the availability of nutrients, presence of beneficial microorganisms, water infiltration and retention, and then aggravating drought, plant nutrient deficiencies and disease problems in several food and cash crops. Mulch and drip irrigation is used in some cash crops, even common beans, in reduced areas due to installation costs, expensive maintenance and impractical use with actual machines used along the growth period

(sowing, mechanical weeding, cutting and threshing process). Reduced tillage also has been implemented in small areas of Durango, but then abandoned due to specialized machinery requirements, increment in weed populations and excessive herbicide use; as well as long periods required before registering yield increments.

The use of the biological pests control has been implemented releasing natural predators of plague-insects causing production problems, but no clear results were observed. Cultivar choice is present in common bean including several landraces (Negro San Luis, Bayo Rata, Canario), improved cultivars (Pinto Saltillo, Pinto Centauro, PID 1 and NOD 1) and breeding lines (PT14053, NGO14013), although low genetic variation has been observed for resistance to *Fusarium* and other fungi included in the root-rot complex. However, differences have been observed for plant surviving or escape strategies avoiding severe problems caused by root and aerial plant diseases.

In chili peppers difficulties have been observed for cultivar change, due to traditional use of specific open-pollinated cultivars (landraces), high seed prices for commercial hybrids and specific traits observed in landraces for fresh and dried fruits, as well as for processed fruit (chile pasado) flavor. Similar traits were considered in other chili pepper cultivars (puya, güerito) used for specific preparations included in Durango's cousine (chile con queso and frijoles charros). However outstanding results have been observed by using tomato 'big plant', produced under nursery conditions [50], and technology could be used for producing pathogen-free chili seedlings.

4. Conclusions

The *Fusarium* genus causes significant reductions in yield and seed or fruits quality in common bean, chili pepper and other crops sown in North-Central México. Low income for farmers and total crop losses are also observed in *Fusarium* infested plots affecting food availability and the local economy. Modern agricultural practices should be validated and implemented for sustainable production in common bean, chili and other important crops used in the Mexican highlands. Breeding for plant adaptation, disease resistance, water productivity and product quality are the main concepts in modern and sustainable agriculture.

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Author details

Julio César Ríos Saucedo[1], María Gabriela Ramírez-Valadez[2], Saúl Santana Espinoza[3], Maihualy Martínez-Fernández[2] and Rigoberto Rosales-Serna[4*]

1 Sustainable Forest Management and Environmental Services, INIFAP - Campo Experimental Valle del Guadiana, Durango, México

2 Horticulture Program, INIFAP - Campo Experimental Valle del Guadiana, Durango, México

3 Forest and Agriculture Health Program, INIFAP - Campo Experimental Valle del Guadiana, Durango, México

4 Common Bean and Chickpea Program, INIFAP - Campo Experimental Valle del Guadiana, Durango, México

*Address all correspondence to: rosales.rigoberto@inifap.gob.mx

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