Cultivos Tropicales, 2019, vol. 40, no. 4, e10
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

octubre-diciembre
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

http://ediciones.inca.edu.cu

Bibliographic review

Generalities of the cultivation of chickpea and biological alternative for the control of the Marchitez

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ABSTRACT

The chickpea (*Cicer arietinum* L.), is one of the main sources of human and animal food and is placed on the list of the most cultivated legumes in the world, after soybeans (*Glycine max*), beans (*Vicia faba)*, beans (*Phaseolus vulgaris*) and peas or pea (*Pisum sativum*). Among the biotic factors that limit a high production of chickpea, are the diseases produced by different pathogenic microorganisms of the soil. The Withering or Fusariosis, the causative agent *Fusarium* spp., Is the most important disease of the chickpea crop, it negatively affects the components of the yield and grain quality. This disease occurs ascending and descending according to the species, sick plants abort their flowers and the few grains that manage to form are of smaller caliber. To control this disease, agro-technical, chemical and biological estrategies are used, the latter being very effective from the economic, social and environmental point of view. The biological fight with products based on *Trichoderma* strains has had a favorable acceptance, and generalized in Cuban agriculture, for showing several mechanisms of action with direct and indirect effects in the reduction of phytopathogenic microorganisms. In this sense, the Wilt is a disease of difficult control, so knowing about the biology of the pathogen and its interaction with the host are important aspects to establish a more efficient control.

Key words: *Cicer arietinum* L, *Trichoderma*, legumes, microorganisms, fusariosis

INTRODUCTION

Origin of the chickpea. Background of the culture. Use and importance

Chickpea is native to the southern Caucasus region and northern Persia, mainly the territory currently occupied by Iran (1). From there it spread to Europe, especially the Mediterranean region, and Africa, mainly Ethiopia, which was its secondary diversification center (2).

Different authors cite the existence of 40 spp. of chickpea preferentially located in the Middle East, Turkey, Israel and Central Asia (3). Of these a single species is cultivated, C. arietinum (4). The Spanish colonizers introduced it in Latin America, successfully implanting in California, Mexico and in dry climate regions throughout the Americas (5).

Chickpea is an improver of soil structure and fertility thanks to the symbiotic fixation of atmospheric nitrogen, which can exceed 70 kg ha^{-1}. In rotation with cereals it makes possible the breaking of the biological cycle of pathogens whose habitat is the soil such as: *Sclerotium rolfsii* Sacc, *Rhizoctonia solani* Künh and *Macrophomina phaseolina* [Tassi] Goidanich (6,7).

World production and trade

The world trade of chickpeas is around 1,100,000 tons, but it records large fluctuations between crop cycles. In the last 12 years the rate of increase of chickpea production in the world was 1.4 %. The main chickpea producing countries are: India, which participates with 68.5 % of the world total, is followed by Australia and thirdly Pakistan. Other countries of importance in production are Turkey, Myanmar, and in the Americas, Mexico and Canada (8). The main exporters are Spain, Jordan and Bangladesh (9).

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Taxonomic classification and botanical characteristics of the chickpea culture

According to Mateo-Box J M, cited by Del Moral [10]; the taxonomic location of the chickpea crop is:

Class: Angiospermae

Subclass: Dicotyledoneae

Order: Leguminosae

Family: *Fabaceae*

Genre: *Cicer*

Species*: Cicer arietinum* L.

This legume develops like a small shrub, an annual, diploid plant, with a chromosomal number of 2n = 16, with the presence of pubescences throughout the plant, glandular and non-glandular hairs. The roots are strong and the root system is deep, reaches 2 m deep, but the largest proportion is up to 60 cm. The stems are branched flexible or straight, erect or creeping crawlers and can reach up to 1 m tall. The leaves are pseudoimparipinate. Floral clusters usually have a flower, rarely two. The flowers are typically papilliated. The pods are pubescent, pointed and swollen, reaching up to 3 cm in length, which can contain up to three seeds. The seeds have shapes that vary between globular and bilobular, some being almost spherical, of various colors that can range from black to ivory white. The reproduction system is fundamentally autogamy; placing the level of autogamy around 1 % [11,12].

Edafoclimatic requirements

Chickpea is a legume well adapted to dry and cool weather. It is generally grown in the winter of tropical regions and in the spring and summer of temperate regions. Among the optimal conditions for cultivation are: temperatures between 21-29 °C during the day and 18-26 °C at night, average annual rainfall of 600 to 1000 mm. Hail, excessive rainfall and heat damage the crop. The development of cold-resistant cultivars allowed to change the sowing season in temperate zones towards winter, with cycles from sowing to 180-day harvest. The low relative humidity is optimal for seed set. Chickpea behaves like a neutral day plant, however, physiological studies characterize it as a quantitative long day plant, although it blooms in all photoperiods [13].

Factors that influence in the production of chickpea

Numerous factors, both abiotic and biotic, can limit chickpea production. Among the first are climatic factors, such as temperature, which can affect germination, plant growth and physiological processes related to plant development. Another factor is the soils. The crop prefers siliceous-clay or silt-clay soils, which do not contain aerated plaster. Chickpea is sensitive to salinity, both soil and irrigation water. The ideal pH for its development is between 6 and 9 [14].

Among the limiting biotic factors for chickpea production, there are diseases caused by different soil-borne microorganisms [15]. Therefore, 115 pathogens affecting chickpea were reported, including fungi, virus bacteria, phytoplasms and nematodes [16]. From these, only a few cause economic damage, which become limiting production in some areas [17]. It is reported that most fungal diseases of the crop can be controlled with fungicides, but their use is sometimes not economical, hence appropriate phytotechnical measures and resistant cultivars are used as more profitable measures [18].

The crop of chickpea in cuba

Chickpea productions in Cuba until the 1990s were of little consideration, and although they have increased, they do not meet national demand, so it was necessary to make imports, mostly from Mexico, Canada and Spain [11,12].

In Cuba, the crop develops in the dry winter period. When temperatures are high and there is a water deficit, the period of vegetative growth is reduced, causing early maturity, with considerable damage to grain production and their size. Temperatures above 30 °C accelerate the fall of flowers, limit symbiotic fixation and accelerate senescence of the crop. The optimum temperatures for seed germination are between 20 and 30 °C, seedlings emerging between 5 and 8 days after sowing. Irrigation improves nodulation and increases yield and number of pods [14]. The yield is favored by low rainfall during maturation, since high volumes of water during this period can cause losses of pods, since it favors the attack of fungal diseases, which affect the yield [19].

The sowing period runs from November 15 to December 30. In the case of short-cycle cultivars (100 days), in some locations with low rainfall or in years of late rains, it can last until January 15. The crop is demanding with the humidity in the stages of germination, pre-flowering, flowering and filling of the pods [(11)].

The wilt in the chickpea crop

The most important diseases in chickpea cultivation in Cuba and the world are those caused by soil fungi [(20)]. Among these are: *Fusarium* spp., *S. rolfsii*, *R. solani*, *M. phaseolina*, the most important being Fusarium spp. for the damages it produces and the frequency with which it occurs [(6)]. The disease appears mainly in clay soils or with drainage problems, a condition that favors the development of these pathologies [(21)].

In the world, this disease was named according to the symptomatology produced by the pathogen species; Yellowness and Vascular Wilt, caused *by F. oxysporum* f. sp. *Ciceri* [(22)]; Black root rot or dry rot, caused by *Fusarium solani* (Martius) [(23)]. In the investigations carried out in Cuba, on the identification of these species, the name of ascending Wilt was given to the symptoms caused by F. oxysporum and descending Marchitez, to the symptomatology caused by *F. solani* [(12)].

SYMPTOMATOLOGY

Infected plants have necrotic roots and brown spots on the neck. Inside the affected roots there are reddish brown colorations along the vascular system, an injury that extends from the root to the neck and to the area on the neck [(21)]. The fungus obstructs the transfer of the sage through the vessels and destroys the roots [(24)].

The aerial symptoms correspond to a gradual development of chlorosis in the foliage, growth arrest and lack of vigor. Sick plants abort their flowers and the few grains that form are smaller. Plants affected early in their development end up dying and those that are attacked later tend to mature early. These symptoms appear randomly, forming irregular circles around those that were first affected or in entire areas when it comes to poorly drained soils.

Causal organism

Fusariosis in chickpea is caused by several species of *Fusarium* [23]. This fungus is located in the:

Fungi kingdom

Phylum: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Nectriaceae;

Genus: *Fusarium*

Species: *Fusarium* spp.

Some researchers have reported in this crop the following *Fusarium* species: *F. oxysporum*, *F. solani* and *F. redolens* [25].

In Cuba there is little information on the diseases that affect this crop, however, *Fusarium* species that affect chickpea have been characterized, in the productions obtained in the provinces of Havana City and Havana, identified as *Fusarium oxysporum* Schlechtend. Fr.f. sp. *Ciceri* (Padwik) Matuo & K. Sato and *Fusarium solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen [26]. *Fusarium oxysporum* f. sp. *Ciceri* is a fungus whose habitat is the soil, an obligate necrotroph, pathogenically specialized in species of the *Cicer* genus, which infects their vascular system and can be transmitted by infected seeds [27]. However, *F. oxysporum* can asymptomatically invade roots of other plants, both leguminous and non-leguminous [22], where it survives, in the absence of its cultivated host. In addition, like the causative agents of other vascular fusariosis, it has the ability to survive inactively in the soil in the form of clamidospores for 6 years [27–30]. The germination of these is stimulated by the exudates of the roots of numerous plants, including non-host species [31].

On the other hand, *F. solani* remains saprophytic in the soil, on the residues of infected plants. In this way, the fungus can develop in the rhizosphere of susceptible plants, before invading their roots. The dissemination of spores and mycelium occurs with the transfer of infected and / or entrained water [32].

Soil compaction favors the invasion process and the development of symptoms. The persistence of this pathogen in the soil is very long and can last several years, so once it has been established in the soil, it becomes necessary to adopt very long rotations of 5-6 years [(23)].

Development of the disease

These fungi are transmitted by conidia and clamidospores, through irrigation water or contaminated soil. These can live in crop residues or decomposing organic matter, forming part of the community of organisms in most of the soils dedicated to horticultural crops. Also, they can spread through contaminated seeds, settling in soil free of the pathogen. They colonize the roots taking advantage of some stress condition of the plant and/or the presence of wounds in roots. The pathogen when it enters the root, grows, multiplies and causes rot that covers the entire root.

Control measures for the causant pathogen of the chickpea´s wilt

Among the measures used for the control of Fusarium in chickpea are: agrotechnical measures (use of chickpea cultivars with a certain degree of resistance to disease, adjustment of planting dates, biofumigation, use of high planting beds, rotation of crops), chemical (fungicide seed treatments) and biological (use of biological control agents as isolates of the genus *Trichoderma*). The best control measures are preventive, since there is no curative control that is effective and economically profitable [(33)].

Trichoderma as a biological control agent

Trichoderma species are characterized as saprophytic fungi and are widely distributed in different types of soils, especially those that contain organic matter or decaying plant wastes. They cover a group of filamentous fungi, facultative anaerobes, of more than 100 species [(34)].

The taxonomic location of this fungus is [(35)]:

Fungi kingdom

Division: Mycota

Subdivision: Eumycota

Class: Hyphomycetes

Order: Moniliales

Family: Moniliaceae

Genus: *Trichoderma*

Species: *Trichoderma* spp.

The genus *Trichoderma* in its vegetative state presents mycelium at the beginning of white, which turn dark green or yellowish, with dense sporulation [(36)]. The mycelium is mostly thin, and seen under a microscope it is fine, the conidiophores are branched and look like a small tree. They are presented as compacted tufts that form rings with a system of irregular branches in a pyramidal manner. These end up in phialides where asexual or conidia spores are formed, of great importance for taxonomic identification at the species level and to ensure fungus generations during a large part of the vegetative period of plants [(36)]. They are haploid and their wall is composed of chitin and glucans [(37)]. In conidiophores, they can occur on phyloids that emerge directly from the mycelium. It produces unicellular clamidospores. These structures are of vital importance for the survival of the fungus in the soil under adverse conditions [(37)].

The relationship between the temperature and the development of *Trichoderma*, depends on the species and the isolation itself, although in general this varies between 25 and 30 ºC (38). However, at 30 °C, the antagonistic activity of this species is almost nil (38). All of which constitute evidence that the optimum temperature for growth does not necessarily coincide with that of its antagonistic activity, and that there is a close relationship between isolation, antagonism and temperature [(39)].

The most commonly used species as biological control agents until a few years ago were *Trichoderma virens* (Miller, Giddens & Foster) Arx, *T. viride* and fundamentally *T. harzianum*, which is an antagonist of phytopathogenic fungi and virus vector fungi [(40,41)]. Currently several species of the genus, and among them *T. asperellum* [(42)], are used in the management of diseases caused by phytopathogenic fungi in various crops of economic importance (rice, beans, tomatoes, among others), with positive results on some pathogens from the soil (*R. solani* and *S. rolfsii*), in farmhouses and in the countryside [(43–45)].

Trichoderma action mechanisms as a biological control agent

Trichoderma presents different mechanisms of action, with direct and indirect effects. Among the main ones are competition for space and nutrients, mycoparasitism and antibiosis, which have a direct action against the phytopathogenic fungus (39–46). In addition, others are reported, whose bioregulatory action is indirectly, such as the induction of physiological and biochemical defense mechanisms, related to the activation in the plant of compounds related to resistance (Resistance Induction) (37), toxin detoxification excreted by pathogens and the deactivation of these enzymes during the infection process; the solubilization of nutritional elements, which in their original form are not accessible to plants. They have the capacity, in addition to creating a favorable environment for radical development, which increases the plant's tolerance to stresses (37–39).

Direct action mechanism

Competition by space or by nutrients

Competition is defined as the unequal behavior of two or more organisms in the same requirement, which reduces the amount of nutrients and / or space available to others. Hence, an essential factor in competition is the limitation of an element (47).

The high versatility in the use of substrates by *Trichoderma* species, allows you to rapidly colonize the medium and prevent the proliferation of other microorganisms in the same habitat. It has a capacity to mobilize and take nutrients from the soil superior to that of other organisms, based fundamentally on the ability to obtain ATP from the metabolism of different sugars or numerous polymers, present at different concentrations in environments where fungi develop. One of the explanations in this regard is due to the presence of the high affinity glucose transporter (Gtt1) in *T. harzianum* (CECT 2413), which enables it to obtain energy from hydrolyzed polymers and the rapid transport of sugar to the interior of the cells, when glucose concentrations are very low (48).

Antibiosis

Antibiosis takes place through interactions that involve diffusible components of low molecular weight or antibiotics produced by *Trichoderma* isolates, which inhibit the growth of other microorganisms [(39,49,50)].

Most strains of this genus produce volatile and non-volatile toxic metabolites, very diverse in structure and function, which prevent the colonization of pathogenic microorganisms [(51)]. Among these compounds are alkyl-pyrones (6-pentyl-α-pyrone), isonitriles (isonitrine), polyketides (harzyanolide), peptabioles (trichodermine, atroviridine, alameticine, suzucacillin and trichorzianine), diketopiperacins (gliovirin, and gliotoxin (sestoxin) heptelidic acid) and steroids (viridine), among others. The same strain can simultaneously produce several antibiotics, which reduces the risk of resistant microorganisms.

Micoparasitism

Mycoparasitism consists of a direct attack from one fungus to another, and leads to the modification or destruction of some of the host structures (mycelium, spores, sclerotia or others), with the consequent use of its components as a source of nutrients.

The mycoparasitic process is complex and may involve the chemotherapy growth of *Trichoderma* towards the host, stimulated by molecules from it, such as amino acids and sugars; lectin-mediated recognition; the formation of appressorium-like structures that contain high concentrations of osmotically active solutes, such as glycerol, and that facilitate penetration; the secretion of extracellular hydrolytic enzymes; and finally the penetration and death of the host [(41,52,53)]. Structural changes can be seen at the cellular level such as vacuolization, granulation, cytoplasm disintegration and cell lysis [(46,54,55)].

Enzymes that degrade the cell walls of phytopathogenic fungi can often act synergistically in combination with some toxic compounds and/or secondary metabolites such as peptabioles, which facilitates the entry of the *Trichoderma* hypha into the lumen of the parasitized fungus and the assimilation of cell wall content [(56)]. These enzymes are grouped into various families of chitinases, glucanases and proteases.

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Mechanisms of indirect action

Indirect action is based, both on the ability of the biocontroller to stimulate the growth and development of plants, and on the induction of plant defense mechanisms that increase their resistance to a wide range of phytopathogenic microorganisms (37– 39).

Trichoderma promotes plant growth, manifested in the empowerment of seminal germination, radical growth and development, nutrient intake and use, resistance to abiotic stress, a more abundant and early flowering, increase in height and weight of plants, even an increase in yields (56,57). These processes are mediated by the synthesis or stimulation of the production of phytohormones by the plant due to the interaction with some strains of *Trichoderma*, such as cytokinin-like molecules (zeatin) and gibberellin GA3; as well as the acidification of the surrounding environment due to the excretion of organic acids: gluconic, citric and fumaric, which allow the solubilization of phosphates, micronutrients and mineral traces (iron, manganese and magnesium) (37).

Induction of local or systemic resistance by *Trichoderma* is effective for many crops. It has similarities with the hypersensitivity response (HR) and acquired systemic resistance (SAR). *Trichoderma* species have been widely used in the control of soil pathogens, foliage and seeds. Among the controlled phytopathogens are: *R. solani*, *Fusarium* Schlecht. oxysporum f. sp. *dianthi* (Prill. & Del.) Snyder. & Hans, *Sclerotinia sclerotiorum* Lib., *Colletotrichum gloeosporioides* (Penz.) Sacc., *S. rolfsii*, *Rosellinia bunodes* (Berk. & Br.) Sacc., *Phytophthora* sp. Heinrich Anton de Bary, *Phytophthora cinnamomi* Rands, *Phytophthora cactorum* (Lebert et Cohn) Schröter, *B. cinerea*, *Armillaria mellea* (Vahl: Fr.) Kumm., *Pythium* sp. Pringsh, and parasitic *Cryptonectria* (Murr.) Barr., among others (36,55).

CONCLUSIONS AND PERSPECTIVES

Wilt or fusariosis of chickpea, is the most economically important disease in this crop, and is distributed worldwide. This is caused by different species of the genus Fusarium, so having a correct identification of the pathogen and the precise selection of biological control agents as alternatives for its control, and they are essential elements for the performance of a more efficient management, which can be combined with the remaining measures of disease management. Taking into account that in order to reduce the effects of Wilt, repeated and insufficient pesticide applications are recommended, having *Trichoderma asperellum* strains as an effective antagonistic microorganism, in addition to greatly reducing the effects of the disease, resource savings are achieved by concept of application of chemical products and in turn, the workforce of personnel dedicated to these tasks is reduced, which leads to a lower degree of toxicity in human health, improving the quality of life and therefore, allows us to adjust to the development of a more ecological and sustainable agriculture, considering that *Trichoderma* not only has the function of acting as an antifungal, but also, it is a stimulator of the growth and development of the crop and therefore the yields would be highly considerable.

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