Article

Cabbage residues for biocontrol of *Fusarium* spp. in tomato cultivation

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Abstract

One of the main phytosanitary problems in the production of tomato (tomate or jitomate in spanish) (*Solanum lycopersicon* Mill.), where soil is used as a growing medium, is *Fusarium* spp., chemical control is the method frequently used for its management; with important economic and environmental impacts. The objectives of this study were: to evaluate cabbage as a rotation crop and the incorporation of residues into the soil in the incidence of permanent wilting on tomato plants and the incidence of *Fusarium* spp., in the soil and evaluate the suppression of *Fusarium* spp., by applying by-products of cabbage leaves and stems. The study was carried out in two stages: in the first the evolution of *Fusarium* spp., was documented in a commercial tomato cultivation, established under anti-aphid mesh conditions and cabbage as a rotation crop, during the period 2012 to 2019. In the second stage, an aqueous extract and a dehydrated powder, obtained from residual cabbage leaves and stems, were evaluated. The results show that cabbage used as a rotation crop and the incorporation into the soil of residual leaves and stems reduced the incidence of permanent wilting on tomato cultivation and effectively suppressed *Fusarium* spp. As well, the dehydrated powder obtained from these residues showed a similar effect on the control of *Fusarium* spp., to that obtained in treatments with sodium methyldithiocarbamate.

Keywords: biofumigation, fusariosis, incidence.

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Introduction

In the production of tomato (*Solanum lycopersicon* Mill.), using soil as a growing medium, one of the main phytosanitary problems is vascular wilt or fusariosis caused by fungi of the genus *Fusarium* spp. (Ma *et al*., 2013). The incidence and severity of the disease is increased when the crop develops under greenhouses or meshes, due to the monoculture and intensity of the exploitation. For the control of this disease, the most common is the application to the soil of fumigants and chemical fungicides, such as sodium methylditiocarbamate, commercially called: Metam, Busan, Fumisol, Nemasol, Raisan.

These products have allowed to maintain the production of this crop; however, they cause a significant economic and environmental impact. The economic impact ranges from $7 000.00 to $40 000.00 pesos per hectare, depending on the quantity applied, recommendations usually start with 125 L ha^{-1} and increase to 800 L ha^{-1}, in addition to the increase of fungicides and additional actions, such as sanitary mats and elimination of diseased plants to reduce the spread of the pathogen, once the crop is established (Carmona and Sautua, 2017). The environmental impact is due to a significant decrease in the native microbiota of soil and the subsequent increase in pathogen populations, which begin with damage to some plants and subsequently expand, to cover entire regions (Abdel-Monaim *et al*., 2011).

On the other hand, there is a growing demand for safe foods produced without the use of agrochemicals or with the least use of them (Gottschalk and Leistner, 2012; Bryla, 2016). All this has led to the search for alternatives for disease management through a control more compatible with the environment, safer and viable (Zavaleta-Mejía, 1998; Ram *et al*., 2018).

An alternative to chemical control of this disease could be the use of natural substances with antagonistic properties to pathogens, especially of soil. Cruciferous are a clear example of this, because they have antimicrobial properties, related to the high content of sulfur compounds in their tissues called glucosinolates (Brown and Morra, 2005; Rodríguez *et al*., 2013), in addition to the enzyme thioglucoside glucohydrolase, called myrosinase (Morra and Kirkegaard, 2002), this enzyme hydrolyzes glucosinolates by transforming them into an unstable aglycone, which subsequently undergoes modifications to result in toxic volatile compounds such as sulfhydryl, nitriles, thiocyanates, isothiocyanates, among others. Each species of cruciferous has different classes and concentrations of glucosinolates (Rosa, 1997; Brown and Morra, 2005; Campas-Baypoli *et al*., 2009; Rodríguez *et al*., 2013), which are kept even in dehydrated residues (Lazzeri and Dallavalle, 2004).

The effectiveness of residues from different plants of the genus *Brassica* incorporated into the soil for the suppression of *Fusarium* spp., has been documented in the field (Gilardi *et al*., 2016; Prasad and Kumar, 2017; Campanella *et al*., 2020). The use of this alternative involves using cruciferous plants as a rotation crop, which is difficult in the protected agriculture system, due to the low availability of time, so it is proposed to evaluate other options, especially liquid and dehydrated extracts obtained from residual cabbage leaves and stems, as an option to chemical control and those who cannot or do not wish to use rotational crops for the management of *Fusarium* spp.

Based on the above, the objectives of this research were: To evaluate the effect of the use of cabbage as a rotation culture, and the incorporation of the residues into the soil, in the incidence of permanent wilting on tomato plants and on the populations of *Fusarium* spp., in the soil and evaluate the suppression of permanent wilting in tomato plants and the incidence of *Fusarium* spp., in the soil by applying by-products of cabbage leaves and stems.

Materials and methods

The study was conducted in two stages

First stage: evaluation of cabbage as a rotation crop and the incorporation of the residues into the soil in the incidence of permanent wilting on tomato plants and incidence of *Fusarium* spp. in the soil. The experiment was conducted for seven years (2012-2019), on a commercial planting of tomato type saladette F1 (of indeterminate growth), planted in bare soil, on an area of 6 ha, covered with anti-aphid mesh, located in Rancho Poca Luz 1, in San Cristóbal, municipality of Catorce, San Luis Potosí, Mexico. Tomato production cycles were from April to September, and cabbage (rotation crop) from October to February. In February cabbage was harvested, the extended leaves and stems were incorporated into the soil as harvest residues.

Plants with permanent wilting were given fungicides (tiabendazole, carbendazim, methyl thiophanate) directly to the plant neck, only to contain the spread of the pathogen. Prior to the start of each tomato production cycle, the population of *Fusarium* in soil was estimated, in a certified laboratory (CIFEF, 2018), from 2011 to 2016, a sample was made for the entire area and from 2017 to 2019 four samplings were carried out. The incidence of fusariosis on plants was assessed and verified by CIFEF. From 2012 to 2016 only one register of plants with wilting and dead in the whole area was carried out at the end of the production cycle, while from 2017 to 2019, plants with wilting in the same area, but divided into four sections, were sampled. The data from these samples were used for the incidence analysis of *Fusarium* spp.

Second stage: it consisted of evaluating the suppression of *Fusarium* spp., in the soil by applying by-products of cabbage leaves and stems. It was held in the facilities of the horticulture department of the Universidad Autónoma Agraria Antonio Narro (UAAAN), in Saltillo, Coahuila, Mexico. To obtain dehydrated powder, leaves and stems were collected from the cabbage (70-30 p/p), at the end of the cabbage harvest, the plant was cut from the base of the stem, and both tissues were exposed directly to the sun until they registered a constant weight (approximately 8 days), then they were ground with a hand mill and screened using a 3 mm mesh. The aqueous extract: was obtained from fresh leaves and stems, the tissues were liquefied with a household blender, for three min at 23 °C, at the rate of 200 g of fresh leaves and stems per 100 ml of distilled water, then it was filtered using standard filter paper, with a pore size of 10µm, the filtrate was preserved in a flask in refrigeration at 4 °C until use.

In addition, in this test a treatment with segmented leaves and stems, similar to that obtained in commercial planting, was added. For which fresh leaves and stems were used, that were chopped to a size of 10 cm, with a stainless-steel knife. The treatments evaluated at this stage are described in Table 1. Each treatment consisted of 24 polyethylene pots with 10 L (repeats) with infested soil and each pot contained one plant.

Table 1. Treatments based on aqueous extract and dehydrated powder from residual cabbage leaves and stems, evaluated in the suppression of *Fusarium* spp., on tomato plants.

Number	Treatment description
T1	Sterilized floor (absolute witness)
T2	Soil infested with *Fusarium* spp. (1 000 ufc kg^{-1}1) (CIFEF, 2018).
T3	Infested soil + Sodium methyldithiocarbamate* (1 cm^3 L^{-1} water)
T4	Infested soil + fresh cabbage segments (150 g $pot^{-1)}$
T5	Infested soil + aqueous cabbage extract (210 cm^3 pot^{-1})
T6	Infested soil + dehydrated cabbage powder (15 g $pot^{-1)}$

*= commercial product Busan 1020. (Metam sodium at 33%). Summit Agro, Mexico.

For the application of treatments with dehydrated powder and segments (pieces) of cabbage, previously the pots were filled up to half with soil infested, then the treatments were added, and the pots were covered with soil until filled, for treatments of aqueous extract and sodium methylditiocarbamate (MDTCNa), they were applied in irrigation water, dissolving the treatments in 2 L of water. MDTCNa was applied 21 days before transplantation, for the rest of the treatments the transplantation was carried out immediately after application. A Yamato SQ810C autoclave was used for soil sterilization.

At this stage it was evaluated: incidence, measured as plants with symptoms of fusariosis (permanent wilting and dead plants), additionally at the end of the cultivation a disk was taken from the base of the stem, this disk was washed with distilled water on the outside to avoid contamination with other microorganisms, subsequently they were sectioned into four parts, to expose the inside of the stem, and put in a PDA culture medium, at 72 h growths were reviewed to check for the presence of *Fusarium* spp. To estimate productivity and quality of tomato, number and weight of fruits were measured in each cut and for quality the average weight of the fruits was determined.

The data obtained were subjected to a variance analysis (Andeva)($p \geq 0.05$ and 0.01) under a random complete block design and a Tukey's mean separation test ($p \geq 0.05$), (Zar, 2010), using Statistica version 7.0 software.

Results and discussion

First experimental stage: evaluation of cabbage as a rotation crop and the incorporation of the residues into the soil in the incidence of permanent wilting on tomato plants and incidence of *Fusarium* spp., in soil.

Population of *Fusarium* in the soil. Based on soil analyses at the beginning of each tomato cycle, the species of *F. oxysporum* and *F. solani* were identified (CIFEF, 2018) and a downward trend of both *Fusarium* populations in the soil was generally observed, when using cabbage as a rotation crop and the incorporation of residual stems and leaves into the soil. As well, in general, the largest reduction was observed in the first two production cycles, and from the third cycle the population remained at average values of 1 000 ufc kg^{-1} (Figure 1).

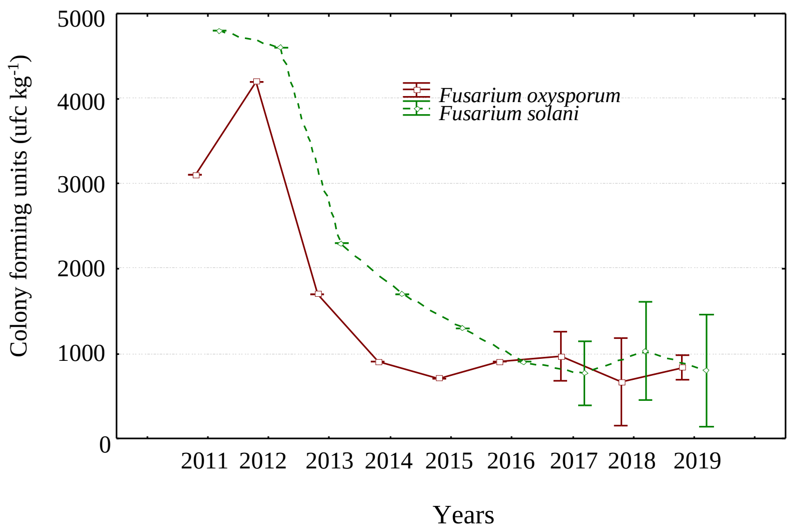

Figure 1. Population dynamics of *Fusarium oxysporum* and *Fusarium solani* in the soil used for the cultivation of tomato and cabbage as a rotation crop.

The decline in the populations of *F. oxysporum and F. solani* appears to be related, on the one hand, to the use of cabbage as a rotation crop, which limits the availability of food, taking advantage of the documented specificity of these *Fusarium* species towards solanaceous and not so for cruciferous (Gil, 1972; Fahey, 2001), being the availability of food the main condition for the growth of the populations of any organism (Bell *et al*., 1991).

On the other hand, the populations of *Fusarium* could be affected by products derived from the decomposition of the cabbage leaves and stems incorporated into the soil as residues or esquilmos, because glucosinolates and myrosinase when contacted result in a number of compounds such as: sulfhydryl, nitriles, thiocyanates and isothiocyanates, the latter with biofumigant or biocidal capacity against soil fungi including *Fusarium* spp. (Fahey *et al.*, 2001; Morra and Kirkegaard, 2002; Lazzeri *et al*., 2004; Pérez, 2014).

Incidence of *Fusarium* spp. The initial incidence of plants with permanent wilting or dead was 11.3%. After the first rotation with cabbage and the incorporation of the residues, this value decreased significantly to 6.8%. In subsequent cycles this incidence was variable, but with a downward trend. During the 2017 to 2019 cycles, the incidence remained more stable and below close to 4% (Figure 2).

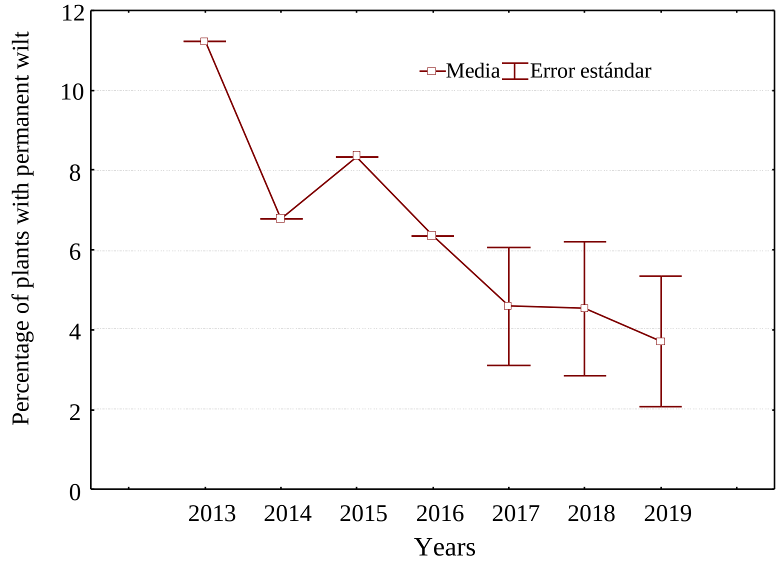

Figure 2. Incidence of permanent wilting caused by *Fusarium* spp., in tomato cultivation, using cabbage as a rotation crop and incorporating its residues into the soil. Over a seven-year sampling period.

The incidence of permanent, wilting through the 2017-2019 tomato growing cycles (Figure 3), showed low percentages (< 0.3) in the first months after tomato cultivation was established and increased rapidly in June, and remained until August. The above may have been due to rains usually recorded in June, coupled with the fact that June to August are the warmest months of the year in the region where the experiment was conducted, which favors the development of *Fusarium* spp., as it has been documented that this pathogen grows optimally in warm climates and moist soils (Agrios, 1985). While the incidence tends to drop in September and October, possibly to the decrease in temperature.

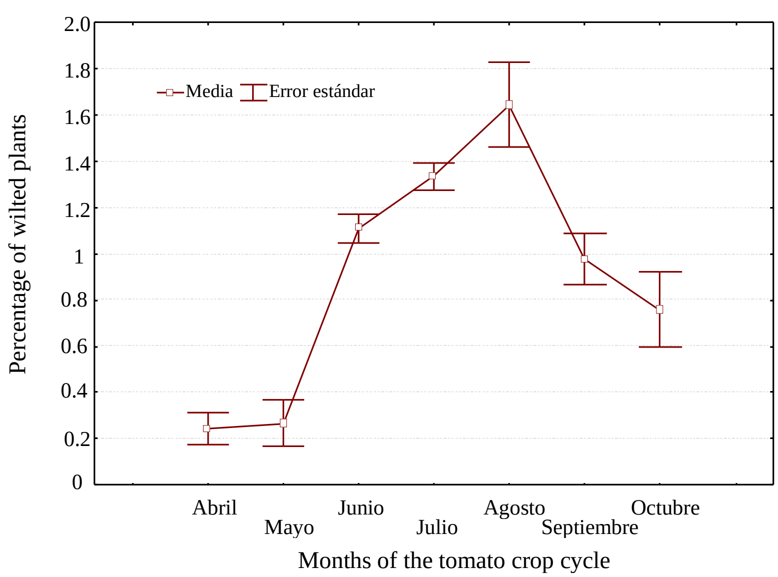

Figure 3. Dynamics of the incidence of permanent wilting in tomato plants during the growing cycle. The three-cycle average (2017-2019) is shown.

On the other hand, the tomato grown under the conditions described above begins to produce at 65 days after transplantation, at this stage the plant increases the accumulation of carbohydrates and as a consequence the susceptibility of the plant to the attack of phytopathogenic fungi, which could be reflected in the increase in the incidence of permanent wilting by *Fusarium* from June, with an upward trend until August, in each cycle of cultivation.

Part two: evaluation of an aqueous extract and a dehydrated powder, obtained from residual cabbage leaves and stems.

Dehydrated cabbage leaves and stems significantly reduced the incidence of the disease and the population of *Fusarium* spp., in soil (Table 2), attributed to the fact that both glucosinolates and myrosinase remained in dehydrated tissues and that the synthesis of thiocyanates and isothiocyanates started when hydrating with soil moisture (Sarwar *et al*., 1998; Caballero, 2017). The incidence values obtained with this treatment were similar to those obtained with conventional MDTCNa fumigant (Table 2). The aqueous extract had a limited effect respect to untreated soil (T2), possibly due to the liquefaction process, which might have accelerated the process of synthesis of thiocyanates and isothiocyanates, by allowing rapid contact of glucosinolates with myrosinase, in addition, as they were volatile compounds, it may have drastically decreased their concentration (Wu *et al*., 2015).

Table 2. Incidence of permanent wilting by *Fusarium* spp., on tomato plants and colonization of *Fusarium* in soil treated with by-products of cabbage leaves and stems.

Treatments	Wilted plants (%)	Dead plants (%)	Colonization Soil ufc kg^{-1}
T1	4 d	0 d	33 c
T2	40 a	40 a	2400 a
T3	4 d	4 c	300 ab
T4	12 c	8 c	566.66 ab
T5	28 b	20 b	1833 a
T6	8.8 cd	4 c	500 ab

Different letters in the same column indicate statistical difference in the means according to the Tukey test ($p \geq 0.05$). ufc= colony forming units.

The low presence of plants with wilting is possibly related to the synchrony of thiocyanate and isothiocyanate synthesis and the increase in the population of *Fusarium* spp., since the infection is not immediate, so for the synthesis of isothiocyanates to occur, the process of hydration of cabbage powder must occur, since there must be an aqueous means for the enzyme to meet glucosinolates.

Effect on yield and quality

The yield and quality of the treated tomato plants are shown in Table 3. The yield was different, due to the elimination of dead plants or due to the reduction of productivity of the plants with wilting.

Treatment with aqueous cabbage extract was the least efficient, possibly attributed to the volatilization of thiocyanates and isothiocyanates (Wu *et al*., 2015; Hashimoto *et al*., 2020), so the plant was most severely affected by the disease and consequently its productivity decreased (Rodríguez-Araujo *et al*., 2010).

Treatment based on powdered cabbage (T6) showed the highest yield, even higher than that obtained in plants treated with MDTCNa (Table 3). This response may be related, in addition to the reduction of plants with wilting, to the addition of organic matter, which occurs when incorporating dehydrated powder from cabbage leaves and stems. By adding 15 g per 10 L of soil pot, it is equivalent to supplying 4 500 kg $ha^{-1}$of dry organic matter, which is similar to the amount of harvest residues that is incorporated into the soil when cabbage is used as a rotation crop.

Cabbage produces from 80 to 100 tons of green matter, about half of which are residues left in the field, this amount of organic matter is sufficient to induce significant changes in the chemical-physical properties and fertility of soils and therefore in the improvement of crops (Salas-Pérez *et al*., 2016; Oldfield *et al*., 2017; Pinedo *et al*., 2018).

Table 3. Effect of the by-products of cabbage leaves and stems applied for the suppression of *Fusarium* spp., and its impact on the yield and quality of tomato.

Treatments	Number of fruits	Average fruit weight (g)	Yield (kg)	Reduction in yield (%)
T1	202.34 a	114.16 ab	23.09 a	+5.19 a
T2	103.5 b	101.87 b	10.54 a	-51.85 a
T3	195.54 ab	111.95 ab	21.89 a	0 a
T4	175.13 ab	109.54 b	19.18 a	-12.38 a
T5	145.79 ab	108.7 b	15.84 a	-27.63 a
T6	175.42 ab	127.54 a	22.37 a	+2.19 a

Different letters in the same column indicate statistical difference in the means according to the Tukey test ($p \geq 0.05$). ufc= colony forming units.

Conclusions

Cabbage (*Brassica oleracea* var. *capitata* L.), used as a rotation crop and the incorporation of its residual leaves and stems into the soil, decreased the population of *Fusarium oxysporum* and *Fusarium solani* in the soil and reduced the incidence of plants with permanent wilting caused by *Fusarium* spp., in tomato (*Solanum lycopersicon* Mill.) grown in soil and ant-aphid mesh. As well, the dehydrated powder obtained from the residual cabbage leaves and stems reduced the incidence of fusariosis on tomato plants and suppressed the population of *Fusarium* spp., in the soil, in a similar way to sodium methylditiocarbamate.

Cited literature

Abdel-Monaim, M. F.; Abo-Elyousr, K. A. M. and Morsy, K. M. 2011. Effectiveness of plant extracts on suppression of damping-off and wilt diseases of lupine (*Lupinus termis* Forsik). Crop Protec. 30(2):185-191. https://doi.org/10.1016/j.cropro.2010.09.016.

Agrios, N. G. 1985. Fitopatología. Marchitamientos por *Fusarium*. Editorial Limusa. México, DF. 371-375 pp.

Bell, G. and Koufopanou, V. 1991. The architecture of the life cycle in small organisms. Philosophical Transactions of the Royal Society of London. Series B. Biological Sci. 332(1262):81-89.

Brown, J. y Morra, M. J. 2005. Glucosinolate-containing seed meal as a soil amendment to control plant pests:2000-2002. (No. NREL/SR-510-35254). National Renewable Energy Lab. Golden. CO (US). 199 p.

Bryła, P. 2016. Organic food consumption in Poland: motives and barriers. Appetite. 105:737-746. https://doi.org/10.1016/j.appet.2016.07.012.

Caballero, B. L.; Márquez, C. J. y Betancur, M. I. 2017. Efecto de la liofilización sobre las características caracteristicas físico-químicas del ají rocoto (*Capsicum pubescens* R & P) con o sin semilla. Bioagro. 29(3):225-234.

Campanella, V.; Mandal, C.; Angileri, V. and Miceli, C. 2020. Management of common root rot and *Fusarium* foot rot of wheat using *Brassica carinata* break crop green manure. Crop Protection. 130:105073. https://doi.org/10.1016/j.cropro.2019.105073.

Campas-Baypoli, O. N.; Bueno-Solano, C.; Martínez-Ibarra, D. M.; Camacho-Gil, F.; Villa-Lerma, A. G.; Rodríguez-Núñez, J. R. y Sánchez-Machado, D. I. 2009. En vegetales crucíferos. Archivos Latinoam. Nutrición. 59(1):95-100.

Carmona, M. y Sautua, F. 2017. La problemática de la resistencia de hongos a fungicidas. causas y efectos en cultivos extensivos. Rev. Facultad de Agronomía UBA. 37(1):1-19.

CIFEF. 2018. Centro Internacional de Servicios Fitosanitarios, SA de CV. Laboratorio integral de diagnóstico fitosanitario. Cedula de aprobación de SAGARPA: 97-705-002 DFG. Saltillo, Coahuila, México.

Gilardi, G.; Pugliese, M.; Gullino, M. L. and Garibaldi, A. 2016. Effect of different organic amendments on lettuce fusarium wilt and on selected soilborne microorganisms. Plant Pathol. 65(5):704-712.

Gottschalk, I. and Leistner, T. 2012. Consumer reactions to the availability of organic food. Inter. J. Consumer Studies. 37(2):136-142. https://doi.org/10.1111/j.1470-6431.2012.01101.x.

Hashimoto, Y.; Sakamoto, H.; Asai, H.; Yasoshima, M.; Min, H. and Koichi, L. 2020. The effect of fumigation with microencapsulated allyl isothiocyanate in a gas barrier bag against *Solenopsis invicta* (Hymenoptera : Formicidae). Appl. Entomol. Zoology. 55(3):345-350. https://doi.org/10.1007/s13355-020-00684-9.

Lazzeri, L.; Curto, G.; Leoni, O. and Dallavalle, E. 2004. Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode Meloidogyne incognita (Kofoid et White) Chitw. J. Agric. Food Chem. 52(22):6703-6707. https://doi.org/10.1021/jf030776u.

Ma, L. J.; Geiser, D. M.; Proctor, R. H.; Rooney, A. P.; O’Donnell, K.; Trail, F. and Kazan, K. 2013. Fusarium pathogenomics. Annual Review Microbiol. 67(1):399-416. https://doi.org/10.1146/annurev-micro-092412-155650.

Morra, M. J. and Kirkegaard, J. A. 2002. Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biol. Biochem. 34(11):1683-1690. https://doi.org/10.1016/S0038-0717(02)00153-0.

Oldfield, E. E.; Wood, S. A. and Bradford, M. A. 2017. Direct effects of soil organic matter on productivity mirror those observed with organic amendments. Plant and Soil. 423(1):363-373.

Pinedo, M.; Abanto-Rodríguez, C.; Oroche, D.; Paredes, E.; Bardales-Lozano, R. M.; Alves, E. y Vargas, J. 2018. Mejoramiento de las característias agronómicas y rendimiento de fruto de camu-camu con el uso de biofertilizantes en Loreto, Perú. Scientia Agropecuaria, 9(4):527-533.

Prasad, P. and Kumar, J. 2017. Management of fusarium wilt of chickpea using brassicas as biofumigants. Legume Res. 40(1):178-182. https://doi.org/10.18805/lr.v0i0.7022.

Ram, R. M.; Keswani, C.; Bisen, K.; Tripathi, R.; Surya, P. and Singh, H. B. 2018. Biocontrol technology: eco-friendly pproaches for sustainable agriculture. omics technologies and bio-engineering. Academic Press. 177-190 pp. https://doi.org/10.1016/B978-0-12-815870-8.00010-3.

Rodríguez-Araujo, E. A.; Bolaños-Benavides, M. M. and Menjivar-Flores, J. C. 2010. Effect of the fertilization on the nutrition and yield of the red pepper (*Capsicum* spp.) in the Valley of the Cauca, Colombia. Acta Agronómica. 59(1):55-64. http://www.redalyc.org/pdf/1699/169916223005.pdf.

Rodríguez, M. K. A.; Monreal,V. C. T.; Huerta, D. J.; Soria, C. J. C. y Jarquín, G. R. 2013. Aporte de microorganismos benéficos por la incorporación al suelo de residuos deshidratados de col (*Brassica oleracea* var *capitata*) y su Efecto en el pH. Rev. Mex. Fitopatol. 31(1):29-44.

Rosa, E. A. S. 1997. Daily variation in glucosinolate concentrations in the leaves and roots of cabbage seedlings in two constant temperature regimes. J. Sci. Food Agric. 73(3):364-368. https://doi.org/10.1002/(SICI)1097-0010(199703)73:3<364::AID-JSFA742>3.0.CO;2-O.

Salas-Pérez, L.; González, J. A.; Garcia, M.; Sifuentes, I. E.; Parra, T. S. y Preciado, R. P. 2016. Calidad biofísica y nutracéutica de frutos de tomate producido con sustratos orgánicos. Nova Sci. 8(17):310-325.

Sarwar, M.; Kirkegaard, J. A.; Wong, P. T. W. amd Desmarchelier, J. M. 1998. Biofumigation potential of brassicas III. *In vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. Plant and Soil. 201(1):103-112. https://doi.org/10.1023/A:1004381129991.

Wu, H.; Xue, N.; Hou, C.; Feng, J. and Zhang, X. 2015. Microcapsule preparation of allyl isothiocyanate and its application on mature green tomato preservation. Food Chem. 175:344-349. https://doi.org/10.1016/j.foodchem.2014.11.149.

Zar, J. H. 2010. Biostatistical analysis (5th Edition). Prentice Hall, New Jersey. 472-478 pp.

Zavaleta-Mejía, E. 1999. Management alternatives for plant diseases. Terra Latinoam. 17(3):201-207.