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Invitro Evaluation of Bio-control Agents against Fusarium wilt and Ascochyta Blight of Chickpea

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A B S T R A C T

Four fungal bio-control agents, *viz.*, *Trichoderma harzianum, T. viride, Aspergillus* and *Tricothecium* were evaluated *invitro* through dual culture technique against *Fusarium oxysporum* f. sp. *ciceri*, the causative agent of wilt of chickpea and *Ascochyta rabiei*, the causative agent of Ascochyta blight of chickpea. The observations were taken after fourth and seventh day for both the pathogens. After fourth day, observations recorded in case of *Fusarium oxysporum* f. sp. *ciceri*, the minimum colony diameter (1 cm) and maximum inhibition percentage over control (85.71%) was observed in *Trichoderma harzianum* which were statistically at par with *T. viride*, *Aspergillus* and *Tricothecium* while after seventh day, the result was more or less similar. The minimum colony diameter (1.2 cm) of pathogen was observed in *Trichoderma harzianum* with maximum inhibition per cent (86.66%) over control, which differed statistically from *T. viride, Aspergillus. Tricothecium* showed least effectiveness against the target pathogen showing colony diameter (4.1 cm) and inhibition (41.42%) after fourth day and colony diameter (4.6 cm) and inhibition (48.88%) after seventh day over control. After fourth day, the observations taken in case of *Ascochyta rabiei*, the minimum colony diameter (0 cm) and maximum inhibition per cent over control (100%) were observed in *T. harzianum* and *T. Viride* which were statistically at par with *Aspergillus* and *Tricothecium.* After seventh day the result was more or less similar. The minimum colony diameter (0 cm) of pathogen was observed in *T.harzianum* and *T. viride* with maximum inhibition percentage (100%) over control, which differed statistically from *Aspergillus. Tricothecium* showed least effectiveness against the target pathogen showing colony diameter (1 cm) and inhibition per cent (66.66%) after fourth day and colony diameter (1.2 cm) and inhibition per cent (82.85%) after seventh day over control.

Introduction

Chickpea (*Cicer arietinum* L) is a cool season legume crop belongs to family Fabaceae and subfamily Papilionaceae (Varshney *et al.*, 2013). Chickpea is the second most important pulse crop worldwide in terms of area under cultivation, and ranks third in production followed by beans (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.). Chickpea is a self-pollinated crop (Toker *et al.*, 2006). It is also known as King of pulses. There are two

main types of chickpea germplasm, namely "Kabuli" and "Desi". The Kabuli (macrosperma) chickpea has relatively large creamy colored seeds, white flowers and do not contain anthocyanin. In contrast the Desi (microsperma) chickpea has small seeds of various colors, purplish flowers and contain anthocyanin. In India chickpea is grown on about 8.11 million hectares area with a production of 5.90 million tons, respectively (Anonymous, 2016).

In India, chickpea (*Cicerarietinum.* L.) is grown for dal making, culinary and for table purpose. It constitutes the main source of protein and several amino acids, therefore; it is useful diet for human beings and occupies a prominent place and is popular due to high nutritional value. It is considered as an alternate to meat for it contains 38-59 per cent protein, 3 per cent carbohydrates, 4.8-5.5 per cent ash fiber, 3 per cent oil, 0.3 per cent phosphorus, 6.29-6.99 per cent crude lipid and amino acids and poly phenol contents with some per cent of minerals (Iqbal *et al.,* 2006; Amjad *et al.,* 2006). Chickpea is a good source of minerals such as Ca, P, Mg, Fe, K and β-carotene. Chickpea has higher content of manganese, zinc and phosphorous than other legumes (Wang *et al.,* 2010).

Chickpea often experiences both abiotic and biotic stress that hampers its growth and decreases its yield. The abiotic stresses like heat, salinity, drought and cold are the major adverse environmental factors that limit plant productivity (Ahmad *et al.,* 2014). While as, biotic stresses include diseases, insects, pests and plant-parasitic nematodes that drastically affect chickpea yield (Basandrai *et al.,* 2011). The major diseases are *Fusarium* wilt (*Fusarium oxysporum*f. sp. *ciceri*), Ascochyta blight (*Ascochytarabiei*), Phytophthora root rot (*Phytophthor amedicaginis*) and Botrytis grey mould (*Botrytis cinerea*) (Ahmad *et al.,* 2005; Knights *et al.,* 2008). *Fusarium* wilt and Ascochyta blight are two of the most important diseases responsible for reducing the yield of chickpea.

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*(Foc) is responsible for wilting, flagging and consequently loss of the yield of the affected plants. Pathogen causes mortality of young seedlings and adult plants to death (Haqqani *et al.,* 2000). *Fusarium* wilt infested seedling collapse and lie flat on the ground retaining their dull green color. Adult plants show typical wilt symptoms of drooping of petioles, rachis and leaflets (Mahmood *et al.,* 2011).

Fusarium wilt is very common seed/soil borne disease causing 10 to 12 per cent annual loss in India. It is a typical vascular disease causing xylem necrosis. The disease is systemic in nature and plants get infected at any stage. The fungus survives in soil up to six years even in absence of host. It is widely spread disease covering in all major chickpea growing areas.

Wilt pathogen destroy the crop completely which cause significant annual yield loss; nevertheless, its prevalence is less common where cold temperature persists for longer period. Presence of sufficient soil moisture and 20-30ºC temperature cause fast spread of disease. Early wilting is more detrimental than late wilting. The most practical, efficient, economical and ideal way of managing chickpea wilt is the use of resistant cultivars (Iqbal *et al.,* 2005; Bakhsh *et al.,* 2007).

Fusarium oxysporum f. sp. *ciceri* produces three types of asexual spores, macroconidia, microconidia and chlamydospores. The teleomorph of *Fusarium oxysporum*is unknown (Leslie and Summerell, 2006).

Ascochyta rabiei, the causal agent of *Ascochyta* blight of chickpea, exists both as

an anamorph and a teleomorph. The anamorph, *A. rabiei*, is characterized by the formation of spherical or pear-shaped black fruiting bodies called pycnidia. A pycnidium contains numerous hyaline unicellular and occasionally bicellular spores, pycnidiospores, or conidia, developed on short conidiophores (stalks) embedded in a mucilaginous mass. Pycnidiospores are oval to oblong, straight, or slightly bent at one or both ends and measure 6–12 by 4–6µm (Nene 1982). The teleomorph, *Didymellarabiei* (Kovacheski) var. Arx (Syn. *Mycosphaerella rabiei* Kovacheski), is a bipolar heterothallic ascomycete and is characterised by pseudothecia developing on chickpea crop residues that have over-wintered in the field. For successful sexual reproduction, the telomorph requires pairing of 2 compatible mating types (MAT1-1 and MAT1-2), which are widely distributed in several major chickpea-growing areas of the world (Armstrong *et al.,* 2001).

Symptoms of AB can develop on all aerial parts of a plant. Seed-borne infection leads to brown lesions at the stem base of emerged seedlings. Subsequently, the lesions enlarge in size, and girdle the stem causing its breakage and death of the plant. Numerous pycnidia develop on the necrotic lesions. In the field, AB may initially appearas small patches (foci) of blighted plants, but can rapidly spread across an entire crop under favourable temperature and rainfall. Plants are attacked at any growth stage, depending on the inoculums availability. However, AB is most prominent during the flowering to early podding growth stages. Air-borne conidia and ascospores infect younger leaves and produce small water-soaked necrotic spots that rapidly enlarge and coalesce. Conidia may also be water-borne and splash dispersed to infect foliage tissue on the same or nearby plants. Subsequently, symptoms spread rapidly to all aerial parts including leaves, petioles, flowers, pods, branches, and stems, which lead to rapid collapse of tissues and death of the plant. Development of pycnidia in concentric rings on lesions is the characteristic symptom of *A. rabiei* infection. Lesions that develop on leaves and pods appear circular with brown margins and a grey centre that contains pycnidia, whereas lesions developing on petiole, stems, and branches are elongated. The lesions that develop on apical twigs, branches, and stems differ in size and in later stages girdle the affected plant parts. The regions above the girdled portion are killed and may break off. Diseased pods with visible blight symptoms often fail to develop any seed. Pod infection often leads to seed infection through the testa and cotyledons. Infected seed can be discoloured and possess deep, round or irregular cankers, sometimes bearing pycnidia visible to the naked eye. Infection during the pod maturation stage often results in shriveled and infected seed (Singh and Sharma 1998; Akem 1999).

Materials and Methods

Biocontrol agents *viz., T. harzianum, T. viride, Aspergillus* and *Trichothecium* were screened for their efficacy against *Fusarium oxysporum* f. sp. *cicero* the causative agent of wilt of chickpea and *Ascochyta rabiei*, the causative agent of *Ascochyta* blight of chickpea in the Division of Plant Pathology, SKUAST of Kashmir. The bioassay was done by the dual culture technique given by Morton and Stroube (1955). Seven day old culture discs of 5 mm diameter each of fungal antagonist and the pathogen were placed at equidistance from the margin of Petri plates (90 mm) containing Potato Dextrose Agar medium on opposite sides. The plates were incubated for 7 days at 25 ± 2^{0}C in the BOD incubator after inoculation of the pathogen (Wang *et al.,* 2003). A control having only test pathogen was also kept side by side for comparison. Each treatment was replicated

thrice. Observations on linear growth (cm) of the test organism up to the inhibition zone were recorded after fourth and seventh day and the per cent growth inhibition of test pathogen over control was calculated. Radial growth of the mycelium in Petri plates was measured in two directions at right angles to each other and average of two was expressed as diameter of colony (Lilly *et al.*, 1951). In case of wavy, irregular growth of colony average of the largest and shortest diameter was taken as the growth diameter (Brown, 1923). The radial growth of mycelium was recorded in each treatment after the pathogen covered the media in control and per cent inhibition over control was calculated using the formula (Vincent, 1947).

$$\text{Per cent growth inhibition} = \frac{C-T}{C} \times 100$$

Where, C= Colony diameter of pathogen in control.

T= Colony diameter of pathogen in treatment.

Results and Discussion

Four bio agents *viz.*, *Trichoderma harzianum*, *T. viride*, *Aspergillus* and *Tricothecium* were evaluated by dual culture technique against *Fusarium oxysporum* f. sp. *ciceri.* The data revealed that all bioagents inhibited the growth of *Fusarium oxysporum* f. sp. *ciceri* varied from 48.88 per cent to 86.66 per cent (Table 1). Minimum colony diameter (1 cm) and maximum inhibition per cent over control (85.71 %) was observed after fourth day in case of *T. harzianum* and which were found statistically at par with each other while *T. viride* showed 76.28 per cent inhibition (1.4 cm), *Aspergillus* 72.85 per cent inhibition (1.9 cm diameter) and *Tricothecium* 41.42 per cent inhibition (4.1 cm diameter). After seven days the results were more or less similar to the results of fourth day. The minimum colony diameter (1 cm) of pathogen was observed in case of *T. harzianum* with the inhibition percentage of 86.66 per cent over controls which were observed statistically different from *T. viride, Aspergillus* and *Tricothecium*. The data also revealed that *T. harzianum* and *T. viride* were actively inhibiting the growth of pathogen in dual culture up to seven days and also the inhibition was more or less same to the inhibition after four days. *Tricothecium* was least effective biological agent against the target pathogen with 4.1 cm diameter and 41.42per cent inhibition after fourth day and with 4.6 cm diameter and 48.88per cent inhibition after seventh day over control (Plate 1). This may due to the slow growth of biocontrol agent. The present findings are in accordance with other workers like Subhani *et al.* 2013.

The data revealed that all bioagents inhibited the growth of *Ascochyta rabiei* varied from 82.85 per cent to 100 per cent (Table 2). Minimum colony diameter (0 cm) and maximum inhibition per cent over control (100 %) were observed after fourth day in case of *T. harzianum* and *T. viride*w hich were found statistically at par with each other while *Aspergillus* showed 83.33 per cent inhibition (0.5 cm diameter) and *Tricothecium* 66.66 per cent inhibition (1 cm diameter). After seven days the results were more or less similar to the results of fourth day. The minimum colony diameter (0 cm) of pathogens was observed in case of *T. harzianum* and *T. viride* with the inhibition percentage of 100 per cent over controls which were observed statistically different from *Aspergillus* and *Tricothecium*. The data also revealed that *T. harzianum* and *T. viride* were actively inhibiting the growth of pathogen in dual culture up to seven days and also the inhibition was more or less same to the inhibition after four days. *Tricothecium* was least effective biological agent against the target pathogen with 1 cm diameter and 66.66per cent inhibition after fourth day and

with 1.2 cm diameter and 82.85per cent inhibition after seventh day over control (Plate 2). This may due to the slow growth of biocontrol agent. The present findings are in accordance with other workers like Kumar *et al.*, 2005.

Table.1 *In-vitro* evaluation of bio-control agents against *F. oxyporum*f.sp. *cicero*

Treatments	After 4 Days		After 7 Days	
	Colony Diameter (cm)	% Inhibition over control	Colony Diameter (cm)	% Inhibition over control
Trichodermaharzianum	1	85.71	1.2	86.66
Trichodermaviride	1.4	76.28	1.5	83.33
Aspergillus	1.9	72.85	2.0	77.77
Tricothecium	4.1	41.42	4.6	48.88
Control	7.0	--------	9.0	----------

Table.2 *In-vitro* evaluation of bio-control agents against *Ascochyta rabiei*

Treatments	After 4 Days		After 7 Days	
	Colony Diameter (cm)	% Inhibition over control	Colony Diameter (cm)	% Inhibition over control
Trichoderma harzianum	0	100	0	100
Trichoderma viride	0	100	0	100
Aspergillus	0.5	83.33	1	85.71
Tricothecium	1	66.66	1.2	82.85
Control	3	--------	7	----------

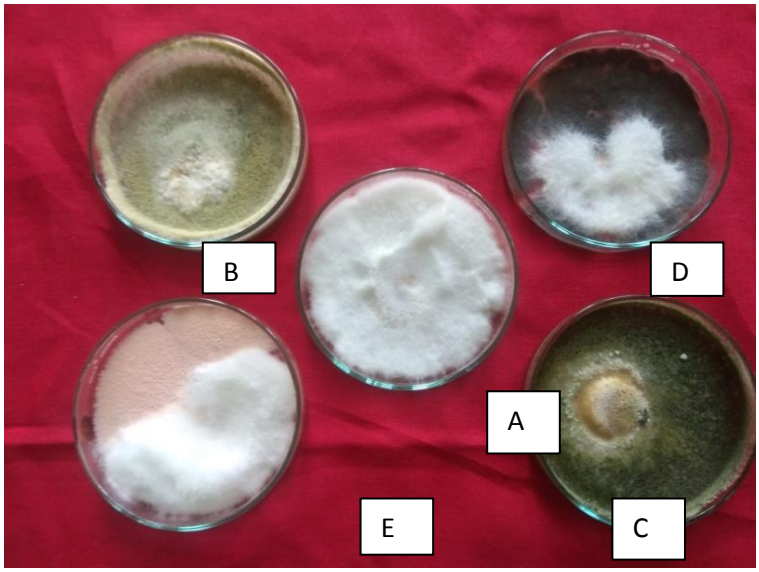

Plate.1 *In-vitro* evaluation of Bio-control agents against *F.oxysporum* f. sp. *ciceri*
A) Control (Pathogen) B) *T. viride* C)*T. harzianum* D) *Aspergillus* spp. E) *Tricothecium* spp.

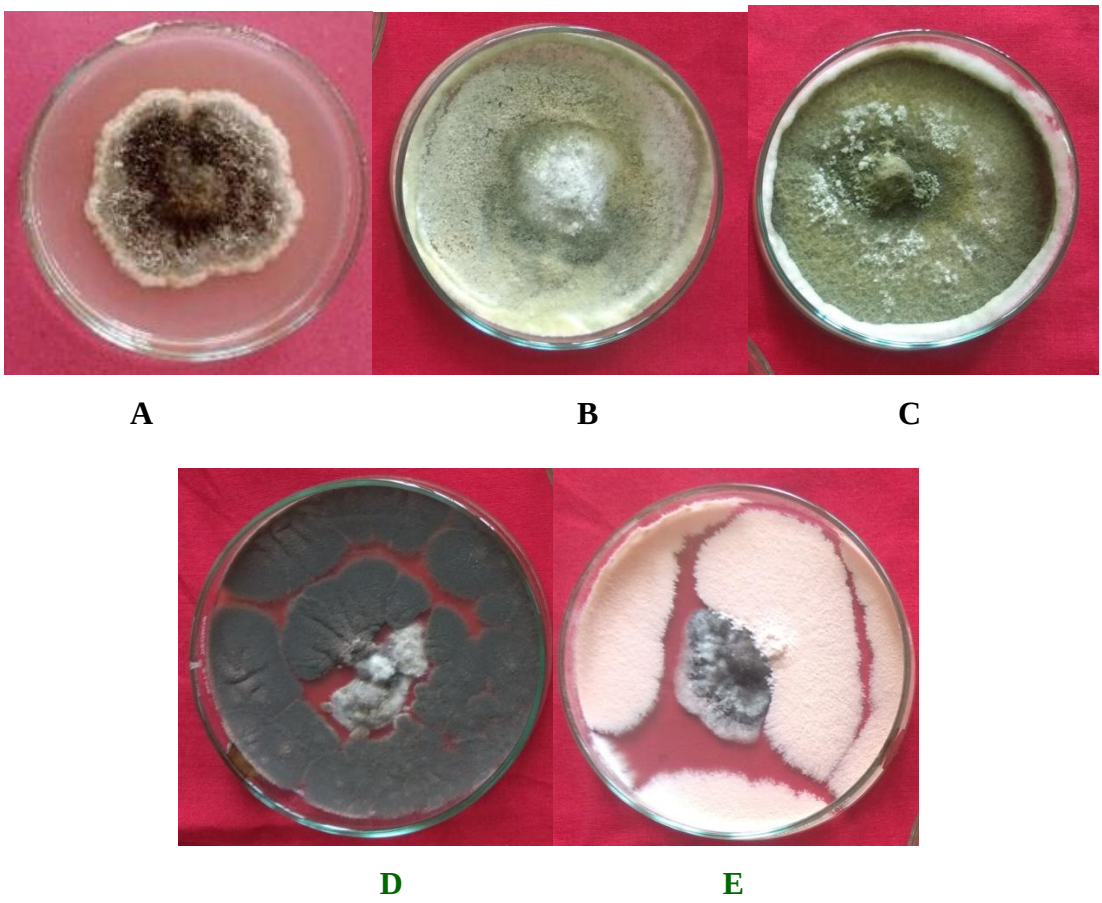

Plate.2 *In-vitro* evaluation of Bio-control agents against *Ascochyta rabiei*
A) Control (Pathogen) B) *T. viride* C) *T. harzianum* D) *Aspergillus* spp. E) *Tricothecium* spp.

References

Ahmad, F., Gaur, P. and Croser, J. 2005.*Chickpea* (*Cicer arietinum* L.). In: Genetic Resources, Chromosomes Engineering and Crop Improvement - Grain Legumes Volume J. (Eds. R. Singh and P. Jauhar.). CRC Press, USA.pp. 185-214.

Ahmad, P., Jamsheed, S., Hameed, A., Rasool, S., Sharma, I., Azooz, M. M. and Hasanuzzaman, M. 2014.*Drought stress induced oxidative damage and antioxidants in plants*. In: Oxidative Damage to Plants: Antioxidant Networks and Signalling. (Ed. P. Ahmad.). Academic Press, Elsevier, NY, USA. pp. 345-367.

Akem, C. 1999.*Ascochyta* blight of chickpea: present status and future priorities. *International Journal of Pest Management* 45: 131–137.

Amjad, I., Khalil, I. A., Nadia, A. and Muhammad, K. S. 2006.Nutritional quality of important food legumes .*Food Chemistry* 97: 331-335.

Anonymous, 2016. Project coordinator's Report, AICRP on Chickpea. *ICAR-IJPR* Kanpur. *http://www.apydacnet.nic.in/cropfryrtoyraspx/*.

Armstrong, C. L., Chongo, G., Gossen, B. D. andDuczek, L. J. 2001, Mating type distribution and incidence of the

teleomorph of *Ascochyta rabiei* (*Didymella rabiei*) in Canada. *Canadian Journal of Plant Pathology* 23: 110–113.

Bakhsh, A., Iqbal, S. M. and Ikram-UL-Haq 2007. Evolution of chickpea germplasm for wilt resistance. *Pakistan Journal of Botany* 39: 583-593.

Basandrai, A. S., Basandrai, D., Duraimurugan, P. and Srinivasan, T. 2011. *Breeding for Abiotic Stress*.In: Biology and Breeding of Food Legumes. (Eds. A. Pratap and J. Kumar.). CAB International, Wallingford, UK. pp. 220-240.

Brown, W. 1923. Experiments on the growth of fungi in culture media. *Annals of botony*, 37:105-109.

Haqqani, A. M., Zahid, M. A. and Malik, M. R. 2000. *Legumes in Pakistan*. In: Legumes in Rice and Wheat Cropping Systems of the Indo-Gangetic Plains constraints and Opportunities. (Eds. C. Johansen, J. M. Duxbury, S. M. Virmani, C. L. L. Gowda, S. Pandes and P. K. Josh). International Crops Research Institute for the Semi-arid Tropics and Cornel University Ithaca New York, USA. pp. 98-128

Iqbal, S. M., Haq, I. U., Bukhari, A., Ghafoor, A. and Haqqani, A. M. 2005.Screening of chickpea genotypes for resistance against *Fusarium* wilt. *Mycopathology*, 3: 1-5.

Iqbal, A., Ateeq, N., Khalil, I. A., Perveen, S. and Saleemullah, S. 2006. Physicochemical characteristics and amino acid profile of chickpea cultivars grown in Pakistan. *Journal of Food Service* 17: 94-101.

Knights, E.J., Acikgoz, N., Warkentin, T., Bejiga, G., Yadav, S. S. and Sandu, J. S. 2008. *Area, Production and Distribution*. In: Chickpea Breeding and Management (Eds. S. Yadav., R. Redden., W. Chen. and B. Sharma.). CAB International, Wallingford, UK. pp. 167-178.

Kumar, E. R., Aggarwal, R. and Singh, B. 2005. Fungal Antagonists for the Biological Control of *Ascochyta* Blight of Chickpea. *Acta Phytopathologicaet Entomologica Hungarica* 40: 35-42.

Leslie, J. F. and Summerell, B. A. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing: Iowa, USA. pp. 369.

Lilly, V. G. and Barnett, H. L. 1951. *Physiology of Fungus.* McGraw Hill Books Inc. Co., New York, USA.

Mahmood, K., Saleem, M. and Ahsan, M. 2011. Inheritance of resistance to *Fusarium* wilts in chickpea. *Pakistan Journal of Agricultural Sciences* 48: 55-58.

Morton, D. T. and Stroube, W. H.1955.Antagnostic and stimulatory effects of micro-organisms upon *Sclerotiumrolfsii*.*Phytopathology*45: 419-420.

Nene, Y. L. 1982. A review of *Ascochyta* blight of chickpea. *Tropical Pest Management* 28: 61–70.

Singh, G. and Sharma, Y. R. 1998.*Ascochyta* blight of chickpea. In 'IPM system in agriculture: pulses'. (Eds. R. K. Upadhyay, K.G. Mukherji and R.L. Rajak) (Aditya Books Pvt. Ltd., New Delhi, India). pp. 163–195.

Subhani, M. N., Sahi, S.T., Ali, L., Hussain, S., Iqbal, J. and Hussain N. 2013. Management of Chickpea wilt caused by *Fusarium oxysporum* f. sp. *cicero* through antagonistic microorganisms. *Canadian Journal of Plant Protection* 1: 1-6.

Toker, C., Canci, H. and Ceylan, F. O. 2006. Estimation of out-crossing rate in chickpea (*Cicer arietinum* L.) sown in autumn. *Euphytica*, 151: 201-205.

Varshney, R. K., Song, C., Saxena, R. K., Azam, S., Yu, S., Sharpe, A. G.,

Cannon, S., Baek. J., Rosen, B. D. and Taran, B. 2013. Draft genome sequence of chickpea (*Cicer arietinum* L.) provides a resource for trait improvement. *Nature Biotechnology* 31: 240-256.

Vincent, J. H. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159: 850.

Wang, H., Hwang, S. F., Chang, K. F., Turnbull, G. D. and Howard, R. J. 2003. Supression of important pea diseases by bacterial antagonists.*Biocontrol*48: 447-460.

Wang, N., Hatcher, D. W., Tyler, R. T. Toews, R., and Gawalko, E. J. 2010. Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.).*Food Research International* 43: 589-594.

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