

Seed-borne fungi of chilli pepper in the Coastal savannah zone of the central region of Ghana

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Abstract

Many chilli pepper (*Capsicum frutescens*) growers in the region have moved from chilli cultivation to other crops due to the continuous decline in yields as a result of disease problems, of which fruit rot is paramount. The study was carried out to identify the mycoflora associated with pepper cultivation in the Coastal Savannah Zone of Ghana. The purposive sampling technique was used to select farms from which samples were collected during the major rains (August – September 2012). Fruits at different stages of maturity were collected and the seeds were extracted and plated for seed health testing using the blotter paper method. The experimental design used was the completely randomized design. Data collected were analyzed using Genstat discovery edition 3. In all, a total of 35 species of fungi belonging to 17 genera were isolated from the samples. Chi-square test results of mean percent seed mycoflora infection gave the following test statistic values ($\chi^2 = 7012.17$, $df = 29$, and $sig. = 0.00$) and ($\chi^2 = 10067.26$, $df = 33$, and $sig. = 0.00$) for within pretreated and untreated seed samples, respectively. Unripened fruits recorded the highest mean percent infection by each fungi. The three most prevalent fungi were *Fusarium species*, *Alternaria species* and *Acremonium strictum*. These rot-causing fungi may be a contributing factor to the declining yields being recorded by the farmers and hence partly explains their shifting to other crops.

Key words: Blotter paper, rot-causing fungi, unripened fruits

Résumé

Beaucoup de producteurs de piments -poivrons (*Capsicum frutescens*) de la région sont passés de la culture du piment à d'autres cultures en raison de la baisse continue des rendements à cause de problèmes de maladies, dont la pourriture des fruits est manifeste. L'étude a été effectuée afin d'identifier la mycoflore associée à la culture du poivron dans zone de la savane côtière du Ghana. La technique de l'échantillonnage raisonné a été utilisé pour sélectionner les exploitations à partir de laquelle les échantillons ont été recueillis lors des grandes pluies (août-septembre 2012). Les fruits à différents stades de maturité ont été recueillies et les graines ont été extraites et déposées pour les tests de santé des semences en utilisant la méthode du papier buvard. Le dispositif expérimental utilisé était complètement aléatoire. Les données recueillies ont été analysées à l'aide de l'édition 3 de la découverte Genstat. En tout, un total de 35 espèces de champignons appartenant aux 17 types ont été isolés à partir des échantillons. Les résultats des tests de chi-carré de moyenne pour cent d'infection de semence myco-flore ont donné les valeurs suivantes de test statistiques $\chi^2 =$

7012,17, df = 29, et sig.= 0,00) et ($\chi^2 = 10067,26$, df = 33, et sig.= 0,00) pour l'intérieur des échantillons de semences prétraitées et non traitées, respectivement. Les fruits non murs enregistraient la plus forte infection pour cent en moyenne par chaque champignon. Les trois champignons les plus fréquents étaient des espèces de *Fusarium*, *Alternaria* et *Acremoniumstrictum*. Ces champignons responsables de la pourriture peuvent être un facteur contribuant à la baisse des rendements étant enregistrées par les agriculteurs et par conséquent explique en partie leur déplacement vers d'autres cultures.

Mots clés: papier buvard, les champignons responsables de la pourriture, fruits non murs

Background

The volume of chilli production in Ghana remained relatively constant (about 270,000 mt) between 2000 and 2005 however, total production increased marginally to 277, 000 in 2006 (FAOSTAT). The volume or quantity of pepper exported as part of Ghana's non-traditional export commodities has ranged from 2,088 mt in 1998, reaching its peak at 5,281 mt in 2001. However, the value exported has been on the decline since 2001, reaching its barest minimum of 282 mt in 2005 (MoFA, 2006). Volume exported between 2005 and 2007 was reported to have increased about 60% due to introduction of the Legon 18 variety which was more resistant to most of the chilli diseases by the time. However, the volume exported began to decrease from 2008 (ADRA Ghana, cited by the Millenium Challenge Corporation). The Ministry of Food and Agriculture, MoFA (2010) reported that the average yield of pepper under rain fed conditions was 6.5 mt ha⁻¹ with achievable yields standing at 32.3 mt ha⁻¹ depending on the variety. The decline in yields of the harvested produce with the subsequent decline in the total export has been attributed to many factors, paramount among them are diseases. Fungi are one of the major causes of diseases of pepper. Within the fungi group, *Colletotrichum* is one of the most important plant pathogens worldwide causing the economically important disease anthracnose in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits (Bailey & Jeger, 1992). The diseases are mainly problematic on mature pepper fruits, causing severe losses due to both pre- and post-harvest fruit decay (Bosland & Votava, 2003). Various control strategies have been employed to reduce losses caused by anthracnose and fruit rot. These include; crop rotation; removal and destruction of infected fruits; and spraying with recommended fungicides (Obeng-Ofori *et al.*, 2007); however, despite these control measures, there has not been significant improvement in the elimination of the diseases because the pathogens responsible are seed-borne.

A survey of the seed-borne fungi associated with chilli pepper production in the Coastal Savannah zone was conducted by plating seeds using the blotter paper method to help classify the fungi found within the agro-ecological zone and to find the rates of infection on chilli fruits at different stages of maturity.

Literature summary

Fungi comprise one of the largest groups of organisms causing diseases among chillies. The most important fungal diseases are: Anthracnose (*Colletotrichum* spp.); Early blight

(*Alternaria solani*); Cercospora leaf spot (*Cercospora capsici*); Damping-off (*Pythium*, *Rhizoctonia* and *Fusarium*); Gray mould (*Botrytis cineria*); Phytophthora rot (*Phytophthora capsici*); Southern blight (*Sclerotium rolfsii*); Verticillium wilt (*Verticillium dahliae*); and White mould (*Sclerotinia sclerotiorum*) (Bosland, 1999). The following fungal disease pathogens are known to be seed-borne and seed transmitted; *Colletotrichum spp.*, *Alternaria solani*, *Fusarium*, *Cercospora capsici*, *Botrytis cineria*, and *Sclerotinia sclerotiorum* (Neergaard, 1979; Bosland, 1999). For example, anthracnose disease caused by *Colletotrichum* species is one of the most economically important diseases reducing marketable yield from 10 to 80% of the crop in some developing countries, particularly in Thailand (Poonpolgul and Kumphai, 2007).

Measures such as crop rotation and early harvesting of fruits at the green unripe stages have been adopted to avoid the fruits being on the field to full maturity and ripening. These have been done in order to avoid the fungal infection but have proven to yield little or no success even though the pathogens which cause fruit rot and anthracnose are mainly problematic on mature pepper fruits (Surianingsih, 1991). An effective control measure is yet to be developed to contain *Colletotrichum spp.* because it is seed-borne, as well as transmitted through the system of the plant to the seeds in the new fruits produced (Kulshrestha *et al.*, 1976). According to Singh and Mathur (2004), fungal infection of seed-borne pathogens may reach the ovule and seed at any stage from the initiation of ovule to mature seed. Also, Singh and Mathur (2004) stated that the germination of propagule and initiation of hyphal growth are important factors that determine the entry of fungal pathogens in any tissue, including the fruit and seed. Neergaard (1979) has listed eight disease cycles for seed-borne pathogens taking into consideration the location of primary inoculum in seed, course of disease development, and reinfection of ovule and seed. According to Neergaard (1979), the infection may be systemic, local, or organospecific and the systemic infection may follow a vascular or a nonvascular course.

Study description

The variety of chilli used for the study was the Scotch bonnet locally known as 'Mako hwam'. This is a late variety and its harvesting starts 3 months after planting (MoFA, 2005). This variety was used because it is widely cultivated by chilli pepper farmers within the study area. Fruit samples were collected from a total of six farms. Three different types of fruits were taken from each farm. These were Physiologically Matured Unripened Fruits, Ripened Fruits, and Dropped Fruits, thus giving a total of eighteen (18) fruit samples. A final sample (Ripened Fruits) was obtained from four (4) different chilli sellers at a major market centre (Mankessim market) and bulked into one sample, thus, making the number of samples nineteen (19). The last sample from the Mankessim market was taken because all the six (6) farmers visited said they purchased pepper fruits from the Mankessim market to extract their own seeds for planting purposes due to absence of certified chilli pepper seeds.

Seeds were extracted from each of the three types of chilli fruits collected from the study area. Each of the samples was divided into two parts and each half was pretreated with 1% Sodium hypochlorite (NaOCl) solution for 10 minutes to prevent the growth of saprophytic

fungi to enable the free growth of pathogenic fungi (Dhingra and Sinclair, 1995; ISTA, 2003a), while the other half was not pretreated. The health of the seeds was tested using the standard blotter method (ISTA, 2003a) in the seed Pathology laboratory of the Crops

Table 1. Comparison of individual mycoflora infection on pre-treated and untreated seeds.

Fungi	Percent seed infection (%)	
	Pre-treated	Untreated
<i>Acremonium strictum</i>	6.21 (14.43)	6.29 (15.22)
<i>Alternaria circinans</i>	2.05 (8.23)	1.37 (6.72)
<i>Alternaria alternata</i>	3.92 (11.42)	4.47 (12.21)
<i>Alternaria sesami</i>	0.42 (3.72)	0.13 (2.07)
<i>Alternaria sesamicola</i>	0.11 (1.90)	0.18 (2.43)
<i>Alternaria solani</i>	2.55 (9.19)*	1.08 (5.97)*
<i>Aspergillus flavus</i>	2.63 (9.33)	3.55 (10.86)
<i>Aspergillus niger</i>	0.50 (4.05)*	1.39 (6.77)*
<i>Bipolaris spp</i>	0.34 (3.34)	0.13 (2.07)
<i>Botrytis cineria</i>	0.32 (3.24)	0.21 (2.63)
<i>Cercospora sojina</i>	0.00 (0.00)	0.03 (0.99)
<i>Cladosporium</i>	1.66 (7.40)	2.37 (8.86)
<i>Colletotrichum coccodes</i>	0.05 (1.28)	0.03 (0.99)
<i>Colletotrichum dematium</i>	0.05 (1.28)	0.03 (0.99)
<i>Colletotrichum gloeosporioides</i>	0.08 (1.62)	0.39 (3.58)
<i>Colletotrichum lindemuthianum</i>	0.00 (0.00)	0.03 (0.99)
<i>Colletotrichum acutatum</i>	0.13 (2.07)	0.05 (1.28)
<i>Corynespora cassiicola</i>	3.71 (11.11)	3.24 (10.37)
<i>Curvularia geniculata</i>	0.05 (1.28)	0.00 (0.00)
<i>Curvularia lunata</i>	1.45 (6.92)*	0.68 (4.73)*
<i>Curvularia pallescens</i>	0.11 (1.90)	0.13 (2.07)
<i>Curvularia trifolii</i>	0.03 (0.99)*	0.34 (3.34)*
<i>Exserohilum turcicum</i>	0.00 (0.00)	0.03 (0.99)
<i>Fusarium avenaceum</i>	0.08 (1.62)	0.24 (2.81)
<i>Fusarium equiseti</i>	0.05 (1.28)	0.11 (1.90)
<i>Fusarium moniliforme</i>	3.24 (10.37)	4.63 (12.43)
<i>Fusarium oxysporium</i>	15.97 (23.55)*	20.47 (26.90)*
<i>Fusarium pallidoroseum</i>	12.87 (21.02)	11.95 (20.22)
<i>Fusarium solani</i>	5.79 (13.92)	6.32 (14.56)
<i>Glomerella</i>	0.00 (0.00)	0.11 (1.90)
<i>Myrothecium leucotrichum</i>	0.03 (0.99)	0.03 (0.99)
<i>Phoma exigua</i>	1.21 (6.32)	1.68 (7.45)
<i>Phoma lingam</i>	0.21 (2.63)	0.03 (0.99)
<i>Phomopsis vexans</i>	0.82 (5.20)	0.87 (5.35)
<i>Rhizopus spp</i>	0.00 (0.00)	0.11 (1.90)
Total	66.63 (54.71)	73.29 (58.89)
Mean	8.37 (16.82)	9.95 (18.38)
Standard Error of Mean	4.31	3.46

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Research application

In all, a total of 35 species of fungi belonging to 17 genera were isolated from the chilli seed samples. Table 1 shows results of the percent seed infection for pre-treated and untreated seed samples. Some of the fungi isolated were identified as *Acremonium strictum*, *Alternaria alternata*, *A. solani*, *Aspergillus flavus*, *A. niger*, *Bipolaris cynodontis*, *Botrytis cineria*, *Cercospora sojina*, *Colletotrichum dematium*, *C. gloeosporioides*, *Curvularia geniculata*, *C. lunata*, *Fusarium moniliforme*, *F. oxysporium*, *F. pallidoroseum*, *F. solani*, *Glomerella cingulata*, *Phoma exigua*, *P. lingam*, *Phomopsis vexans* and *Rhizopus* spp. The rest are as presented in the Table 1.

Chi-square test results of percent seed mycoflora infection gave the following test statistic values ($\chi^2 = 7012.17$, $df = 29$, and $sig. = 0.00$) and ($\chi^2 = 10067.26$, $df = 33$, and $sig. = 0.00$) for within pretreated and untreated seed samples respectively. From the test statistic values obtained, there were significant differences ($P < 0.05$) in percent seed mycoflora infection within treatments. Therefore, H_{0a} was rejected and H_{1a} was accepted. Also in Table 1, a comparison of the percent seed mycoflora infection between pretreated and untreated seeds showed significant differences ($P < 0.05$) in the percent seed infections of *Alternaria solani*, *Aspergillus niger*, *Curvularia lunata*, *Curvularia trifolii* and *Fusarium oxysporum*. *Alternaria solani* recorded at 2.55% (9.19) and 1.08% (5.97) for pretreated and untreated seeds; *Aspergillus niger* 0.50% (4.05) and 1.39% (6.77), *Curvularia lunata* at 1.45% (6.92) and 0.68% (4.78), *Curvularia trifolii* at 0.03% (0.99) and 0.34% (3.34), and *Fusarium oxysporum* at 15.97% (23.55) and 20.47% (26.90) respectively, for pretreated and untreated seed samples.

The three most prevalent fungi were *Fusarium species*, *Alternaria species* and *Acremonium strictum*. These rot-causing fungi may be a contributing factor to the declining yields being recorded by the farmers and hence partly explains their shifting to other crops as well as the decline in the tonnage of chillies exported from Ghana due to latent infections in seeds (Manandhar *et al.*, 1995).

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References

Bailey, J.A. and Jeger, M.J. (Eds.). 1992. *Colletotrichum*: Biology, Pathology and Control. Wallingford: Commonwealth Mycological Institute. p. 388.

- Bosland, P. 1999. Disorders, pests and diseases of Chilles. In: Chille peppers, hot tips and tasty picks for gardeners and gourmets. Marinelli, J., Ludlam, J., Lindstrom, B. and Tebbitt, M. (Eds.). Brooklyn Botanic garden Inc. Science Press. pp. 41-55.
- Bosland, P.W. and Votava, E.J. 2003. Peppers: vegetable and spice *Capsicums*. England: CAB International. p. 233.
- Dhingra, O.D. and Sinclair, J.B. 1995. Basic Plant Pathology Methods (2nd ed.). CRC Press, Inc. 434 pp.
- ISTA. 2003a. Common laboratory seed health testing methods for detecting fungi. 1st ed. Mathur, S.B. and Kongsdal, O. (Eds.). Danish Government Institute of Seed Pathology for Developing Countries. Copenhagen, Denmark. pp. 182-183.
- Kulshrestha, D.D., Mathur, S.B. and Neergaard, P. 1976. Identification of seed-borne species of *Colletotrichum*. *Friesia*. 11:116-125.
- Manandhar, J.B., Hartman, G.L. and Wang, T.C. 1995. Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. *Plant Disease* 79:380-383.
- Ministry of Food & Agriculture (MoFA). 2005. Chilli peppers. In: Good agricultural practices and crop protection recommendations. Kyofa-Boamah, M., Blay, E., Braun, M. and Kuehn, A. (Eds.). Vol. 5. Pp. 113-135.
- Ministry of Food & Agriculture (MoFA). 2006. Agriculture in Ghana: Facts and figures (2006). Statistics, Research and Information Directorate (SRID) – Ministry of Food and Agriculture. pp. 11, 34 and 36.
- Ministry of Food & Agriculture (MoFA). 2011. Agriculture in Ghana: Facts and Figures (2010). Statistics, Research and Information Directorate (SRID) – Ministry of Food and Agriculture. May 2011. p. 12.
- Neergaard, P. 1979. Seed Pathology. Vols. 1 and 2. Macmillan Press, London, UK. 1191 pp.
- Obeng-Ofori, D., Danquah, E.Y. and Ofori-Anim, J. 2007. Vegetable and spice crop production in West- Africa. Ofori, K (Ed.). City Publishers Limited, Accra, Ghana. pp. 72.
- Poonpolgul, S. and Kumphai, S. 2007. Chilli pepper anthracnose in Thailand. Country Report. In: Abstracts of the First International Symposium on Chilli Anthracnose. Oh, D.G. & Kim, K.T. (Eds.) Republic of Korea: National Horticultural Research Institute, Rural Development of Administration. p. 23.
- Singh, D. and Mathur, S.B. 2004. Histopathology of Seed-Borne Infections. CRC Press LLC 2000 N.W. Corporate Blvd., Boca Raton, Florida 33431. pp. 282.
- Surianingsih, E. 1991. 'Resistance of pepper to anthracnose caused by *Colletotrichum* species'. In: ARC Training, Widjata, Indonesia. pp. 1-2.
- Millenium challenge corporation of the United States of America. Investment opportunity Ghana - Chilli pepper. Millenium Development Authority. pp. 22. Available online: <https://www.mcc.gov/documents/investmentopps/bom-ghana-english-chili.pdf>