CHARACTERIZATION OF STEMPHYLIUM BLIGHT SYMPTOMS IN LENTIL

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ABSTRACT
Stemphylium blight caused by *Stemphylium botryosum* infected lentil plant (*Lens culinaris*) were collected from farmer's field of major lentil growing areas of Bangladesh during 2012-2013 cropping season. Morphological symptoms on leaf were characterized during this period. The development of stemphylium blight of lentil first appears on leaflet as small pin-headed light brown spots which smaller lesions later irregularly enlarged, covering the surface of the leaflet within 2 to 3 days and killed the single leaflet or in presence of more inoculums more than one leaflet infected. The disease gradually increased by showing symptoms on shoot or twig which showed special type symptoms like fishing hook. Later rapidly spread over the leaf, shoots or twigs infection but pod remained green. In epidemic condition of the disease, farmers' field might be possible to reach brownish color and looking just burn by fire within 7 to 10 days. Stemphylium blight could not occur at seedling stages and should not confuse with foot rot occurring at seedling stages. V-6 juice media (modified of V-8 agar media) were used for isolation and identification of the pathogen. Pathogenicity test was done under laboratory condition and produced same symptoms.

Key words: Characterization, Lentil, *Lens culinaris*, Stemphylium blight, Identification

1. INTRODUCTION

The lentil plant (*Lens culinaris* Medikus) is the fourth most important pulse (legume) crop in the world [1]. Lentil is the second most important pulse crop in Bangladesh in terms of both area and production [2]. Although the total cultivated area and production of lentil in our country have increased gradually over last 5 years but productivity is still very low compared to average yield of the world [3]. Among the factors responsible for low yield of lentil, diseases are considered to be the most serious one. Globally lentil is susceptible to more than 35 diseases [4]. So far, 15 pathogens causing 17 diseases have been recorded in Bangladesh, among them stemphylium blight caused by *Stemphylium botryosum* Walr. is considered as the most devastating one [5]. In Bangladesh, the disease was first recorded during 1981 and was further confirmed in1986 [6]. The disease was also observed as a major disease in 1990 decades to till date [7]. Since then it has gained importance due to its increased severity with reports of more than 80% crop loss "[8], [9]". Preliminary studies in Bangladesh and India estimated yield losses of 62% and total crop failure have been reported in some cases where the disease defoliated the crop in the early pod setting stage "[10], [11]". More than 50 species of *Stemphylium* (sexual state: *Pleospora*) have been described and they are commonly isolated from a range of plants [12]. Disease symptoms have been well characterized in South Asia where *Stemphylium botryosum* has caused a great devastation to the lentil crop. Bakr [10] reported that the symptoms of the disease in Bangladesh include the appearance of small pin-headed light brown to tan colored spots on the leaflets which later enlarge, covering the leaf surface within 2 to 3 days. The symptoms differ from other foliar lesions by being larger and spreading across or

along the entire leaflet. Bayaa and Erskine [13] reported that massive defoliation and stem bending was observed in leaf blight of lentil caused by *Stemphylium botryosum*. Bakr [14] has reported that *Stemphylium botryosum* commences infection when the ambient night temperature remains above 8^0C; and the mean day temperature exceeds 22^0C as well as the relative humidity inside the canopy must also reach 94%. The study of research work has been undertaken to isolate and identify the causal pathogen of blight of lentil causing different types of symptoms on plant parts.

2. MATERIALS AND METHODS

Different samples of stemphylium blight infected lentil plant were collected from farmer's field during cropping season of 2012-2013 throughout the major lentil growing area of Bangladesh. Lentil growing 11 districts *viz*. Jessore, Kushtia, Faridpur, Pabna, Rajshahi, Maherpur, Madaripur, Barisal, Jhalokathi, Khulna and Satkhira in Bangladesh were included in this study. The experiment was conducted at Plant Pathology Division, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Rahmatpur, Barisal, Bangladesh.

2.1. Preparation of culture media

V-6 juice agar media was used in this experiment for culture the *Stemphylium botryosum*. V-6 juice agar media was made by extract of six vegetables that media were basically modified of V-8 juice agar media described by Alam [15]. Media preparation procedure were: 1) Equal amount (34 g) of six vegetables i.e. total 200 g vegetable was blended with blender machine. 2) Extract of six blended vegetables (tomato, carrot, potato, lettuce leaves, cabbage leaves and Indian spinach) was made by boiling in water. Sieving the extract was done with fine cotton fabric. 3) Then the extract was taken in a 1000 ml conical flask and adjusted the volume up to 1000 ml by adding distilled water. 4) 20 g dextrose and 20 g agar were dissolved in the extract. 5) In order to overcome the problem of overgrowth by other fast growing fungi, Rose Bengal 0.3 g was added as a growth inhibitor that can slowdown the radial growth of the fungal colony. 6) The extract was autoclaved at 121^0C under15 psi pressure for 30 minutes. After autoclaving the medium was kept few minutes for cooling. Then 20 ml medium was poured into each sterile petridish for use. Similarly, test tube slants were prepared pouring 10 ml medium in each 15x2 cm test tube.

2.2. Isolation of the pathogen

S. botryosum was isolated by tissue planting methods from diseased samples. Then the samples were cut into small pieces (0.5-1.0cm). Infected leaflets were surface sterilized with 1% sodium hypochlorite solutions for two minute and then rinsed in sterile distilled water for three times. After washing, cut pieces were transferred to the surface of the medium aseptically. The inoculated petri dishes were then incubated at 25^0C under 12-hour darkness alternate with 12-hour florescence light or near ultra-violate light (NUV light) for 10 days for growing or sporulating the pathogen.

2.3. Purification and Identification of the pathogen

Purification was carried out using single spore isolation technique [16]. Spore suspension of 10 days old culture was prepared in the test tube (15x2cm) containing 10 ml of sterilized water. The spore density in suspension was observed under compound microscope taking a drop of suspension on a glass slide. The spore density was adjusted with the addition of sterile water to get 1-5 spores per microscopic field (10x). One drop of the suspension was then poured on water agar medium (2%) in petri dishes and spread over the medium thoroughly with a sterilized needle and the petri dishes were then incubated for 16-20 hr at 25 ± 1^0C and allowed the spores to germinate. A germinated spore was then picked up under a microscope and transferred to a slant culture in the tube and incubated for 10 days at 25 ± 1^0C under 12 hr darkness alternate with 12 hr florescence light or near ultra-violate light (NUV light) for 10 days for growth and sporulation. The pure culture of the pathogen thus obtained was then preserved at 5^0C in the refrigerator. For identification, conidia were taken from mature colonies and examined for size, shape and color. Details of the cultural characters and microscopic details were noted and the fungus was identified following Ellis [17].

2.4. Pathogenicity test of Stemphylium blight of lentil

Pathogenicity test of Stemphylium blight of lentil was followed by Koch's Postulates [18].

3. RESULTS

3.1. Symptoms of the disease

The symptoms clearly differ from other foliar lesions of lentil. These findings were recorded by frequently field visit with closed observation. Stemphylium blight symptoms were started at flowering stage in most of the cases. Spore of *Stemphylium botryosum* landing and infected the leaflet and formed of small pinhead gray spots or light brown on the leaflets (Plate 1.a) which smaller lesions later irregularly enlarged, covering the surface of the leaflet within 2 to 3 days and killed single leaflet (Plate 1.b) or in presence of more inoculums more than one leaflet infected (Plate 1.c). The disease gradually increased by showing symptoms on shoot or twig which showed special type symptoms like fishing hook (Plate 1.d). Later rapidly spread over the leaf, shoots or twigs infection but pod remained green (Plate 1.e). During favorable environment of the pathogen, plant became in severe condition, leaflet blighted and plant become defoliated leaving a few green leaves and some immature fruits (Plate 1.f). In the presence of susceptible condition of the disease, farmers' field might be possible to reach brownish color and looking just burn by fire within 7 to 10 days (Plate 1.h). When plants were dense populated, infection occurred lower parts of the plants (Plate 1.g) but it should not have confused with healthy mature plants at later growth stage (Plate 2.e). Stemphylium blight could not occur at seedling stages and should not confuse with foot rot occurring at seedling stages, foot rot shown brown or light yellow colored leaf looking stemphylium blight (Plate 2.a). Several symptoms collected from seedling stage and isolated following standard procedure but there was no *S. botryosum* conidia found. Some other symptoms were observed on lentil plant at different growth stages but after isolation it was confirmed that were not stemphylium blight (Plate 2.b, c, d and e).

(a) Small pinhead gray spot

(b) Single leaflet infected

(c) More than one leaflet infected

(d) Infected shoot or twig


(e) Leaf infected but pod remain green


(f) Severely infected plants


(g) Infected the lower parts of the plant


(h) Severely infected farmers field

Plate 1: Stemphylium blight infected lentil plant showing different kind of symptoms


(a) Foot rot infected at seedling stage


(b) Leaf rot

(c) Nutrient deficiency (d) Red color leaf (e) Crop maturity stage

Plate 2: Lentil plant showing different kinds of symptoms but that were not infected by *S. botryosum*

3.2. Isolation of the pathogen

Dotted symptoms was found on leaflet of lentil and plate 3.a was shown just little enlarged view under microscope. Huge amount of conidia was found on infected leaflet of lentil and enlarged view of leaflet was shown directly under microscope (Plate 3.b). Conidia of *S. botryosum* were isolated directly from leaf by scraping with needle and then prepared slide and later observed under compound microscope. After isolation conidia and conidiophore were shown in the plate 3.c and d. On the other hand, *S. botryosum* were isolated by using V-6 media (modified of V-8 media) and found pure culture.

3.3. Morphology of the pathogen

Conidia: Morphological characters were found under compound microscope. Conidia oblong rounded at the ends, broadly ellipsoidal or sub-spherical, with usually 3 transverse and 1-3 longitudinal septa; constricted at the median transverse septum; pale to mid dark brown or olivaceous brown; minutely verrucos or echinulate and muriform; average size of conidia 18-28 × 11-17 µm and length to breadth ratio (L/B) was about 1.0 to 1.5.

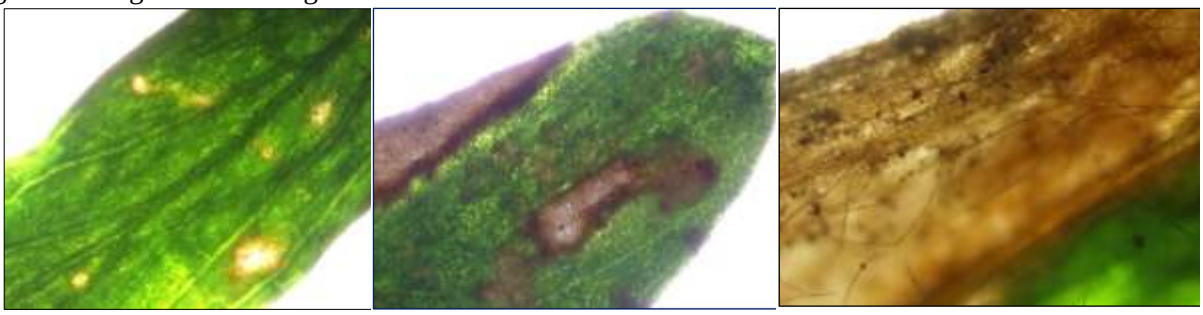
Conidiophores: Conidiophores were brown with terminal swellings which become through percurrent proliferation intercalary 7-11µ diam, dark verrucose band a little way below the apex.

Colony characteristics

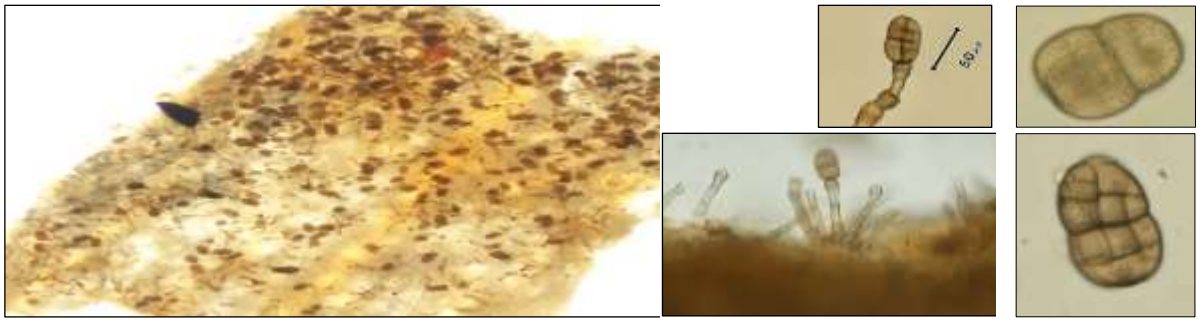
S. botryosum colonies were grown rapidly on V-6 media. The colonies were velvety to cottony in texture with a grey, brown or brownish-black color and black pigmentation on the colony.

3.4. Effect of light

Pure cultures were found at 25^0C under 12hr darkness alternating with 12 hr light (Fluorescence light or Near Ultra Violate, NUV light) (Plate 4.). But pure culture also found without alternating darkness and light i.e. using continuous light or continuous darkness.

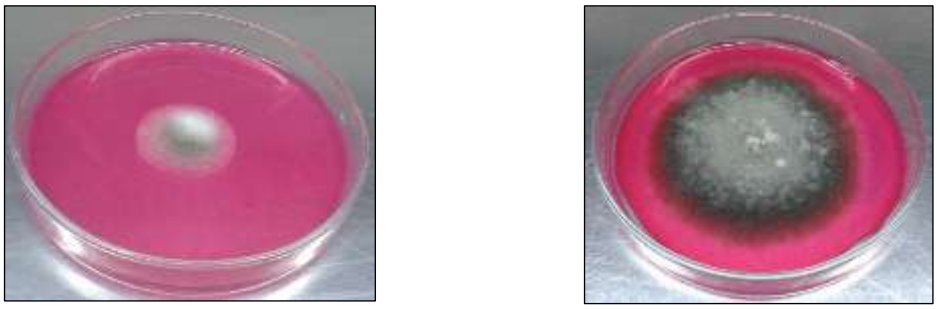


(a) Dotted symptoms on leaflet with just little enlarged view under microscope



(b) Enlarged view of leaflet under microscope showing huge amount of conidia (c) Conidia with conidiophore (d) Conidia with different view

Plate 3: Enlarged view of lentil leaf and also showing conidia with conidiophore



(a) Pure culture on five days (b) Pure culture on fifteen days

(c) Pure culture under fluorescence light (d) Pure culture under NUV light

Plate 4: Different types of pure culture of *S. botryosum* from lentil

3.5. Pathogenicity test

Samples were collected from the stemphylium blight infected plants and isolation and purification were done following standard procedure. From the pure culture spore suspension were prepared and inoculated the healthy plant by using small spray machine. After few days same symptoms were developed as like as previously collected lentil plant. Finally infected leaves were collected and isolated again from the infected leaves and found *S. botryosum* spores (Plate 5.).

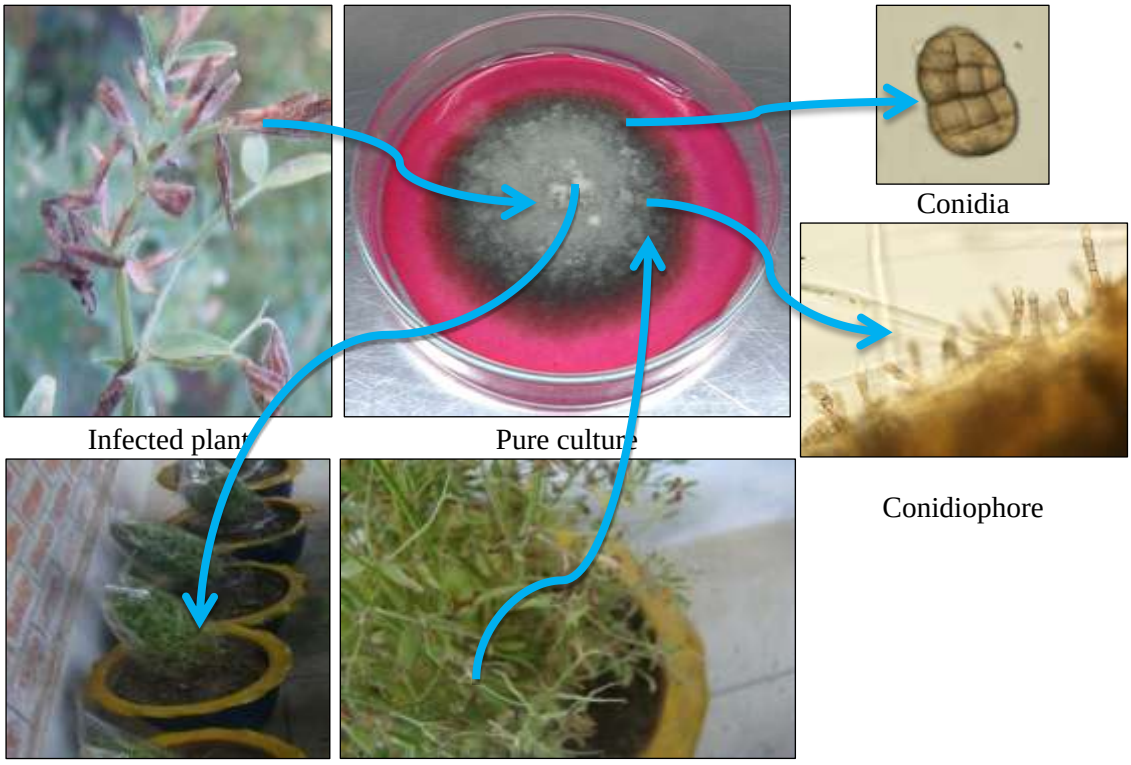


Plate 5: Koch's postulates for proof of pathogenicity

4. DISCUSSION

The symptoms of stemphylium blight disease of lentil were well characterized with chronological photography of the disease in this investigation. Because of chronological photography of the disease symptoms might be helpful to the farmers or researchers. This study also helpful for identification of the disease correctly. Stemphylium blight disease of lentil could not occur at seedling stages and should not confuse with root rot occurring at seedling stages. Root rot shown brown or light yellow colored leaf looking stemphylium blight.

The results i.e. characterization of the stemphylium blight disease symptoms were in agreement with the findings of Bakr [10] who reported that the symptoms of the disease in Bangladesh included the appearance of small pin-headed light brown to tan colored spots on the leaflets which later enlarged. A blighted dull yellow appearance was observed with infected foliage and branches. Defoliation occurred rapidly, leaving the branches with terminal leaves. The stems and branches also bend down and dry up but the pods remain green. White mycelia growth could also be observed on the infected stems. Barker [19] again confirmed the symptoms. There were many researchers agreed with the present findings "[11], [13], [20], [21]". But Morrall *et al*. [22] reported that in Saskatchewan, it was suspected that stemphylium blight has not been correctly identified in the field, as the lesions closely resembled those of ascochyta blight. Isaacs [23] also similar commented that stemphylium blight caused by the fungal pathogen *S. botryosum*, is a lentil disease that has only been identified as a problem in recent years and according to Sabine Banniza, a researcher at the University of Saskatchewan this might be due to misdiagnosis in the past.

5. CONCLUSION

Spore of *Stemphylium botryosum* landing and infect the leaflet of lentil (*Lens culinaris*) and showed different kinds of symptoms. The above symptoms clearly differ from other foliar lesions of lentil. During susceptible condition of the disease within 7 to 10 days farmers' field might be possible to reach brownish

color and looking just burn by fire. Stemphylium blight could not occur at seedling stages and should not confuse with foot rot occurring at seedling stages.

REFERANCES

[1] L. Szilagyi, I. Alabboud and G.V. Roman, "Stability analysis for seed yield in lentils (*Lens culinaris* medik.)", Scientific papers, UASVM Bucharest, Series A, Vol. LIV. pp. 338-343, 2011.
[2] KD (Krishi Diary), "Acreage and Production of Pulse Crops", Krishi Diary, Agricultural Information Service, Department of Agricultural Extension, Ministry of Agriculture, Bangladesh. p. 14, 2016.
[3] FAOSTAT, "Food and Agriculture Organization of the United Nations", Statistical Databases: FAOSTAT 2015. Rome, 2015.
[4] Wikipedia, "List of lentil diseases", Available on https://en.wikipedia. org/wiki /List_of_lentil_diseases, 2016.
[5] M.H. Rashid, M.O. Ali, M.A. Bakr and A. Sarker, "Botrytis gray mold: a new foliar disease of lentil in Bangladesh", *Bang. J. Pl. Pathol*., 23 (1&2): 93-94, 2007.
[6] M.A. Bakr and M.A. Zahid, "Stemphylium blight: a new foliar disease of lentil in Bangladesh", *Bang. J. Plant Pathol.*, 2: 69-70. 1986.
[7] M.A. Bakr, M.H. Rashid, M.S. Hossain, and A.U. Ahmed, "Effect of climatic changes on the incidence of disease of winter pulses", In: R. Lal *et al.* (eds.), Climate Change and Food Security in South Asia, 397-406. DOI 10.1007/978-90-481-9516-9_23, © Springer Science+Business Media, 2011.
[8] M.A. Bakr and F. Ahmed, "Development of stemphylium blight of lentil and its chemical control (Abst.)", *Bang. J. Plant Pathol.*, 8: 39-40, 1992.
[9] E. Mwakutuya and S. Banniza, "Influence of temperature and wetness periods on the development of stemphylium blight on lentil", *Pl. Dis*., 94: 1219-1224, 2010.
[10] M.A. Bakr, "Plant protection of lentil in Bangladesh", In: Lentil in South Asia. Edited by Erskine W. and M.C. Saxena, International Center for Agricultural Research in the Dry Areas, ICARDA, 1991.
[11] W. Erskine and A. Sarker, "Bangladesh in a big way and the results has been satisfying", ICARDA has been helping breed the varieties of the future. ICARDA Caravan. 6(6): 8-10. Available on http://www.icarda. org/Publications/Caravan/Caravan6/Cara6, 1997.
[12] D.F. Farr and A.Y. Rossman, "Fungal databases, Systematic Mycology and Microbiology Laboratory", ARS, USDA, Beltsville, MD, USA. http://nt.ars-grin.gov/fungaldatabases, 2014.
[13] B. Bayaa and W. Erskine, "Lentil Pathology", In: Pathology of food and pasture legumes (eds., D. Allen and J. Lenne), Commonwealth Agricultural Bureaux International, U.K in association with: International Crop Research Center for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. pp. 423-472, 1998.
[14] M.A. Bakr, "Plant protection of lentil in Bangladesh", Lentil in South Asia (Erskine, W. and M. C. Saxena, eds.). ICARDA, Aleppo, Syria. pp. 177-186, 1993.
[15] K.H. Alam, "Studies on stemphylium blight of lentil (*Lens culinaris*) and its management practices", PhD thesis, Department of Crop Science and Technology, University of Rajshahi, Rajshahi, Bangladesh, 2016.
[16] T.A. Toussoun and P.E. Nelson, "A Pictorial guide to the identification of *Fusarium* species", Second Edition. Pennsylvania State University Press, University Park. 43 p., 1976.
[17] M.B. Ellis, "Dematiaceous Hyphomycetes", Kew, UK: Commonwealth Mycological Institute. 608 p., 1971.
[18] G.N. Agrios, "Plant pathology", 5th Ed., Elsevier academic press, Boston. Mass, 2005.
[19] B. Barker, "Stemphylium blight of lentil on the radar screen", Top Crop Manager. 105, Donly Drive South, Simcoe, Ont. Canada-N3Y 4K5, 2009.
[20] W. Chen, A.K. Basandrai, D. Basandrai, S. Banniza, B. Bayaa, L. Buchwaldt, J. Davidson, R. Larsen, D. Rubiales and P.W.J. Taylor, "Diseases and their management", In: The Lentil: Botany, production and uses. (Erskine, W., F.J. Muehlbauer, A. Sarker and B. Sharma, Eds.). CAB Int. Wallingford, UK. Pp. 269-270, 2009.
[21] T.D. McGreevy, "Stemphylium blight in lentil", Take your pulse, USA Dry Pea & Lentil Council, 2780 W. Pullman Road, Moscow, ID 83843, USA. 2 (2): 1-24, 2013.
[22] R.A.A. Morrall, A. Vandenberg and S. Banniza, "Recent developments in lentil pathology in Canada", [Online] In Proceedings of the 5th Canadian pulse research workshop. London, ON. 28-30 November, 2004.
[23] J. Isaacs, "Lentils with blight can be misdiagnosed", Grainews, Farm Business Communications, part of the Glacier Farm Media family, 1666 Dublin Avenue, Winnipeg. Web on www.farmmedia.com, 2014.