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Incidence of bean anthracnose in Western Kenya and its management using aqueous extract of *Aloe vera*

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ABSTRACT

High seed exchange and frequent resistance breakdown continue to be a challenge in managing seed borne fungi Colletotrichum lindemuthianum in Western Kenya farm saved bean seeds. Continuous pathogen surveillance can enable timely development and application of effective environment friendly mitigation strategies. The objective of this study was to determine the presence C. lindemuthianum on farm-saved bean seeds collected from seven different regions of Western Kenya and its possible control using aqueous plant extracts at different concentration in comparison to traditional fungicides. Systematic random sampling method was used to collect 196 farms saved bean seeds from farmers in the agro-ecological zones within the regions for four seasons between 2008 and 2010. Incidence was scored for each sampled seed lot. Water extract of Aloe vera was screened in vitro for antifungal activities against C. lindemuthianum using poison food technique. Both incidence and inhibitory data were subjected to analysis of variance using GenStat® computer package. The sampled eight seed varieties namely Red haricot (GLP- 585), Mwitemania GLP X92, Yellow (KAT B 1), Rose coco (GLP-288), White, Rose coco (GLP-2), Brown and Mwezi moja (GLP-1004) had high and significantly difference ($p \leq 0.05$) anthracnose incidences. Variety GLP 585 had the highest incidence (53.1%) whereas GLP 2 had the lowest (28.7%). Aqueous extract from Aloe vera plant exhibited a higher antifungal activity at all concentration levels (30, 25 and 20%) tested and compared well with the standard synthetic fungicide; Mancozeb. There is high disease incidence in the farm saved seeds in Western Kenya and this could be due to high seed exchange and the warm, humid environment. However, farmers should be encouraged to use environment friendly, affordable and effective aqueous plant extract of Aloe vera.

Key words: *Bean anthracnose, Colletotrichum lindemuthianum, Incidence, Aqueous plant extract, Aloe vera.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important legume food in Kenya (FAO, 2008). Its per capita consumption is estimated to be 14 Kg per year in Kenya, but can be as high as 66 per year in Western Kenya (Buruchara, 2007; FAOSTAT, 2013). The total area under common bean cultivation in Kenya is estimated to be an average of 910,478 hectares with yield potential of 1-2 tonnes ha^{-1} (FAOSTAT, 2013). Unfortunately there has been yield decline at an average rate of 6.8% (FAO, 2007; FAOSTAT, 2008) due to different constraints including diseases.

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Lams. – Scrib. (Barrus, 1911) is one of the important diseases of common bean in Kenya. The disease symptoms include dark brown sunken lesions on all above ground parts including the seeds (Jerba *et al*., 2005). The pathogen is seed-borne and seed transmitted (Yesuf and Sangchote, 2007). It survives as a dormant mycelium within the seed parts including seed coat and cotyledon and therefore infected seed is of low quality and contributes in the long distance distribution of the pathogen (Zaumeyer and Meiners, 1975; Tesfaye, 2003). The results in the introduction of the disease or new races into new regions (Agrios, 2000). Studies show that for each 1 percent increase in anthracnose incidence, seed yield decreases by 9 Kg/ha (Allen *et al.,* 1998). Susceptible bean varieties succumb earlier to anthracnose than the resistant (Nkalobo et al., 2007). Seed infections as low as 0.01-0.1% can cause an epidemic leading to a yield loss exceeding 90% on favourable conditions (Fernandez *et al.,* 2000; Sharma *et al.,* 2004; Yesuf and Sangchote, 2007). Quality of

seed is strongly linked to presence or absence of the pathogen in seed. Seventy five percent of farmers in Western Kenya use farm saved seeds and exchange seed among themselves. The farm-saved seed appear apparently healthy to farmers but the seed could be of low quality as it could have originated from an infected pod. Tu (1992) observed that 10% of cultivated seeds without anthracnose symptoms but originating from infected pods produced infected seedlings. Unfortunately only 18% farmers are keen on control of diseases during the growing period (Opole *et al*., 2003).

The current control methods include use of certified seeds, synthetic fungicides and host resistance. However, these methods reduce profitability as they are expensive, threaten the environment and induce development of fungicide resistant biotypes (Milkas *et al*., 2006). Studies have shown that use of plant extracts with active antifungal property as alternative fungicide could be an effective control method because they are affordable and environmentally friendly. *Aloe vera* L., a member of liliaceae family, contains aloine, an anthracnose heroside which is the active constituent of various drugs (Bruneton, 1993). *In vitro* experiments show that at 0.35% concentration *Aloe vera* gel had strong antifungal activity which completely eradicated seed borne fungi *Drechslera hawaiensis* and *Alternaria alternate* (Uzma *at al*., 2011). The objective of this study is to investigate the anthracnose incidence and management using *Aloe vera* in Western Kenya.

MATERIALS AND METHODS

Study area

The study was carried out in seven regions namely, Bungoma, Kakamega, Busia, Trans Nzoia Nandi, Kisii and Siaya. They were selected purposely to represent the major common dry bean growing regions of Western Kenya. The areas have an elevation of between 1,200 and 2,100 meters above sea level. They receive bimodal rainfall pattern with an average annual rainfall of 750 - 2,100 mm. The first rains (long rains season) start in March and end beginning of June while the second rains (short rains season) start in August and last end of November. Temperatures range from a minimum of 14 ^{o}C to 18 ^{o}C and to a maximum of 30 ^{o}C to 36 ^{o}C throughout the year. The regions are found in different agro ecological zones in Kenya.

Collection of Farm-Saved Seed Samples

Farm-saved seed samples were collected between November 2008 and July 2010 from bean farmers along accessible routes in the study regions. The most common varieties collected were Red haricot (GLP- 585), Mwitemania GLP X92, Yellow (KAT B 1), Rose coco (GLP-288), White, Rose coco (GLP-2), Brown and Mwezi moja (GLP-1004). Systematic random sampling was used to select farmers at regular intervals of 4-10 km. Averages of 10 farmers were sampled in a given region and from each 250 grams of available farm-saved seed varieties was purchased. During sample collection information on the region agro-ecological zones, climate, bean variety and season were observed and recorded. Certified seed of the same varieties, as those obtained from farmers were purchased from a seed company shop. The collected samples were placed in a labelled paper bag, packed in a sack and stored at a room temperature of 20-22 ^{o}C at University of Eldoret Seed laboratory for immediate study of incidence of anthracnose disease. In the laboratory all the seed samples of the same variety collected from the same season and region were mixed to form a composite seed sample of the variety. Eight common varieties were obtained from each of the seven regions in four different rains seasons (short rain season 2008, long rain season 2009, short rain season 2009 and long rain season 2010) making a total of 224 submitted samples from all bean growing regions in the study period. Certified seed were treated as a region (C0) and used as control.

Assessment of Anthracnose Incidence on Farm-Saved Seed

Standard blotter test (ISTA, 2008) was used to determine anthracnose incidence in the seed samples. A working sample of 400 seeds was obtained from each seed sample cluster using random-cup method (ISTA, 2008) and divided into four replicates of 100 seeds each. The seeds were surface sterilized in a solution of 0.5% sodium hypochlorite for 10 minutes and rinsed with water. They were spread out on moistened double sheets 350 x 450 mm Whatman No. 1 filter paper soaked with water. They were then covered with a lid to maintain moisture during incubation. The seeds were incubated for seven days in darkness at 20 ^{o}C before emergence of seedlings.

Seedlings were moistened at 90- 95% moisture content after every 12 hours to enhance disease symptom development. After seven days the cotyledons and hypocotyls were examined for black depressed spots with well delimited outlines. To confirm presence of anthracnose each spot was checked for presence of acevuli with dark brown setae and conidia using 25 X magnification then 200 X magnification electron microscope. The number of

anthracnose infected seedlings were counted and recorded. Anthracnose incidence was determined in percentage using the following formula:-

$$I = \frac{\sum n}{\sum N} \quad X\ 100$$

I= Incidence
∑N= Total number of seeds
∑n=Total number of seeds infected

Collection of *Aloe Vera* Plant Leaves

In this study *Aloe vera* extract was prepared from samples of older leaves of *Aloe vera* plant collected from a natural field in Baringo County, Kenya (0°28'0" N, 35°59, 0"E). The leave samples were identified, given voucher number, Mogita G. W./Marigat/06/11/007, and deposited at a herbarium in the School of Natural Resources, University of Eldoret. The leaves were taken to University of Eldoret Seed laboratory in the School of Agriculture and Biotechnology for analysis of their antifungal potential against bean anthracnose disease.

Preparation of Aqueous Plant Extract

Aqueous extract of the *Aloe vera* leaves was prepared as described by Satish *et al.,* (2007). The leaves were washed thoroughly under running tap water then in sterilized distilled water and surface dried with blotting paper. One hundred grams were chopped, blended in a surface sterilized blender (LG model) with 100 ml sterile water (1:1 w/v) for 10 minutes and left to stand for 48 hours at room temperature. The extract was filtered through two layer of muslin cloth and the final filtrate obtained was used as stock solution. Mancozeb, one of the synthetic fungicides recommended for control of bean anthracnose, was used as a positive control, applied at recommended dosage (2gm l^{-1}). Mancozeb has chemical component of Zn-manganese ethylene bisdithiocarbamate and its common name is Dithane M-45 80WP.

Antifungal bioassay

The *in-vitro* tests were conducted to determine effect of *Aloe vera* extracts on *C. lindemuthianum* mycelia growth using poison food technique (Begum and Bhiyan, 2006). Extract stock solution of 20 ml, 25 ml and 30 ml were mixed with 80, 75 and 70 ml of the sterilized molten (45°C) PDA media, respectively to obtain 20, 25 and 30 percentage concentrations. To avoid bacterial contamination 1% streptomycin was added. On cooling, 15 ml of the amended medium was poured into each of the 9 ml petridish and labeled. After solidification, the plates were inoculated by placing at the centre, 5 mm discs of the 14 days old PDA cultures of *C. lindemuthianum* obtained from anthracnose infected bean pod collected from a farmer's field in Uasin Gishu county (0°34'28.47"N, 35°18'00.33"E. The disc was cut out from periphery of actively growing mycelium using cork borer. The inoculated plates were incubated at 22 ± 2 °C for seven days. The zero percentage treatment served as negative control while Dithane M-45 80WP (2 gm l^{-1}) was used as a positive control. For each of the five treatments three replicates were maintained. Data on inhibition of the mycelia growth was recorded on the 14th day after incubation when growth on control plates completely covered the plate. Diameter of the colonies on PDA with and without extract was measured in mm from the bottom side of the petridish.

The fungi toxicity of the extracts in terms of percentage mycelia growth inhibition was calculated by using the following formula (Singh and Tripathi, 1999; Tegegne *et al*., 2008):

$$\% \text{ mycelia growth inhibition} = \frac{dc - dt}{dc} \quad X\ 100$$

Where dc = Average diameter increase in mycelial growth in control,
dt = Average diameter increase in mycelial growth in treatment.
The data obtained was subjected into the following 0-5 scale:- (source:- own composition)

Scale 0:- 0% - No mycelia growth inhibition
Scale 1:- 1-25%- slight mycelia growth inhibition
Scale 2:- 26-50%- lower mycelia growth inhibition
Scale3:- 51-75%- intermediate mycelia growth inhibition
Scale 4:- 76-95 %- higher mycelia growth inhibition
Scale 5:- 95-100%- complete mycelia growth inhibition

Based on the effectiveness of the plant extracts the scale was grouped as:-

Scale 0 & 1- Not effective treatment

Scale 2&3- Averagely effective treatment
Scale 4 &5- Effective treatment

Data Analysis
All data obtained from the experiments were analyzed statistically by analysis of variance (ANOVA) procedure using GenStat computer package (VSN International 2008) after angular transformations of the data. Separation of means was done using Turkeys Test at p≤0.05.

RESULTS

Incidence of Anthracnose on Bean Varieties Grown in Western Kenya
Variety GLP- 585 was the most predominant variety among the farmers sampled. Results showed that Western Kenya farm-saved seed had significantly (P≤ 0.05) high anthracnose incidence compared to certified seed (C0) which had no pathogen in all varieties. The GLP 585 variety collected from Bungoma had the highest anthracnose incidence. Table 1 shows that disease incidence in all the seed samples were significantly (P≤ 0.05) different in varieties and seasons. The highest disease incidence was recorded in GLP 585 variety followed by GLP X92, white, Brown, Yellow and GLP 1004 while the lowest was recorded in variety GLP 2 followed by variety GLP 288. However, incidence in varieties GLP 1004, Yellow, Brown, GLP X92 and white was not significantly (P>0.05) different.

Seed samples collected from Bungoma had highest incidence (Table 2) followed by Kakamega, Busia, Trans Nzoia and Nandi while those collected from Kisii had lowest incidence followed by Siaya. However, there were no significant (P > 0.05) differences among the mean incidence in the seed from Kakamega and Busia; Nandi and Trans Nzoia or from Kisii and Siaya. Significant (P ≤ 0.05) differences were observed for the anthracnose incidence in the seed on interaction of region by season by variety. GLP 585 variety, from Bungoma region in both seasons, recorded the highest disease incidence while long rains season, variety GLP 2 from Kisii had the lowest incidence.

Table 1: Incidence (%) of anthracnose observed on seed varieties collected from W. Kenya.

Varieties	Anthracnose incidence (%)				Variety
	SR 2008	LR 2009	SR 2009	LR 2010	Mean
GLP2	26.8 a	25.7 a	29.8 a	30.0 a	28.7
GLP 288	29.1 ab	32.9 a	44.8 ab	30.9 a	37.0
GLP 1004	32.3 ab	35.7 ab	49.1 b	38.0 ab	40.0
Brown	35.7 ab	36.1 ab	51.1 b	42.7 ab	43.5
Yellow	44.3 bc	39.5 ab	51.4 b	44.5 ab	43.5
White	45.0 bc	41.3 ab	54.1 b	45.5 ab	44.4b
GLP X92	45.2 bc	50.0 b	54.5 b	48.2 ab	47.4
GLP 585	59.0 c	51.1 b	54.5 b	51.3 b	53.1
Season mean	39.7	39.0	48.7	41.4	
Grand mean	42.2				
S.E.D	2.9				
CV%	52.2				

Means sharing the same letters are not significantly different at p≤ 0.05 according to Turkey's test SR- Short Rains season; LR- Long Rains season.

Table 2: Incidence of anthracnose observed on seeds collected from farms in different regions of W. Kenya.

Region	Anthracnose incidence (%)				region
	SR 2008	LR 2009	SR 2009	LR 2010	Mean
KISII	28.91 a	23.28 a	30.94 a	18.91 a	25.5
SIAYA	27.50 a	25.00 a	36.88 a	27.97 ab	29.3
KITALE	39.22 ab	43.75 b	53.91 bc	42.34 bc	44.8
NANDI	42.66 ab	42.19 b	44.53 ab	44.84 cd	43.6
KAKAMEGA	47.66 b	52.66 b	54.84 bc	47.97 cd	50.7
BUSIA	45.94 b	40.00 b	54.22 bc	49.06 cd	47.3
BUNGOMA	45.78 b	46.25 b	65.31 c	58.59 d	54
Season mean	39.7	39.0	48.7	41.4	
Grand mean	42.2				
S.E.D	2.6				
CV%	49.3				

Means sharing the same letters are not different at p≤ 0.05 according to Turkey's test
SR- Short Rains season; LR- Long Rains season

Fungal Inhibitory Activity of Aloe Vera Extract

The results showed that *A. vera* had ability to inhibit the mycelial gowth of *C. lindemuthianum*. There was no significant (P≤ 0.05) difference in mycelia growth inhibition for the extract and positive control (Dithane M-45 80WP) while there was signifacant difference on the inhibotory effect of the extract and negative control (water). The positive control exhibited maximum growth inhibition of 92.6%, followed by Aloe extract 90% while negative control had no mycelia growth inhibition (0.0%).

However, there was significant (P≤ 0.05) difference in the mycelia growth inhibition by the plant extract at different concentrations. At 30 % concentration, *Aloe vera* had significantly higher mycelia growth inhibition followed 25% least mycelia growth inhibition exhibited at 20% concentration.

DISCUSSION

Incidence of Anthracnose on Farm-Saved Bean Seed

Results from this study showed that anthracnose incidence was generally higher in farm-saved seed than in the certified seed which was used as control. This implies that the apparently clean healthy farm-saved seed was of low quality in terms of its health status due to anthracnose infection. These variations in the disease incidence in different regions of study could be attributed to the fact that the regions had diverse seed varieties, agro-climatic conditions, agronomic practices and agro-ecological conditions which bring about different influences on disease development. GLP 285 variety had the highest disease incidence while GLP 2 had the lowest. This study shows differences in susceptibility of different varieties to the disease as also observed by Ombiri *et al* (2003) and Nkalubo (2007). They concluded that variety susceptibility depends on type and number of resistance genes present in each cultivar. This encourages cultivation of many bean varieties on the same farm since different varieties have different resistance genes to the disease leading to production farm-seed of low disease incidence (Mwaniki, 2002). GLP 2 variety is known to have genes resistant to anthracnose although still there was incidence of infection by the disease. Farmers in Kisii predominantly planted GLP 2 and GLP 288 varieties which had low disease incidence. This led to low disease incidence in the region.

In this study it has been observed that variation in climatic conditions from one season to another influences the occurrence and severity of the disease. During the collection of farm-saved seed the temperature ranged between 15 and 18 ^{o}C in the short rains season and 24 and 30 ^{o}C in the long rain season. Rainfall ranged between 1600 and 2100 mm in short rain season and 1200 and 1800 mm in the long rain season. Seed collected during long rains season had generally lower anthracnose incidence level while short rains season had higher incidence in all the study regions. It confirms that rainfall and temperature contribute to the level of infection and development of anthracnose. Anthracnose is reported to develop well in high altitudes, moderate temperature ranges of 18 - 24 ^{o}C, more rainy days and high relative humidity of above 95% (Yesuf and Sangchote, 2007).

Variation of disease incidence during the collection period could also be attributed to observed different agronomic practices in different seasons. Most Western Kenya farmers predominantly practiced pure stand cropping during the

short rain season while in long season they practiced intercropping with other crops especially maize. In mono cropping practice bean crops are very closely spaced than in the intercrop. Closely spaced crops retain high humidity and temperature in between their population, a condition conducive for the diseases to develop and spread from one plant to another. Close spacing of bean plants increase chance of contact within plant populations or use of farm implements by farmers during different agronomic practices in the field such as weeding leading to spread of the disease from infected plants to health plants. Rain splashes between closely spaced plants are other factors that could increase dissemination of the pathogen in the field. This contributes to high incidence of anthracnose in seeds harvested on short rain season than long rain season seeds. However, farmers in Kisii regions predominantly practiced intercropping in both seasons. This could also explain why Kisii consistently had lower incidence in both seasons.

Efficacy of Plant Extracts Antifungal Activity against Bean Anthracnose

Current investigation clearly indicates that the antifungal activity *A. vera* against *C. lindemuthianum* because the extract inhibitory of mycelia radial growth compared with the control as also observed by Wokocha and Okereke, (2005). This indicates that *Aloe* extract induce toxic effects on mycelia growth and proliferation of *Colletotricum lindemuthianum*. This study is in agreement with several other workers who tested the activities of *A. vera* with the aim of assessing their activity against *Colletotrichum gloeosporioides* and *Colletotrichum capsici* using food poison technique. The extracts showed higher activity against the *Colletotrichum* species. They identified that aloine and aloe-emodin chemical components in *Aloe vera* plant were the active reagents with activity against *Colletotrichum gloeosporioides* and *Colletotrichum capsici*. Also Jaya and Dhananjay (2012) worked on six plants, *Azadirachta indica, Aloe vera, Ocimum sanctum, Ocimum basilicum, Lantana camara and Asparagus* antifungal activity against the *Aspergillus niger, Aspergillus flavus, Rhizoctonia solani, Rhizoctonia bataticola* and found that *Aloe vera* was the most effective.

A. vera extract had high inhibitory activity at 30% concentration than at 25% and 20%. This indicates that there was an increase in inhibition of fungal mycelia growth as the extract concentrations increased. This could be due to increased availability of the antifungal chemicals in the media. Other studies have also shown that there was increase in inhibition of fungal colony when extracts concentrations increased (Cao and van Bruggen, 2001; Shovan *et.al*,. 2008; Masangwa *et al*., 2012). Masoko *et al*., 2005 speculated it to be due to solubility of compounds in water and stated that water fails to extract non-polar active compounds in plant materials and hence need for higher plant extracts concentration to achieve high fungal toxic level.

Use of *A.vera* plant extracts as an alternative control method if integrated in anthracnose management could reduce over reliance on the synthetic fungicides by the farmers, as well as cut down cost of bean production and thus reduce anthracnose incidence in farm saved seeds in Western Kenya.

RECOMMENDATION

Further work should be done on the *Aloe vera* plant extract to investigate its durability as systemic fungicide in the plants for increased efficiency against *C. lindemuthianum*.

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