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RESEARCH ARTICLE

OCCURRENCE OF COMMON BACTERIAL BLIGHT CAUSED BY *Xanthomonas axonopodis* pv. *phaseoli* ON COMMON BEAN AND THE INFLUENCE OF DIFFERENT FACTORS ON IT'S INCIDENCE IN UASIN GISHU COUNTY.

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Abstract

Beans (*Phaseolus vulgaris*) is a popular legume crop in Kenya, ranking first among the legumes in importance. The production of this crop in Kenya has not kept pace with the demand. Among the main causes for poor yields in common beans are bacterial diseases of which common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a major seed-borne disease of common bean worldwide and can cause 10 to 40% loss in yield. A survey was carried out in 60 farms in three sub counties of UasinGishu county, to establish occurrence of common bacterial blight, during the rainy seasons between April - June and September – December, which are the main bean production seasons. The disease incidence was determined in each farm by taking four quadrants at random, in each quadrant the number of plants showing disease symptoms were counted against the total number of plants in the quadrant and the means were used as the disease incidence of each farm. The altitude which determines the agro ecological zone was established from the reading of Geographical Positioning System equipment (GPS). Diseased plant parts were collected and the bacteria isolated. The isolates obtained were confirmed through gram staining, microscopy, biochemical and pathogenicity tests. Farmer practices were determined through questionnaires and farmers' interview. Descriptive statistics, Chi-square tests and spearman's correlation coefficient analysis were used for data analysis and correlations. Common bacterial blight disease incidence in the individual farms varied from 13.2 to 32.5%. There was a significant correlation between altitude and common bacterial blight incidence. The Pearson's correlation coefficient ($p < 0.01$) indicated a reduction in common bacterial blight incidence as altitude increased. The disease also reduced with an increase in the number of seasons the field was occupied by other crops besides beans. There was a wide spread occurrence of the disease, and major contributing factors to common bacterial blight in the region include; seed source, bean variety, lack of seed renewal and the method of production. The survey provides background information useful in the disease management in the region in order to improve bean production consequently increasing the yields.

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Introduction:-

Common bean (*Phaseolus vulgaris* L.) is an important grain legume, most consumed worldwide and a major protein source to most households in Kenya (Munyasa, 2013 and Kiptoo *et al.*, 2016). It follows maize in ranking of staple food crops grown by thousands of Kenyan households (Mureithi *et al.*, 2003 and Kiptoo *et al.*, 2016). It is considered as a perfect food by the fact that it's content of protein is high, rich in complex carbohydrates, folic acid, iron and other essentials of diet (Gichangi, 2012). Apart from the contribution in terms of human nutrition, it also provides income for the farmers with small farms through the sale of their beans (Mwaniki, 2002). Having a short growth cycle and moderate rainfall requirements allows it to be produced during seasons of less rain (Bitocchi, *et al.*, 2012). It is also consumed by people from all income levels (Kara *et al.*, 2009) and therefore plays a big role in terms of food security (Kabutbei, 2014).

Over the last few years, bean production in Kenya has been on the decline (Katungi *et al.*, 2010). Fungal, viral and bacterial diseases are considered as part of the most important causes of low yield in common beans (Ferreira *et al.*, 2003). Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a major seed-borne disease of common bean worldwide (Popovic *et al.*, 2012). The severity of the disease, environmental factors favouring the onset and development of the disease and how much susceptible a cultivar is, determines the amount of yield loss (Asensio *et al.*, 2006).

In Kenya, common bacterial blight (*Xap*) constraints the production of beans. It has been reported to cause losses of between 10 and 75% (Katungi *et al.*, 2009). In Uasin Gishu county, most farmers plant seeds that are not certified, seeds saved from the previous harvest, seeds obtained from other farmers or bought from local markets which tend to promote the spread and the development of the disease (Anon, 2010 and Gicharu *et al.*, 2013). The main objective of this study was therefore to establish the occurrence of common bacterial blight disease infecting common bean in Uasin Gishu county and the factors that affect the disease incidence.

Materials and Methods:-

Determination of incidence of common bacterial blight

Field surveys were carried out in UasinGishu county, Kenya, which lies between longitudes 34 degrees 50" east and 35 degrees 37" West and latitudes 0 degrees 03" South and 0 degrees 55" North. UasinGishu experiences high and reliable rainfall which is evenly distributed throughout the year. Common bacterial blight surveys were conducted in three sub counties in Uasin Gishu county namely; Turbo, Moiben and Soy, (Fig 1) having diverse altitudinal gradients. From the selected areas, 60 bean farms having beans at different development stages were surveyed during the rainy seasons, (April - June and September – December). A distance of not less than 5 kilometres between each bean producing farm was maintained to determine the disease occurrence.

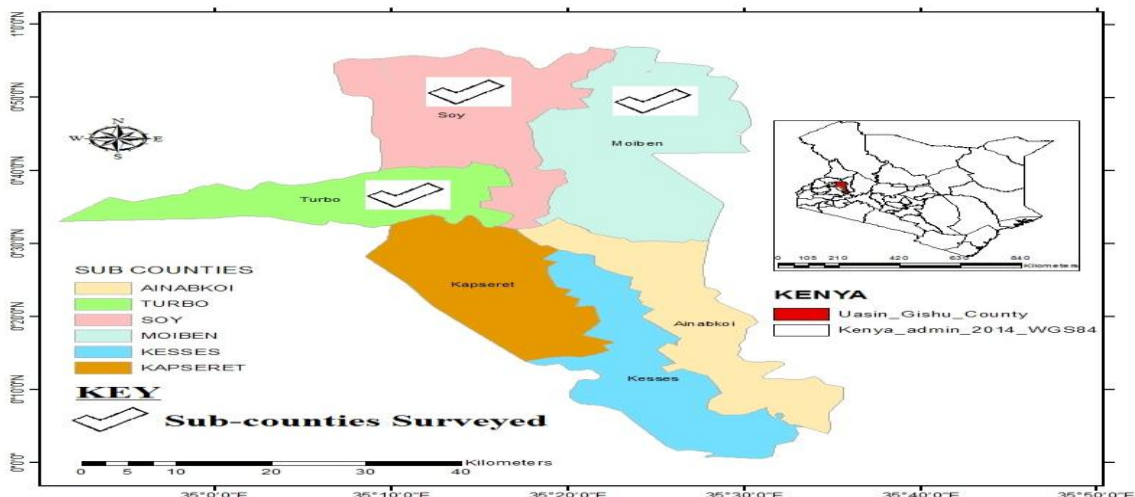


Figure 1:-Surveyed areas in Uasin Gishu county to establish occurrence of common bacterial blight

The disease incidence was determined by drawing four random quadrants, (1m²) in each bean field. From each quadrant, plants showing typical common bacterial blight disease symptoms (lesions that are necrotic and irregular having borders that are yellow and water-soaked spots), were counted and recorded against the total number of plants in each quadrant, while noting the seed variety.

Common bacterial blight incidence was calculated as the number of plants infected expressed as percent of total count of the plants observed in each quadrant using the following formula as described by Sharma, (2012).

$\% \text{ CBB incidence} = \frac{\text{No. of plants with symptoms in quadrant}}{\text{Total no. of plants in the quadrant}} \times 100$

Collection of diseased plants

During the survey, diseased plants were collected from each farm and taken to laboratory for the isolation of the bacterium.

Isolation of *Xanthomonas axonopodis* pv. *phaseoli* and cultural identification

At the laboratory a small section at boundary of the blight lesion from each leaf sample was cut, (0.2-0.5 mm) and surface sterilized in 2.5% sodium hypochlorite for 2 minutes and rinsed in three changes of sterile distilled water, then macerated. A loopful of suspension was streaked on Yeast Dextrose Calcium carbonate agar (YDC) and incubated at 28°C for 2 days. They were then confirmed through colony growth on Sucrose Peptone Agar (SPA) which is a semi selective medium as described by Mwangi *et al.*, (2008). The plates were then examined for yellow, circular, smooth and mucoid colonies which provided preliminary identification. Isolates from the different farms were designated T 01, T 02, T 03up to T20 for Turbo, S21, S22, S23.....S40 for Soy and M41, M42, M43.....M60 for Moiben sub counties. Each of these isolates was stored in labelled universal bottles in nutrient broth at 4°C for other analysis.

Gram-staining test

A fairly turbid suspension of the testing bacteria was made from a solid medium in sterile water (24-48 hrs old culture). A loopful of the suspension was smeared on a microscope slide. The slide was air-dried and fixed by passing rapidly two or three times through a bunsen burner flame. The slide was then flooded with crystal violet solution for 1 minute. The smear was then washed in a gentle stream of tap water until no more stain remained on the smear. The smear was flooded with lugol's iodine for one minute and washed again in a gentle stream of tap water and then blot dried. It was then decolourized by washing in a gentle stream of 95% ethanol for not more than 30 seconds to remove stain and blot dried. It was then counterstained by flooding with safranin for 20 seconds then washed with tap water, blot dried and a drop of immersion oil was added and observed under the compound microscope's oil immersion objective as described by Abd-Alla, (2010).

Biochemical tests

Characterization of the presumptive pathogen was carried out by subjecting the isolated bacterial colonies to various biochemical tests. The following biochemical tests were undertaken to confirm *Xap* from bacterial isolates. Asculin hydrolysis, Starch hydrolysis, Catalase test, Oxidative/Fermentative (O/F) test and KOH Solubility test as described by Karavina, (2011).

Pathogenicity test

The *Xap* isolates were confirmed further through pathogenicity tests by inoculating on young bean plants of Mwitmania – (GLPX92 - Kenya seed company), a bean variety that is susceptible to CBB. The plants were grown in pots with soil that had been sterilized and kept in glass house till first visible trifoliate leaves that had fully expanded appeared (15 days from planting). Two hours before the inoculation was done, water was sprayed on the plants to generate appropriate humidity for the pathogen to cause an infection. A sterilized toothpick was dipped into the bacterial culture of the targeted strains that had been grown on Sucrose Peptone Agar medium. The toothpick that had been dipped into bacterial culture was pushed into the plant tissues until the tip emerged on the other side. The toothpick while inside the plant tissues was turned slightly meanwhile, being pulled out so as to leave the bacteria inside the tissues. Four replicates were maintained per isolate. The negative controls were inoculated with

sterile distilled water. The seedlings which had been inoculated were then allowed to grow in the glass house at the temperatures between 25°C and 32°C at all the time the floor of the glass house was made wet to generate humidity so as to favour the infection. The inoculated plants were checked on daily basis for appearance of symptoms. The symptoms that developed as a result of inoculation were recorded from 10 days from the date of inoculation. Bacteria showing the same symptoms like those observed in the field were re-isolated from the symptomatic tissues.

The influence of different factors on the incidence of common bacterial blight in Uasin Gishu county.

During the field survey on individual farmers, semi-structured questionnaires were administered to obtain data on the physical, economic and the social aspects practiced by farmers that could influence common bacterial blight status in the area under bean production. Source of bean seed and bean production season, varieties produced and their susceptibility to disease and cropping practices, farmer knowledge on pests and diseases affecting beans and method of beans storage and usage was collected. Geographical location and elevation (altitudes, latitudes and longitudes) of the fields were recorded using Geographical Positioning System equipment (GPS; Garmin eTrex 30).

Results:-

The occurrence and incidence of common bean blight

Common bacterial blight occurred in all the farms surveyed in the three sub counties (Table 1). The individual farm incidence recorded was higher in farms 8 and 14 (32.5%) located in Turbo sub county, while the least disease incidence was recorded in farm 49 (13.2%) located in Moiben Sub county. When the sub counties mean was compared, it was noted that Turbo sub-county had the highest mean disease incidence of 28.2% followed by Soy sub county at 22.5% and the least was Moiben sub county at 15.9% (Figure 2). The analysis of variance showed a significant difference in disease incidence in the three sub counties. Further, it was noted that high disease incidence was recorded mainly in the areas of the upper midlands and lesser in the upper highlands (Table 1).

Table 1:- Common bean blight incidence in various bean farms in Uasin Gishu county

Farm / Isolate No.	Sub county	Agro ecological Zone	Altitude (masl)	Mean % CBB incidence
T 01	Turbo	Upper midlands	1610	31.9
T 02	Turbo	Lower highlands	2011	24.4
T 03	Turbo	Lower highlands	1957	25.5
T 04	Turbo	Upper midlands	1893	26.7
T 05	Turbo	Upper midlands	1712	28.7
T 06	Turbo	Upper midlands	1592	32.3
T 07	Turbo	Upper midlands	1823	27.4
T 08	Turbo	Upper midlands	1500	32.5
T 09	Turbo	Upper midlands	1742	28.6
T 10	Turbo	Lower highlands	1991	24.6
T 11	Turbo	Upper midlands	1670	30.9
T 12	Turbo	Upper midlands	1874	27.2
T 13	Turbo	Lower highlands	2033	21.9
T 14	Turbo	Upper midlands	1543	32.5
T 15	Turbo	Lower highlands	1939	25.6
T 16	Turbo	Upper midlands	1638	31.8
T 17	Turbo	Upper midlands	1885	26.7
T 18	Turbo	Lower highlands	2025	22.9
T 19	Turbo	Upper midlands	1679	31.6
T 20	Turbo	Upper midlands	1683	30.4
S 21	Soy	Lower highlands	2070	22.3
S 22	Soy	Lower highlands	1964	20.5
S 23	Soy	Lower highlands	2009	23.7
S 24	Soy	Upper midlands	1874	25.6
S 25	Soy	Lower highlands	1920	18.4
S 26	Soy	Lower highlands	2095	21.1

S 27	Soy	Lower highlands	1939	19.7
S 28	Soy	Upper midlands	1742	29.9
S 29	Soy	Lower highlands	2025	22.9
S 30	Soy	Lower highlands	2090	21.4
S 31	Soy	Upper midlands	1850	17.8
S 32	Soy	Upper midlands	1781	29.5
S 33	Soy	Upper midlands	1815	16.4
S 34	Soy	Lower highlands	1983	20.9
S 35	Soy	Lower highlands	1968	24.2
S 36	Soy	Lower highlands	1951	19.9
S 37	Soy	Upper midlands	1813	26.6
S 38	Soy	Lower highlands	1992	20.7
S 39	Soy	Lower highlands	2057	22.8
S 40	Soy	Lower highlands	1911	25.5
M 41	Moiben	Lower highlands	2155	15.6
M 42	Moiben	Upper midlands	1800	20.1
M 43	Moiben	Lower highlands	2240	14.3
M 44	Moiben	Lower highlands	2495	13.3
M 45	Moiben	Lower highlands	1994	16.9
M 46	Moiben	Upper highlands	2420	13.9
M 47	Moiben	Lower highlands	1930	18.3
M 48	Moiben	Lower highlands	2198	15.1
M 49	Moiben	Upper highlands	2500	13.2
M 50	Moiben	Lower highlands	2290	14.2
M 51	Moiben	Upper midlands	1875	19.7
M 52	Moiben	Lower highlands	2021	16.2
M 53	Moiben	Upper highlands	2480	13.5
M 54	Moiben	Lower highlands	2210	14.4
M 55	Moiben	Lower highlands	1970	17.2
M 56	Moiben	Upper midlands	1826	18.7
M 57	Moiben	Lower highlands	2066	16.1
M 58	Moiben	Upper highlands	2450	13.7
M 59	Moiben	Lower highlands	2187	15.3
M 60	Moiben	Lower highlands	2395	14.2
MEAN				22.1
CV(%)				5.99587
SE(I)				0.77406
LSD				0.00001
(0.05)				($p < 0.05$)

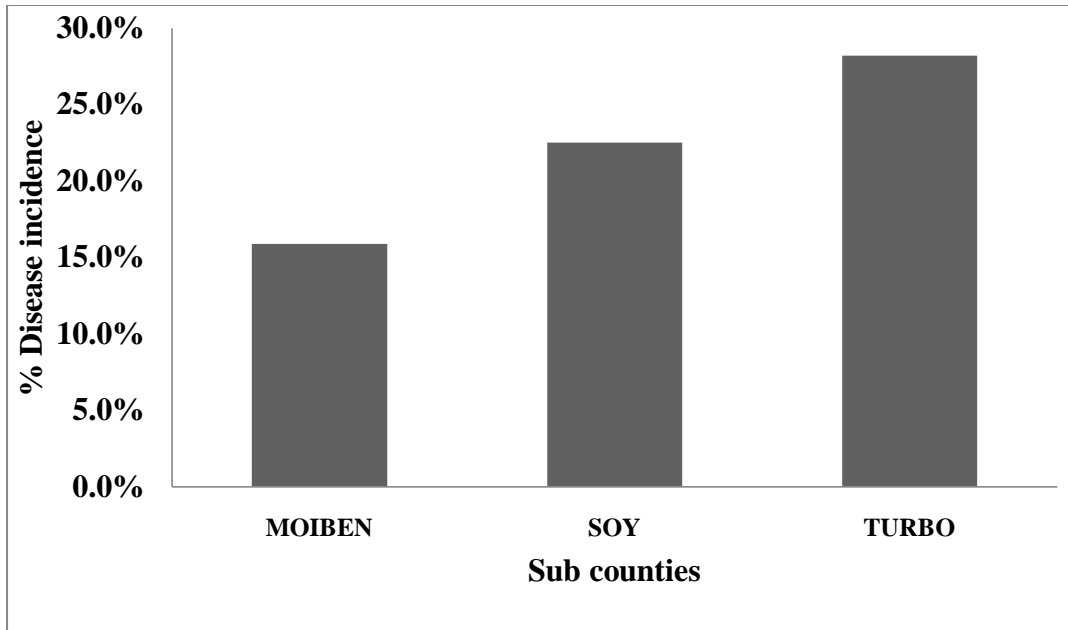


Fig 2:-Mean incidence of common bacterial blight across the sub-counties

Cultural and morphological characterization of *Xap* isolates

The bacterial colonies isolated were mucoid, yellow, convex and shining when grown on yeast dextrose calcium carbonate (YDC) medium, after two days of incubation (Plate 1A). On Sucrose Peptone Agar (SPA), colonies had a yellow pigmentation, were smooth, mucoid and circular (Plate 1B). On Nutrient Agar (NA), colonies were mucoid, yellow, with entire margins, glistening and convex (Plate 1C).

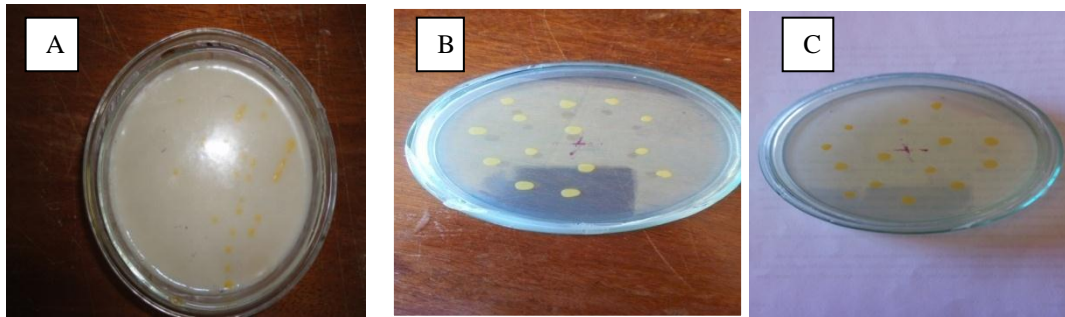


Plate 1:-*Xanthomonas axonopodis* pv. *phaseoli* colonies grown on YDC (A), SPA (B) and NA (C) culture media.

Biochemical identification of the bacterial pathogen.

The biochemical tests showed similarities among all the isolates. Asculin hydrolysis, starch hydrolysis, catalase test, oxidative/fermentative test and KOH solubility test were positive for all isolates. The bacterium appeared as single cells that are straight rods, and were also gram negative, when subjected to the gram- staining test.

Pathogenicity test.

All the *Xanthomonas axonopodis* pv. *phaseoli* isolates were pathogenic on common bean showing small water-soaked spots that developed 10-12 days from inoculation. The necrotic symptoms developed at the margins of the diseased leaves 20 days after inoculation confirming that all isolates were pathogenic (Plate 2).

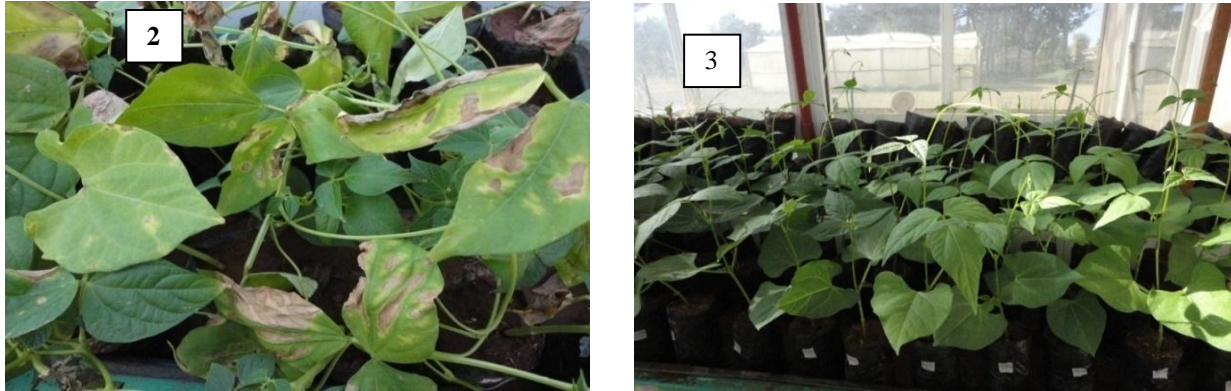


Plate 2:-Common bean leaves showing common blight symptoms 20 days from inoculation of *Xanthomonas axonopodis* pv. *phaseoli* bacterial isolates. **Plate 3:** Uninfected common bean plants (negative control)

The lesions became large and developed into brown, dry spots with distinct, rather narrow, yellow borders. These lesions consisted of irregular parts of brown dry tissues that frequently occurred on leaf margins. The plants used as negative control (with sterile distilled water) had no symptom development (Plate 3).

The influence of different factors on the incidence of common bacterial blight of common bean in Uasin Gishu county.

When altitude was considered and the variance analysed, it showed a significant difference in altitudes in the three sub counties, ($p < 0.05$). Multiple comparisons between them also indicated a significant difference ($p < 0.05$). On the other hand, there is also a relationship between disease incidence and the agro-ecological zone of the sub county. There was a higher disease incidence in the upper midlands of Turbo sub county, having an altitude range of between 1500 and 1900m above sea level, followed by the lower highlands (altitude 1900-2400masl) and the least disease incidence was reported in the upper highlands of Moiben sub county (altitude 2400-2700masl), as seen in Table 1 above.

Bean varieties grown by farmers in Uasin Gishu county and susceptibility to disease.

There were 12 bean varieties grown by farmers in Uasingishu county. More than one variety of beans was grown by most farmers in their farms. In fact all of them had planted KATX69 bean variety, (100% of all the surveyed farms). The second most popular bean variety was KK8 (78.9%) followed by Yellow variety at 68.4%.

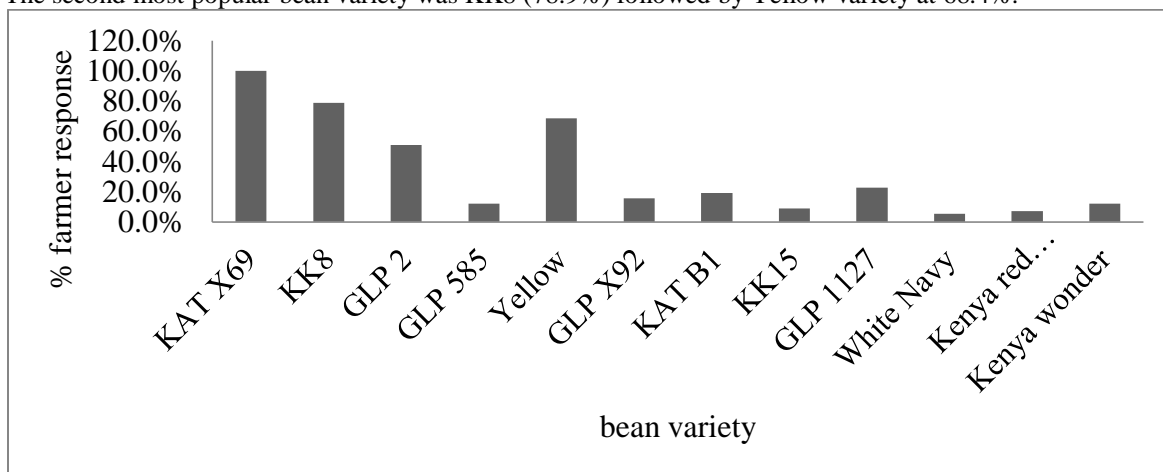


Fig3:-Bean varieties grown by farmers in UasinGishucounty

The least popular common bean variety planted by farmers was the White Navy variety (5.3%), Kenya red kidney (7.0%) and KK15 (8.8%) as shown in Figure 3. There was a significant difference ($p \leq 0.05$) in responses pertaining to bean varieties planted by farmers in Uasin Gishu county ($\chi^2 = 348.122$, $df=11$, $p\text{-Value} = 0.00001$ ($p < 0.05$)). GLPX92 (Mwitmania) bean variety was reported by 59.7% of the farmers as the most susceptible bean variety to disease, and had the highest disease incidence, followed by White Navy (50.9%).

The least susceptible bean variety to CBB was KK15 (3.5%) and KATX69 (5.3%), as reported by farmers during the survey (Table 2). KK15 variety had the least disease incidence. The difference in bean variety against disease incidence was significant ($\chi^2= 140.219, p= 0.00001 (p<0.05)$).

Table 2:-Bean variety against disease incidence

BEAN VARIETY	% DISEASE INCIDENCE	Percent frequency of farmers growing the particular bean variety in their farms
KAT X69	5.3%	60
KK8	12.3%	47
GLP 2	15.8%	31
GLP 585	21.1%	7
Yellow	26.3%	41
GLP X92	59.7%	9
KAT B1	14.0%	12
KK15	3.5%	5
GLP 1127	21.0%	14
White Navy	50.9%	3
Kenya Red Kidney	15.8%	4
Kenya Wonder	24.6%	7

Bean planting seasons and sources of seed

Majority (91%) of the farmers planted beans during the long rains of April to July. Smaller significant proportion of the farmers (7%) planted beans between September and December. Only a few farmers planted beans in their farms in all seasons as shown in Figure 4. The goodness of fit chi square test showed that planting seasons was significant ($\chi^2= 150.02, p = 0.00001 (p< 0.05)$). All the farmers that planted beans during the long rains and those that planted during both seasons indicated that the disease incidence was higher during the long rains.

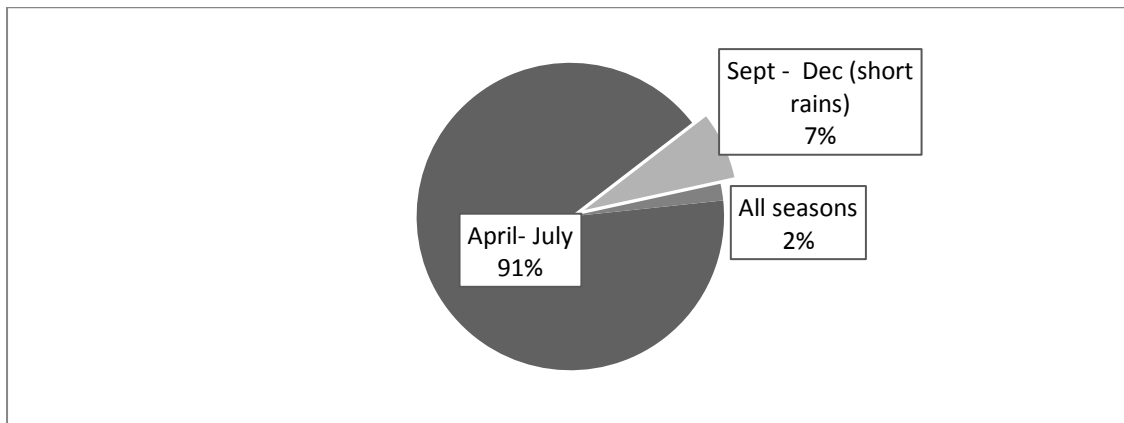


Fig 4:-Bean planting seasons by farmers in Uasin Gishu county

Most of the farmers (64.9%) planted seeds sourced from nearby markets followed by those using their own saved seed from the preceding harvest (38.5%). Only 1.8% of the farmers planted certified seeds sourced from agro dealers (Fig. 5). There was a significant difference ($p< 0.05$) on seed sources in the sub counties surveyed. The farmers that sourced seeds from the market, own-saved seed from previous crop and from neighbours reported high disease incidence than those that planted certified seeds from agro-dealers.

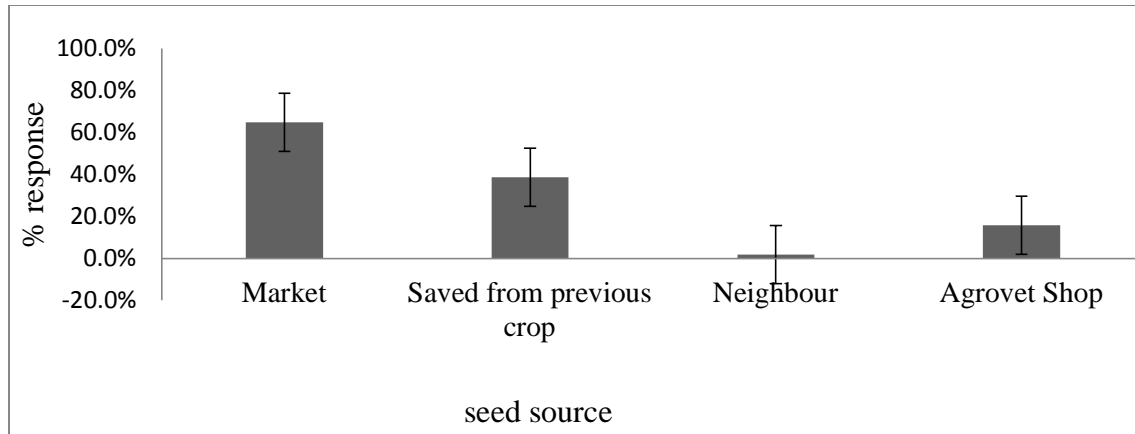


Fig 5:-Sources of bean seed planted by farmers of the county of Uasin Gishu

Discussion:-

Incidence of common bean blight caused by *Xanthomonas axonopodis* pv. *phaseoli*.

Common bacterial blight incidence was high in the sampled areas of Turbo and Soy. However, the disease occurrence was least reported in Moiben. There was observed significant difference in correlation between disease incidence and altitude in the three sub counties in Uasin Gishu county. Research findings by Sahile, *et al.*, (2008) indicated a correlation between disease parameters and altitude. Oshone, *et al.*, (2014) reported varying proportions of *Xanthomonas axonopodis* pv. *phaseoli* from bean samples obtained from small scale farmers in different cropping systems in Ethiopia. This variation could be attributed to disease predisposing conditions in the wetter and warmer upper midland and lower midland zones relative to the cooler lower highland zones of UasinGishu county. This observation could therefore be linked to favourable climatic conditions for disease development and establishment in the wetter warmer upper midland areas of UasinGishu. In this case there was a correlation between common bacterial blight caused by bacteria and the prevailing weather conditions, which could have been predisposing conditions for *Xanthomonas axonopodis* pv. *phaseoli* pathogen. This is the first documented report indicating the occurrence and the level of incidence of common bacterial blight disease in Uasin Gishu county.

Xanthomonas axonopodis pv. *phaseoli* when present on or in the seed provides potentially dangerous inoculum source. The quality and the source of seeds are important factors that determine an outbreak of common bacterial blight. The seeds infected by the pathogen acts as primary inocula sources for seed borne disease such as common bacterial blight, as explained by Hall, (1994) as the bacteria is seed-borne, occurring internally and externally on the bean seeds which incite disease.

Cultural, morphological and biochemical identification of the isolates

The bacterial colonies present showed mucoid, smooth, circular and a pigment that is yellow in colour called xanthomonadin. The *Xanthomonas axonopodis* pv. *phaseoli* bacterium was isolated using YDC medium and confirmation was done through appearance of colony growth on sucrose peptone agar medium which is deemed as semi selective medium, as described by Mwangi, *et al.*, (2007). The colonies were yellow, circular, smooth and mucoid. The bacterium appeared as single cells, straight rods and were also gram negative to the gram- staining test. The biochemical tests showed similarities among all the isolates. Asculin hydrolysis, starch hydrolysis, catalase test, oxidative/fermentative test and KOH Solubility test were positive to all isolates selected, confirming that they were *Xanthomonas axonopodis* pv. *phaseoli* isolates.

Bean variety susceptibility to disease and seed sources

In this study, the finding showed that twelve bean varieties were grown in Uasin Gishu county. KATX69 commonly referred as 'Nyayo', KK8 called 'Saitoti', Yellow and GLP2 (Rosecoco) were the main varieties produced. The farmers planted more than one variety of beans in their farms, and KATX69 was their favourite, followed by KK8, Yellow and GLP2 varieties respectively. Mugisha, (2008) reported that farmers choose bean varieties based on their ability to resist diseases and their marketability. Farmers in southern western Uganda shifted from large bean variety to small bean variety because of their ability to resist diseases. In this study, it was established that most farmers chose KATX69 variety rather than KK15 though KK15 is less susceptible to diseases as compared to KATX69.

This agrees to the finding of Mugisha, (2008) who reported that farmers from the eastern part of Uganda grew large bean varieties due to their disease tolerance and marketability attributed to consumers' preferences.

Nearly all sampled farmers` inter-cropped beans during production with maize, bananas etc. and only a small proportion of farmers planted beans in pure stand. These finding agrees with earlier research findings by Katungi, *etal.*, (2009) and ECABREN, (2003) who reported that, majority of the small scale bean farmers produced beans under multiple inter-cropping with cereals, bananas and coffee among other crops. This could be attributable to the need by the famers to maximize on the limited farming space as well as take advantage of nitrogen fixation by beans as reported by Thiong`o, *et al.*, (2007) and Birachi, *et al.*, (2011).

The results from the farmers showed that the difference in bean variety susceptibility to diseases was significant ($p<0.05$). GLPX92 (Mwitmania) bean variety was reported as the most susceptible to disease followed by White Navy. They observed that the least susceptible bean variety to diseases was KK15 and KATX69. The bean susceptibility characteristic can be attributed to breeding management since most of these varieties were the latest release to farmers thus therefore the availability of recycled seed or saved seed was minimal as compared to other varieties which have been in the market. This study agrees with the study carried out by Schneider, *et al.*, (2001) where they observed that absence of resistance to common bacterial blight over years may be due to concentration of both breeding and management practices on other factors such as seed size and growth habits rather than pest and disease resistance. In this case GLPX92 (Mwitmania) is most susceptible due to its wide growth habit (Mugisha, 2008). Saving bean grain for seed use in the preceding season was an important use of the harvested beans. These findings are in consistence with Opole, *et al.*, (2006) who reported that many of the farmers kept their own bean for seed use in the subsequent season. Similarly, Muthii, (2014) collaborated the use of saved seeds for planting on the following season. The results could explain the prevalence of CBB in the surveyed areas as farmers saved and shared seed during the preceding season. Therefore management of common bacterial blight should be given a priority because of its seed borne nature and research on breeding for resistance should be enhanced to enable release of common bacterial blight resistant/tolerant varieties to farmers.

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