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Effect of Seed Potato Tuber Storage Methods on Occurrence of Potato Diseases

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Abstract **– Many small holder farmers recycle farm saved seed potato and store the seed tubers under sub-optimal conditions. This leads to accumulation and spread of tuber-borne diseases in storage and in the field. This study was carried out with the objective of determining effect of seed potato storage methods on occurrence of diseases and tuber yield. Storage methods evaluated were diffused light, heap, jute bag, dark storage and treatment of seed tubers with gibberellic acid (GA_3). The seed tubers were stored for three months after which the tubers were analysed for bacterial wilt and viruses by NCM-ELISA and DAS ELISA, respectively. The tubers were then planted in the field for evaluation of occurrence of tuber-borne diseases. Certified seed tubers were included in the field trials as standard check. Diseases evaluated in the field included bacterial wilt, late blight and viruses. Tubers stored in diffused light and in jute bags resulted in the highest incidence of bacterial wilt. After storage, there was 88.8% bacterial wilt infection in all treatments except in dark storage (100%). At harvest, highest bacterial wilt infection was 100% and 88.8% in symptomatic and non-symptomatic plants respectively. Storage conditions had no significant effect on concentration of potato viruses after storage and on prevalence of viruses in symptomatic and non-symptomatic leaves after planting. The storage methods significantly varied in infection of symptomatic and non-symptomatic leaves with PLRV, PVX, PVS and PVM at up to 100%, 55%, 22% and 44%, respectively. Only certified seed potato had significant effect on yield which was up to 36t/ha. Methods of storage had no significant effect on yield and diseases during plant growth and after ELISA tests. Non-symptomatic tissues tested positive due to latent infection and certified seed was the least affected. Farmers should use recommended methods of storage and certified seed potato for increased yields.**

Keywords **– Bacterial Wilt, Late Blight, Potato Viruses, *Solanum tuberosum* L.**

I. INTRODUCTION

Potato (*Solanum tuberosum* L.) is the second most important food security crop in Kenya after maize and its production is on an increase with increasing human population [1, 2]. Despite the high potential and increase in land area under cultivation, there is declining productivity of the crop [2, 3]. Limited supply of certified seed potato tubers is the main cause for reduction in yields among small scale farmers in Kenya since over 97% use seed potato from informal supply sources [4-6]. Due to inadequate supply of quality seed tubers, farmers use farm saved seed potato from the previous harvest which is recycled across seasons without renewal [4, 5, 7]. Farm saved seed potato is of inferior quality status since it is highly degenerated and contaminated with many seed borne diseases especially viruses [8, 7]. Major seed borne diseases such as bacterial wilt (*Ralstonia solanacearum*), potato late blight (*Phytophtora infestans*) and potato viruses are highly prevalent in farm saved seed [9-11].

Farmers select seed potato from the previous harvest which is stored at the farm level for the next planting season [4]. Due to limited availability of recommended storage structures such as cold storage and diffused light stores, most farmers in East Africa store seed potato tubers in poor storage conditions leading to seed quality and quantity losses [12]. Faster disease spread mainly bacterial wilt, *Fusarium* dry rot and storage pest attack are common in seed potato tubers stored under poor storage conditions [12, 13, 15]. The method and duration of seed potato storage affects the quality of tubers eventually affecting the performance of the crop [10]. Methods of storage used by farmers include gunny and polythene bags, heaps, delayed harvest, field pits and diffused light stores [4, 10, 12].

Storage conditions such as relative humidity, temperature and light affect weight of seed tubers, disease spread, water loss and sprouting [13, 15]. Most traditional methods of storage used by farmers can lead to over 50% seed quality and quantity losses [10]. Diffused light stores are low cost structures recommended for seed potato storage since there is maintenance of seed quality and there are reduced storage losses but few farmers have taken up this technology [10, 16]. Poor storage structures cause seed quality loss leading to poor crop performance since storage conditions cannot be regulated [17]. Poor storage structures also increases pest and disease attack both in stores and in the field thus lowering yields [14]. Disease accumulation is high in poor storage structures and its spread further in the field thus lowering seed quality and yields [12, 18]. This study, therefore, aimed at determining the effect of methods of storage on potato diseases and yield.

II. MATERIALS AND METHODS

A. Set up of Laboratory Experiment

Fresh mature seed potato tubers (variety Shangi) of uniform size, damage free and with diameter range of 25-35 mm were collected from a model farmer's field. Storage methods evaluated included diffused light store, open heap storage, open dark storage, jute storage and jute bags with a seed treatment of Gibberellic Acid (GA_3). Each treatment had 48 Kg of tubers in three replications of 16 kgs each.

Modified diffused light store was set up using crates and tubers were arranged in layers and covered with translucent polythene. Heaps were mounted in open away from disturbance while dark storage was set up at room temperature (23 ± 2ºC) in darkness. Gibberellic acid ($C_{19}H_{22}O_6$) was applied by dissolving 3g of the hormone in five litres of distilled water and the solution sprayed evenly on tubers and allowed to dry before storage. The experiment was laid in Completely Randomised Design (CRD) with storage methods as treatments each replicated three times. The seed potato tubers were stored for three months after which they were planted in the field at two sites.

B. Set up of the Field Experiment

Field experiments were established at the University of Nairobi Field Station farm and in a farmer's field in Tigoni, Kiambu County. The University Field Station lies in Lower Highland Zone II while Tigoni is in Lower Highland Zone I [19]. The seed potato tubers from the storage experiment were planted in plots measuring 4.5M by 3M with spacing of 75 cm between rows and 30 cm within rows (NPCK, 2013). Certified seed was introduced as a positive check. Each treatment was replicated thrice and laid in a Randomised Complete Block Design (RCBD). A guard row consisting of potato of the same variety was planted around the experimental plots. Di-Ammonium Phosphate (46% P_2O_5, 18% N) fertilizer was applied at a rate of 300 Kg/Ha [20] and standard agronomic practices including weeding, and pest control were carried out as when necessary [20]. Data collected included incidence of bacterial wilt, late blight, virus infection, tuber yield, incidence of bacterial wilt and virus infection in the harvested tubers.

C. Assessment of Disease Incidence and Tuber Yield

Incidence of bacterial wilt, late blight and virus was determined on weekly basis commencing four weeks after planting until maturity. Symptoms observed during disease assessment were wilting for bacterial wilt, irregularly water soaked brown lesions on leaves for late blight while yellowing, leaf curling, stunted growth and leaf rolling for viruses [5, 11, 21]. Disease incidence was assessed by counting the number of plants showing characteristic disease symptoms in each plot and the percent incidence calculated as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants per plot}} \times 100$$

Three plants with bacterial wilt and virus infection symptoms and three non-symptomatic plants in each plot were tagged. Two leaves from top, middle and bottom of each tagged plant were plucked off and placed in labelled self-lock transparent sampling bags and stored overnight in a refrigerator before analysis for virus infection. Tubers from each of the tagged plants were harvested separately for *R. solanacearum* detection. At physiological maturity the aboveground biomass (haulm) were cut off and harvesting was done two weeks later. At harvest, the tubers were separated into four grades based on size as follows: chatts (<20 mm), small (20-25 mm), medium (25-35 mm) and large (>35 mm) [22]. The weight of each grade was determined and the total yield for each treatment was computed in tonnes per hectare.

D. Detection of Bacterial wilt in Potato Tubers

Bacterial wilt infection in tubers was detected by Enzyme Linked Immunosorbent Assay on Nitrocellulose Membrane (NCM ELISA) described by Priou [23]. The NCM ELISA kits were sourced from the International Potato Centre (CIP), Lima, Peru. Three tubers from each replicate of each treatment were tested. The tubers were washed in tap water and dipped in 1% NaOCl for one minute. The stolon end of each tuber was cut a transversally and the vascular ring scooped out and crushed in a crushing buffer. The extract was enriched overnight by adding 500µl of enrichment broth. The extracts were loaded alongside positive and negative controls on nitrocellulose membranes soaked in a buffer solution and allowed to dry. Blocking solution added and allowed to react for an hour with continuous agitation. Binding of *R. solanacearum* antibodies were done by soaking the membranes in an antibody solution for two hours with continuous agitation. Membranes were soaked in a solution with conjugated antibodies for an hour and washed three times using a washing buffer. Colour development was done by adding colour development solution and reaction allowed to run for 30 minutes with formation of purple colour in samples similar to the positive controls. Data was collected on samples testing positive for *R. solanacearum*.

E. Detection of Potato Viruses in Tubers and Leaves

Detection of potato viruses in tubers and leaves was done using Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS ELISA) as described by Clark and Adams [24] and Priou [25]. The DAS ELISA kit was sourced from International Potato Centre (CIP) Lima, Peru (Priou, 2001b). The DAS-ELISA kit can detect six potato viruses: Potato Leaf Roll Virus (PLRV), Potato Virus A (PVA), Potato Virus M (PVM), Potato Virus S (PVS), Potato Virus X (PVX) and Potato Virus Y (PVY). The tubers were allowed to sprout before analysis for virus infection. For each virus, 20µl of antibody were dissolved in 20µl of coating buffer, loaded on microtiter plates and incubated for four hours. The leaf and sprout samples were crushed in a crushing buffer and this extract loaded on washed coated plates. Three healthy controls specific to each virus were filled in the last three wells of each plate and loaded plates were incubated overnight. Plates were washed three times in a washing buffer and conjugate solution added followed by incubation for five hours. Washing was done thrice and substrate solution added until development of yellow colour with healthy controls remaining clear. Concentration and interpretation of virus infection calculated using the formula:

$$x \geq \bar{x}h \times 2$$

Where x is threshold value of the samples and $\bar{x}h$ is the average values of healthy controls.

F. Statistical Data Analysis

Data on incidence of potato late blight, incidence and percentage infection with of bacterial wilt and viruses after ELISA, and yield was subjected to analysis of variance using GENSTAT© statistical package. Mean separation done using Fisher's protected Least Significant Difference at 5% level of significance [26].

III. RESULTS

A. Effect of Methods of Storage on Bacterial Wilt

Storage methods had a significant effect on incidence of bacterial wilt in both sites. Seed tubers stored under diffused light and in jute bags resulted in the highest infection levels with bacterial wilt while the certified seed tubers resulted in the least bacterial wilt incidence (Table I). Crop planted at Kabete had higher levels of infection compared to the crop at Tigoni. Seed tubers stored in heaps, in the dark and treated with gibberellic acid had significantly lower bacterial wilt incidences. There were no significant differences in bacterial wilt incidence among the storage methods at the fifth and seventh weeks after planting at Kabete. Bacterial wilt was detected in significantly higher number of tubers harvested from plots planted with seed tubers stored in the different methods compared to plots planted with certified tubers and treated with gibberellic acid (Table II). Bacterial wilt was detected in up to 100% of tubers under various methods of storage compared to 55% in the tubers harvested from plots planted with certified seed tubers. Tubers harvested from symptomatic and non-symptomatic plants had similar percentage mean infection in both sites. The differences in the percentage of tubers infected with bacterial wilt were not consistent among the different storage methods for both symptomatic and non-symptomatic plants at both sites. However, tubers harvested from the potato crop planted at Kabete had up to 66% and 44.5% higher infection with bacterial wilt in symptomatic and non-symptomatic plants respectively (Table II). Tubers treated with gibberellic acid resulted in the highest infection levels in tubers harvested from symptomatic plants at Tigoni (Table II).

Table I: Percentage incidence of bacterial wilt at various weeks of plant growth at two sites in a potato crop established from seed potato tubers stored under different methods of storage

	Weeks after planting					
	4	5	6	7	8	Mean (%)
Kabete						
Diffused light	3.0b	6.0a	10.5a	9.4a	3.0ab	6.3b
Heap storage	2.4b	2.9ab	3.3cd	4.8b	0.9b	2.8e
Dark storage	3.2b	4.6ab	5.8bc	3.4b	1.8ab	3.8d
Jute storage	13.6a	8.1a	9.7ab	1.9b	5.8a	7.8a
Gibberellic acid (GA_3)	7.1ab	5.2a	6.0bc	1.8b	3.6ab	4.7c
Certified seed	0.0b	0.0b	0.5d	1.5b	0.0b	0.4f
Mean	4.9	2.5	6.0	3.8	2.9	4.3
P(0≤0.05)	7.1	4.8	3.8	3.6	4.2	0.14
CV (%)	80.1	58.7	35.4	52.1	90.9	1.9
Tigoni						
Diffused light	7.5ab	2.8a	5.3a	4.7a	3.0ab	4.6b
Heap storage	4.3c	1.4a	5.6a	5.8a	2.0ab	3.8d
Dark storage	4.9bc	0.0a	2.1ab	3.8a	0.6ab	2.2e
Jute storage	8.4a	2.3a	6.1a	5.5a	1.7ab	4.8a
Gibberellic acid (GA_3)	5.8abc	2.8a	2.7ab	6.4a	3.2a	4.1c
Certified seed	0.0d	0.0a	0.0b	0.0a	0.0b	0.0f
Mean	5.2	1.6	6.6	4.4	1.7	3.2
LSD (P0≤0.05)	1.1	3.1	4.4	5.8	2.8	0.07
CV (%)	28.2	10.71	66	72.8	87.1	1.3

Means in the same column followed by the same letter are not significantly different at P ≤0.05.

Table II: Percentage bacterial wilt infection in tubers harvested from symptomatic and non-symptomatic plants at two sites in potato crop established from seed tubers stored under different storage methods

	Kabete		Tigoni	
Method of storage	Symptomatic tubers	Non symptomatic tubers	Symptomatic tubers	Non symptomatic tubers
Diffused light	100.0a	100.0a	56.1c	44.7d
Heap storage	100.0a	100.0a	54.5cd	88.8a
Dark storage	100.0a	77.7b	53.0d	55.5c
Jute storage	100.0a	66.5c	66.6b	66.6b
Gibberellic Acid (GA_3)	77.0b	100.0a	88.8a	55.4c
Certified seed	44.0c	55.5d	44.4e	44.4d
Mean	86.8	83.2	60.5	59.2
LSD (P0≤0.05)	1.3	1.3	2.1	1.6
CV(%)	0.8	0.9	1.9	1.6

Means in the same column followed by the same letter are not significantly different at P ≤0.05.

B. Effect of Methods of Storage on Late Blight of Potato

Significant differences among the storage methods on the incidence of late blight were only observed in Kabete (Table III). However, there were significant differences among the storage methods in the mean late blight incidence in both sites. Crop from certified seed tubers and tubers treated with gibberellic acid showed significantly lower incidence of late blight. There was higher up to 7.7% higher late blight infection in Tigoni compared to Kabete (Table III).

Table III: Percentage incidence of late blight at various weeks of plant growth at two sites in a potato crop established from seed potato stored under different methods of storage

Kabete	Time in weeks after planting				
Method of storage	8	9	10	11	Mean (%)
Diffused light	17.0a	22.7a	21.5a	27.3a	22.1a
Heap storage	13.8ab	20.1a	21.6a	27.0a	20.6b
Dark storage	19.1a	20.8a	19.5a	21.5ab	20.2c
Jute storage	14.2ab	19.5ab	22.0a	25.3a	20.2c
Gibberellic acid (GA_3)	12.9ab	17.9ab	21.3a	24.1a	19.0d
Certified seed	7.6b	12.1b	8.5b	10.4b	9.6e
Mean	14.1	19.1	19.1	22.7	18.6
P(0≤0.05)	7.6	7.1	7.0	11.2	0.19
CV (%)	29.7	20.5	20.2	27.2	0.6
Tigoni					
Diffused light	5.7a	23.7a	44.2a	44.1a	29.4b
Heap storage	4.8a	23.5a	38.0a	53.3a	29.9a
Dark storage	7.0a	21.4a	26.5a	40.8a	23.9d
Jute storage	3.8a	28.4a	42.7a	40.1a	28.7c
Gibberellic acid (GA_3)	0.9a	21.8a	30.1a	41.2a	23.5e
Certified seed	0.0a	14.7a	31.9a	44.1a	22.6f
Mean	3.7	22.3	35.6	43.8	26.3
LSD (P0≤0.05)	6.6	18.3	20.5	19.6	0.19
CV (%)	98.1	45.2	31.3	24.7	0.4

Means in the same column followed by the same letter are not significantly different at P ≤0.05

C. Effect of Methods of Storage on Potato Viruses

The seed tubers from different methods of storage resulted in plants showing significantly different incidences of virus infection (Table IV). Tubers stored in heap storage resulted in the highest incidence of virus infection in both sites while tubers treated with gibberellic acid resulted in the least virus infection incidence. Heap storage had the highest and significant effect on incidence in both sites which was up to 3.6% and 7.9% more than the incidence observed in certified seed potato in Kabete and Tigoni respectively. Potato viruses were detected in leaves from both symptomatic and non-symptomatic plants. The potato viruses detected were PLRV, PVM, PVS and PVX (Tables V and VI). However, only PLRV, PVX and PVS were detected in symptomatic leaves. There was no consistent pattern in incidences of the detected viruses among the different seed tuber storage methods and between the two experimental sites. Higher virus infection incidences of up to 100% were detected in the symptomatic in leaves from the symptomatic plants than the non-symptomatic ones. Viruses PLRV and PVS were only detected in plants from seed tubers stored under diffused light. There were no significant differences among treatments on infection of PVS and PVX in Tigoni in symptomatic leaves in Tigoni. Diffused light storage had the highest proportion of samples infected with PLRV in Tigoni unlike certified seed and jute storage which had no infected samples. There was higher prevalence of PLRV in Kabete unlike PVX in Tigoni (Table VI).

Table IV: Incidences of potato viruses at various weeks of plant growth at two sites in a potato crop established from seed tubers stored under different methods of storage

	Kabete					Tigoni				
	Weeks after planting					Weeks after planting				
Storage method	8	9	10	11	Mean	8	9	10	11	Mean
Diffused light	6.6a	11.8a	18.4bc	17.7b	13.6d	5.2b	6.5b	16.2bc	23.4abc	12.8c
Heap storage	9.5a	18.5a	21.6ab	22.1ab	17.9a	9.6a	23.5a	24.0a	33.6a	22.6a
Dark storage	8.9a	13.6a	17.7bc	21.7ab	15.4c	3.0c	10.3b	19.4ab	26.8ab	14.8b
Jute storage	4.9a	17.0a	23.9a	23.3a	17.2b	4.3bc	12.5b	15.4bc	26.7abc	14.7b
Gibberellic acid	6.0a	10.7a	18.4bc	17.7b	13.2e	4.8bc	8.2b	9.6cd	19.4bc	10.5d
Certified seed	7.1a	12.4a	13.5c	9.8c	10.7f	3.1c	6.7b	8.6d	12.6c	7.7e
Mean	7.2	14.1	18.9	18.7	14.6	5.0	11.3	15.5	23.8	13.8
P (0≤0.05)	4.7	9.5	4.8	4.7	0.18	1.8	8.5	6.5	10.5	0.19
CV (%)	36.1	37.4	14.1	13.8	0.7	20.1	41.5	22.9	24.4	0.8

Means in the same column followed by the same letter are not significantly different at P ≤0.05.

Table V: Percentage incidences of different potato viruses in leaves harvested from non-symptomatic plants at two sites in seed potato tubers stored under different methods of storage

	Kabete			Tigoni			
Storage method	PLRV	PVS	PVX	PVM	PLRV	PVS	PVX
Diffused light	11.1a	22.2a	22.2b	0.0b	11.1a	0.0a	66.6a
Heap storage	0.0a	0.0a	44.3b	11.1b	0.0a	0.0a	100.0a
Dark storage	0.0a	0.0a	66.6ab	33.3ab	0.0a	0.0a	11.1b
Jute storage	0.0a	0.0a	33.3b	44.3a	0.0a	0.0a	0.0b
Gibberellic acid (GA_3)	0.0a	0.0a	77.7ab	10.9b	0.0a	0.0a	0.0b
Certified seed	0.0a	0.0a	100.0a	0.0b	0.0a	11.1a	0.0b
Mean	1.8	3.7	57.4	16.6	1.8	1.8	29.6
P(0≤0.05)	14.8	14.2	51.5	31.2	14.8	14.2	47.8
CV (%)	424.1	212.1	49.4	103.2	424.1	423.1	88.7

Means in the same column followed by the same letter are not significantly different at P ≤0.05. PLRV=Potato Leaf Roll Virus, PVM= Potato Virus M, PVS=Potato Virus S, PVX=Potato Virus X.

Table VI: Percentage incidences of different potato viruses in leaves harvested from symptomatic plants at two sites in seed potato tubers stored under different methods of storage

	Kabete		Tigoni		
Storage method	PLRV	PVX	PLRV	PVS	PVX
Diffused light	100.0a	55.3a	100.0a	0.0a	100.0a
Heap storage	55.5b	55.5a	77.6a	0.0a	100.0a
Dark storage	33.3bcd	44.4ab	22.2a	11.1a	100.0a
Jute storage	22.2cd	0.0c	0.0b	0.0a	100.0a
Gibberellic acid (GA_3)	11.1d	22.2bc	11.1b	0.0a	100.0a
Certified seed	44.4bc	11.1a	0.0b	11.1a	88.9a
Mean	44.4	31.5	35.2	3.7	98.2
P(0≤0.05)	29.3	28.6	26.3	18.0	14.2
CV (%)	36.2	50.0	41.2	268.3	8.0

Means in the same column followed by the same letter are not significantly different at P ≤0.05. PLRV = Potato Leaf Roll Virus, PVS = Potato Virus S, PVX = Potato Virus X.

D. Effect of Methods of Storage on Tuber Yield

Certified seed tubers resulted in significantly higher yield of all the three tuber grades (Table VII). There were no significant differences among storage methods in mean tuber yield for crop planted at Tigoni. However, at Kabete significantly lower mean tuber yield was harvested from plots planted with tubers stored under diffused light and in jute bags. Significant differences among the yield for all tuber grades were observed only on crop planted at Kabete. There were no significant differences among the storage methods for the medium grade tubers from potato crop planted at Tigoni (Table VII).

Table VII: Yield of potato (T/Ha) at two sites from seed potato stored under different methods of storage

Kabete	Grades of potato				
Method of storage	Chatts	Small	Medium	Large	Total
Diffused light storage	2.2a	1.5b	9.5d	1.2b	14.7c
Heap storage	0.4b	1.9ab	17.4b	2.2b	21.1b
Dark storage	1.6a	1.4b	15.9bc	3.5ab	22.5b
Jute storage	1.9a	1.1b	12.4cd	1.2b	16.7bc
Gibberellic acid (GA_3)	1.9a	1.4b	14.5bc	3.4ab	21.3b
Certified seed	0.3b	2.6a	28.0a	5.0a	36.0a
Mean	1.0	1.7	16.1	2.8	22.8
LSD(P≤0.05)	1.4	0.7	4.2	2.4	5.4
CV (%)	41.8	24.2	14.3	47.8	13.5
Tigoni					
Diffused light storage	2.7a	2.8ab	8.5b	0.5a	14.6b
Heap storage	2.4a	3.0ab	9.9b	0.3ab	15.3b
Dark storage	1.4a	3.9a	10.0b	0.3ab	15.7b
Jute storage	2.4a	2.7ab	9.5b	0.07ab	14.9b
Gibberellic acid (GA_3)	1.5a	2.0b	10.2b	0.2ab	14.1b
Certified seed	2.3a	3.9a	17.8a	0.6a	24.8a
Mean	2.2	3.1	11.0	0.3	16.6
LSD(P≤0.05)	1.7	1.3	4.5	0.5	6.3
CV (%)	42.8	23.8	22.8	53.9	20.8

Means in the same column followed by the same letter are not significantly different at P ≤0.05

IV. DISCUSSION

Seed tubers from diffused light storage had the highest bacterial wilt disease incidence unlike certified seed potato. These results agree with the findings of Hirpa et al.[10] who reported increased disease infection under diffused light storage and the success of the this storage structure depended on sources of the seed tubers and the variety. In addition similar observations were made by Mulatu et al.[12], Elphinstone [18]and Wustman [13] who reported increased disease build up both in storage and in the field due to use of poor storage methods. Studies in India showed lower bacterial wilt infection levels in fields established using certified seed potato unlike those established from informally sourced seed tubers as also revealed in this study [27]. The higher incidence of bacterial wilt disease in all seed storage methods unlike certified seed could be attributed to latent infections in tubers before storage which led to pathogen accumulation during storage and faster spread in the field [11, 18]. Bacterial wilt pathogen (*Ralstonia solanacearum*) is seed borne and it is carried across cropping seasons in tubers [28-30]. Some of the other possible sources of bacterial wilt inocula that could have led to increased field spread include presence of alternate hosts, favourable climate at experimental sites predisposing the crop to pathogen attack and infected plant debris. Since farm saved tubers were used in the setup of storage experiment, latent infection with bacterial wilt in stored seed tubers could have acted as sources of inoculum inducing secondary infection in plots planted with certified seed through handling, tools and runoff water [31]. Small experimental plots such as the ones used in this study do not guarantee field hygiene that minimizes spread of bacterial wilt inocula which is common among small scale potato farmers [1]. Studies have shown that high infection levels with bacterial wilt are due to use of seed tubers latently infected [9, 31, 32]. Treating seed tubers with gibberellic acid did not reduce disease prevalence in the field. Pre-planting seed treatments have been shown to only improve vigour unlike disease resistance [33]. High prevalence of bacterial wilt in Kabete was probably due to favourable environmental conditions at the site predisposing the crop to *R. solanacearum* attack. Studies by Srinivasamurthy et al. [34] revealed high bacterial wilt infection in areas with high temperatures (24-36ºC) and moisture of 50-00%. This is because high temperatures promote survival, reproduction, infectivity and spread of the pathogen [29]. In addition, the pathogen is highly adapted under favourable tropical climate and can survive in deep soils with low microbial competition [29]. High prevalence of bacterial wilt can cause total crop loss due to faster pathogen spread in soil and infected tubers [12, 28, 35]. Poor storage conditions leads to accumulation of pathogens causing seed rot which further spreads in the field upon planting [18]. Apart from yield reduction, bacterial wilt is of economic importance in seed certification schemes and trade since there are zero tolerance levels to the disease [36].

At harvest, methods of storage had a highly significant effect on percentage number of tubers infected with bacterial wilt except in symptomatic plants in Kabete. Certified seed had the least infection among the treatments in both sites and bacterial wilt infection was higher in Kabete compared to Tigoni in both symptomatic and non-symptomatic tubers. Serological results from NCM ELISA revealed high percentage of positive samples compared to the visual incidence observed. Some tubers from non-symptomatic plants tested positive for the disease. These results agree with findings by Sagaret al. [28] who reported high bacterial wilt infection levels in India and lower incidences were reported in farms established using quality seed potato. In addition, studies by Bekele et al. [11] revealed high bacterial wilt incidences in Ethiopia after detection through post-enrichment NCM ELISA like the one used in this study. Studies by Gildemacher et al. [5, 32] revealed high disease incidence including that of bacterial wilt in Kenyan potato farms. High prevalence of bacterial wilt has also been reported in Ethiopia among small-scale potato farmers [37, 38]. However, Mwangi et al. [9] reported comparatively lower bacterial wilt infection levels after detection in stems. Despite certified seed having negligible incidence based on observation of visual symptoms in the field, ELISA results revealed positive samples for *R. solanacearum* hence might have got infected in the field during plant growth. Bacterial wilt infection can occur in the field since the pathogen is also soil borne [18, 28, 39]. Additional sources of viable *R. solanacearum* inocolum include infected runoff water, tools and volunteer plants that act as alternate hosts [29]. Seed tubers from non-symptomatic plants tested positive for the disease due to latent infection. Muthoni and Nyamongo [1] also revealed that latently infected seed potato tubers are sources of bacterial wilt inoculum among small scale potato farmers in Kenya. Latent infections pauses a threat in use of quality seed tubers since most farmers in developing countries use informal seed supply channels and tubers are selected based on visual appearance which does not take into account for latent infection [10]. This is worsened by poor storage conditions that lead to pathogen accumulation and spread [12, 18]. Even though some of the symptomatic tubers tested negative for the *R. solanacearum,* Bekele et al. [11] suggested that negative results in symptomatic tissues can be attributed to low bacterium concentrations in tubers extracts below the detection limit by post enrichment NCM ELISA. Due to presence of other biotic and abiotic factors causing wilt like symptoms similar to those of bacterial wilt in the field, some symptomatic tubers might have not been really infected with *R. solanacearum* even though they showed disease symptoms in the field [11]. Poor farming practices such as irregular crop rotation patterns, poor field sanitation and excessive application of salty fertilizers have been reported to aggravate the spread and accumulation of *R. solanacearum* in the soil [9, 29, 39, 40]. Bacterial wilt can cause total yield loss and leads to production of poor quality tubers [21, 31]. In Kenya and most European countries, *R.solanacearum* is treated as a quarantine pest with zero tolerance in seed certification schemes [18, 21, 41].

The study showed that methods of storage did not have significant effect on incidence of potato late blight in the field. There was an increase in late blight infection in all

treatments during the period of evaluation and the incidence was higher in Tigoni compared to Kabete. High late blight disease incidence and severity was also reported from studies conducted in India by Lal et al. [42] despite application of different fungicides. These results also concur with the findings of Fry [43] who reported high late blight infection in cooler and areas with high relative humidity. In addition, Agajie et al. [44] reported high late blight infection in potato sourced from informal supply channels in Ethiopia. Studies by Ayalew [33] revealed that dormancy breaking seed treatments such as gibberellic acid do not reduce disease infection but just improve crop vigour hence treated seed tubers were affected by the disease in this study. High prevalence of late blight in Tigoni compared to Kabete could be due to favourable environmental conditions at the site mainly lower temperatures of 15 to 25ºC and high humidity that predisposed the crop to *Phytopthora infestans* infection since these conditions that are favourable for pathogen reproduction and dispersal [43]. Late blight pathogen (*P. infestans*) produces sporangia asexually which are dispersed by wind and water while oospores are produced sexually and have longer survival rates in the soil lasting many cropping seasons leading to faster disease build up and spread [45]. Certified seed potato was therefore, probably infected with the disease inoculum present in the soil, plant debris and volunteer plants [43, 46]. Infected seed tubers in poor storage structures are also major source for increased spread of late blight leading to reduction in seed quality and yield loss [32, 46]. Late blight of potato is of phytosanitary importance in seed certification schemes and trade [21, 30].

Only certified seed had significant lower incidence of potato viruses in the field and there was higher virus infection in Kabete compared to Tigoni. These results are contrary to the findings by Njukeng et al. [47] who reported significant effect of different potato stores and tubers sizes on prevalence of potato viruses in Cameroun. In addition, Gunadi et al. [48] reported significant effect of seed generations on incidence of viruses in the field. Low infection with viruses in certified seed potato was due to the seed being virus free at planting or had low levels of latent infection but got infected in the field. The generation of seed tubers influences virus infection since highly degenerated tubers such as farm saved seed have higher viral loads [8]. Potato viruses are by spread by vectors such as aphids and latently infected tubers hence these could have led to infection of certified seed [48]. In addition, stored seed tubers may be infested by aphids on sprouts during storage [50]. Potato crops established from degenerated tubers are the most susceptible to aphid infestation [7, 8, 51]. Viruses increase degeneration of seed tubers and under favourable tropical climate for potato pests, disease pressure of viral diseases high [52]. Volunteer crops and other *Solanacea* plants are alternate hosts that harbour viruses and become sources of inoculum for fresh infections. High infection levels with viruses leads to seed tuber degeneration, production of small and deformed tubers and eventually low yields [5, 21].

Methods of storage had no significant effect on proportion of seed tuber samples infected with viruses in both symptomatic and non-symptomatic leaves. Only PLRV, PVS, PVX and PVM were detected in leaves. Potato Virus X was the most dominant virus detected followed by PLRV, with the highest infection under diffused light storage. These results concur with the findings by Abbas and Hameed [53] who reported PLRV and PVX as the most prevalent potato viruses in Pakistan. In addition despite a higher visual incidence of virus like symptoms in the field, not all viruses were detected. In addition, similar findings were reported in Ethiopia in which PVA was not detected in leaves despite collection of samples from symptomatic plants [11]. Chandra-Sarker et al. [54] also reported positive samples for PVY and PLRV in certified seed potato tubers as revealed in this study. However, Njukeng et al. [47] and Gunadi et al. [48] reported significant effect of seed stores and generations on incidence of viruses unlike no significant effect in this study. Prevalence of viruses in leaves could be due to use of latently infected tubers before storage and infestation by aphid pests during storage [54, 55]. Use of contaminated seed tubers, presence of vectors and volunteer plants cause high virus infection with observable symptoms on leaves [47, 51]. Some of the non-symptomatic leaves tested positive due to latent infection while symptomatic ones tested negative despite showing virus symptoms suggesting presence of virus like disease symptoms that could not be detected by the antibodies used in this study [11]. Negative results obtained through DAS ELISA cannot be used to exhaustively conclude that plants were free from virus infection with other viruses such as PVY [56]. Low concentration of viruses has been reported to reduce the detection efficiency through DAS ELISA necessitating use of molecular markers and other serological techniques alternative to ELISA especially in seed certification schemes [56-58]. Infection of potato crop with viruses can lead to total yield loss, with PLRV, PVS and PVX being the among the most lethal ones [7, 59]. Even though viruses may lead to yield loss, production of infected and deformed tubers due to virus attack leads to rejection of certification of the seed crop [21].

Methods of seed tuber storage had no significant effect on potato grades and only certified seed had significant effect on total yield. Medium sized tubers were the largest proportion of harvested grades and certified seed had the highest yield in both sites. Contrary observations were made by Rojoni et al. [60] who reported significant effect of seed tuber sizes on yield of potato unlike no significant effect revealed in this study. Studies by Njukeng et al. [47] showed that due to high virus concentration in farm saved seed tubers, the yield obtained was of small to medium sized ware potato. High proportion of chatts could be attributed to virus infection in stored farm saved seed potato [5, 8, 47]. Certified seed potato produced the highest yield in both sites due to low infection with disease during plant growth [27]. The results of the present study agree with findings by Yadavi et al. [61] who reported 39.8t/ha as the highest yield attained resulting from different cropping densities and emergence of pigweed. In addition, Das et al. [62] reported 25.7t/ha obtained when new potato varieties

were treated with different nitrogen levels. The non-significant effect of methods of storage on yield was due to use of the same source and variety of seed potato tubers in storage. Infection with diseases such as late blight lowers yield because 40-80% yield reduction can be incurred [63, 64] while bacterial wilt of potato can cause total yield loss [30]. Despite yield reduction, seed borne disease such as bacterial wilt, late blight and viruses lower seed quality and this limits the use of the crop to ware potato only unlike being used as seed tubers [8, 29, 30].

V. Conclusion and Recommendations

Methods of storage had no significant effect on disease infection in the field during plant growth and also on total yield. Non-symptomatic tissues tested positive for bacterial wilt and viruses indicating latent infection with seed borne disease causing pathogens. Therefore, to manage seed tuber transmitted diseases, farmers should be encouraged to use certified seed potato and appropriate methods of seed tuber storage. The use of clean, disease-free certified seed potato tubers will curb the accumulation and spread of the disease-causing pathogens during storage and in the field.

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