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Research paper

Slow mildewing in Pumpkins: an opportunity to reduce Pumpkin yield losses caused by *Erysiphe cichoracearum*

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Abstract

Pumpkin powdery mildews, a disease caused by fungus *Erysiphe cichoracearum*, is one of the major diseases that reduce yield and quality of pumpkins. A field screening study involving fifty two pumpkin accessions was carried out at three powdery mildew hot spot sites in Malawi. The main objective was to assess the reactions of pumpkin accessions to *E. cichoracearum*. The experiment was laid out in a randomized complete block design replicated three times. Plot size was 3m x 3. 7m. There were statistically significant differences in apparent infection rates (P<0.001), area under disease progress curve, AUDPC (P<0.001), percent disease index, PDI (P<0.001) among the accessions across all sites. Pumpkin accessions 6 and 42 consistently showed slow rate of powdery mildew development at 4^{th}, 6^{th}, and 8^{th} week after germination. A strong positive correlation (R= 0.7 at 8^{th} week, and 0.97 at 12^{th} week after germination) was observed between the number of rotten fruits and AUDPC. This study showed that out of the fifty two pumpkin accessions tested, none was immune to *Erysiphe cichoracearum*. However the accessions that recorded very slow rate of powdery mildew development showed high potential to maintain viable vines and support their fruits up to physiological maturity. We concluded that promoting slow mildewing pumpkin genotypes is appropriate solution to fruit yield and quality losses caused by *E. cichoracearum*.

Keywords: *Accession; Powdery Mildew; Pumpkin; Erysiphe Cichoracearum; Screening; Malawi.*

1. Introduction

Pumpkin (*Cucurbita maxima L.*) belongs to family *Cucurbitaceae*, tribe *Cucurbitanae* and Genus *Cucurbita* and has about 27 species all of which originated from North, Central and South America [23]. The cultivated *cucurbita* include five main species: *Cucurbita moschata* (Butternut squash) *Cucurbita maxima* (pumpkin), *Cucurbita mixta* (Winter squash), *Cucurbita pepo* (summer squash) and *Cucurbita ficifolia* (guard). All these five species are widespread throughout the tropical, subtropical and milder temperate regions [13]. In Malawi, the most widely grown are *C. moschata* and *C. maxima* [16]. Pumpkins are mainly grown for their leaves as a vegetable and fruit as food supplement.

Despite being widely grown throughout Malawi, pumpkin production is limited by a number of biotic stresses such as insects and diseases [26]. Pumpkin diseases include powdery mildews (*Erysiphe Cichoracearum*), root knot (*Meloidogyne species*) [21]; bacterial wilts, cucumber mosaic (cucumber mosaic virus), water melon mosaic virus (WMV-2), Zucchini yellow mosaic (ZYMV) and Squash leaf curl (SLCV) [5].

Powdery mildews or *Erysiphaceae* are those fungi with white hyphae and colorless one-celled ascospores born in asci enclosed in black cleistothecia on surface of living plants (perfect or sexual stage) or those fungi with white superficial hyphae on aerial parts of living plants with large one-celled conidia produced terminally on isolated aerial unbranched conidiophores and with haustoria in epidermal cells of their hosts [9].

Pumpkin Powdery mildews occur globally in both green houses and in the field [4]. This fungus attacks leaves, vines, flower buds and fruit stalk [6]. Under severe attack, the disease may cause untimely death of leaves and vines; otherwise, the most striking aspect of disease loss is a reduction in leaf and fruit quality.

The widely used powdery mildew control measures include use of sulphur based fungicides at field application rate of 4. 5kg elemental sulphur per hectare applied when the fungus is first observed [10] and use of resistant varieties [29]. This study was therefore done to identify genotypes that are resistant to cucurbit powdery mildews.

2. Materials and Methods

2.1. Sites description

The field study was done both under rain fed and sprinkler irrigation conditions. Sites under rain fed conditions were: Chitekwere, south east of Lilongwe in Central Malawi; mean daily temperatures (^{0}C) of 21, 21,21 and 19 for cropping months of January, February, March and April, respectively; mean daily relative humidity, RH (%) of 82, 85, 80 and 77 and daily mean rain fall (mm) of 10, 12, 5 and 1.2 for cropping months of January, February, March and April, respectively and Enfeni in the North; mean daily temperatures (^{0}C) of 20, 20, 20 and 20 and mean daily RH (%) of 83, 84,83 and 79 and mean daily rain fall (mm) of 6, 5.5, 2and 1.3 for cropping months of January, February, March and April, respectively. The conditions at Bunda where the trial was done under sprinkler irrigation: daily mean temperatures (^{0}C) were 19, 22, 23 and 22 and daily mean RH (%) of 55, 51, 60 and 77 for the months of September, October, November and December respectively.

2.2. Experimental design

Fifty two pumpkin accessions, sourced from Chitedze Gene Bank were used as test host plants. These were collected from eleven districts of Malawi namely Chitipa, Likoma, Nkhata Bay and Mzimba in the North, Nkhotakota, Dedza and Ntcheu in the Centre and Nsanje, Chiradzulu, Zomba and Machinga in the South. The experiment was laid out in a randomized complete block design replicated three times. The total land size on each site was 0.4 hectare. Plot size was 3m x 3.7m.(11.1m^2). Each plot had three ridges of 3.7m long spaced at 1m apart. Three seeds per planting station were planted at 0.9m apart and a week after germination thinned to one seedling per station.

At all the field sites, basal dressing was done one week after germination using 89 kg D compound (10 : 24 : 20 : + 6S + 0.1B) fertilizer/ha. Thus the elemental basal application rates used were 8.65kg N/ha; 9.02kg P/ ha; 14.41kg K/ha; 5.19kg S/ha and 0.086kg B/ha. Two weeks later CAN (27% N) was applied as top dressing at the rate of 23.4kg N/ha [25]. First weeding was done four weeks after planting using a hand hoe followed by hand weeding four weeks later.

In all the field trials, pathogen introduction to the test plants was based on natural infestation. Scoring for powdery mildew severity started five weeks after germination on a 1-5 rating where 1 = no disease; 2 = light infection; 3 = moderate infection; 4 = heavy infestation and 5 = extremely severe and test pumpkin plants completely dry or dead. Eight test plants were randomly selected in each plot and tagged. On each test plant nine leaves: three from lower, middle and tip of the vine were rated for powdery mildew severity. To monitor disease progress over time, scoring was done at fortnightly interval. The disease rating scores were consistently assigned and mean disease severity was calculated arithmetically and converted to percentage of maximum disease score of 5. A total of four assessments were done at 4th week, 6th week, 8th week and 12th week after germination of test pumpkin plants. The disease severities recorded at these different time intervals were converted to area under disease progress curve (AUDPC) following procedure by Tooley and Grau, [18], as follows:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(X_{i+1} + X_i) / 2] [t_{i+1} - t_i]$$

Where

n = total number of observations, t_i = time (days after planting) at the ith observation, X_i = the cumulative disease incidence expressed as a proportion at the ith observation.

Apparent infection rate, r, (rate of progress of the disease based on proportional measures of extent of infection at different times), was determined by dividing mean AUDPC by total duration (days) under disease observation as described by Parry and David, [12], using the formula:

$$r = \frac{1}{t_2 - t_1} \log_e \left[\frac{x_2 (1 - x_1)}{x_1 (1 - x_2)}\right]$$

Where:

r is the apparent infection rate, t_1 is the time of the first measurement, t_2 is the time of the second measurement, x_1 is the proportion of infection measured at time t_1, x_2 is the proportion of infection measured at time t_2.

At 12th week after germination, peak percent disease index (PDI) was determined by converting the 1-5 point disease severity scores to PDI using formula by Wheeler, [22].

Data was analyzed on GenStat 15th Edition. Analytical tools used included general analysis of variance (ANOVA) for comparison of means, [24].

Correlation coefficient (R) was used for measuring the degree of relationships between the measured variants. Means for AUDPC and number of rotten fruits were subjected to GenStat in a pairwise approach to determine R values. The level of relationship was based on a -1 to +1 index scale where -1 = perfectly negative relationship and +1 = perfectly positive relationship. The correlations were used as identifiers of traits to be used as selection indices for responses to pathogen under study [14], [17].

3. Results and Discussion

3.1 Effect of accession on apparent infection rate (r) and area under disease progress curve (AUDPC)

There were highly significant differences across the three sites (P <0.001) in the overall site means of area under disease progress curve (AUDPC), measure used to evaluate disease progress rate in different crops. Bunda, where the trial was done under sprinkler irrigation conditions, registered the highest AUDPC value (Table 1). This shows that the environment under sprinkler irrigation at Bunda was more favourable for powdery mildew development and spread. This environment was characterized by low relative humidity ranging from 51% to 59% and average temperature ranging from 16^{o}C to 23^{o}C.

There were highly significant differences in apparent rates of infection, r, (P < 0.001) which tallied with AUDPC site means. Overall mean "r" value was highest at Bunda, (0.021) followed by Emfeni (0.021) and lowest at Chitekwere (0.018) (Table 3). The differences in site means of AUDPC and "r" values across sites were attributed to differences in environmental factors such as rainfall (Figure 4) temperature and relative humidity. Chitekwere registered higher mean daily rainfall figures with shorter intermittent dry spells of not more than one week during critical period of crop development in January, February and March (Fig.4) but the mean values of AUDPC and 'r' were lower than those of Emfeni where the mean daily rainfall figures were lower. This means that powdery mildew change in severity over time increased with decrease in amount of rainfall. These observations conform to those of Yarwood [27], who reported lower progress of powdery mildews under high rainfall conditions. The environment at Bunda site favoured powdery mildews most (Table 1). Relative humidity was low. District relative humidity means for Lilongwe were 55%, 51% and 60% for September, October and November, respectively and corresponding mean temperatures were 19^{0}C, 22^{0}C and 23^{0}C, respectively. Comparatively, relative humidity under rain fed conditions at Chitekwere and Emfeni were higher (January, February and March, 2010) than at Bunda (September, October and November, 2009).This conforms to observations by Yarwood [27] who reported that germination of powdery mildew pathogens favours dry conditions. Therefore, use of slow mildewing pumpkin land races has the potential to mitigate the effects of this disease on fruit/leaf yield and quality.

Analysis of final powdery mildew peak severity scores at 12th week after germination supported the trend of AUDPC mean values across sites. There were highly significant site differences in overall mean peak powdery mildew severity scores (P <0.001) across the three sites. Overall, mean powdery mildew severity was higher under dry and irrigated conditions at Bunda (4.364) than the two sites under rain fed conditions namely Chitekwere (3.370) and Emfeni (3.417) (Table 1). This means dry conditions under irrigation conditions provided most favourable conditions for disease spread and development. However, the difference in overall site means of peak powdery mildew severity for the two sites under rain fed conditions is largely attributed to differences in mean monthly rainfall figures i.e. higher mean site scores under lower rainfall at Emfeni than under comparatively higher rainfall at Chitekwere (Fig. 4).

Highly significant differences in means of apparent infection rate "r" were observed among the pumpkin accessions (P<0.001).

Comparatively lower means of “r” values were registered in accession 6 (0.015), 42 (0.010), 27(0.016), 8(0.016) and 43(0.015) the rest had their mean “r” values ranging from 0.023to 0.025. Accession 22 gave highest mean “r” value (0.025), (Table 3).

The mean “r” values, calculated with formula proposed by Van der Plank, [19] are widely used to detect partial resistance to powdery mildew pathogens. This form of resistance is also known as slow mildewing resistance, [15]; [1]; [2].The current study has shown that accessions 42, 27, 8, 43 and 6 are the slow mildewing genotypes. None of the pumpkin accessions tested showed complete resistance. Therefore, the slow mildewing pumpkin accessions can be promoted in the absence of complete resistance.

These findings are in conformity with those of Ashok and Mishira [1] who reported that five out of twelve pea cultivars studied showed slow mildewing trait characterized by longer latent period and reduced number and area of mildew lesions compared to highly susceptible ones characterized by relatively high “r” values.

Gupta [8] also studied pea powdery mildew and showed that slow mildewing cultivars had lower apparent infection rates. Ashok and Mishira [1] reported pea cultivars with slow mildewing characterized by retarded mycelial growth, smaller specks and longer latent period compared to fast mildewing ones.

3.1. Effect of host growth stage on powdery mildew development

There were significant differences in area under disease progress curve (AUDPC) among the pumpkin accessions at 4th week ($P<0.001$); 6th week ($P<0.001$) and 8th week ($P<0.001$) after germination. However, at 12th week, there were no significant differences ($P<0.214$) in mean AUDPC (Figure 1). The slow mildewing accessions registered comparatively lower AUDPC means at 4, 6 and 8 weeks after germination (Figure 1.). This means that the slow mildewing mechanisms in accessions 6 and 42 were demonstrated during the early stages of development.

At 4 weeks after planting, there was significant interaction between accession and site over AUDPC means ($P<0.001$). The interaction was, however, not significant at 6 weeks (P= 213) and 8 weeks (P= 1.00). This implies that site differences in disease progress at 4th week were partly due to differences to environmental factors. Positive but no significant correlations were observed between AUDPC at 4th week and 6th week (R = 0.34); AUDPC at 6th week and 8th week (R = 0.38) and a highly significant positive correlations were observed between AUDPC at 6th week and 12th week (R = 0.50); AUDPC at 8th week and 12th week (R = 0.94). These observations support the fact that the amount of initial inoculum determines extent of disease development over time. Giladi [7] showed that inoculum doses of 50, 100 and 200 conidia per leaf of chilli resulted in 12, 33 and 71 per cent, respectively, of ventral leaf area covered by the powdery mildew six days after inoculation. Some of the possible reasons contributing to the differences in disease progress under natural setting include availability, quantity and infectivity of initial inoculums and host susceptibility. The variation in susceptibility is also due to differences in physiological factors such as host age, level of susceptibility at an early age and microclimatic factors resulting from greater density of foliage in older crops. All these factors may also be of some importance for infection [11]. However, the most important observation from the current study is the slow rate of powdery mildew development as shown by accessions 6 and 42 during early growth stages at 4, 6 and 8 weeks after germination (Fig. 1).

3.2. Effect of pumpkin accession on peak powdery mildew severity (Per cent disease index, (PDI)

Peak per cent disease index (PDI) was recorded at 12th week after germination by following a 1-5 point scale. Further, the scales were converted to percent disease index (PDI) using formula given by wheeler [22].There were significant differences in mean peak PDI among the pumpkin accessions ($P<0.001$) and further, significant interaction ($P<0.001$) was observed between location and genotype over peak PDI. This implies that location had an influence on accession peak powdery mildew severity. Mean peak PDI across sites ranged from 41.1% to 67.2% among slow mildewing accessions. Accession 42 registered the lowest mean peak PDI (41.1%) at 12th week after germination.

Wide variation in PDI means across sites suggests that disease intensity depends on location factors such as rainfall (Figure 4), temperature and relative humidity. Lowest mean PDI value was registered at Chitekwere (23.34%) for accession 42, while the same genotype registered comparatively higher mean PDI at Emfeni (66.66%), (Table 2). The variation in peak mean PDI values may also indicate possible existence and role of physiological races of cucurbit powdery mildew pathogen or may be attributed to extreme dryness under irrigated conditions. These findings are in conformity with those of Yarwood [28], who reported role of physiological races in case of powdery mildews of different crops and the decline in powdery mildew prevalence with increase in rainfall and decline in sporulation of the powdery mildew fungi with increase in temperature above the optimum of around 21^{o}c. These results further indicate dependence of disease intensity on factors like cultural practices. For example, higher PDI range (33.34% to 96.66%) was registered under sprinkler irrigation conditions compared to the range of 23.34% to 78.34% under rain fed conditions. The higher peak PDI under irrigation conditions suggests that this environment was more favourable for powdery mildew. These results agree with earlier reports by Biju [3] in powdery mildew of peas and Venkatrao [20] in powdery mildew of green grams where comparatively higher PDI values were reported under relatively lower rainfall conditions than under higher rainfall conditions.

3.3. Effect of powdery mildew on fresh fruit yield

Highly significant differences were observed in fresh fruit yield ($P<0.001$) among the accessions at all sites. There was highly significant interaction between site and accession over fresh fruit yields ($P<0.001$). This implies that location contributed to differences in fresh fruit yield among the pumpkin genotypes. This may be attributed to differences in environmental factors such as rainfall (Figure 4.) and temperatures.

The current study has shown that fruit yield reductions are mainly due to powdery mildew induced pre-harvest fruit rots. This was supported by positive correlations observed between powdery mildew induced pre-harvest fruit rot counts and AUDPC at 8th week (R = 0.7) and 12th week after planting (R = 0.97). This period was observed to be critical for flowering, fruit setting and development. It was further noted that earlier powdery mildew induced vine wilts contributed to pre-harvest fruit rots (Fig.2 and Fig.3)

There was variation in fresh fruit yield between rain-fed conditions and sprinkler irrigation conditions. Yields for the two sites under rain fed conditions were comparable for the slow mildewing genotypes 6 and 42. However, fast mildewing accessions registered extremely low or no fresh fruit yield at all under irrigated conditions characterized by extremely hot and dry air in September to November, 2009). Pumpkins in Malawi are widely grown under small scale overhead irrigation using watering cane primarily for the leaf as a vegetable. Therefore identification of genotypes that mildew slowly and produce fruit yield levels comparable to those attainable under rain fed conditions would add value to what farmers are already practicing and contribute to food diversification and source of income through leaf and fruit sales.

It is worth noting that the differences in fresh fruit yield between the two slow mildewing genotypes (6 and 42) were largely due to differences in mean fruit volume which were 12,774cm^3 and 1,742cm^3, respectively.

Early pre-harvest fresh fruit losses in fast mildewing genotypes were associated with increased counts of immature fruit rots due to untimely powdery mildew induced vine wilts (Fig.2 and Fig.3). These results have revealed that powdery mildews contribute to low pumpkin fruit yields particularly under irrigated conditions.

However, relatively high fruit yields obtained from slow mildewing genotypes under irrigated conditions suggest that slow mildewing trait is essential for selecting pumpkin accessions for fruit production under irrigated conditions where cucurbit powdery mildew is prevalent.

Table1: Area under Disease Progress Curve (AUDPC) Means of Eight Selected Pumpkin Accessions with Differential Reactions at Chitekwere, Emfeni and Bunda in 2009/2010

	AUDPC (%)			
Accession	Chitekwere	Emfeni	Bunda	Means
22	9.44[a]	9.54[a]	10.57[a]	9.85[a]
25	8.60[b]	8.88[b]	9.86[a]	9.12[b]
45	8.34[b]	8.57[c]	9.53[ab]	8.81[b]
6	5.24[cf]	7.50[d]	4.84[d]	5.86[c]
42	3.29[d]	5.71[f]	2.89[e]	3.96[d]
27	5.66[c]	5.66[g]	6.91[c]	6.08[c]
8	5.41[c]	6.94[e]	6.34[c]	6.23[c]
43	5.51[c]	5.81[f]	6.55[c]	5.96[c]
$LSD_{(0.05)}$	0.30	0.02	0.94	0.42
CV%	17.50	26.00	26.00	23.20
Significance	***	***	***	***

Means within column followed by the same letter are not significantly different.
*** = Significant at P <0.001. LSD = Least Significant differences. CV = Coefficient of variation.

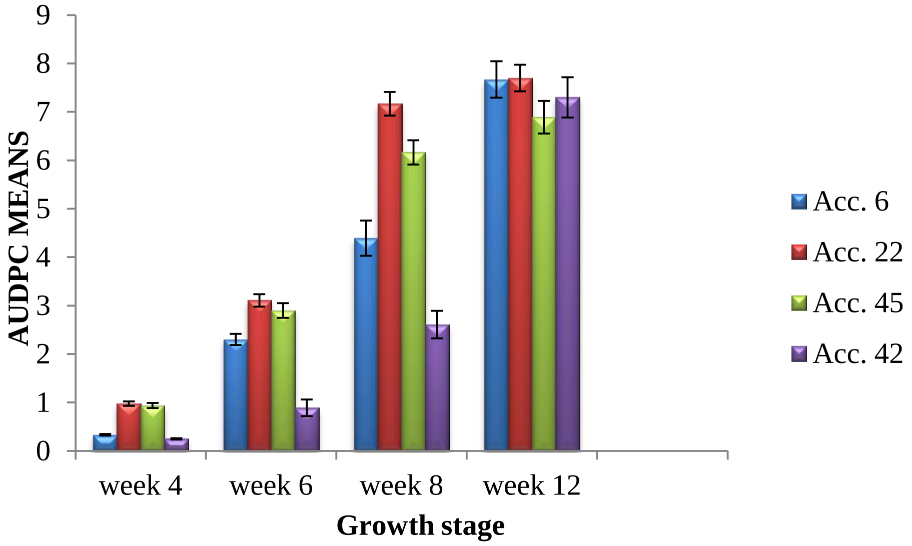

Fig. 1: Area under Disease Progress Curve Means at Different Growth Stages of the Host

Table 2: Means of Peak Powdery Mildew Severity Scores of Six Selected Pumpkin Accessions with Differential Reactions (1-5 Scale) at Chitekwere, Emfeni and Bunda in 2009/2010 Growing Season

	Peak powdery mildew severity scores (score and %)			
Accession	Chitekwere	Emfeni	Bunda	Mean
22	3.83[a] (76.66)	3.83[a] (76.66)	4.83[a] (96.66)	4.17[a] (83.33)
25	3.83[a] (76.66)	3.92[a] (78.34)	4.83[a] (96.66)	4.19[a] (83.89)
45	3.92[a] (78.34)	3.58[a] (71.66)	4.92[a] (98.34)	4.14[a] (82.78)
6	2.33[c] (46.66)	2.42[c] (68.34)	3.00[c] (60.00)	2.92[b] (58.33)
42	1.17[d] (23.34)	3.33[ab] (66.66)	1.67[d] (33.34)	2.06[c] (33.34)
27	2.83[b] (56.66)	2.92[b] (58.34)	3.83[b] (76.66)	3.19[b] (63.89)
$LSD_{(0.05)}$	0.376	0.467	0.745	0.529
CV%	19.6	16.0	28.7	21.43
Significance	***	****	****	***

Means within column followed by the same letter are not significantly different.
*** = Significant at P <0.001. LSD = Least Significant differences. CV = Coefficient of variation
Figures in parenthesis are means of percentage (%) disease index (PDI)

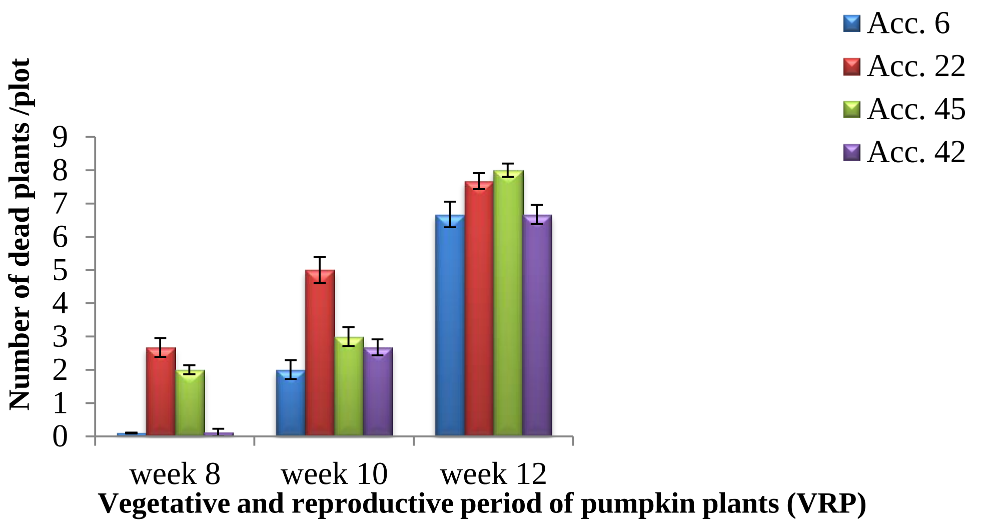

Fig. 2: Means of Dead Plants/Plot of Four Selected Pumpkin Genotypes with Differential Reactions to Powdery Mildews at Different Stages of Plant Development

Table 3: Apparent Infection Rates for Selected Eight Pumpkin Accessions with Differential Reactions to Powdery Mildews at Chitekwere, Emfeni and Bunda in 2009/2010 Growing Season

	Apparent Infection Rates (AUDPC /day) Site			
Accession	Chitekwere	Emfeni	Bunda	Mean
22	0.024[a]	0.024[a]	0.027[a]	0.025[a]
25	0.022[a]	0.023[a]	0.025[a]	0.023[a]
45	0.021[ab]	0.022[ad]	0.024[a]	0.023[a]
6	0.013[c]	0.019[b]	0.012[bd]	0.015[b]
42	0.008[d]	0.015[c]	0.007[c]	0.010[c]
27	0.015[c]	0.015[c]	0.018[b]	0.016[b]
8	0.014[c]	0.018[b]	0.016[b]	0.016[b]
43	0.014[c]	0.015[c]	0.017[b]	0.015[b]
$LSD_{(0.05)}$	0.002	0.001	0.004	0.002
CV%	17.50	26.00	26.00	23.17
Significance	***	***	***	***

Means within column followed by the same letter are not significantly different.
*** = significant at P <0.001. LSD = Least Significant differences. CV = coefficient of variation

Table 4: Area under Disease Progress Curve (AUDPC) Means of Eight Selected Pumpkin Accessions with Differential Reactions at Chitekwere, Emfeni and Bunda In2009/2010

	AUDPC (%)			
Accession	Chitekwere	Emfeni	Bunda	Means
22	9.44[a]	9.54[a]	10.57[a]	9.85[a]
25	8.60[b]	8.88[b]	9.86[a]	9.12[b]
45	8.34[b]	8.57[c]	9.53[ab]	8.81[b]
6	5.24[cf]	7.50[d]	4.84[d]	5.86[c]
42	3.29[d]	5.71[f]	2.89[e]	3.96[d]
27	5.66[c]	5.66[g]	6.91[c]	6.08[c]
8	5.41[c]	6.94[e]	6.34[c]	6.23[c]
43	5.51[c]	5.81[f]	6.55[c]	5.96[c]
$LSD_{(0.05)}$	0.30	0.02	0.94	0.42
CV%	17.50	26.00	26.00	23.20
Significance	***	***	***	***

Means within column followed by the same letter are not significantly different.
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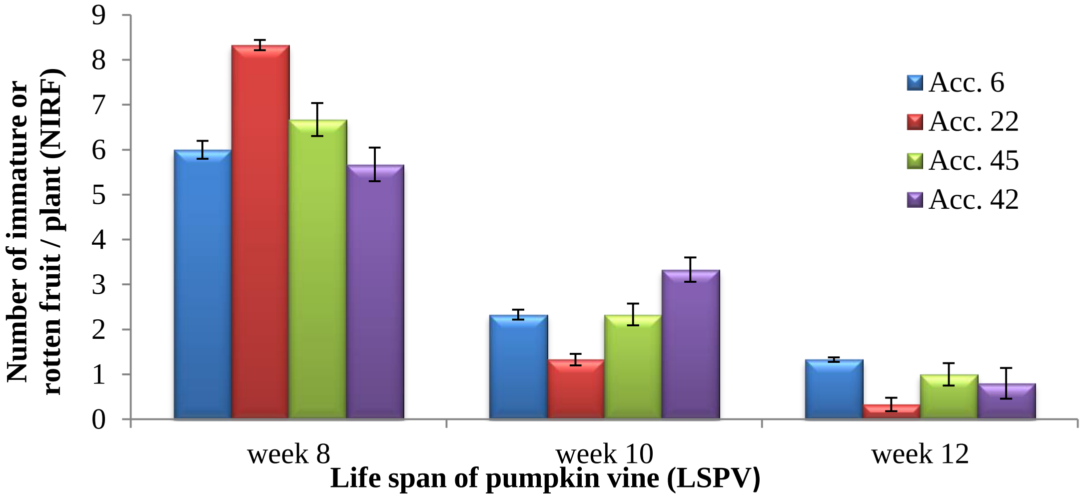

Fig. 3: Relationship between Vine Life Span and Number of Immature and Rotten Fruits

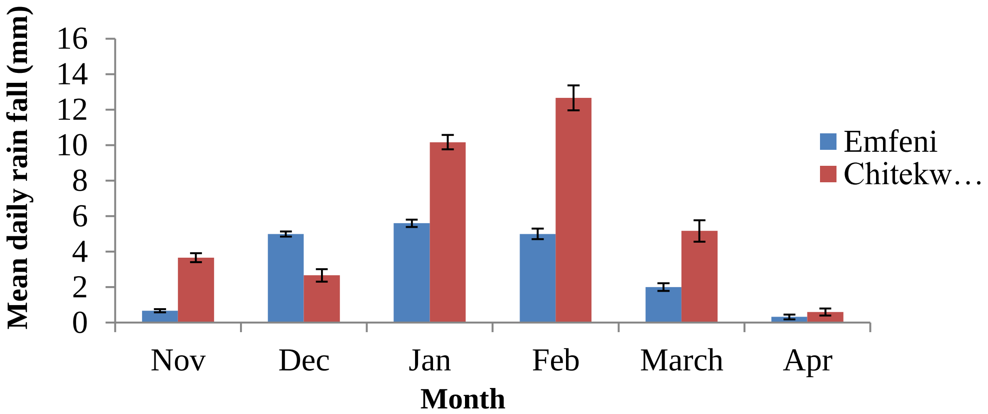

Fig. 4: Mean Daily Rain Fall for Emfeni and Chitekwere for November to April for 2009/2010 Cropping Season

4. Conclusion

Different pumpkin accessions differed in their response to *Erysiphe cichoracearum*. Accessions 6, 42, 27, 8 and 43 showed slow mildewing type of resistance at all the three sites. The rest were susceptible. Under irrigated conditions, Accessions 6 and 42 produced fruit yield levels comparable to those obtained under rain fed conditions. This suggests that powdery mildews limit pumpkin fruit production during the dry season production under small scale irrigation farming. Therefore planting resistant genotypes is one way to promote pumpkin fruit production under irrigation conditions. This means slow mildewing trait in pumpkins can be useful in reducing both pre and post-harvest fruit losses by reducing untimely wilting of pumpkin vines due to powdery mildew attack thereby allowing fruits to achieve physiological maturity and enable the fruits to withstand opportunistic pathogens responsible for fruit rots

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