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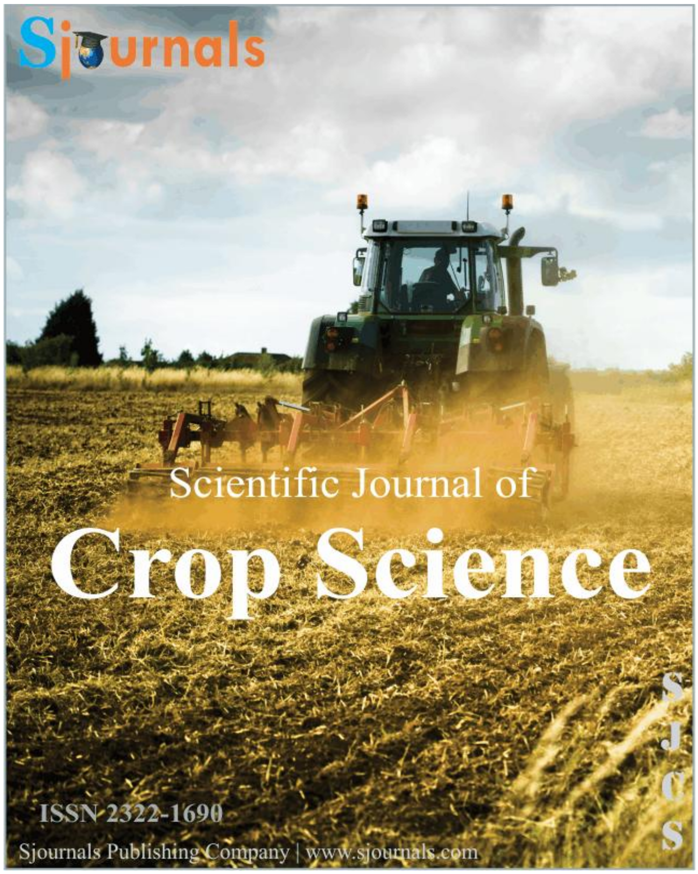

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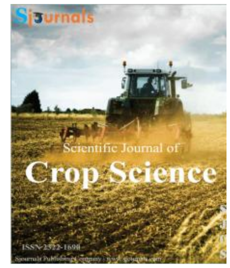

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Original article

Efficacy of locally available fungicides in controlling powdery mildew of Butternut squash (*Cucurbita moschata* Duchesne ex Poiret) in greenhouse at Sebele, Botswana

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A B S T R A C T

The powdery mildew of butternut squash (*Cucurbita moschata* Duchesne ex Poiret) caused by *Podosphaera xanthii* (Castagne) U. Braun and Shiskoff is the most severe disease in Botswana. The disease causes a significant reduction in quality and quantity of butternut fruits. Greenhouse experiments were conducted during 2007 and 2008 at the Department of Agricultural Research Station, Sebele, Botswana to evaluate the efficacy of locally available protectant and systemic fungicides on the control of the powdery mildew of butternut squash. In 2007 treatment trial included a protectant fungicide, chlorothalonil and two systemic fungicides, benomyl and bupirimate which were used separately, and tank mixes of each of the systemic fungicide with the protectant fungicide. Fungicidal treatment in 2008 included an additional protectant fungicide, mancozeb which was used alone and also tank mixed with systemic fungicides used in 2007. Chlorothalonil and mancozeb when used alone were not effective in controlling the disease with the disease severity (DS) of 23.52% and 9.43% respectively over 31.45 % disease severity in non-treated plants. Out of the two systemic fungicides, bupirimate (1.21% DS) was more effective over benomyl (2.89% DS). The data on systemic - protectant fungicide treatment were not significantly different (P<0.05). Bupirimate when combined with protectant fungicide either chlorothalonil (0.46% DS) or mancozeb (0.34% DS), and tank mixes of benomyl with chlorothalonil (0.91% DS), and with mancozeb (0.34% DS) proved to be very effective in the control of powdery mildew of butternut squash as compared to all other fungicidal treatments.

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1. Introduction

Butternut squash (*Cucurbita moschata* Duchesne ex Poiret) is one of the commercial crops grown in Botswana. Butternut squash is a vine plant producing fruits which have hard tan yellow skin, inside with orange flesh which is delicious and sweet. The fruits have a compartment of seeds at the bottom. The fruits are used as vegetable in boiled or roasted form and also as soup. The fruits are good source of vitamin A and C, and other important minerals.

Powdery mildew of butternut squash caused by *Podosphaera xanthii* (Castagne) U. Braun and Shiskoff is the most common, conspicuous, widespread and severe disease in Botswana. The pathogen was reported to cause powdery mildew on other cucurbits (Sherf and McNab, 1986; McGrath, 1996b; Tomason and Gibson, 2006; Hafez, 2008; Perez-Garcia et al., 2009). The disease affects butternut squash grown in the field and in greenhouses producing white, powder like conidia and mycelia particularly on leaf surfaces (Fig. 1). The sexual stage of the pathogen was not observed at any stage of the disease. The butternut powdery mildew in Botswana results in a significant reduction in yield and market quality of fruits. Yield losses may be through a reduction in the number of fruits brought about by reduced photosynthetic potential of infected leaves. Poor market quality on the other hand is mainly due to premature senescence of infected leaves resulting in fruits sun-burning or ripening prematurely or incompletely (McGrath, 1996a). Such fruits have low soluble solids and consequent poor flavour. The fungus utilises the plant nutrients, reduces photosynthesis, causes yellowing and sometimes the death of leaves (Perez-Garcia et al., 2009).

Fig. 1. Powdery mildew symptoms on butternut squash in the greenhouse.

Fungicides play a major role in the control of powdery mildew. Although plant breeders are developing powdery mildew resistant cultivars, it is not anticipated that genetic control will eliminate the need for fungicides (McGrath et al., 1996). However, fungicide resistance may be a major factor with which to contend in managing the causal fungi (Reuveni et al., 1994; McGrath, 1996a). Systemic fungicides play a crucial role in the suppression of the pathogen on the lower leaf surface where powdery mildew occurs most extensively due to favorable growth conditions (McGrath, 1996b; McGrath and Shishkoff, 2001). On the other hand, systemic fungicides tend to be site -specific inhibitors and have high potential for developing resistance (Schroeder and Provvidenti, 1969; McGrath and Staniszewska, 1996; McGrath, 1996a; Culbreath, 2002). In contrast, most protectant fungicides are less likely to develop resistance due to their multi-site mode of action (McGrath, 1996b; McGrath, 2001). Hence, they play an important role in delaying resistance development through reduction in the overall size of the population subjected to selection action of the systemic fungicides (McGrath, 1996b).

Various timing regimes and methods of application were proposed in efforts to maximise control of multiple diseases and to minimise the risks of developing pathogen populations with reduced sensitivity to these fungicides (McGrath, 1996a; Culbreath, 2002). Full growing season level of control was assessed for two methods namely preventative (before symptoms appear) and threshold based (after appearance of symptoms) fungicide treatment programmes (McGrath, 1996a; McGrath and Staniszewka, 1996). These timing regimes were investigated using tank mixes of systemic and protectant fungicides (McGrath, 1996a; Culbreath, 2002), and alternation of two systemic fungicides with different modes of action, both combined with protectant fungicides (McGrath and Staniszewska, 1996). Both application methods were found to be quite effective in controlling powdery mildew with no/little indication of resistance development (McGrath, 1996a; McGrath and Staniszewska, 1996).

The powdery mildew of butternut squash was indicated as one of the major diseases of cucurbits, including butternut squash, in Botswana (Anonymous, 1991). Currently the powdery mildew of butternut squash in Botswana is so severe that farmers are finding it difficult to control it on their produce. However, there is no documented information on the efficacy of locally available fungicides in controlling the disease under Botswana conditions and a study to investigate this is warranted. Therefore, the present study was undertaken to assess the efficacy of locally available systemic and protectant fungicides and their tank mixes in control of powdery mildew of butternut squash using the threshold based fungicide programmes.

2. Materials and methods

2.1. Experimental design

"Waltham" cultivar of the Butternut squash was selected for the experiments on the fungicide treatment. Treatments were arranged in a randomised complete block design with four replicates and two plants per treatment in each replication. Plants were arranged in lines in the greenhouse and the space between treatments was 70 cm while block spacing was 125 cm. Plants were direct seeded in 20 cm diameter pots 24th May 2007 and 5th June 2008. Two wires approximately 30 cm apart from each other were supported horizontally on the roof of the greenhouse. Training strings connected to each pot were connected to the overhead wire and plants supported up the strings. Single superphosphate (10.5%) was pre plant incorporated at 5.6 g per pot. Top dressing was carried out twice a month using liquid NPK at the ratio of 3:2:1. The plants were exposed to natural infection by placing infected plants in the middle of the blocks.

2.2. Treatments

2007 trial treatments consisted of (i) non-treated control (ii) a protectant fungicide, chlorothalonil (Bravo 720 g/L) at 5 ml/5L sprays (iii) Bupirimate (Nimrod 250 g/L) applied at 2 ml/5L sprays and (iv) Benomyl (500 WP) applied at 2.5 g /5L sprays; tank mixes of each of the systemic fungicide with the protectant in the following combinations: (v) Benomyl + chlorothalonil (2.5 g + 5 ml/5L) and (vi) Bupirimate + chlorothalonil (2 ml+5 ml/5L). 2008 treatments included an additional protectant fungicide, mancozeb which was used alone ((80% WP) at 12.5 g/5L) as well as tank mixes of this with the two systemic fungicides; (viii) Benomyl + mancozeb (2.5 g + 12.5 g/5L) and (ix) Bupirimate + mancozeb (2 ml+12.5 g /5L).

2.3. Treatment applications and disease assessment

In 2007 greenhouse fungicide applications were made 32, 42, 56, 70, 84, and 98 days after planting (DAP) and 48, 62, 76, 90 and 104 DAP in 2008. Fungicide applications were made using 5L Knapsack sprayers in a 14 day schedule. The first application was made when 1 out of 50 randomly examined leaves showed symptoms of the disease. Powdery mildew severity assessment was made 39, 46, 53, 60, 67, 73, 80, 87, 94 and 102 DAP in 2007 and 54, 60, 67, 74, 81, 88, 95, 102, 109 DAP in 2008 for the greenhouse trials. Disease severity was calculated as percentage of leaf area covered by powdery mildew on both the lower and the upper leaf surfaces as per McGrath

(1996b). Initially, only six big leaves could be assessed per treatment. Once the disease had spread throughout the whole plant assessment was made on a total of 30 leaves selected from all growth stages (old, middle, new-10 per age class). Data from these groups was then averaged to get severity value for each leaf surface for each plot.

2.4. Data analysis

A randomised complete block design (RCBD) with four replicates was used in the study. Statistical Analysis System (SAS) was used to analyse the statistical significance of the data. PROC ANOVA was performed for analysis of variance. Comparisons of the effect of the different treatments over time were done using Duncan's Multiple Range Test.

3. Results and discussion

3.1. Disease severity-2007

Results indicated that there was a significant difference (P< 0.05) between efficacy of protectant and that of systemic fungicides. The protectant fungicide, chlorothalonil when used alone had disease severity of 22.1%, and thus was not effective in controlling the powdery mildew on both leaf surfaces (Table 1, Fig. 2). The results obtained for this fungicide did not differ with those of the control treatment (24.3%). Eighty - eight DAP most leaves on these treatments (control and chlorothalonil) were completely covered with the mildew and already turning yellow. At the end of the experiment (102 days after planting) most leaves on both treatments (chlorothalonil and control) were completely defoliated.

Table 1
Mean percentage disease severity of powdery mildew of butternut squash cultivar "Waltham" during 2007 and 2008 trials.

	Mean severity	Treatment (2008 trials)	Mean severity	Avg mean severity
Control	24.33a	Control	38.58a	31.45
Chlorothalonil	22.14b	Chlorothalonil	24.95b	23.54
Benomyl	5.47c	Benomyl	0.32c	2.89
Benomyl & chlorothalonil	1.00d	Benomyl & chlorothalonil	0.82c	0.91
Bupirimate	0.91d	Bupirimate	1.52c	1.21
Bupirimate & chlorothalonil	0.46d	Bupirimate & chlorothalonil	1.77c	1.11
-	-	Mancozeb	9.43c	9.43
-	-	Bupirimate & mancozeb	0.34c	0.34
-	-	Benomyl & mancozeb	0.34c	0.34

Means followed by different letters are significantly different (P<0.05).

The systemic fungicide, benomyl and bupirimate when used alone conferred better control than the protectant fungicide with the disease severity of 5.47% and 0.91% respectively. Although benomyl was quite effective at the beginning of the study, its efficacy decreased with treatment time. This decline became more apparent on the upper leaf surfaces first (67 DAP), with a similar trend observed 81 DAP on the lower leaf surfaces (Fig 3). On the last sampling day, most of the older leaves under this treatment were completely covered with powdery mildew. Bupirimate was the most effective and disease development was suppressed substantially through the study period. In all the treatment diseases severity was more pronounced on the upper leaf surfaces (Table 1).

All the combinations used in this study period; benomyl/chlorothalonil and bupirimate/chlorothalonil significantly suppressed mildew development with the disease severity of 0.1% and 0.46% respectively. The mean severity of the tank mixes were far lower than that of either fungicide used alone. However, the mean severity of the tank mixes did not differ significantly with that bupirimate used alone (Table 1). For both tank mixes a very gradual increase in the severity levels was only evident toward the end of the growing period (Fig. 3).

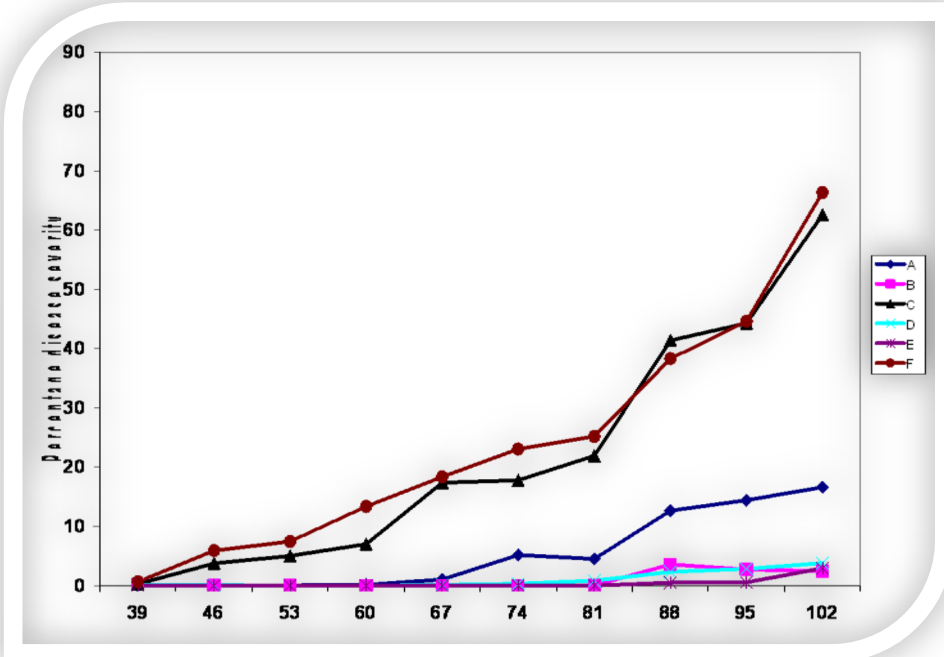

Fig. 2. Mean powdery mildew severity progress as brought about by different fungicides control in the year 2007.

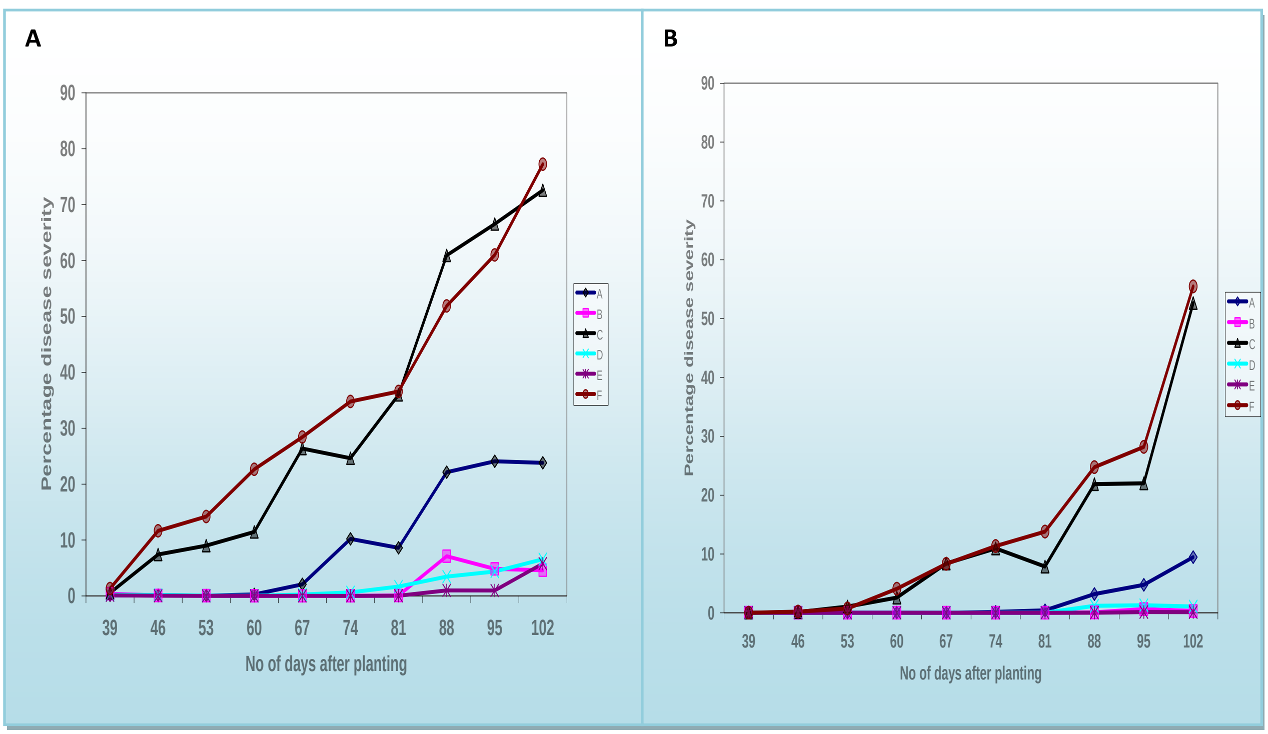

Fig. 3. Powdery mildew severity progress as brought about by different fungicides control. A- Trend on the upper leaf surface and B- Trend on the lower leaf surface.

A = Benomyl
B = Bupirimate
C = Chlorothalonil
D = Benomyl and Chlorothalonil
E = Bupirimate and Chlorothalonil
F = Control

3.2. Disease severity-2008

A trend similar to 2007, was also observed in the 2008 greenhouse trial in that fungicide control efficacy of the systemic fungicides was higher than that of the protectant fungicides. Chlorothalonil was the least effective of the four fungicides assessed (Table 1). At the end of the study period most of the leaves under this treatment were defoliated or completed covered by powdery mildew. Mancozeb performed significantly ($p<0.05$) better than chlorothalonil although its efficacy was still lower than that of the systemic fungicides, benomyl and bupirimate.

Benomyl was observed better than bupirimate in the year 2008 with the disease severity of 0.32% over 1.52% of the latter after 109 DAP. Although not significantly different ($P<0.05$), table 1 shows that the efficacy of the tank mixes of the systemic fungicides with mancozeb (benomyl+mancozeb, 0.34%; bupirimate+mancozeb, 0.34%), was higher than that of their combinations with chlorothalonil (benomyl+chlorothalonil, 0.82%; bupirimate + chlorothalonil, 1.77%). In all the treatment powdery mildew was most severe on the upper leaf surface and quite predominant on the bigger leaves (Fig. 4).

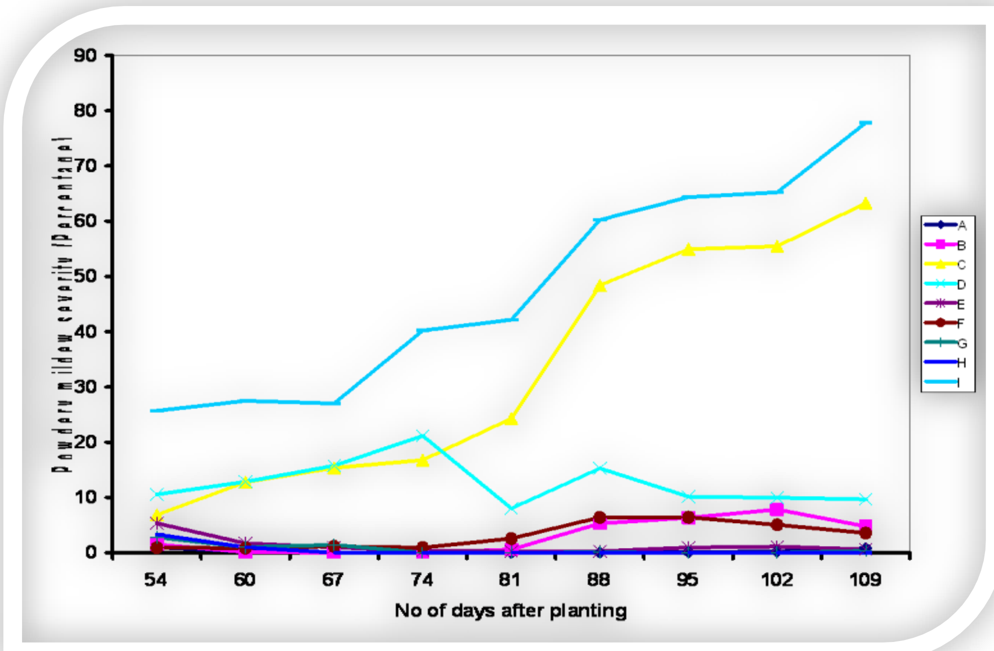

Fig. 4. Mean powdery mildew severity progress as brought about by different fungicides control in the year 2008.

A = Benomyl	B = Bupirimate	C = Chlorothalonil
D = Mancozeb	E = Benomyl and Chloro	F = Bupirimate & Chloro
G = Benomyl and mancozeb	H= Bupirimate & mancozeb	I = Control

3.3. Yield

In the year 2007, there was only one marketable fruit produced per treatment. Any second fruit that was produced got aborted before it reached maturity. However, in the 2008, the number of marketable fruits was quite higher with an average of 9 fruits per treatments. None the less, there was still no notable effect of the different treatment on yield and fruit quality.

The results revealed that the best control against powdery mildew of butternut squash was brought about by tank mixes of systemic, bupirimate or benomyl and each with protectant fungicides, either chlorothalonil or mancozeb. Powdery mildew severity for the tank mixes remained at levels below 1.5% throughout the study period, which is an indication of high efficacy of the combinations used. These findings corroborate with that of McGrath and Staniszewska (1996) and McGrath (1996a) that systemic fungicides applied at the same time with a protectant fungicide are more effective than each of the fungicide applied alone. Effectiveness of the tank mixes is probably brought about by multiple site mode of action of protectant fungicides delaying development of resistance which is likely to occur when systemic fungicides are used alone rendering them ineffective.

Protectant fungicides confer most protection where they are deposited and are quite effective in controlling the disease in the upper surfaces (McGrath, 2001; McGrath and Staniszewska, 1996). Chlorothalonil is a broad spectrum fungicide recommended for control of powdery mildew hence, lower disease severity was expected on the upper leaf surfaces. McGrath (1996b) observed high chlorothalonil efficacy on the adaxial compared to the abaxial leaf surface. However, in this study it was observed that chlorothalonil applied alone failed to control powdery mildew on both leaf surfaces. Biological activity of fungicides is however, influenced by several parameters, such as physiochemical characteristics of the active ingredient, the environmental conditions and the application techniques (Karaoglanidis and Karadimos, 2006). Since this study was carried out under controlled conditions using knapsack sprayers which could effectively reach both leaf surfaces, poor performance of chlorothalonil, suggests a need to increase its rate/dosage.

The two systemic fungicides, bupirimate and benomyl used in this study, the former had high control efficacy against powdery mildew. Unfortunately, these fungicides have a high risk of resistance development because they have specific modes of action, and powdery mildew fungi have a high potential for resistance development (McGrath, 2001). In 2007, benomyl was able to suppress powdery mildew for 4 and 6 weeks only in the upper and lower leaf surfaces respectively. Thereafter there was a gradual increase in the disease severity on both surfaces. Benomyl is a benzimidale fungicide with a single site mode of action (McGrath, 2001). This increase in disease incidence/severity may be due to development of resistance to benomyl by the fungus. Selection pressure was indicated to be sufficient to bring about large increases in the number of isolates resistant benomyl resulting with a decline in the fungicide efficacy (McGrath and Staniszewska, 1996). Effective control of the disease brought about by bupirimate alone however is probably due to the fact that it has got both a systemic with curative and protectant action.

Powdery mildew develops most extensively on the lower leaf surface where conditions are more conducive for disease development (McGrath, 1996a). In contrary, it was observed in the present study that powdery mildew growth was more intense on the upper than the lower leaf surface. In the field, the leaves are in contact with the soil impeding on effective fungicide control. There is also higher moisture content on the lower side of the leaf as well as reduced light intensity brought about by a dense plant canopy, which encourages powdery mildew development. However, in the greenhouse whereby the plants were trained up, there is increased ventilation and the fungicides can effectively reach both sides which may account for the low severity levels.

4. Conclusion

The present study demonstrated that two protectant fungicides, chlorothalonil and mancozeb when used alone were not effective for the control of powdery mildew of butternut squash. The two systemic fungicides, bupirimate and benomyl when used alone, the former was more effective than benomyl. The tank mixes of protectant and systemic fungicides in combinations of bupirimate+chlorothalonil, bupirimate+mancozeb, benomyl +chlorothalonil and benomyl+mancozeb proved to be very effective in the control of the disease.

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