# MANAGEMENT OF PHAEORAMULARIA FRUIT AND LEAF SPOT DISEASE OF CITRUS IN ETHIOPIA

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***Abstract***

*In Ethiopia, production of sweet orange (Citrus sinensis) is threatened by the devastating leaf and fruit spot disease caused by Phaeoramularia angolensis. This study was undertaken to evaluate fungicides singly and in combination in controlling the disease and to assess the reaction of five sweet orange cultivars to the disease under field conditions in northwest Ethiopia. Three sprays of benomyl, chlorothalonil, copper hydroxide, benomyl +chlorothalonil and benomyl + copper hydroxide were applied at 15 day interval to sweet orange cultivar, Washington Naval. Among the fungicide treatments, the application of benomyl @ 0.039 % plus chlorothalonil @ 0.09 % proved most effective in controlling the disease on the leaves. Application of benomyl alone @ 0.078% proved next best and was significantly better over mixture of 0.039% benomyl + 0.056% copper hydroxide, chlorothalonil alone (0.18%) and copper hydroxide alone (0.115%). The cultivars, Jaffa and Campbell Valencia, were classified as moderately resistant while the cultivars Washington Naval, Pineapple and Hamlin were classified as moderately susceptible. Moderately resistant cultivars need to be considered for new plantations. This must be augmented with three sprays of a mixture of benomyl and chlorothalonil at 15-day interval before fruit set to effectively manage the disease and avoid development of resistance against the systemic fungicide.*

**Key words:** fungicides; sweet orange resistance; phaeoramularia leaf spot.

## INTRODUCTION

Citrus is among the most important fruit crops of Ethiopia. Its cultivation started in Upper Awash valley and Melkassa areas in southeast Ethiopia. Upper Awash eco-conditions proved best for orange, mandarin, tangor and tangelo, while middle Awash was appropriate for grape fruit, lemon and lime (Herath *et al*., 1994). Citrus occupied 7290 hectares of land with production of 230,970 m tonnes in 1985, but area and production have been reduced to 5380 hectares and 33,500 metric tonnes, respectively in the country. Presently 2200 ha are under orange, 1750 ha under mandarin, 1100 ha under lemon and limes (FAO, 2004).

In 1990, a new and devastating disease of citrus, phaeoramularia leaf and fruit spot caused by *Phaeoramularia angolensis* (Car.& Men.) P.M. Kirk, (syn. *Cercospora angolensis* Car.& Men.) appeared in south-eastern region of Ethiopia (Eshetu, 1999; Kirk,1985). This disease results in premature leaf defoliation, loss of tree vigour and greatly affects market value of attacked fruits. Currently it has spread over vast areas including the northwest citrus production regions of the country where citrus cultivation has been promoted in the last 20 years (Eshetu, 1999). The disease has become so destructive in this part of the country that some of the farmers are compelled to up-root sweet orange trees and replace them with other crops. Kuate *et a*l. (1997) also reported that this disease could cause 50 – 100% loss in yield. Based on the information obtained from other African countries, three fungicides namely benomyl, copper hydroxide and chlorothalonil, were recommended in Ethiopia for the management of this disease (Seif, 1995; Eshetu, 1999). However, extensive and repeated use of benomyl may lead to the development of resistance in *P. angolensis* population as has been observed for many other pathogens with this chemical (Hewitt, 1998). *Cercospora beticola* developed resistance to benzimidazole compounds including benomyl within two years of their use on sugar beets (Hewitt, 1998). Problem of development of resistance can be managed by combinations of products with different modes of action either in mixture (co-formulations or tank mixtures) or as alternations in a spray programme (Hewitt, 1998). This study was undertaken with the objective of evaluating the efficacy of benomyl, chlorothalonil and copper hydroxide singly and in combinations in northwest Ethiopia, where ecological conditions vary considerably from southeastern parts of the country. In addition, natural incidence and severity of disease on established five sweet orange cultivars in the area was also investigated to find out cultivars suitable for the region.

## MATERIALS AND METHODS

### Fungicidal control of Phaeoramularia leaf spot

The treatments were:

1. Benomyl (Benlate 50 WP) 0.078 % a.i
2. Chlorothalonil (Daconil 75 WP) 0.18 % a.i.
3. Copper hydroxide (Kocide 50 WP) 0.115 % a.i
4. Benomyl 0.039 % + Chlorothalonil 0.09 % a.i
5. Benomyl 0.039 % + Copper hydroxide 0.057 % a.i
6. Control (water)

Four replications, each with one tree were maintained for all treatments arranged in randomized complete block design (RCBD).

**Evaluation of sweet orange cultivars for resistance to phaeoramularia leaf spot**

Five popular sweet orange cultivars viz., Washington Navel, Pineapple, Hamlin, Campbell Valencia, and Jaffa established at Chagni were evaluated for their reaction to *P. angolensis* under prevailing natural conditions. Trees of same age and relatively same canopy size (2.3-m in diameter and 3-m height) of each cultivar were marked for the studies. Four trees of each cultivar served as replications of the cultivar.

**Disease assessment**

Disease severity was assessed using a 1-5 disease scoring scale where: 1=0 %; 2= <5 %; 3= 5-20 %; 4 = 21-50 %; and 5= > 51 % of the leaf area affected (Seif and Hillocks, 1998). Percent premature defoliation of leaves was calculated from the number of labeled and subsequently present leaves after last assessment on each tagged shoot. Area under disease progress curve (AUDPC) was computed from severity data using the formula suggested by Shaner and Finney (1977).

$$AUDPC = \sum_{i=1}^{n-1} [(x_{i+1} + xi)] / 2[ti + 1 - ti]$$

Where xi = the disease severity (%) at $i^{th}$ observation, $t_i$ = time (days after disease occurrence) at the $i^{th}$ observation and n = total number of observations.

Disease severity index was also computed using the formula suggested by Chaube and Singh (1990) as given below:

$$\text{Disease severity index} = \frac{\text{Sum of all numerical ratings x 100}}{\text{Total number of observations x maximum disease score}}$$

In the second experiment assessment of natural incidence and severity of the disease on leaves was carried out 7-times at 15-days interval. AUPDC, disease severity index and premature leaf defoliation were also derived. Cultivars with scores of 1-2 were rated resistant; 3 moderately resistant; 4 moderately susceptible and with 5 score susceptible, respectively.

Data in each experiment were subjected to analysis of variance using MSTAT-C computer statistical package. Data on disease incidence and premature leaf defoliation were transformed using arc-sin transformation to achieve homogeneity of variance. The least significant difference (LSD) test ($p \leq 0.05$) was used to find the significance of treatment mean difference (Gomez and Gomez, 1984).

## RESULTS

**Evaluation of fungicides against phaeoramularia leaf spot**

Phaeoramularia leaf spots that were 6-9 mm in diameter and often surrounded by yellow halo started appearing naturally on both sides of leaves of sweet orange trees during late September 2003. At later stages, spots developed light brown or greyish centre and eventually leaves were shed prematurely.

Application of different fungicidal treatments reduced the disease incidence significantly over control (Table 1). Highest significant disease control ($p \leq 0.05$) was achieved with a mixture of benomyl (0.039%) and chlorothalonil (0.09%), which reduced the disease incidence to 3 % as compared to 36% in control trees. This was significantly superior over all the fungicidal treatments. Application of benomyl alone @0.078% proved next best (7% disease incidence) and was significantly better over mixture of 0.039% benomyl + 0.056% copper hydroxide, 0.18% chlorothalonil alone and 0.115% copper hydroxide alone, which resulted in 14, 17 and 20 % disease incidence, respectively and were at par with each other. All the fungicides also reduced the disease severity significantly over control. Disease severity index was also found to be significantly lowest (2%) on trees, where mixture of benomyl and chlorothalonil was sprayed, followed by benomyl alone (5%), mixture of benomyl and copper hydroxide (10%), chlorothalonil alone (15 %) and copper hydroxide alone (23%). Highest disease severity index (50%) was observed in control trees. Mixture of benomyl and chlorothalonil was significantly better over benomyl alone, which in turn was significantly better over mixture of benomyl and copper hydroxide. Copper hydroxide proved significantly inferior to chlorothalonil when both were applied alone.

Area under disease progress curve and premature leaf defoliation followed similar trend. Mixture of benomyl and chlorothalonil, benomyl alone, mixture of benomyl and copper hydroxide, chlorothalonil alone, copper hydroxide alone and control had 11, 35, 67, 103, 153 and 322 per cent day AUPDC, respectively, while per cent premature defoliation was 3, 5, 16, 20, 29 and 44%, respectively. From the data it is apparent that mixture of benomyl and chlorothalonil not only reduced disease incidence, but also reduced disease progress and subsequent premature leaf fall. In addition, a strong positive correlation ($R^2$ = 0.96) was observed between phaeoramularia leaf spot severity index and premature leaf defoliation of sweet orange trees (Fig.1).

**Reaction of citrus cultivars to phaeoramularia leaf spot**

Te five tested cultivars of sweet orange varied in their susceptibility to Phaeoramularia leaf spot under natural conditions (Table 2). There was significant difference ($p \leq 0.05$) among the treatments for all the parameters studied. Comparison of the test cultivars with the susceptible check (Washington Naval) indicated that the cultivar Jaffa scored the lowest mean disease incidence (11%), severity index (9%), AUDPC (58%) and premature leaf defoliation (12%) followed by the cultivar Campbell Valencia that scored 17% disease incidence, 15% disease severity, 105 per cent day AUDPC and 19% leaf defoliation. Both these cultivars scored 3 on the 1-5 scoring scale and were classified as moderately resistant. Washington Navel, Pineapple and Hamlin showed relatively high scores of mean disease incidence, severity index, AUDPC and premature leaf defoliation and scored mean value of 4. Therefore, they were grouped as moderately susceptible cultivars (Table 2).

## DISCUSSION

In the present study, heavy incidence of leaf spots occurred on the cultivar Washington Naval and other cultivars of sweet orange. The systemic fungicide benomyl and non-systemic fungicides chlorothalonil and copper hydroxide reduced the incidence and severity of Phaeoramularia leaf spots over control significantly, when applied singly or in mixtures as also reported by Seif (1995). However, the level of reduction varied among treatments. Application of benomyl @ 0.039 % plus chlorothalonil @ 0.09 % was the most effective fungicidal treatment for reduction of foliar symptoms of the disease as evidenced by reduced disease incidence, severity index and lower AUDPC values. Individually benomyl @ 0.078% and chlorothalonil @ 0.18% were significantly less effective in reducing the disease over their combination at half the concentrations. Mixtures are often used to broaden the activity spectrum of fungicides and they perform usually additively against a target pathogen. However, mixtures may exhibit antagonistic or synergistic interactions between their components Chlorothalonil has earlier been found to interact synergistically with fenpropimorph. Differences in uptake, mobility and persistence largely contribute to synergistic interaction between fungicides. It was also reported that combination of chlorothalonil and benomyl gave better control of gummy blight of watermelon caused by *Didymella bryoniae.* Combinations might be used to delay the development of resistant strains of pathogens to benomyl and other high-risk fungicides (Hewitt, 1998).

Out of the two non-systemic fungicides tested chlorothalonil proved better in reducing disease incidence, severity index, AUDPC and premature leaf defoliation over copper hydroxide singly as well as in combination with benomyl. Eshetu (1999) also reported that this disease was controlled with chlorothalonil better than other tested contact fungicides. Thus the present study clearly suggests that the application of benomyl in mixture with chlorothalonil or copper hydroxide can effectively manage Phaeoramularia leaf spot disease of sweet orange. These mixtures may also reduce the chances and delay the development of resistant strains of *P. angolensis* as selection pressure will be much lesser as compared to that exerted by the repeated application of benomyl alone.

Susceptibility of the five tested sweet orange cultivars differed significantly to Phaeoramularia leaf spot disease. Jaffa scored the lowest mean disease incidence and severity index, AUDPC value and premature leaf defoliation followed by the cultivar Campbell Valencia and thus clearly showed moderately resistant behaviour. Disease incidence in cultivars Washington Naval, Pineapple and Hamlin ranged between 32 to 37% and severity index between 45 and 49 %, which reflected moderately susceptible reaction under Chagni conditions. Cultivars Pineapple and Washington Navel were earlier reported to be susceptible to the disease in Kenya Marsh seedless grapefruit was most susceptible in Kenya, while Tahiti lime (*Citrus latifolia*) was least ((Seif , 1995). The moderately resistant cultivars Jaffa and Campbell Valencia not only showed less disease severity but also lesser AUDPC and premature leaf defoliation and the reverse were true for the moderately susceptible cultivars. The mechanism of this variability in susceptibility is not yet determined and further investigations could be required.

In the present study, a strong positive correlation between disease severity and premature leaf defoliation ($R^2 = 0.93$) was observed. In the fungicidal control trial, premature leaf defoliation was only 3 % in trees sprayed three times with mixture of benomyl and chlorothalonil, where least disease occurred in comparison to 44 % defoliation in control trees with high level of disease. Similarly, among the cultivars, least defoliation (12 %) was observed in cultivar Jaffa, where disease incidence and severity were 11 and 9 %, respectively. On the other hand 44% defoliation was recorded in Washington Naval, where disease incidence and severity were 37 and 49 %, respectively. It can be concluded that Phaeoramularia leaf spot disease is responsible for premature leaf defoliation.

## CONCLUSION

In the present study, the disease affected the five sweet orange cultivars to a certain degree and none of them was found to be completely free of disease. Resistance alone does not seem to offer a desirable solution to the disease problem. Therefore, incorporation of chemical intervention with moderately resistant cultivars, such as Jaffa and Campbell Valencia seems promising in the management of phaeoramularia leaf and fruit spot disease in northwest Ethiopia.

**ACKNOWLEDGEMENT**

We are thankful to the Amhara Agricultural Research Institute and the Amhara Regional Bureau of Agriculture for funding this research work.

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*Received for publication on September 18 , 2006*
*Accepted for publication on November 22 , 2006*

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**Tab. 1. :** Effect of some fungicides on phaeoramularia leaf spot disease incidence, severity index, and AUDPC

| Treatment | Dosage (a.i) | Disease incidence (%) | Disease severity index (%) | AUDPC |
|---|---|---|---|---|
| Benomyl | 0.078 % | 14.9c (6.7) | 12.7e (4.9) | 34.56e |
| Chlorothalonil | 0.18 % | 23.8b (16.6) | 22.8c (15.3) | 103.18c |
| Copper hydroxide | 0.115 % | 26.4b (20.2) | 28.7b (23.0) | 153.19b |
| Benomyl + Chlorothalonil | 0.039%+0.09 % | 9.7d (2.7) | 7.3f (1.6) | 11.11f |
| Benomyl + Copper hydroxide | )39%+0.056% | 21.4b (13.5) | 18d (9.6) | 67.07d |
| Control | - | 36.6a (35.6) | 44.7a (49.5) | 322.4a |
| Mean | | 22.1 (23.8) | 22.4 (26.0) | 115.25 |
| S. E M | | 11.5 | 2.1 | 222.71 |
| LSD (0.05) | | 5.1 | 2.22 | 22.49 |
| CV (%) | | 15.3 | 6.53 | 12.95 |

Means followed by the same letter in a column are not significantly different from each other

($p \leq 0.05$)

Percent values were transformed using arc- sign transformation.

Values in parenthesis are original values

**Tab. 2. :** Reaction of five sweet orange cultivars to phaeoramularia leaf spot disease at Chagni Orchard site in northwest Ethiopia, 2003

| Treatment Cultivar | Disease incidence (%) | Disease severity index (%) | AUDPC | Ppld (%) | Reaction |
|---|---|---|---|---|---|
| Washington Navel | 37.43a (37.0) | 48.7a | 324.19a | 41.49a (44.0) | MS |
| Pineapple | 35.69ab (34.1) | 47.3ab | 306.8ab | 39.77a (41.0) | MS |
| Hamlin | 34.1b (31.5) | 45.0b | 282.43b | 35.04b(33.0) | MS |
| Campbell Valencia | 24.24c (17) | 15.2c | 105.42c | 26.39c (19.1) | MR |
| Jaffa | 19.47d (11.2) | 8.6d | 58.26d | 20.02d (11.8) | MR |
| Mean | 30.18 (26.13) | 33.0 | 215.42 | 32.55 (29.7) | |
| S.EM | 3.77 | 2.008 | 311.0 | 2.118 | |
| LSD (0.05) | 2.991 | 2.623 | 27.17 | 2.242 | |
| CV (%) | 6.43 | 4 .30 | 8.19 | 4.47 | |

Values followed by the same letter in a column are not significantly different ($p \leq 0.05$)
1-5 scoring system: 1=0 %; 2=<5 %; 3=5-20 %; 4= 21-50 %; 5= >51 %
Values for disease incidence and premature leaf defoliation were transformed using arc sin transformation
Values in parenthesis are original values
Pld (%) = Per cent premature leaf defoliation

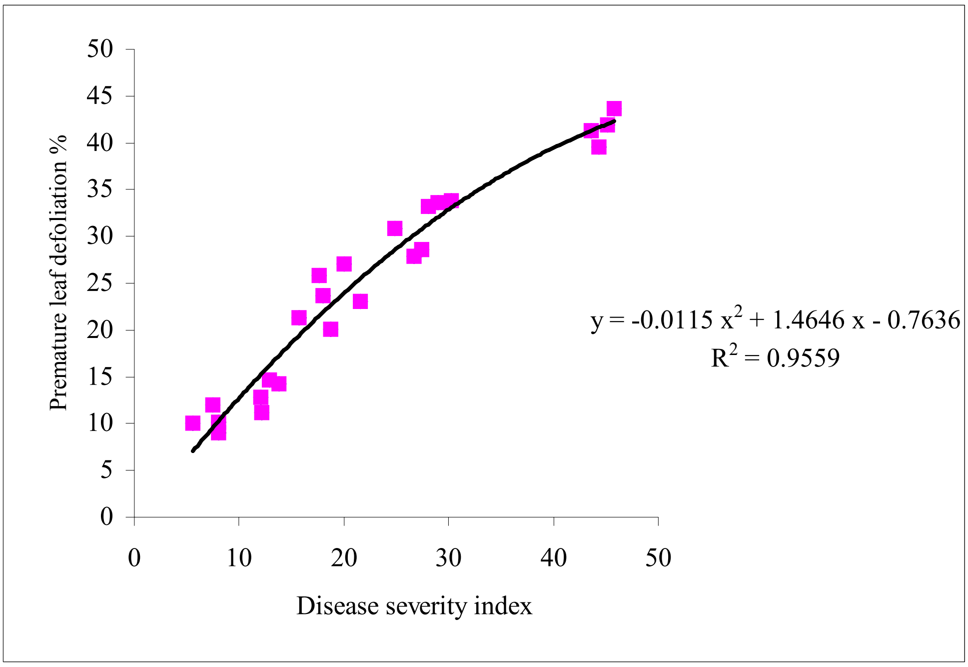


**Fig. 1.:** Relationship between disease severity index and premature leaf defoliation based on coefficient of determination