

Management of pepper powdery mildew caused by *Leveillula taurica* (Lev.) Arn. using fungicides and plant extracts

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ABSTRACT

Sweet pepper (*Capsicum annuum* L.) is one of the most valuable vegetable crops grown in newly reclaimed land in Egypt. Pepper plants are attacked by several fungal, bacterial and viral diseases which cause great losses in yield. Powdery mildew disease, caused by *Leveillula taurica* anamorph *Oidiopsis taurica* is one of the most serious disease attacking pepper plants under greenhouse and open field conditions. The seven fungal isolates were varied in their virulence to pepper cv. California winder. The efficiency of some systemic fungicides and plant extracts on management of pepper powdery mildew disease was evaluated under greenhouse and field conditions. Greenhouse experiments revealed that application of the tested systemic fungicides, *i.e.* Leader 45%, Penazole 10%, Daconil 75%, Topas 10% and Flint 50% as well as garlic and thyme extract at the rate of 6%, significantly reduced the disease severity, meanwhile plant length and foliage fresh weight were increased as comparison with check treatment. However, systemic fungicides were more efficient in this concern than the tested plant extracts. Under field conditions, application of either Leader 45%, Penazole 10% or Flint 50% followed by spraying of garlic and thyme extract at the rate of 6% caused significant decrement in the disease severity with significant increment in the fruit yield when compared with check treatments. However, these treatments were, to somewhat, less efficient than application of systemic fungicides only. Garlic and thyme extract at the rate of 6%, gave the highest increased in chlorophyll content.

Keywords: Pepper, chemical control, fungicides, powdery mildew and plant extracts

Introduction

Powdery mildew caused by *Leveillula taurica*, anamorph *Oidiopsis taurica* (Braun, 1987), is one of the most important diseases of pepper (*Capsicum annuum*) grown as a greenhouse and open field in Egypt. The pathogen is associated with more than 1000 plant species, including hot capsicum peppers and tomato (*Lycopersicon esculentum*), and is spreading quickly in Egypt and around the world. *Capsicum annuum* was described as the most susceptible *solanaceous* host crop for this mildew (Dixon, 1978). Pepper (*Capsicum annuum* L.) is one of the most popular and highly remunerative, annual herbaceous vegetable crop (de Souza and Cafe-Filho, 2003). In Egypt pepper is among the most important *solanaceous* crops and leading export vegetable. Under protected cultivation, and open fields capsicums are widely grown due to higher productivity and economic feasibility. Production of vegetables results in effective use of the land resources, besides being able to increase the production of quality vegetables both for the export and domestic markets by offsetting biotic and abiotic stresses to a great extent that otherwise is prevalent in open cultivation. Diseased crops give lower yield and fruit, a reduced harvesting period and poor quality marketable produce due to various constrains like diseases, insect pests, lack of planting and management technology (Cerkaukas and Brown, 1999 and Amar and Banyal, 2011).

Several researchers reported that, use foliar application of some systemic fungicides, as propiconazole, were found to be effective in reducing powdery mildew incidence in different crops (Sharmila *et al.*, 2004, Singh, 2006, Pramod Prasad and Dwivedi, 2007, Akhileshwari *et al.*, 2012, Raju *et al.*, 2017 and Daunde *et al.*, 2018). It is well known that chemical control of plant diseases mostly causes environmental pollution and increase the accumulated toxic substances in human food chain. On the other hand, using alternative disease management, *e.g.* biological control, plant extracts, antioxidants and agricultural practices, are not enough to obtain efficient results (Hillal-Mervat, 2004 and Abada *et al.*, 2008). Alternations between the systemic fungicides and resistance inducing

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chemicals gave intermediate effect on disease reduction and the produced fruit yield, but it could be of great interest, where the produced fruits are of low fungicides residue, which the long period after the latter fungicides spray is capable to cause metabolic changes to be not poisoned (Ashour, 2009). Plant extracts such as neem (*Azadirachta indica*) and garlic (*Allium sativum*), for controlling powdery mildew fungi of several plants were reported by Ehteshamul *et al.*, (1998), Abd-El- Sayed, (2000) and Tohamy *et al.* (2002). Morsy *et al.*, (2009) reported that, natural infection by cucumber powdery mildew was decreased by spraying garlic or onion extracts at concentration of 9% and increased of length, fresh and dry weight of shoots and roots as well as number of flowers/plant compared with control. Rettinassababady *et al.* (2000) found that, significant reduction in blackgram powdery mildew incidence due to foliar spray of neem oil (3%) might be due to the presence of sulphur containing compounds viz., nimbidin and azadirachtin. Ebrahim, and Helmy-Karima (2016) found that, thyme essential oil completely prevented the sugar beet powdery mildew incidence in the field.

The present study, was designed to study, (1) isolate the pathogen of pepper powdery mildew (2) evaluate the five systemic fungicides and/or two different concentrations of garlic and thyme extract against powdery mildew disease under greenhouse and field conditions (3) study the effect of applications the best tested fungicides followed by spraying pepper plants with garlic and thyme extract on powdery mildew incidence.

Materials and Methods

Isolates source and identification of the causal pathogen:-

Powdery mildew isolates were collected during autumn growing season (2016) from naturally infected leaves samples of diseased pepper showing powdery mildew symptoms which collected from different localities of Nubaryia region, *i.e.* Belal, Adam and Aboel-Youser villages. Microscopic perorations for identification of the causal pathogen were made by placing epidermal strips, taken from the infected pepper leaves, (bearing the fungal conidiophores and conidia) on glass slides then stained with lactophenol cotton blue and covered with cover glass then examined using light microscopic at 10, 20 and 40x.

Pathogenicity test:

Inoculation technique:-

Pepper seeds (cv. California winder), obtained from Veg. Res. Depts., Horticulture Res. Inst., Agric. Res. Center, were sown in ordinary seed boxes filled with peat moss-vermiculate (1:1, w/w) for 35 days under greenhouse conditions (25±5 °C and 75-90% R.H.), then transplanted in pots (30 cm. diam.) filled with sterilized sandy-clay soil (1: 1, w/w), at the rate of 3 seedlings/pot. The growing plants (7-week-old) were inoculated by dusting conidia from naturally infected pepper leaves. The inoculated plants were then incubated under greenhouse conditions (25±5°C and 70-80% R.H.). After inoculation, the plants were covered with polyethylene bags for 24 hours to maintain enough moisture necessary for the successful infection and kept under greenhouse conditions at 25 ± 5°C. Other healthy plants were kept without inoculation to serve as control. The grown plants were irrigated when necessary and fertilized twice, *i.e.* three and five weeks after sowing, by the crystalon compounded 18/18/18 (1g/pot). Inoculated plants were examined daily for disease symptoms and powdery mildew severity was calculated 10, 20 and 30 days later.

Stock cultures of the causal pathogen:-

Pepper plants, cv. California winder were transplanting in pots as mentioned before at the rate of (3 seedlings/pot). Five weeks after transplanting, seedlings were artificially inoculated by dusting conidia from naturally infected pepper leaves, and incubated under greenhouse conditions (25±5°C and 75-90% R.H.). Diseased leaves were used as inoculum source in the following experiments.

Disease assessment:-

Powdery mildew severity was determined using the proposed scale by Ullasa, *et al.* (1981), of which: Resistant: no symptoms, moderately resistant: with 10% of the leaf area affected, moderately susceptible: with 11-20% of the leaf area affected, Susceptible: with 21-50% of the leaf area affected

and highly susceptible: with 51% or more of the leaf area affected. The percentage of disease severity was calculated using the equation suggested by Townsend and Heuberger (1943) as follows:

$$P = \frac{\{a \text{ (rating no.)} \times b \text{ (no. leaves in rating category)}\}}{N \text{ (Total no. leaves)} \times K \text{ (Highest rating value)}} \times 100$$

Greenhouse experiments:

Five systemic fungicides, *i.e.*, Leader 45% EC (prochloraz), Penazole 10% EC (Penconazol), Daconil 75% SC (Chlorothalonil), Topas 100 10% EC (Penconazole) and Flint 50% WG (Trifloxystrobin) as well as two different concentrations (3, and 6%) of garlic and thyme extracted according to Baiuomy, (1997). During mid of April 2016 the growing pepper plants (21 days after transplanting) were sprayed with either recommended doses of tested fungicides or 3 and 6% of tested plant extracts, three days before inoculated by dusting conidia from naturally infected pepper leaves of *L. taurica* isolate (No. 2). The inoculated plants were incubated under greenhouse conditions (25±5°C). The grown plants were irrigated when necessary and fertilized twice, *i.e.* three and five weeks after sowing, by the crystalon compounded 18/18/18 (1g/pot) Four replicates inoculated with the tested pathogen only, were sprayed with water only and served as control. Disease severity was assessed 10, 20 and 30 days after spray of either fungicides or plant extract. Also, plant length (cm) and foliage fresh weight (g/plant) were determined 8 weeks after transplanting.

Field experiments:

Field experiments were conducted at Nobaryia locality (Beheira governorate) during Autumn 2016 and 2017 growing seasons to evaluate the effect of three fungicides, *i.e.* Leader 45%, Penazole 10%, and Flint 50%, as well as garlic and thyme extract at the rate of 6%, against the natural infection of pepper powdery mildew. Pepper seedlings (cv. California winder), 35-day-old, were transplanted on first of Augustus under open field conditions. All agricultural practices, *i.e.* fertilization as well as weeds and pests control, were applied according to the standard recommendations of Ministry of Agriculture. When disease symptoms initially appeared (3-weeks-old after transplanting) on naturally diseased plants, the tested fungicides were sprayed at concentrations of 75ml (Leader 45%), 25ml (Penazole 10%) and 25g (Flint 50%) per 100 liter water, as well as garlic and thyme extract at the rate of 6%. The applied treatments were as follows: 1) Plants received four sprays, with the tested fungicides at aforementioned doses, at 10 days intervals. 2) Plants received four sprays, with of each garlic or thyme extract at the rate of 6%, at 10 days intervals. 3) Plants received two sprays, with of each aforementioned fungicide, at 10 days intervals (until two weeks before first harvesting of fruits) and then received another two sprays with of each garlic or thyme extract at the rate of 6% at ten days intervals). 4) A set of plants, unsprayed by fungicides or garlic and thyme extract, was served as check treatments. Four plots (21m) were used as replicates for each treatment. The disease severity was assessed for each growing season. Also, fruit yield (kg/plot) and its components, Number of fruits/plant, average of fruits weight per plot were calculated.

Chemical properties:

Chlorophyll A and B contents:

According to Holden (1965), chlorophyll A and B were estimated. Potato leaf samples were cut into small pieces and 0.25g were grinded in acetone to extract pigments and then filtered to get rid of debris. The volume of acetone is restored to 25 ml. Chloroplast pigments were determined by measuring the optical density at 663 and 645 nm and calculated using the formula devised by Arnold (1949). Chlorophyll A mg/L = 12.7* O.D. at 663 nm- 2.9*O.D. at 645nm Chlorophyll B mg/L = 22.4* O.D. at 645 nm- 4.68*O.D. at 645nm Chlorophyll A+B mg/L = 8.02*mg/L* O.D. at 663nm +20.20*O.D. at 545nm where O.D was the optical density reading.

Statistical analysis:

Obtained data were statistically analyzed using the standard procedures for complete randomized block and split designs as mentioned by Snedecor and Cochran (1980). The averages were compared at 5% level using least significant differences (L.S.D.) according to Fisher (1948)

Results and Discussion

1. Morphological characters of the causal pathogen

When pepper infected leaves were examined with the microscopically examination to the epidermal strips, it showed that long septate conidiophores were observed arising from the endophytic hyphae branched and emerging singly or in cluster forms or bundles through the stomata of the lower leaf surface. Microscopic examinations revealed that endophytic mycelium of *L. taurica* consists of hyphae that enter the host plant tissues through stomata and produce haustoria in the mesophyll cells of the plants, long and often branched conidiophores are formed from internal mycelia and emerge through stomata to produce conidia borne singly. Two kinds of conidia are produced. The first-formed conidium, sometimes termed the primary (pyriform) or lanceolate conidium, exhibits a narrowed apex. The other kind of conidium, termed the secondary or cylindrical conidium, is formed following production of the primary conidium and lacks the narrowed apex. Appresoria developed on endogenous mycelia within leaf tissue. On the other hand, the sexual stage of *Leveillula taurica* was not noticed during the present investigation. These results are in agreement with those obtained by Mohan (2005) and Little (2006).

2. Pathogenicity tests:

Pathogenicity test of *L. taurica* isolates, collected from different localities of Nubaryia sector, *i.e.* Belal, Adam and Aboel-Youser villages were tested for their virulence, under greenhouse conditions, on highly susceptible pepper plants (cv. California winder). Data presented in Table (1) show that, all tested isolates were pathogenic to pepper plants under greenhouse conditions but differed significantly in their virulence.

Table 1: Pathogenic ability of *L. taurica* isolates to induce powdery mildew symptoms on pepper plants under greenhouse conditions

Village	Isolate No.	Disease severity (%)			Mean
		10 days	20 days	30 days	
Belal	1	27.0	32.7	37.4	32.4
	2	35.4	38.8	44.5	39.6
	3	24.9	33.5	30.0	29.5
	4	23.4	29.7	34.1	29.1
Adam	5	32.4	34.5	36.6	34.5
	6	32.6	35.4	37.5	35.2
	7	28.2	30.9	34.9	31.3
Aboel-Youser	8	29.4	34.1	35.4	33.0
	9	17.1	21.5	25.9	21.0
* Control		00.0	00.0	00.0	00.0

L.S.D at 5% for: Isolates (I) = 1.9, Time (T) = 1.1, I x T = 3.4

* Plants un-inoculated.

The disease severity, 10 days after inoculation, Belal, village isolate (No.2) was the most aggressive one (35.4% disease severity), followed by Adam village isolate No.6 and No.5 (32.6 and 32.4%, respectively). Both isolates Nos. 8, 7, 1, 3 and 4 showed moderate aggressiveness (29.4 and 28.2, 27, 24.9 and 23.4% respectively). Meanwhile, the third isolate of Aboel-Youser (No.9) was of lowest effect in this concern (17.1%). The same trend of results was recorded 20 and 30 days after inoculation. The morphological characteristics of the pepper powdery mildew fungus were stable within the ranges described by recent reports from elsewhere in Europe, North America and Australia (Ahila Devi and Prakasam, 2014, Daunde *et al.*, 2018) and agreed with the description of these characters in earlier UK studies (Fletcher *et al.*, 1988, Cook & Fox, 1992, Whipps *et al.*, 1998). These data indicate that the natural population of the pepper powdery mildew pathogen (*Leveillula taurica*) has different pathotypes differed from locality to another. These results are in agreement with (Mohamed, 1994).

3. Greenhouse experiment:-

Data presented in Table (2) show that all tested fungicides and tested plant extract caused significant reduction in the severity of powdery mildew with significant increase in plant length and foliage fresh weight of pepper plants in comparison with check treatments. However, tested fungicides were more efficient in these regards than tested plant extract. Flint 50% was the most effective fungicide followed by Penazole10%, and Leader 45%, as they reduced the disease severity to 2.4, 2.5 and 2.8% and increased plant length to 42.3, 42.1 and 41.1 cm and foliage fresh weight to 134.7, 134.3 and 130.3 g/plant, respectively. Meanwhile, garlic extract at the rate 6% was the most effective plant extract followed by thyme extract at the rate 6%, as they reduced disease severity to 14.2 and 15.0% and increased the plant length to 35.3 and 35.2 cm and the foliage fresh weight to 110.7 and 110.6 g/plant, respectively. These results are in agreement with Sharmila *et al* (2004), Karaogluidi & Karadimo (2006) and Ashour (2009).

Table 2: Effect of systemic fungicides and two plant extracts on powdery mildew severity and some vegetative characters of pepper plants under greenhouse conditions.

Treatments		Disease severity (%)	Average plant length (cm)	Average foliage fresh weight (g/plant)
Fungicide	Leader 45%	2.8	41.1	130.3
	Penazole10%	2.5	42.1	134.4
	Daconil 75%,	3.6	41.1	129.5
	Topas 10%	3.4	41.0	129.5
	Flint 50%	2.4	42.3	134.7
Plant extract	Garlic 3%	16.9	34.3	105.0
	Garlic 6%	14.2	35.3	110.7
	Thyme 3%	18.9	33.8	105.7
	Thyme 6%	15.0	35.2	110.6
Check	43.0	27.6	86.5	
L.S.D at 5%		2.95	3.16	2.95

4. Field experiment:-

On the basis of the highly efficient, three fungicides, i.e. Penazole10%, Flint 50% and Leader 45%, as well as garlic and thyme extract at the rate 6% were chosen to evaluate their efficiency in managing the natural disease infection under field conditions. Data in Table (3) indicate that, in comparison with check treatments, all tested the fungicides and/or plant extract caused significant reduction in the natural infection of powdery mildew on pepper plants, and enhanced the average fruit yield in both growing seasons. In this respect, tested fungicides were also more efficient than plant extract. Moreover, application of tested fungicides only was more effective in this regard than the application of them followed by tested garlic and thyme extract at the rate 6%.

On the average, application of Penazole10% alone recorded the highest values' either in reducing the disease severity or in rising the average fruit yield (being 2.7% and 130.8 kg/plot, respectively) followed by Flint 50% (being 3.0% and 128.2 kg/plot, respectively) then Leader 45% (3.1% and 127.7 kg/plot, respectively). On the other hand, applying any tested plant extract alone recorded, on the average, the lowest efficiency in reducing disease severity (being 14.8 and 15.0%) and low values of fruit yield (being 104.8 and 104.4 kg/plot) for garlic and thyme extract at the rate 6%' respectively. Chemicals are the most common and practical method for the management of powdery mildew. These results may be due to the strobilurins form an important new class of fungicides, with a unique mode of action that targets mitochondrial respiration by blocking electron transfer in cytochromes b and c. Trifloxystrobin (Flint) is a new strobilurin fungicide, active as a foliar spray against a wide range of fungal plant pathogens under both greenhouse and field conditions. It has shown excellent prophylactic activity against powdery mildews in grapevines, apple, mango and nectarine trees (Tally *et al.*, 1998) and has suppressed sporulation and further development of *U. necator* on grape leaves bearing sporulating colonies, or on mildewed clusters in the field (Reuveni, 2000).

Table 3: Effect of fungicides and/or two plant extracts on powdery mildew mildew of pepper plants (cv. California winder) under field conditions during 2016 and 2017 growing seasons

Treatments	Disease severity (%)		Mean
	during		
	2016	2017	
Penazole 10%	2.7	2.6	2.7
Flint 50.0%	3.1	2.8	3.0
Leader 45%	3.2	2.9	3.1
Garlic at 6%	15.3	14.8	14.8
Thyme at 6%	15.0	14.4	15.0
Penazole 10% then garlic at 6%	5.6	5.4	5.5
Penazole 10% then thyme at 6%	6.1	5.9	6.0
Flint 50.0%, then garlic at 6%	5.4	5.1	5.3
Flint 50.0%, then thyme at 6%	5.9	6.1	5.8
Leader 45%, then garlic at 6%	6.1	5.9	6.0
Leader 45%, then thyme at 6%	6.7	6.7	6.7
* Control	47.5	45.2	46.6
Mean	6.8	6.7	
	Treatment (T) =		2.92
	Season (S) =		n.s.
	T x S =		3.39

* Values of the control treatments were not calculated in the mean of the treatments.

Data presented in Table (4) show that all tested fungicides and tested plant extract caused significant increase in the No. of fruits/plant, average fruit weight and total yield in the two seasons compared with check treatment, the data illustrated that, all tested the fungicides and/or plant extract caused significant enhanced the No. of fruits/plant and average fruit yield in both growing seasons. In this respect, tested fungicides were also more efficient than plant extract. Moreover, application of tested fungicides only was more effective in this regard than the application of them followed by tested garlic and thyme extract at the rate 6%. Overall, the use of systemic fungicides for the management of powdery mildew found to be very effective than spraying of non-systemic fungicide.

Table 4: Total yield and its components of pepper plants (cv. California winder) as affected with systemic fungicides and two plant extracts under field conditions during 2016 and 2017 growing seasons

Treatments	Yield parameters							
	2016				2017			
	Fruits /plant		Fruits /plot		Fruits /plant		Fruits /plot	
No.	Increase %*	Kg.	Increase %	No.	Increase %	Kg.	Increase %	
Penazole 10%	28.5	93.9	560.0	128.9	29.9	100.6	585.5	124.4
Flint 50.0%	28.6	94.6	543.0	122.1	28.0	88.1	509.1	95.1
Leader 45%	25.4	72.8	487.5	99.3	26.0	74.5	499.1	91.3
Garlic 6%	16.6	12.9	305.5	24.9	17.0	14.1	327.3	25.4
Thyme 6%	19.1	30.1	303.1	23.9	19.2	28.9	309.6	18.7
Penazole 10%, then garlic 6%	24.3	65.3	482.6	97.3	25.5	71.1	502.9	92.8
Penazole 10%, then thyme 6%	18.1	23.1	338.6	38.4	19.2	28.9	358.7	37.5
Flint 50.0%, then garlic 6%	24.2	64.6	504.0	106.1	24.3	63.1	498.2	91.1
Flint 50.0%, then thyme 6%	23.6	60.5	462.7	89.1	25.4	70.5	498.2	91.1
Leader 45%, then garlic 6%	23.4	59.2	468.9	90.4	23.7	59.1	476.6	82.7
Leader 45%, then thyme 6%	24.5	66.7	366.4	49.8	21.5	44	330.9	26.8
Control	14.7	—	244.6	—	14.9	—	260.9	—
LSD at 0.05%	0.562		0.634		0.426		0.575	

*Comparing with untreated control.

These results may be due to the strobilurins form an important new class of fungicides, with a unique mode of action that targets mitochondrial respiration by blocking electron transfer in cytochromes b and c. Trifloxystrobin (Flint) is a new strobilurin fungicide, active as a foliar spray

against a wide range of fungal plant pathogens under both greenhouse and field conditions. It has shown excellent prophylactic activity against powdery mildews in grapevines, apple, mango and nectarine trees (Tally et al 1998) and has suppressed sporulation and further development of *U. necator* on grape leaves bearing sporulating colonies, or on mildewed clusters in the field (Reuveni, 2000).

These results are in agreement with those obtained by Sharmila *et al* (2004) and Karaoglanidi & Karadimo, 2006. In this regard, spraying the onion plants or soaking seeds with licorice extract gave the highest yield as mentioned by Al-Marsumy and Al- Sahaaf (2001), Hamood (2010). Moreover, Abd-Moniam *et al.* (2011), Zuhair *et al.* (2011) and Faraj *et al.* (2012) on strawberry found that, licorice root extract increase significantly some yield characters as pod weight and length, plant yield and total yield of kidney bean.

Several workers reported that, propiconazole, myclobutanil, penconazole, triadimefon and hexaconazole were found to be effective in reducing powdery mildew incidence in different crops (Singh *et al.*, 2000, Sharmila *et al.*, 2004, Singh, 2006, Pramod Prasad and Dwivedi, 2007 and Akhileshwari *et al.*, 2012) and some of them found that the efficacy of penconazole against *E. cichoracearum* was due to reduction of ergosterol biosynthesis in pathogen, which interferes with haustoria formation.

Chlorophyll contents:

Effect of some organic acid and mineral salts:

Data in Table (5) reveal that treating pepper plants with all tested fungicides and tested plant extract increased their contents of chlorophyll A, chlorophyll B and chlorophyll A+B contents in leaves of treated plants. Garlic at 6% was the highest effective treatment among the tested treatments in raising the leaves contents of chlorophyll B (16.9 mg/L) and chlorophyll A+B (26.34 mg/L) whereas, the highest content of chlorophyll A was recorded with garlic at 6% compared with control. As well as, all tested fungicides then garlic at 6% and/or thyme at 6% increased also the total chlorophyll A+B in leaves of treated plants comparing with check treatment. Amooaghaie and Golmohammadi (2017) found that 25% vermicomposting substitution promoted the best seedling emergence indices, chlorophyll and carotenoid contents in of *Thymus vulgaris* and the highest essential oil content were observed in 50% vermicomposting substitution. The use of plant extracts was previously reported as alternative method for controlling many powdery mildew diseases including powdery mildew of pepper Ehteshamul *et al.*, (1998), Abd-El- Sayed, (2000), Rettinassababady *et al.* (2000), Tohamy *et al.*, 2002) and Ebrahim, and Helmy-Karima (2016).

Table 5: Effect of systemic fungicides and two plant extract on chlorophyll contents of pepper plants (cv. California winder) under field conditions

Treatments	Chlorophyll (mg/g fresh weight)		
	Chlorophyll A	Chlorophyll A	Chlorophyll A
Penazole10%	9.45	10.33	21.50
Vectra 10%	8.76	8.76	17.44
Leader 45%	9.45	11.45	21.87
Garlic at 6%	15.99	16.90	26.34
Thyme at 6%	14.75	14.35	25.55
Penazole10%, then garlic 6%	15.88	17.2	26.55
Penazole10%, then thyme 6%	14.88	14.77	26.00
Flint 50.0%, then garlic 6%	15.00	16.88	26.99
Flint 50.0%, then thyme 6%	14.15	14.95	26.55
Leader 45%, then garlic 6%	14.44	15.88	26.55
Leader 45%, then thyme 6%	15.15	15.00	25.95
Check	7.21	9.32	16.53

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