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Studies of Climate Impact on Occurrence of Bunch Rot Disease Caused by *Botrytis cinerea* on Grapevine Using Some Weather Parameters and Some Controlling Programs

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ABSTRACT

In Egypt, grapes are one of most broadly grown fruit crops, it comes second only to citrus. Egypt is ranked fourth worldwide in the global production volume of grapes (FAOSTAT). *Botrytis cinerea* causing grapevine grape bunch rot is one of the most hazardous diseases, which may cause enormous damage both during plant growth and in post-harvest phase causing great losses. Disease incidence % and disease severity % of bunch rot were assessed on Thompson seedless grape during 2019 and 2020 seasons in three different governorates in Egypt. Disease occurrence was more prevailing in Gharbia governorate than Giza and Qalyoubia. Pathogenicity test on Thompson seedless grape bunches showed that *B. cinerea* which isolated from bunch rot are pathogenic. 70% and 80% disease severity of grape bunches with *B. cinerea* was estimated at end of cold storage after 25 and 30 days respectively. Five fungicides *viz.*, ABASH 50%WG,Amistar top 32.5% EC, Topsin M70WP, NO ROT 38%WG and Imazalil 50%EC were used to control bunch rot disease under field conditions. Trial were conducted by spraying with compounds at commercial recommended dose each alone at the three different time, first of April or first of May and or first of June.The highest percentage of reduction happened, when grape clusters sprayed with Amistar top and Topsin M70 in the first application time which fungicides sprayed at the first of April.Disease development was monitored on grapes in *vitro and vivo*.In vitro, under controlled conditions in a growth chamber, we observed the disease severity at different temperature degrees of 10, 15, 20, 25 and 30 ˚C each with different relative humidity (RH) levels of 70, 80, 90 and 100% and presence or absence of wetness. Optimal conditions for highest disease severity (DS) were at temperature between 15 ˚C and 25 ˚C with RH greater than 90% and precipitation > 0.5 mm. In vivo, this study was conducted in Gharbia Governorate to study the Disease severity of *B. cinerea* causing grape bunch rot of grapevine in several district of Gharbia Governorate during season 2019 / 2020. In all study areas, 100 grapevines were examined against infection of bunch rot involving various climatic parameters collected by Agro-weather stations. Collected data was then used for assessment of relations between effect of different temperature degrees and Relative humidity percentages on the disease incidence and severity.

Keywords: Gray mold of grape; *Botrytis cinerea*; early earning.

INTRODUCTION

In Egypt, Grapevine (*Vitis vinifera* L., family *Vitaceae*) is one of most widely grown fruit crops, secondly after citrus. Egypt's grape production is spread from Alexandria to Aswan, enabling the continued availability of fresh table grapes from May to November. Egypt is ranked fourth world wide in global production volume of table grapes. In 2018, Egypt producedabout 1.75million tons of grapes from 78,853 ha (FAOSTAT). From 2017 to 2018, Egypt exported grapes with 221.5$ million representing 2.5% share of the international exportation (HITAC trading). Grape agriculture is affected by various disease problems all over the world and in Egypt.

Botrytis cinerea is a wide range host fungus, where it is accountable for economic losses in fruit, vegetables and flowers (Droby and Lichter 2004;Wahab 2012).Gray mold of grape, also known as Botrytis bunch rot, is a fungal disease caused by the fungus *B. cinerea*. It's one important of grapes diseases in the world, which can cause serious losses in grape yields. Fungus can occur anytime during the growing season, but most commonly occurs near the harvest time. All aerial parts of host plant can be infect by fungus, Flower infections are the main reason for latent infections leading to damages during the storage. Usually, *B. cinerea* infects ripe berries. First infected berries become soft and watery, which under high relative humidity and moisture become covered with the grey sporulating growth of the fungus.Wet and humid environmental conditions increase the disease progress. Optimal conditions for disease development are at temperatures between 20 and 25˚C (Ciliberti, 2015), free moisture, and high relative humidity (above 90%). While Fedele, (2020) concluded that it is at temperature between at 18 and 32 ˚C.
Considerable losses in crop yield led to rigorous management for a high value crop like grapes in order to sustain production. Neglectful usage of chemicals can cause human diseases, environmental problems and pollution. Subsequently, effect of pesticide overuse is internationally concerned leading to more attentionfor production of crops free from chemical substances. Using disease management programs helps in lowering fungicide applications number, which decreases production costs, chemical residues in crops produced, and risk of development of fungicide resistance in pathogens. There has been a noteworthy effort in studying climatic conditions effect on important diseases and crops worldwide. Consequently, Objective of this study was studying weather parameters and correlating them with the disease incidence and disease severity in order to produce a preciseand effective disease warning model.Study also focused on determine the best chemical control time for bunch rot disease, according to optimal application times of some fungicides.

MATERIALS AND METHODS

1. Survey of bunch rot disease incidence and severity in grapevine orchards.

Survey was conducted after the first disease symptoms were observed during seasons 2019 and 2020 on Thompson seedless grapevine orchards in three Egyptian governorates, *i.e*. Gharbia, Qalyoubia and Giza. Trees were normal growth, uniform in vigor as possible. Common agriculture practices were treated in both seasons. The trees were left to the natural infection by bunch rot disease and fifteen trees were used as three replicates. Five bunches were selected randomly from each tree. Disease incidence % was assessed as infected bunches relative number to total bunches. Disease severity % was estimated depending on modified scale (0-3) by Townsend and Heuberger, 1943 as follows:

0 = No bunch rot observed (healthy).
1 = 1-10% of bunch rot affected.
2 = 11 - 20% of bunch rot affected.
3 = up to 20 % of bunch rot affected.
Disease severity was calculated using formula:

$$\text{Disease severity\%} = \frac{\sum(n \times v)}{3N} \times 100$$

That:
n = Number of the infected bunches in each category.
v = Numerical values of each category.
N = Total number of the examined bunches.

2. Isolation and Identification of Causal Agent.

Small pieces of grape bunches, visually observed to contain both diseased and healthy tissues transferred into 9 cm diameter Petri dishes containing potato dextrose agar (PDA) medium and incubated at 24 ± 1^{o}C for 3-5 days in the dark**.** Purified fungus were identified on the basis of their morphological characteristics, according to Barnett and Hunter (1986).

3. Pathogenicity test.

Fungus isolate obtained from grape bunch rot disease was tested for its ability to cause the same disease condition in a healthy grape bunch to prove the Koch’s postulate. Thompson seedless were collected from farm of Gharbia governorate maturity, and free from visible wounds, defects rots and decay before inoculation, Thompson seedless grape bunches were thoroughly washed under tap water , surface sterilized for 30 sec in a 1 % NaOCl solution followed by washing three times in sterilized water then were prepared for inoculation using the isolated fungus. Each bunch wounded by small scratch, then inoculated by spore suspension of the fungus isolate (1×10^{6} spores/ml) from 7-days old cultures following the methods of Lachhab *et al*., (2015). Inoculated and control bunches were placed on cardboard box so that they did not touch each other, and the cardboard boxes were enclosed in polyethylene bags to maintain high humidity. Each treatment contained three replicates (one kilogram grape bunches for each replicate). Disease severity of grape bunches with *B. cinerea* was estimated at end of cold storage. The disease severity % was estimated depending on the modified scale (0-3) by Townsend and Heuberger, 1943 as mentioned above.

4. In field.

Disease control.

In 2019 and 2020 growing seasons, field trials were carried out at the experimental grape orchard (cv. Thompson seedless, 12-year old) Gharbia governorate to compare the effect of three application times according to the time of application on the effectiveness of fungicides for controlling grape bunch rot disease to choose one treatment program in order to reduce the amount of fungicide required. Also, this study was undertaken to evaluate the efficiency of five fungicides for controlling grape bunch rot disease. This experiment depended on the time of application during the two successive seasons and was divided into three groups (three programs) of application to reduce the number of the fungicide treatments. In the first application group, the tested fungicides were sprayed at the first of April, in second application group, the tested fungicides were sprayed at the first of May, in the third application group, the tested

fungicides were sprayed at the first of June. In the control treatment the trees were sprayed with water. Three replicates were used for each treatment and five trees were used as replicates in each group.Tested fungicides separately were sprayed three times at the 15-day interval. Fungicides in Table 1 were used. The trees were left to the natural infection by bunch rot disease. Grape tree was carefully examined, five bunches were selected randomly from four geographical directions of each tree in the end of June. Disease severity % was estimated depending on modified scale (0-3) by Townsend and Heuberger, 1943 as mentioned above.
Efficiency of spray treatments was estimated using the following formula**:**

$$\%\ \text{Efficiency} = \frac{\text{Disease severity in control} - \text{Disease severity in treatment}}{\text{Disease severity in control}} \times 100.$$

5. Disease early warning model: In laboratory (Growth chamber):

The grey mold disease model was designed and tested for several times under laboratory conditions using growth chamber before switching to the validation experimental phase in field. Developed model is based on short-term observations (hourly weather data) analyzing the correlation between microclimatological factors such as (temperature, relative humidity, wetness) and disease severity. Then, model is computerized as a software that provides a warning message to indicate whether fungicides application is needed or not in appropriate time for disease infection.

The model is based on the rules of system analysis to identify events that triggers perfect conditions for infection which are Event A and B (Figure2).We observed that Event A is triggered when model identifies at least X accumulated dynamic summation hours of RH > 90% and temperature between Y and Z according to data tabulated in (Table 2). Also, it looks for Event B which is precipitation > 0.5 mm. Furthermore, this model doesn't stop at this point, it keeps working all through growing season to give warnings for any secondary disease infection possibilities.

Weather data collection:

Automated agro-weather stations (Metos) was used to monitor and collect microclimatic data in the field, Station collects data like temperature, relative humidity, wind speed, leaf wetness, precipitation and global radiation. Station is provided with a built-in hard drive that saves the collected data and also the data is uploaded to the domain website to ease access.

Model validation

This study was conducted in Gharbia Governorate to study *Botrytis cinerea* Disease severity causing grape bunch rot of grapevine in several district of Gharbia Governorate during seasons 2019 / 2020. In all study areas, 100 Thompson seedless grapevines were examined against infection of bunch rot.

Fungicide application was applied when software indicated an alarmin comparison with standard time-table application recommendations. An inspection was done at least once a week for the appearance of disease symptoms. Bunch rot disease was evaluated on the bunch using the following scale on 15 bunches for each replicate, three replicates of each treatment. Disease Severity (DS) was calculated according to the following formula:
Disease severity (%) = $\sum$ (ni × vi) ×100 (V × N)
That:

(ni) = the number of bunches with disease rating.
(vi) = disease score (0 = no symptoms).
1= 1-10% infection on the bunch.
2= 11-20% infection on the bunch.
3= up to 20 % infection on the bunch.
(N) = the total number of bunches investigated.
(V) = the highest disease score (3).

Experimental design and statistical analysis: Experiments were adopted according to Complete Randomized Block Design (CRBD) and data were statically analyzed using the Fisher's LSD according to Gomez and Gomez (1983). Duncan's Multiple Range tested (Duncan, 1955) were used to compare differences among means.

RESULTS

1. Disease Survey.

Survey during 2019 and 2020 growing seasons in Figure 1 show that the highest percentage in disease incidence and severity were recorded in Gharbia governorate the two seasons, respectively, followed by Giza governorate, while Qalyoubia governorate recorded the lowest disease occurrence.

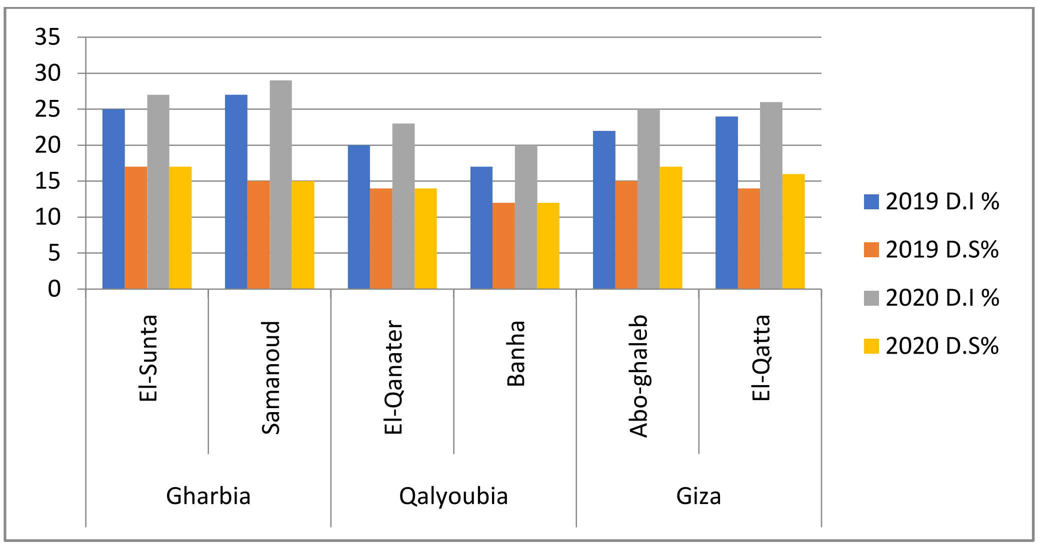


Figure 1. Disease incidence and severity % of bunch rot diseasein the six sites in the three governorates during 2019 and 2020 seasons.

2. Isolation and identification of the causal agent.

Botrytis cinerea was isolated and purified from grape bunch rot disease using single spore method. Pure culture stocks of the isolated fungus were kept on PDA slants at 4°C for further study.

3. Pathogenicity test.

Koch's postulates were fulfilled by artificially infecting Thompson seedless cultivar. after ten days, tests confirmed that pathogenicity of the fungus isolate *B. cinerea* able to cause grape bunch rot disease compared with control treatment. Generally, grape bunch rot was recorded maximum percentage on bunches inoculated with *B .cinerea*, at 70% and 80% disease severity of grape bunches with *B. cinerea* was estimated at the end of cold storage after 25 and 30 days respectively.

4. Disease control.

Grape bunch rot disease developed early and quickly eventually reaching moderate to severe levels and usually lasts six to seven weeks starting approximately at first week of April and ending around the end of June in field. One of most important factors to consider when making fungicide application is timing of the application and how long can you expect protection from each fungicide. Fungicides were applied three times in an effort to reduce losses due to this disease. During seasons 2019 and 2020 were favorable for the development of infection grape bunch rot disease caused by *B. cinerea* which resulted in 12, 14 and 15% disease severity in the first year and 13, 15 and 15% disease severity in the second year in control treatment which estimated in April, May and June. Five fungicides were evaluated for their efficiency for controlling grape bunch rot disease. Also, the experiments were undertaken to compare the effect of three application programs according to the time of application on effectiveness of fungicides for controlling grape bunch rot disease under field conditions in Gharbia Governorate. Data in Table 3 mentioned that all tested fungicides significantly reduced disease severity of bunch rot disease during 2019 and 2020 growing seasons. In this respect, the highest percentage of reduction happened, when grape clusters sprayed with Amistar top and Topsin M70 in the first application group which fungicides sprayed at first of April 10-days interval. Also, Imazalil and NOROT gave moderate disease reduction when used at the same time of application. Data also show that, the efficiency of tested fungicides strongly depended on the time of application and time of application have markedly affected efficiency of the fungicides.

Table 3. Effect of different application times of treatments on grape bunch rot disease severity of Thompson seedless cv. during season 2019 and 2020.

Treatments	Severity of natural infection (%)											
	Season 2019						Season 2020					
	First application group (April)		Second application group (May)		Third application group (June)		First application group (April)		Second application group (May)		Third application group (June)	
	%D.S	%E.F	%D.S	%E.F	%D.S	%E.F	%D.S	%E.F	%D.S	%E.F	%D.S	%E.F
ABASH 50% WG	3.0 B	75	5.0 B	64.28	7.0 B	53.33	3.0 B	76.92	4.0 B	73.33	8.0 B	46.66
Amistar top 32.5% EC	0.0 E	100	2.5 D	82.14	4.5 E	70.0	0.0 D	100	2.5 D	83.33	5.5 E	63.33
Topsin M70WP	0.0 E	100	2.5 D	82.14	5.5 D	63.33	0.0 D	100	2.5 D	83.33	5.5 E	63.33
NOROT 38% WG	2.0 D	83.33	3.0 C	78.57	7.0 B	53.33	2.0 C	84.61	3.0 C	80.0	7.0 C	53.33
Imazalil 50% EC	2.5 C	79.16	2.5 D	82.14	6.5 C	56.66	0.0 D	100	2.5	83.33	6.5D	56.66
Control	12.0 A	--	14.0 A	--	15.0 A	--	13.0 A	--	15.0 A	--	15.0 A	--

% D.S = % Disease severity. % E.F = % Efficiency.

Mean numbers within columns followed by different letters are significantly different *at P* <0:05 according to Duncan's Multiple Range Test.

5. Weather data collection and Model validation.

Grape bunch rot disease early warning software (GBR) Figure 3 was tested successfully for first time in Egypt. Results shown in Figure 4 showed that GBR model has detected the correct time for first application on 7th and second application on 3rd of May 2019. Then again, results displayed in Figure 5 verified that the GBR model precisely estimated correct time for first application in 4th and second application on 8th of May 2020. Disease severity in control reached 38 in the first season and 42 for the second. Therefore, three sprays were applied in both seasons of evaluation according to standard routine system while two sprays were applied according to the recommendation of GBR warning system.Therefore experimental treatments successfully controlled bunch rot disease in 2019 and 2020 growing season by 30% reduction in fungicide application.

Figure 3. Interface of grape bunch rot disease early warning software (GBR)

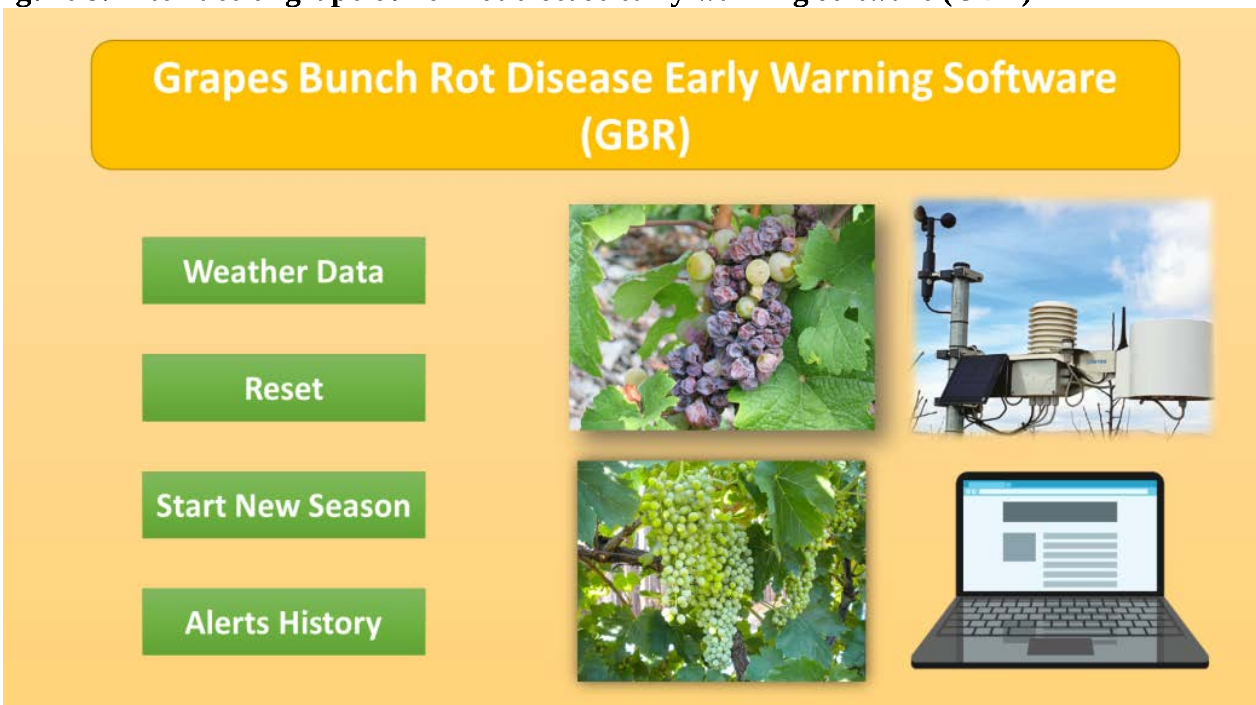
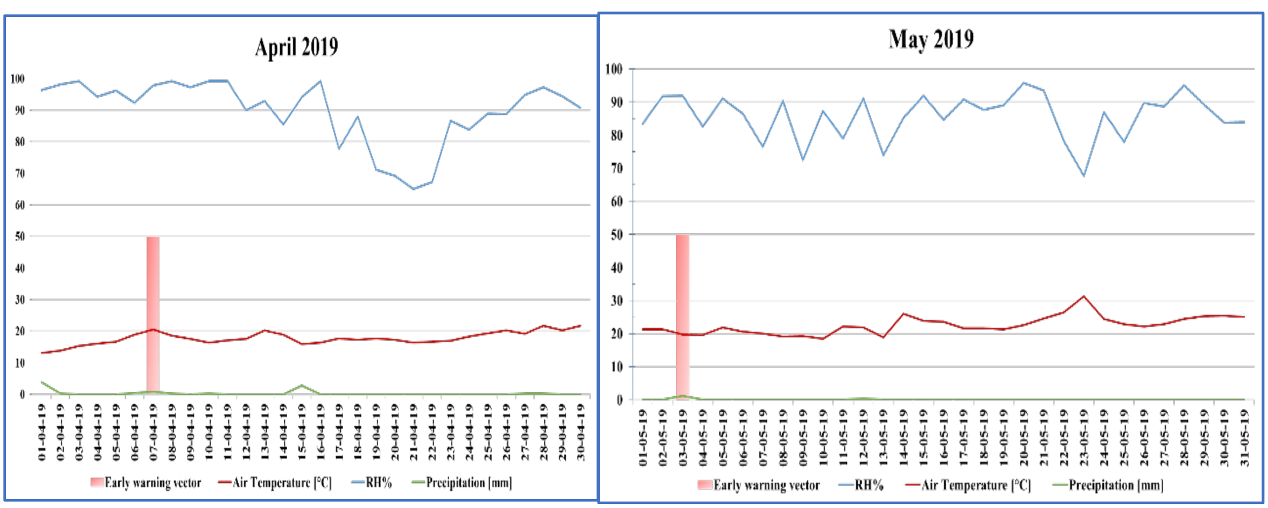


Figure 4. The daily microclimatic data for April, May and June 2019 growing season with the early warning vector indicator.



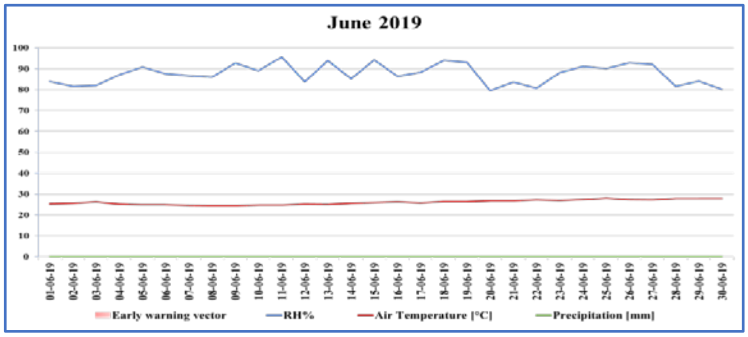
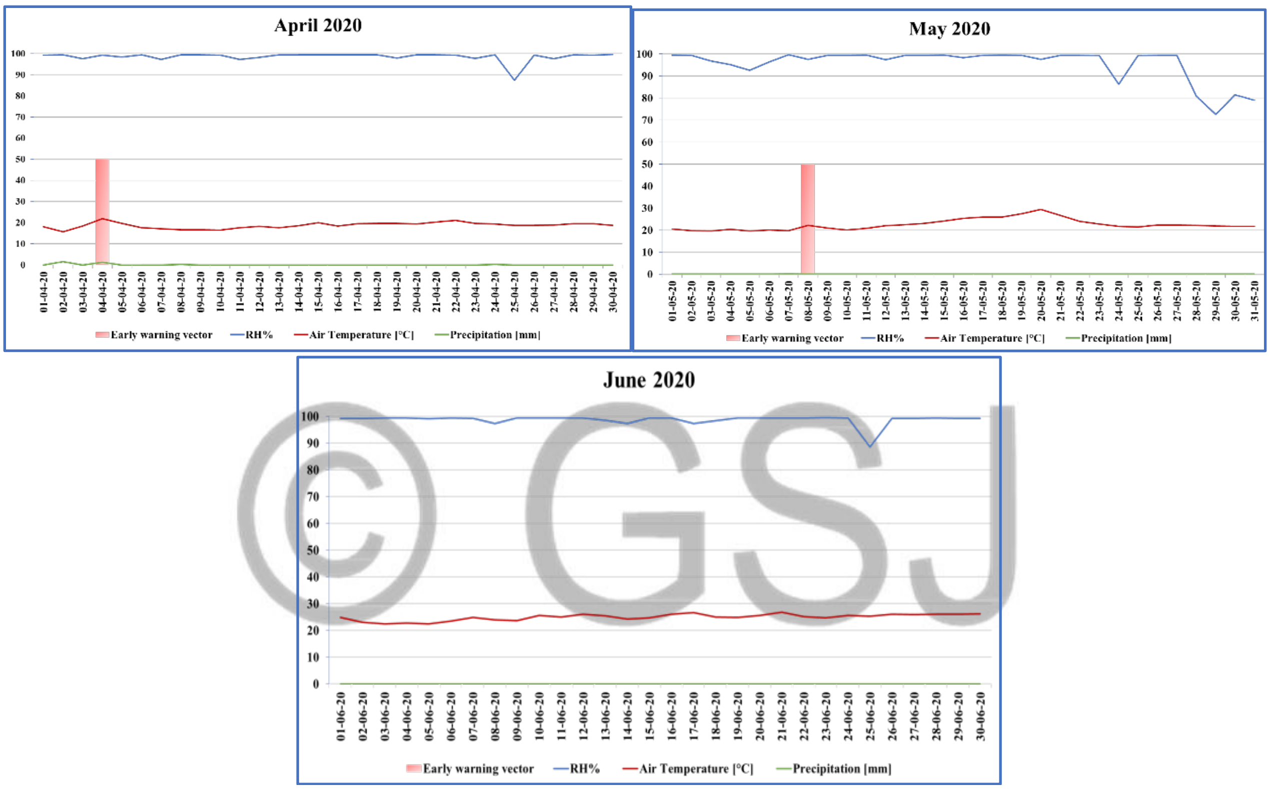


Figure 5. The daily microclimatic data for April, May and June 2020 growing season with the early warning vector indicator.



DISCUSSION

Grape orchards are increased rapidly as more desert areas are being planted every year for local market consumption and exportation. Grapes are subjected to the infection with several pre and postharvest diseases. The bunch rot disease caused by *Botrytis cinerea* is serious fungus on grape bunches all over the world where it cause great losses and also affect grape quality, especially during picking, packing, marking, exporting and storage. fungus *B. cinerea* is considered one of most serious fungi that attack grape berries during their progressive stages of development, especially when shipped at 0-1^{o}C by the sea for exportation or during cold storage for local market (Soltan *et all*.,2016). Survey during 2019 and 2020 growing seasons showed that the highest percentage in disease incidence and severity were recorded in Gharbia governorate in the two seasons, respectively, followed by Giza governorate, while Qalyoubia governorate recorded the lowest disease occurrence. A survey was conducted to observe postharvest diseases on locally grown grapes in Pakistan. Some grapes were observed showing gray mold or Botrytis bunch rot symptoms. Patches of soft brown tissue were observed on approximately 40% of the fruit Javed *et al*., (2017). The causal agent of gray mold has long been identified as *B. cinerea* (Chen *et al*., 2006:

Zhou *et al*., 2014). Pathogenicity tests on Thompson seedless grape bunches showed that *B. cinerea* which isolated from bunch rot are pathogenic. 70% and 80% disease severity of grape bunches with *B. cinerea* was estimated at end of cold storage after 25 and 30 days respectively. A survey of vineyards in 14 provinces of China was carried out to identify the causal agent of Botrytis bunch rot. A phylogenetic analysis showed that *B. cinerea* is the main causal agent of grape bunch rot disease in China. Pathogenicity tests showed that wounded grape berries are more susceptible to *B. cinerea* infection than non-wounded berries Jayawardena *et al*.,(2018). Grape bunch rot caused by *B. cinerea* is one of the important disease affecting Thompson seedless grapevines in Egypt. In Egypt, the routine spraying application is mainly based on studies forthe fungicide management scheme. Use of synthetic fungicides has been the traditional option to control plant diseases, but the extensive and prolonged use of synthetic fungicides has resulted in the resistance development of the fungi. Furthermore, the residual effect on the crop and environmental pollution are other problems associated with the use of these chemicals. Therefore, the use of pre- and postharvest chemical treatments is increasingly limited due to consumer concerns. Present focuses on the development of alternative means of controlling fungal in the field and during storage for local market. This alternative means, are safe to human and environment, have been initiated (Soltan *et al*., 2008; Abd El ghany *et al*., 2007; Abo Rehab *et al*., 2007; Rushed, 2001). Using great amount of fungicides affects the environment and human health on addition to its expensive costs which affects the farmer. It also affects the chances of the produced crop for exportation. Therefore lowering the number of fungicide application is a target for all beneficiaries. The highest percentage of reduction happened, when grape clusters sprayed with Amistar top and Topsin M70 in the first application time which fungicides sprayed at the first of April and three times 15-days interval. Imazalil and NO ROT gave moderate disease reduction. The efficiency of the tested fungicides strongly depended on the time of application and time of application have markedly affected the efficiency of the fungicides. Radwan & Hassan, (2019) found that time of application have markedly affected the efficiency of the fungicides that used. In most of the vineyards, a recommended program for the control of downy mildew, powdery mildew, and *B. cinerea* was generally followed. Sprays against downy mildew started at 10- to 15- cm shoot length and were applied until pea size. Fungicides used were Folpet, mancozeb (Dithane M45), and mancozeb/oxadixyl (Recoil 56/8 WP). Applications against powdery mildew started at 2- to 5-cm shoot length and were applied until 3 weeks before harvest. Fungicides used were penconazole (Topaz 10 EC), Pyrifenox (Dorado 48 EC) and triadimenol (Bayfidan 25 EC). Sprays against *B. cinerea* were applied at flowering, bunch closure, véraison, and 2 weeks before harvest. Fungicides used were iprodione (Rovral Flo 25 EC) and pyrimethanil (Scala 40 EC) De Klerk, (1985). An early warning software was designed and evaluated for two successive grape growing seasons in Egypt in order to manage Grape bunch rot with lower dosage of fungicides. Some other papers used different techniques for early detection of *B. cinerea* (Ciliberti, 2016 and Wahab, 2012). The achieved results were promising and proved that grapevine bunch rot can be controlled successfully in Egypt with smaller amount of fungicide applications using early warning models compared to standard schedule application. The number of sprays was reduced by 30 % during 2019 and 2020 grape-growing seasons.

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Table 1. Trade names, active ingredients and application rates of fungicides.

Trade name	Active ingredient	Dose/ 100 L water
ABASH 50%WG	**Boscalid**	**100 g**

Amistar top 32.5% EC	20% Azoxystrobin +12.5% Difenoconazole	60 ml
Topsin M70WP	Thiophanate methyl 70%	60 g
NO ROT 38%WG	Pyraclostrobin + Boscalid	50 g
Imazalil 50%EC	Imazalil	50 ml

Table 2. The basic rules of system analysis to identify event A.

Y = Temp from:	Z = Temp to:	X = 6 hours of RH	X = 12 hours of RH
21 °C	25 °C	Event Adetected	
15 °C	20 °C		Event Adetected

Figure 2. Grapes bunch rot disease model flowchart.