Pathological Studies of Some Local Isolates of S. sclerotiorumondifferent crops and Evaluation the Efficacy of Some Fungicides toControlLettuce White Mold.

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المستخلص:
أجريت هذه الدراسة لتسجيل حدوث مرض العفن الابيض المتسبب عن Sclerotiniasclerotiorum على محاصيل مختلفة وتقدير نسبة الإصابة Disease incidence (DI) و شدة الإصابة (disease severity (DS بسبعة مواقع في مدينة طرابلس وهى(عين زارة ، وادي الربيع ، جنين ، المضل ، القيو ، الباعيش ، النشيع).
تم جمع ثمانية وأربعون عينة نباتية خلال هذه الدراسة وأظهرت النتائج أن نسبة الإصابة بالمرض تفاوتت بين مواقع المسح حيث تبين أن عزلاتSS8,SS3 كانت أكثر ا ضراوةوأشارت النتائج المتحصل عليها أن أصناف الخس الخمسة أظهرت حساسية مختلفة اتجاه مرض العفن الأبيض وكان الصنف Crisphead منالأصناف عالية الحساسية.تم استخدام خمس مبيدات فطرية لمكافحة فطر S. sclerotiorum المسبب لمرض العفن الأبيض في الخس بالجرعة الموصى بها.
أظهرت النتائج إن المبيد الفطريRovral كان الأكثر فعالية بنسبة 89٪ في تثبيط الممرض يليه Swith WG 62.5 بنسبة 80٪ بينما كان Topsin M 70الأقل فاعلية بنسبة 50٪ مقارنة بمعاملة الشاهد .

الكلمات الدليلية: مرض العفن الأبيض ، نبات الخس ، شدة الإصابة ، نسبة الإصابة، مبيد روافرال .

Abstract:

This study was conducted to record the disease incidence (DI%) and disease severity (DS%) of the white rot disease caused bySclerotiniasclerotiorumon different cropsat seven sites of Tripoli; Ain Zara, Wadi Al Rabee, Jenin, Al-Mudhal, Al-Qiu, Al-Baish, Nashiaa.A total of 48 plant samples were collected during this study.Results revealed that percentage of disease incidence varies among the surveyed locations.

S. sclerotiorum lettuce isolates SS8 and SS3 were the most destructive isolates.Results indicated that the five selected lettuce varieties showed different susceptibility to white rot.Crisphead was the high susceptible cultivar. Five fungicides were used at recommended dosein vivo to control S. sclerotiorumonlettuce.Resultsshowed that Rovralwas the most effective with (89%) of disease inhibition, followed bySwith WG 62.5 at 80%,whiletheTopsin M 70% WP was the least effective with 50% compared tocontrol.

Key words: White rot disease ,Lettuce, Disease severity, Disease incidence , Rovral.

Introduction:

Sclerotiniasclerotiorum (Lib.) de Bary is a facultative parasitic ascomycetes fungus **(Kirk et al., 2001**) an internationally important plant pathogen that causes serious diseases known as white mold, Sclerotinia stem rot, wilt or stalk rot, or Sclerotinia head rot on a wide variety of broadleaf crops. This pathogen is known to infect about 500 plants species**(Saharan and Mehta, 2008).** It is generally prefer cool climates, moist conditions.Butit is surprisingly shows broad ecological distributions, and can grow well even in an unfavorable environment and survive for up to 8 years in soil in the phasesclerotia form **(Adams and Ayers, 1979).**White mold disease caused losing in production up to 95% worldwide **(Hao and Subbarao, 2005; Chitrampalam et al., 2010).**It was also noted that the pathogen affect the quality market value due to losses in crop size and weight **(Clarkson et al., 2014).**Sclerotia play an important role in disease cycle by germinating either vegetatively for local colonization or carpogenically to initiate the sexual cycle, including the production of

apothecia from which ascospores are released **(Bardin and Huang, 2001; Bolton et al., 2006).**
The formation of apothecia and subsequent ascospore production depends on soil temperature and moisture conditions which infrequently encountered in desert production areas**(Matheron and Porchas 2004).** The symptoms caused by this disease are: watery soft rot with development of white cottony mycelia on different parts of lettuce plants **(Abawi and Grogan, 1979;Bolton et al., 2006).**
In many lettuce-growing areas, small numbers f S. sclerotiorum sclerotia lead to high levels of disease incidence**(HaoandSubbarao, 2005).** This makes control measures for individual plant pathogens impractical. The availability of commercially desirable varieties that have resistance to specific diseases is limited or unknown for many of the crops grown in pots and in-ground; therefore, broad-spectrum control measures are more feasible, both technically and economically. Achieving good control of Sclerotinia white mold is difficult and a challenge in all crops. Management of the disease requires the use of a wide range of strategies. Fungicide application can decrease the disease incidence and increase the yield, on both susceptible and resistant cultivars **(Woodward et al., 2015)**.
For many years, both potting soil and field soil were fumigated with methyl bromide, a cost-effective, broad-spectrum biocide, which controlled soil-borne fungal and bacterial plant pathogens, plant-parasitic nematodes, and weeds. The loss of this versatile compound, due to its negative impact on the ozone layer, has led to the development of new soil disinfestation approaches as well as renewed interest in improving technologies used in the past. The tools that are currently available for soil disinfestation include fumigants, nonfumigant soil applied chemicals, steam, solarization, and anaerobic soil disinfestation.**(Rosskopf et al.,2018).**
In Libya, there are few fungicides registered for white rot in lettuce and for the consideration of losses and importance of the disease. Thus, it is important to evaluate commercial fungicides and their potency for controlling S. sclerotiorum.
Therefore, thisstudy was conducted to investigate the white rot disease in different locations in Tripoli, Libya, investigate the

pathogenicity with different S. sclerotiorum isolates, study the susceptibility of some commercial Lettuce varieties to S.sclerotiorumand evaluate the efficacy of five commercial chemical fungicides for inhibiting and control S. sclerotiorumin-vivo.

Materials and Methods

Thisresearchwas conductedin 2018 to study the extent of white rot disease of different Plants.Samples were collected during growing season in winter and springfrom seven areas insouth of Tripoli;Ain Zara, Al-Baish, Wadi Al-Rabee, Al-Mudhal, Al-Qiu, Al-Jenin, and Al-Nashiaa..The percentage of disease incidence (DI%) was calculated according to **Reznikov et al.,(2018)**using the following formula:

Disease incidence (DI%) = $\frac{\text{Number of infected plants}}{\text{Total number of observed plants}} \times 100$

Severity of the disease was rated in terms of percentage of infected plants among 100 plants collected randomly from the three fields in each area,with three replicates in each field. The disease severity of white rot that caused by S. sclerotiorum was assessed using disease scale proposed by **Grauet al. (1982)** consisted of three categories: 0 to 3, where; (0= no detectable symptoms1= appearance of a 1-2 cm water-soaked lesion on the crown region of the plant, 2= appearance of a 2 cm water-soaked lesion covering the stem base of the plant, 3= plant completely dead).

Disease Severity(DS%) = Σ (a x b) / N x K x 100

Where: a = Number of infected leaves in each category.

b = Numerical value of each category.

N = Total number of examined leaves.

K = The highest degree of infection category.

Isolation, and identification of S.sclerotiorum isolates.

S. sclerotiorum were isolated fromdifferentvegetables crops;lettuce(Lactuca sativa), green beans(Phaseolus vulgaris), cabbage(Brassica oleracea var. capitata), Fennel (Foeniculumvulgare), pepper(Capsicumannuum)and Eggplant (Solanummelongena)at the main cultivation areas in south Tripoli. The disease symptoms appeared on infected plants, whilesclerotia were collected from some infected plants.

Infected plant parts of stems and leaveswere cut into small pieces (1cm)using sterilized scalpel,thensterilized using NaOcl solution

(0.1%) for 30 sec. These pieces were washed with sterilized water to remove the traces of NaOcl solution. Sterilized pieces of plant tissue placed at equal distance in Petri dishes containing potato dextrose agar (PDA 2%) medium under aseptic conditions. The Petri dishes were incubated at 23±1 ^{0}C for 5 days. The tips of the hyphal from advancing mycelium were transferred aseptically into fresh agar plates. The fresh grown of the hyphal tips were transferred into culture tubes. Pure culture was maintained during the study period on PDA at 5-8^{0}C and sub-cultured at fortnightly intervals according to**Pandey et al., (2010).**

Isolated fungi were examinedmicroscopically and identified according to their morphological characters, referred to them fromSS-1 to SS-16.Isolateswere purified by transferring the single hyphal tip on to the fresh medium and prepared pure culture of each isolate **(Tutte, 1969).**

Pathogenicity test.

Pathogenictest were carried out forproveing Koch postulate to confirm the ability of S. sclerotiorum the causal agent of white rot disease to infect lettuce. In this study 4 weeks old seedlings of lettuce weresowingin plastic pots (35 cm in diameter) filled with sterile sandy soil under greenhouse conditions in Al-Mudhal in January 2018, then artificially infested individually (at the rate of 5sclerotia per plant) with the inoculum of each tested isolate, which previously grown for two weeks on sand barley medium. Five isolates were tested,the pots of tested plants were placed in a chamber with 90% relative humidity at 22 ° C for 48 hrbefore moved togreenhouse**.**Three pots each containing five transplants were used as replicates for each tested isolate as well as control. (**Abdel-Kader et al.,(2012**).Thirty days after inoculation disease incidence was recorded. At the end of the experiment re-isolation of the fungus was done from symptomatic tissues for comparison withthe original isolate obtained from plant tissues.

Susceptibility of some lettuce cultivars to S.sclerotiorum

Thisexperiment was established to study the susceptibility of some lettuce toinfectionwithS. sclerotiorum**,**fivecultivars of lettuce;CudVoorburgu, Butterhead,Krolowalata, Crisphead and Maugli,were evaluated by artificial inoculation under greenhouse conditions.Seeds of each variety were sown in a plastic bags (30 cm high, 25 cm in diameter) filled with sterile soil at 18 – 30̊ C. Seedlings of each cultivar were transferred to new plastic pots filled with sterile soil 18 days after sowing and cultivated in the greenhouse in Al-Mudhal. Fresh fungal mats of 6 mm in diameter from PDA culture were placed on leaves or stems 5 to 10 cm above ground for one-month-old host plants after the seedlings were planted in plastic pots. PDA disks of the same size were placed on the leaves or stems of control plants. The pots with inoculated plants were placed in a chamber with 90% relative humidity at 22˚C for 48 hr and then moved into the greenhouse. **Kim and Cho(2002).**Plants were watered and fertilized with a nutrient solutionasneeded.Ten replicates were used for each treatment.Fifteen days after inoculation, the incidence of disease, was recorded.

Evaluation of the effectiveness of some fungicides against S.sclerotiorum

The experiment was conducted in WadiRabee, to evaluate the efficacy of five different fungicides belonging to various chemical groups;namelySumisclex, Rovral, Rizolex – T, Topsin -M, and SwitchagainstS. sclerotiorumon the lettuce cultivarCrisphead (Table.2)**(Abied 2019)**.All these fungicides were sprayedat the recommended dose (Table.1). One hundred lettuce plants were treated with tested fungicides in the soil,whichwasnaturally infested with S.sclerotiorum. Lettuce seedlings are 20 days oldwhere they weresoakedwith the aforementioned fungicides before planting, the distance between plants was25 cm.The plants were sprayedtwice,and the period between the twotreatments was 15 days. Plants were regularly watered and fertilized. The growth parameters were recorded after 30 days.

Table(1): Commercial, common names and active ingredient of Fungicides used in the study:

Commercial name	Common name	Active ingredient	Application rate / Liter
Sumisclex 50 WP	Procymidone	3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione	**1g/l**
Rovral WP50%	Iprodione	3-(3,5-Dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide	**1.5 g/l**
Rizolex T WP 50%	Telcolofos-methyl/ thiram	20% Telcolofos-methyl (0,2,6 dichloro-4-methylphenyl 0,0 dimethyl phosphorothioate) and 30% thiram.	**2 g/l**
Topsin M 70% WP	Thiophanate-methyl	Dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate)	**1g/l**
Switch WG 62.5	37.5%Cyprodinil 25% Fludioxoni	4-cyclopropyl-6-methyl-N-phenylpyrimidin-2-amine 4-(2,2-difluoro-1,3-benzodioxol-4-yl)pyrrole-3-carbonitrile	**1g/l**

Results:

Survey of white rot disease in different locations in south of Tripoli. In this study 48 samples of lettuce were collected from the sites of study in the south of Tripoli. Examined samples showed that 15 of them were infected with white rot disease (Table2.) It can be seen from data in the Table 2 that PDI%fluctuated from 13 up to 80 in different sites were studiedSS8 isolate in WadiRabee Site recorded the highest 80%, while SS12 and SS14 from Al- Qiu and Al-Jenin recoded the lowest 13%.On the other hand, if it turned to DS% was fluctuate in different sites of study from 5.66% caused by isolate SS15 from Al-Nashiaa site up to 30.33% that caused by SS1 from Ain Zara site (Table2).Overall, these results indicate that PDI% and DS% were differed between different sites, crops and isolates.

Table (2): White rot disease in different areas in the south of Tripoli.
Isolation and identification of S.sclerotiorum

Infected plant material were collected in sterile polyethylene bags from various fields in the lettuce cultivation areas south Tripoli(Table2).

No	Area	Isolate	Common Name	Scientific Nam	Soil Type	DI %	DS %
1	Ain Zara Collected in Winter season	SS1 SS2	Lettuce Fennel	Lactuca sativa L . Foeniculumvulgare	Sandy red sandy	77 55	30.33 20.66
2	Al-Baish Collected in Spring season	SS3 SS4 SS5	Lettuce Cabbage Green Bean	Lactuca sativa L . var. capitata PhaseolusvulgarisL.	sandy sandy clay Sandy clay	75 35 44	28.33 17.66 20.66
3	WadiRabee Collected in Winter season	SS6 SS7 SS8	Cabbage fennel Lettuce	Brassica oleracea var. capitata ılgare L .	Sandy loam sandy Sandy loam	50 43 80	16.66 16.33 53.33
4	Al-Mudhal Collected in Spring season	SS9 SS10	Lettuce fennel	. Miller Foeniculumvulgare	clay sandy	48 30	20.00 11.33
5	Al- Qiu Collected in Spring season	SS11 SS12	Pepper fennel	Capsicumannuum Foeniculumvulgare.	Sandy Sandy red	35 13	18.33 6.33
6	Al-Jenin Collected in Winter season	SS13 SS14	Green Bean Lettuce	PhaseolusvulgarisL. LactucasativaL.	Sandy red sandy	17 13	7.00 6.00
7	Al-Nashiaa Collected in Spring season	SS15 SS16	Eggplant pepper	Solanummelongena Capsicumannuum .	Light sand sandy	15 23	5.66 9.66

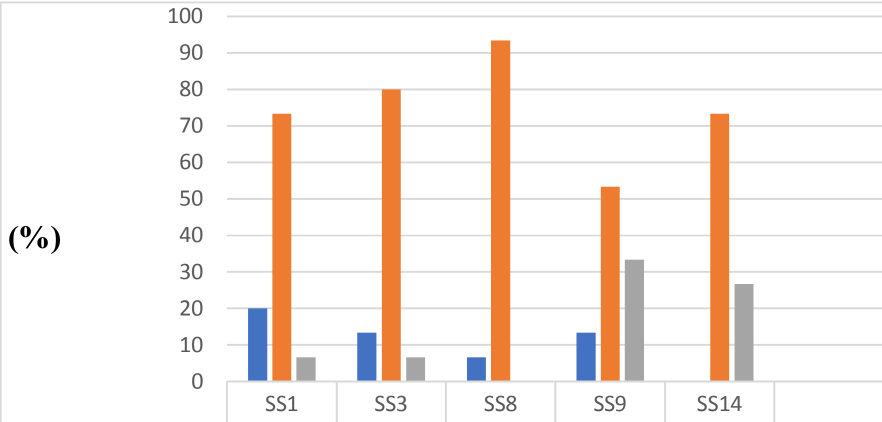
Five isolates of S. sclerotiorum were isolated referred to them as (SS1,SS3,SS8,SS9,SS14) from different lettuce cultivars; Crispheadcultivar was the most affected, followed by Królowalata , Cud voorburgu, Maugli and Butterhead.

Pathogenicity test:

Pathogenicity of the S. sclerotiorumwas tested on five isolates; SS1, SS3, SS8,SS9 and SS14 on the Crispheadlettuce cultivar.

Difference was observed between tested isolates on lettuce cultivars compared to control.

The Fig (1)showed that, the most pathogenic isolates were SS3, SS8 producing the highest percentage of infected plants (100%), followed by SS1 and SS14 isolate at 93.33%, while the SS9 isolate was the least pathogenic compared to control plants According to the results, the selected isolates may be divided indifferent groupsdue to its pathogenicity i.e.: Moderately pathogenic isolate (SS9), highly pathogenic isolate (SS14), aggressive isolates (SS3, SS1) and destructive isolate (SS8).



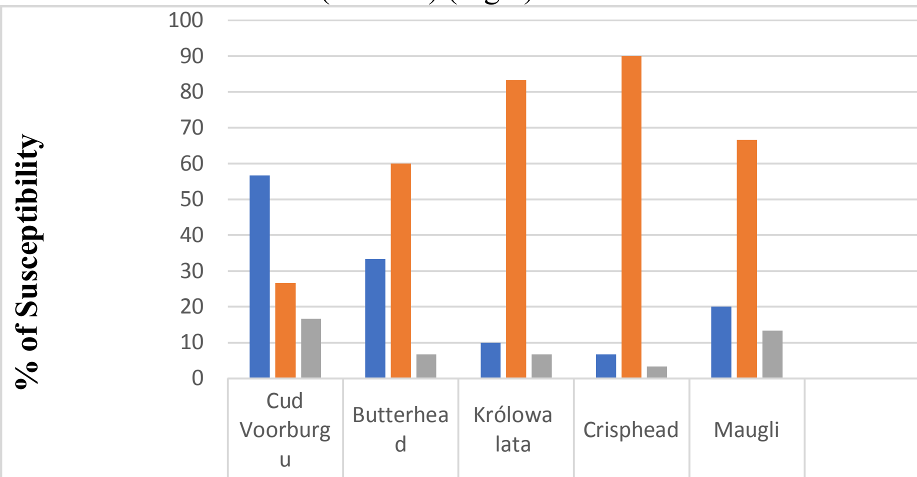
	SS1	SS3	SS8	SS9	SS14
Initial symptoms	20	13.33	6.66	13.33	0
Collapsed plants	73.33	80	93.33	53.33	73.33
Survival Plants	6.66	6.66	0	33.33	26.66

Fig (1):Pathological study of Sclerotiniasclerotiorum fungi on lettuce plant cv. capitata under greenhouse conditions.

Susceptibility of some lettuce cultivars towards S.sclerotiorum

There was difference between lettuce cultivars; Crisphead ,Królowalata , Cud Voorburgu , Maugli and ButterheadtowardsS. sclerotiorum isolate SS8.

The cultivar Crispheadwas the most susceptible with 90% of disease severity, followed by Królowalata with 83.33% and Mogli at 66.66% and Butterhead 60.00%. Cud voorburgu had the lowest probability of occurrence of white rot (26.66%) (Fig.2).

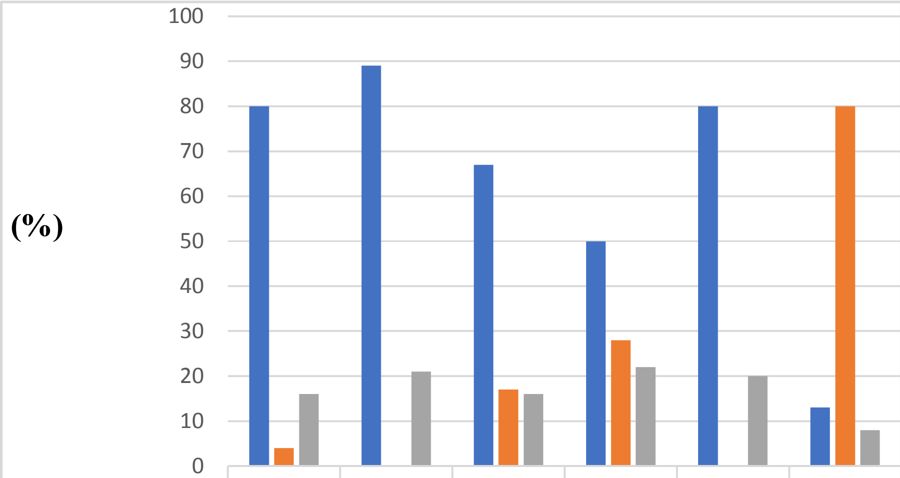


	Cud Voorburgu	Butterhead	Królowalata	Crisphead	Maugli
Initial symptoms	56.66	33.33	10	6.67	20
Collapsed plants	26.66	60	83.33	90	66.66
Survival Plants	16.67	6.67	6.67	3.33	13.33

Fig (2):Susceptibility of lettuce cultivars to S.sclerotiorum under greenhouse conditions.

Evaluation of the effectiveness of some fungicides onS.sclerotiorum

Fivefungicides were used at recommended dosein vivo to control S. sclerotiorum in lettuce. Results showed that Rovral was the most effective with 89%, followed by Swith WG 62.5 at 80%, while Topsin M 70% was the least effective with 50% compared to the control (Fig.3).



	Sumisclex	Rovral	Rizolex -T	Topsin - M	Switch	Control
Initial symptoms	80	89	67	50	80	13
Collapsed plants	4	0	17	28	0	80
Survival Plants						

Fig (3): Effect of some fungicides on Sclerotiniasclerotiorumin vivo .

Discussion

The plant pathogen Sclerotiniasclerotiorum can cause serious losses on lettuce crops and as for most other susceptible crops.

Throughout the study, incidence of white rot was reported in the lettuce fields and the incidence was varies in production districts.Although this fungus is most frequently found in regions tending to be cool and moist in the winter season**(Purdy, 1979).**The lower temperatureseasonsmake favorable conditions for the development and reproduction of S. sclerotiorum, especially in the intensive agricultural system. This confirms the presence of Sclerotiniasclerotiorum during late winter / early spring when disease progression is much faster at 80-100% RH at 20̊ C.**(Clarksonet al, 2014).**Therefore, the main cause for the decline of lettuce in the southern regions of Tripoli may be due to a high sclerotia population and the intensive lettuce-cropping systems that occur in the winter. These results further support the idea of**(Clarksonet al, 2014)** who pointed out thatthe lower temperature seasons make favorable conditions for the development and reproduction of S.

sclerotiorum,especially in the intensive agricultural system. This confirms the presence of S.sclerotiorum during late winter / early spring when disease progression is much faster at 80-100% RH at 20̊ C.

Distribution of S. sclrotiorum in different fieldsin the south of Tripoli, this may be because the inoculum potential of sclerotia germinating carpogenically is much higher than for those germinating to produce mycelium due to the large number of airborne ascospores released, which dramatically increases the spatial spread from a single sclerotium. S. sclerotiorumwhich dramatically increases the spatial spread from a single sclerotium. S. sclerotiorum also causes lettuce 'drop', where plants quickly wilt in the field due to infection of the stem base and lower leaf axils of infected plants, followed by rapid rotting of the tissue **(Young et al., 2004; Clarkson et al., 2014).**

In the present study S. sclerotiorum was isolated from the collected plants and the aggressiveness of the fungus isolates in commercial lettuce cultivars showed big differences among all tested isolates in disease severity.

The results revealed that the tested lettuce cultivars differed in their susceptibility to the infection with, Sclerotiniasclerotiorum . Crisphead was the most susceptibile to white rot followed by Królowalata and Mogli while Cud voorburgu was the least susceptible one. Similar results previously obtained by**(Azis et al., 2016).**who found that cultivar Crisphead was susceptible to S. sclerotiorum . The varieties of lettuce show different susceptibility to white rot caused by S. sclerotiorumaffected by differed in their factors such as, differences in the pathogenicity of the fungus isolate and plant genotype.**(Hayes et al., 2010; Elia and Piglionica, 1964).** In addition to that, the variation among S. sclerotiorum isolates pathogenicity can be referred to the high genetic, and the diversity in the genotypes can refer to a phenotypes differences between these isolates. Therefore affect their virulence**(Leyronas et al.2018; Denton-Giles et al., 2018).**S.sclerotiorum showed various sensitivity to the tested chemicals fungicide. All chemical fungicides prevented the growth of S. sclerotiorumin vivo but the inhibition rate was different between selected fungicides. The fungus genetics can affect its sensitivity toward the fungicides as a sensitive or

insensitive to a chemical molecule. The sensitivity means fungi toxicity but insensitivity means as no fungal toxicity. **(McGrath, 2009;Reis et al. 2010),**also the differences in the chemical group and the active ingredient of the used fungicides are much supported for the potential variation.The obtained results concerning to the efficacy of the tested fungicides against the fungus
S. sclerotiorumin vivo are in the same line with previous researchers **(Subbarao, 1998;Matheron and Porchas, 2004).**Thesefindings are in agreement with those obtained by**(Cotes et al., 2007; Elsheshtawi et al. 2017).** as they found that Iprodione was the most efficient fungicide in inhibition the mycelia growth of S. sclerotiorum, while the fungus was insensitive to the active ingredients of Thiophanate-methyl. It was acceptable that not all chemicals are causing toxicity to fungi and therefore, there is no fungicide control forusedfungi**(Reis et al., 2010).**

Conclusion

The most obvious finding to emerge from this study is that percentage of disease incidence varies among the surveyed locations. S. sclerotiorum isolates SS8 and SS3 were the most destructive isolates. Results of this study indicated that the five selected lettuce varieties showed different susceptibility to white rot. Crisphead was the high susceptible cultivar. Five fungicides were used at recommended dosein vivo to control S. sclerotiorum in lettuce. Results showed theatRovral was the most effective with 89%, followed by Swith WG 62.5 at 80%, while theTopsin M 70% was the least effective with 50% compared to control.

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