

PATHOLOGICAL STUDIES ON DOWNY MILDEW OF PEA CAUSED
BY *PERONOSPORA VICIAE*
(BERK.) CASP.F.SP.*PISI* (SYDOW) IN EGYPT

[30]

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Keywords: Downy mildew, *Peronospora viciae* f. sp. *pisi*, Oospores, Artificial inoculation, Tenderls, Host range and Survival

ABSTRACT

Six pea cultivars including those commercially important in Egypt were tested for their susceptibility to infect with *Peronospora pisi*. Sugar pea was the most susceptible cultivar to *P. pisi* followed by Mange tout and Little marvel cultivars. On the other hand, the lowest infection was found on Master B cultivar followed by Lincoln and Relavil cultivars. Examination of senescent foliage of severely mildewed plants of Sugar, the highly susceptible pea cultivar, indicated the presence of oospores stage of this pathogen under greenhouse and open field conditions in Egypt. Microscopic examination indicate that the oospores were observed in naturally and artificially infected leaflets, stems, petioles and tenderls. Artificial inoculation with conidia suspension of *P. pisi* on Broad bean (*Vicia faba* L.) cv. Giza 153, onion (*Allium cepa*) cv. Giza 1 and Green bean (*Phaseolus vulgaris* L.) cv. Branco proved to be immune to infect by pea downy mildew throughout the whole growing period, as no symptoms were noticed. So, the pathogen causing downy mildew on pea was identified as *Peronospora viciae* f.sp. *pisi*. Conidia survived better at lower temperature than at higher temperatures when an artificial inoculation was used in survival tests. Conidia deposited on pea leaves stored at lab temperature lost their viability after a week. However, conidia deposited on pea leaves

stored in refrigerator carried viable conidia for 3 weeks. This means that *P. pisi* conidia survived best at 10°C and poorest at 25-28°C. Oospores either stored at lab conditions or in refrigerator recorded higher percentages of disease severity during winter season and survived during summer season, host - free periods, and recorded new infection on pea plants after a year. This means that conidia lost their viability during summer season and it is not responsible for disease occurrence from season to another. So, oospores formed in infected pea plants are important for the survival of the pathogen during host - free periods. Effect of spraying different fungicides for controlling pea downy mildew disease under field conditions in two locations during 2007-2008 seasons indicated that Privicure - N, Ridomil Gold plus 42.5% and Ridomil Gold Mz 68 % were the most effective fungicides in reducing percentage of infection and disease severity of downy mildew.

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important economic legume crops in Egypt for local consumption and exportation. During the last few years, downy mildew incited by *Peronospora viciae* f.sp. *pisi*, has been found to attack seriously pea plantations all over the country. This disease can cause different kinds of damage to the crop (Dixon, 1981). Infections of pods cause the most serious type of symptoms, leading to disturbed growth and brown discoloration of the pea.

As far the authors are aware, few papers were published on downy mildew of pea in Egypt since it was recorded by Briton-Jones (1925). Zayan, Sahar (1995) and Attia *et al* (1997) reported that downy mildew of pea has become one of the most important problems which face pea growers in Egypt, especially in the northern governorates. Control of pea downy mildew by the adoption of some agricultural practices and fungicides was investigated.

The present investigation aimed to study the reaction of some pea cultivars to downy mildew infection. Occurrence of oospores in naturally and artificially infected pea tissues, host range in relation to downy mildew infection and survival of *P. viciae* f. sp. *pisi* in pea infected tissues. Application of fungicides for disease control was also evaluated.

MATERIALS AND METHODS

Source of diseased samples and propagation of mildew inoculum

Pea plants, heavily infected with downy mildew pathogen, *Peronospora pisi*, were collected during February - March 2007, from the farms of agricultural companies in Sinai, Ismailia Governorate. This was the original source of infection. The conidia of *P. pisi* were then transferred to Sugar pea plants previously grown in pots 30 cm in diameter under greenhouse conditions. The newly infected plants provided the conidial inoculum for further experiments.

Method of inoculation

The pathogen is an obligate parasite which can only grow on living plant tissues. Spores of the pathogen collected from surface of the tendrils, stems, pods and under surface of the leaflets with hair brush and suspended in distilled water were used for artificial inoculation. Fresh spore suspensions (10^3 spores /ml distilled water) were applied on healthy plants using hand atomizer. However, check plants were sprayed with sterile water. Inoculated and non-inoculated plants were covered with polyethylene bags for the duration of 24 hrs and observed until disease assessment was undertaken.

Reaction of cultivars susceptibility and host range

Seeds of six pea (*Pisum sativum* L.) cultivars named : Master B., Sugar, Mange tout, Little Marvel, Lincoln and Relavil used in this study were

kindly obtained from Vegetable Department, Horticulture Research Institute, Dokki, Giza.. Seeds of the host range included: Broad bean (*Viciae faba* L.) cv. Giza 153, Onion (*Allium cepa*) cv. Giza 1 and Green bean (*Phaseolus vulgaris* L.) cv. Branco were obtained from commercial suppliers. The experiment was carried out under greenhouse conditions. Seeds of each cultivar were sown in clay soil in pots, 30 cm in diameter, each seeded with 8 seeds. Four pots were set for each particular cultivar. The seedlings were inoculated three weeks after sowing as previously mentioned. Data were recorded at different plant ages till the mildew reached its maximum (when plants became 75 - days old).

Disease assessment

Reaction of varietal susceptibility was determined according to. The disease severity key for downy mildew of pea described by Falloon *et al* 1995 as the following categories:

0 = No downy mildew.

1 = Less than one quarter of blade under surface mildewed.

2= More than one quarter, but less than one half of the undersurface of blade mildewed.

3 = More than one half, but less than three quarters of blade undersurface mildewed

4 = More than three quarters of blade surface mildewed.

The percentage of disease severity for each particular treatment was calculated by using the following formula:

$$\% \text{ of disease severity} = \frac{\text{Sum of } (nxv)}{5N} \times 100$$

Where: N = Number of leaflets in each category.

V = Numerical value of each category

N = Total number of leaflets in sample

The percentage of disease severity was determined on the lower surfaces and the average was calculated.

Survival of *Peronospora viciae* f. sp. *pisi* the causal of pea downy mildew disease

The diseased parts of pea plants were stored in paper bags at lab conditions (25 -28°C and can raise up till 35°C) during summer season and in a refrigerator (10°C).

Conidia of the pathogen deposited on pea infected tissues, stored at lab conditions or in refrigerator were collected one, two, three and four weeks intervals after storage with hair brush and

suspended in distilled water were used for artificial inoculation during October 2006 as mentioned before. Moreover, oospores embedded in infected tissues stored at lab conditions or at refrigerator were extracted in a blender and sieved through a muslin sheet. Spore suspensions, 10^3 oospores per ml distilled water, were applied on Sugar pea plants at different intervals.

Artificial inoculation with oospores and pathogen mycelium stored at lab conditions or in refrigerator were repeated and applied on pea plants 40-50 days - old, cv. Sugar, previously grown in pots 30 cm .in diameter under greenhouse conditions during October 2007. The percentage of disease severity was determined according to **Falloon et al (1995)** on the lower blade surfaces and the average of four replicates was calculated.

Chemical control

Field experiments were carried out in two locations: EL- Wasfia and Seuz Canal University, Faculty of Agricultural Farm, Ismailia Governorate during 2007-2008 where downy mildew is severe and caused great losses in the two locations during the last few years. The experiments in both two locations were carried out in the end of October 2007. In each location plots of 4x5m (each consisted of 4 rows) were sown with pea seeds cv. Sugar pea.

One month after sowing, the growing plants in the two locations were sprayed four times at 12 days intervals. Each fungicide was sprayed using the recommended dose (**Table.3**). Control treatment consisted of plots sown with pea seeds and the emerged plants were not sprayed with any fungicide, but with water only. Three replicated plots were used for each treatment. Disease incidence and average percentage of disease severity were recorded 3 days after the last spray.

Statistical analysis

The collected data were statistically analyzed using two factors completely Randomized block Design. Treatments were compared at 0.05 and 0.01 level of probability LSD (**Steel and Torrie1960**).

RESULTS AND DISCUSSION

1- Symptomatology

Downy mildew causes different kinds of symptoms on pea plants .Typical symptoms of the disease were observed in the surveyed cultivated areas.

Systemic infection in seedlings causes stunted growth with conidia sporulation, which often covers a major part of the plant surface .Later in the season, top systemic symptoms can develop with stunting and sporulation occurring over the entire surface of the top of plants. **Taylor et al (1990)** showed that systemic infection could originate from leaf infection. They also induced systemic infection by inoculating conidia into the apical bud of young plants.

Local infection on leaves or tendrils develop from conidia present on the plant surface. Pod infection causes yellow lesions on the pod surface (**Fig. 1**). Pod infection often causes distorted pods, seed abortion and brown discoloured small peas. Pod infection directly affected pea quality. Oospores are formed within the yellow lesions observed on infected pods, leaflets, stems, petioles and tendrils. Leaves of infected plants were initially slightly chlorotic, especially near the central vein. Within 2to 3 days, a characteristic gray, furry growth was evident on the lower leaf surface and sometimes on the upper leaf surface .Basal leaves were severely affected.

Microscopic observations revealed conidiophores branching two to five times. Conidiophores ended with sterigmata bearing single conidia (**Fig. 2**) .Conidia were elliptical and grayish in mass. It is well known that *Peronospora* produce sporangia do not contain zoospores, and germinate by germ tubes (**Show, 1981**). *P. viciae* also produces oospores, which have a typical reticulate pattern of the exosporium.

2- Reaction of some pea cultivars to infect by downy mildew

Six pea cultivars including the commercially important in Egypt were inoculated with *Peronospora pisi* three weeks after sowing. The percentage of mildew infection on the six cultivars was estimated at different growth periods.

Results presented in **Table (1)** indicate that these cultivars differed in their susceptibility to downy mildew infection. It is also clear that infection increased with the increase of plant age. However, no symptoms were noticed on plants of 21 - days old. The first symptoms were observed when the plants reached 25 days after sowing. Sugar pea was the most susceptible to *Peronospora pisi* followed by Mange tout and little marvel cultivars. On the other hand, the lowest infection by downy mildew during the growing period was found on Master B cultivar followed by Lincoln and Relavil

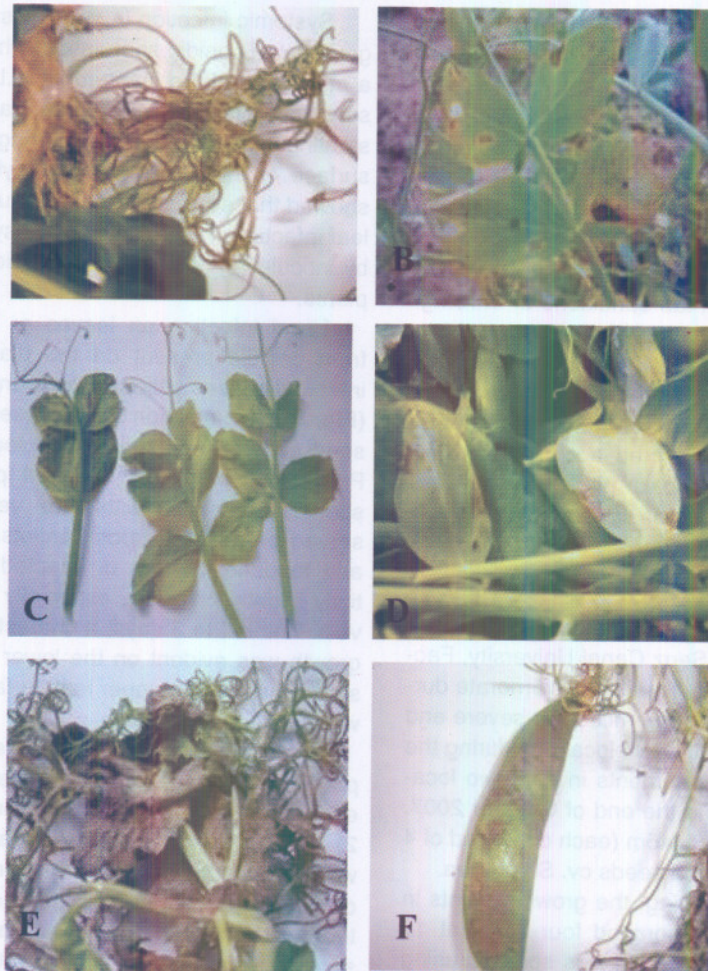


Fig. 1. Typical symptoms of downy mildew developed on tendrils (A), leaflets (B,C,D), stem (E) and pod (F) of pea plants naturally infected with *Peronospora vicia* f.sp.*pisii*.

Table 1. Percentage of infection with downy mildew on different pea cultivars and host range plants grown under greenhouse conditions

Inoculated plant : species and varieties	Percentages of infection at different plant ages (days)						
	21	25	35	45	55	65	75
Sugar pea	0.0	5.2	10.5	14.4	22.1	31.4	45.2
Mange tout	0.0	3.4	6.8	11.6	13.5	25.5	39.5
Little marvel	0.0	2.1	3.7	5.9	10.2	14.5	27.8
Relavil	0.0	1.4	2.9	4.9	8.5	12.4	18.5
Lincoln	0.0	1.2	2.7	3.4	6.5	9.6	13.5
Master B	0.0	0.0	0.0	0.7	0.9	1.1	1.4
<i>Vicia faba</i> cv. Giza 157	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaseolus vulgaris</i> cv. Branco	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Allium cepa</i> cv. Giza 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0

cultivars. It may be concluded that variation in susceptibility among the tested pea cultivars in this investigation and those tested by various authors may be due to the genetic structure of the different tested cultivars or to the variation among the pathogen strains, beside the chemical compositions of the cultivars under study. Results of the relative susceptibility of pea cultivars under study are of interest.

Variation in the susceptibility among different pea cultivars to downy mildew are of great importance as they provide basis for national crop important program of breeding for disease resistance (Attia *et al* 1997). Improved resistance against this disease is therefore desired. Stegmark (1991) indicated that a significant genetic variation among pea lines was found for infection of seedlings with downy mildew in greenhouse tests.

3- Host range in relation to downy mildew infection, at 75 - days after sowing

Artificial inoculation with spore suspension of *Peronospora pisi* on the host range which included Broad bean (*Viciae faba* L) cv Giza 153, onion (*Allium cepa*) cv. Giza 1 and green bean Bean (*Phaseolus vulgaris* L.) cv. Branco proved to be immune to downy mildew infection throughout the whole growing period, as no symptoms appeared (Table 1). It may be concluded that variation in susceptibility among the tested plants in this investigation may be due to the variance in genetic structure among the different tested cultivars. Stegmark (1994) reported that forma specialis *psii* can only infect *Pisum* species and not species of the genus *Viciae* within the tribe *viciae*. So, the pathogen caused downy mildew on pea was identified as *Peronospora viciae* f. sp. *psii*.

4- Occurrence of Oospores of *Peronospora pisi* in naturally and artificially infected tissues

Due to the importance of the perfect stage in the life cycle of the downy mildew, investigations were carried out under greenhouse conditions to elucidate this point.

Examination of senescent foliage of severely mildewed plants (artificially and naturally infected) of the highly susceptible pea cultivar Sugar was carried out by the light microscope. Results obtained indicate the presence of oospores stage (Fig. 2) of this pathogen under greenhouse and open field conditions in Egypt. Microscopic examination indicate that the oospores were observed in

naturally and artificially infected leaflets, stems, petioles and tenderls. Stegmark (1994) explained the disease cycle of downy mildew on pea and mentioned that oospores in the soil are the primary inoculum early in the season and oospores can survive for a long time in the soil.

5- Survival of *Peronospora viciae* f.sp. *psii* the causal of pea downy mildew

Data presented in Table (2) indicate that conidia survived better at lower temperature than at higher temperature when an artificial inoculation was used in survival tests.

It is also clear that conidia deposited on pea leaves stored at lab temperature lost their viability after a week. However, conidia deposited on pea leaves, stored in refrigerator carried viable conidia for 3 weeks. This means that *P. psii* conidia survived best at 10 °C and poorest at 25-28 °C±5.

On the other hand, viability of *P. psii* oospores decreased very little after exposure to high temperature under lab conditions compared with those stored in refrigerator. It is also clear that Oospores either stored at lab conditions or at refrigerator still survive during summer season, host free periods, and recorded new infection on pea plants during October 2007. This means that conidia lose its viability during summer season and it is not responsible for disease occurrence from season to another. So, Oospores deposited in pea infected plants are important for the survival of the pathogen during host - free periods. It is well known that the pathogen disperses by conidia formed on infected host tissues which survived better at cool temperature than at higher temperature. These results are in agreement with those reported by Bashi and Aylor (1983) who found that *P. destructor* conidia survived best at 10 °C and poorest at 35 °C. These results are also in agreement with those reported by Pegg and Mence (1970) who reported that *P. viciae* infected pea leaves could produce conidia up to 6 weeks after infection and sporulating lesions carried viable conidia for 3 weeks. In the same time, Wu *et al* (2000) reported that *Bremia lactucae* produces oospores, secondary spread of the disease in the field exclusively results from infection by air - borne conidia.

Survival of oospores population of *Peronospora viciae* f.sp. *psii* embedded in pea plant tissue stored at lab conditions or in refrigerator indicates that surviving oospores decreased to less than 3% after a year (Table 2). Olofsson (1966) reported that oospores of downy mildew of pea in Western



Fig. 2. Artificial inoculation with *Peronospora viciae* f. sp. *pisi* on leaflets and leaves of Sugar pea cv. (A), conidiophores and conidia (B, C and D), and abundant Oospores in infected pea stem tissues (E) and Oospores showing outer thick wall (F) and magnified oospores showing the clear envelope sheet surrounding it (G).

Table 2. Survival of conidia and Oospores of *P. viciae* f.sp.*psii* deposited on and in pea leaves stored at lab conditions and in the refrigerator for different periods

Inoculation with	Stored under conditions of	Artificial inoculation on pea plants during				
		October 2006				October 2007
		Average % of disease severity after storage for				
		One week	Two weeks	Three weeks	Four weeks	One year
Conidia	Lab 25-28°C±5	8.1	0.0	0.0	0.0	0.0
	Refrigerator 10°C	12.3	7.7	4.1	0.0	0.0
Oospores	Lab 25-28°C±5	9.1	9.3	8.7	6.8	1.7
	Refrigerator 10°C	9.2	9.8	9.5	8.7	2.3

Table 3. Effect of spraying different fungicides for controlling pea downy mildew disease under field conditions during 2007-2008 seasons in two locations EL-Wasfia and Suez Canal Univ. Farm)

The tested fungicides	Recommended dose /100L.water	% of infection			% of disease severity		
		EL-Wasfia	S.C. Univ Farm	Mean	EL-Wasfia	S.C. Univ Farm	Mean
Privicure – N	250ml	16.0	12.0	14	7.3	4.2	5.75
Ridomil Gold	200g	17.3	12.3	15	6.7	5.3	6
Plus 42.5 % Ridomil Gold	200g	17.6	12.7	15.15	7.5	5.5	6.5
MZ 68% Acrobat copper	250g	19.7	13.2	16.5	8.5	5.7	7.1
46% WP Folio Gold 537.5	300ml	19.1	13.5	16.3	8.7	5.7	7.2
Dithane M-45	250g	20.2	13.8	17.05	8.8	6.3	7.6
Control	--	64.3	39.9	51.8	46.9	36.1	41.5
LSD at 0.05		Fungicides =3.42 Location = 1.82			Fungicides =0.77 Location = 0.41		

Europe can survive for 10-15 years in the soil. Stegmark (1994) reported that oospores can survive for a long time and infections are common in south Sweden where a 6 year crop rotation is common practice. Oospores of *P. destructor* in onion debris showed good viability after 25 years of outdoor storage (Mckay, 1957).

6- Effect of spraying different fungicides for controlling pea downy mildew disease under field conditions in two locations during 2007-2008 season

Data presented in Table (3) indicate that all tested fungicides showed significant reduction in both percentage of infection and the disease severity as compared with the untreated control.

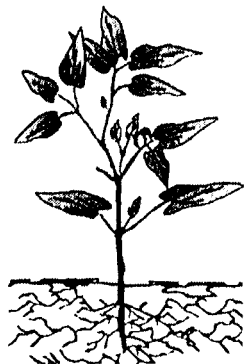
Privicure-N, Ridomil Gold Plus 42.5 % and Ridomil Gold Mz 68 % were the most effective in reducing percentage of infection and disease severity of downy mildew. While considerable reduction in percentage of infection and disease severity took place with Acrobat copper 46% WP and Folio Gold 537.5. Dithane-M-45 was the least effective fungicides when compared with the tested fungicides or untreated plants. This agrees with results obtained by Attia *et al* (1997) who reported that Ridomil-Mancozeb 72%, Privicure-N and Sandofan M-8 were the most effective fungicides against pea downy mildew in Egypt. It could be concluded that application of fungicides can reduce disease incidence and disease severity.

It is also clear that the percentage of infection and disease severity was higher in EL- Wasfia ,the first location compared with S.C.Univ – Farm, -the second location .The high relative humidity at EL- Wasfia may favours greatly the development of pea downy mildew disease under field conditions when compared with the second location which little bit dry than EL- Wasfia.

Therefore, the results of this investigation may help pea growers to minimize the substantial losses caused by *P. viciae* f. sp. *lisi* Sydow.

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دراسات مرضية علي البياض الزغبي في البازلاء المتسبب عن

Peronospora viciae f.sp.*psi* في مصر

[٣٠]

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الوريقات والمحاليق المصابة طبيعياً والمعدية صناعياً بجراثيم الطفيل. العدوى الصناعية بمعلق جراثيم الطفيل *Peronospora pisi* علي العوائل النباتية والتي تشمل صنف الفول جيزة ١٥٣ والبصل جيزة ١ والفاصوليا برونكو ، أثبتت أن هذه العوائل منيعة للإصابة بمرض البياض الزغبي طوال فترات النمو لهذه العوائل حيث لم تظهر عليها أي أعراض للمرض وعليه فان المسبب لمرض البياض الزغبي في البازلاء تم تعريفه علي انه الطفيل *Peronospora viciae* f.sp.*psi*. الاختلافات في مدي قابلية أصناف البازلاء المختبرة في هذا البحث قد ترجع الي الاختلافات في العوامل الوراثية بين هذه الأصناف المختلفة . الجراثيم الكونيدية تستطيع أن تحتفظ بحيويتها تحت درجات الحرارة المنخفضة (الثلاجة) أفضل من تخزينها تحت درجات الحرارة المرتفعة (تحت ظروف المعمل) حينما استخدمت تجارب العدوى الصناعية كاختبار لمدي حيويتها. الكونيديات المخزنة بحرارة المعمل فقدت حيويتها بعد أسبوع واحد بينما ظل الطفيل المحمول علي اوراق البازلاء المخزنة بالثلاجة علي درجة ١٠°م قادراً علي احداث العدوى الصناعية حتى ثلاثة أسابيع من التخزين . بينما الجراثيم البيضية سواء المخزنة تحت

تم اختيار ستة أصناف من البازلاء تشمل الأصناف ذات الأهمية التجارية في مصر، ثم تم حقنها بالطفيل *Peronospora pisi* بعد ثلاثة أسابيع من الزراعة . تختلف أصناف البازلاء فيما بينها من حيث درجة قابليتها للإصابة بمرض البياض الزغبي . تزداد الإصابة بالمرض بزيادة عمر النبات . في حين لم تشاهد أي أعراض للإصابة علي النباتات التي عمرها اقل من ٢١ يوم . تم ملاحظة أول أعراض الإصابة بالمرض عندما بلغ عمر النباتات المعدية بجراثيم الطفيل ٢٥ يوم بعد الزراعة . كان صنف البازلاء Sugar أكثر الأصناف قابلية للإصابة بالطفيل يليه الصنف Mange tout والصنف Little marvel . علي الجانب الآخر اظهر الصنف Master B يتبعه الصنف Lincoln والصنف Relavil اقل إصابة بمرض البياض الزغبي أثناء فترة النمو . أوضح فحص المجموع الخضري لأوراق شديدة الإصابة علي النباتات المقبلة علي الشبخوخة للصنف البازلاء السكرية ، اشد الأصناف قابلية للإصابة ، وجود الطور الجنسي للطفيل الممرض (الجراثيم البيضية) تحت ظروف الصوبة والحقل المكشوف في مصر . الفحص الميكروسكوبي أوضح وجود الجراثيم البيضية في كل من الوريقات والسوق وأعناق

تحكيم: أ.د مديح محمد علي

أ.د محمد فاروق عطيه

بحيويتها لمدة عام ذات أهمية في بقاء الطفيل حي أثناء فترة غياب العائل الذي تتطفل عليه .
تأثير الرش بالمبيدات الفطرية المختلفة لمقاومة مرض البياض الزغبي في البازلاء تحت ظروف الحقل في منطقتين أثناء موسم ٢٠٠٧-٢٠٠٨ أشار إلي أن المبيدات Privicure-N, Ridomil Gold Mz 68% و Ridomil Gold plus 42.5% كانت أكثر المبيدات فاعلية في خفض النسبة المئوية بالبياض الزغبي.

ظروف المعمل أو الثلاجة وظلت محتفظة بحيويتها في فصل الصيف ، الفترة التي لا يوجد فيها العائل ، وسجلت إصابات جديدة علي نباتات البازلاء في الموسم التالي خلال شهر أكتوبر ٢٠٠٧ . هذا يعني ان الجراثيم الكونيدية فقدت حيويتها أثناء موسم الصيف وأنها غير مسؤولة عن حدوث المرض وانتقاله من موسم الي آخر (الموسم التالي) . وعليه فان الجراثيم البيضية المتكونة في نباتات البازلاء المصابة والتي أوضحت النتائج انها ظلت محتفظة