

Management of Pea Powdery Mildew Disease using some Resistance Inducing Chemicals and Systemic Fungicides

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The efficiency of some inducing resistance chemicals (IRC) and systemic fungicides were evaluated *in vitro* on suppressing the germination of conidiophores of *Erysiphe pisi* as well as controlling powdery mildew of pea under greenhouse and field conditions.

Different degrees of inhibition to the germinated conidiospores of *E. pisi* occurred when the tested IRC, *i.e.* Bion, potassium monobasic phosphate, salicylic acid and zinc sulphate and the systemic fungicides, *i.e.* fenarimol 12% (Rubigan), diconazole 5% (Sumi-8), deconazole 10% (Topas) and bromuconazole 10% (Vectra). The systemic fungicides were more efficient than IRC in this regard.

Spraying pea plants with the tested IRC and fungicides reduced significantly the severity of powdery mildew disease, as well as, they increased plant height, number of green pods/plant and weight of green pods/plant compared with control treatment under greenhouse conditions. On the other hand, spraying pea plants under field conditions during 2008 and 2009 growing seasons with any of the fungicides Topas and Rubigan, each alone or in alternation with any of salicylic acid and zinc sulphate resulted in a significant decrease in the severity of the disease with significant increment in the produced green pods yield compared with the control treatment. In addition, alternations between the tested IRC and fungicides were less efficient comparing to treatments sprayed with the tested fungicides only. Meanwhile, they were more effective than those sprayed with the tested IRC only.

Keywords: Chemical control, pea, powdery mildew disease, resistance inducing chemicals, systemic fungicides.

Pea (*Pisum sativum* L.) is one of the most popular leguminous crops in Egypt for local consumption and exportation due to their high content from vitamins and protein. Pea plants are liable to attack by bacterial, fungal and viral diseases in addition to nematode infection and physiological disorders (Osman, 1966; Reeser *et al.*, 1983; Abada, 1996; Abada *et al.*, 1996 and 1997 and Ahmed, 2009). Under Egyptian conditions, powdery mildew disease caused by *Erysiphe pisi* is one of the most serious diseases affecting pea production. The disease is widespread and of economic importance in semi-arid regions (Reeser, *et al.*, 1983 and Abada *et al.*, 1996 and Richardson, 2006). The best favourable conditions for disease development are 15-25°C and over 70% RH during flowering and pod filling stages

in the growing season. Heavy rainfall is not favourable for the disease, as it will actually wash spores off plants and night time dews are sufficient for the disease to develop (Reeser *et al.*, 1983 and Richardson, 2006).

Chemical control is the short way to obtain sufficient control to plant pests including plant diseases. However, using of pesticides mostly cause environmental pollution and accumulated as toxic substances in human food chain, especially in case of fresh vegetables and fruits. On the other hand, using other trials of disease management, e.g., plant extracts, antioxidants, biological control and agricultural practices are not enough to obtain efficient results, especially under the absence of the resistant cultivars (Abada, 1996 and El-Shahawy, 2009).

This work aimed to evaluate the inhibitory effect of some resistance inducing chemicals (IRC) and systemic fungicides on the germinated conidia of *E. pisi*. Also, studying their effect on the severity of pea powdery mildew in a greenhouse experiment to select the most efficient treatments to apply them in alternation, under field conditions.

Materials and Methods

1- Effect of some inducing resistance chemicals (IRC) and fungicides on conidial germination:

The tested IRC, *i.e.* Bion, potassium monobasic phosphate, salicylic acid and zinc sulphate as well as the systemic fungicides, *i.e.* fenarimol 12% (Rubigan), diclazoprim 5% (Sumi-8), deconazole 10% (Topas) and bromuconazole 10% (Vectra) were used to test their inhibitory effect on the *in vitro* germination of *Erysiphe pisi* conidia. In this regard, stock solution was prepared from the tested IRC (depending on their molecular weight) and fungicides (depending on their active ingredient). Serial concentrations, *i.e.* 0.0, 10, 25, 50, 100, 150, 200 and 250 mM. of IRC and ppm of fungicides were prepared and sprayed individually using atomizer on clean glass slides and left to air dry. Infected pea leaves showing obvious formation of powdery mildew infection were shaking on the glass slides to deposit the conidia. Glass slides were put on glass rods in Petri-dishes (two in each dish) containing distilled water (about 90% relative humidity). Conidial germination percentages were assessed at zero time of the experiment, and then the dishes were sealed with para-film and incubated at 20±1°C for 24 hours (Reeser *et al.*, 1983). Two drops of lactophenol-cotton blue were put on each slide (to kill, fix and stain the conidia and germ tubes) just before examining the conidial germination, then the germinated conidia on each slide were counted (ten fields), using light microscope. Five dishes were used for each concentration.

2- Greenhouse experiments:

Four inducing resistance chemicals (IRC) as well as four systemic fungicides were individually tested for their effects on the severity of pea powdery mildew. Plants were artificially inoculated with *E. pisi* under greenhouse conditions, using techniques adopted by Abada *et al.* (1996).

Pots (25 cm in diameter) containing formalin-disinfested soil were sown with pea seeds. Seven seeds (cv. Master pea) were sown in each pot, irrigated and left

to grow then thinned into five plants, 2 weeks after sowing. The grown plants (aged 6 weeks) were artificially inoculated by shaking of naturally infected young pea leaves by powdery mildew on the grown plants. The tested fungicides were sprayed on pea plants one week after artificial inoculation by the causal fungus. Meanwhile, IRC were sprayed one week before artificial inoculation by the fungus. Five pots were used for each treatment. The grown plants were irrigated when it was necessary and fertilized by adding one gram for each pot from the Crystalon compounded fertilizer (1:1:1; N:P:K), three weeks after sowing and two weeks later.

The disease severity was assessed weekly and the average was recorded. Also, hundred days after sowing plant foliage fresh weight (g), number and weight of green pods (g)/plant were estimated and recorded.

3- Field experiments:

Field experiments were conducted during 2008 and 2009 growing seasons at Alayiat County, Giza governorate to evaluate the effect of spraying pea plants with the systemic fungicides Rubigan 12 % and Topas two sprays then spraying salicylic acid and zinc sulphate as IRC on controlling the natural infection of powdery mildew.

Soil was prepared for sowing pea (cv. Master pea) at the end of January during 2008 and 2009 growing seasons using Herati planting method on rows in plots of 42 m² (8 rows). All agricultural practices, *i.e.* irrigation, weeds and pests control and fertilization were applied according to the standard recommendations of Ministry of Agriculture.

Two fungicides, *i.e.* Rubigan 12% and Topas as well as the two IRC, *i.e.* salicylic acid and zinc sulphate, were evaluated under field conditions, against the natural infection by powdery mildew. In this regard, the grown plants were left to the natural infection by the causal fungus then sprayed at the first appearance of powdery mildew symptoms by the tested fungicides (20 ml/100 l water) two times at two week-intervals (until flowering stage and beginning of forming small green pods). In addition, the two selected IRC were also sprayed at 50 mM, one week after the latter spray with the tested fungicides, two times at 10 day-intervals in different combinations with the tested fungicides until harvesting the green pods. Unsprayed plants with the tested fungicides or IRC served as control treatment. Five plots were used for each treatment. Green pods of each plot were harvested 100 days after sowing and the average weight was recorded.

4- Disease assessment:

Both artificially and naturally inoculated plants were carefully examined to estimate the severity of the infection by powdery mildew depending on the devised and modified scale (0-5) by Townsend and Heuberger (1943) using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (nxv)}{5N} \times 100$$

Whereas: n = Number of infected leaves in each category.

v = Numerical values of each category.

N= Total number of the infected leaves.

Statistical analysis:

The obtained data were statistically analyzed using the standard procedures for split designs as mentioned by Snedecor and Cochran (1967).

Results

Effect of different IRC and fungicide concentrations on conidial germination of Erysiphe pisi:

All the tested IRC and fungicides resulted in different degrees of suppression to the germinated conidia of the causal fungus compared with control treatment. This reduction was gradually increased by increasing the used concentration (Table 1). However, the fungicides were more efficient than IRC in this respect, where no one of the tested IRC caused complete inhibition to the germinated conidia even at 250 mM. On the other hand, the fungicides Topas and Rubigan 12% caused complete inhibition to the germinated conidia at 50 ppm, Sumi-8 at 100 ppm and Vectra at 150 ppm.

Table 1. Effect of different RIC and fungicide concentration on conidial germination of *E. pisi*, 24 hours after incubation at 20±1°C and 90% relative humidity

Treatment	Conidial germination (%) at * (mM or ppm)							Mean
	10	25	50	100	150	200	250	
Bion	86.2	81.8	76.6	72.8	62.0	52.2	39.8	67.3
Potassium monobasic phosphate	84.6	80.4	74.8	61.2	48.6	38.2	29.8	59.7
Salicylic acid	80.8	76.8	70.2	58.6	43.8	35.4	24.4	55.7
Zinc sulphate	80.0	75.0	68.0	54.8	40.6	31.0	22.6	53.1
Rubigan 12%	40.2	16.8	0.0	0.0	0.0	0.0	0.0	8.1
Sumi-8	48.4	31.6	12.0	0.0	0.0	0.0	0.0	13.1
Topas	38.0	14.2	0.0	0.0	0.0	0.0	0.0	7.5
Vectra	52.8	34.4	20.0	7.8	0.0	0.0	0.0	16.4
Mean	63.9	51.4	40.2	31.9	24.4	22.4	14.6	----

L.S.D. at 5% for: Treatments (T)= 2.2, Concentrations (C)= 2.9 and TxC= 3.3

* Average percentage of conidial germination at zero time recorded 2.4%; meanwhile it was 89.6% in check (fungicide and IRC free) treatment, after 24 hours of incubation at 20±1°C.

Greenhouse experiments

The tested IRC and fungicides resulted in significant reduction to the severity of powdery mildew with significant increase in the plant height, number of green pods and weight of green pods /plant (Table 2). In addition, the tested fungicides, *i.e.* Rubigan 12%, Sumi-8, Topas and Vectra were more efficient in this regard, being 3.1, 4.0, 2.8 and 4.7% of disease severity, 54.7, 53.2, 55.0 and 54.0 cm for plant height, 16.2, 15.8, 16.4 and 15.4 for green pods /plant, 112.8, 110.7, 115.9 and 109.0 g for green pods yield /plant, respectively, than tested IRC, *i.e.* Bion, potassium monobasic phosphate, salicylic acid and zinc sulphate, being 17.8, 18.7, 15.8 and 16.3% disease severity, 46.1, 45.0, 50.4 and 50.0 cm for plant height, 11.0, 10.0, 12.0 and 12.4 for green pods /plant, respectively. In addition, control treatment recorded 44.8%, 38.2 cm, 8.6 pod and 53.9g, respectively. Due to the high efficiency of the two fungicides Rubigan 12% and Topas and the two IRC salicylic acid and zinc sulphate, therefore, they were tested for their efficiency in managing the natural disease infection in field experiments, in alternation.

Table 2. Effect of spraying the resistance inducing chemicals and fungicides on the severity of pea powdery mildew as well as some crop parameters, under greenhouse conditions

Treatment	Disease severity (%)	Average plant height (cm)	Average number of pods /plant	Average weight of green pods (g/plant)
Bion	17.8	46.1	11.0	85.5
Potassium monobasic phosphate	18.7	45.0	10.0	83.8
Salicylic acid	15.8	50.4	12.0	89.0
Zinc sulphate	16.3	50.0	12.4	90.8
Rubigan 12%	3.1	54.7	16.2	112.8
Sumi-8	4.0	53.2	15.8	110.7
Topas	2.8	55.0	16.4	115.9
Vectra	4.7	54.0	15.4	109.0
Control	44.8	38.2	8.6	53.9
L.S.D at 5%	2.1	2.6	2.5	1.9

Field experiments

Spraying the two fungicides, *i.e.* Rubigan 12% and Topas on pea plants were more efficient in reducing the natural infection by powdery mildew, being 2.2 and 2.1% disease severity, on the average and resulted in producing the highest values of green pods yield, being 55.0 and 56.0 kg/plot (42 m²) on the average, respectively (Table 3). On the other hand, tested IRC, *i.e.* salicylic acid and zinc sulphate recorded, on the average, low efficiency in reducing disease severity being 14.0 and 13.4% and low values of green pods yield, being 40.1 and 41.8 kg/plot (42 m²), respectively. Meanwhile, spraying these fungicides two times followed by two

Table 3. Effect of spraying pea plants with two systemic fungicides and IRC on the severity of powdery mildew as well as pods yield of pea plants (cv. Master pea) under field conditions during 2008 and 2009 growing seasons

Treatment	Disease severity (%) during		Mean	Average green pods yield (kg/plot)* during		Mean
	2008	2009		2008	2009	
Salicylic acid (SA)	13.8	14.1	14.0	40.2	40.0	40.1
Zinc sulphate (ZS)	13.1	13.6	13.4	42.0	41.6	41.8
Rubigan 12% (R)	2.1	2.3	2.2	55.8	54.2	55.0
Topas (T)	2.0	2.1	2.1	56.6	55.4	56.0
R then SA	4.4	4.3	4.4	46.7	46.0	46.4
R then ZS	3.9	3.9	3.9	47.1	46.8	47.0
T then R	3.7	3.9	3.8	48.0	47.5	47.8
T then ZS	3.6	3.7	3.7	48.8	48.0	48.4
Control *	53.4	53.0	53.2	33.1	32.3	32.7
Mean	7.6	7.3	-----	217.7	219.5	-----
L.S.D. at 5% for: Treatment (T)=			2.5			3.3
Season (S) =			n.s			n.s
T x S =			3.7			4.2

* Each plot equal 42 m².

sprays of IRC in alternation, resulted in intermediate values of disease severity and green pods yield. In addition, unsprayed plants (control) recorded 53.4% disease severity and produced 32.7 kg/ plot (42 m²).

No significant differences were detected between the values of disease severity and green pods yield due to the effect of the growing season.

Discussion

Pea plants are liable to infection by powdery mildew and the peak of infection reaches its maximum at the time of green pods harvesting.

During the last decades, the world is suffering great pollution by many pollutants including agrochemicals including pesticides and fungicides. Therefore, the current strategy of management pests, especially of vegetables and fruits depends on using alternative methods other than pesticides, fungicides and/or using these chemicals at the first periods of plant growth prior to fruit maturity. Hence, this work aimed to using IRC, as safe chemicals, in alternation with systemic fungicides, in which the fungicides spray at the first period of infection (before green pods harvesting) to minimize the infection to low level for a period of about 45 days (the time of flowering and green pods formation until pre-maturity) then spraying IRC just before and during harvesting the green pods in order to obtain green pods of permitted ratio and / or free from fungicides residue.

All the tested IRC and fungicides caused different degrees of suppression to the germinated conidia of the causal fungus compared with control treatment. This suppression was gradually increased by increasing the tested concentration. However, fungicides were more efficient than IRC in this respect, where none of the tested IRC caused complete inhibition to the germinated conidia even at 250 mM.

The obtained data of pot experiment in the greenhouse showed that the tested IRC, *i.e.* Bion, potassium monobasic phosphate, salicylic acid and zinc sulphate caused significant reduction to the disease with significant increase to the plant height, number of green pods and weight of green pods yield / plant in comparison with the control treatment. On the other hand, fungicides were more efficient than IRC in this regard. It is well known that fungicides, especially systemic ones, are more efficient in management of many fungal diseases including pea powdery mildew (Abada *et al.*, 1996; McGrath, 2001 and Richardson, 2006). Also, IRC were reported as alternative and/ or safe management of many diseases, especially those of vegetable crops (Metranx and Boller, 1986; Fouly, 2004, Abada *et al.*, 2008 and Ashour, 2009a and b).

It has been found from field experiment that spraying pea plants twice with any of Rubigan and Topas fungicides in alternation with another two sprays of salicylic acid or zinc sulphate as IRC, resulted in significant reduction in disease severity with a marked increase in the productivity of green pods yield compared with unsprayed (control) plants. However, these treatments were of low efficiency when compared with the tested fungicides only, and still of high efficiency when compared with spraying IRC only. Although, the alternation between the tested fungicides and RIC gave intermediate effect on disease reduction and the produced green pods yield. The produced green pods are of low fungicides residue, which the long period after the latter fungicides spray may cause metabolic changes to become safe or less toxic compound.

The reduction in pea powdery mildew may be due to the effect of the tested fungicides and RIC each alone or in alternation. In addition, the role of fungicides in reducing the disease is well known (McGrath, 2001 and Richardson, 2006) and the role of RIC is explained by many hypothesis, where acquired resistance induced by restricted infection is not due to a specific component of the pathogen, but rather to gradual appearance and persistence of a level of metabolic disturbance leading to stress on the host.

It has been mentioned that inducing acquired resistance is persistent and generally is pathogen nonspecific (Doubrava *et al.*, 1988). Moreover, Larcke (1981) reported that unlike elicitors of phytoalexins accumulations, which are elicited at the site of application, may be responsible for localized protection and induces systemic acquired resistance that sensitizes the plant response rapidly after infection. These responses inducing phytoalexins accumulation and lignifications and induce enhance activities of chitinase and β -glucanase (Dean and Kuc, 1985 and Metranx and Boller, 1986, Abd El-Kareem *et al.*, 2001). Kessmann *et al.* (1994) reported that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable broad spectrum disease control and they could be used

preventatively to bolster general plant health, resulting in long lasting protection. In addition, Vernooij *et al.* (1994) mentioned also that salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance to plant pathogens, but is required in signal transduction. So, resistance might be correlated with the production of oxidative enzymes in the treated healthy and diseased plant tissues (Wen *et al.*, 2005). In this respect, Melo, *et al.* (2006) mentioned that polyphenoloxidase and peroxidase are enzymes of broad spectrum among plants catalyze the hydroxylation of monophenols to O-diphenols and their oxidation to O-diquinones. He added that quinines are highly reactive molecules that can spontaneously complex various types of molecules into large types

The use of RIC was previously used as alternative method for controlling many powdery mildew diseases including powdery mildew of cantaloupe (Larcke, 1981; Ibrahim, 1998; Fouly, 2004; Abada *et al.*, 2008 and Ashour, 2009a).

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استخدام بعض الكيماويات المستحثة للمقاومة والمبيدات الفطرية الجهازية في مكافحة مرض البياض الدقيقي في البسلة

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تم اختبار فعالية بعض الكيماويات المستحثة للمقاومة وكذلك بعض المبيدات الفطرية الجهازية علي تثبيط إنبات الجراثيم الكونيدية لمكافحة مرض البياض الدقيقي في البسلة المتسبب عن الفطر ايرسيفي بيسي في تجارب أجريت بالصوبة والحقل.

أحدثت كل الكيماويات المستحثة للمقاومة وهي البيون ، فوسفات الكالسيوم ، فوسفات البوتاسيوم الأحادية القاعدية ، حامض الساليسيك ، كبريتات الزنك والمبيدات الفطرية الجهازية وهي الروبيجان ، سومي-8 ، توباس ، فيكترا درجات مختلفة من التثبيط لإنبات الجراثيم الكونيدية. بالإضافة لذلك ، فقد كانت المبيدات الفطرية أكثر فعالية عن الكيماويات المستحثة للمقاومة.

أوضحت نتائج تجربة الصوبة أن رش نباتات البسلة بأى من الكيماويات المستحثة للمقاومة والمبيدات الفطرية تسببت في إحداث انخفاض معنوي للمرض مع حدوث زيادة ملحوظة في المحصول من حيث ارتفاع النبات وعدد القرون الخضراء ووزن القرون الخضراء لكل نبات مقارنة بنباتات المقارنة.

أوضحت تجارب الحقل خلال موسمي ٢٠٠٨، ٢٠٠٩ أن رش نباتات البسلة بأى من المبيدين روبيجان أو توباس متبوعا برش أى من حامض الساليسيك أو كبريتات الزنك كل على حده أو بالتبادل أحدث انخفاض معنوي لشدة الإصابة بمرض البياض الدقيقي مع حدوث زيادة معنوية لمحصول القرون الخضراء مقارنة بنباتات المقارنة ، ومع ذلك كانت معاملات تبادل الرش بين المبيدين المختبرين والكيماويات المستحثة لحد ما أقل فعالية من رش المبيدات الفطرية ولكن كانت أكثر فعالية في خفض الإصابة بالمرض وزيادة محصول القرون الخضراء مقارنة باستخدام الكيماويات المستحثة للمقاومة بمفردها.