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Effectiveness of some biological control agents and agricultural practices in controlling pea leaf blight caused by *Ascochyta* spp. under field conditions

Ahmed I. S. Ahmed*; Mohamed K. M. Agha

Plant Protection Department, Desert Research Center, Cairo, Egypt.
*Corresponding author: ahmed_drc@yahoo.com

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Abstract— *Ascochyta blight is one of the most common diseases that threaten pea and cause severe crop losses. The research is concerned with the integrated control of this disease by studying the effect of biological control agents with inorganic salts, planting dates and planting distances, especially in light of climate change and the impact of these factors on the spread of the disease. In this study, two bacterial strains Bacillus megaterium and Pseudomonas fluorescens as biocontrol agents; potassium carbonate and sodium carbonate were evaluated with the effect of planting dates, where the first date was at the beginning of October, and the second date was at the beginning of November. Also the distance between the irrigation lines (0.75 and 1.5 meter) in two successive seasons 2020/2021 and 2021/2022 under the conditions of the Dakhla Oasis, the New Valley Governorate. The best results were with the first date of planting and a planting distance of 1.5 meters, which led to a significant reduction of disease severity, with a significant increase in traits associated with vegetative growth. Also, treatment with Pseudomonas fluorescens led to an increase in vegetative growth and plant height compared to treatment with Bacillus megaterium. By studying the interaction between planting dates with biocontrol agents, it led to a significant decrease in disease incidence and severity, with a significant increase in vegetative growth. Also, there was no significant effect with interaction between planting dates and planting distances, while it had a significant effect on the incidence and severity of infection. Bacterial isolates used in this study with mineral salts contributed to increasing plant growth rates and reducing ascochyta blight infection rates. Further studies can be conducted to include these treatments within the integrated control programs for Ascochyta blight on pea.*

Keywords— ***field pea; biological control; Bacillus megaterium; Pseudomonas fluorescens; potassium carbonate; sodium carbonate***

I. INTRODUCTION

Field pea (*Pisum sativum* L.) is an annual, cool-season legume native to northwest to southwest Asia. It was among the first crops cultivated. Pea is a winter season vegetable crop. Master B. is an old cultivar has been cultivated for more than 23 years ago in Egypt because of its superior features. Otherwise, this cv. has decline in its certain good attributes, many reasons are behind this decline some this reasons is wrong agricultural practices) Elsadek, *et al.*, 2017). Pea are affected by many fungal diseases, the most important group of these diseases are leaf spots. Ascochyta blight disease is one of the most important diseases affecting field pea. The disease occurs in almost all pea-growing regions of the world and can cause significant crop losses when conditions are favorable for an epidemic (Bretag, *et al.*, 2006). The extent and severity of disease depend on the cropping system and weather conditions. The most favorable conditions for the pathogen are frequent rainfall, high relative humidity, and leaf wetness duration (Roger *et al.,* 1999, Tivoli and Banniza, 2007). Ascochyta blight is a destructive disease in many field peas (*Pisum sativum* L.) growing regions and it causes significant losses in grain yield. It caused by a complex of fungal pathogens, it is commonly referred to the Ascochyta complex, including *Ascochyta pinodes* L.K. Jones (teleomorph: *Mycosphaerella pinodes*; Berk and Blox, Vestergr.), *Phoma medicaginis* var. *pinodella* (Jones) Morgan-Jones and K. B. Burch, *Ascochyta pisi* Lib.(teleomorph: *Didymellapisi* sp.nov.) and *Phoma koolunga* (Davidson *et al.*, 2009; Liu *et al.*, 2013; Liu *et al.*, 2016). This blight complex causes range of different symptoms, including, Ascochyta blight, foot rot, black stem, leaf and pod spot. Seed quality may also be reduced through seed discoloration or retardation of seed development. *A. pinodes* can infect seedlings and all aerial parts of pea plants, causing necrotic leaf spots, stem lesions, shrink age and dark-brown discoloration of seeds, blackening the base of the stem, and foot rot in seedlings. The mycelia of *A. pinodes* can penetrate pea leaves across the stomas, and formed specific penetration structures (Liu *et al.*, 2016). The disease symptoms caused by *P. pinodella* are similar to those observed with *A. pinodes*. However, *P. pinodella* infection can result in more severe foot rot symptoms that can extend below ground, while causing less damage to the leaves, stems and pods. *A. pisi* causes slightly sunken, circular, tan-colored lesions with dark brown margins that occur on the leaves, pods, and stems (Chilvers *et al.*, 2009). Ascochyta blight accelerates the maturity of affected pea crops, the plants lose water in stems, leaves, accelerate seed desiccation, reduce seed weight, disturbs nutrient metabolism and reduces photosynthetic potential of plants (Garry *et al.,* 1998). The severity of disease can differ due to the temperature and duration of leaf wetness. Even a low level of infection can cause significant losses in both production and quality (Kraft and Pfleger, 2001). Usually fungicides are used to control Ascochyta blight, seedling blight, and root rots. Treating seeds with fungicides is one time application, it's activity is too short-lived to protect the plants throughout the growing season. Multiple fungicide applications are often required to manage foliar diseases. However, each application results need additional expenses, carries risks to the environment, and repeated applications may lead to reduced fungicide efficacy due to pathogen evolution, i.e. the development of genetic resistance to frequently applied fungicides (Jones and Ehret, 1976; Cook and Zhang, 1985). This requires the evaluation of safe alternatives in disease control, such as biological control to avoid environmental pollution and avoid adverse effects on public health. The key to achieving successful, reproducible biological control is gradual appreciation that knowledge of the ecological interactions in soil and root environments is required to predict the conditions under which biocontrol can be achieved (Deacon, 1994; Whipps, 1997a). Bennett *et al.,* 2019 showed that time of sowing had a greater impact on yield, emphasize the need for adopting biological methods for the control of plant diseases. Bacterial antagonists offer a promising alternative to existing chemical control practices and have great market potential for disease management. The use of bacterial antagonists as biofungicides for the control of various plant diseases has attracted considerable interest (Baker and Cook, 1982; Cook and Baker, 1983). Many bacteria, including *Bacillus subtilis* (Ehrenberg) Cohn, *B. megaterium* de Bary, and *Pseudomonas fluorescens* Migula, have been studied as biocontrol agents for plant pathogens of food legumes. Many researchers using inorganic salts carbonate and bicarbonate as alternative disease control strategies it is proved to be cost effective and eco-friendly for the management of many plant diseases due to the development of resistant fungal strains. Türkkan *et al.* (2017) reported that using carbonate and bicarbonate salts completely inhibited the mycelial growth of *Botrytis cinerea* under *in vitro* conditions. Carbonate and bicarbonate salts had the highest efficacy on powdery mildew of Tomato plants, (Bakeer, *et al.,* 2012). Sodium carbonate (SC), potassium carbonate, sodium bicarbonate (SBC), and potassium bicarbonate inhibit spores germination of *Penicillium digitatum* (causal agent of citrus green mold), and SC and SBC were equal and superior for control green mold on lemons and oranges (Smilanick *et al.,* 1999). In same trend (Ahmad *et al.*, 2019) revealed that inorganic salts sodium bicarbonate followed by potassium carbonate have shown significant inhibition to mycelial growth of *Alternaria solani* and proved to be cost effective and eco-friendly for the management *A. solani* in comparable with fungicides. Ascochyta ascospores are released into the air from infested residue at certain times of the year, depending on environmental conditions, sowing date, and row spacing of crops can be manipulated to avoid the maximum risk period when airborne ascospores are at their highest numbers (Bretag, 1991; Jacobson and Backman, 1993). Wide row spacing and low seeding rate reduced Ascochyta blight severity and increased seed yield per plant, also reduced plant population density could be effective factor in a program to manage Ascochyta blight of chickpea (Chang *et al.,* 2007). In addition, these methods were useful in combating several other diseases, for example, delaying in sowing date increasing the disease incidence of rust and powdery mildew on pea varieties, and early or late planting dates causing losses in seed yield (Sangar and

Singh, 1994; Singh and Singh, 2011). Early sown in the season caused minimum disease incidence while maximum disease severity was observed when the crop was sown late in the season (Kumar *et al.*, 2022). Plant density is an important agronomic factor that affects crop growth, development, and yield. The optimum plant density to attain the highest yield can vary with genotype, production and, environmental factors, also plant density is one of the most effective agronomic factors for determining optimum plant nutrient uptake (Asik *et al.*, 2020).

The objectives of this current study were to investigate the use of two strains of *Bacillus megaterium*, *Pseudomonas fluorescens* antagonistic bacterial agents for disease control, and effects of Potassium carbonate, Sodium carbonate as inorganic salts for disease management, as well as investigate effect of different practices such as the difference in sowing dates and row spacing on *Ascochyta* blight diseases control of pea as one of the new crops that were recently introduced in New Valley Governorate, and some diseases have appeared under these conditions, such as *Ascochyta* blight.

II. MATERIALS AND METHODS

Isolation, purification and identification of Ascochyta Blight-Associated Fungal Isolate

Infected pea plant tissues with typical ascochyta blight symptoms were collected from fields in Dakhla Oasis, The new valley governorate and from extension fields implemented by the agricultural clinic project for desert and reclaimed lands. Infected plant parts were cut into small pieces and surface sterilized in 70% ethanol with a 30s treatment followed by sodium hypochlorite with a 10 min, then washed for three times with sterile water. Sterilized samples were placed onto potato dextrose agar (PDA) plates (200g potato, 20g glucose, 15g agar, and 1L water) supplemented then incubated at 25°C for 4 days. After incubation, the culture were purified with single conidium and saved at 4°C for further Experiments.

Source of seeds, salts and bioagents

The source of pea cv. Master B was obtained from Horticulture Research Institute, Agricultural Research center, Egypt. The source of tested mineral was Al-Gomhoria Company for medicines and medical supplies, and bio agents were obtained from microbiology and soil fertilizing unit, Desert Research Center.

Agricultural Practices

Sowing Date (SD): Pea seeds were sown in two dates, First date on the first of October (SD1); Second date on the first of November (SD2). The planting was carried out at two Row spacing (RS), using GR irrigation lines; as 1.5 m between the two lines (RS1); 75 cm between the two lines (RS2).

Pea Foliage Sprayed with (FS): *Bacillus megaterium*, (B1); *Pseudomonas fluorescens* (B2); *Potassium carbonate* (Pc); *Sodium carbonate* (Sc) were used to evaluate their effects on ascochyta leaf blight. Bacterial culture grown on nutrient broth medium then incubated on 28°C±2 for 4 days and resuspended at a concentration. Bacterial isolate *P. fluorescens* was grown on King's medium B (KMB) broth (per liter, Proteose peptone 20g, Glycerol10 mL, K_2HPO_4 1.5g and $MgSO_4$ 1.5g). The flasks were placed on a rotary shaker to grow at 120 rpm for 60 hrs at 24±1°C. Plants were inoculated with 48h bacterial suspension diluted to give approximately 10^8 cfu/ml.

Experimental layout and growth of plants

The experiment was conducted at Dakhla Oasis, the New Valley Governorate during two successive seasons 2020-2021 and 2021-2022 to evaluate the efficacy of two biological control strains (*Bacillus megaterium* and *Pseudomonas fluorescens*), two mineral salts (potassium carbonate and sodium carbonate). Role of planting dates and spacing between irrigation lines in reducing disease incidence and severity of natural infection of Ascochyta blight, and improving pea productivity. Experiment was conducted in 3 replications, each replicate is 6 m long, (each meter has 3 drippers, and each replicate has 18 drippers. The treatments were on the first two true leave stage, and repeated every 10 days until the beginning of flowering stage. All other agriculture practices were used as recommended.

Data recorded:

Disease incidence and severity; Ten plants per plot were randomly taken for evaluation of the following traits:

Disease severity, was assessed visually on a 0–9 scale, where: 0 = no infection, 1 = 1–9% of foliage area affected per plot, 2 =10–19%, 3 = 20–29%, 4 = 30–39%, 5 = 40–49%, 6 =50–59%, 7 = 60–69%, 8 =70–79% and 9 ≥ 80% of the foliage area affected per plot according to (Chang *et al.*, 2007). First assessment was done 10 days after treatment and the next at two weeks intervals. The severity of infection was estimated on 10 plants in each replicate, and the percentage of infection severity was calculated based on the formula:

Total (the number of plants in each degree of the scale × the degree of the scale) ÷ (the number of plants in which the estimate was taken×5). Diseases incidence recorded on the basis of number of infected plants in each treatment relative to the number of plants sown in each treatment.

Growth parameters

Total Fresh weight of plant, plant length, No. of branches per plant, No. of flowers per plant, No. of pods per plant, were recorded at the end of the season and the average results of the two seasons were analyzed.

Data analysis

Data were statistically analyzed by ANOVA, differences between treatment means were considered significant at P< 0.05. All values presented are the averages of each season.

III. RESULTS AND DISCUSSION

One of the reasons responsible for the low productivity of peas is fungal diseases that cause large annual losses in the grain yield; from the main disease that affected peas is Ascochyta blight which more commonly known as black spot disease, and it is distributed worldwide (Bretay *et al.,* 2006). The control of Ascochyta blight on pea is a complex process that is affected by many factors which related to environment, other are related to the host plant, and the pathogen. This study aims to reduce the disease incidence and severity under natural infection conditions by using treatments that do not have a polluting effect on the environment or plants.

Identification of fungal pathogen

The infection of ascochyta blight in field peas is a complex of different fungi including *Ascochyta pinodes*, *A. pisi* and *Phoma glomerata*. Sometimes these pathogens can occur together in one field and on one the same host plant (Liu *et al.,* 2016). While the *Ascochyta pisi* was the main pathogen infecting peas in the cultivation sites from which the fungi were isolated in this study, where symptoms of infection were included lightly sunken, circular, tan-coloured lesions with a dark brown margin on the leaves, pods, and stems (Chilvers *et al.* 2009). This fungus usually does not attack the base of pea plants or cause foot rot. Single spore fungal isolates were grown on pea agar medium (2% pea powder, 1.5% agar, w/w) for 15 days with a 16-h photoperiod under fluorescent light at 20±2 °C. Colony characteristics were assessed with a stereo microscope, and the shape and size of conidia were determined with a compound microscope *A. pisi*. The color of the spore masses was observed with a stereo microscope since the production of carrot-red spore masses is the principal characteristic according to (Jones *et al.,* 1927 and Dokken *et al.*, 2007).

Effect of bioagents and tested salts on Ascochyta blight and growth of peas

The results in Table (1) showed that effects of spraying treatments, planting dates and row spacing were significant, whether between treatments or treatments compared with control. Spraying with bacteria *B. megaterium* was the most efficient in reducing the incidence and severity of infection (20.83 %,12.92 %) in first season (46.67 %,30.08 %) in second season, whether from other treatments or control(50.50 %,45.67) for S1 and (72.00 %,66.5 %) for S2 then followed by *Pseudomonas fluorescens* and spraying with potassium carbonate. Spraying with sodium carbonate was the least efficient than other treatments (36.42 %, 31.50 %) for S1 and (59.58 %, 56.17 %) for S2 in reducing incidence and severity of infection, but it was still more efficient than control. The difference was significant between the treatments and treatments compared with control in terms of the effect on growth parameters, as the spraying with bacteria *P. fluorescens* had the highest effect on fresh weight (69.6g), plant length (74.5cm), on other hand *B. megaterium* had the highest effect on other growth parameters, whether number of leaves (22.8), number of pods (35) and number of branches (3.1) per plant, followed by bacteria and potassium carbonate. Hence, the use of sodium carbonate was the least effective among the treatments. The results also showed that the planting date and row spacing had significant effects on the incidence and severity of infection, as well as on the growth parameters of (fresh weight, plant length, and number of leaves, pods and branches). Where the incidence and severity were lower in the case of the first planting date first of October and RS1 with long row space 1.5 m than the second planting date (first of November) and RS2 the (short row space 75cm) in each season. So the fresh weight, plant height, number of leaves, number of pods, and number of plant branches were significantly affected by the sowing dates and row spacing. These results in the same line with Bretag *et al.* (1995) where reported that, infection level with ascochyta differed from year to year and region to region depending on local climatic conditions. El-Mohamedy *et al.* (2013) indicated that, using biological control agents is an active and non-toxic approach to decrease crop loss initiated by plant pathogens. *Bacillus megaterium* was reduced severity of *septoria tritici* blotch (STB) of wheat caused by the fungal pathogen *Mycosphaerella graminicola* by combination of different mechanisms (Kildea, *et al.*, 2008). Ganeshan and Manoj (2005) reported that *Pseudomonas fluorescens* are known to enhance plant growth promotion and reduce severity of various diseases, and induced systemic resistance. Chang *et al. (*2007) found that wide row spacing reduced ascochyta blight severity and increased seed yield per plant. Bennett *et al.* (2019) revealed that sowing date had a greater impact on growth parameters than repeated application of fungicide.

Table (1): Effect of foliar spraying with bio-agents, mineral salts, sowing dates and Row spacing on Ascochyta blight and growth parameters of peas (average of combined data over 2020/2021 and 2021/2022 seasons) of pea

Traits / Treatments	DI		DS		Fresh weight (g)	Plant length (cm)	Leaf No.	Pod No.	Branches No.
	S1	**S2**	**S1**	**S2**					
B1	20.8	46. 7	12.9	30.8	69.6	74.5	22.8	35	3.1
B2	24.6	52	18.4	37.8	64	68.7	18.8	28.3	2.7
PC	31.1	54	26. 7	50	63.3	65	17.8	24.9	2.1
SC	36.4	59.6	31.5	56.2	63. 7	53.3	15.8	20.8	1.7
C	50.5	72	45. 7	66.5	44.8	38.2	14.2	13.5	1.6
LSD at 0.05	1.9	2.8	2.4	1.6	0.98	1.3	1.5	0.7	0.4
SD1	24.1	51.6	18.5	46.3	62.9	61.9	18.9	26.2	2.4
SD2	41.3	62. 7	35.6	49.9	59.2	57.9	16.8	22.8	2.3
LSD at 0.05	1.2	0.8	1.8	0. 8	0.7	0.7	0.5	0.5	0.2
RS1	28.4	51.9	22.9	44.8	62.6	61.3	18.8	25.8	2.4
RS2	36.9	61.7	31.2	51.4	59.5	58.5	16.7	23.2	2.7
LSD 0.05	1.2	0.9	1.8	0.9	0.6	0.4	0.4	0.5	0.3

- DI, disease incidence ; DS, disease severity; S1, season 2020/2021; S2, season 2021/2022.
- B1, *B. megaterium;* B2, *Pseudomonas ;* PC, potassium carbonate; SC, sodium carbonate; C, control.
- SD1, first of October; SD2, first Of November; RS1, row space 1.5m; RS2, row space 75 cm.

Effect of interaction between foliar spraying and sowing dates on Ascochyta blight and growth of peas

The results in Table (2) revealed that, the effect of interaction between foliar spraying (FS) and sowing date (SD) on the total vegetative growth, diseases incidence and diseases severity were reduced significantly, in comparing with control. The first sowing date in the (first of October) showed the lowest in the percentage of incidence and severity of infection during the two seasons. Plant lengths, fresh weight, number of leaves, number of pods and branches per plant, at the same time are higher in SD1 than SD2 (first of November). FS with B1in SD1 recorded lowest percent of DI (11%) and DS (5.17%) in S1 and gave superior number from leaf (24.5), No. of pod (37.0) and No. of branches (3.3). These obtained data in the same trend with Singh and Singh (2011) they revealed that early sowing dates resulted in poor seed yield and delayed sowing dates resulted in poor seed yield and quality.

Effect of interaction between foliar spraying and row spacing on Ascochyta blight and growth of peas

Data in table (3) showed significant effect in interaction between foliar spraying (FS) and row spacing (RS) on DI, DS, plant length, and leaf number while data recorded for fresh weight, pod numbers, and branches numbers were not significant. Percentage of DI and DS were lower in RS1 in the two seasons with all FS treatments, otherwise percentage of DI and DS were higher in RS2. FS with B1 gave the highest effect in suppressing DI (16.7%), DS (8.8%) in case of RS1 followed by B2, PC, and SC gave the lowest effect DI (32.8%), DS (26%) in comparison with control DI (48%), DS (40%) in season 1. Also in RS1 Foliar spraying with B2 gave good effect on fresh weight (70.5g) and plant length (75.3cm) comparing with control which gave fresh weight (46g) and plant length (40cm). Lowest growth parameters date were recorded in control treatment in RS2 which average was (43.5g, 36.4cm, 12.8, 12.5and 1.33) for fresh weight, plant length, No. of leaves, No. of pods, and No. of branches respectively. Decreasing in disease incidence and severity in large row spacing may be due to dispersal of Ascochyta conidia which may be more effective via narrow row spacing than wide rows, also when plants are grouped more tightly together in a row than if they are widely spaced. Second, air movement within a dense canopy is reduced, thus maintaining a more humid microclimate that supports blight development (Burdon and Chilvers 1982; Boudreau and Mundt 1997). Jaccoud-Filho *et al.* (2016) reported that, reduced row spacing increase severity of *Sclerotinia sclerotiorum* in soybean, while increase row spacing decreased disease severity.

Table (2): Interaction between foliar spraying and sowing dates on Ascochyta blight incidence, severity and some growth parameters of pea (average of combined data over 2020/2021 and 2021/2022 seasons)

Treatments		**DI**		**DS**		**Fresh weight (g)**	**Plant length (cm)**	**Leaf NO.**	**Pod No.**	**Branc hes No.**
		S1	**S2**	**S1**	**S2**					
B1	SD1	11	41.3	5.7	28	66.5	70.8	24.5	37	3.3
	SD2	30.7	52	20.7	32.2	61.5	66.6	21	33	3
B2	SD1	16	48.3	10.3	36.8	72.2	75.8	20.2	31.2	3.2
	SD2	33.2	55.7	26.5	38.7	67.1	73.2	17.4	25.5	2.2
PC	SD1	19.7	48.5	16.7	49	65	67.5	17.7	25.3	2.2
	SD2	42.5	59.5	37.7	51	61.5	62.5	16.5	24.5	22
SC	SD1	27.7	52	20.3	54.8	64.2	565	16.7	22.5	1.7
	SD2	45.2	67.7	42.7	57.5	63.2	50.6	15	19.2	1.7
C	SD1	46.2	68	40.3	63	47	39.6	15.8	15	1.8
	SD2	54.8	76	51	70	42.5	36.9	12.5	12	1.3
LSD 0.05		2.7	1.8	3.9	1.8	1.6	1.6	1.2	1.1	0.5

- DI, disease incidence ; DS, disease severity; S1, season 2020/2021; S2, season 2021/2022; FS, foliar spray; SD, sowing date.
- B1 *B. megaterium* , B2 *Pseudomonas* , PC potassium carbonate, SC sodium carbonate, C control.
- SD1, first of October; SD2, first of November.

Table (3): Effect of interaction between foliar spraying and row spacing on Ascochyta blight incidence, severity and growth of pea (average of combined data over 2020/2021and2021/2022 seasons).

Treatments		**DI**		**DS**		**Fresh weight (g)**	**Plant length (cm)**	**Leaf No.**	**Pod No.**	**Branches No.**
		S1	S2	S1	S2					
B1	RS1	16.7	40.8	8.8	25	66	69.7	24.2	36	3.3
	RS2	25	52.5	17	35.2	62	67.8	21.3	34	2.8
B2	RS1	18.5	46	16.8	33.3	70.5	75.5	19.7	29.5	2.8
	RS2	30.7	58	20	42.2	68.8	73.8	17.9	27.2	2.5
PC	RS1	26	48	22.8	47.2	65	66.5	17.6	26.8	2.2
	RS2	36.2	60	30.5	52.8	61.5	63.5	16.6	23	2
SC	RS1	32.8	56	26	55	65.5	55.3	17	22.2	1.7
	RS2	40	63.2	37	57.4	61.8	51.3	14.7	19.5	1.7
C	RS1	48	69	40	63.5	46	40	15.5	14.5	1.8
	RS2	53	75	51.3	69.5	43.5	36.4	12.8	12.5	1.3
LSD 0.05		2.6	1.9	3.9	2.1	NS	0.8	0.9	NS	Ns

- DI, disease incidence ; DS, disease severity ; S1, season 2020/2021; S2, season 2021/2022.
- B1, *B. megaterium ;* B2, *Pseudomonas ;* PC, potassium carbonate; SC, sodium carbonate; C control.
- RS1, row space 1.5m; RS2, row space 75 cm.

Effect of interaction between Row spacing and sowing dates on Ascochyta blight and growth of peas

Data in table (4) revealed that (RS)×(SD) significantly effected on DI, DS, and fresh weight while there were no significant differences between data recorded for plant length, leaf, pod, and branches numbers. The good suppressing for percentage of DI and DS were recorded in SD1 with both RS1 (18.8% DI, 15.87 DS) and RS2 (29.4%DI, 21.1%DS) for S1. On the other hand good fresh weight were obtained in SD1 with both RS1 and RS2 (64.8g and 61.1g) respectively. These data in line with Migawer and Bakeer (2003), they reported that interaction between sowing date × plant spacing significantly effected on disease severity.

Table (4): Effect of interaction between row spacing and sowing dates on Ascochyta blight and growth of peas (average of combined data over 2020/2021and2021/2022 seasons)

Characters / Treatments		DI		DS		Fresh weight (g)	Plant length (cm)	Leaf No.	Pod No.	Branches No.
		S1	S2	S1	S2					
RS1	SD1	18.8	47.2	15.8	43.7	64.8	63.4	20	27.5	2.5
	SD2	38	56.7	29.9	45.9	60.4	59.3	17.9	24.1	2.2
RS2	SD1	29.4	56.7	21.7	49	61.1	60.5	17.5	24.9	2.3
	SD2	44.5	67.4	41.3	53.8	57.9	56.6	15.4	21.6	1.9
LSD 0.05		1.6	1.2	2.5	1.3	0.8	NS	NS	NS	Ns

- DI, Disease incidence; DS, disease severity; S1, season 2020/2021; S2, season 2021/2022.
- B1, *B. megaterium;* B2, *Pseudomonas;* PC, potassium carbonate; SC, sodium carbonate; C, control.
- SD1, first of October; SD2, first Of November; RS1, row space 1.5m; RS2, row space 75 cm.

Effect of interaction between foliar spraying, sowing date and row spacing on Ascochyta blight and growth of peas

Data in table (5) clarified that the interaction between (FS)×(SD)×(RS) gave significant effect in suppressing both DI, DS and increased the average of fresh weight in the two seasons, otherwise the interaction increased plant length, leaf number, pod number, and branches number than control treatment but without significant deference. The good result in suppressing percentage of DI, DS comes out when spraying pea plants with B1 in SD1 on RS1 (6% DI& 2.7%DS for S1) followed by spraying with B2 (8.7% DI& 8.3%DS for S1) also B2 increased average of fresh weight (73g, 68g) (71.3g, 66.2g) and plant length (76.7cm, 73,8cm) (75cm, 72.5cm) in both (SD1, SD2) RS1, and (SD1, SD2) RS2, respectively. Data recorded in SD1 were superior for all FS whether RS1and RS2.The lowest data were recorded with control treatment in SD2 in both seasons and RS1 and RS2.

Table (5): Interaction between foliar spraying, sowing date and row spacing on Ascochyta blight incidence and growth of peas (growth parameters of pea (average of combined data over 2020/2021 and 2021/2022 seasons)

Treatments		DI				DS				Fresh weight (g)		Plant length(cm)		Leaf No.		Pod No.		Branches No.	
		S1		S2		S1		S2											
		RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2
B1	SD1	6	16	36	46.7	2.7	7.7	24	32	69	64	72.17	69.5	26	23	38	36	3.3	3
	SD2	27.3	34	45.7	58.3	15	26.3	26	38.3	63	60	67.2	66	22.3	19.7	34	32	3.3	2.7
B	SD1	8.7	23.3	44	52.7	8.3	12.3	32.7	41	73	71.3	76.7	75	21	19.3	32	30.3	3.3	3

2	SD2	28.3	38	48	63.3	25.3	27.7	34	43.3	68	66.7	73.8	72.5	18.3	16.5	27	24	2.3	2
PC	SD1	12	27.3	44	53	15.7	16.7	46	52	68	62	69	66	18.2	17.2	27.7	23	2.3	2
	SD2	40	45	52	67	30	44.3	48.3	53.7	62	61	64	61	170	16	26	23	2	2
SC	SD1	23.3	32	48	56	18	22.7	53.7	56	66	62.3	58	54	18	15.3	24	21	1.7	1.7
	SD2	42.3	48	64	70.3	34	51.3	56.3	58.7	65	61.3	52.5	48.5	16	14	20.3	18	1.7	1.7
C	SD1	44	48.3	64	72	34.7	46	62	64	48	46	41	38	17	14.7	16	14	2	1.7
	SD2	52	57.7	74	78	45.3	56.7	65	75	44	41	39	34.8	14	11	13	11	1.7	1
LSD 0.05		3.7		2.7		5.6		2.9		1.8		NS		NS		NS		NS	

- DI, disease incidence; DS, disease severity ; S1, season 2020/2021; S2, season 2021/2022; FS, foliar spray; SD, sowing date.
- B1, B. megaterium ; B2, Pseudomonas; PC, potassium carbonate; SC, sodium carbonate; C, control.
- SD1, first of October; SD2, first Of November; RS1, row space 1.5m; RS2, row space 75 cm.
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IV. CONCLUSION

Data obtained from this research indicated that, the use of biological control strains and mineral salts as a foliar spray had a significant effect in reduction of incidence and severity of Ascochyta blight natural infection and improving vegetative growth, with significant differences between treatments also between treatments and control. The effect of *Bacillus* strain was most effective isolate in reducing incidence and severity of infection followed by *Pseudomonas* strain, then potassium carbonate, and sodium carbonate had the least effect on infection. Bacterial isolates used in the study with mineral salts also contributed to increasing plant growth rates and reducing ascochyta blight infection rates. Further studies can be conducted to include these treatments within the integrated control programs for ascochyta blight on pea.

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REFERENCES

[1] Ahmad, S., Aslam, A., Iqbal, Z., Ghazanfar, M. U., Ahmad, I., Ashraf, E. and Asim, M. (2019). Different Methods used to Control Early Blight of Potato in Laboratory Conditions. Asian Journal of Biological and Life Sciences, 8(2), 77.

[2] Ahmad, S., Khan, M. A., Ahmad, I., Iqbal, Z., Ashraf, E., Atiq, M. and Naseer, S. (2021). Efficacy of fungicides, plant extracts and biocontrol agents against Ascochyta blight (*Ascochyta rabiei*) of chickpea (*Cicer arietinum* L.) under field conditions. Plant Science Today, 8(2), 255-262.

[3] Asik, B. B., Uzun, A., and Acikgöz, E. (2020). Seeding rate and cultivar impacts on nutrient uptake of field pea under fertile soil condition. Chilean journal of agricultural research, 80(1), 11-20.

[4] Bakeer, A. R. T., Abdel-latef, M. A., Afifi, M. A., and Barakat, M. E. (2012). Application of microelements and sodium and potassium salts on tomato plants and their role in suppressing powdery mildew disease. World, 1(1), 01-10.

[5] Baker, K.F. and Cook, R.J. (1982). Biological Control of Plant Pathogens. American Phytopathological Society, St Paul, MN, USA.

[6] Begum, M. M., Sariah, M. Puteh A. and Siddiquiy B. (2011). Field performance of bio primed seeds to suppress *Colletotrichum truncotum* causing damping off and seedling stand of soybean. Biological Control 53(1):18-23.

[7] Bennett, S., Lee, R. C., and Sharma, A. (2019). Management of field peas to reduce Ascochyta blight and maximise yield. In 19th Australian Agronomy Conference: Cells to Satellites. www.agronomyaustralia.org/conference-proceedings.

[8] Boros, L., and Marcinkowska, J. (2010). Assessment of selected pea genotypes reaction to ascochyta blight under field conditions and the impact of disease severity on yield components. Journal of Agricultural Science, 2(3), 84.

[9] Boudreau, M. A. and Mundt, C. C. (1997). Ecological approaches to disease control. Pages 33–62 in N. A. Rechcigl and J. E. Rechcigl, eds. environmentally safe approaches to crop disease control. CRC Press, Boca Raton, FL.

[10] Bretag, T. W. (1991). Epidemiology and control of ascochyta blight of field peas. PhD. Thesis, La Trobe University.

[11] Bretag, T. W., Keane, P. J., and Price, T. V. (2006). The epidemiology and control of ascochyta blight in field peas: a review. Aust. J. Agr. Res. 57, 883–902. doi: 10.1071/AR05222

[12] Burdon, J. J. and Chilvers, G. A. (1982). Host density as a factor in plant disease ecology. Annu. Rev. Phytopathol. 20: 143–166.

[13] Chang, K. F., Ahmed, H. U., Hwang, S. F., Gossen, B. D., Howard, R. J., Warkentin T. D., Strelkov, S. E. and Blade, S. F. (2007). Impact of cultivar, row spacing and seeding rate on ascochyta blight severity and yield of chickpea. Can. J. Plant Sci. 87:395–403.

[14] Chilvers, M.I., Rogers, J.D., Dugan, F.M., Stewart, J.E., Chen, W.D., and Peeve, L. (2009). *Didymella pisi* sp *nov*.,the teleomorph of *Ascochyta pisi*. Mycol. Res. 113,391–400.doi:10.1016/j.mycres.2008.11.017.

[15] Chilvers, M.I.; J.D. Rogers; F.M. Dugan; J.E. Stewart; W.D. Chen; T.L. Peever (2009). *Didymella pisi* sp. nov., the teleomorph of *Ascochyta pisi*, Mycol. Res. 113 391–400

[16] Cook, R. J. and Zhang, B.-X. (1985). Degree of sensitivity to metalaxyl within the *Pythium* spp. pathogenic to wheat in the Pacific Northwest. Plant Disease 69: 686–688.

[17] Cook, R.J. and Baker K.F. (1983). The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St Paul, MN, USA.

[18] Deacon, J .W. (1994). Rhizosphere constraints affecting biocontrol organisms applied to seeds. In: Seed treatment: prospects and progress. BCPC Monograph 57. Thornton Heath: British Crop Protection Coucil, 315-327.

[19] Dokken, L. F.; Banniza, S., Warkentin, T., Morrall, R.A.A. (2007). Occurrence of Ascochyta pisi on field pea in Saskatchewan, Can. J. Plant Pathol. 29: 218.

[20] El-Mohamedy, R.S.R and AbdAlla, M.A. (2013). Bio-priming seed treatment for biological control of soil borne fungi causing root rot of green bean (*Phaseolus vulgaris* L.). Journal of Agricultural Technology. 9(3):589-599.

[21] Ganeshan, G., and Manoj Kumar, A. (2005). *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. Journal of Plant Interactions, 1(3), 123-134.

[22] Garry G., Jeuffroy M.H., Ney B. and Tivoli, B. (1998). Effects of ascochyta blight (*Mycosphaerella pinodes*) on the photosynthesizing leaf area and the photosynthetic efficiency of the green leaf area of dried-pea. Plant Pathology, 47: 473–479 http://dx.doi.org/10.1046/j.1365-3059.1998.00259.

[23] Jaccoud-Filho, de Souza; Sartori, D., Manosso-Neto, F. F., Vrisman, M., da Cunha C. M., Pierre, M. L., Berger-Neto, A. and Zanon, S. (2016). Influence of row spacing and plant population density on management of white mould in soybean in southern Brazil. Australian Journal of Crop Science, 10(2), 161-168.

[24] Jacobson, B.J. and Backman, P.A. (1993). Biological and cultural plant disease controls: Alternatives and supplements to chemicals in IPM systems. Plant Disease 77: 311–315.

[25] Jones, A.L. and G.R. Ehret, 1976. Isolation and characterization of benomyl-tolerant strains of Monilinia fructicola. Plant Disease Reporter 60: 765–769.

[26] Jones, L. (1927). Studies on the nature and control of blight, leaf and pod spot, and foot rot of peas caused by species of Ascochyta, Research Report. No. 547. New York State Agric. Exp. Stn., Geneva, NY.

[27] Kildea, S., Ransbotyn, V., Khan, M. R., Fagan, B., Leonard, G., Mullins, E., and Doohan, F. M. (2008). *Bacillus megaterium* shows potential for the biocontrol of Septoria tritici blotch of wheat. Biological control, 47(1), 37-45.

[28] Kraft, J. M., & Pfleger, F. L. (2001). Compendium of pea diseases and pests (No. Ed. 2). American Phytopathological Society (APS Press).

[29] Kumar, Y., Singh, S. N., and Patel, K. K. (2022). Effect of sowing dates on severity of the pathogen Myrothecium leaf spot of sesame (*Sesamum indicum* L.). (TPI) The Pharma Innovation Journal; 11(1): 504-506.

[30] Lejeune-Hénaut, I., Bourion, V., Etévé, G., Cunot, E., Delhaye, K.and Desmyter, C. (1999). Floral initiation in field-grown forage peas is delayed to a greater extent by short photoperiods, than in other types of European varieties. Euphytica, 109 (3), 201-211.

[31] Lejeune-Henaut, I., Hanocq, E., Bethencourt, L., Fontaine, V., Delbreil, B., Morin, J., and Giauffret, C. (2008). The flowering locus Hr colocalizes with a major QTL affecting winter frost tolerance in *Pisum sativum* L. Theoretical and Applied Genetics, 116(8), 1105-1116.

[32] Liu, N., Xu, S., Yao X., Zhang G., Mao W., Hu Q., Feng, Z. and Gong, Y. (2016). Studies on the Control of Ascochyta Blight in Field Peas (*Pisum sativum* L.) Caused by Ascochyta pinodes in Zhejiang Province, China. Front. Microbiol. 7:481. doi: 10.3389/fmicb.2016.00481.

[33] Migawer, E., and Bakeer, A. (2003). Influence of sowing dates, plant spacings, cultivars and their interactions on wilt disease incidence, seed yield and yield components of lupins. Departamento de Agronomía, departamento de Botánica Agrícola. El Cairo. Universidad de Fayoum. 17p.

[34] Nasir, M. and Hoppe, H. H. (1991). Studies on pathotype differentiation within *Mycosphaerella pinodes* (Berk. & Bloxam) Vestergren, a component of the Ascochyta-disease-complex of peas (*Pisum sativum* L.)/Untersuchungen zur Pathotype differenzierung an *Mycosphaerella pinodes* (Berk. and Bloxam) Vestergren, einer Komponente des Ascochyta-Krankheitskomplexes der Erbsen (Pisum sativum L.). Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection, 619-626.

[35] Roger C, Tivoli B, Huber L. (1999). Effects of temperature and moisture on disease and fruit body development of *Mycosphaerella pinodes* on pea (*Pisum sativum*). Plant Pathol. 48:1–9.

[36] Sangar, R. B. and Singh, V. K. (1994). Effect of sowing dates and pea varieties on the, severity of rust, powdery mildew and yield. Indian J. Pul. Res, 7(1), 88-89.

[37] Singh, R. and Singh, P.M. (2011). Effect of sowing dates and varieties on yield and quality of garden pea seed. Vegetable Science, 38(2): 184-187.

[38] Singh, R., and Singh, P. M. (2011). Effect of sowing dates and varieties on yield and quality of garden pea seed. Vegetable Science, 38(2), 184-187.

[39] Skoglund, L. G., Harveson, R. M., Chen, W., Dugan, F., Schwartz, H. F., Markell,S. G., Porter, L., Burrows, M. L., and Goswami, R. (2011). Ascochyta blight of peas. Online. Plant Health Progress doi:10.1094/PHP-2011-0330-01-RS.

[40] Smilanick, J. L., Margosan, D. A., Mlikota, F., Usall, J., and Michael, I. F. (1999). Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis. 83:139-145.

[41] Tivoli B, Banniza S. (2007). Comparative epidemiology of ascochyta blights on grain legumes. Eur. J. Plant Pathol. 119:59–76.

[42] Tivoli, B., Beasse, C., Lemarchand, E., & Masson, E. (1996). Effect of ascochyta blight (*Mycosphaerella pinodes*) on yield components of single pea (*Pisum sativum*) plants under field conditions. Annals of Applied Biology, 129(2), 207-216.

[43] Türkkan, M., Özcan, M. and Erper, İ. (2017). Antifungal effect of carbonate and bicarbonate salts against *Botrytis cinerea*, the causal agent of grey mold of kiwifruit. Akademik Ziraat Dergisi, 6(2), 107-114.

[44] Waseem, K., Ghafoor, A., Khan, R. U. and Nadeem, M. A. (2000). Effect of sowing dates and row spacing on the yield of spinach (*Spinacia oleracea* L.). Pakistan Journal of Biological Sciences (Pakistan); 3, (5), 822-823.

[45] Whipps JM. (1997). Developments in the biological control of soil-borne plant pathogens. Advances in Botanical Research 26,1-134.

[46] Zhang, J. X., Fernando, W. G. D., and Xue, A. G. (2003). Virulence and genetic variability among isolates of *Mycosphaerella pinodes*. Plant Dis. 87, 1376–1383.doi: 10.1094/PDIS.2003.87.11.1376.