

USE OF SOME *STREPTOMYCES* ISOLATES AND THEIR MIXTURES AS BIOLOGICAL CONTROL AGENTS AGAINST POWDERY MILDEW OF FLAX CULTIVAR MARYLIN R3 IN AN OUTDOOR POT EXPERIMENT

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ABSTRACT

In this study, the effect of some isolates of *Streptomyces* and their mixtures was evaluated as a biological control agent against powdery mildew (PM) of flax cv. Marlin R3, its effect on disease incidence (DI), disease severity (DS), seed yield (SY) and straw yield (StY) R3 in an outdoor pot experiments of two seasons (2016 and 2017). Four *Streptomyces* isolates (SI) of SI-3, SI-5 and SI-11 with its mixture and 15 treatments. The individual isolates (SI-1), SI-3, and SI-5 were the most effective in the resistance of mildew disease with efficiency of 86.96, 69.57 and 86.96%, respectively. In contrast, individual isolates (SI-11) had no effect on the disease. On the other hand, there was a difference in the effect of the isolate's mixture in the resistance of the PM, as demonstrated by the efficiency of the three mixtures (SI-1, SI-3, SI-5, SI-11) and SI-1 (SI-3) and SI-3, SI-5, SI-11), 86.96, 73.33 and 56.67%, respectively. The rest of the mixtures did not have any effect on the resistance of the PM disease. Linear correlation analysis showed a significant positive correlation between DI and disease intensity at a significant level ($r = 0.985$, $p \leq 0.1$) in 2016 and 2017 ($r = 0.960$, $p \leq 0.1$). In 2016 there was a significant negative correlation between DI, DS, SY and StY. While in 2017 there was a negative correlation between the severity of the disease and the occurrence of the disease and SY only. Cluster analysis was only partially successful in the separation of the potent and ineffective strains of *Streptomyces* in the resistance to PM disease.

Key words: *Streptomyces*, Biological control, Powdery mildew, Flax, Disease incidence, Disease severity.

INTRODUCTION

The powdery mildew (PM) diseases are major foliar diseases have an important economic impact on a variety of plant species (Aly et al. 1994, Mansour and Mohamed 2007, Micali et al. 2008, Hegazi and El-Kot 2010). Chemical application is still the main strategy to decrease the risk of most crop diseases caused by fungi and other pathogens (Zaid et al. 2002, Saeed et al. 2016). One of the most important factors that have led to the control of plant pathogens is the use of bio-control agents (BCAs) which can minimize the use of chemical pesticides and its harmful side effects to human health (Bardin et al. 2015, Saeed et al. 2017).

Locci and Sharples (1984) showed that actinomycetes are an important bacterial group differs from other rods and cocci microorganisms, found in habitats such as soils, composts, freshwater, seawater and cold and warm-blooded animals (Williams and Wellington 1982). Genus *Streptomyces* spp. is the most dominant genera of order Actinomycetales, because of their dominance, ease of isolation and antibiotics production (Goodfellow and Williams 1983). At the level of their activities as BCAs, they were effective against many plant pathogens, in particular, fungi as shown by Lee et al. (2005) and El-Tarabily et al. (2009). This was due to producing a number of bioactive metabolites that can be employed to manage plant pathogens and improves crop produ-

ctivity ((El-Tarabily and Sivasithamparam 2006, Mansour et al. 2010, Verma et al. 2011, Baltz 2016).

Several investigators (Singh et al. 2000, Abd El-Moneim 2001, Mosa 2002, El-Gamal 2003, Kamel 2003, Saber et al. 2003, McGrath 2004, Hussein et al., 2007, El-Tarabily et al. 2009, Mansour et al. 2010, AbuQamar et al. 2017) reported that bio-control, could be considered as an effective alternatives for limiting or spurring the populations of plant pathogens under greenhouses and/or field trials.

Microorganisms as BCAs typically have a relatively narrow spectrum of activity compared with synthetic pesticides (Baker 1991, Janisiewicz 1996), and soil actinomycetes particularly *Streptomyces* spp. has antagonistic activity against wide range of plant pathogens (Zarandi et al. 2009). Therefore, this study aimed to determine the effect of some *Streptomyces* isolates (SIs) and their mixtures as BCAs on disease intensity variables of PM of flax cultivar Marylin R3 in an outdoor pot experiment over two seasons.

MATERIALS AND METHODS

Materials source: Four SIs coded SI-1, SI-3, SI-5, and SI-11 having antifungal activities were kindly provided by Department of Agricultural Micro-biology, Soil and Water and Environment

Research Institute; Agricultural Research Center (ARC), Giza, Egypt. Seeds of flax cultivar Marilyn R3 and bunch fungicide, which used as a positive control compared to the *Streptomyces* spray, were obtained from Plant Pathology Research Institute, ARC, Giza, Egypt.

Antagonistic activities: The antibiosis activity of the four SIs against the fungus causing the PM of flax was determined according to the method described by Mansour and Mohamed (2007). The *Streptomyces* inocula (individual or mixtures) were prepared as mentioned by Mansour *et al.* (2010).

The outdoor pot experiment: The four SIs as well as their mixtures were used against the PM of flax cultivar Marilyn R3 in an outdoor experiment was carried out over the two seasons of 2016 and 2017. A number of 15 treatments were designed as follows: four *Streptomyces* individual treatments (SI-1, SI-3, SI-5, SI-11), 11 *Streptomyces* mixture treatments (SI-1, SI-3), (SI-1, SI-5), (SI-3, SI-5), (SI-1, SI-3, SI-5), (SI-1, SI-11), (SI-3, SI-11), (SI-1, SI-3, SI-11), (SI-5, SI-11), (SI-1, SI-5, SI-11), (SI-3, SI-5, SI-11), (SI-1, SI-3, SI-5, SI-11) as previously described by Mansour and Mohamed (2007) in a final concentration of 1 mL/50 mL water for each spraying treatment. At the concentration level three mL/ 100 mL water of Bunch fungicide was used (Treatment 16). As a negative control, the plants were sprayed with only water (Treatment 17). For each treatment, there were three replicates (pots) with a diameter of 25 cm containing sterilized soil and planted with flax seeds in the first week of December. On germination of flax seeds, the only plants were left in each pot. The flax plants were sprayed twice: the first spray was carried out in mid-March and the second one was carried out 15 days after the initial spray.

Disease variables: Four parameters of intensity variables, *i.e.*, disease incidence (DI, % of plants infected with powdery mildew for each pot), disease severity (DS, number of infected leaves in each plant), seed yield (SY, weight of produced seeds/plant) and straw yield (StY, weight of produced straw/plant) were determined. Both of DI and DS were assessed 15 days post the second spray with either *Streptomyces* or Bunch fungicide compared with control. SY and StY were measured at the beginning of May.

Statistical analyses: Data were subjected to analysis of variance (ANOVA), and Duncan's multiple range test was used to compare treatment means. Linear correlation coefficient (r) was calculated to evaluate the degree of association between disease intensity variables and yield. ANOVA and correlation analyses were performed with MSTATC statistical package. *Streptomyces* isolates were also clustered as phenogram by unweighted pair group method with arithmetic mean (UPGMA) (Sokal and Michener 1958). Cluster analysis was performed with the software package Statistical Package for the Social Sciences (Spss) 6.0.

RESULTS AND DISCUSSION

Four *Streptomyces* isolates coded SI-1, SI-3, SI-5 and SI-11 and their mixtures containing fifteen treatments as shown in Table (1) were used to evaluate the effect of *Streptomyces* isolates (Individual or mixtures) on powdery mildew of flax cultivar Marilyn R3 intensity variables (disease incidence, (DI) and disease severity (DS)), seed and straw yields in two growing seasons 2016 and 2017 in outdoor experiment. LSD was used to compare the means of individual streptomycete treatments in 2016 (Table 1). Data show that *Streptomyces* treatment isolates 1(SI-1), 4(SI-5) and 15(SI-1, SI-3, SI-5, SI-11) significantly decreased DI compared to control. The efficiency of the treatment isolates was 86.96% for these three treatments. Similarly, treatments 2(SI-3), 3(SI-1, SI-3) and 14 (SI-3, SI-5, SI-11) were significantly decreased DI by 69.51, 65.21 and 43.49%, respectively.

Treatments 1(SI-1), 15(SI-1, SI-3, SI-5, SI-11), 2(SI-3), 3(SI-1, SI-3), 4(SI-5) and 14(SI-3, SI-5, SI-11) significantly decreased DI with efficiency 70.37, 70.37, 62.97, 59.26, 59.26%, respectively, while, one treatment 2(SI-3) significantly increased seed yield. None of *Streptomyces* treatments (Individually or mixture) significantly affected on straw yield. Referring to the results in Table 2 treatments 1(SI-1), 15(SI-1, SI-3, SI-5 and SI-11), 2(SI-3), 3(SI-1, SI-3) and 4(SI-5) significantly decreased DI with efficiency 70.37, 70.37, 62.97, 59.26 and 59.26% respectively. Treatments 1(SI-1), 2(SI-3), 3(SI-1, SI-3), 4(SI-5), 15(SI-1, SI-3, SI-5, SI-11) and 5(SI-1, SI-5) were significantly decreased DS with efficiency 96.07, 95.37, 94.55, 94.55, 83.90 and 80.11%, respectively. In 2017 none of the *Streptomyces* treatments significantly affected both seed and straw yields.

Table 1: Effect of *Streptomyces* isolates (individual or mixture) on disease intensity variables (DI and DS), SY/plant and StYstraw yield/plant of flax cultivar Marilyn R3 in outdoor experiment in 2016.

| Treatments | <i>Streptomyces</i> isolates | Intensity variables | | | | | | | |
|------------|------------------------------|---------------------|----|-------|-----|------|---|------|----|
| | | DI | | DS | | SY | | StY | |
| T1 | SI-1 | 10.00 | E* | 04.40 | F | 0.17 | B | 0.40 | AB |
| T2 | SI-3 | 23.33 | D | 13.90 | E | 0.27 | A | 0.50 | A |
| T3 | SI-1, SI-3 | 26.67 | D | 16.23 | D | 0.15 | B | 0.40 | AB |
| T4 | SI-5 | 10.00 | E | 12.63 | E | 0.13 | B | 0.40 | AB |
| T5 | SI-1, SI-5 | 76.67 | AB | 72.80 | ABc | 0.12 | B | 0.40 | AB |
| T6 | SI-3, SI-5 | 76.67 | AB | 64.00 | Bc | 0.16 | B | 0.40 | AB |
| T7 | SI-1, SI-3, SI-5 | 73.33 | B | 61.07 | c | 0.09 | B | 0.40 | AB |
| T8 | SI-11 | 76.67 | AB | 67.77 | Bc | 0.09 | B | 0.30 | AB |
| T9 | SI-1, SI-11 | 86.67 | A | 66.73 | Bc | 0.11 | B | 0.20 | AB |
| T10 | SI-3, SI-11 | 80.00 | AB | 73.27 | ABc | 0.12 | B | 0.30 | AB |
| T11 | SI-1, SI-3, SI-11 | 80.00 | AB | 81.73 | A | 0.15 | B | 0.30 | AB |
| T12 | SI-5, SI-11 | 73.33 | B | 72.33 | ABc | 0.12 | B | 0.30 | AB |
| T13 | SI-1, SI-5, SI-11 | 86.67 | A | 76.13 | AB | 0.12 | B | 0.30 | AB |
| T14 | SI-3, SI-5, SI-11 | 43.33 | C | 36.70 | D | 0.14 | B | 0.30 | AB |
| T15 | SI-1, SI-3, SI-5, SI-11 | 10.00 | E | 03.97 | F | 0.12 | B | 0.30 | AB |
| T16 | Bunch | 00.00 | F | 00.00 | G | 0.16 | B | 0.40 | AB |
| T17 | Control | 76.67 | AB | 71.7 | Bc | 0.10 | B | 0.30 | AB |

DI: Disease incidence (Percentage of infected plants/pot). DS: Disease severity (Percentage of infected leaves/plant). SY: Seed yield (g/plant). StY: Straw yield (g/plant). * Means followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 2: Effect of *Streptomyces* isolates (individual or mixture) on disease intensity variables (DI and DS), SY/plant and StYstraw yield/plant of flax cultivar Marilyn R3 in outdoor experiment in 2017.

| Treatments | <i>Streptomyces</i> isolates | Intensity variables | | | | | | | |
|------------|------------------------------|---------------------|----|-------------------|-----|-------|-----|-------|------|
| | | DI | | DS | | SY | | StY | |
| T1 | SI-1 | 26.67 | E* | 3.37 ^b | GH | 0.153 | A | 0.557 | AB |
| T2 | SI-3 | 33.33 | DE | 3.97 | GH | 0.083 | Bc | 0.393 | BcD |
| T3 | SI-1, SI-3 | 36.67 | DE | 4.67 | GH | 0.093 | Bc | 0.377 | cD |
| T4 | SI-5 | 36.67 | DE | 4.67 | GH | 0.073 | Bc | 0.333 | D |
| T5 | SI-1, SI-5 | 53.33 | cD | 16.53 | F | 0.083 | Bc | 0.437 | ABcD |
| T6 | SI-3, SI-5 | 70.00 | BC | 45.30 | E | 0.060 | c | 0.280 | D |
| T7 | SI-1, SI-3, SI-5 | 83.33 | AB | 76.83 | D | 0.063 | Bc | 0.353 | Cd |
| T8 | SI-11 | 83.33 | AB | 79.10 | cD | 0.060 | Bc | 0.327 | D |
| T9 | SI-1, SI-11 | 83.33 | AB | 86.47 | BcD | 0.083 | Bc | 0.397 | BcD |
| T10 | SI-3, SI-11 | 90.00 | A | 88.80 | ABc | 0.037 | c | 0.253 | D |
| T11 | SI-1, SI-3, SI-11 | 86.67 | A | 93.50 | AB | 0.063 | Bc | 0.297 | D |
| T12 | SI-5, SI-11 | 90.00 | A | 90.27 | AB | 0.053 | c | 0.330 | D |
| T13 | SI-1, SI-5, SI-11 | 93.33 | A | 91.47 | AB | 0.060 | Bc | 0.283 | D |
| T14 | SI-3, SI-5, SI-11 | 93.33 | A | 95.47 | A | 0.050 | c | 0.273 | D |
| T15 | SI-1, SI-3, SI-5, SI-11 | 26.67 | E | 13.80 | FG | 0.067 | Bc | 0.330 | D |
| T16 | Bunch | 06.67 | F | 2.30 | H | 0.173 | A | 0.603 | A |
| T17 | Control | 90.00 | AB | 85.70 | BcD | 0.120 | ABc | 0.520 | ABc |

T: Treatment. DI: Disease incidence (Percentage of infected plant/pot). DS: Disease severity (Percentage of infected leaves/plant). SY: Seed yield (g/plant). StY: Straw yield (g/plant). * Means followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Mansour and Mohamed (2007) mentioned that when percentages of disease incidence and disease severity increased, the seed yield and straw yield decreased. In other word, a negative relationship of powdery mildew of flax disease incidence, disease severity, seed yield and straw yield was noted. Results in Table 3 showed linear correlation coefficients shown that a highly positive correlation coefficients ($p < 0.01$) between DI and DS in both of two years, 2016 and 2017. In 2016 the relationship between DI and straw yield

showed negative correlation ($p < 0.05$) also DS shown negative correlation coefficient on both of seed yield and straw yield. These results are in agreements with that found by Mansour (1998), who found that it was evident that powdery mildew intensity variables (DI and DS) reached maximum levels on young plants (late sowing dates) and decline considerable on adult plants (early sowing dates). The intensity of disease on young plants was significantly reflect on deteriorate of seed and straw production.

Table 3: Correlation among disease intensity variables (DI and DS), SY and StY for evaluating effect of individual and mixture of some *Streptomyces* isolates on control of powdery mildew on flax in two seasons (2016 & 2017) under outdoor conditions.

| Years | Variables | | Variables | | | | | | | | | | |
|-----------------|-----------|-----|-----------------------------|---------|---------|----------|-----------------------------|---------|----------|-------|--|--|--|
| | | | 1 st Year (2016) | | | | 2 nd Year (2017) | | | | | | |
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | | |
| 1 st | 1 | DI | 1.000 | | | | | | | | | | |
| | 2 | DS | 0.985*** | 1.000 | | | | | | | | | |
| | 3 | SY | -0.416* | -0.488* | 1.000 | | | | | | | | |
| | 4 | StY | -0.540* | -0.534* | 0.601* | 1.000 | | | | | | | |
| 2 nd | 5 | DI | 0.886** | 0.883** | -0.494* | 0.633** | 1.000 | | | | | | |
| | 6 | DS | 0.812** | 0.815** | -0.515* | -0.767** | 0.960** | 1.000 | | | | | |
| | 7 | SY | -0.557** | -0.546* | 0.211 | 0.347 | -0.644** | -0.536* | 1.000 | | | | |
| | 8 | StY | 0.141 | -0.107 | -0.023 | 0.158 | 0.112 | -0.038 | -0.966** | 1.000 | | | |

^a: Linear correlation coefficient (rs) is significant at $P < 0.05$ (*) or $P < 0.01$ (**).

On the other hand, relationship between seed yield and straw yield shown positive correlation ($r = p < 0.05$). In 2017, highly positive correlation coefficient ($r = p < 0.01$) between DI in both two years which means that the infection stable by DI, and this agree with that reported by Mansour (1998) and Aly *et al.* (1994). Results represented in Fig. 1 showed that the tested isolates were placed in three subclusters, the first one included three groups. The first subgroup (Distance = 0.0) included very highly susceptible treatments (T8,

T13, T10, T17, T12, T11, T9). However, the susceptible treatment (T6) (Distance 5.0) made up a separate group (subcluster) unrelated to the other susceptible treatments. The third subgroup included (T14, T15) made up a separate subcluster unrelated to the other treatments in the susceptibility which treatment (T15) considered very highly resistance to the disease (Tables 1 and 2). Treatment (T5) separated in subcluster (distance = 2.5) although it belongs to the susceptible subgroup.

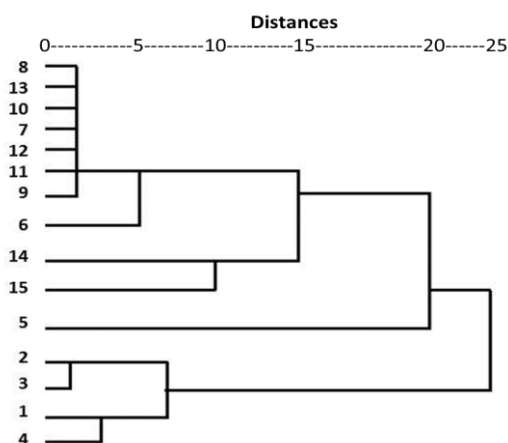


Figure 1: Phenogram based on average linkage cluster analysis of *Streptomyces* isolates (individual or mixtures) on disease intensity variables (disease incidence and disease severity) of flax powdery mildew, seed yield and straw yield. The isolates individual and mixture of *Streptomyces* were T1(SI-1), T2(SI-2), T3(SI-1, SI-3), T4(SI-5), T5(SI-1, SI-5), T6(SI-3, SI-5), T7(SI-1, SI-3, SI-5), T8(SI-11), T9(SI-1, SI-11), T10(SI-3, SI-11), T11(SI-1, SI-3, SI-11), T12(SI-5, SI-11), T13(SI-1, SI-5, SI-11), T14(SI-3, SI-5, SI-11), and T15(SI-1, SI-3, SI-5, SI-11).

The third subcluster included four treatments T2, T3 (Distance = 0.0) and treatments T1, T4 (Distance = 2.5) which exhibit highly resistance treatments. In general, the dendrogram has failed in differentiate between the resistance and susceptible treatments. This study suggested that antagonist treatments are more effective in biological control of powdery mildew of flax when used individually. Results showed that individual treatments were very effective (Tables 1 and 2). Dandurand and Kundsén (1993), Hubbard *et al.* (1983) reported that of combinations of biological

control agents did not improved on suppression of disease compared with separate antagonists. Only one mixture treatment gave an effective result in controlling powdery mildew in this study 15 (SI-1, SI-3, SI-5, SI-11). Schisler *et al.* (1997) mentioned that the still very limited number of compatible and effective mixtures of BCAs is due to several factors. The majorities of mixtures have no benefit or are determinate to biocontrol activity. Further, a mixture that improves activity less than one set of conditions or on a different host. From an economical point of view, a bio-

control product composed of a mixture of strains has a potential draw back, because producing and registering such a product will likely be more costly than a product composed of a single or individual strain.

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