Application of Bio P60 and Bio T10 Alone or in Combination against Stem Rot of Pakcoy

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ARTICLE HISTORY

Received : 9 August 2019

Revised : 12 September 2019

Accepted : 3 October 2019

KEYWORDS

Bio P60

Bio T10

pakcoy

Sclerotium rolfsii

stem-end rot

ABSTRACT

Application of Bio P60 and Bio T10 alone or in combination in suppressing stem rot of pakcoy and on pakcoy growth has been demonstrated. The research was carried out at the Plant Protection Laboratory and Screen House, Faculty of Agriculture, Jenderal Soedirman University. A split-plot design was used with application time (before and after inoculation of *Sclerotium rolfsii*) as main plot and Bio P60, Bio T10, and Bio P60 + Bio T10 as sub-plot. Observed variables were incubation period, disease intensity, crop height, number of leaves, and crop fresh weight. Results of the research showed that single and combined application of Bio T10 and Bio P60 did not differ in the suppression of stem-end rot in pakcoy. The combination of Bio T10 + Bio P60 was able to control the disease by delaying the incubation period and suppressing the disease intensity respectively by 37.48-39.16% and 54.77-61.91% compared to controls. Combined Bio T10 + Bio P60 was able to improve plant height, number of leaves, and fresh weight of plants as 29.99-46.62, 24.39-35.07, and 71.17%, respectively, compared to controls. The results of this study suggest that the raw secondary metabolites of Bio P60 and Bio T10 either alone or in combination could be applied for the prevention or treatment of the diseases in pakcoy.

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1. INTRODUCTION

Pakcoy is one type of leafy vegetable used by people as food, both fresh and processed, and contains complete nutrition (Chu and Seltzer, 2010; Di Noia, 2014). Based on the data from Central Bureau of Statistics and Directorate General of Horticulture (2015), Pakcoy crop production decreased by around 5.23%, from 635,728 tons/year in 2013 to 602,468 tons/year in 2014 with a decrease of 1.89% in productivity. Pakcoy cultivation suffers from many disorders, one of which comes from biotic factors, in the form of pathogenic fungal attacks that cause huge crop losses. One of the pathogenic fungi that can cause stem rot is *Sclerotium rolfsii* Sacc. (Nahar *et al.*, 2013; Akhtar *et al.*, 2017).

Rakholiya (2010) reports that *S. rolfsii* is a soil-borne pathogen that is difficult to control because it can live for years in the soil in the form of sclerotium and has extensive host range. *Sclerotium rolfsii* is a facultative parasitic fungus that grows and survives saprophytes in the soil. According to Noble (2011) and Zachow *et al.* (2011), this fungus attack is more severe in sandy soils because the *S. rolfsii* fungus requires aerobic oxygen (O₂), and in soil conditions with low nitrogen content and high

temperatures. Therefore, another alternative is needed to control the stem rot disease such as using fungicides (Bhuiyan *et al.*, 2012; El-Mohamedy *et al.*, 2014), organic amendments (Hadar, 2011; Lahre *et al.*, 2012; Paramasivan *et al.*, 2013), soil solarization (Raj and Sharma, 2009; Lee *et al.*, 2016), botanical pesticides (Amin *et al.*, 2013), rhizobacteria (Hapsah, 2019), and bio-agents (Rakh *et al.*, 2011; Lahre *et al.*, 2012). Each method has its advantages and disadvantages.

The agents used in this research were Bio P60 and Bio T10. Bio P60 is a liquid formula containing raw secondary metabolites from *Pseudomonas fluorescens* P60 (Soesanto *et al.*, 2013). *P. fluorescens*, apart from being an antagonistic bacterium, affects plant resistance and can increase plant growth. This is because *P. fluorescens* as plant growth-promoting rhizobacteria (PGPR) can produce plant hormones, including indole acetic acid (IAA) (Ardebili *et al.*, 2011). Meanwhile, Bio T10 active ingredient is raw secondary metabolites from *Trichoderma harzianum* T10 (Soesanto *et al.*, 2019). Tran (2010) stated that *Trichoderma* sp. plays a crucial role in controlling plant fungal pathogens, especially soil-borne

pathogens.

The purpose of this research was to determine the ability of single and combined Bio P60 and Bio T10 to control stem-end rot and the growth of pakcoy.

2. MATERIALS AND METHODS

2.1 Preparation of the fungal pathogen

Sclerotium rolfsii was isolated from diseased legume stem from the field by tissue segment method and was multiplied by inoculating PDA and WA in a 9 cm diameter glass petri dish. The stem sections had been sterilized by using NaOCl (Songvilay *et al.*, 2012; Kumar *et al.*, 2014a).

2.2 Preparation of pakcoy seeds

Pakcoy seeds were soaked according to treatment in 5 mL/L of Bio P60 and 10 mL/L of Bio T10 single or combined, and Some seeds were also soaked in sterile water for control. Seeds were soaked for more than one hour then they were grown on a mixed seedling soil and manure medium(1:1). Seeds were sown for 3-6 days until the seeds germinate (around seven days).

2.3 Cultivation

Pakcoy seedlings were then transferred to the planting medium that has been inoculated with sclerotia in the polybag. In the third week, the plants were sprayed with Bio P60, Bio T10, and combined Bio T10 + Bio P60 in the amount of 1000 mL each. Observations were made after the plants reached the age of 2-3 weeks after transplanting. A split-plot design was used with the main plot of application time (before and after inoculation of *S. rolfsii*) and the subplot consisted of

three secondary metabolites treatments (Bio P60, Bio T10, and a combination of Bio T10 + Bio P60) and were repeated four times. Pakcoy plant maintenance conducted including manual weeding and watering.

2.4 Variables observed

The observed variables were incubation period, disease intensity, crop height, leaves a number, and crop fresh weight. Disease intensity was measured using the formula of $DI = [\sum(n \times v) / Z \times N] \times 100\%$, where DI = percentage of disease intensity, n = the value (score) of crop damage is based on the leaf area of all affected plants, v = the number of plants that have the same value v (crop damage), Z = highest score (v = 5), and N = number of plants observed. According to Kator *et al.* (2015), disease assessment key was 0 –5 where 0 = no infection, 1 = 1–20% of plant tissue damage, 2 = 21 –40% of plant tissue damage, 3 = 41 –60% of plant tissue damage, 4 = 61 –80% of plant tissue damage, 5 = 81 –100% of plant tissue damage.

2.5 Statistical Data Analysis

Data were analyzed by F-test at an error rate of 5%. If there are significant differences, then further tests are carried out by DMRT (≤ 0.05).

3. RESULTS AND DISCUSSIONS

3.1 The effect of the treatments on main plot

The statistical analysis results of the observed variables are presented in Table 1.

Table 1. The effect of application time on incubation period, disease intensity, crop height, number of leaves, and crop fresh weight

Application time	Incubation period (dai)	Disease intensity (%)	Crop height (cm)	Number of leaves	Crop fresh weight (g)
Before	22.75 a	42.138 a	18.747 a	11.25 a	23.583 a
After	22.43 a	41.985 a	19.314 a	12.90 a	28.687 a

Note: Mean followed by different letters in the same column show significantly different in DMRT with an error level of 5%. Dai = days after inoculation.

The results of the analysis showed that there was no significant effect between the time of pathogen inoculation to the incubation period, disease intensity, crop height, number of leaves, and crop fresh weight. Before and after inoculation of pathogenic fungi had a similar effect in appearing the disease symptom and resulting in the disease intensity. This is in line with Junsopa *et al.* (2016) that the difference inoculation method of *S. rolfsii* was not significant in disease incidence. The application time of inoculation did not differ pakcoy growth in crop height, number of leaves, and fresh weight of the crop. This is presumably because pathogenic fungi cannot interfere with water and nutrient transport in pakcoy which affects photosynthesis and *S. rolfsii*, which can be less virulent to attack pakcoy plants.

According to Thiessen and Woodward (2012), the development of a disease is supported by three factors, namely vulnerable hosts, virulent pathogens, and a supportive environment. Pathogens in their development are influenced by several abiotic factors, namely temperature, humidity, oxygen, degree of acidity (pH), and light. According to Chaurasia *et al.* (2013), the process of infection of a pathogen and the development of a disease are greatly influenced by many factors such as temperature, humidity, light intensity, concentration of inoculum and isolates of the pathogen, individual, age, and variety of plants, as well as other genetic factors.

3.2 The effect of the treatments on sub-plot

DMRT results on the influence of sub-plot on the

observed variables are presented in Table 2.

Table 2. The effect of the treatments on the incubation period and disease intensity

Treatments	Incubation period (dai)	Delay (%)	Disease intensity (%)	Decrease (%)
Control	17.208 a	-	73.843 b	-
Bio P60	24.083 c	27.7	31.131 a	57.84
Bio T10	21.958 b	21.6	33.398 a	54.77
Bio P60 + Bio T10	27.125 d	36.6	29.874 a	59.55

Note: Mean followed by different letters in the same column show significantly different in DMRT with an error level of 5%.

1. Incubation period

Based on the results, the secondary metabolite treatments had a significant effect on the incubation period and disease intensity (Table 2). The raw secondary metabolites either from *P. fluorescens* P60 (Bio P60) or *T. harzianum* T10 (Bio T10) alone or in combination gave similar effect on lengthening incubation period in between 37.48 and 39.16% compared to control. This was in line with decreasing the disease intensity. This is because the raw secondary metabolites can suppress the disease.

This is presumably that the raw secondary metabolites could suppress pathogen development because of their substance content. The raw secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 could enter the crop and influence crop resistance. The raw secondary metabolites of *P. fluorescens* P60 contained several substances such as chitinase, protease, and antibiotic. Neidig *et al.* (2011) and Shahid *et al.* (2016) suggested that many strains of *P. fluorescens* produce secondary metabolites, such as 2,4-diacetylphloroglucinol (DAPG), phenazines and hydrogen cyanide, thereby inhibiting soil-borne pathogens. Besides that, Bansal and Mukherjee (2016) stated that *Trichoderma* spp. are the rich source of secondary metabolites with a variety of biological activities. According to Li *et al.* (2019), the secondary metabolites per *Trichoderma* species elaborate on approximately 390 non-volatile compounds from 20 known species and various unidentified species.

2. Disease intensity

The results of the analysis showed that secondary metabolites were able to suppress the intensity of the disease by 54.77 and 59.55% compared to control (Table 2). This is presumably because both Bio P60 and Bio T10 contain raw secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10, respectively. The raw secondary

metabolites consisted of substances that could inhibit pathogen growth and suppress disease development.

P. fluorescens has the ability to form siderophore but not all are toxic to plant pathogens (Luján *et al.*, 2015; Trapet *et al.*, 2016). This causes *P. fluorescens* to suppress the intensity of the disease. The main mechanism of *P. fluorescens* biocontrol is producing antibiotics such as 2,4-diacetyl phloroglucinol (PHL), pyoluteorin (PLT), pyrrolnitrin and phenazine 1-carboxylate (Shahid *et al.*, 2016). Meanwhile, the biocontrol mechanism of *Trichoderma* species is a complex process mediated by the secretion of extracellular enzymes, such as chitinases, β -glucanases, and proteinases, as well as secondary metabolites (Kumar *et al.*, 2014b). Integrated treatment comprising of soil application of combined biocontrol agents was found very effective in reducing the plant mortality, promoting the plant growth, and increasing the yield (Jambhulkar *et al.*, 2018; Kabdwal *et al.*, 2019).

3. Crop height

The analysis showed that the raw secondary metabolites alone or in combination were able to increase crop height between 37.48% and 39.18% compared to control (Table 3). This is presumably because both Bio P60 and Bio T10 contain raw secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10, respectively, that consisted of growth hormones that affect crop growth. *P. fluorescens* is known as Plant Growth Promoting Rhizobacteria (PGPR) (Soesanto *et al.*, 2010; Dorjey *et al.*, 2017). Besides, according to Latifah *et al.* (2011), *Trichoderma* sp. has a PGPF mechanism to plant growth. Raw secondary metabolites of *Trichoderma* sp. (Reino *et al.* 2008; Vinale *et al.* 2008) and *P. fluorescens* (Heydari and Pessarakli, 2010; Soesanto *et al.*, 2013) may have a role in both plant growth regulation and activation of plant defense responses.

Table 3. The effect of the treatments on crop height, number of leaves, and crop fresh weight

Treatments	Crop height (cm)	Increase (%)	No of leaves	Increase (%)	Crop fresh weight (g)	Increase (%)
Control	12.933 a	-	9.125 a	-	3.217 a	-
Bio P60	21.254 b	39.15	13.125 b	30.46	5.636 b	42.91
Bio T10	20.675 b	37.48	13.583 b	32.55	5.304 b	39.36
Bio P60 + Bio T10	21.262 b	39.18	12.458 b	26.48	5.855 b	45.05

Note: Mean followed by different letters in the same column show significantly different in DMRT with an error level of 5%.

4. Number of leaves

The raw secondary metabolites alone or in combination could also increase the number of pakcoy leaves between 26.48 and 32.45% compared to control (Table 3). Raw secondary metabolites alone or in combination can also increase the number of pakcoy leaves between 26.48 and 32.45% compared to controls (Table 3). This proves that the disease can be suppressed by applying Bio P60 and Bio T10 either individually or in combination. *P. fluorescens* suppress fungal pathogen (Luján *et al.*, 2015) and *Trichoderma* sp. could also control the soil-borne pathogen (Vinale *et al.*, 2008). On the other hand, *P. fluorescens* and *Trichoderma* spp. could produce growth hormones (Heydari and Pessarakli, 2010; Dorjey *et al.*, 2017). The crop could grow better resulted in the fungal pathogen control and growth regulators.

5. Crop fresh weight

The results of the analysis showed that secondary metabolites were able to increase the pakcoy fresh weight in between 39.36 and 45.051% compared to control (Table 3). This was in line with increasing drop height and

number of leaves. This is because the raw secondary metabolites of *P. fluorescens* and *Trichoderma* sp can increase growth and yield and to inhibit the growth of pathogens, so pathogens require more time to infect plants.

In accordance with the opinion of Dorjey *et al.* (2017), *P. fluorescens* can produce 2,4-diacetylfloroglucinol antibiotics, which have antibacterial properties and can activate the host plant's defense system. Heydari and Pessarakli (2010) and Kumar *et al.* (2014b) state that *Trichoderma* spp. have a role in both plant growth regulation and activation of plant defense responses.

3.3 The effect of application time and the treatment interaction

DMRT results on the effect of application time and the raw secondary metabolites combination on the incubation period and disease intensity are presented in Table 4.

Table 4. The effect of application time and the treatments combination on incubation period and disease intensity

Treatments	Incubation period (dai)	Lengthen	Treatments	Incubation period (dai)
Control >< Before	17.25 a	-	70.73 a	-
Control >< After	17.12 a	-	76.95 a	-
Bio P60 >< Before	24.42 b	29.36	31.42 b	55.58
Bio P60 >< After	23.75 b	27.92	30.84 b	59.92
Bio T10 >< Before	22.00 b	21.59	35.96 b	49.16
Bio T10 >< After	21.92 b	21.90	30.84 b	59.92
Bio P60+Bio T10 >< Before	27.33 c	36.88	30.44 b	56.96
Bio P60+Bio T10 >< After	26.92 c	36.40	29.31 b	61.91

Note : Mean followed by different letters in the same column show significantly different in DMRT with an error level of 5%.

1. Incubation period

The interaction treatments alone or in combination could lengthen incubation period, and the combination gave a better result. Application of the combined treatment was not affected by pathogen attack indicated by lengthening incubation period between 36.40% and 36.88%. The combined treatment gave better results than a single treatment (Table 4).

According to Chandrashekara *et al.* (2012), the degree of virulence plays a role in causing wilting symptoms rather than population density, while the virulence mechanism is closely related to several factors, such as host range, geographic distribution, physiological characteristics, and its adaptability to environmental temperature. The combined biocontrol agents were found very effective in reducing plant mortality (Kabdwal *et al.*, 2019).

2. Disease intensity

Decreasing the disease intensity was influenced by application the raw secondary metabolites alone or in combination (Table 4). The raw secondary metabolites of *P. fluorescens* P60 (Bio P60) and *T. harzianum* T10 (Bio T10) either alone or in combination, could decrease the disease intensity between 49.16 and 61.91% compared to control. This was in line with the lengthening incubation period (Table 4). This is presumably that because the pathogenic fungi could not grow and develop well in soil, the disease intensity in pakcoy decreased. The raw secondary metabolites could inhibit the pathogen growth and induce resistance in pakcoy (Reino *et al.*, 2008; Vinale *et al.*, 2008; Heydari and Pessarakli, 2010; Soesanto *et al.*, 2013).

3. Crop height

Based on Table 5, the interaction of application time and the raw secondary metabolites alone or in combination

differed significantly in crop height, number of leaves, and crop fresh weight compared to control. Increasing pakcoy height was affected by the application of Bio P60 and Bio T10 either before or after the pathogen attack in between 29.99 and 46.62% compared to control (Table 5). Bio P60 and Bio T10 contain raw secondary metabolites that consisted of several substances such as growth hormones.

Bio P60 derived from *P. fluorescens* P60 produced IAA. Raw secondary metabolites of *Trichoderma* sp. (Reino et al. 2008; Vinale et al. 2008) and *P. fluorescens* (Heydari & Pessarakli, 2010; Soesanto et al., 2013b) may have an important role in both plant growth hormones and activation of plant defense responses.

Table 5. The effect of application time and the combination of the treatments on the incubation period and disease intensity

Treatments	Crop height (cm)	Increase (%)	Number of leaves	Increase (%)	Crop fresh weight (g)	Increase (%)
Control >< Before	14.03 a	-	8.33 a	-	9.42 a	-
Control >< After	11.83 a	-	9.92 a	-	11.17 a	-
P60 >< Before	20.48 b	31.49	12.08 b	31.04	29.50 b	68.07
P60 >< After	22.03 b	46.30	14.12 b	29.75	32.75 b	65.89
T10 >< Before	20.04 b	29.99	12.83 b	35.07	25.92 b	63.66
T10 >< After	21.31 b	44.49	14.33 b	30.77	30.08 b	62.87
P60+T10 >< Before	20.44 b	31.36	11.75 b	29.11	29.50 b	68.07
P60+T10 >< After	22.08 b	46.42	13.12 b	24.39	38.75 c	71.17

Note: Mean followed by different letters in the same column show significantly different in DMRT with an error level of 5%.

4. Number of leaves

The interaction of application time and the raw secondary metabolites (Bio P60 and Bio T10) was significantly different in the number of pakcoy leaves (Table 5). The interaction either alone or in combination could increase the number of leaves between 24.39% and 35.07% compared to control. Increasing the number of leaves was presumably affected by decreasing the disease intensity (Table 2) and improving crop height (Table 3 and 4).

Suppression of the plant pathogen could influence healthy plants and increasing growth components such as number of leaves. Application of raw secondary metabolites could increase growth components because of the growth regulator contained. *P. fluorescens* could produce growth hormones such as IAA so known as PGPR (Dorjey et al., 2017). *T. harzianum* was known as PGPF as well because of hormone content (Reino et al., 2008).

5. Crop fresh weight

Application of the treatments either alone or in combination, influenced pakcoy fresh weight compared to control but there was no differences among the treatments (Table 5). The treatments could increase crop fresh weight in between 62.87% from Bio T10 after inoculation and 71.17% from Bio P60 + Bio T10 after inoculation. The application time of the fungal inoculation did not differ significantly in crop fresh weight. This is in line with crop height and number of leaves. Bio P60 and Bio T10 could be applied before or after plant pathogens attack. The raw secondary metabolites containing Bio P60 and Bio T10 has an important role as a plant regulator because of growth hormones (Reino et al. 2008; Dorjey et al., 2017).

4. CONCLUSION

Single and combined application of Bio T10 and Bio P60 did not differ in the suppression of stem-end rot in pakcoy. The combination of Bio T10 + Bio P60 was able to suppress the disease by delaying the incubation period and containing the disease intensity respectively by 37.48-39.16% and 54.77-61.91% compared to controls. Combined Bio T10 + Bio P60 is able to increase growth and yield components (plant height, number of leaves, and fresh weight of plants) by increasing crop height, number of leaves, and fresh weight of plants as 29.99-46.62, 24.39-35.07, and 71.17%, respectively, compared to controls. Recommendations from the results of this study are the raw secondary metabolites of Bio P60 and Bio T10 either alone or in combination can be applied for the prevention or treatment of the diseases in pakcoy.

REFERENCES

- Akhtar, J., Singh, B., Kandan, A., Kumar, P., Maurya, A. K., & Dubey, S. C. (2017). Interception of pathogens during quarantine processing: An effort towards safe import of oilseed and vegetable Brassicas germplasm in India. *Journal of Oilseed Brassica* 8 (2): 120-130.
- Amin, R., Sarker, B. C., Adhikary, S. K., Sultana, S., & Zubair, T. (2013). Effect of some botanical extracts and cow's urine on *Sclerotium rolfsii* causal agent of foot and root rot of betel vine. *The International Journal of Engineering and Science (IJES)* 2(9): 77-82.
- Ardebili, Z. O., Ardebil, N. O., & Hamdi, S. M. M. (2011). Physiological effects of *Pseudomonas fluorescens* CHAO on tomato (*Lycopersicon esculentum* Mill) plants and its possible impact on *Fusarium oxysporum* f.sp *lycopersici*. *Aus. J. Crop Sci.* 5(12):1631-1638.
- Badan Pusat Statistik dan Direktorat Jendral. (2015). Produksi Pakcoy. Kementerian Pertanian Republik Indonesia.
- Bansal, R., & Mukherjee, P. K. (2016). Identification of novel gene clusters for secondary metabolism in *Trichoderma* genomes. *Microbiology* 85(2): 185-190. Doi: 10.1134/S002626171602003X.

- Bhuiyan, M.A.H.B., Rahman, M. T., & Bhuiyan, K. A. (2012). In vitro screening of fungicides and antagonists against *Sclerotium rolfsii*. *African Journal of Biotechnology* 11(82): 14822-14827. Doi: 10.5897/AJB12.1463.
- Chandrashekara, K.N., Kumar, M. K. P., & Saroja, S. (2012). Aggressiveness of *Ralstonia solanacearum* isolates on tomato. *J of Experimental Sciences* 3 (9): 5-9.
- Chaurasia, S., Chaurasia, A. K., Chaurasia, S., & Chaurasia, S. (2013). Factors affecting the growth and sclerotial production in *Sclerotium rolfsii* causing foot rot of brinjal. *Indian Journal of Fundamental and Applied Life Sciences* 3(2): 73-84.
- Chu, M., & Seltzer, T. F. (2010). Myxedema coma induced by ingestion of raw bok choy. *N Engl J Med.* 362(20): 1945-6. Doi: 10.1056/NEJMc0911005.
- Di Noia, J. (2014). Defining powerhouse fruits and vegetables: A nutrient density approach. *Preventing Chronic Disease* 11: 130390. DOI: 10.5888/pcd11.130390.
- Dorjey, S., Dolkar, D., & Sharma, R. (2017). Plant Growth Promoting Rhizobacteria *Pseudomonas*: A Review. *Int. J. Curr. Microbiol. App. Sci.* 6(7): 1335-1344.
- El-Mohamedy, R.S.R., Jabnoun-Khiareddine, H., & Daami-Remadi, M. (2014). Control of root rot diseases of tomato plants caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* using different chemical plant resistance inducers. *Tunisian Journal of Plant Protection* 9: 45-55.
- Hadar, Y. (2011). Suppressive compost: when plant pathology met microbial ecology. *Phytoparasitica* 39(4): 311-314. Doi: 10.1007/s12600-011-0177-1.
- Hafsah, S., Rahayu, A., Syamsuddin, S., & Firdaus, F. (2019). Induced Resistance against Fusarium Wilt Disease caused by *Fusarium oxysporum* in Local Red Peppers (*Capsicum annum* L.) in Aceh using Rhizobacteria Isolates. *Journal of Tropical Horticulture*, 2(1), 6-10.
- Hartati, S.Y. & Nuri. 2014. Teknik inokulasi *Ralstonia solanacearum* untuk pengujian ketahanan nilam terhadap penyakit layu. *Bul. Littro.* 25 (2) : 127-135.
- Heydari, A. & Pessarakli, M. (2010). A review on biological control of fungal plant pathogens using microbial antagonists. *Journal of Biological Sciences*, 10, 273-290. DOI: 10.3923/jbs.2010.273.290.
- Jambhulkar, P.P., Sharma, P., Manokaran, R., Lakshman, D. K., Rokadia, P., Jambhulkar, N. (2018). Assessing synergism of combined applications of *Trichoderma harzianum* and *Pseudomonas fluorescens* to control blast and bacterial leaf blight of rice. *European Journal of Plant Pathology* 152(3): 747-757. Doi: 10.1007/s10658-018-1519-3.
- Junsopa, C., Jogloy, S., Saksirirat, W., Songsri, P., Kesmala, T., Shew, B. B., & Patanothai, A. (2016). Inoculation with *Sclerotium rolfsii*, cause of stem rot in Jerusalem artichoke, under field conditions. *European Journal of Plant Pathology* 146(1): 47-58. Doi: 10.1007/s10658-016-0890-1.
- Kabdwal, B.C., Sharma, R., Tewari, R., Tewari, A. K., Singh, R. P., & Dandona, J. K. (2019). Field efficacy of different combinations of *Trichoderma harzianum*, *Pseudomonas fluorescens*, and arbuscular mycorrhiza fungus against the major diseases of tomato in Uttarakhand (India). *Egyptian Journal of Biological Pest Control* 29:1. Doi: 10.1186/s41938-018-0103-7.
- Kator, L., Oche, O. D., & Hosea, Z. Y. (2015). Incidence and severity of *Sclerotium rolfsii* disease on tomato farms in Chile Island (Makurdi), Benue State, Nigeria. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)* 8(11): 97-103. Doi: 10.9790/2380-0811297103.
- Kumar, M.R., Santhoshi, M. V. M., Krishna, T. G., & Reddy, K. R. (2014a). Cultural and morphological variability *Sclerotium rolfsii* isolates infecting groundnut and its reaction to some fungicidal. *Int. J. Curr. Microbiol. App. Sci.* 3(10): 553-561.
- Kumar, V., Shahid, M., Srivastava, M., Pandey, S., Singh, A., & Sharma, A. (2014b). Role of secondary metabolites produced by commercial *Trichoderma* species and their effect against soil-borne pathogens. *Biosensors Journal* 3:108. Doi: 10.4172/2090-4967.1000108.
- Lahre, S.K., Khare, N., Lakpale, N., & Chaliganjewar, S.D. (2012). Efficacy of bio-agents and organic amendments against *Sclerotium Rolfsii* in chickpea. *Journal of Plant Disease Sciences* 7 (1): 32-34.
- Latifah, A., Kustantinah, & Soesanto, L. (2011). Pemanfaatan beberapa isolat *Trichoderma harzianum* sebagai agensia pengendalian hayati penyakit layu fusarium pada bawang merah in planta. *Eugenia* 17(2): 86-95.
- Lee, S.W., Lee, S. H., Lan, J. M., Park, K. H., Jang, I. B., & Kim, K. H. (2016). Control of soil-borne pathogens in ginseng cultivation through the use of cultured green manure crop and solarization in greenhouse facilities. *Korean Journal of Medicinal Crop Science* 24(2): 136-142. Doi: 10.7783/kjmcs.2016.24.2.136.
- Li, M.-F., Li, G.-H., & Zhang, K.-Q. (2019). Non-Volatile Metabolites from *Trichoderma* spp. *Metabolites* 9(3): 58. Doi: 10.3390/metabo9030058.
- Luján, A.M., Gómez, P., & Buckling, A. (2015). Siderophore cooperation of the bacterium *Pseudomonas fluorescens* in soil. *Biol Lett.* 11(2): 20140934. Doi: 10.1098/rsbl.2014.0934.
- Nahar, M., Rahman, M., Kibria, M., Karim, A., & Miller, S. (2013). Use of tricho-compost and tricho-leachate for management of soil-borne pathogens and production of healthy cabbage seedlings. *Bangladesh Journal of Agricultural Research*, 37(4): 653-664. Doi: 10.3329/bjar.v37i4.14390.
- Neidig, N., Paul, R. J., Scheu, S., & Jousset, A. (2011). Secondary metabolites of *Pseudomonas fluorescens* CHA0 drive complex non-trophic interactions with bacterivorous nematodes. *Microbial Ecology* 61(4): 853-859. Doi: 10.1007/s00248-011-9821-z.
- Noble, R. (2011). Risks and benefits of soil amendment with composts in relation to plant pathogens. *Australasian Plant Pathology* 40(2): 157-167. Doi: 10.1007/s13313-010-0025-7.
- Paramasivan, M., Mohan, S., Muthukrishnan, N., & Chandrasekaran, A. (2013). Degradation of oxalic acid (OA) producing *Sclerotium rolfsii* (Sacc.) by organic biocides. *Archives of Phytopathology and Plant Protection* 46(3): 357-363. Doi: 10.1080/03235408.2012.738520.
- Raj, H. & Sharma, S. D. (2009). Integration of soil solarization and chemical sterilization with beneficial microorganisms for the control of white root rot and growth of nursery apple. *Scientia Horticulturae* 119(2): 126-131. Doi: 10.1016/j.scienta.2008.07.025.
- Rakh R.R., Raut, L., S., Dalvi, S. M., & Manwar, A. V. (2011). Biological control of *Sclerotium rolfsii*, causing stem rot of groundnut by *Pseudomonas cf. monteilii* 9. *Recent Research in Science and Technology* 3(3): 26-34.
- Rakholiya, K.B. (2010). Efficacy of fungicides against *Trichoderma harzianum* and *Sclerotium rolfsii*. *International Journal of Plant Protection* 3(2): 406-407.
- Reino, J.L., Guerrero, R. F., Hernández-Galán, R., & Collado, I. G. (2008). Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochem Rev.* 7: 88-123. DOI: 10.1007/s11101-006-9032-2.
- Shahid, I., Rizwan, M., Baig, D. N., Saleem, R. S., Malik, K. A., & Mehnaz, S. (2016). Secondary Metabolites production and plant growth promotion by *Pseudomonas chlororaphis* and *P. aurantiaca* strains isolated from cactus, cotton, and para grass. *J. Microbiol. Biotechnol.* 27(3): 480-491. Doi: 10.4014/jmb.1601.01021.
- Soesanto, L., Mugiastuti, E., & Rahayuniati, R. F. (2010). Kajian mekanisme antagonis *Pseudomonas fluorescens* terhadap *Fusarium oxysporum* pada tanaman tomat in vivo. *J. HPT Tropika* 10(2): 108-115.
- Soesanto, L., Mugiastuti, E., & Rahayuniati, R. F. (2013). Application of *Pseudomonas fluorescens* P60 liquid formulation to suppress viral diseases on red chili. *Jurnal Fitopatologi Indonesia* 9: 179-185. Doi: 10.14692/jfi.9.6.179. [In Bahasa Indonesia].
- Soesanto, L., Mugiastuti, E., & Manan, A. (2019). Raw secondary metabolites of two *Trichoderma harzianum* isolates towards vacuolar streak dieback on cocoa seedlings. *Pelita Perkebunan* 35(1): 22-32.
- Songvilay, P., Groenewald, J. Z., Vongphachanh, P., Sayapaththa, S., Chittarath, K., Crous, P. W., & Burgess, L. W. (2012). First report of *Sclerotium rolfsii* in the Lao PDR. *Australasian Plant Dis. Notes*. Doi:

- 10.1007/s13314-012-0085-3.
- Stolp, H., & Gadkari, D. (1983). Nonpathogenic members of the genus *Pseudomonas*. In: Starr, M. P., Troper, H. G., Ballows, A., & Schiegel, H. G. (eds.). *The Prokaryotes A Handbook on Habitat, Isolation and Identification of Bacteria*. Springer-Verlag. New York.
- Shukla, P., Walia, S., Ahluwalia, V., Parmar, B.S., Nair, M.G. (2012). Activity of alkanediol alkanoates against pathogenic plant fungi *Rhizoctonia solani* and *Sclerotium rolfsii*. *Nat Prod Commun.* 7(9): 1219-22.
- Thiessen, L.D., & Woodward, J. E. (2012). Diseases of peanut caused by soilborne pathogens in the Southwestern United States. *International Scholarly Research Notices Agronomy*, Article ID 517905, 9 pages. Doi: 10.5402/2012/517905.
- Tran, N. H. (2010). Using *Trichoderma* species for biological control of plant pathogens in Vietnam. *J. ISSAAS.* 1 (16) : 17-21.
- Trapet, P., Avoscan, L., Klinguer, A., Pateyron, S., Citerne, S., Chervin, C., Mazurier, S., Lemanceau, P., Wendehenne, D., & Besson-Bard, A. (2016). The *Pseudomonas fluorescens* siderophore pyoverdine weakens *Arabidopsis thaliana* defense in favor of growth in iron-deficient conditions. *Plant Physiol.* 171(1): 675-693. Doi: 10.1104/pp.15.01537.
- Vinale, F., Flematti, G., Sivasithamparam, K., Lorito, M., Marra, R., Skelton, B. W., & Ghisalberti, E. L. (2009). Harzianic acid, an antifungal and plant growth promoting metabolite from *Trichoderma harzianum*. *J. Nat. Prod.* 72: 2032-2035. DOI: 10.1021/np900548p.
- Zachow, C., Grosch, R., & Berg, G. (2011). Impact of biotic and a-biotic parameters on structure and function of microbial communities living on sclerotia of the soil-borne pathogenic fungus *Rhizoctonia solani*. *Appl Soil Ecol.* 48(2): 193-200. Doi: 10.1016/j.apsoil.2011.03.006.