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In vitro, Evaluation of Organic and Mineral Treatments Against Potato Black-leg Disease (*Pectobacterium atrosepticum*)

Sondos G Nagdy[1], Afaf ZA El-Meneisy[3], Naglaa M Balabel[1,2], Nagy Y Abd El-Ghaffar [3*]

1- Potato Brown Rot Project (PBPR), Ministry of Agriculture and Land Reclamation, Dokki, Egypt
2- Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt
3- Plant Pathology Dept, Fac of Agric, Ain Shames Univ, P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

*Corresponding author: elghaffar3339@agr.asu.edu.eg

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Abstract: Potato (*Solanum tuberosum* L.) is considered one of the main food crops in many countries worldwide. The present work was planned to manage potato black-leg disease using organic treatments as well as mineral-nitrogenous treatments, in addition to examining their effects on the pathogenic bacterium (*Pectobacterium atrosepticum*) population under artificial inoculation conditions. The obtained data indicated that applications of cabbage, onion and peppermint dry ground leaves as organic matters, and applications of ammonium super-phosphate, potassium sulfate and urea as mineral-nitrogenous fertilizers reduced *P. atrosepticum* population and black-leg disease of potato compared with the control treatment. In addition, these treatments led to increasing potato yield, while the beneficial effects increased with increasing their doses compared with the control treatment. Meanwhile, the organic matters appeared highly effective compared with mineral-nitrogenous fertilizers in disease reduction. However, mineral-nitrogenous fertilizers appeared highly effective in increasing potato yield. Meanwhile, onion dry ground leaves (organic matter) and urea (nitrogenous fertilizer) were the most effective on the pathogenic bacterium population and severity of the disease compared with other treatments.

1 Introduction

Potato is an important economic cash crop in Egypt, where exportation rates are constantly increasing. Potato plants are attacked by many diseases, which affect potato yield (Elhalag 2019). Potato plants are attacked by 40 soil-borne diseases causing high damage to tubers (Fiers et al 2012). *Pectobacterium* bacterium is considered one of the main bacterial pathogens, and that bacterium led to discord Potato tubers (van der Wolf and De Boer 2007). In addition, *Pectobacterium carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliensu* and *Dickey* spp. can cause black leg and soft rot diseases, under different environmental conditions (Duarte et al 2004, Ngadze et al 2012).

Potato black leg and soft rot diseases caused by Pectobacterium spp. and *Dickey* sp. bacteria pectin lytic and caused important loss for potato production, in Egypt (Ashmawy et al 2015). *Pectobacterium* and *Dickeya* species induce black leg disease on stems and soft rot on tubers of potatoes (Pérombelon 2002, de Werra et al 2020, Dupuis et al 2021). Worldwide, *Dickey* and *Pectobacterium* species are considered as causative pathogens for soft rot and black-leg diseases which caused great losses in potato production. Also, control procedures for these pathogens are not yet available (Motyka-Pomagruk et al 2020).

Fertilization Treatments indirectly affected the development of diseases (*Pectobacterium* spp.) pathway enhancing foliar growth because of the high level of humidity which is necessary for bacterium growth. Amendment contributed to disease control pathway changing soil properties i.e. increasing activity of microbial and pH soil or both (Termorshuizen et al 2006, Steinberg et al 2006, Lazarovits et al 2001). Bio-fertilizers have many microorganisms that facilitate supplying nutrients for plant roots by converting ingredients into a simple form. Sustainable agriculture used bio-fertilizers for the production of economically important crop yields and because of their beneficial effects on plant growth, and the nodulation process (Htwe et al 2019).

Plant growth-promoting microorganisms (PGPM) play a significant role in competition for nutrients (eg. production of 125 siderophores), by activating and systematic resistance to pathogens or stimulating the growth of other beneficial microorganisms useful for plant development (Shahab et al 2009). Soil amendment is a requirement for biological control, holistic disease management and integrated pest management (Mathre et al 1999). Larkin et al (2021) reported that various soil amendments materials led to modifying physical, biochemical and microbial properties. Plant-based amendments, organic and inorganic matter amendments and microbial amendments were categorized as amendments. Partially living plant materials, green manures and rotation crops are considered as organic amendments. Potato yield increased with the application of plant-based amendments, due to increasing biomass input and suppresses the disease at various levels (Larkin and Lynch 2018).

This work was carried out to control potato black-leg disease, using some organic matters and mineral fertilizers treatments, under artificial inoculation conditions.

2 Materials and Methods

2.1 Inoculums preparation and soil infestation

Infected potato plants and tubers were collected from different Governments (Behaira, Kalubia and Scharkia). The causal organism was isolated according to Potrykus et al (2016) and Zoledowska et al (2018) and identified according to Czajkowski et al (2009) and Zaczek-Moczydlowska et al (2019). Isolation and identification procedures were carried out in the Potato Brown Rot Project (PBPR), Ministry of Agriculture and Land Reclamation, Dokki, Egypt. Selected virulent isolate was grown at 28°*c* for 48hr in plates containing nutrient agar medium. The bacterial suspension was prepared and adjusted at 6.4x 10^8 Colony Forming Units (CFU) as optical density at 600 nm with 0.1 (Ngadze 2018). The soil infestation procedure was carried out by adding 750 ml of the previous suspension/pot (30 cm, diam.), each pot containing 10 Kg sterilized clay soil. Germinated potato tubers (containing 2-3 sprouts, diamond, cv.) were stored at 4ºC and afterward placed at room temperature in the dark into trays for three weeks for stimulation of potato germination. These tubers were sowing in Soil infestation with the pathogen. The pots were irrigated at equal intervals.

2.2 Organic matters and mineral-nitrogenous fertilizers

Organic matters i.e. cabbage, onion and peppermint dry ground leaves were used at rates of 5, 10 and 15 g/pot, thirty days before the planting process. Besides, mineral-nitrogenous fertilizers (ammonium super-phosphate (46% P_2O_2 + 16% nitrogen), potassium sulfate (52% K O_2 +17-18% sulfate) and urea (46% nitrogen) were applied at the rates of 0.50, 1.0 and 1.5; 0.25, 0.50 and 1.0 and 1.0, 2.0 and 3.0 g/pot, respectively. Where, ammonium super-phosphate was added to the soil 20 days before the sowing, while potassium sulfate and urea were added to the soil 20 days after the sowing.

2.3 Population of *Pectobacterium atrosepticum* bacterium in soil potato plants

Approximately 10 grams of potato plants soil/treatment were collected at zero, 30, 60 and 90 days after planting. Each sample was mixed with 90 ml of sterile distilled water into a sterilized flask (250 ml) and shaken at 3000rpm for 30 min. using a horizontal shake. One ml of 10^7dilution was poured into sterilized Petri dishes with melted crystal violet pectate medium as a selective medium for *Pectobacterium atrosepticum* (Potrykus et al 2016). These plates were gently rotated and incubated for one to three days. After the incubation period, appearance colonies were estimated and purported per gram soil. Four plates were used as a replicate per treatment.

2.4 Assessment of disease severity and potato yield

Disease severity was calculated as the following: 1) disease index (DI) was assessed according to the disease rating scale for the individual plant after 90 days from planting (Hélias et al 2000). The scale was defined as (1) wilting/colors; (2) black-leg infection; (3) haulm desecration corresponding to death of plant stems; (4) death of plant with desiccation of all stems, and (5) complete plant death; where disease index (DI) was calculated according to the following formula: DI=$\sum R.T \div 5 \times N \times 100$, T = total number of plants / category, R = disease severity scale (R=1- 5) and N = number of tested plants. The percentage of infected tubers was estimated after 105 days from planting. Collected tubers per treatment were placed in meshed black polythene material; the tubers were stored at 25°c and 60% relative humidity for eight weeks and assessed fortnightly for rotting. The number of rotten tubers was estimated and related to total potato numbers (Ngadze 2018). The percentage of disease reduction (PDR) was calculated according to the following formula: PDR= (Dck – Dtr ÷ Dck) x100, where Dck = disease severity in the control treatment and Dtr = disease severity in the treated samples. Potato yield was estimated as the number and weight of tubers/treatment at 105 days after sowing.

2.5 Statistical analysis

Statistical differences were calculated using an ANOVA according to pairwise multiple comparisons (Duncan's method and Kruskal-Wallis tests). Analysis was performed using the Excel Add-in XISTAT software program (Addinsoft 2021).

3 Results

3.1 Population of *Pectobacterium atrosepticum* in soil potato plants.

Obtained data in **Tables 1 and 2** showed that applications of dry ground leaves of cabbage, onion and peppermint (organic matters) and ammonium super-phosphate, potassium sulfate and urea (mineral-nitrogenous fertilizers) decreased pathogenic bacterium (*P. atrosepticum*) population compared with the control. Besides, the population of pathogenic bacterium was reduced with increased rates of organic matter, while the population was increased with increasing rates of mineral-nitrogenous fertilizers. Meanwhile, organic matters were more effective than mineral-nitrogenous fertilizers on *P. atrosepticum* bacterium population, where the populations were 4.7 – 7.5 and 6.4 – 9.0 × 10^8CFU per g soil, respectively.

However, onion dry ground leaves as the organic matter was the most effective on the pathogenic bacterium population compared with cabbage and peppermint dry ground leaves, where bacterium population were 4.7-6.1, 5.3-6.8 and 5.5-7.5×10^8 Colony Forming Units (CFU)/g soil respectively. However, urea as mineral-nitrogenous fertilizer was the most effective on the pathogenic bacterium population followed by potassium sulfate and ammonium superphosphate; where bacterium populations were 6.4-8.3, 6.4-8.8 and 6.4-9.0×10^8 CFU/g soil respectively. While bacterium population ranged from 6.4 to 9.2× 10^8 CFU/g soil in the control treatment.

3.2 Disease severity and yield of potato

Amendments by organic matter and/or mineral-nitrogenous fertilizers decreased the severity of potato black-leg disease and increased the yield of potatoes compared with the control. Where the efficiency of organic additives ranged from 20.2 to 28.8% for disease index and from 7.3 to 16.8% for infected tubers. Meantime, the efficiency of mineral-nitrogenous additives ranged from 17.1 to 20.2 % for the disease index and from 6.3 to 16.8 % for infected tubers. However, the mean number of tubers ranged from 3.8 to 6.0 per pot for organic matter treatments and from 6.0 to 8.4 per pot for mineral-nitrogenous fertilizer treatments. Meantime, the weights of tubers ranged from 120.5 to 172.8 g/pot for organic matter and fall in the range of 182.7-238.2 g/pot for mineral-nitrogenous fertilizer treatments, while the control treatment was 3.0 tuber/plot and the weight was 62.4 g/plot. Therefore, the application of organic matter was more effective than the application of mineral-nitrogenous fertilizers to disease reduction. In addition, mineral-nitrogenous fertilizers were more effective than organic matter to increase potato yield. However, onion dry ground leaves (organic matter) was the most effective treatment followed by cabbage and peppermint dry ground leaves to reduce black-leg disease and increase potato yield. Where, the percentage of disease reduction were 26.0-28.8, 25.2-28.6 and 20.2-23.7 % for disease index and 10.2-16.8, 7.9-14.3 and 7.3-11.1 for infected tubers. However, the yields as an average number of tubers were 4.5-6.0, 4.3-5.0 and 3.8-4.6 tuber/pot and the weights of tubers were 145.2-172.8, 136.4-163.4 and 120.5-149.1 g/pot respectively. Meantime, the urea (mineral-nitrogenous fertilizer) was the most effective treatment against

Table 1. Effect of some organic matters, at several rates, against pathogenic bacterium (*Pectobacterium atroseptium)* population, in soil potato plants, under artificial inoculation conditions

Treatment	Dose (g/pot)	Count of Pathogenic bacteria (10^8) / g soil after			
		Zero*	**30 ***	**60 ***	**90 ***
Cabbage	5	6.4	5.7	6.2	6.8
	10	6.4	5.6	5.9	6.5
	15	6.4	5.3	5.6	6.1
Onion	5	6.4	5.4	5.6	6.1
	10	6.4	5.1	5.3	5.8
	15	6.4	4.7	5.0	5.4
Peppermint	5	6.4	5.9	6.2	7.5
	10	6.4	5.8	6.0	7.3
	15	6.4	5.5	5.9	6.6
Control	0	6.4	7.5	8.3	9.2

* Time sample after planting (day).

Table 2. Effect of some mineral-nitrogenous fertilizers, at several rates, against pathogenic bacterium (*Pectobacterium atrosepticum)* population, in soil potato plants, under artificial inoculation conditions

Treatment	Dose (g/Pot)	Count of Pathogenic bacteria (10^8)/g soil after			
		Zero*	**30 ***	**60 ***	**90 ***
Ammonium super-phosphate	0.50	6.4	6.8	7.6	8.5
	1.0	6.4	6.9	7.7	8.7
	1.5	6.4	7.2	8.0	9.0
Potassium sulfate	0.25	6.4	6.8	7.3	8.4
	0.50	6.4	7.0	7.4	8.5
	1.1	6.4	7.5	7.6	8.8
Urea	1.0	6.4	6.5	7.0	7.6
	2.0	6.4	6.7	7.3	7.9
	3.0	6.4	7.0	7.6	8.3
Control	0.0	6.4	7.5	8.3	9.2

* Time sample after planting (day)

disease severity followed by potassium sulfate and ammonium superphosphate treatments. Where the disease reduction (%) was 18.4-20.2, 17.2-19.1 and 17.1-18.9 % for disease index and 10.2-16.8, 8.6-12.4 and 6.3-12.1% for infected tubers. Moreover, the application of potassium sulfate was the most effective to increase potato yield compared with ammonium superphosphate and urea. Where, the average numbers of tubers were 7.7-8.4, 6.0-6.9 and 5.5-6.2 tuber/pot and the weights of tubers were 211.9-238.2, 182.2-218.6 and 180.4-209.7 g/pot by using potassium sulfate, ammonium superphosphate and urea respectively (**Tables 3 and 4**).

4 Discussion

Amendments by organic matter or mineral-nitrogenous fertilizers led to a decreasing pathogenic bacterium (*Pectobacterium atrosepticum*) population and severity of black-leg disease but increasing yield tubers under artificial inoculation conditions compared with the control. Increasing tested fertilizer doses increased their effectiveness; besides, organic matters were more effective than mineral-nitrogenous fertilizers. These findings are in agreement with that obtained by Bonanomi et al (2018) who reported that organic amendments affected the severity of soil–borne diseases in the following ways: (1) soil biological buffering capacity is increased, (2) the population of the pathogen is reduced

Table 3. Effect of some organic matters, at several rates, against the severity of black-leg disease after 90 days from planting and infected tubers with soft rot disease and potato yield after 105 days from planting, under artificial inoculation conditions

Treatment	Dose (g/Pot)	Disease severity (%)				Yield / Pot	
		Disease Index	Efficiency	Infected tubers	Efficiency	No.	Weight (g)
Cabbage	5	57.8a	25.2	29.0ab	7.9	4.3ab	136.4b
	10	56.6a	26.7	28.2ab	10.5	4.6ab	151.3bc
	15	55.2a	28.6	27.0a	14.3	5.0b	163.4bc
Onion	5	57.2a	26.0	28.3ab	10.2	4.5ab	145.2b
	10	56.0a	27.5	27.4a	13.0	5.0b	151.8bc
	15	55.0a	28.8	26.2a	16.8	6.0b	172.8bc
Peppermint	5	61.7ab	20.2	29.2ab	7.3	3.8ab	120. 5b
	10	60.8ab	21.3	28.6ab	9.2	4.1ab	131.2b
	15	59.0ab	23.7	28.0ab	11.1	4.6ab	149.1b
Control	0	77.3b	0.0	31.5b	0.0	3.0a	62.4a

Table 4. Effect of some mineral-nitrogenous fertilizers, at several rates, against severity of black-leg disease after 90 days from planting and infected tubers with soft rot disease and potato yield after 105 days from planting, under artificial inoculation conditions

Treatment	Dose (g/ Pot)	Disease severity (%)				Yield / Pot	
		Disease Index	Efficiency	Infected tubers	Efficiency	No.	Weight (g)
Ammonium super-phosphate	0.5	64.1ab	17.1	29.5ab	6.3	6.0b	182.7b
	1.0	63.5a	17.9	28.8ab	8.6	6.4b	201.7b
	1.5	62.7a	18.9	27.7a	12.1	6.9bc	218.6bc
Potassium sulfate	0.25	64.0ab	17.2	28.8ab	8.6	7.7bc	211.9b
	0.50	63.1a	18.4	28.2ab	10.5	8.0bc	225.4bc
	1.0	62.5a	19.1	27.6a	12.4	8.4bc	238.2c
Urea	1.0	63.1a	18.4	28.0ab	11.1	5.5b	180.4b
	2.0	62.5a	19.1	27.1a	14.0	5.9b	191.0b
	3.0	61.7a	20.2	26.2a	16.8	6.2b	209.7b
Control	0.0	77.3b	0.0	31.5b	0.0	3.0a	62.4a

during organic matter decomposition, and (3) nitrification process is affected by the soil nitrogen. Several green manures (organic matter and organic nitrogen) were associated with disease suppression and increased yield of potatoes (Bakker et al 2010). On the other hand, some organic amendments contain a high level of carbon and nitrogen contents which could include some toxic compounds e.g. ammonia and nitrous acid produced during a series of biochemical and microbial activities (Lazarovits et al 2001). Organic amendments interacted with soil properties i.e. pH and structure of soil (Ninh et al 2015). Plant-based amendments have several functions i.e. allelopathic production, specific toxic compounds production, inhibiting pathogens, and refreshing beneficial microorganisms (Wiggins and Kinkel 2005, Larkin et al 2010). Recheigl (1994) reported that organic soil amendments affected physical soil properties including the reduction of soil bulk density; increasing water holding capacity; increasing water infiltration and drainage, enhancing fine texture, and improving soil aggregation. Noble (2011) reported that several organic fertilizers and compost have been efficacy used for the suppression of diseases, depending on their degree of maturity. Soil texture was affected by humidity and soil amendment impact which affected pH and chemical element availability. In addition, the development of pathogens inoculums was affected in soil or on tuber surfaces, under optimal climatic and edaphic conditions (Fiers et al 2012). The application of biotic and abiotic compounds, used to control soil-borne diseases, is dependent on the density of inoculum pathogens, their intrinsic aggressiveness and severe soil factors (Steinberg et al 2006).

The application of onion and potassium sulfate was the most effective treatment against the population of *P. atrosepticum*; the severity of black-leg disease of potatoes and the crop yield was improved compared with other treatments. Adding urea as soil amendment decreased the population of the pathogen due to ammonium or nitrate accumulation formed from urea decomposition: besides, soil pH rises (Akanonu et al 2021). Usually, fertilization and amendments gave strong and healthy plants less susceptible to pathogens (Epelde et al 2018). Urea induces a high increase in soil pH and better management of several diseases (Noble 2011). Application of brassica plants during crop rotation and as green manure led to reducing soil-borne pests and pathogens, due to volatile sulfur compounds production, bio-fumigation process and changes in soil microbial community structure (Janvier et al 2007). Composed applications improved plant defense abilities against pathogens and increased higher crop yields (Viti et al 2010).

5 Conclusion

Organic matters i.e. cabbage, onion and peppermint dry ground leaves as well as mineral-nitrogenous, i.e. ammonium-super phosphate, potassium sulfate and urea treatments, as soil amendments, were highly effective against the population of *Pectobacterium atrosepticum* bacterium, severity of black-leg disease and potato yield compared with the control. Organic treatments showed a higher effect than mineral treatments against pathogenic bacterium populations and disease severity. However, mineral treatments were highly effective than organic treatments against potato yield. Onion as an organic amendment and urea as nitrogenous amendment were the most effective treatments compared with other amendments.

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