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DEVELOPMENT OF INTEGRATED MANAGEMENT PACKAGE FOR BLACKLEG OF POTATO AND EVALUATION OF POTATO VARIETIES FOR RESISTANCE TO BACTREIAL SOFT ROT CAUSED BY *PECTOBACTERIUM CAROTOVORUM* SUBSP. *CAROTOVORUM*

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ABSTRACT

Elahi F (2020) Development of integrated management package for blackleg of potato and evaluation of potato varieties for resistance to bactreial soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum*. *Int. J. Expt. Agric*. 10(1), 13-19.

To develop an integrated management package for the management of potato blackleg disease, chitosan (3% and 5%) combined with of gypsum ($CaSO_4$) fertilizer (3g/3kg soil and 5g/3kg soil) and *Trichoderma harzianum* BTH-N1 (10g/kg tuber seed) were tested in pothouse trials in the division of Plant Pathology, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. BARI Alu 7 (Diamant) was used as the test variety *T. harzianum* BTH-N1 reduced blackleg severity compared to the non-treated control when applied alone or in combination with chitosan (3%) or gypsum (3g/3kg soil). The highest level of disease reduction was recorded in chitosan 3% + *T. harzianum* BTH-N1 treated plants. Ten BARI-released potato varieties showed different levels of reactions to soft rot causing bacterium strain *Pectobacterium carotovorum* ssp. *carotovorum* Pki2 in artificial tuber inoculation. BARI Alu 25 was the resistant to soft rot, BARI Alu 72 and BARI Alu 41 were the susceptible. Calcium ($p<0.05$) content but not dry matter percentage of tubers was correlated with soft rot severity of the tested potato varieties.

Key words: *Trichoderma harzianum, BARI Alu 7 (Diamant), Chitosan, Gypsum ($CaSO_4$)*

INTRODUCTION

Potato tuber soft rot causes yield losses both in field and storage in Bangladesh Rasul *et al.* (1999). This disease is characterized by tuber and/or stem degradation by pectinolytic bacteria of the genera *Pectobacterium, Dickeya*. These pathogens can survive in the soil for several months, and transform from a latent stage to the active stage depending on temperature and moisture (Perombelon 2002). Rotted tissue is initially white in creamy in color, later turn to brown due to oxidation. A characteristic foul odor typically develops, related to secondary bacterial colonization (Perombelon *et al.* 1979). The three main blackleg-causing pathogens are *Pectobacterium carotovorum* ssp. *carotovorum, Pectobacterium atrosepticum and Dickeya* spp. (van der wolf and De Boer, 2007). These gamma-proteobacteria bacteria are pectinolytic, gram negative, facultative anaerobic, and rod shaped. *Pectobacterium carotovorum* ssp. *carotovorum* has a wide host range and infects both seedlings and tubers in tropical and temperate regions, whereas *P. atrosepticum* only infects plants in temperate regions. Blackleg disease of potato has been reported in potato growing areas of Bangladesh. Blackleg causes inky black discoloration and soft rot in the stem of potato seedlings. Other symptoms of blackleg include yellow wilted leaves and stunting. The leaves of infected plants may also be stiff and small. Pathogens can be transmitted from infected mother tubers to daughter tubers and may remain latent until environmental conditions are favorable for bacterial growth Reiter *et al.* (2002).

Several attempts have been made to develop effective strategies to manage blackleg in the field. Seed certification approaches can be highly effective; however certification systems are lacking in many countries. Some other approaches such as environment management in storage and sanitation in the field reduce bacterial inoculum from the tubers. Crop rotations for up to 3-8 years can eliminate pathogens from the soil Czajkowski *et al.* (2012). Effective management requires reliable, inexpensive tactics readily available to growers. However, due to lack of availability of appropriate inputs and information, growers in Bangladesh have few options to manage blackleg bacteria in the field and storage. Physical seed treatments such as hot water treatment have been used to kill superficial pathogens but may negatively affect tuber health and shoot emergence. According to Mackay and Shipton (1983), *P. carotovorum* ssp. *carotovorum* and *P. atrosepticum* were not detected in tuber peels after treatment of potato tubers in water for 10 min at 55ºC, and in a field experiment plants from treated tubers did not show blackleg symptoms. However, depending on potato variety and physiology, sprouting could be delayed or tubers could be killed outright; as a result, yield reduction might occur (Robinson and Foster, 1987). Researchers in many countries have started applying new biochemical and biocontrol agents along with certain elements to manage this disease in the field and in storage (Colyer and Mount, 1984). Chitosan, made from chitin of crab shells, supplies secondary metabolites to plant organs (O'Herlihy *et al.* 2003). Chitosan has been shown to be effective against soft rot of stored potato tubers (Makhlouf and Abdeen, 2014). Species of the biocontrol fungus *Trichoderma* also have been used to control post-harvest diseases of fruits and vegetables. According to Abd-El-Khair and Karima (2007) *Trichoderma harzianum* reduced soft rot on potato tubers and also increased vegetative characteristics such as plant height, number of leaves per plant and yield. This species of *Trichoderma,* along with other *Trichoderma* species such as *T. atroviridae,* increased potato growth parameters such as stolon number and yield and reduced the percentage of *Rhizoctonia*-infested solons (Hicks *et al*. 2014). To manage blackleg and soft rot in the field and storage, sustainable integrated management strategies including biocontrol as a component should be developed. Calcium fertilizer strengthens the texture and

structure of potatoes in the growing period of tubers. It can significantly reduce blackleg and soft rot of tubers (McGuire and Kelman, 1984). However, calcium is not equally distributed through potato plants, and tubers especially have low levels of calcium (Dunn and Rost, 1945). Gypsum ($CaSO_4$) added to soil-increased resistance against not only blackleg of plants but also soft rot of daughter tubers (Bain *et al.* 1996). Some studies have shown that tubers with higher calcium content have lower soft rot potential than tubers with lower calcium content (McGuire and Kelman, 1984). Dry matter content is an important factor that determines the resistance to soft rot bacteria in tubers (McGuire and Kelman, 1984). Earlier studies showed that increased calcium content reduced the probability of potato soft rot incidence in storage (Tzeng *et al.* 1990). However, little is known about the resistance of Bangladeshi potato varieties and there are no soft rot-resistant commercial varieties available on the market. The objectives of this study were: 1) to identify the effective treatments for the management of blackleg of potato; 2) to assess the level of resistance of BARI released potato varieties to soft rot disease caused by *P. carotovorum* ssp. *carotovorum.*

MATERIALS AND METHODS

Integrated management of blackleg disease of potato

Isolation of bacterial strains: Two strains of *P. carotovorum* ssp. *carotovorum* isolated from potato tubers in Munshigonj and Kishoregonj, Bangladesh in 2015. The identity of the strains as *P. carotovorum* ssp. *carotovorum* was confirmed using a combination of biochemical, molecular and plant-based tests (Elahi 2018). Strain *P. carotovorum* ssp. *carotovorum* Pmu6 (KU945635) causes both soft rot of potato tubers and blackleg and was selected for blackleg management experiments. Strain *P. carotovorum* ssp. *carotovorum* Pki2 (KX098357) is highly virulent on potato tubers, causing soft rot, and was selected to evaluate potato varieties for soft rot resistance.

***In vitro* antagonism assay:** Three *Trichoderma* spp., namely *T. harzianum* BTH-N1, *T. viridae* BTV-N1 *and T. virens* BTVI-N1) were provided by the Plant Pathology section of the Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI). Antagonism of these biocontrol agents against *P. carotovorum* ssp. *carotovorum* Pmu6 was evaluated using a confrontation assay (Arunachalam and Sharma, 2012). *Pectobacterium carotovorum* ssp. *carotovorum* Pmu6 was grown on Luria-Bertani agar (LBA) medium for 2 days. Bacterial suspensions were prepared and adjusted with sterile water to an optical density (OD) of 0.2 at 600nm (10^8 Colony Forming Units (CFU)/mL) and concentrations were checked by dilution plating on LBA medium (Subedi 2015). Bacterial lawns were prepared by spreading 50 µL bacterial inoculum on LBA medium. *Trichoderma* spp. were grown on potato dextrose agar (PDA) medium for 7 days, then 5-mm disks were cut from the periphery of the colonies. After bacterial lawns were dried in a laminar flow hood for 5 min, one disk of *Trichoderma* spp. was placed in the center of each lawn and incubated in the dark for 5 days at 28ºC. Bacterial lawns without *Trichoderma* served as controls. After 5 days of incubation, the diameter of the clear inhibition zone around the *Trichoderma* plug was measured. Each assay was replicated three times and the experiment was repeated once.

Preparation of *Trichoderma harzianum* inoculum: *Trichoderma* isolate BTH-N1 was grown on PDA medium. Three-days-old mycelial plugs were added into sterilized wheat grains and incubated for 7 days at 28°C. When *Trichoderma* grown well, wheat grain dried and grounded for the application on tuber. Prior to inoculation potato tubers were surface sterilized with 0.5% sodium hypochlorite and dried for 2 hrs. Pre-sprouted potato tubers were cut and tubers were mixed with *T. harzianum* BTH-N1. The inoculum was mixed with potato tubers at the rate of 10g BTH-N1/kg tuber where the concentration of *Trichoderma* was 10^7conidia/mL (Srivastava *et al.* 2016).

Preparation of chitosan solution: Sterilized distilled water was used to make 3% and 5% chitosan solutions (3g/100mL, 5g/100mL), which were kept for 12 hours at room temperature to form a uniform solution (pH 5.5). Full size sprouted potato tubers were surface sterilized (using 70% ethanol for two min) and were dried under laminar air flow for 10 min. Potato tubers were cut into half and dipped into the chitosan solution for 30 min, air-dried and sown in steam-sterilized loamy soil.

Application of different treatments: Different doses (3g and 5 g) of gypsum ($CaSO_4$) fertilizer were added to 3 kg soil per pot along with basal doses of fertilizers (1.2 g N, 1.2 g P and 1.2 g K). The treatments were as follows: T1= gypsum 3g/3kg soil; T2= gypsum 5g/3 kg soil; T3= *T. harzianum* BTH-N1; T4= gypsum 3g/3 kg soil+ BTH-N1; T5= chitosan 3%; T6= chitosan 5%; T7= chitosan 3% + BTH-N1; T8= gypsum 3g/3 kg soil + BTH-N1 + chitosan 3% and T9= Non-amended control.

Inoculation procedure: Potato variety BARI Alu 7 (Diamant) were planted in 15cm diameter pots containing steam-sterilized field soil. Plants were watered twice daily. Bacterial suspensions were prepared in sterilized distilled water from 2-day-old cultures of each isolate growing in LB broth and adjusted to an optical density of 0.2 (OD_{600}). Bacterial concentrations were confirmed by dilution plating (10^8 CFU/mL). A drop (15 µL) of aqueous cell suspension (10^8 CFU/mL) of each isolate was placed in the lowest leaf axil. After 4 weeks of sowing tubers, all the seedlings in each pot were inoculated at the base of the stem with *Pectobacterium*

carotovorum ssp. *carotovorum* strain Pmu6. Sterilized 200 µL pipette tips were inserted through the drops into the center of the stem of the lowest leaf axil. The tips were removed after introducing the inoculum into the stems. Plants were maintained for two weeks more to observe the symptoms of blackleg.

Disease incidence and severity: Disease severity was scored based on blackleg symptoms: blackening from the lower part of the seedlings and progressing upward, yellow leaves and vascular discoloration. Disease severity percentage was assessed based on a scale developed by Wright *et al.* (2005) and data were recorded from 3 to 12 days after pathogen inoculation at 3 day intervals. In the scale 0= no symptoms of blackening on the stem, 1= less than 50% of the stems have blackening symptoms, 2= more than 50% of the stems have blackening symptoms, and 3= plants are dead. Area under the disease progress curves (AUDPC) was calculated based on blackleg percent severity according to the formula: $\sum([\frac{(xi+xi-1)}{2}])\ (ti - ti - 1)$, where xi is the rating at each evaluation time and (ti-ti-1) is the number of days between evaluations (Madden *et al.* 2007).

Evaluation of potato varieties for resistance to soft rot caused by *Pectobacterium carotovorum* ssp. *Carotovorum*

Bacterial inoculum preparation and tuber inoculation: *Pectobacterium carotovorum* ssp. *carotovorum* Pki2 was streaked on LBA medium and incubated at 28°C for 48 hours. Several single colonies were picked from pure cultures and inoculated into 5mL LB broth medium and incubated at 28°C for 48 hours. The concentrations of bacterial suspensions were adjusted to 10^8 CFU/mL and checked by dilution plating as described above. Tubers of ten BARI potato varieties harvested at the same time in February 2017 were collected from storage, where the potatoes are kept at 2.5°C, in June 2017. Twelve randomly selected tubers from each variety were washed with 0.5% sodium hypochlorite. Bacterial inoculum was stirred with a magnetic stirrer to avoid sedimentation. Two wells, 5 mm in diameter x 5 mm deep, were made in different sites on each tuber using a sterilized metal screw. A 50 µL aliquot of *P. carotovorum* ssp. *carotovorum* Pki2 inoculum was placed in each well and the wells were wrapped with parafilm. Four randomly selected tubers from each variety mock-inoculated with sterilized distilled water served as a negative control. After inoculation, tubers were placed in moistened plastic containers in the dark for 3 days at 28°C (Pasco *et al.* 2006). After incubation tubers were weighed, rotted tuber tissue was separated from healthy tissue with a spatula and the fresh weight of the rotten portion was measured in grams and later converted to percentage. The experiment was replicated three times and repeated once.

Determination of calcium and dry matter content of potato tubers: Four tubers from each potato variety were peeled, cut into thin slices, weighed and dried at room temperature. Potato slices were oven dried at 65°C for 2 days and mixed all the replications sample of each variety together to make sufficient sample. Samples were digested with H_2SO_4 and H_2O_2 (Thomas *et al.* 1967) weighed. Dry matter percentage was calculated following the formula:

Dry matter % = (dry weight/fresh weight) x100

Dried potato samples were sent to the Department of Soil Science, BARI, Bangladesh to measure the percentage of calcium in medullar tissues using flame atomic absorption spectrometry following a wet digestion method (Habib *et al.* 2004).

Experimental design and site: This experiment was conducted in the pothouse of the Division of Plant Pathology, BARI, Gazipur in January 2017. The pot experiment was conducted following randomized complete block design (RCBD) with three replications where four pots containing three seedlings/pot in each replication. The pots were overhead irrigated twice regularly using a hose during the growing period.

Data analysis: MINITAB 16 software was used for the general linear model where, AUDPC and rotted tissue were the response variables and different treatments and potato varieties were independent variable. Differences in means were assessed using Tukey's range test with an error rate of α=0.05.

To see the correlation between percent tuber calcium concentration/percent dry matter of tuber and soft rotted potato tuber (%), Pearson's correlation coefficient was done. Regression analysis equation was generated considering both percent dry matter and percent calcium conc. on soft rot severity. Data from the two experiments of *in-vitro* assay and blackleg severity, were combined since in Levene's test for equality of variances was not significant ($p>0.05$).

RESULTS AND DISCUSSION

***In vitro* antagonism of *P. carotovorum* ssp. *carotovorum* by *Trichoderma* spp. and integrated management of blackleg of potato:** *Trichoderma harzianum* BTH-N1, *T. viridae* BTV-N1 and *T. virens* BTVI-N1 produced zones of inhibition (18.57mm, 16.20mm and 14.41mm respectively) significantly larger than the non-challenged control (0.0mm) on lawns of *P. carotovorum* ssp. *carotovorum* Pmu6 *in vitro* (Fig. 1). Inhibition of Pmu6 was significantly higher by *T. harzianum* BTH-N1 than by *T. virens* BTVI-N1 but similar to that of *T. viridae* BTV-N1.

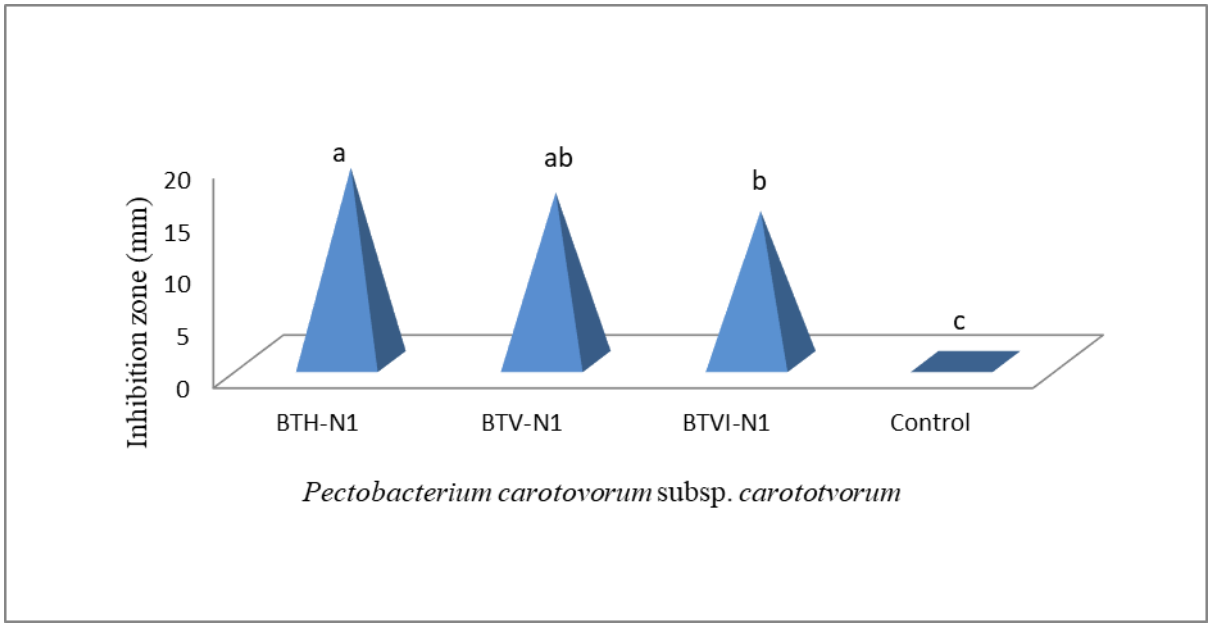

Fig. 1. Mean diameter of zones of inhibition produced by three *Trichoderma* spp. against *Pectobacterium carotovorum* ssp. *carotovorum* strain Pmu6 in an overlay method. Bars with different letters are significantly different based on Tukey's method at $P \leq 0.05$

In the pot experiment, gypsum (3g/3kg soil and 5g/3kg soil), *T. harzianum* BTH-N1 alone (T3), *T. harzianum* BTH-N1 + gypsum 3g/3kg soil, *T. harzianum* BTH-N1+ chitosan 3% significantly reduced the AUDPC of blackleg compared to the non-treated control (Figure 2). However, integrated application of *T. harzianum* BTH-N1 + chitosan 3% + gypsum 3g/3kg was non-significant in reducing AUDPC compared to control plants.

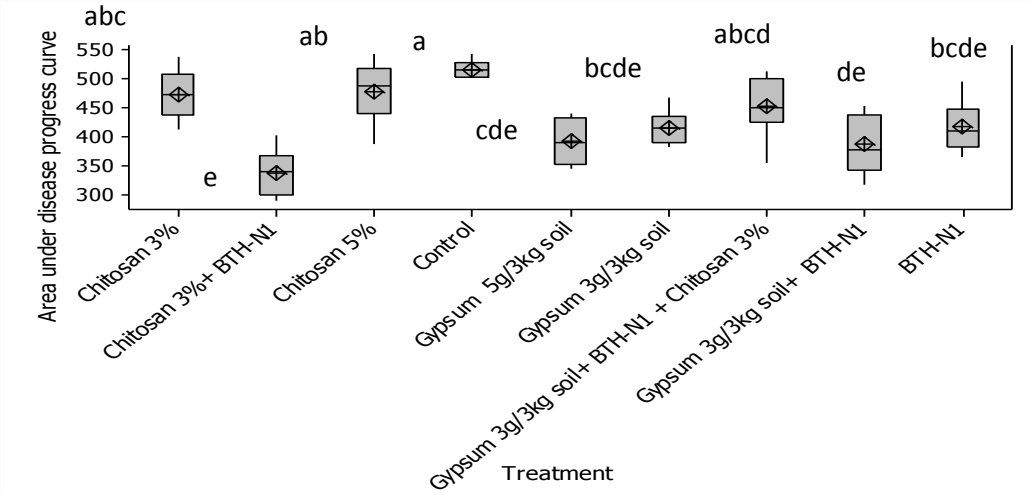

Fig. 2. Blackleg disease progression in seedlings of the potato variety BARI Alu 7 (Diamant), shown as area under the disease progress curve (AUDPC) values

In this study, *Trichoderma harzianum* strain BTH-N1 was used but little information is available about its mode of action. This isolate has been shown to reduce losses in cabbage seedbeds to soil borne fungal and oomycetes pathogens when applied with compost, but the mechanism of biocontrol was not determined (Nahar *et al.* 2012). Since a significant reduction in blackleg severity was demonstrated in plants inoculated with *P. carotovorum* ssp. *carotovorum* Pmu6 in stems that emerged from tubers previously inoculated with *T. harzianum* BTH-N1, it is possible and perhaps likely that induced resistance is also involved in the disease suppression observed. According to Lorito *et al.* (2010), *Trichoderma* enhances nutrient availability in soil and helps with nutrient uptake for the plants. It also increases plant respiration and thus increases photosynthetic activity. *Trichoderma* spp. were also found to be effective against bacterial species at various concentrations (Leelavathi *et al.* 2014). In this study no treatments containing chitosan reduced blackleg severity compared to the non-treated control while all the treatments containing gypsum alone or in combination with *T. harzianum* BTH-N1 reduced disease severity compared to non-amended control. This finding is supported by those of Berry *et al.* (1988), in which the severity of bacterial canker of tomato was negatively correlated with calcium at 100 ppm or higher dose in the nutrient solution.

Screening of BARI potato varieties to soft rot of potato: All ten potato varieties tested showed tuber soft rot damage 3 days after inoculation. There were statistically significant differences among the potato varieties in soft rot disease severity ($p<0.05$) (Table 1). Mean soft rot of potato tuber tissue ranged from 7.7% to 38.5%, BARI Alu 25 (7.7) had significantly lowest damage than BARI Alu 53, BARI Alu 63, BARI Alu 41 and BARI Alu 72. The percentage of tuber calcium ranged from 0.8%–1.2% in the tested ten potato varieties, and differences among varieties were significant ($p=0.001$) (Table 1). Eight varieties contained statistically similar calcium percentages ranging from 0.9–1.2%, while calcium content was lowest in BARI Alu 63 and BARI Alu 46 (0.8%). The relationship of percentage of rotted tissue and percentage of tuber calcium the correlation coefficient ($r=-0.506$), was statistically significant ($p<0.05$). Percentage tuber dry matter ranged from 15.0% (BARI Alu 62)–22.3% (BARI Alu 41), and differences among varieties were significant. BARI Alu 7, BARI Alu 63, BARI Alu 25, BARI Alu 8 and BARI Alu 72 were relatively high in dry matter percentage, whereas the BARI Alu 53, BARI Alu 46 and BARI Alu 73 were relatively low in dry matter percentage. However, the correlation coefficient ($r=0.278$) for the relationship of rotted tissue and dry matter was not statistically significant ($p=0.137$). The regression model considering both calcium and dry mater percentage was significant ($p<0.05$) at 5% significance level.

The regression model is: Rotted tissue (%) = 22.7 + 0.819 Dry matter (%)-23.6 Ca%

Table 1. Percent rotted tissue of Bangladesh Agricultural Research Institute (BARI)- released potato varieties after inoculation with *Pectobacterium carotovorum* ssp. *carotovorum* strain Pki2, and the percentages of calcium and dry matter for each variety

Variety	Percent rotted tissue	Percent calcium	Percent dry matter
BARI Alu 63	21.3 bc	0.8 c	21.2 ab
BARI Alu 53	18.4 bcd	1.0 abc	18.7 bc
BARI Alu 08	10.1 de	1.2 a	20.7 ab
BARI Alu 62	11.9 cde	1.0 abc	15.0 d
BARI Alu 25	7.7 e	1.1 ab	20.9 ab
BARI Alu 41	26.5 b	1.0 abc	22.3 a
BARI Alu 72	38.5 a	0.9 abc	20.4 ab
BARI Alu 46	12.6 cde	0.8 bc	17.3 cd
BARI Alu 73	13.9 cde	1.2 a	16.9 cd
BARI Alu 07	10.2 de	0.9 abc	21.5 a
p value	<0.05	<0.05	<0.05

We observed variation in the susceptibility of tubers of ten different varieties of potatoes released from the BARI breeding program. This variation in susceptibility is supported by the previous work of Reeves *et al.* (1999) who reported that different level soft rot incidence and weight reduction in potato tubers when they were inoculated with soft rot pathogens. In our study, no varieties were fully resistant to soft rot. Naturally soft rot-immune potato varieties are not known; rather some partially resistant potato cultivars are available (Lyon 1975). In this study, a significant negative correlation was found between tuber susceptibility and calcium percentage in tubers. This observation is supported by those of Pagel and Heitefuss (1989), who indicated that there is a consistent relationship between calcium content of tissues and soft rot susceptibility of potato cultivars. Dry matter percentage of potato tubers may be a source of variability in the evaluation process of potato varieties for resistance to soft rot (Tzeng *et al.* 1990). Biehn *et al.* (1972) reported that potato varieties with a relatively high amount of dry matter were generally less susceptible to soft rot than those with lower dry weights. A multiple regression analysis was generated including both percentage of calcium conc. and percentage of dry matter. Here we found that percent calcium conc. has negative significant association whereas percent dry matter has positive non-significant association with percent rotted tissue. This study was conducted twice in one season with a limited number of potato varieties. Nevertheless, as there is no previous information regarding the resistance of BARI potato varieties against soft rot of tubers, the information will be useful to potato growers in Bangladesh.

CONCLUSION

For the management of blackleg disease of potato, single application of chitosan, gypsum, *Trichoderma* and their combined treatments were tested in laboratory and in pot experiment. From the study, it was revealed that single application of *harzianum* BTH-N1 isolate, gypsum (3g/3kg soil 5g/3kg soil) and integrated application of *T. harzianum* BTH-N1+ gypsum 3g/3kg soil and *T. harzianum* BTH-N1+ chitosan 3% reduced AUDPC of potato blackleg. Ten BARI released potato varieties were screened to find out resistant source to soft rot disease. BARI alu 25 had the lowest tissue damage due to soft rot disease.

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REFERENCES

Abd-El-Khair H, Karima HEH (2007) Application of some bactericides and bioagents for controlling the soft rot disease in potato. *Res. J. Agril. and Biol. Sc*. 3, 463-473.

Arunachalam MK, Sharma P (2012) Confrontation assay for *Trichoderma* as a potential biocontrol agent against *Pythium aphanidermatum* and *Sclerotinia sclerotiorum. Pest Management in Horticultural Ecosystem* 18, 74-77.

Bain R, Millard P, Perombelon M (1996) The resistance of potato plants to *Erwinia carotovora* ssp. *atroseptica* in relation to their calcium and magnesium content. *Potato Res.* 39,185–193.

Berry SZ, Madumadu GG, Uddin RM (1988) Effect of calcium and nitrogen nutrition on bacterial canker disease of tomato. *Plant and Soil*. 112,113–20.

Biehn WL, Sands DC, Hankin L (1972) Relationship between percent dry matter content of potato tubers and susceptibility to bacterial soft rot. *Phytopathology* 62, 747 (Abstract).

Colyer PD, Mount MS (1984) Bacterization of potatoes with *Pseudomonas putida* and its influence on postharvest soft rot diseases. *Plant Dis*. 68,703-706.

Czajkowski R, Perombelon MCM, van Veen JA, van der Wolf JM (2012) Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. *Plant Pathol.* 60, 999-1013.

Dunn L, Rost C (1945) Effect of fertilizers on the quality of potatoes grown in the Red River Valley of Minnesota. *Am. J. Potato Res*. 22, 173–187.

Elahi F (2018) Identification of the Causal Agent of Bacterial Soft Rot of Potato and its Management in Bangladesh (Doctoral dissertation, The Ohio State University).

Habib A, Abdulnour J, Donnelly DJ (2004) *Potato Res*. 47:139. https://doi-org.proxy.lib.ohio-state.edu/10.1007/BF02735980.

Hicks E, Bienkowski D, Braithwaite M, Mc Lean K, Falloon R, Stewart L (2014) *Trichoderma* strains suppress Rhizoctonia diseases and promote growth of potato. *Phytopathologia Mediterranea* 53, 502-514.

Leelavathi MS, Vani L, Reena Pascal (2014) Antimicrobial activity of *Trichoderma harzianum* against bacteria and fungi. *Intl. J. Cur. Micro. and Appl. Sc*. 3, 96-103.

Lorito M, Woo SL, Harman GE, Monte E (2010) Translational research on *Trichoderma*: from 'omics to the field. *Annu. Rev. Phytopathol*. 48, 395–417.

Lyon GD, Lund BM, Bayliss CE, Wyatt GM (1975) Resistance of potato tubers to *Erwinia carotovora* and formation of rishitin and phytuberin in infected tissue. Physiol. *Plant Pathol*. 6, 43-50.

Mackay JM, Shipton PJ (1983) Heat treatment of seed tubers for control of potato blackleg *(Erwinia carotovora* ssp. *atroseptica*) and other diseases. *Plant Pathol*. 32, 385-393.

Madden LV, Hughes G, van den Bosch F (2007) The study of plant disease epidemics. St. Paul: *American Phytopathological Society*.

Makhlouf AH, Abdeen R (2014) Investigation on the effect of chemical and biological control of bacterial soft rot disease of potato in storage. *J. Biol. Agri. and Healthcare*. 4, 31-44.

McGuire RG, Kelman A (1984) Reduced severity of *Erwinia* soft rot in potato tubers with increased calcium content (Doctoral dissertation, University of Wisconsin Madison).

Nahar MS, Rahman MA, Kibria MG, Rezaul Karim ANM, Miller SA (2012) Use of Trichocompost and Tricho-leachate for management of soil-borne pathogens and production of healthy cabbage seedlings. *Bangladesh J. Agril. Res*. 37, 653-664.

O'Herlihy EA, Duffy EM, Cassells AC (2003) The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from microplants. *Folia Geobotanica* 38, 201-207.

Pagel W, Heitefuss R (1989) Calcium content and cell wall polygalacturonans in potato tubers of cultivars with different susceptibilities to *Erwinia carotovora* ssp. *atroseptica*. *Physiol. Mol. Plant Path*. 35, 11-21.

Pasco C, Bozec M, Ellisseche D, Andrivon D (2006) Resistance behavior of potato cultivars and advanced breeding clones to tuber soft rot caused by *Pectobacterium atrosepticum*. *Potato Res*. 49, 91-98.

Perombelon MCM (2002) Potato diseases caused by soft rot *Erwinias*: an overview of pathogenesis. *Plant Pathol*. 51, 1–12.

Perombelon MCM, Gullings-Handley J, Kelman A (1979) Population dynamics *of Erwinia carotovara* and pectolytic *Clostridia* in relation to decay of potatoes. *Phytopathology* 69, 167-173.

Rasul MG, Islam MS, Sheikh MHR (1999) Storability of different potato varieties under natural condition. *Bang. J. Sc. and ind. Res*. 34, 86-90.

Reeves AF, Olanya OM, Hunter JH, Wells JM (1999) Evaluation of potato varieties and selections for resistance to bacterial soft rot. *Am. J. Potato Res*. 76, 183-189.

Reiter B, Pfeifer U, Schwab H, Sessitsch A (2002) Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* ssp. *atroseptica. Appl. and Environ. Microbiol.* 2261-2268.

Robinson K, Foster G (1987) Control of potato blackleg by tuber pasteurisation: the determination of time-temperature combinations for the inactivation of pectolytic *erwinia. Potato Res*. 30, 121–125.

Srivastava M, Kumar B, Shahid M, Pandey S, Singh A (2016) *Trichoderma*- a potential and effective bio fungicide and alternative source against notable phytopathogens: *A review. Afr. J. Aca. Res*. 11, 310-316.

Subedi N (2015) Characterization and management of *Ralstonia solanacearum* populations in South Asia (Doctoral dissertation, The Ohio State University).

Thomas RL, Sheard RW, Moyer JR (1967) Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. *Agronomy Journal* 59, 240-243.

Tzeng KC, McGuire R, Kelman A (1990) Resistance of tubers from different potato cultivars to soft rot caused by *Erwinia carotovora* ssp. *atroseptica*. *Am. J. Potato Res*. 67, 287–305.

van der Wolf JM, De Boer SH (2007) Bacterial pathogens of potato. In: Vreugdenhil D, ed. Potato Biology and Biotechnology: Advances and Perspectives. Delft, the Netherlands: *Elsevier*. 595-617.

Wright PJ, Triggs CM, Anderson JAD (2005) Effects of specific gravity and cultivar on susceptibility of potato (*Solanum tuberosum*) tubers to blackspot bruising and bacterial soft rot. *New Zeal. J. Crop and Hort*. 33, 353-361.