INFLUENCE OF ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF POTATO BLACKLEG DISEASE

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Abstract. Climatic conditions play a crucial part regarding the development of blackleg caused by *Pectobacterium atrocepticum (Pa)*, which is one of the major threats the potato industry faced. These factors affect the stage of growth, host susceptibility, succulence, vigor, survival, rate of multiplication, direction of pathogen dispersion, rate of spore penetration and germination. In the present experiment, effect of these environmental variables like maximum and minimum temperature (max and min T), relative humidity (RH), rainfall (RF), and wind speed (WS) on disease development were studied. Significant positive correlation was observed for all twenty-five varieties and a multiple regression model ($Y = + 24.382 + 0.3592X_1 + .0970X_2 - 0.2551X_3 + 1.982X_{4)}$ based on the two-year study was developed to find out the relationship between environmental parameters and disease projection. Goodness of model was indicated by coefficient determination value (97.5%). Correlation of these environmental factors on the development of disease on twenty-five potato varieties during 2018, 2019 was done. Significant positive correlation was observed on all twenty-five varieties. In conclusion, it was established that environmental factors, including max. T (28 °C), min. T (15 °C), RH 70%, and WS 3.5 Km/h were conducive for the development of blackleg. Current study would be helpful for researchers in designing better disease management strategies under changing climatic conditions in the future.
Keywords: *Pectobacterium atrocepticum, blackleg, environmental variables, regression model, correlation*

Introduction

Potato (*Solanum tuberosum*) belongs to the family of *Solanaceae*. It is a perishable food crop. It is a tuber bearing plant, that is cultivated worldwide and ranked 4th after maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*). In temperate regions, it is a major field crop (Kroschel et al., 2020). Potato tubers are not only used as a seed but also used as a food source for market and processed products. In developing countries, potato crop is a major source of employment and income (Horten et al., 2019). It has become the staple food of Irish people (De Jong, 2016). It contains water (70-80%) and organic matter (20%) (Nyawade et al., 2018). It has dietary materials (22%), food energy (325 kcal), protein (7.6 g), fat (0.04 g), carbohydrates (72.8 g), calcium (42 mg), phosphorus (213 mg), iron (2.7 mg), vitamin A (70 IU), riboflavin (0.15 mg), niacin (4.4 mg), ascorbic acid (64 mg) and also rich in antioxidants (Tunio et al., 2020). Potato crop is facing several biotic and abiotic constraints, in which blackleg caused by *Pectobacterium atrosepticum (Pa)* is a major threat among all (Rivedal et al., 2021). In Pakistan it was firstly reported in 1984 from swat valley. In India, losses due to this disease recorded up to 45% (Raza et al., 2021). When disease incidence is above 5-10%, Pakistan economy damaged due to yield reduction and potato degradation (Sharma et al., 2017). Characteristic symptom of this

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disease is a slimy, wet, black rot lesion appearing on tuber and spreading from infected tuber up to stem (Elhalag et al., 2020). Maceration occurs due to enzymes (pectinase, protease and cellulose) released by bacteria. Then the stem turns into inky black color and becomes soft. Above ground parts of plant drop down because plant becomes infected from the soil. Shoots become stunted and stem turns into black color at the bottom and hence it is called blackleg (Aboshama et al., 2019) (*Fig. 1*).

Figure 1. *Characteristic symptoms of black leg disease of potato*

Epidemiology of any disease is necessary to be studied to gain knowledge about all environmental factors which directly or indirectly affect the development of epidemic. Environmental factors have a critical role in blackleg disease development (Skelsey et al., 2018). Temperature is a key factor in this disease, and cause rotting of tubers. *Pa* is more vigorous at temperature lower than <25 °C (Kaczynska et al., 2019). In rotting of mother tubers, if more than one pathogen species is present, it will depend on temperature that which pathogen will predominate. A species gains maximum growth rate at its ideal temperature. Moisture is also an important factor for tuber rotting.

There is dire need to study these epidemiological factors and understand the characterization of these factors for disease management. Present study will be designed to assess the epidemiological factors like max and min temperature, rainfall, wind speed, relative humidity etc.) and their impact on disease development. By following this information, researchers develop disease predictive model to minimize the losses and to guide the farmers that how they can tackle these situations and get maximum yield.

Material and methods

Data collection

A comprehensive study was designed for two years to collect disease incidence data from the experimental area of Department of Plant Pathology, University of Agriculture Faisalabad. For pathogenicity, three weeks after stem emergence, 100 uL of bacterial inoculum at 10^8 CFU/mL was injected in the collar region of the stem. Five genotypes (Harmony, Lady rosetta, Faisalabad white, Accent and Desiree) were tested for the assessment of different environmental factors playing role in disease development. All

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were susceptible against the black leg disease. Environmental factors data including max and min temperatures (°C), wind speed (Km/h), rainfall (mm) and relative humidity (%) was obtained from meteorological station located at the Agronomy research area, UAF for the whole duration of crop (from sowing to harvesting) and their conduciveness with the disease development was checked by regression and correlation analysis and a predictive model was developed based upon the results.

Regression analysis

For determining the relationship b/w the climatic/environmental factors and disease development/incidence, regression analysis was used. There are actually two types of regression models that were used, first one is a simple regression model, and the other is multiple regression model (*Eqs. 1* and *2*). Regarding the development of simple linear regression models, Y act as response variable in case the of disease while X will be worked as explanatory variable which represent the environmental/climatic factors. The equation for simple linear regression is:

$$Y = \beta 0 + \beta 1X \qquad \text{(Eq.1)}$$

where β0 denotes as intercept and β1 is the slope.

In the case of multiple linear regression models, more than one explanatory or predictor variables (X) are included as compared to the simple linear regression analysis. The equation of multiple regression model with relationship of variables is described by:

$$Y = \beta 0 + \beta 1x1 + \beta 2x2 + \cdots + \beta i\ xi + \in \qquad \text{(Eq.2)}$$

Here, x characterizes as the compilation of predictors x1, x2... xi in the model, and β1, β2...βi act for the corresponding regression coefficients and ∈ is the random error or interruption in the experiment.

Characterization of environmental factors conducive for black leg of potato

All environmental data as well as disease incidence (%), and alterations in the environmental factors and disease incidence observed on potato cultivars, were checked by (LSD at $P < 0.05$) least significance difference test. The effect of environmental factors on blackleg disease was studied by correlation. Mean square error (MSE) Mallows Cp and R2 were criteria used for selecting the best models (Gottwald and Irey, 2007).

Goodness of model

Correlation was used for determining goodness of the fit model (Steel and Torrie, 1997) and varieties/cultivars in which, more than 50% of the environmental variables exert significant effects which were plotted and the most conducive environmental factors for development of blackleg disease was determined with the help of regression analysis. The manipulation of these factors on the establishment of blackleg was tested by drawing a comparison in between the recorded and predicted values of disease incidence with the help of multiple regression models. Furthermore, disease predictive model depending on environmental conditions was developed which has significant influence on blackleg disease development.

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Results

Development and evaluation of blackleg predictive model based on two years data

Regression analysis expressed that relative humidity exhibited statistically non-significant response. So, it is removed from the model. The multiple regression model based on data of two years: $Y = 24.382 + 0.3592X_1 + .0970X_2 - 0.2551X_3 + 1.982X_4$ (X_1 = Max.T, X_2 = Mini.T, X_3 = Rainfall X_4 = Wind speed) explained 97% variability. The normal probability plot showed (*Fig. 2*) that most points were present on the reference line at different points. In the residual verses fitted value, all the points were distributed within range -1 to +1. Only four points were present along the reference line.

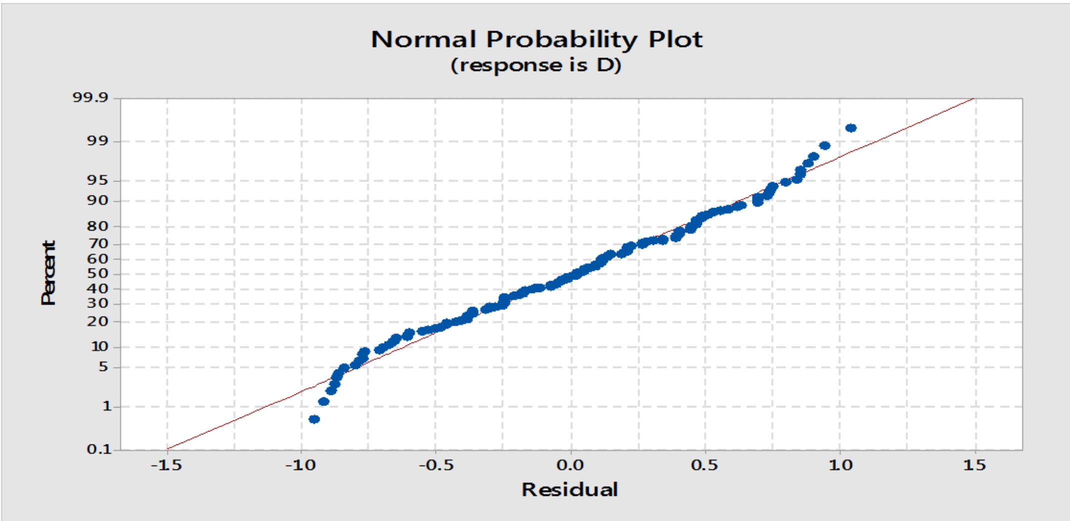

Figure 2. *Normal probability plot for the model of blackleg disease of potato based on data of two years*

Assessment of disease predictive model by comparing the dependent variables with regression coefficient through physical theory

Disease predictive model expressed $R^2 = 0.97$ coefficient of determination which showed that the model is good for disease prediction with low standard error. Temperature (Max. and min.) rainfall (mm) and wind speed (km/h) have significant contribution in the development of blackleg disease of potato (*Table 1*).

Table 1. *Regression model's coefficients of variables, standard error, t stat and P-value for blackleg*

Parameters	Coefficients	Standard error	t Stat	*P*-value
Intercept	24.382	0.456	53.41	0.000*
Max.T (°C)	0.3592	0.0255	14.06	0.000*
Mini. Temp.	0.0970	0.0461	2.10	0.037*
Rainfall	0.2551	0.0652	-3.92	0.000*
Wind speed	1.982	0.115	17.30	0.000*

*Significant at $P < 0.05$

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Estimation of model on the basis of predicted and observed values

For assessing the reliability of model value differences of observed and predicted data points were estimated. Among the observed values fourteen data-points were beyond reference line (standard error = 0.485174) and created an error in the experiment. According to the graphs, maximum prediction values having differences (less than 5) were consolidated between 95% interval of confidence (C.I) and 95% predictive interval (P.I) showed that there was a good fit in between predictive and observed values (*Fig. 3*).

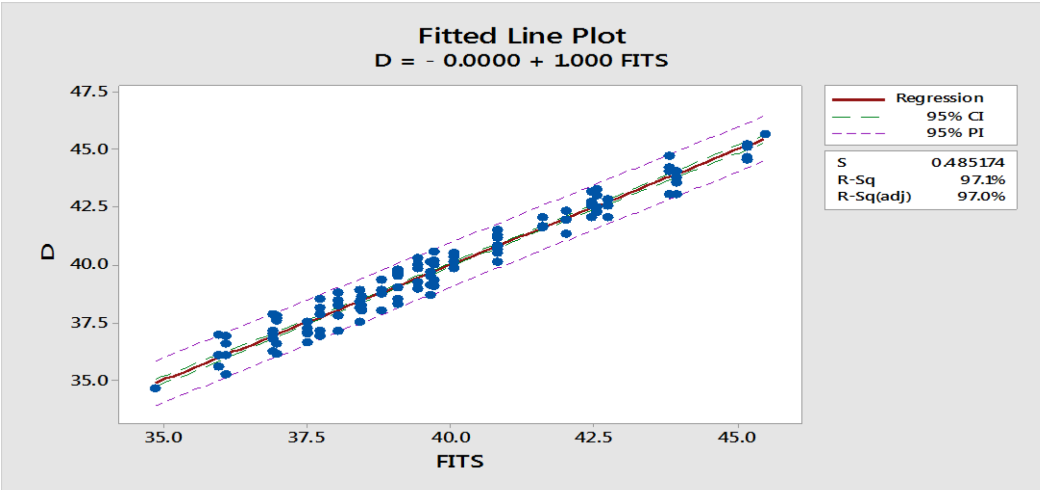

Figure 3. *A fitted line plot for blackleg with observed and predicted data points at 95% confidence and predictive interval*

Correlation of environmental factors for the development of blackleg on various varieties during 2018 and 2019

The environmental dependent variables i.e. maximum temperature (°C), minimum temperature (°C), relative humidity (R.H %), rain fall (mm) and wind speed (Km/h) expressed significant positive correlations with all varieties Cardinal, Vales Everest, Paramount, Atlantic, Orla, Melody, Patrones, Harmony, Multa, SH70, Desiree, Hermes, Diamant, Raja symphonia, Ajax, Fontane, Lal-e-Faisal, Ultimas, FSD white, FSD red, Sante, Lady Rosetta, Agria, Accent and Prima during 2018 (*Table 2*) and similar results were obtained during the year of 2019 (*Table 3*).

Discussion

Numerous environmental factors influenced the successful growth of plants (Li et al., 2020). Environmental factors viz. maximum air temperature, minimum air temperature (°C), moisture (%), relative humidity (%), rainfall (mm) and wind speed (km/h) are the imperative sources that predispose the plants for the development of infection (Saharan et al., 2016). Abrupt changes in the climatic conditions can impose an effect on plant diseases (Serdeczny et al., 2017). Environmental factors play an influential role in the resistance and susceptibility of the host plant. They can also alter rate of pathogen growth, reproduction, dissemination, infection, survival, and host pathogen interaction. So, in present study impact of environmental factors on the development of disease, was observed.

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Table 2. *Correlation of environmental factors with blackleg disease on different varieties of potato for 2018*

Sr#	Variety name	Max. T (°C)	Min. T (°C)	RH (%)	RF (mm)	WS (km/h)
1	Cardinal	0.960 0.000**	0.849 0.000**	0.894 0.000**	0.602 0.023*	0.960 0.000**
2	Vales Everest	0.966 0.000**	0.913 0.000**	0.922 0.000**	0.703 0.005*	0.983 0.000**
3	Paramount	0.967 0.000**	0.887 0.000**	0.913 0.000**	0.656 0.011*	0.986 0.000**
4	Atlantic	0.956 0.000**	0.827 0.000**	0.918 0.000**	0.555 0.039*	0.955 0.000**
5	Orla	0.970 0.000**	0.889 0.000**	0.923 0.000**	0.663 0.010*	0.980 0.000**
6	Melody	0.964 0.000**	0.820 0.000**	0.944 0.000**	0.535 0.048*	0.951 0.000**
7	Patrones	0.980 0.000**	0.933 0.000**	0.930 0.000**	0.737 0.003*	0.993 0.000**
8	Harmony	0.987 0.000**	0.899 0.000**	0.918 0.000**	0.671 0.009*	0.985 0.000**
9	Multa	0.951 0.000**	0.859 0.000**	0.897 0.000**	0.609 0.021*	0.967 0.000**
10	SH70	0.965 0.000**	0.865 0.000**	0.939 0.000**	0.628 0.016*	0.978 0.000**
11	Desiree	0.955 0.000**	0.795 0.001*	0.925 0.000**	0.548 0.042*	0.940 0.000**
12	Hermes	0.942 0.000**	0.842 0.000**	0.886 0.000**	0.586 0.028*	0.952 0.000**
13	Diamant	0.977 0.000**	0.912 0.000**	0.912 0.000**	0.693 0.006*	0.989 0.000**
14	Raja symphonia	0.966 0.000**	0.804 0.001*	0.931 0.000**	0.527 0.053*	0.950 0.000**
15	Ajax	0.968 0.000**	0.848 0.000**	0.934 0.000**	0.614 0.019*	0.961 0.000**
16	Fontane	0.961 0.000**	0.825 0.000**	0.920 0.000**	0.561 0.037*	0.561 0.037*
17	Lal-e-faisal	0.960 0.000**	0.843 0.000**	0.902 0.000**	0.587 0.027*	0.970 0.000**
18	Ultimas	0.960 0.000**	0.849 0.000**	0.894 0.000**	0.602 0.023*	0.960 0.000**
19	FSD white	0.966 0.000**	0.913 0.000**	0.922 0.000**	0.703 0.005*	0.983 0.000**
20	FSD red	0.967 0.000**	0.887 0.000**	0.913 0.000**	0.656 0.011*	0.986 0.000**
21	Sante	0.958 0.000**	0.868 0.000**	0.902 0.000**	0.652 0.02*	0.962 0.000**
22	Lady Rosetta	0.955 0.000**	0.895 0.000**	0.897 0.000**	0.672 0.009*	0.973 0.000**
23	Agria	0.991 0.000**	0.906 0.000**	0.938 0.000**	0.678 0.008*	0.990 0.000**
24	Accent	0.982 0.000**	0.860 0.000**	0.932 0.000**	0.608 0.021*	0.980 0.000**
25	Prima	0.970 0.000**	0.872 0.000**	0.908 0.000**	0.638 0.014*	0.983 0.000**

Upper values indicated Pearson’s correlation coefficient. Lower values indicated level of significance at 5% probability. Ns = non-significant; *Significant ($P < 0.05$); **Highly significant

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Table 3. *Correlation of environmental factors with blackleg disease on different varieties of potato for 2019*

Sr#	Variety name	Max. T (°C)	Min. T (°C)	RH (%)	RF (mm)	WS (Km/h)
1	Cardinal	0.837 0.000**	0.965 0.000**	0.951 0.000**	0.688 0.007	0.954 0.000**
2	Vales Everest	0.911 0.000**	0.963 0.000**	0.980 0.000**	0.780 0.001	0.990 0.000**
3	Paramount	0.885 0.000**	0.961 0.000**	0.983 0.000**	0.764 0.001*	0.989 0.000**
4	Atlantic	0.820 0.000**	0.952 0.000**	0.948 0.000**	0.655 0.011	0.954 0.000**
5	Orla	0.878 0.000**	0.977 0.000**	0.967 0.000**	0.745 0.002	0.000 0.979
6	Melody	0.891 0.000**	0.977 0.000**	0.964 0.000**	0.765 0.001	0.970 0.000**
7	Patrones	0.866 0.000**	0.969 0.000**	0.970 0.000**	0.705 0.005	0.964 0.000**
8	Harmony	0.870 0.000**	0.975 0.000**	0.972 0.000**	0.706 0.005	0.972 0.000**
9	Multa	0.836 0.000**	0.944 0.000**	0.955 0.000**	0.674 0.008	0.958 0.000**
10	SH70	0.931 0.000**	0.955 0.000**	0.993 0.000**	0.784 0.001	0.986 0.000**
11	Desiree	0.875 0.000**	0.982 0.000**	0.975 0.000**	0.733 0.003	0.974 0.000**
12	Hermes	0.900 0.000**	0.966 0.000**	0.972 0.000**	0.786 0.001	0.970 0.000**
13	Diamant	0.879 0.000**	0.959 0.000**	0.973 0.000**	0.752 0.002	0.982 0.000**
14	Raja symphonia	0.889 0.000**	0.978 0.000**	0.982 0.000**	0.744 0.002	0.981 0.000**
15	Ajax	0.842 0.000**	0.974 0.000**	0.966 0.000**	0.699 0.005	0.961 0.000**
16	Fontane	0.856 0.000**	0.982 0.000**	0.947 0.000**	0.711 0.004	0.950 0.000**
17	Lal-e-Faisal	0.900 0.000**	0.963 0.000**	0.978 0.000**	0.776 0.001	0.982 0.000**
18	Ultimas	0.933 0.000**	0.964 0.000**	0.981 0.000**	0.807 0.000**	0.991 0.000**
19	FSD white	0.898 0.000**	0.990 0.000**	0.972 0.000**	0.748 0.002	0.974 0.000**
20	FSD red	0.874 0.000**	0.970 0.000**	0.981 0.000**	0.728 0.003	0.980 0.000**
21	Sante	0.854 0.000**	0.956 0.000**	0.956 0.000**	0.723 0.004	0.955 0.000**
22	Lady Rosetta	0.829 0.000**	0.970 0.000**	0.953 0.000**	0.679 0.008	0.946 0.000**
23	Agria	0.820 0.000**	0.952 0.000**	0.948 0.000**	0.655 0.011	0.954 0.000**
24	Accent	0.878 0.000**	0.977 0.000**	0.967 0.000**	0.745 0.002	0.979 0.000**
25	Prima	0.909 0.000**	0.971 0.000**	0.983 0.000**	0.740 0.002	0.972 0.000**

Upper values indicated Pearson’s correlation coefficient. Lower values indicated level of significance at 5% probability. Ns = Non-significant; *Significant ($P < 0.05$); **Highly significant

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Present study was designed to check the collective effect of environmental factors including temperature, rain fall, relative humidity and wind speed which showed positive correlation with the blackleg disease incidence. The results of present study are hand in line with the findings of Agrios (2005) and it was concluded that various environmental factors have great impact on blackleg disease development, maximum and minimum temperature is the most serious factor in the disease development. Favorable conditions for blackleg development included cool and wet conditions (Charkowsky et al., 2020). In this current study, maximum number of cultivars showed positive correlation with the blackleg disease development and collectively all environmental factors including maximum, minimum temperature, wind speed, rain fall and relative humidity. The highest disease incidence was observed at 9-15 °C minimum temperature, 20-28 °C maximum temperature, 65-70% relative humidity, 3.5 km h^{-1} wind speed. The results of this study are supported by the findings of Quiroz et al. (2018) who concluded that temperature within 15-22 °C with high air moisture are the most crucial for blackleg disease development. Dispersal of disease significantly increased by warm and wet climatic conditions. In the favorable atmospheric conditions, the entire potato crop may be destroyed within short time periods. Ideal conditions for disease spread are high relative humidity of more than 75% and temperature above 10 °C. Rainfall had great impact in infecting young tubers. The potato crop is significantly affected in warm temperature (15-20 °C) and high humidity (80-100%) (Rykaczewska, 2017).

Conclusion

All the environmental factors (Max. T, Min. T, RH, WS and RF) have significant positive correlation on all varieties of potato for two years. Due to sudden fluctuations in the climatic conditions, continuous monitoring of environmental variables is necessary for accurate prediction of blackleg and its management. Installation of weather stations in the major potato growing areas would be helpful in risk assessment and forecasting systems in a specific area. On the basis of data collected from different areas, a Decian Support System can be developed for precise management of disease.

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