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A Logistic Regression Model for Predicting *Sclerotinia* Stem Rot in Egyptian Clover (*Trifolium alexandrinum* L.)

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ABSTRACT

Background: Stem rot caused by *Sclerotinia trifoliorum* is the most damaging disease of Egyptian clover (popularly called as berseem), which is widely grown as a leguminous, winter season fodder crop in India. Stem rot management currently relies on fungicides which have negative effect on livestock and environmental health. In this study, in order to rationalize the fungicide use for stem rot management, a prediction model which assesses the high risk of stem rot (>20% incidence) was developed.

Methods: The disease and weather data was collected from week-50 (second week of December) to week-14 (first week of April) during 2010-11 to 2019-20. The model was developed using logistic regression modeling approach.

Result: The model included increasing weekly average temperature (between 8-25°C) and wind speed (between 1-7 km/hr) as key predictor variables. Goodness of fit statistics such as number of concordant pairs (83.8%), discordant pairs (16.1%), Somers' D (0.68), Gamma (0.68) and Tau-a (0.33) indicates high accuracy of the model. The model had high area under receiver operating characteristic curve value of 0.84 during development and 0.82 on cross validation indicating that it will perform fairly well on an independent dataset. Thus, these macro climatic-weather variables can be used to predict high risk (>20% incidence) of stem rot, which ultimately will rationalize use of fungicides for stem rot management. This is the first model to predict *Sclerotinia* stem rot in Egyptian clover based on weather variables in India and probably around the world.

Key words: Clover rot, Egyptian clover, Prediction model, *Sclerotinia,* Weather.

INTRODUCTION

Egyptian clover (*Trifolium alexandrinum* L.) (commonly called as berseem clover), is the most popular leguminous fodder crop among livestock farmers of India. It is cultivated as an annual crop during winters in various countries including India, Pakistan, Turkey, Egypt, eastern Australia, South Africa, southern Europe and the southeast USA (Frame, 2005). India ranks first in area under Egyptian clover cultivation (around 2 million ha) followed by Egypt (1.1 million ha) and Pakistan (0.71 million ha) (Muhammad *et al*., 2014). Egyptian clover gives an average green fodder yield of 80-90 t/ha (Yadav *et al*., 2015) whereas, a maximum potential yield of 100-150 t/ha could be achieved. This difference in the actual yield and potential yield is due to the adverse effect of various biotic and abiotic stresses. Among biotic stresses, stem rot caused by *Sclerotinia trifoliorum* Erikks. is the major constraint. Stem rot reduces crop establishment, forage quality and green fodder as well as seed yield. Incidence of stem rot can vary from 0-60% and average yield damage reported due to this disease is around 20-25% (Rathi *et al*., 2007). Plants affected by stem rot develop characteristic cottony mycelial growth and sclerotia are formed both inside the stem and on the plant surface. Affected plants are completely bleached and rotted in severe infections (Abawi and Grogan, 1979). Hence, management of stem rot is of utmost importance as this disease directly impairs the crop stand. Varieties with complete resistance to stem rot are lacking. Various breeding and biotechnological efforts have been made to develop disease tolerant genotypes through interspecific hybrids of *T. alexandrinum* with wild species such as *T. apertum* (Malaviya *et al*., 2004), *T. constantinopolitanum* (Roy *et al*., 2004), *T. resupinatum* (Kaushal *et al*., 2005), *T. lappaceum* (Malaviya *et al*., 2018). This has resulted in creating genetic variability and identifying lines for disease tolerance and other agronomic traits. However, still no immune variety for this disease has been identified till date. Other alternative measures involving fungicides for management of stem rot (Bhaskar *et al*., 2003; Pande *et al*., 2008; Iqbal and Iqbal, 2014) have been developed. But to reduce the yield loss, timely and need based application of fungicides is important. The use of fungicides can be more rationalized, if farmers have access to a timely and reliable disease-warning system (DeWolf *et al*., 2003; Paul and Munkvold, 2004). However, such a disease warning system for predicting the risk of stem rot in Egyptian clover is lacking. Among different plant disease prediction approaches, logistic regression which is

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a nonparametric method has been used for predicting several diseases in the past including head blight in wheat (De wolf *et al*., 2003), stem rot in soybean (Mila *et al*., 2004), gray leaf spot in maize (Paul and Munkvold, 2004), Stewart's wilt in maize (Esker *et al*., 2006), late blight of potato (Henderson *et al*., 2007) and white mold in dry bean (Harikrishnan and del Rio, 2008). In most of these models, key predictors are identified based on previous weather and disease data. Because there is a need to provide Egyptian clover growers with timely risk information about stem rot disease, the objective of this study was to develop a prediction model based on logistic regression using weather variables as predictors and stem rot incidence data collected through a 10-year study (2010-11 to 2019-20) as a response variable.

MATERIALS AND METHODS

Disease and weather data

Experiment was conducted during winter season at Ludhiana (30.9010° N, 75.8071° E), India during 2010-11 to 2019-20 as part of All India Coordinated Research Project (AICRP) on Forage Crops and Utilization. Ludhiana is characterized by humid subtropical climate and Egyptian clover growing season spans from first week of November (week-45) to first week of May (week-18). Egyptian clover cultivar BL-42 was grown in 4 x 4 m plots in four replications. This cultivar is of multicut nature and has high fodder as well as seed yield. This cultivar was released in 2005 and notified during 2007 (S.O. No. 1178 (E), dated 20.07.2007) for cultivation in whole of India and Punjab state in particular. Ten plants per replication were selected and stem rot disease incidence (percentage of plants infected per replication) was assessed at weekly interval from second week of December (week-50 in a year) till the disease presence for ten consecutive years (2010-11 to 2019-20). Total numbers of observations recorded were 132. Data regarding five weather variables *viz*, temperature (°C) [TEMP], relative humidity (%) [RH], rainfall (mm) [RF], sunshine (hours/day) [SNS] and wind speed (km/hr) [WS] was collected daily from meteorological observatory of PAU, Ludhiana and converted to weekly average value in each year of the study period. The weekly average value of each weather variable along with weekly stem rot incidence was further subjected to statistical analysis and used for development of prediction model. The average value of all the weather variables for each week in the disease observation period from 2010-11 to 2019-20 was also calculated in order to get an overall variation in weather variables.

Development of model

Correlation between stem rot incidence and weather variables

Kendall Tau-b non-parametric correlation coefficient (r_k) between stem rot incidence and weather variables (TEMP, RH, RF, SNS and WS) was estimated to assess the level of relationship between disease incidence and weather variables. Predictor variables were checked for multicollinearity by calculating the variance inflation factor and tolerance limit. Tolerance value of <0.1 and variance inflation factor of >10 was considered as an indicator of existence of multicollinearity among the predictor variables (Joshi *et al*., 2012).

Model fitting

Logistic regression (maximum likelihood method) was used for model fitting. Stem rot incidence (response variable) was binary coded as 0 (<20% incidence) and 1 (>20% incidence). Our event of interest was 1 (>20% incidence). The 20% cutoff was selected because field studies have shown that lower disease incidence does not affect the yield levels, however, as the incidence level goes beyond 20% corresponding effect on fodder as well as seed yield is quite significant (unpublished data). The whole stem rot incidence data along with weekly average values of significantly associated weather variables was used for model development using stepwise selection. Model selection was done based on goodness-of-fit criteria such as concordant and discordant pairs, Sommer's D, Gamma, Tau-a, odds ratio and Hosmer-Lemeshow test. The biological significance of the selected predictor variables was tested through area under receiver operating characteristics curve (AUC) indicating sensitivity and specificity of the model and χ^2 test which indicates significance of deviance between the predicted and observed stem rot incidence. Scatter plots with penalized B- spline curves were drawn between model incorporated weather variable on the *X-axis* and disease incidence on the *Y-axis* in order to find out effect of selected weather variables on the stem rot disease.

Model validation

Cross validation technique was used for model validation. In cross validation, the cross validated predicted probability for an observation is calculated by simulating the process of fitting the stem rot model ignoring that observation and then the model fit is used on the remaining observations in order to compute the predicted probability for the ignored observation. In this way, model was fitted to the complete data set and cross validated predicted probabilities were used to provide a receiver operating characteristic (ROC) curve analysis. The cross validated ROC statistics was compared with the original model in order to assess the accuracy of the model.

Data analysis

The analysis was done in SAS university edition software (SAS Institute, Cary, NC). Kendall Tau-b non-parametric correlation coefficient was calculated using PROC CORR procedure. Multicollinearity was calculated using TOL and VIF function of PROC REG procedure. Scatter plots were fitted using PROC SGSCATTER procedure. Analysis related to model fitting and validation was done using PROC LOGISTIC procedure.

RESULTS AND DISCUSSION

Stem rot incidence and weather conditions

Stem rot appeared as early as week-51 (third week of December) and it was recorded to occur as late as up to week-14 (first week of April). Maximum incidence observed was 64.8, 30, 43.9, 44.4, 38.4, 55.6, 61.4, 55.9, 55.0 and 65.3% respectively from 2010-11 to 2019-20 (Fig 1). More than 20% stem rot incidence was observed in all the years (Fig 2). During the disease observation period from 2010-11 to 2019-20, average weekly temperature ranged from 11.4 to 22.8°C, average weekly relative humidity from 59.0 to 80.1%, average weekly rainfall from 0.0 to 28.3 mm, average weekly sunshine hours from 3.1 to 10.1 and average weekly wind speed from 2.7 to 4.2 km/hr (Fig 3). Kendall tau-b correlation coefficient (r_k) between stem rot incidence and weather variables shows that weekly average temperature (TEMP) had strong positive correlation (r_k = 0.48) with stem rot. Correlation of all other variables with stem rot incidence was low and positive [RF (r_k = 0.13); SNS (r_k = 0.31); WS (r_k = 0.20)] except RH which had low negative correlation (r_k = -0.23) (Table 1). Multicollinearity was lacking among predictor variables as highest variance inflation factor value was 3.49 and lowest tolerance value was 0.29 (Table 1). High incidence (> 20%) of stem rot in all the years was probably due to the fact that weather conditions were quite congenial for disease development. Temperature, wind speed and sunshine hours increased as the clover growing season progressed and had positive correlation with the disease, while relative humidity decreased along the season and had negative correlation with stem rot. Rainfall was not uniform and hence had lowest correlation with the disease. Stem rot incidence was low during early observation period (week-50 onwards), however, it progresses gradually. The peak level varied during different years due to variation in weather conditions. Earlier reports in a different clover species (red clover) also suggest that depending on the prevailing weather conditions, stem rot can be almost absent or can create havoc by destroying entire fields. Temperature is considered as the most important weather variable which affects the stem rot development. The most favorable condition for disease spread is a humid autumn facilitating ascospore germination followed by a warm humid winter with intermittent periods of frost. Extremely cold and dry winters restrict the mycelial growth and thereby preventing disease spread (Dillon *et al*., 1946; Marum *et al*., 1994). It was also reported that since incidence of stem rot is dependent on weather conditions, the magnitude of yield loss can vary from year to year (Loveless, 1951; Dijkstra, 1964).

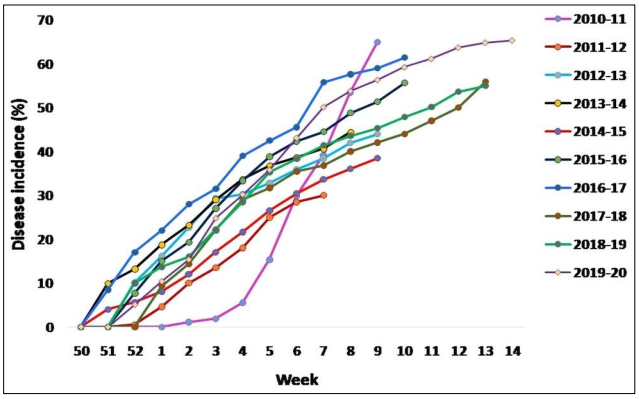


Fig 1: Disease incidence (%) of stem rot in Egyptian clover during the years 2010-11 to 2019-20.



Fig 2: Egyptian clover severely infected with stem rot disease (a) and close-up view of infected plants showing severe rotting and death of plants (b).

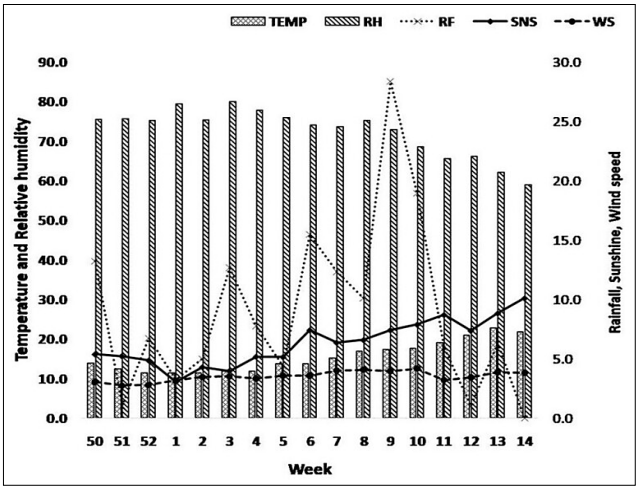


Fig 3: Average values of five weather variables (temperature, relative humidity, rainfall, sunshine hours and wind speed) for ten years in a particular week during disease observation period from 2010-11 to 2019-20.

Table 1: Kendall Tau-b correlation coefficient (r_k) among weather variables and stem rot incidence in Egyptian clover along with multicollinearity diagnostics values of weather variables.

Variable	TEMP	RH	RF	SNS	WS
RH	-0.34 (*P*<.0001)	-	-	-	-
RF	0.06 (NS)	0.26 (*P*<.0001)	-	-	-
SNS	0.36 (*P*<.0001)	-0.52 (*P*<.0001)	-0.19 (*P*=0.003)	-	-
WS	0.13 (*P*=0.02)	0.18 (*P*=0.002)	0.28 (*P*<.0001)	-0.06 (NS)	-
DI (n=132)	0.48 (*P*<.0001)	-0.23 (*P*=0.0001)	0.13 (*P*=0.04)	0.31 (*P*<.0001)	0.20 (*P*=0.0009)
*VIF	3.49	3.24	1.11	2.23	1.43
#Tolerance	0.29	0.31	0.90	0.42	0.70

TEMP= Weekly average temperature; RH= Weekly average relative humidity; RF= Weekly average rainfall; SNS= Weekly average sunshine hours; WS= Weekly average wind speed; DI= Disease Incidence (%); NS= Non-significant; n= 132 indicates total number of observations during 2010-11 to 2019-20.

*Variance inflation factor value of >10 indicates multicollinearity.

#Tolerance value of <0.1 indicates multicollinearity.

Table 2: Parameter estimates of the logistic regression model used to explain stem rot incidence (>20%) in Egyptian clover.

Variable	Estimate	Standard error	Wald Chi-Square	Pr > χ^2	Odds ratio point estimate
Intercept	-8.98	1.73	26.86	<.0001	
TEMP	0.53	0.11	22.19	<.0001	1.70
WS	0.69	0.24	8.46	0.0036	1.99

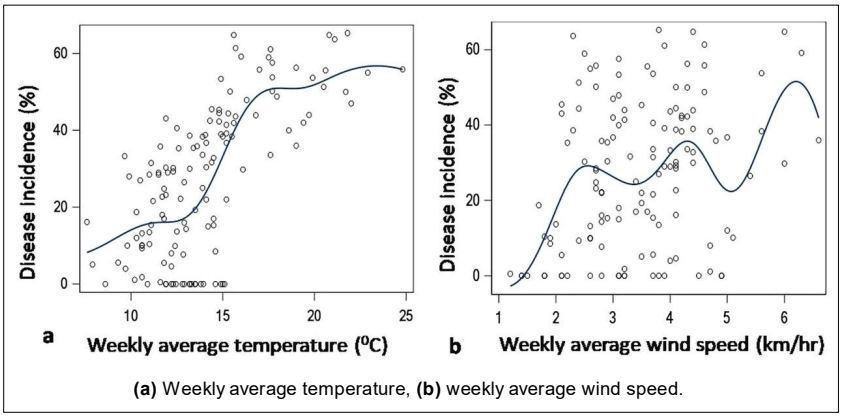
TEMP= Weekly average temperature; WS= Weekly average wind speed.

Model development and validation

The prediction model included weekly average temperature and wind speed as key predictor variables (Table 2). The model indicates that likelihood of stem rot incidence of >20% is governed by increasing weekly average temperature (between 8-25°C) and wind speed (between 1-7 km/hr) during week-50 (second week of December) to week-14 (first week of April) of a calendar year (Fig 4). Percentages of concordant pairs were 83.8% and percentages of discordant pairs were 16.1%. The value of Somers' D, Gamma, Tau-a, was 0.68, 0.68 and 0.33 respectively. Point estimates of odds ratio of temperature and wind speed were 1.70 and 1.99 respectively. Hosmer and Lemeshow test statistics indicates that there is no evidence of lack of fit in the selected model (p=0.71) (Table 3). Cross validated predicted probabilities were almost similar to the model predicted probabilities (Fig 5). The value of AUC was 0.84 during development (Fig 6a) and was 0.82 on cross validation (Fig 6b). The value of Somers' D, Gamma and Tau-a, on cross validation was 0.65, 0.65 and 0.31 respectively (Table 4). No prediction model for stem rot in Egyptian clover based on weather variables is available till now. However, disease prediction models have been developed for other diseases caused by *Sclerotinia* sp. especially *S. sclerotiorum* (in rapeseed, lettuce, carrot) in order to determine the need for or deciding the timing of fungicide application. All these models utilize different variables such as rainfall, soil moisture, canopy density, petal infestation, crop history, air temperature, relative humidity and sunshine duration, in order to predict whether inoculum is present or not, what is the risk of disease outbreak for better timing of fungicides (Turkington and Morrall, 1993; Twengstrom *et al*., 1998; Bom and Boland, 2000; Clarkson *et al*., 2007). The inclusion of only two predictors (temperature and wind speed) in the model indicates that these predictors contribute to stem rot development and have biological significance. High percentage of concordant pairs indicates that the model predicted high probabilities when incidence of stem rot was truly >20%. Less percentage of discordant pairs indicates that only a few probabilities were falsely predicted. The point estimates of >1 indicates that increasing value of predictor variable will lead to increasing odd of >20% stem rot incidence. Variability in stem rot incidence over the years affected the level of accuracy of the selected model. However, AUC value of 0.84 during development and 0.82 on cross validation indicates high accuracy of the model as models having AUC value between 0.8-0.9 are considered as having excellent discriminating and prediction power (Hosmer *et al*., 2013). Both temperature and wind speed play important roles in lifecycle of diseases caused by *Sclerotinia* sp. An initial low temperature over a period of time followed by an increase in temperature along with adequate soil moisture results in production of apothecia (Bardin and Huang, 2001). From apothecia, ascospores are produced which can travel for several kilometers with the help of wind (Li *et al*., 1994) and initiates the infection after coming in contact with plants. Under north Indian conditions, temperatures are quite low during December to February followed by gradual increase in temperature from March onwards. As a result of this, stem rot incidence also starts slowly during month of December-January and as the temperature rises, disease incidence also rises. Wind helps

Table 3: Goodness of fit statistics of the logistic regression model used to explain stem rot incidence (>20%) in Egyptian clover.

Per cent concordant	Per cent discordant	Per cent Tied	Somers' D	Gamma	Tau-a	Hosmer and Lemeshow test (Pr > ChiSq)
83.8	16.1	0.0	0.68	0.68	0.33	0.71



(a) Weekly average temperature, **(b)** weekly average wind speed.

Fig 4: Scatter plot with penalized B-spline curves showing influence of key predictor variables on stem rot incidence in Egyptian clover.

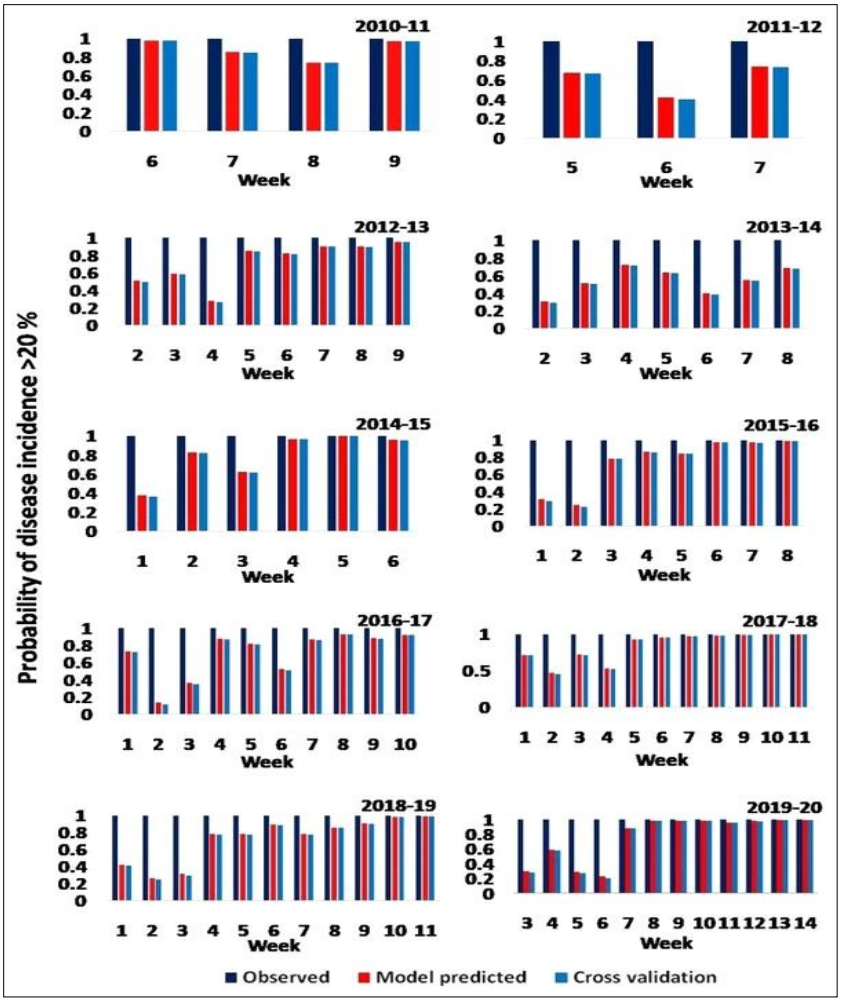
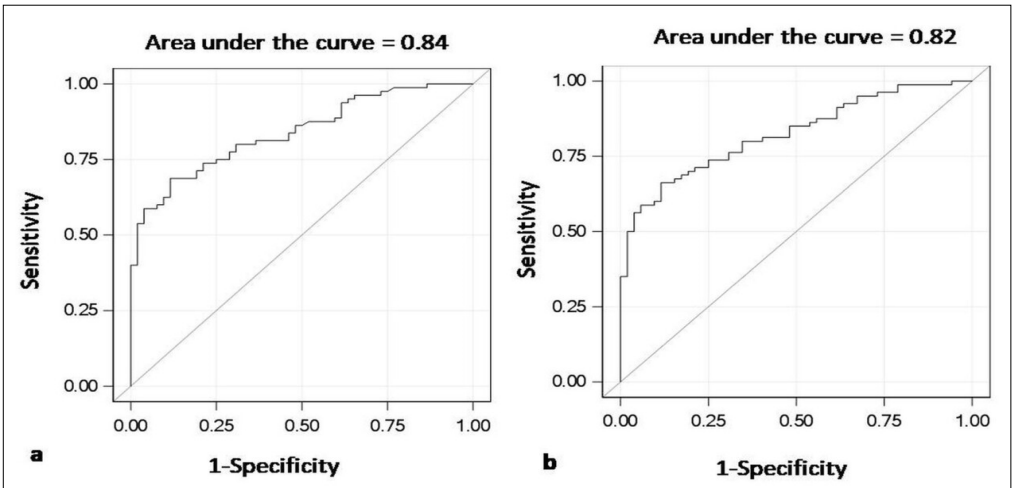


Fig 5: Observed, model predicted and cross validation predicted probabilities of stem rot incidence (>20%) in Egyptian clover during 2010-11 to 2019-20.



(a) ROC curve along with area under curve (AUC) value for the developed model, **(b)** ROC curve along with area under curve (AUC) value for the cross validated model.

Fig 6: Receiver operating characteristics (ROC) curve of the prediction model for stem rot in Egyptian clover.

Table 4: Cross validation association statistics of the logistic regression model used to explain stem rot incidence (>20%) in Egyptian clover.

Model	Mann-Whitney				Somers' D	Gamma	Tau-a
	*AUC	Standard error	95% Wald confidence Limits				
Selected Model	0.84	0.03	0.77	0.90	0.68	0.68	0.32
Cross validated model	0.82	0.03	0.75	0.89	0.65	0.65	0.31

* AUC= Area under receiver operating characteristic curve.

by carrying the ascospores released from apothecia to the plant surface and thus helps in initiating and spreading the infection. Hence, the developed model has picked up the right weather variables which contribute to higher incidence of stem rot. This is the first prediction model for stem rot in Egyptian clover in India and probably around the world. The model will help in rationale, need based use of fungicides in Egyptian clover. The model can predict stem rot incidence in regions having climatic conditions similar to Ludhiana, however its applicability in areas with different climatic conditions remains to be proved. This model will be applicable to other Egyptian clover cultivars as most of the Egyptian clover cultivars in cultivation are derived from Miscavi ecotypes and have similar phenology and growing season. However, the applicability of proposed models to other clovers remains to be proved as they have different growing period and climate. Additional research with regard to factors such as soil moisture and soil temperature, which might affect pathogen survival and development in the soil can be carried out as it will likely lead to further improve the proposed model.

CONCLUSION

In this study, a logistic regression model for predicting high risk (>20%) of *Sclerotinia* stem rot in Egyptian clover during week-50 (second week of December) to week-14 (first week of April) was developed using macro-climatic weather variables as predictors. The model incorporated weekly average temperature and wind speed as key predictor variables. As the model depends on weekly weather values, it is easy to predict the probability of high risk of stem rot one week in advance and hence, appropriate management decisions regarding preventive spray of fungicides can be made. The use of this model will likely lead to reduction in the number of fungicide applications for stem rot management. The model will also help in reducing the stem rot incidence and consequent loss in fodder and seed production by timely management.

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Conflict of interest

All authors declared that there is no conflict of interest.

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