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INFLUENCE OF EDAPHOLOGICAL FACTORS ON *SCLEROTIUM ROLFSII* SACC., CAUSING COLLAR ROT OF BETELVINE (*PIPER BETLE* L.) UNDER COASTAL SALINE ZONE OF WEST BENGAL

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Abstract

Betelvine (*Piper betle* L.) is an important cash crop in the coastal saline zone of West Bengal, which often suffers serious crop loss due to soil borne disease-collar rot, incited by *Sclerotium rolfsii* Sacc. Influence of edaphological factors were studied on incidence of the disease and its perpetuation through sclerotia in the soil. On surveying 40 locations in 20 villages, under four betelvine growing blocks, *viz*, Sagar, Namkhana, Kakdwip and Patharpratima of South 24 Parganas District in West Bengal, a low (6.67%) to moderate (17.67%) level of collar rot incidence was witnessed. The collar rot incidence and sclerotial population were positively correlated with the available Nitrogen (N) and Organic Carbon (OC) content of the respective soils. Whereas, soil Potassium (K) content, Clay percentage and soil pH had an inverse influence on the disease incidence and sclerotia population. Through stepwise multiple regression analysis, a model was developed for the prediction of sclerotia population in soil. Soil Potassium and Nitrogen contents were found to be two important predictors for prediction of sclerotia population in the soil.

Key words: Edaphological factors, pH, Nitrogen, Potassium, *Sclerotium rolfsii*, Collar rot, Sclerotial population, Betelvine.

Introduction

India is the leading producer of Betelvine (*Piper betle* L.) in the world, where it is grown on 55,000 ha area with an annual production worth about 9000 million (Guha, 2006). Though the crop is believed to be originated from Malaysia, it is more popular in India than in any other country, where, 15-20 million people consume the betel leaves daily. Among the Indian States, West Bengal alone shares 35% of total cultivated area and 66% of the total country's production. It is an important cash crop for 5 lakh farm families in the state (Guha and Jain, 1997; Anonymous, 2015). This perennial crop is mostly cultivated inside a protected shade house, known as 'boroj', in this state. The confined structure provides required shade to the vines by allowing diffused sunlight through it and stops easy escape of moisture from it.

However, cultivation of Betelvine is highly risky and returns are often uncertain because of its proneness to several diseases, aggravated by the moist and humid conditions of the protected chamber. The mild temperature, high humidity and diffused light prevailing inside the boroj not only favour vine growth but also provide a congenial microclimate for the growth of many pathogens (Sengupta *et al*., 2011). The collar rot disease, caused by soil borne pathogen *Sclerotium rolfsii* Sacc., is one such menace that is very difficult to manage and eradicate, once established in the boroj. The pathogen infects the collar region, resulting in wilting and rapid mortality of the vine. Maiti and Sen, (1982) recorded 25-90% yield loss due to this disease from different districts of West Bengal. The pathogen is reported to infect more than 600 plant species and can survive in soil from 2 months to 7 years, by producing sclerotia (Aycock, 1966, Farr *et al*., 2006). The sclerotia, present in the soil inside boroj, acts as the major source of primary inoculum to initiate collar rot disease in betelvine.

However, the survival and population of the sclerotia and incidence of collar rot disease in a particular soil system is governed by many soil physiochemical factors. Sclerotial germination and pathogenicity of *Sclerotium*

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rolfsii is greater in acidic soil than in alkaline soil (Punja and Grogan, 1982; Shim and Starr, 1997). Banyal *et al.*, (2008) found significant positive correlation between collar rot incidence in Tomato and available soil Nitrogen, Organic Carbon, Soil pH and pathogen population in soils collected from different locations of Himachal Pradesh, whereas negative correlation was recorded with Phosphate and Potassium content in the soil. He also reported that lighter soils were more favourable to *Sclerotium* collar rot than the heavy textured soils. El-Abyad *et al*., (1988), while studying the pathogenicity of three soil borne pathogens on sugarbeet root rot disease under different salinity stresses, found that *Sclerotium rolfsii* was most aggressive due to its certain tolerance to NaCl. Khalili and Kamyab, (2016) studied the relationship between soil properties and sclerotial

Table 1: Soil physiochemical parameters, sclerotial population and collar rot disease incidence (DI).

Block	Village	Loc-ation	DI (%)	pH (1:2.5)	EC (dSm^{-1})	OC (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)	Clay (%)	Sclerotia (no. per 100g soil)
Sagar	Khansaheb Abad	1	9.33	8.1	0.15	0.58	301.29	92.71	290.72	47	6.44
		2	11.67	6.5	0.32	0.74	422.35	72.54	416.74	35	8.57
	Harinbari	1	11.33	7.7	0.36	0.74	365.32	47.37	252.67	31	7.89
		2	12.23	6.7	0.45	0.59	341.51	99.68	176.85	39	8.90
	Bamankhali	1	9.00	7.87	0.28	0.62	264.13	35.18	325.38	46	7.11
		2	9.33	7.3	0.16	0.55	275.18	42.25	390.46	42	7.30
	Rudranagar	1	9.67	8.05	0.35	0.46	206.48	98.72	217.72	45	6.22
		2	13.33	7.8	0.55	0.49	312.47	59.82	146.39	31	10.20
	Haradhanpur	1	8.67	8.01	0.19	0.6	201.00	52.78	206.36	46	6.78
		2	8.00	7.9	0.47	0.41	205.55	90.5	326.38	44	6.33
Namkhana	Narayanganj	1	14.33	5.4	0.42	0.72	411.16	40.15	245.34	27	9.44
		2	14.33	6.4	0.24	0.72	583.47	68.87	156.75	31	10.30
	Debnagar	1	6.67	7.75	0.28	0.46	128.51	93.38	509.60	48	3.67
		2	8.67	7.1	0.72	0.56	265.22	44.65	381.48	45	6.30
	Madanganj	1	9.67	7.45	0.85	0.37	169.00	117.62	378.76	38	5.89
		2	9.00	7.9	0.12	0.44	217.36	65.24	342.55	35	7.10
	Namkhana	1	17.67	5.56	0.47	0.88	549.78	51.41	135.25	26	11.56
		2	15.33	7.2	0.44	0.77	432.46	39.55	165.24	29	9.67
	Shibnagar Abad	1	7.67	7.3	0.08	0.34	131.76	115.37	562.03	42	3.44
		2	11.33	7.7	0.76	0.29	182.84	84.2	212.39	34	7.11
Kakdwip	Shibkalinagar	1	11.67	7.5	0.07	0.48	205.55	74.41	244	39	6.78
		2	8.40	6.5	0.24	0.61	306.25	90.6	234.52	35	7.30
	Bamnagar	1	14.67	5.24	0.21	0.75	534.18	94.74	185.71	27	10.78
		2	13.67	6.1	0.37	0.81	487.38	49.4	261.75	37	8.90
	Akshaynagar	1	12.67	6.3	0.24	0.67	380.15	39.25	137.94	33	9.00
		2	13.33	6.2	0.31	0.42	266.72	34.8	139.78	26	8.10
	Gangadharpur	1	13.33	6.5	0.64	0.74	353.73	49.81	201.16	31	6.56
		2	12.67	5.9	0.45	0.59	321.45	88.65	249.3	25	7.22
	Budhakhali	1	9.33	7.7	0.6	0.38	134.07	97.90	262.58	45	6.33
		2	9.67	6.8	0.09	0.32	216.92	54.8	278.65	43	7.30
Patharpratima	Keorakhali	1	13.67	6.5	0.66	0.42	424.60	86.68	262.69	35	9.22
		2	14.33	6.1	0.28	0.82	528.36	41.5	301.42	33	10.30
	Dakshin Raipur	1	11.33	7.13	0.49	0.47	169.00	14.88	291.00	32	7.89
		2	10.67	6.9	0.37	0.31	192.32	90.8	157.68	35	6.58
	Digambarpur	1	11.00	7.1	0.81	0.35	205.55	66.31	237.64	30	7.11
		2	12.33	5.5	0.39	0.52	353.74	110.4	184.35	32	8.72
	Dakshin Durgapur	1	12.67	6.4	0.6	0.62	334.41	36.54	227.35	28	7.00
		2	13.00	6.1	0.87	0.35	187.24	50.68	135.75	29	8.50
	Uttar Mahendrapur	1	8.67	7.35	0.2	0.46	228.92	87.20	403.20	43	7.67
		2	9.67	7.6	0.19	0.39	242.88	112.8	389.46	39	5.10

population of *Macrophomina phaseolina*, causing charcoal rot in soyabean and reported a negative correlation with percent clay and soil organic matter but positive correlation with soil pH. However, very little information is available on influence of different edaphological parameters on *Sclerotium rolfsii* collar rot disease in Betelvine under the coastal saline zone of West Bengal. This zone is an important Betelvine growing area, known for the Mitha Pata cultivar, which is the most popular among all betel leaves for its low fibre content, sweet taste and fennel-like odour.

Though there are standard procedures for estimation sclerotial population in soil, it is often very difficult to determine correct sclerotial population over a large field, given the heterogeneous distribution of such tiny propagules in the soil. So it is necessary to develop an easy predictive methodology to forecast sclerotial population in a soil.

In the present investigation attempts were made to understand the relationship between collar rot disease and different soil physiochemical parameters and development of model for prediction of sclerotial population in soil so as to aid in better preparedness in managing the disease before its incidence.

Materials and Methods

To study the relationship between collar rot disease and soil physiochemical factors, soil samples were collected from 40 betelvine boroj located at twenty villages, across four blocks of the district South 24 Parganas under coastal and saline zone of West Bengal (Table 1) in the year 2016. Two boroj (Location 1 & Location 2) were selected from each village. It was ensured that same variety (Mitha pata) was grown following similar agronomic management practices. Composite soil samples were collected at 0-15 cm depth, during the month of March, 2016.

Standard laboratory techniques were followed to determine soil pH and Electrical Conductivity (Jackson, 1979), Organic Carbon (Walkley and Black, 1934), total available Nitrogen (Subbaiah and Asija, 1956), available Phosphorus (Olsen *et al.,* 1954), available Potassium (Jackson, 1973), Clay % (Piper, 1966) and sclerotial population in the soil ('rapid flotation - sieving' technique as described by Rodriguez *et al.,* 1974). For recording collar rot incidence, three rows of vines (each with 100 vines) were marked randomly inside the selected boroj, during March 2016. Number of vines showing wilting symptom in each of the marked rows was counted during June 2016. Disease incidence (%) was calculated for each row, as:

$$\text{Disease Incidence (\%)} = \frac{\text{No. of wilted vines in a row}}{\text{Total no. of vines in a row}} \times 100$$

Observations of the three rows were averaged and expressed as Disease Incidence (DI) of that particular boroj.

To find out the influence of soil physiochemical characteristics on disease incidence and sclerotial population in soil, multiple correlation and regression analysis were done with the data collected at "Location 1" of each village, with the help of statistical software IBM SPSS 20. Pearson correlation analysis was used to examine linear correlations among all variables. Stepwise multiple regression analysis was carried out to determine the most important predictors for sclerotial population in soil. With the help of this model, the sclerotial population was predicted in 20 boroj (Location-2 of the selected villages) and compared with the respective observed sclerotial population. The accuracy of the model was studied by calculating Root Mean Squared Error (RMSE) between the predicted and observed data.

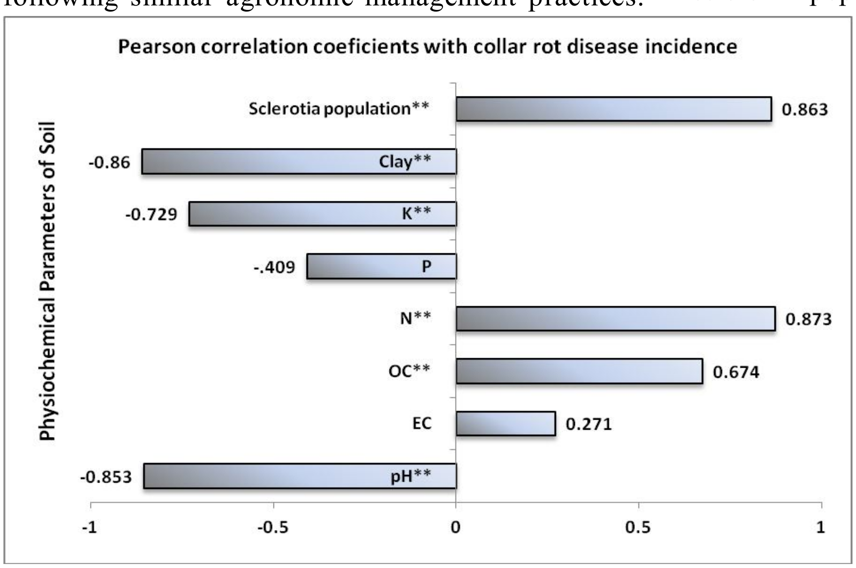

Fig. 1: Correlation of collar rot disease incidence with physiochemical parameters of soil.

Result and Discussion

Relationship between edaphological parameters, collar rot incidence and sclerotial population in soil

The data on collar rot incidence in 40 betelvine boroj, distributed over 20 villages in four important betelvine growing blocks of West Bengal, is presented in Table 1, along with the respective soil physiochemical parameters and initial sclerotial population in the soil. The collar rot incidence during the month of June in 2016 varied between 6.67% to 17.67%, whereas, initial sclerotial population in soil in the month of March of the same year ranged from 3.67 to 11.56 numbers per 100 g of soil. A strong positive correlation (0.863 at $p<0.001$) was found between the initial sclerotial population in soil and disease incidence. The values of soil pH, EC, Organic Carbon (OC), total Nitrogen (N), Phosphorus (P), Potassium (K) and Clay % varied between 5.24 to 8.1, 0.07 to 0.87 dS m^{-1}, 0.29 to 0.88%, 128.1 to 583.47 kg ha^{-1}, 14.88 to 117.62 kg ha^{-1}, 135.25 to 562.03 kg ha^{-1} and 25 to 48%, respectively. The correlation studies conducted between the edaphological parameters and disease data of 'location 1' of each village (Fig. 1) revealed that the collar rot incidence was significantly and positively correlated with the total available Nitrogen and Organic Carbon content of the soil. On the other hand, disease incidence was inversely correlated with Potassium, pH and percentage of Clay in the soil. No significant relation was found between disease incidence, soil salinity (EC) and soil Phosphorus level.

These results indicate that collar rot in Betelvine is favoured by soils high in Nitrogen and Organic Carbon content but low in pH and Potassium content. Light textured soils also favoured the disease incidence. The scatter diagram in fig. 3 represents the linear relationship of collar rot with the soil parameters and their respective regression coefficients. The highest disease incidence (17.67%) was recorded in the boroj (Location 1 in Namkhana village) having initial sclerotial population of 11.56 per 100g soil, 549.78 kg N per ha, 135 kg K per ha, 0.88% Organic Carbon, soil pH of 5.56 and a lighter soil texture with 26% Clay content. Whereas the boroj (Location 1 of Debnagar village) containing soils with initial sclerotial population of 3.67 per 100g soil, 128.51 kg N per ha, 509.6 kg K per ha, 0.46% Organic Carbon, soil pH of 7.75 and a heavier soil texture with 48% Clay content resulted in lowest disease incidence (6.67%).

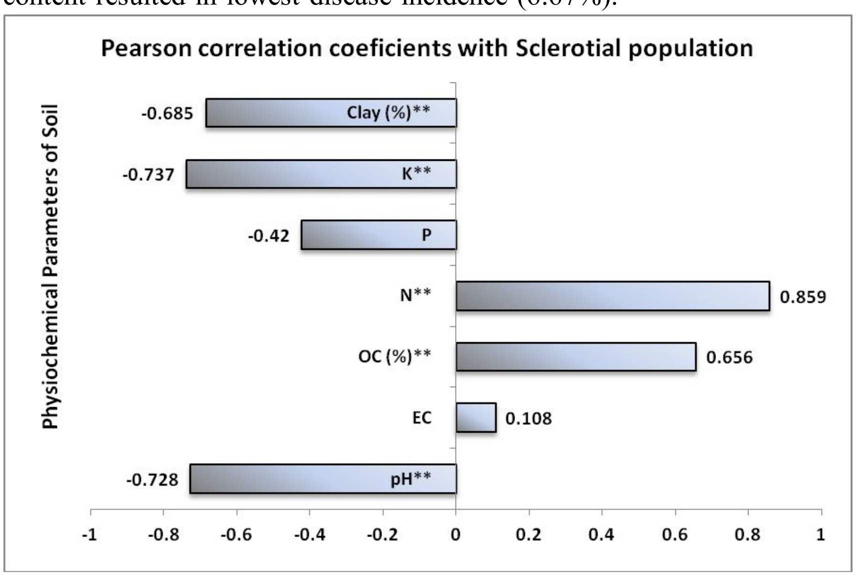

Fig. 2: Correlation of sclerotial population in soil with physiochemical parameters of soil.

Similar relationship were reported in collar rot of Tomato by Banyal *et al*., (2008) from Himachal Pradesh and by Mahato and Mondal, (2014) from red & lateritic zones of West Bengal, in respect of the Nitrogen, Organic Carbon, Potassium content of the soil, soil texture and soil inoculum level. Acidic soils were also reported to favour sclerotial germination and pathogenicity of *Sclerotium rolfsii* over alkaline soils (Punja and Grogan, 1982; Shim and Starr, 1997).

The absence of significant impact of soil salinity on *Sclerotium* collar rot incidence in the present study may be due to the tolerance of the pathogen to salinity stress upto a certain level. This result corroborates with the findings of El-Abyad *et al*., (1988), who found that *Sclerotium rolfsii* infestation in sugarbeet in Iran was not deterred by the increase in soil salinity.

Prediction of sclerotial population in soil

Considering the strong correlation between initial sclerotial population in soil and collar rot incidence, it becomes imperative to develop a model for prediction of sclerotial population in a soil to take up timely preventive measures before the disease onset. As like disease incidence, the sclerotial population in soil also showed significant correlation with various physiochemical properties of the soil (Fig. 2). To reduce the interdependence of some of the soil physiochemical parameters and find out the most critical edaphological predictors of sclerotial population in soil, a stepwise multiple regression analysis was carried out which resulted into the following regression model:

$$Y = 6.079 + 0.01\,(N) - 0.006\,(K) \qquad (1)$$

$$R^2 = 0.801,\ R^2_{adj} = 0.778$$

Where, 'Y' represents predicted value of sclerotial population in soil (no. per 100 g soil), N represents total available Nitrogen (kg ha^{-1}) and K represents total Potassium (kg ha^{-1}) in soil.

This model '1' could able to explain the variation of sclerotial population in soil upto 77.8% accuracy. The two most significant important estimators for estimation of sclerotial population in soil are soil Nitrogen and Potassium level.

Validation of the prediction model

The multiple regression analysis model was validated with the help of a separate data set on sclerotial population in soil & edaphological parameters, collected from the 'Location 2' of each of the twenty villages during the same year 2016 (Table 1). A predicted value of

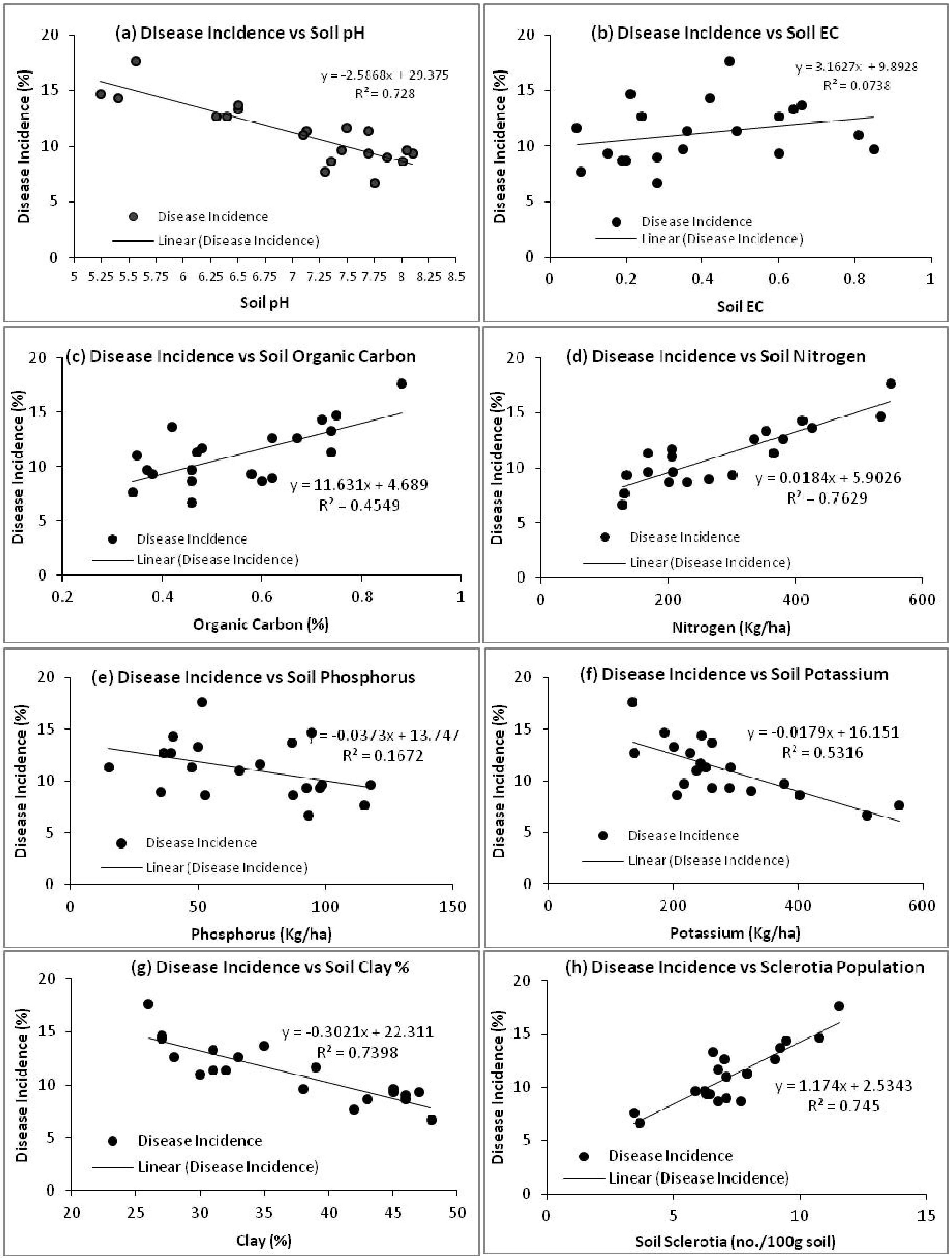

Fig. 3: (a to h) Scatter diagram of collar rot incidence & its regression coefficients with respect to different factors

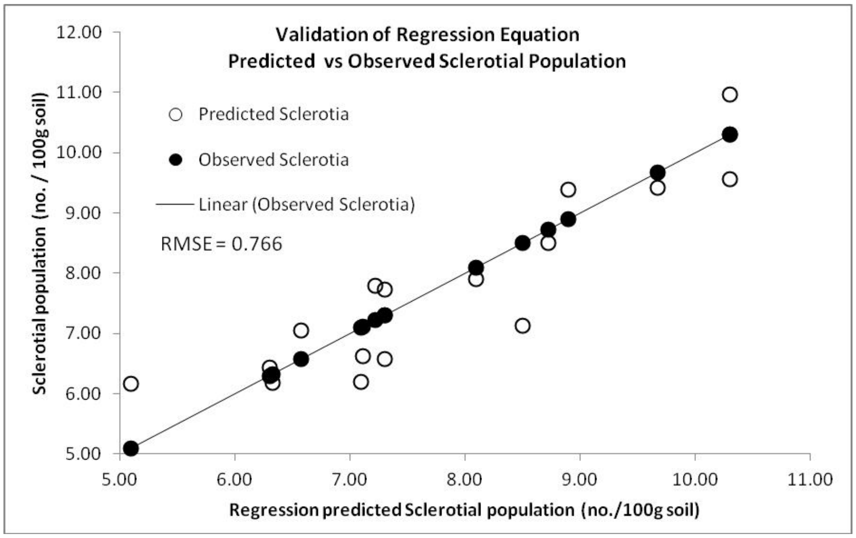

Fig. 4: Scatter diagram of predicted and observed sclerotial population in soil.

sclerotial population of a particular location was generated against the respective predictor values (Nitrogen and Potassium content) of that location, with help of the regression equation '1'. The scatter diagram (Fig. 4) shows the relationship between observed and predicted sclerotial population in soil based on the multiple regression analysis model. The performance of the prediction model was measured in terms of the square root of the variance of the residuals between the predicted and observed values, expressed as Root Mean Squared Error (RMSE). The RMSE value (0.766) gives a good measure of how accurately the model predicted the sclerotial population.

Conclusion

Low to moderate level incidence of *Sclerotium rolfsii* collar rot in Betelvine was found in all the surveyed Blocks of coastal saline zone of West Bengal, India, during 2016. The collar rot incidence and sclerotial population were positively correlated with the available Nitrogen (N) and Organic Carbon (OC) but inversely with Potassium (K) content, Clay percentage and soil pH. Potassium and Nitrogen contents in soil were found to be two important predictors for prediction of sclerotial population in the soil. The findings of the study will be helpful for developing a suitable management practice by incorporating it into the integrated disease management programme.

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