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RELATIONSHIP OF METEOROLOGICAL VARIABLES AND INOCULATION TECHNIQUES CONDUCIVE FOR LOOSE SMUT OF WHEAT DEVLEOPMENT

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A B S T R A C T

Ustilago tritici, a seed borne pathogen, remains dormant in seed embryo and causes loose smut disease. The present study was conducted to evaluate two inoculation methods i.e., dip inoculation and syringe inoculation to find out the better method for efficient and reproducible assessment of disease severity, disease incidence and screening of resistant wheat germplasm. Syringe inoculation was found to be better for the inoculation of heads with higher disease incidence than dip inoculation method. Twenty-seven lines were tested for their resistance against loose smut. Two lines were moderately resistant, nineteen lines susceptible and three lines were highly susceptible. Environmental conditions play an integral role in the development of loose smut disease. The relationship of temperature, humidity and rainfall with loose smut development was studied. An average temperature of 23-27 ˚C, relative humidity 60-90% and rainfall 0.02-1 mm rainfall were statistically most conducive for disease development. The correlation of environmental factors significantly influenced the disease development.

Keywords: *Ustilago tritici*, inoculation, temperature, relative humidity, rainfall, resistant, susceptible.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the staple food crop in Pakistan. Wheat was cultivated on an area of 9199318 hectares with production of 25979399 tones (FAOSTAT, 2014). Smut diseases caused by Basidiomycetes fungi occur throughout the world and reduce the total crop yield (Gondal and Khan, 2012). Numerous species of smut fungi have been reported to cause diseases in cereals, smuts also affect sugarcane, onion and some ornamental plants. Loose smut is an important disease of wheat. The first report about the cereal smuts comes from Theophrastus (332-384 BC). Smut was known to Romans, named as *Ustilago*, which is derived from Latin word "burn". In Pakistan, two physiological races of *Ustilago tritici*, loose smut of wheat were reported in 1966-67. These races were isolated from Sialkot district of Pakistan (Hassan *et al*., 1970). In Pakistan, loose smut of wheat contributes a significant share in total yield loss (Jamil *et al*., 2005). It can cause 5-8% yield loss if farmers recycle the seed from previous crop (Joshi *et al.,* 1985).

Ustilago tritici attacks at heading stage and causes distortion of wheat grains by replacing them with dark teliospores. These spores spread by wind to adjacent fields. An increase (1.2-1.9%) in the incidence of loose smut was reported during a survey in Sangrur, Hoshiarpur and Gurdaspur districts of India (Rewal *et al*., 2001). The marvelous role of breeders to generate disease resistant varieties against different wheat pests is of high significance to combat against the yield loss caused by loose smut pathogen.

Environment plays a significant role in development and spread of this disease, teliospores germination occurs at 22 to 27 ˚C, whereas relative humidity ranges from 60-90% (Druzhin and Krupnov, 2000). Optimum environmental factors i.e., 20-27°C temperature and 60-90% relative humidity, significantly contribute to the development, spread and germination of teliospores.

The project was undertaken to screen out wheat germplasm for resistance against loose smut pathogen

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and to evaluate the correlation of environmental factors and disease development.

MATERIALS AND METHODS

Inoculum preparation: Inoculum was prepared by dissolving 1g of teliospores in 1L of distilled water following the method given by (Mishra *et al*., 1990). Inoculum was also prepared by dissolving 30-35 infected heads in 250L of distilled water, followed by staining via muslin cloth.

Screening of wheat germplasm: Field trial was conducted at Plant Pathology Section, Ayub Agricultural Research Institute, Faisalabad to screen out wheat germplasm against *Ustilago tritici*. Twenty-seven lines were sown in research farm area, each line was sown in a single plot 7x3 ft in size, and plot to plot distance was maintained up to 1.5 ft.

Effect of inoculation methods on loose smut incidence: Two different inoculation methods were compared to find more conducive inoculation method for loose smut development.

Syringe inoculation: Inoculum was stained through dense nylon mesh of 900cm^2 to separate suspended particles. Inoculation was carried out at anthesis stage by injecting 5 mL inoculum via, a clean, sterile syringe with 24 hypodermic gauges. Each floret was inoculated by syringe, and heads were bagged and labeled.

Dip inoculation: Twenty-five wheat heads were selected in each plot, that were slightly cut with the help of scissor, dipped into the inoculum two to three times for few seconds to completely inoculate the ovary. The inoculated heads were bagged and labeled.

To compare the different inoculation methods for successful disease development, a susceptible wheat cultivar WL-711 was sown in a plot measuring same size 7x3 ft as a susceptible check. Disease development was assessed based on visual symptoms regularly. The infected heads were counted, and disease intensity was calculated. The disease rating scale of (Ilyas *et al.,* 1990) which states, 1. No symptoms = Immune (I), 2. 0.1-5% plants with smutted heads = Highly resistant (HR), 3. 6-10% plants with smutted head = Resistant (R), 4. 11-20% plants with smutted heads = Moderately resistant (MR), 5. 21-30% plants with smutted heads= Moderately susceptible (MS), 6. 31-50% plants with smutted heads = Susceptible (S), 7. 51-100% plants with smutted heads = Highly susceptible (HS), was used. Disease incidence was calculated by the following formula.

$$\text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

The AUDPC was calculated by trapezoidal integration of the disease severity over time, considering the whole period and evaluated, as follows:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where, n is number of assessments; X, disease severity and (*ti*+1 – *ti*), time interval between two consecutive assessments. To allow comparison between different treatments that were assessed during different periods of time, the AUDPC integral variable was divided by its respective observation period (*ti*+1 – *ti*), Thus, AUDPC was the standard area under the loose smut of wheat severity progress curve and interpreted as the measure of disease severity (Shaner and Finney, 1977).

Correlation of Environmental Factors with Loose Smut Development: Wheat nursery was planted on 24-11-2011 consisting of 27 wheat lines. V-WL-711 variety was used as a susceptible check. Proper agronomic practices including recommended fertilizer dose and scheduled irrigation were followed. Environmental data consisting of minimum, maximum temperature (°C), relative humidity (%) and rainfall (mm) was recorded by conventional instruments installed in a weather monitoring observatory of Meteorological Department, Ayub Agricultural Research Institute, Faisalabad about 1km from experimental area. Disease intensity was recorded on weekly basis.

STATISTICAL ANALYSIS

Data was subjected to statistical analysis using a statistical software. The influence of each environmental variable on loose smut development was determined by correlation analysis. The weather data and disease incidence data were subjected to correlation and regression analysis to determine the relationship of environment with the disease incidence. Data of loose smut incidence recorded on wheat varieties/lines were processed according to the CIMMYT AUDPC calculating table (Steel *et al.,* 1997).

RESULTS

Syringe inoculation method was found to be more effective for the successful establishment of loose smut

infection than dip inoculation method (Figure 1). Syringe inoculation showed higher disease incidence. The results are supported by the findings of Poehlman (1945) where he reported that syringe inoculation method was more effective inoculation method compared to dip inoculation method for loose smut development. In case of syringe inoculation teliospores directly adhere to ovary and lead to successful infection establishment.

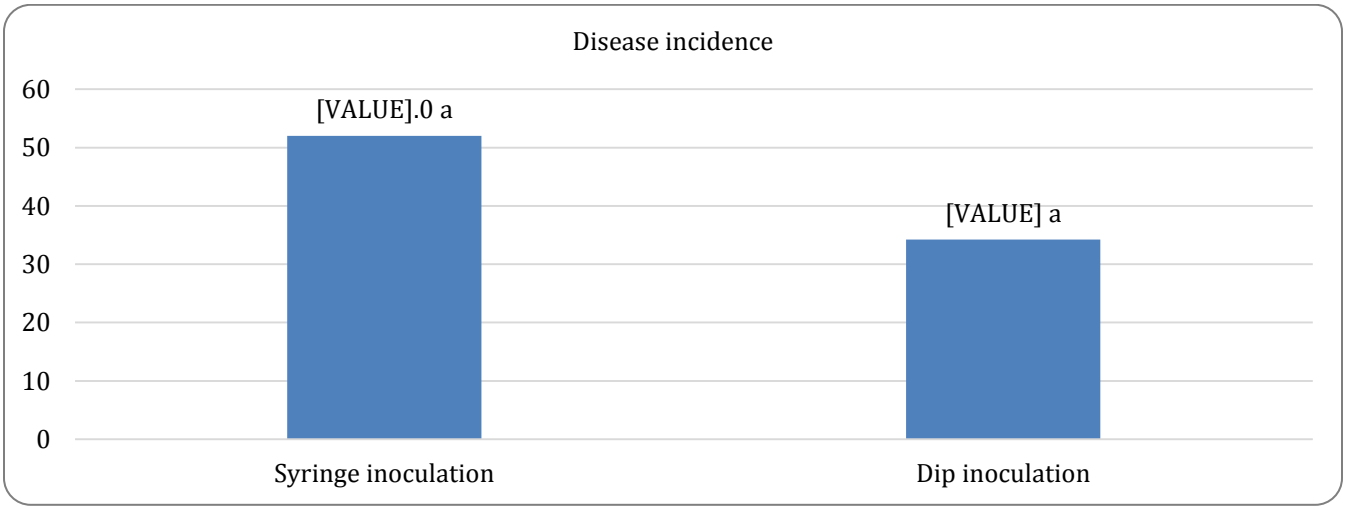

Figure 1. Effect of inoculation methods on disease incidence of loose smut.

The varieties v-WL-711, v-010309, v-09006 were the most susceptible varieties/lines with disease incidence of 80.19, 46.92 and 54.92 respectively whereas, v-09082, v-08212 showed moderately resistant response with 14.69 and 13.47 disease incidence respectively. The value of area under disease progress curve (AUDPC) was maximum in variety v-WL-711 up to 1120.77, while, least in v-08212 up to 188.3 (Table 1). Seth *et al.* (2014) screened 20 wheat varieties, all of them except three were significantly affected by loose smut pathogen with variable response supporting the present study.

Correlation of maximum temperature (°C), minimum temperature (°C), rainfall (mm) and relative humidity (%) with loose smut of wheat was determined by taking meteorological data at seven-day interval. Results revealed that there was positive correlation between maximum, minimum temperature, rainfall and relative humidity. The main cause of the epidemic is the combination of favorable environmental conditions, increasing the chances of disease incidence.

Maximum and minimum temperature gave a positive correlation with loose smut incidence (Table 2). The lines V-WL-711, v-010317, v-09087, v-09006 and v-010309 showed significant increase in loose smut incidence with maximum temperature 23-34 ºC and minimum temperature 10-16 ºC (Figure 2 and 3). Rainfall and relative humidity also revealed a positive correlation with loose smut development. Relative humidity 45-60% and rainfall 0.02-1 mm significantly increased loose smut infection (Figure 4 and 5). The present study is supported by the findings of Paul and Munkvold (2005) who stated that temperature and relative humidity significantly influence the development of fungal infection. The findings of Neelam *et al.* (2012) also strengthened the present investigation where they revealed positive correlation of environmental variables with sugarcane smut development. Different varieties gave different correlation values with environmental factors. Correlation of varieties V-WL-711, v-010317, v-09087, v-09006 and v-010309 was more significant indicating that disease incidence values and environmental correlation values were directly related with each other. Maximum disease incidence was observed in variety V-WL-711 showing the correlation of environmental factors for maximum temperature its value was 0.995 and for rainfall its value was 0.080.

Environmental conditions have a significant role in development and spread of loose smut disease. Minimum, maximum temperature, relative humidity and rainfall play a very important role in disease development. Teliospores germination occurs at 22 to 27 ºC, whereas optimum relative humidity ranges from 60-90% (Druzhin and Krupnov, 2000). Loose smut pathogen stops its development in plant tissues at a temperature of 7 to 8 ºC, therefore, winter wheat is less damaged by smut pathogen than spring wheat (Druzhin and Krupnov, 2000).

Table 1. Response of wheat germplasm against loose smut disease.

Sr. No.	Variety	Disease incidence (%) (mean ± SE)	AUDPC	Response
V1	v-08171	42.75±3.43 B-F[a]	596.715	S[b]
V2	v-09087	36.22±2.33 G	505.47	S
V3	v-09137	25.31±1.03 H	354.165	MS
V4	v-08214	44.08±2.57 B-E	615.72	S
V5	v-010309	46.92±1.40 B	655.76	HS
V6	v-010317	42.88±1.76 B-F	598.22	S
V7	v-010296	38.11±1.40 FG	531.72	S
V8	v-09082	14.69±0.88 I	205.205	MR
V9	v-09091	42.01±1.98 B-F	586.6	S
V10	v-09006	45.92±2.09 BC	639.59	HS
V11	v-08212	13.47±0.55 I	188.3	MR
V12	v-09136	39.54±1.53 EFG	552.195	S
V13	v-09031	41.62±1.15 C-F	581.665	S
V14	v-010306	42.21±1.99 B-F	587.615	S
V15	v-08118	38.31±1.53 FG	534.31	S
V16	v-NN-2	40.03±1.53 D-G	559.825	S
V17	v-NN-1	22.09±1.16 H	308.91	MS
V18	v-9157	38.05±1.77 FG	532.28	S
V19	v-8124	41.88±1.27 C-F	589.89	S
V20	v-6346	24.68±1.32 H	343.91	MS
V21	v-8184	41.76±1.92 C-F	582.225	S
V22	v-9375	44.72±1.35 BCD	624.015	S
V23	v-99199	40.06±1.35 D-G	559.825	S
V24	v-8132	42.71±1.45 B-F	597.555	S
V25	v-6422	45.25±1.32 BC	631.26	S
V26	v-6309	38.39±1.93 FG	534.1	S
V27	v-WL-711	80.19±1.84 A	1120.77	HS

[a]Means sharing similar letter in a column are statistically non-significant (P>0.05).

[b] MR=moderately resistant, MS=moderately susceptible, S=susceptible, HS=highly susceptible.

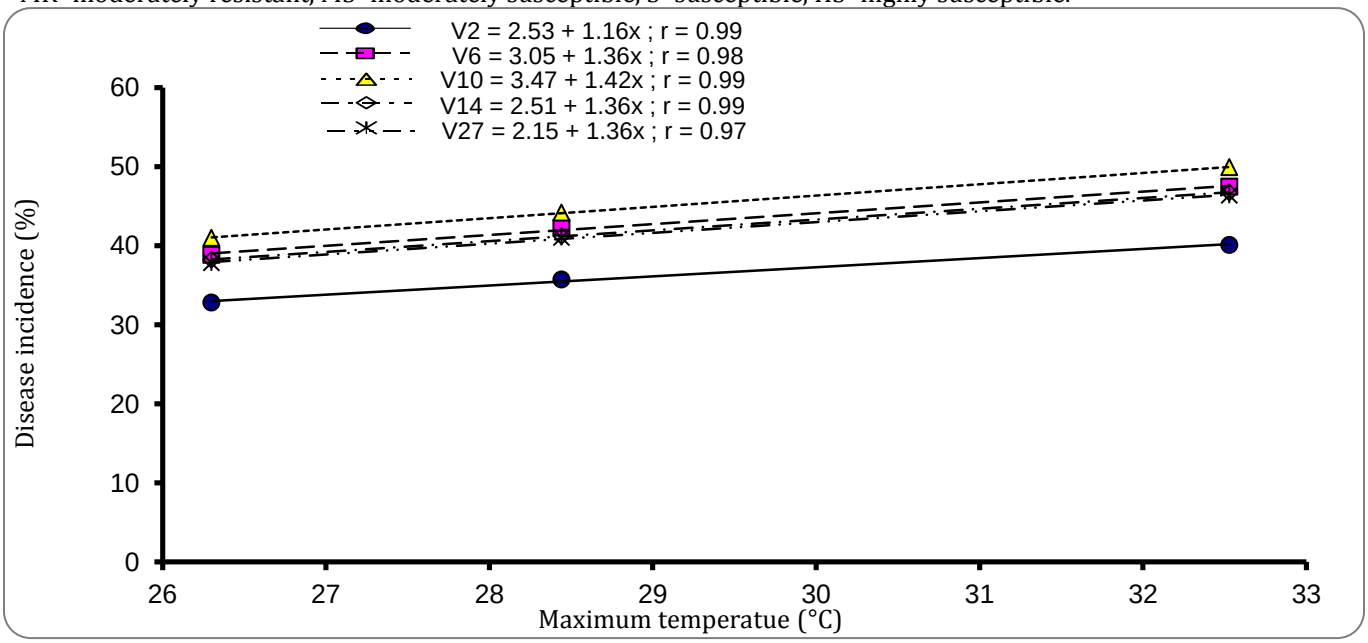

Figure 2. Relationship between maximum temperature and loose smut development for varieties V6:v-010317, V2:v-09087, V10: v-09006, V14:v-010306 and V27:V- WL-711.

Table 2. Correlation between environmental factors and loose smut of wheat.

Sr. No.	Variety	Max. Temp (ºC)	Min. Temp (ºC)	RH (%)	Rainfall (mm)
V1	v-08171	0.997* [a]	0.986	-0.400	0.106
		0.046 [b]	0.107	0.738	0.933
V2	v-09087	0.998*	0.987	-0.395	0.111
		0.042	0.104	0.742	0.929
V3	v-09137	0.987	0.968	-0.477	0.019
		0.101	0.162	0.683	0.988
V4	v-08214	0.995	0.982	-0.421	0.082
		0.061	0.122	0.723	0.948
V5	v-010309	0.994	0.980	-0.431	0.072
		0.068	0.129	0.716	0.954
V6	v-010307	0.998*	0.988	-0.387	0.120
		0.037	0.098	0.747	0.924
V7	v-010296	0.997	0.984	-0.409	0.096
		0.052	0.113	0.732	0.939
V8	v-09082	0.993	0.977	-0.442	0.060
		0.075	0.137	0.709	0.962
V9	v-09091	0.997	0.985	-0.407	0.098
		0.051	0.112	0.733	0.938
V10	v-09006	1.000*	0.992	-0.360	0.149
		0.018	0.080	0.766	0.905
V11	v-08212	0.992	0.975	-0.451	0.049
		0.082	0.143	0.702	0.969
V12	v-09136	0.994	0.979	-0.432	0.071
		0.068	0.129	0.716	0.955
V13	v-09031	0.994	0.979	-0.432	0.070
		0.069	0.130	0.716	0.955
V14	v-010306	1.000**	0.996	-0.328	0.182
		0.003	0.058	0.787	0.884
V15	v-8118	0.998*	0.987	-0.393	0.113
		0.041	0.102	0.743	0.928
V16	v-NN-2	0.990	0.972	-0.463	0.036
		0.090	0.152	0.694	0.977
V17	v-NN-1	0.990	0.972	-0.463	0.035
		0.091	0.152	0.693	0.977
V18	v-9157	0.990	0.972	-0.463	0.036
		0.091	0.152	0.693	0.977
V19	v-8124	0.837	0.781	-0.795	-0.391
		0.369	0.430	0.415	0.745
V20	v-6346	1.000*	0.994	-0.349	0.159
		0.011	0.073	0.773	0.898
V21	v-8184	0.999*	0.991	-0.367	0.141
		0.023	0.084	0.761	0.910
V22	v-9357	0.999*	0.989	-0.382	0.125
		0.034	0.095	0.751	0.920
V23	v-99199	0.993	0.978	-0.438	0.064
		0.073	0.134	0.711	0.959
V24	v-8132	0.989	0.970	-0.471	0.027
		0.096	0.158	0.688	0.983
V25	v-6422	0.999*	0.990	-0.376	0.131
		0.030	0.091	0.755	0.916
V26	v-6309	1.000**	0.995	-0.336	0.173
		0.003	0.064	0.782	0.889
V27	v-WL-711	0.995	0.981	-0.423	0.080
		0.062	0.123	0.722	0.949

[a] Upper values indicating Pearson's correlation coefficient; [b] Lower values indicating level of significance at 5% probability.

* = Significant (P<0.05); ** = Highly significant (P<0.01)

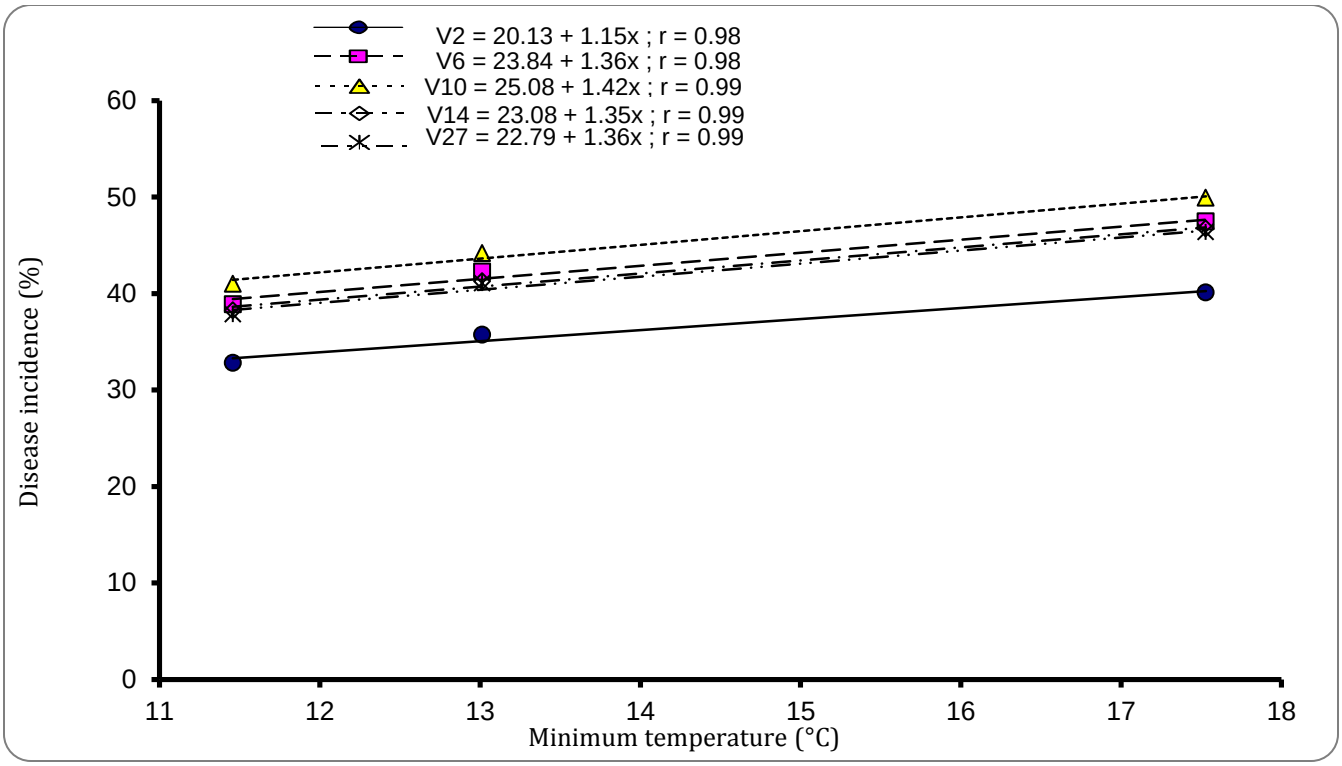

Figure 3. Relationship between minimum temperature and loose smut development for varieties V6:v-010317, V2:v-09087, V10: v-09006, V14:v-010306 and V27:V- WL-711.

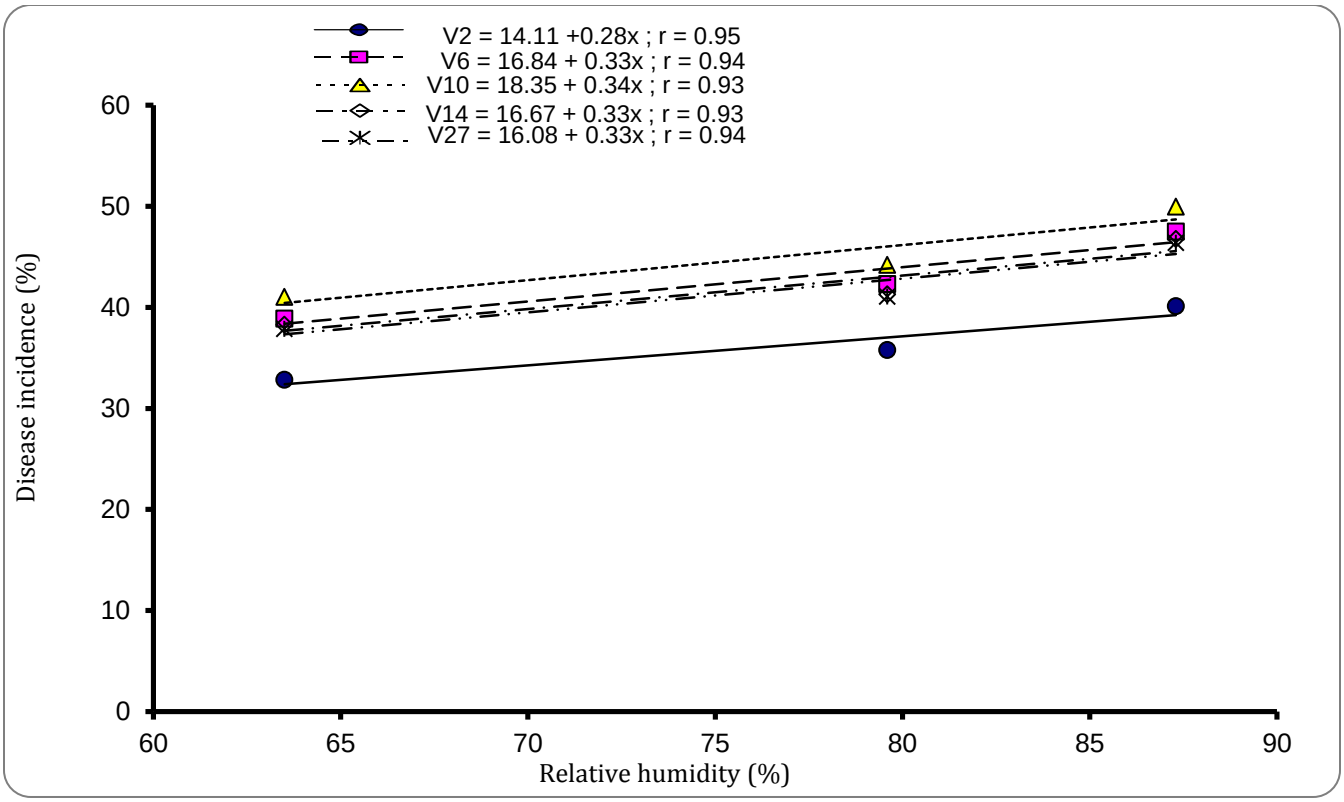

Figure 4. Relationship between relative humidity and loose smut development for varieties V6:v-010317, V2:v-09087, V10: v-09006, V14:v-010306 and V27:V- WL-711.

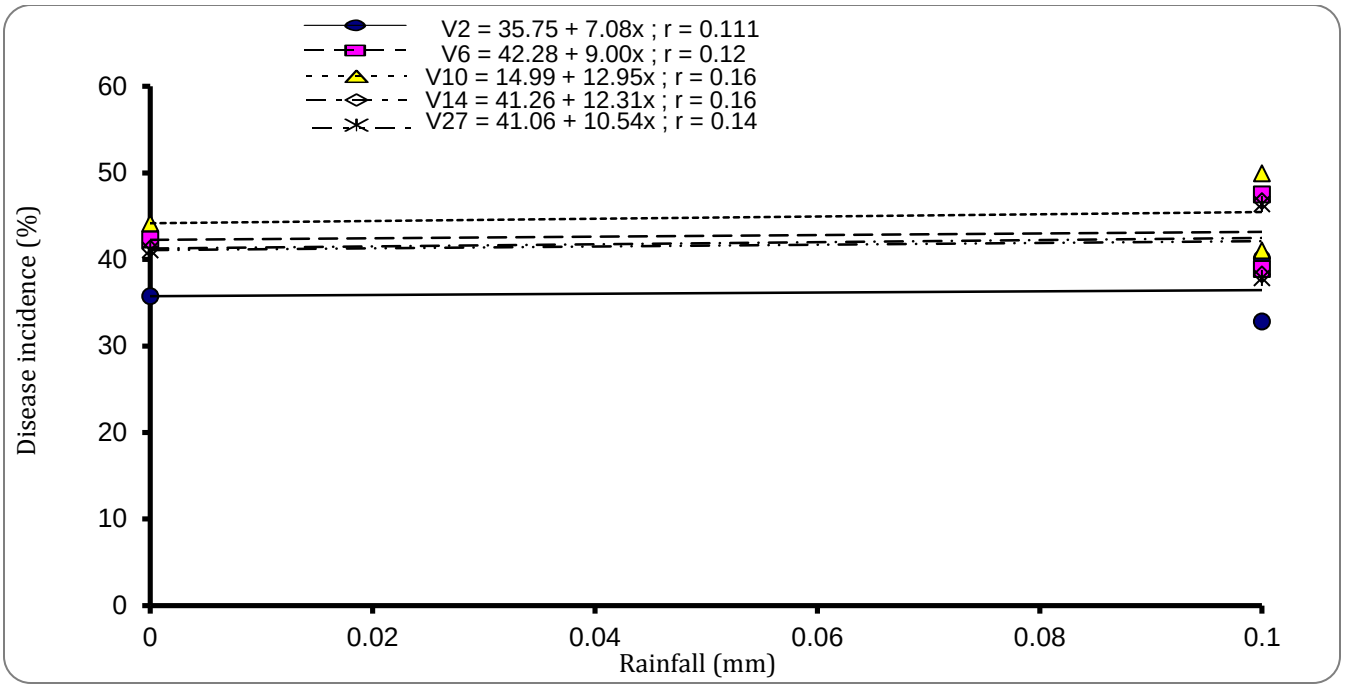

Figure 5. Relationship between rainfall and loose smut development for varieties V6:v-010317, V2:v-09087, V10: v-09006, V14:v-010306 and V27:V- WL-711.

CONCLUSION

This study has concluded that out of twenty-seven wheat cultivars two varieties/lines were found moderately resistant and rest of the varieties/lines were susceptible or highly susceptible. Among artificial inoculation methods, syringe inoculation method was found most effective. Disease incidence of loose smut of wheat varies significantly in relation to environmental conditions Maximum temperature 23-27 ºC, relative humidity 60-90% and rainfall 0.02-1 mm were found to be more conducive for pathogen infection.

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