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Quantification of inoculum load of uredospore for inducing black rust of wheat

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Wheat is primarily threatened by three types of rust pathogens: stem rust, brown rust, and stripe rust, all of which significantly impact its production. Among these, black or stem rust, caused by *Puccinia graminis tritici*, is particularly concerning due to its widespread occurrence and ability to develop new races, leading to substantial yield losses. To investigate the quantification of the inoculum load of uredospores necessary for inducing black rust in wheat, three susceptible genotypes- A-9-30-1, Agra local, and Lok 1 were cultivated in pots. Uredospore concentrations were maintained at 10^2, 10^3, and 10^4 per milliliter, as measured by a hemocytometer. The inoculation of *P. graminis tritici* uredospores was performed using a syringe method on plants that were 15, 30, and 45 days old. Following inoculation, the number of uredia per square centimeter and the number of pustules per square centimeter were recorded for each concentration. An increase in uredospore concentration (10^2, 10^3, and 10^4 uredospores/ml) corresponded with a rise in the number of uredia per square centimeter (2.63×10^4 , 3.64×10^4 , and 4.85×10^4) and pustules per square centimeter (2.28, 3.19, and 4.05) on the inoculated plants at 15, 30, and 45 days, respectively.

Keywords : Haemocytometer, inoculation, pustules, rust, uredospore

INTRODUCTION

Wheat (*Triticum aestivum* L.) ranks as the second most important staple crop in India, following paddy. The cultivation area for this crop spans approximately 30 million hectares, yielding an annual production of around 103 million metric tons. Future production must increase as the global population is estimated to exceed nine billion people by 2050.

As such, it is predicted that annual cereal production must grow by almost one billion tonnes. Furthermore, increases in consumption of wheat products throughout many countries in Asia and changes to the grain quality requirements to meet the hidden hunger objectives demand additional crop production (Anonymous, 2017; Shewry *et al.* 2016). Despite facing numerous biotic challenges, wheat cultivation is particularly threatened by wheat rust, which poses a significant risk not only in India but also in various wheat-producing nations globally. Stem rust, caused by *P. graminis f. sp. tritici*, threatens around 7 million hectares in Central and Peninsular India. It is potentially most dreadful when the variety is susceptible and conditions are favourable for their development and hence, known as 'killer' disease of wheat because it kills wheat plant.

Stem rust occurs mainly on stems but can also be found on leaves, sheaths, glumes and awns. Initial symptoms appear as oval to elongate lesions with reddish-brown in colour. Stem rust pustules on leaves develop mostly on the dorsal

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side, but may penetrate and make limited sporulation on the frontal side. Severe infection of stems interrupts nutrient flow to the developing heads, resulting in shriveled grains and stems weakened by rust infection are prone to lodging. Stem rust is favoured by humid conditions and warmer temperatures of 15 to 35°C. It is the most devastating of the rust diseases and can cause losses of 50 per cent in one month when conditions for its development are favourable. Losses of 100 per cent can occur with susceptible cultivars. Therefore, it is imperative to investigate the quantification of the inoculum load of uredospores required to induce black rust in wheat.

MATERIALS AND METHODS

The present investigations were carried out at Department of Plant Pathology, C. P. College of Agriculture, and Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat during *Rabi* 2021-22 and 2022-23. An experiment was carried out in pot conditions utilizing three susceptible genotypes: A-9-30-1, Agra local, and Lok 1, which were sourced from the Wheat Research Station at Sardarkrushinagar Dantiwada Agricultural University in Vijapur. The cultivation of all varieties took place from November 15 to November 25.

Inoculation with P. graminis tritici

Wheat plants aged fifteen, thirty, and forty-five days were chosen for inoculation. Uredospores were obtained from plants infected with stem rust at the Wheat Research Station, Sardarkrushinagar Dantiwada Agricultural University, Vijapur. The collection of uredospores involved gently brushing the rust-infected plants with a camel hair brush, which was subsequently dipped in sterile distilled water. The resulting inoculum suspension was filtered through a double layer of muslin cloth, and the concentration of uredospores was adjusted to 10^2, 10^3, and 10^4 per ml using a hemocytometer. The inoculation of *P. graminis tritici* uredospores was performed using the syringe inoculation technique.

In each pot, five plants were selected. The syringe inoculation technique was employed on these selected plants. Firstly, a syringe needle was immersed in a suspension containing uredospores and approximately 5 ml of the suspension was drawn into the syringe. The syringe was then inserted into the stem of the plant and observed for bubble formation on the surface of the stem, which indicates successful inoculation. After this, , the syringe was removed and the procedure for the remaining plants was repeated (Fig. 1).

Fig. 1: Syringe Inoculation Method

No. of uredia/cm²

For each genotype, leaf discs exhibiting pustules with a diameter of 1.0 cm were collected and placed into test tubes containing 10 ml of sterilized distilled water. Five measurements were taken regarding the number of uredia per square centimeter. Uredospore counts were conducted using a heamocytometer under a microscope for each genotype at each concentration, and the data were presented as the number of uredia per square centimeter.

No. of pustules/cm²

For each genotype, an area of 1.0 cm² was designated on both halves of the leaf, with one marking on the right side and the other on the left side. Five measurements were taken regarding the number of pustules per cm² for each genotype at each concentration.

RESULTS

No. of uredia/cm²

The objective of this study is to examine the uredospore load generated by the stem rust pathogen *Puccinia graminis f. sp. tritici* across various wheat varieties and to assess its potential impact on crop damage. The number of uredia per square centimeter was recorded from plants aged 15, 30, and 45 days, which were inoculated with different concentrations of uredospores: 10^2, 10^3, and 10^4 uredospores/ml, respectively.

The data presented in Tables 1 and 2 illustrates the number of uredia per cm² in various genotypes inoculated with uredospore suspensions of differing concentrations. It was observed that as the concentration of the uredospore suspension increased (10^2, 10^3, and 10^4), the number of uredia per cm² also rose, specifically to 2.63 × 10t , 3.64 × 10^4 , and 4.85 × 10^4, respectively. Furthermore, the data concerning the number of uredia per cm² in relation to the age of the wheat plants indicated that as the age increased (15, 30, and 45 days), the number of uredia also increased, with values of 2.93 × 10^4 , 3.62 × 10^4, and 4.57 × 10^4, respectively. Notably, the genotype A-9-30-1 exhibited the highest number of uredia per cm² at 4.53 × 10^4 , followed by Agra local at 3.73 × 10^4 and Lok 1 at 2.86 × 10^4 .

Interaction effect of concentration and treatments

The information presented in Table 1 indicated that the interaction between concentration and treatments was statistically significant. The highest count of uredia/cm² (5.67 × 10^4) was recorded in A-9-30-1 at a concentration of 10^4 uredospores/ml, followed closely by Agra local with a count of 4.96 × 10^4 at the same concentration. Conversely, the lowest count of uredia/cm² (1.82 × 10^4) was noted in Lok 1 at a concentration of 10^2 uredospores/ml.

Interaction effect of days and treatments

The information presented in Table 2 indicated that the interaction between various ages of wheat plants (15, 30, and 45 days) inoculated with uredospore and the treatments applied was significant. The highest recorded number of uredia/cm² (5.89 × 10^4) was noted in A-9-30-1 for 45 day old plants, followed by Agra local with a count of 4.64 × 10^4 in 45 day old plants. Conversely, the lowest number of uredia/cm² (2.93 × 10^4) was found in Lok 1 at 15 days of age.

Interaction effect of days and concentration

The data presented in Table 3 revealed that the interaction of different age of wheat plant (15, 30 and 45 days) which inoculated with uredospore were found significant. The highest number of uredia per square centimeter (5.64 × 10^4) was observed in 45 day old plants treated with 10^4 uredospores per milliliter. In comparison, 30 day old plants exhibited a count of 4.76 × 10^4 uredia/cm², which was comparable to the 4.62 × 10^4 uredia/cm² found in 45 day old plants also treated with 10^4 uredospores/ml. Conversely, the lowest count of uredia/cm² (1.87 × 10^4) was recorded in 15 day old plants inoculated with 10^2 uredospores/ml.

Interaction effect of days, concentration and treatments

The data presented in Table 4 revealed that the interaction effect of days, concentration and treatments were found significant. The significantly superior numbers of uredia/cm² (6.60 × 10^4) recorded in A-9-30-1 at 10^4 uredospores/ml on 45 days old plants. The numbers of uredia/cm² recorded in A-9-30-1 (6.13 × 10^4) at 10^3 uredospores/ml on 45 days old plants, which was at par with Agra local (5.87 × 10^4) at 10^4 uredospores/ml on 45 days old plants. The significantly inferior numbers of uredia/cm² (1.47 × 10^4) observed in Lok 1 at 10^2 uredospores/ml on 15 days old plants.

No. of pustules/cm²

The progression of the disease was further investigated by assessing the quantity of pustules formed on the leaf sheath. This assessment was conducted across various plant varieties and quantified as the number of pustules per square centimeter for plants aged 15, 30, and 45 days,

Table 1: Numbers of uredia/cm^{2}of wheat genotypes inoculated with different uredospore concentrations at 15, 30 and 45 days old plants

Treatments	No of uredia/cm^2(× 10^4)			
	Concentration			Mean (T)
	10^2	10^3	10^4	
A-9-30-1	3.51	4.40	5.67	4.53
Agra local	2.56	3.69	4.96	3.73
Lok 1	1.82	2.82	3.93	2.86
Mean (C)	2.63	3.64	4.85	-
	C	T	C × T	
	(Concentration)	(Treatment)	(Concentration × Treatment)	
S. Em. ±	0.03	0.03	0.05	
C.D. at 5%	0.09	0.09	0.15	
C.V.%	4.28			

Table 2: Number of uredia/cm^{2}of wheat genotypes inoculated at 15, 30 and 45 days old plants

Treatments	No of uredia/cm^2(× 10^4)			
	Concentration			Mean (T)
	15 days	30 days	45 days	
A-9-30-1	3.29	4.40	5.89	4.53
Agra local	2.89	3.67	4.64	3.73
Lok 1	2.60	2.80	3.18	2.86
Mean (C)	2.93	3.62	4.57	-
	D	T	D × T	
	(Days)	(Treatment)	(Days × Treatment	
S. Em. ±	0.03	0.03	0.05	
C.D. at 5%	0.09	0.09	0.15	
C.V.%	4.28			

Table 3: Number of uredia/cm^{2}of different uredospore concentrations at15, 30 and 45 days old plants

Concentration	No of uredia/cm^2 (× 10^4)			
	15 days	30 days	45 days	Mean (C)
10^2	1.87	2.58	3.44	2.63
10^3	2.76	3.53	4.62	3.64
10^4	4.14	4.76	5.64	4.85
Mean (D)	2.93	3.62	4.57	-
	D	C	D × C	
	(Days)	(Concentration)	(Days × Concentration)	
S. Em.±	0.03	0.03	0.05	
C.D. at 5%	0.09	0.09	0.15	
C.V.%	4.28			

Table 4: Number of uredia/cm^{2}of wheat genotypes inoculated with different uredospore concentrations at 15, 30 and 45 days old plants

Genotypes	No of uredia/cm^2 (× 10^4)								
	15 days			30 days			45 days		
	10^2	10^3	10^4	10^2	10^3	10^4	10^2	10^3	10^4
A-9-30-1	2.27	2.94	4.67	3.34	4.13	5.74	4.93	6.13	6.60
Agra local	1.87	2.67	4.13	2.47	3.67	4.87	3.33	4.73	5.87
Lok 1	1.47	2.66	3.67	1.93	2.80	3.67	2.07	3.00	4.47
Mean	1.87	2.76	4.16	2.58	3.54	4.76	3.44	4.62	5.65
	D × C × T								
	(Days × Concentration × Variety)								
S. Em.±	0.09								
C.D. at 5%	0.26								
C.V.%	4.28								

Table 5: Number of pustules/cm^2 of wheat genotypes inoculated with different uredospore concentrations at 15, 30 and 45 days old plants

Treatments	No of pustules/cm^2 Concentration			Mean (T)
	10^2	10^3	10^4	
A-9-30-1	2.56	3.64	4.47	3.56
Agra local	2.31	3.27	4.18	3.25
Lok 1	1.98	2.67	3.51	2.72
Mean (C)	2.28	3.19	4.05	-
	C	T	C × T	
	(Concentration)	(Treatment)	(Concentration × Treatment)	
S. Em. ±	0.03	0.03	0.05	
C.D. at 5%	0.09	0.09	0.15	
C.V.%		4.99		

Table 6: Number of pustules/cm^2 of wheat genotypes inoculated at 15, 30 and 45 days old plants

Treatments	No of pustules/cm^2 Concentration			Mean (T)
	15 days	30 days	45 days	
A-9-30-1	2.84	3.38	4.44	3.56
Agra local	2.58	3.11	4.07	3.25
Lok 1	2.18	2.71	3.27	2.72
Mean (C)	2.53	3.07	3.93	-
	D	T	D × T	
	(Days)	(Treatment)	(Days × Treatment	
	0.03	0.03	0.05	
S. Em. ±				
C.D. at 5%	0.09	0.09	0.15	
C.V.%		4.99		

Table 7: Number of pustules/cm^2 of different uredospore concentrations at 15, 30 and 45 days old plants

Concentration	No of pustules/cm^2			
	15 days	30 days	45 days	Mean (C)
10^2	1.93	2.24	2.67	2.28
10^3	2.51	3.16	3.91	3.19
10^4	3.16	3.80	5.20	4.05
Mean (D)	2.53	3.07	3.93	-
	D	C	D × C	
	(Days)	(Concentration)	(Days × Concentration)	
S. Em. ±	0.03	0.03	0.05	
C.D. at 5%	0.09	0.09	0.15	
C.V.%		4.99		

Table 8: Number of pustules/cm^2 of wheat genotypes inoculated with different uredospore concentrations at 15, 30 and 45 days old plants

Genotypes	Number of pustules/cm^2								
	15 days			30 days			45 days		
	10^2	10^3	10^4	10^2	10^3	10^4	10^2	10^3	10^4
A-9-30-1	2.20	2.87	3.47	2.53	3.53	4.07	2.93	4.53	5.87
Agra local	2.00	2.53	3.20	2.20	3.27	3.87	2.73	4.00	5.47
Lok 1	1.60	2.13	2.80	2.00	2.67	3.47	2.33	3.20	4.27
Mean (D)	1.94	2.52	3.16	2.25	3.16	3.94	2.67	3.91	5.20
				D × C × T					
				(Days × Concentration × Variety)					
S. Em. ±				0.09					
C.D. at 5%				0.26					
C.V.%				4.99					

which were inoculated with concentrations of 10^2, 10^3, and 10^4 uredospores per milliliter.

he findings presented in Table 5 and 6 illustrate the number of pustules per square centimeter across different genotypes of wheat plants inoculated with varying concentrations of uredospore suspension. The results indicated that as the concentration of the uredospore suspension increased specifically 10^2, 10^3, and 10^4 , the number of pustules per square centimeter also rose, with values of 2.28, 3.19, and 4.05, respectively. Additionally, the data concerning the number of pustules per square centimeter in relation to the age of the wheat plants demonstrated that as the age increased specifically at 15, 30, and 45 days, the number of pustules also increased, yielding values of 2.53, 3.07, and 3.93. Notably, the genotype A-9-30-1 exhibited the highest number of pustules per square centimeter at 3.56, followed by Agra local at 3.25 and Lok 1 at 2.72.

Interaction effect of concentration and treatments

The data presented in Table 5 revealed that the interaction effect of concentration and treatments were found significant. The significantly superior numbers of pustules/cm^2 (4.47) observed in A-9-30-1 at 10^4 uredospores/ml followed by Agra local (4.18) at 10^4 uredospores/ml. The significantly inferior numbers of pustules/cm^2 (1.98) observed in Lok 1 at 10^2 uredospores/ml.

Interaction effect of days and Treatments

The data presented in Table 6 revealed that the interaction of different age of wheat plants (15, 30 and 45 days) which inoculated with uredospore and treatments were found significant. The significantly superior numbers of pustules/cm^2 (4.44) recorded in A-9-30-1 on 45 days old plants followed by Agra local (4.07) on 45 days old plants. The significantly inferior numbers of pustules/cm^2 (2.18) observed in Lok 1 on 15 days old plant.

Interaction effect of days and concentration

The data presented in Table 7 revealed that the interaction of different age of wheat plant (15, 30 and 45 days) which inoculated with uredospore concentration was found significant. The significantly superior numbers of pustules/cm^2 (5.20) recorded on 45 days old plants with 10^4 uredospores/ml. The pustules/cm^2 (3.91) recorded on 45 days old plants with 10^3 uredospores/ml, which was at par with 3.80 pustules/cm^2 recorded on 30 days old plants with 10^4 uredospores/ml. The significantly inferior numbers of pustules/cm^2 (1.93) recorded on 15 days old plants with 10^2 uredospores/ml.

Interaction effect of days, concentration and treatments

The data presented in in Table 8 indicated that the interaction effects of days, concentration, and treatments were statistically significant. The highest number of pustules per square centimeter (5.87) was noted in the A-9-30-1 treatment at a concentration of 10^4 uredospores/ml on 45 day old plants, followed closely by the Agra local variety, which recorded 5.47 pustules/cm^2 under the same conditions. Conversely, the lowest number of pustules/cm^2 (1.60) was found in the Lok 1 variety at a concentration of 102 uredospores/ml on 15 day old plants.

DISCUSSION

Result of the present study on no. of uredia/cm^2 is similar to the presented by Danelli and Reis (2016) who reported that two soybean cultivars produced highest of 4012.8 spores/cm^2 and 7348.4 uredospores/cm^2, respectively. The concentration of 4×10^4 uredospores/ml resulted in a disease intensity that allows safe differentiation between susceptible and resistant cultivars in the seedling stage (Turra *et al*. 2017). Cheng *et al.* (2019) observed the number of urediniospores/cm^2 on leaves among switchgrass cultivars, which ranged from 44,228.3 (Cloud 9) to 653.1 (Prairie Sky). Sharma *et al.,* (2022) reported that highest mean number of uredia (6.45×10^4 uredospore/cm^2) observed in A-9-30-1. Similarly, result on No. of pustules/cm^2 obtained in this study conformed to those presented by Salih and Al-Hamdany (2012) who reported that under severe artificially induced epiphytotic conditions, pustule density per cm^2 on flag leaves varied from 15 in the mutant SA/12

to 127 in the Golden Melon cultivar and the naked barley strain Aamer. Pustules on moderately resistant genotypes caused by pea rust were relatively small, measuring between 1.5 and 1.7 mm. In moderately susceptible genotypes, pustule sizes exhibited considerable variation, ranging from 1.3 to 4.4 mm, while susceptible genotypes showed sizes between 2.9 and 4.8 mm. The largest pustule sizes, measuring 4.2 to 4.6 mm, were observed in highly susceptible genotypes (Upadhyay *et al*. 2017). Cheng *et al*. (2019) documented that the average number of pustules per cm^2 on host leaf surfaces varied among switchgrass cultivars, ranging from 29.5 in Summer to 3.6 in Prairie Sky. Additionally, Sharma *et al*. (2022) noted that the highest mean number of pustules (25.25 pustules/cm^2) was recorded in the A-9-30-1 treatment.

DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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