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RESEARCH ARTICLE **OPEN ACCESS**

EFFICIENT APPROACHES (IN VITRO) FOR MANAGEMENT OF KARNAL BUNT OF WHEAT AGAINST NEOVOSSIAINDICA

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ABSTRACT

Wheat (*Triticumaestivum* L) is second most important cereal crop of India after rice and it grown throughout the country. It is a major staple crop of India and their quality of grain is reduced through infection of different diseases, among them Karnal bunt (*Neovossiaindica*) is one of major pathogen. It is of high quarantine importance which restrict global trade of wheat between different countries. In the present study, an*in vitro*attempt has been made to correlate effect of temperature (15-30)°C on teliospore germination as well as effect of systemic fungicides on mycelial growth of *Neovossiaindica*. During teliospore germination, maximum germination (90%) recorded at 20ºC after 28 days of incubationwhile minimum germination (32%) recorded at 30ºC after 7 days of incubation. The germination of teliospores increased progressively with an increase in temperature from 15 to 20ºC but decreased further significantly. Different systemic fungicides (Tilt 25EC, Amistar Top 325 SC, Folicur 25 EC, Bavistin 50WP and Vitavax 75 WP) were tested against Karnal bunt of wheat using Poison Food Technique and result revealed thatTilt 25 EC recorded maximum mycelial growth inhibition of (97.21%) followed by Amistar Top 325SC (96.88%) and minimum mycelial growth inhibition was recorded by Bavistin 50WP (94.22%).

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INTRODUCTION

Wheat is an essential food crop of India which play an important role in human diet as it is a rich source of protein, carbohydrate, vitamins and minerals. *Neovossiaindica* causing Karnal bunt of wheat is a seed borne pathogen. It is a heterothallic fungus i.e., producing opposite mating types for sexual reproduction and characterized by presence of individual self-sterility, with bipolar mating system (governed by a single allelic mating locus) (Duran and Cromarty, 1977).There is a need to understand effect of various temperatures encountered in the teliosporic phase of disease cycle during field as well as storage of wheat at new places.Development of Karnal bunt depends on the availability of favourable conditions like optimum temperature. Temperature plays important role in the survival of primary sporidia of *Neovossiaindica* as they are temperature sensitive. The optimal conditions for disease infection that promotes KB development are high relative humidity, cloudiness with mild temperatures and rainfall during anthesis. In addition, daytime temperatures between (18–24)◦C and soil temperatures in the range of (17–21)◦C increase KB severity and are correlated with KB sporidial showering, rendering favorable the KB infection climate for wheat. In Northern India, this condition is usually prevalent during February to March (Rush *et al*., 2005).

Karnal bunt is known to be a quarantine diseaseand cause economic damage by limiting potential export markets. This makes Karnal Bunt a challenge to the grain industry as it constitutes a global non-tariff barrier to the wheat trade (Gurjar*et al.,* 2019). The disease received recognition after becoming major Sanitary and phytosanitary (SPS) measure as stipulated by World Trade Organization (WTO) during 1999 to avoid the spread of infected kernels from wheat exporting countries. The management of Karnal bunt of wheat has become a major concern in India. The gravity of the situation of the disease calls for evaluation of fungicitoxicants against the disease for its management. Epidemiological factors have great influence on the epidemic development of Karnal bunt disease. Wheat is vulnerable to Karnal bunt fungus only during a 2-3 week of favorable weather conditions like temperature and humidity.

MATERIAL AND METHODS

This experiment was conducted during 2022-23 in the laboratory of Department of Plant Pathology, Khalsa College, Amritsar.

Germination of teliosporesat different temperature: Infected kernels of wheat were procured from KVK Nag Kalan, Amritsar and division of Plant Pathology, PAU Ludhiana. Free teliosporeswere obtained by

lightly crushing severely affected seeds, graded from class 3 to class 5 (Warham *et al*., 1986) with a mortar and pestle. Spore suspension of 10mg of teliospores in 100 ml of sterilized water was prepared by centrifuging the solution at 10,000 rpm for 2 minutes which will allow the teliospores to settle at the bottom.

Table 1. Rating scale used to determine level of resistance/ susceptibility

Disease rating scale	% Grain infection	Level of resistance or susceptibility
0	No infection	Highly resistant
1	1 % or less bunted grain	Resistant
2	1.1-2 % bunted grain	Moderately Resistant
3	2.1-5 % bunted grain	Moderately susceptible
4	5.1-10 % bunted grain	Susceptible
5	More than 10 % bunted grain	Highly susceptible

To evaluate the germination, teliospores were spread uniformally on potatodextrose agar mediumon petri plates with the help of macro-pipette and incubated at (15-30)°C in a BOD incubator in darkness by covering them in foil paper. After 7, 14, 21 and 28 days of incubation, the petri plates were examined to record the radial germination.Three series were randomly assigned to each temperature which served as replication. Radial growth of mycelium in petri plates was measured in mm.

In vitro* evaluation of fungicides against *Neovossiaindica: Evaluation of four fungicides viz., Tilt 25 EC, Amistar Top 325SCBavistin 50 WP, Vitavax 75 WP, and Folicur 25 EC was done against radial growth of *Neovossiaindica* at different concentrations by using Poisoned Food Technique (Schmitz, 1930). Each fungicide stock solution was prepared in sterilized distilled water by dissolving double the fungicide required in the measured volume of sterilized distilled water. The calculated volume of stock solution was then added to double strength sterilized PDA, yielding final concentrations of 50, 100 and 200 ppm. The stock solution was mixed with sterilized distilled water to obtain the desired concentrationswhich were calculated using the formula: C1V1= C2V2. The medium containing various fungicide concentrations was poured (20 ml) into each sterilised petri plate and allowed to solidify. Each petri plate was centrally inoculated with a 10 mm mycelial disc cut with a sterilized cork borer from a 15-day-old test fungus culture, and plates without fungicides served as controls. Three replications were maintained for each treatment and incubated at 20±1 °C. Regular observations were recorded and finally the colony diameter was measured 25 days after inoculation. Percent inhibition of mycelial growth was calculated by Vincent (1947) formula:

$$I\ (\%) = \frac{(C - T)}{C} \times 100$$

Where,

I = Percent inhibition
C = Colony diameter in control
T = Colony diameter in treatment

Statistical analysis

The data from laboratoryexperiment was subjected to appropriate statistical analysis using standard procedures and Completely Randomized Design (CRD) in laboratory experiments, described by Gomez and Gomez (1984). Critical difference (CD) among the treatment in various experiment at 5 per cent level was calculated. All the statistical analysis was operated on OPSTAT software. Cluster analysis (Dendogram) was used for analysis of temperature affecting teliospore germination using PAST software.

RESULTS AND DISCUSSION

Effect of different temperature on teliospore germination of *Neovossiaindica*: Germination was determined after various times of incubation.The data on the effect of temperature on germination of teliospores is described in the Table 2, Plate 1 and Fig. 1.

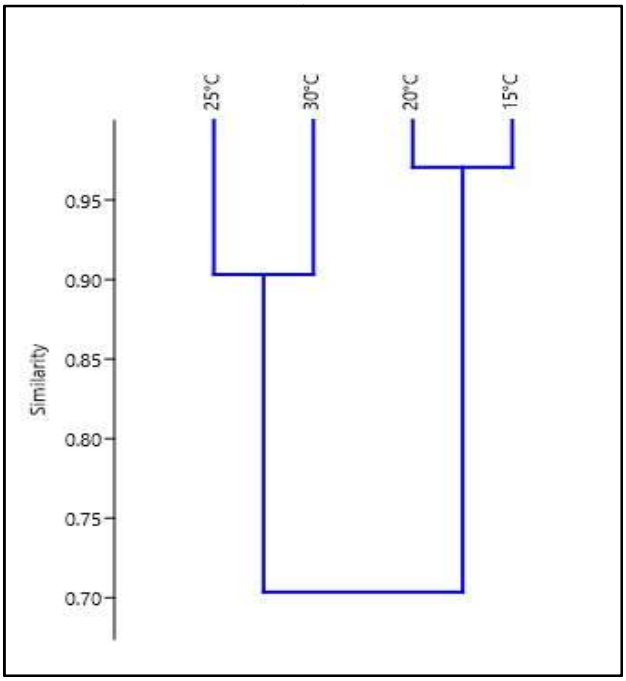

Fig. 1. Dendogram representing relation between different temperature affecting teliospore germination

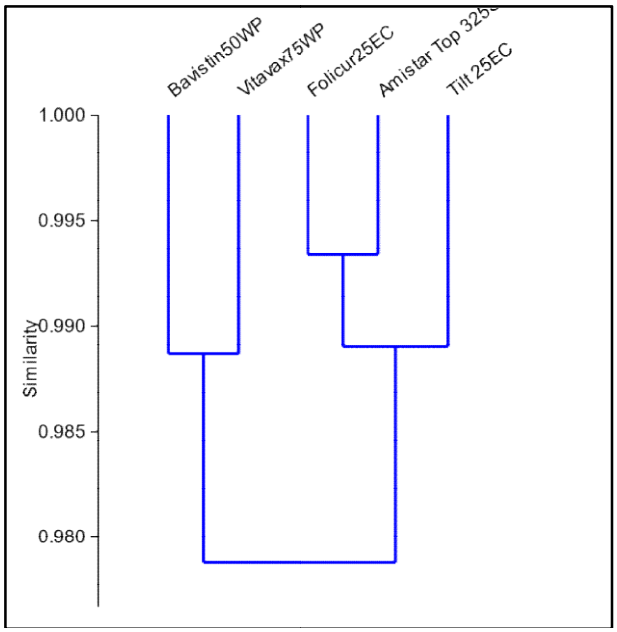

Fig. 2. Dendogram representing the relation between effect of fungicides on mycelial growth inhibition of *Neovossiaindica*

The teliospores of the Karnal bunt germinated at all the temperatures ranging from 15◦C to 30ºC.Maximum germination of teliospores (90%) was observed at 20ºC at 28 DAI, while, minimum germination of the teliospores was recorded at 30ºC at 7 DAI. The germination of teliospores increased progressively with an increase in temperature from (15-20)ºC but decreased further significantly.Nearly 40 to 90 per cent germination of teliospores of KB was obtained between (15-20)ºC. At 15ºC, minimum germination was recorded at 7DAI i.e., 41.7 per cent which gradually increase at 14, 21 and 28 DAI by 57, 72 and 90 per cent, respectively. At 20ºC, 47.2 per cent germination was recorded at 7 DAI followed by 62.3, 77 and 90 per cent germination at 14, 21 and 28 DAI, respectively. At 25ºC, 37.2 per cent teliospore germination was recorded at 7DAI followed by 39.1, 42 and 42 per cent at 14, 21 and 28 DAI, respectively. At 30ºC, germination was minimum at 7 DAI i.e., 32 per cent which slightly increased at 14DAI by 32.6 per cent but stayed constant at 21 and 28 DAI by 33.7 per

cent germination. These findings are in line with that of Joshi (2006) who reported that maximum germination of teliospores 71.1 per cent was observed at 20ºC followed by 68.3 per cent at 25ºC. Critical analysis of data revealed that optimum temperature for germination of teliospores ranges between (15-20)ºC. Similar results were evaluated by More *et al.* (2018) who concluded that maximum teliospore germination was observed at 20ºC temperature.

Tilt 25 EC at 200 ppm resulted in maximum growth inhibition (97.21%) followed by Amistar Top 325SC (96.88%), Folicur 25EC (95.21%), Vitavax 75 WP (94.56%) and Bavistin 50 WP (94.22%). The results of the present study had positive support from the study conducted by Kumar *et al.* (2014) who reported that Tilt 25ECwas found best for mycelial growth inhibition of *N. indica*. Singh *et al.* (2018) observed similar results which depicts that Tilt 25EC showed

Table 2. Effect of Temperature on teliospore germination of *Neovossiaindica*

Temperature (°C)	Teliospore germination (%)				Mean
	7 DAI	14DAI	21DAI	28DAI	
15	41.7	57	72	90	65.18
	(40.22)	(49.02)	(58.05)	(71.57)	
20	47.2	62.3	77	90	69.13
	(43.39)	(52.12)	(61.34)	(71.57)	
25	37.2	39.1	42	42	40.08
	(37.58)	(38.70)	(40.40)	(40.40)	
30	32	32.6	33.7	33.7	33.00
	(34.45)	(34.82)	(35.49)	(35.49)	
C.D(p=0.05)	1.37	1.21	1.6	2.19	51.84
SE(m)	0.41	0.36	0.48	0.66	

DAI: Days After incubation
Figures in parenthesis are the Arc Sine transformed values

Table 3. Effect of fungicides on mycelial growth inhibition of *Neovossiaindica*

Fungicide	Mycelial growth (mm)* at different concentrations (ppm)			Mycelial inhibition (%) at different concentration (ppm)		
	50	100	200	50	100	200
Tilt 25EC	24.83	12.03	2.79	75.17	87.97	97.21
				(60.11)	(69.71)	(80.38)
Folicur25EC	27.97	14.23	4.79	72.03	85.77	95.21
				(58.07)	(67.84)	(77.36)
Bavistin50WP	32.97	18.12	5.78	67.03	81.88	94.22
				(54.96)	(64.81)	(76.09)
Vitavax75WP	29.73	16.13	5.44	70.27	83.87	94.56
				(56.96)	(66.32)	(76.51)
Amistar Top 325SC	26.71	13.79	3.12	73.29	86.21	96.88
				(58.88)	(68.20)	(79.83)
Control	100	100	100	0	0	0
CD (p=0.05)	3.59	3.29	1.83	3.08	3.44	2.88
SE(m)	1.12	1.03	0.57	0.96	1.04	0.88

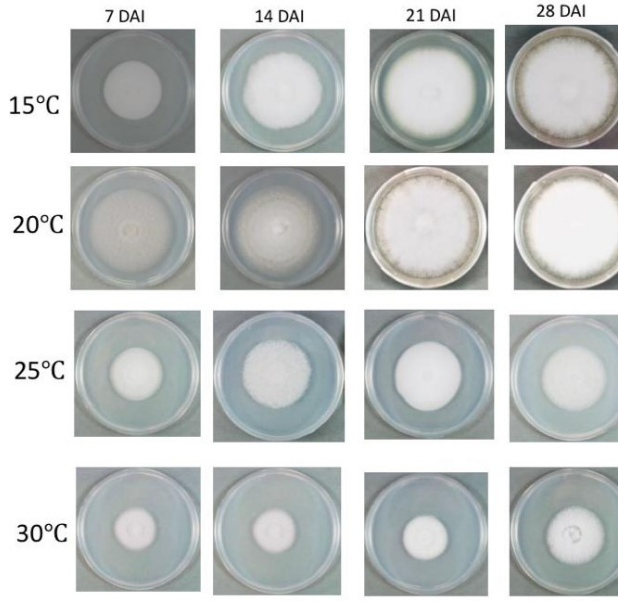

Plate 1. Effect of temperature on teliospore germination of Neovossiaindica

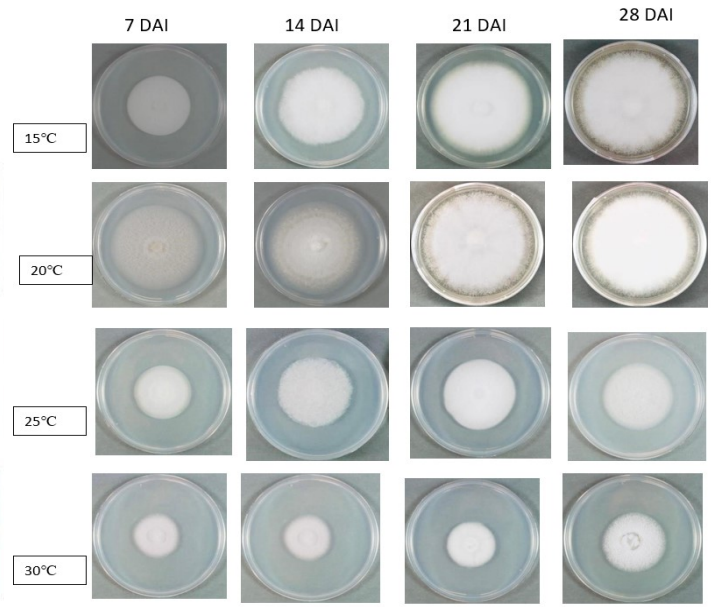

Plate 2. Effect of fungicides on mycelial growth inhibition of *Neovossiaindica*

Effect of test fungicides on mycelial growth of Neovossiaindica: Data of the mycelial growth inhibition of *Neovossiaindica* by four fungicides at five concentrations was recorded and results are presented in Table 3, Plate 2 and Fig 2. Data in the table revealed that, the efficacy of different fungicides at different concentrations on percent inhibition of mycelial growth of *Neovossiaindica* differed significantly.

maximum mycelial inhibition. Dendogram 2 represents that Tilt 25EC, Amistar Top 325SC and Folicur 25EC are in similar cluster, while Bavistin 50WP and Vitavax 75 WP are in similar cluster.

CONCLUSION

From above study, it can be concluded that optimum temperature for teliospore germination of *Neovossiaindica* is 20ºC. Temperature

parameter from figure1 reveals that (15 and 20)ºC are favorable temperature for maximum teliospore germination. From dendogram figure 2 it is clear that Karnal bunt of wheat can be successfully managed by use of single spray of Tilt 25 EC which recorded mycelial inhibition of 97.21 per cent.

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