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In vitro evaluation of fungicides and plant extracts against the mycelial growth of *Neovossia indica*

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Abstract
Karnal bunt (KB) of wheat caused by *Tilletia indica* Mitra (*Nevossia indica* Mundkur), was initially documented in 1931 in Karnal, India and named as 'Karnal bunt'. Karnal Bunt is difficult to control because of its intermittent nature and it has a significant impact on yield and grain quality of wheat which makes it inedible. Foliar fungicides can greatly minimize disease occurrence. The current study focuses on the influence of fungicides and plant extracts on the inhibition of *Neovossia indica* mycelial growth in the laboratory. Four fungicides *viz*., Bavistin 50 WP, Vitavax 75 WP, Tilt 25 EC and Folicur 25 EC and three locally available botanicals *viz.*, Turmeric (*Curcuma longa*), Eucalyptus (*Eucalyptus globulus*) and Lantana shrub (*Lantana camara*) were evaluated against the radial growth of *Neovossia indica* by Food Poison Technique under lab conditions and out of four fungicides, at 200 ppm concentration Tilt 25 EC resulted in maximum mycelial growth inhibition (96.75%) followed by Folicur 25 EC (95.51%), Bavistin 50 WP (94.41%) and Vitavax 75 WP (92.50%). Similarly, in case of plant extracts tested *Lantana camara* show maximum inhibition (92.55%) at 75% concentration followed by *Eucalyptus globulus* (91.76%) and *Curcuma longa* (89.69%).

Keywords: Karnal bunt, *Neovossia indica*, fungicides, plant extracts, mycelial growth

Introduction
Wheat (*Triticum aestivum* L.) is the most important single product cultivated both as a food and the source of income in many countries of the world. India is the second largest wheat growing country in the world after China and contributes one fourth (27%) of the worldwide wheat production and covers one fifth of the total cropped area of the world. Wheat is primarily a Rabi crop and its production is highly concentrated in the north belt of Uttar Pradesh, Punjab and Haryana. Fungal, bacterial and viral diseases are major causes which are responsible for low yield in wheat among which the fungal diseases are found to be most predominant stresses. Wheat is affected by various fungal diseases such as rusts, Powdery mildew, loose smut, leaf blight and Karnal bunt and amongst these Karnal bunt is the major disease of wheat which affects the quality of grains make it unfit for consumption. Generally grain infection more than 3 percent bunted kernels is considered unfit for human consumption. Karnal bunt is a quarantine disease that impacts the worldwide trade of commercial wheat grain. Approximately 70 countries place quarantine restrictions on movement of wheat from countries where Karnal bunt is known to occur (Lari *et al*., 2006) [7]. The rejection of such produce by nations where Karnal bunt is not known to occur, results in significant costs for meeting international quarantine requirements in addition to the direct quantitative and qualitative losses in grain yield and quality. As a result of its sporadic occurrence and the timing of infection and damage, which coincide with late stages of host growth, Karnal bunt is more difficult to control than other systemic smuts.

Material and Methods
Current study emphasizes on the *in vitro* evaluation of fungicides and plant extracts against the mycelial growth of *Neovossia indica* and the experiment was conducted in P.G. Department of Agriculture, Khalsa College Amritsar, Punjab, India. The following materials and methodologies were used during the study.

Isolation and purification of *Neovossia indica*
Neovossia indica was isolated from wheat grains that had been infected by the Karnal bunt. By gently crushing severely damaged seeds, categorised to category 3 to 5 (Warham *et al*., 1986) [12], with a mortar and pestle, free teliospores were obtained.

A suitable teliospore suspension in 200 ml of sterilised water was made for each infected sample. A 120 mesh sieve was used to filter teliospores suspended in water which retained larger debris. The filterate was centrifuged at 12000 rpm for two minute, causing the spores to settle into a pellet and excess of water was decanted out. The teliospores were disinfected with commercial bleach (5% chlorox) for 30 seconds. Once more, the sort of pellet was used to collect the teliospores, and the chlorox was decanted out. The teliospores were once more centrifuged and then rinsed twice in sterile water. To reduce the chance of contamination, the entire procedure was repeated three times. In the test tubes containing the cleaned teliospores, sterile water was added to create a spore suspension. With the aid of a macro-pipette, few drops of the spore suspension were added to each of several petri-plates filled with plain agar medium and spread with L shaped spreader. The composition of plain agar medium was 20 g agar dissolved in one liter of water. Petri plates were incubated at a temperature of 15–20 °C under a 14–10 hour cycle of light and dark. After 25-30 days of incubation, teliospores germinated and primary sporidia were visible on the surface of plain agar medium in petri-plates in the form of thread like or star like structures.

Secondary sporidia were grown on PDA using the primary sporidial culture. To accomplish this, 100 ml of autoclaved PDA was placed in each 250 ml flask, and the media was solidified in a slanting position in the flasks. The slants were then inoculated with a primary sporidial culture by gently placing an inverted culture block size of about 3×3 mm (making angle) on the upper edge of the slants. The inverted block of the culture facilitated the sporidia, frequently shedding on the medium. The culture slants in flasks were incubated at 18-20 °C. Within 5 to 6 days secondary sporidia started to discharge forcibly from primary sporidial culture in the form of brittle, crustaceous, unibonate colonies with dendric margins. Thousands of the sickle shaped and allantoid type secondary sporidia were observed under the microscope.

In vitro* evaluation of fungicides against *Neovossia indica

Evaluation of four fungicides *viz*., Bavistin 50 WP, Vitavax 75 WP, Tilt 25 EC and Folicur 25 EC was done against radial growth of *Neovossia indica* at different concentrations by using Poisoned Food Technique (Schmitz, 1930) [10]. 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 25, 50, 100, 200, and 250 ppm. The stock solution was mixed with sterilized distilled water to obtain the concentrations listed above, which 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) [11] formula.

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

In vitro* evaluation of plant extracts against *Neovossia indica

Evaluation of water extracts of three locally available botanicals *viz.*, Turmeric (*Curcuma longa*), Eucalyptus (*Eucalyptus globulus*) and Lantana shrub (*Lantana camara*) was done against radial growth of *Neovossia indica* at different concentrations by using Poisoned Food Technique (Schmitz, 1930) [10]. The plant's water extracts were made by crushing the leaves and making a stock solution of the extract by combining 200 g of crushed leaves in 200 ml of distilled water using a grinder. The mixture was filtered through muslin cloth and centrifuged for 20 minutes at 3500 rpm. Whatmann filter paper was used to filter the supernatant. The calculated volume of stock solution was then added to double strength sterilised PDA to obtain final concentrations of 25, 50, 75, and 100%. The stock solution was used to sterilise distilled water in order to obtain the above concentrations, which were calculated using the formula: C1V1=C2V2. The medium containing various plant extract concentrations was poured (20 ml) into each sterilised petri plate and allowed to solidify. Each petri plate was centrally inoculated with 10 mm mycelial disc cut with the help of sterilized cork borer from 15 days old culture of test fungus and plates without plant extracts served as check. Three replications were maintained for each treatment and incubated at 20±1 °C. The colony diameter was measured 25 days after inoculation and percent inhibition of mycelial growth was calculated by Vincent (1947) [11] formula.

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

Results and Discussion

Data of the mycelial growth inhibition of *Neovossia indica* by four fungicides at five concentrations was recorded and results are presented in Table 1. Data on the table revealed that, the efficacy of different fungicides at different concentrations on percent inhibition of mycelial growth of *Neovossia indica* differed significantly. All the tested fungicides completely inhibited mycelial growth of *N. indica* at 250 ppm. However, Tilt 25 EC at 200 ppm resulted in maximum growth inhibition (96.75%) followed by Folicur 25 EC (95.51%), Bavistin 50 WP (94.41%) and Vitavax 75 WP (92.50%). The result of the present study has positive support from the study conducted by Kumar *et al*. (2014) [6] who reported that Tilt and Folicur were best for mycelial growth inhibition of *N. indica.* Kapadiya *et al*. (2013) [4] and Balai *et al*. (2013) [1] found that Tilt 25 EC and Folicur at 200 ppm concentrations were the best fungicides for inhibiting *N. indica* mycelial growth. Bavistin 50 WP at 250 ppm concentration inhibited *N. indica* almost completely, according to Krishna (1979) [5].

At 100 percent concentration, all plant extracts resulted in cent percent inhibition of mycelial growth of *N. indica*. However at 75 percent concentration *Lantana camara* show

maximum inhibition (92.55%) followed by *Eucalyptus globulus* (91.76%) and *Curcuma longa* at the same concentration show 89.69% inhibition (Table 2). The results of this study corroborated with the findings of Kumar *et al.* (2014) [6] who recorded that *Lantana camara*, *Eucalyptus globulus* and *Curcuma longa* were equally effective against the mycelial growth of *N. indica* at higher concentration but at lesser concentration *Curcuma longa* and *Eucalyptus globulus* give better results. There have been few reports of botanicals being tested in lab against *Neovossia indica*. However, previous researchers have reported antifungal activity of *Lantana camara* against other plant pathogens (Dutta and Deb 1986, Meena and Muthusamy 1998, El- Mohamedy and Abdalla 2014 and Sales *et al*., 2016) [2, 8, 3, 9].

Table 1: Mycelial growth inhibition of *Neovossia indica* by test fungicides

Fungicides	Mycelial inhibiton (%) at different concentrations (ppm) of fungicides					
	25	**50**	**100**	**200**	**250**	**Mean**
Bavistin 50 WP	39.99 (39.23)	53.31 (46.90)	84.34 (66.96)	94.41 (76.32)	100 (89.96)	74.41 (59.61)
Vitavax 75 WP	41.21 (39.94)	53.62 (47.08)	80.28 (63.64)	92.50 (74.11)	100 (89.96)	73.52 (59.03)
Tilt 25 EC	46.93 (43.24)	54.05 (47.32)	83.54 (66.06)	96.75 (79.61)	100 (89.96)	76.25 (60.83)
Folicur 25 EC	43.80 (41.44)	54.42 (47.54)	82.47 (65.45)	95.51 (77.77)	100 (89.96)	75.29 (60.19)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	34.39 (35.90)	44.08 (41.60)	66.13 (54.41)	75.83 (60.55)	80.00 (63.44)	
C.D. (p=0.05)	Fungicides = 0.15, Concentration= 0.17 Fungicides × concentration = 0.34					

*average of three replications
Figure in parenthesis are arc sine transformed values
**combination fungicides

Table 2: Mycelial inhibition of *Neovossia indica* by plant extracts

Plant extracts	Mycelial inhibition (%)* at different concentrations (%) of plant				
	25	50	75	100	Mean
Curcuma longa	38.37 (38.27)	80.20 (63.58)	89.69 (71.27)	100 (90.00)	77.07 (61.39)
Eucalyptus globulus	46.90 (43.22)	78.60 (62.44)	91.76 (74.16)	100 (90.00)	79.51 (63.09)
Lantana camara	45.87 (42.63)	81.20 (64.30)	92.55 (73.32)	100 (90.00)	79.70 (63.22)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	32.79 (34.93)	60.00 (50.77)	68.50 (55.86)	75.00 (60.00)	
C.D. (p= 0.05)	Plant extracts = 0.26, concentrations = 0.30 Plant extracts × concentrations = 0.53				

*average of three replications
Figure in parenthesis are arc sine transformed values

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