



In Vitro Management of Karnal Bunt of Wheat by Using Fungicides and Botanicals

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Abstract

The study was conducted during November, 2023–April, 2024 at laboratory of Plant Pathology, PG Department of Agriculture, Khalsa College, Amritsar, Punjab, India to investigate the comparative analysis of two different methods of management of Karnal bunt i.e., through botanicals and fungicides. Efficacy of four botanicals viz., lantana (*Lantana camara*), eucalyptus (*Eucalyptus tereticornis*), datura (*Datura stramonium*) and neem (*Azadirachta indica*) was tested at three different concentrations (10, 25, 50%) against *Neovossia indica* the causal agent of Karnal bunt in wheat. Additionally, five fungicides namely, Tilt 25 EC, Antracol 70 WP, Score 25 EC, Nativo 75 WG and Dithane M-45 75 WP was evaluated at three different concentrations (50, 100, 150 ppm) under in vitro conditions on inhibition in mycelial growth of *Neovossia indica*. Results revealed that Datura showed the highest average mycelial inhibition of 55.03% followed by Neem (46.42 %) and Eucalyptus (37.63%) and Lantana was found least effective with average mycelial inhibition of 11.33%. Among the five tested fungicides, Tilt 25 EC was found to be the most effective with maximum average mycelial growth inhibition of 95.40% followed by Nativo 75 WG (87.28%), Score 25 EC (66.51%) and Dithane M-45 75 WP (62%). However, the minimum average mycelial inhibition of 47.69% was recorded in Antracol 70 WP. The study highlighted the potentiality of both botanicals and fungicides in managing Karnal bunt, with varying degrees of efficacy.

Keywords: Botanicals, fungicides, karnal, neovossia, mycelial, inhibition, non-trariff, concentration

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated food grain crop all around the world due to its wider adaptability to different agro-climatic and soil conditions (Vishwakarma et al., 2023). Wheat belongs to one of the most diverse and substantial family, Poaceae (Khalid et al., 2023). Major species grown worldwide are *Triticum aestivum*, a hexaploid species called “common” or “bread wheat” and *T. durum*, a tetraploid species called pasta wheat (Yadav, 2023). In India, wheat is best grown as a *Rabi* or winter season crop since the conditions during that time are conducive for its growth. Geographically, wheat is grown in the Northern Hemisphere between 30–55 °N latitude and in the Southern Hemisphere between 20–40 °S latitude (Kumar et al., 2014). Wheat serves as the main source of nourishment for the majority of the world’s population, however major proportion of the calories and protein is supplied by wheat among all

the cereal crop (Geisslitz et al., 2021). In India, total area under wheat cultivation is around 343.23 lakh ha with annual production of 112 mt (Anonymous, 2023). Uttar Pradesh, Punjab and Haryana are the major wheat growing states in India contributing maximum area and production under wheat cultivation. The other wheat producing states are Rajasthan, Madhya Pradesh, Bihar, Gujarat, Maharashtra and West Bengal. In Punjab, wheat is cultivated over an area of 35.43 lakh ha with annual production of 14.68 mt (Anonymous, 2023). The major wheat producing districts in Punjab are Faridkot, Sangrur, Bathinda, Muktsar sahib and Ludhiana. Karnal bunt is an important and old disease of wheat with restricted occurrences in Africa, America and Asia (Emebiri et al., 2019; Gurjar et al., 2019; Singh et al., 2020). In Punjab, the incidence of Karnal bunt was highest observed in the year 2014–15 (Kaur et al., 2018). Numerous biotic and abiotic factors are responsible for this low productivity. Biotic factors include many important pathogens like fungi, nematodes,



viruses and bacteria. Fungi have become main disease-causing agents in wheat. The diseases caused by fungi include rust, smut and bunt. These diseases cause severe qualitative and quantitative losses in wheat every year (Muhammad et al., 2013; Singh et al., 2020). The pathogen is a soil, seed and air-borne fungus that produces teliospores, which is the main source of inoculum that can resist adverse environments and live for several years in the soil (Manakkatt et al., 2024). Kumar et al. (2021) stated that symptomatology of Karnal bunt in wheat is marked by the presence of partially bunted kernels with a distinctive odour. Additionally, the disease causes changes in the chemical composition of infected grains (Vishwakarma et al., 2023). Karnal bunt is a significant non-tariff trade obstacle (Daloz et al., 2021). Disease management in India is much dependent on the use of fungicides. Chemical fungicides have shown to be quite successful in lowering the incidence and severity of the disease (Bishnoi et al., 2021). But excessive and irrational use of fungicides causes environment deterioration and has non target effects on plants and animals also responsible for residue problem and resistance development in pathogens and different health hazards to human beings (Goswami et al., 2018). Apart from chemicals plant extracts are one of effective methods that incorporate natural antifungal substances. The importance of botanicals is attributed to their efficacy, biodegradability, varied mode of action, low toxicity as well as availability of source material (Neeraj et al., 2017). In this study the comparative analysis of two different methods of management of Karnal bunt was done i.e., through botanicals and fungicides.

2. Materials and Methods

The study was conducted during November, 2023–April, 2024 at laboratory of Plant Pathology, Department of Agriculture, Khalsa College, Amritsar.

2.1. Preparation of plant extracts (Botanicals)

Freshly harvested (200 g) leaves of about 2 months old plants namely lantana (*Lantana camara*), eucalyptus (*Eucalyptus tereticornis*), datura (*Datura stramonium*) and neem (*Azadirachta indica*) was taken. Leaves were then washed under tap water. Each sample was grinded in mixer and blender by adding small quantity of sterilized distilled water. After grinding, 200 ml distilled water was added and homogenized in orbital shaker at 2000 rpm for half an hour to get 100% extract of each plant. The plant material was then filtered through double layered muslin cloth. Tyndallization of the extract of different plants was done in autoclave at pressure 5 or 10 psi for 30 min for 3 consecutive days and then the extracts were kept in refrigerator for further use.

2.2. In vitro evaluation of botanicals

Lantana, Eucalyptus, Datura and Neem were evaluated against mycelial growth of *Neovossia indica* at different concentrations by adopting Poisoned food technique. The efficacy of botanicals was tested at 10, 25 and 50% concentration. Double strength

PDA media was prepared and autoclaved at 15 psi pressure for 20 min. A concentration of 10% was prepared initially. 50 ml of double strength PDA media was filled with a solution made by adding 10 ml of sterilized plant extract into 40 ml of sterilized distilled water. 25 ml of plant extract was combined with 25 ml distilled water to make 25% concentration, which was then added to 50 ml double strength PDA medium. In a similar manner, a 50% concentration was made by adding 50 ml of plant extract into 50 ml of double strength PDA medium. The medium containing different concentrations of plant extract was poured (20 ml) in each sterilized Petri plate (90 mm) and allowed to solidify. Plates without plant extracts served as check. 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. Three replications were maintained for each treatment and incubated at 20±1°C. The colony diameter was measured 15 days after inoculation and per cent mycelial growth inhibition would be calculated by using formula (Vincent, 1947) as given below

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

I=Percent mycelial inhibition
C=Linear growth in control
T=Linear growth in treatment

2.3. In vitro evaluation of fungicides

The experiment was carried out by adopting Poisoned food technique. Five fungicides were evaluated against *Neovossia indica* at three different concentrations viz., 50, 100 and 150 ppm. Double strength PDA medium was prepared and autoclaved at 15 psi pressure for 20 min. The test fungicides were used to prepare 1000 ppm stock solution for fungicide evaluation. For the experiment, the buffer stock was added to sterilized distilled water to further get the different ppm concentrations of the fungicides. 50 ml of fungicide from the stock solution was added to 50 ml of sterilized distilled water. Following thorough mixing, 50 ml of autoclaved double strength PDA was combined with the poisoned water. In the similar manner, 100 and 150 ppm concentration were prepared. The medium containing different concentrations of fungicides was poured (20 ml) in each sterilized Petri plate (90 mm) and allowed to solidify. Plates without fungicides served as control. 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. Three replications were maintained for each treatment and incubated at 20±1°C. Radial growth of the fungal mycelium (mm) was recorded from the incubated Petri plate 15 days after inoculation. Per cent mycelial growth inhibition would be calculated by the following formula (Vincent, 1947):

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



I=Percent mycelial inhibition

C=Linear growth in control

T=Linear growth in treatment

3. Results and Discussion

The efficacy of aqueous extracts from *Eucalyptus tereticornis*

Table 1: List of botanicals used against *Neovossia indica* under *in vitro* conditions

Sl. No.	Botanicals	Scientific name	Plant part used	Concentrations (%)
1.	Lantana	<i>Lantana camara</i>	Leaves	10,25,50
2.	Eucalyptus	<i>Eucalyptus tereticornis</i>	Leaves	10,25,50
3.	Datura	<i>Datura stramonium</i>	Leaves	10,25,50
4.	Neem	<i>Azadirachta indica</i>	Leaves	10,25,50

(*Eucalyptus*), *Lantana camara* (Lantana), *Datura stramonium* (Datura), *Azadirachta indica* (Neem) in inhibiting the mycelial growth of *Neovossia indica* was evaluated under *in vitro* conditions using the Poisoned food technique at three different concentration. The data on the diametric growth of inhibition per cent of *Neovossia indica* by different plant extracts were presented in Table 1.

It was pertinent from the data (Table 1) that there was a significant positive correlation between plant extract concentration and mycelial growth inhibition of *Neovossia indica*. Among the evaluated plant extracts, Datura showed the highest average mycelial inhibition of 55.03% followed by Neem (46.42%) and Eucalyptus with mycelial inhibition of 37.63%. The average mycelial inhibition rate for Lantana found to be the lowest with minimum mycelial inhibition of 11.33%. Our results were in accordance with Gupta and Singh (1983) who reported that phytoextracts from Datura was highly effective against *Neovossia indica* and finally showed that it resulted in maximum inhibition.

3.1. *In vitro* efficacy of fungicides against the *Neovossia indica*

The data on diametric growth of *Neovossia indica* was

Table 2: *In vitro* efficacy of botanicals (Lantana, Eucalyptus, Datura, Neem) against mycelial growth of *Neovossia indica*

Sl. No.	Botanicals	Mycelial growth (mm)			Mean (mm)	Mycelial inhibition (%)
		10%	25%	50%		
1.	<i>Lantana camara</i>	83.70 (66.16)	81.60 (64.50)	74.10 (59.41)	79.80 (63.38)	11.33
2.	<i>Eucalyptus tereticornis</i>	62.43 (52.18)	59.60 (50.51)	46.37 (42.89)	56.13 (48.53)	37.63
3.	<i>Datura stramonium</i>	44.27 (41.69)	40.53 (39.52)	36.60 (37.20)	40.47 (39.47)	55.03
4.	<i>Azadirachta indica</i>	59.77 (50.61)	46.03 (42.70)	38.87 (38.55)	48.22 (43.95)	46.42
	Control	90	90	90	90	
	CD ($p=0.01$)	Botanicals			1.27	
		Concentration			1.10	
		Botanical \times Concentration			2.20	

Figure in parenthesis were arc sine transformed value

Table 3: Efficacy of fungicides (Tilt 25EC, Antracol, Score, Natio, Dithane M-45) against mycelial growth of *Neovossia indica*

Sl. No.	Botanicals	Mycelial growth (mm)			Mean (mm)	Mycelial inhibition (%)
		50 ppm	100 ppm	150 ppm		
1.	Tilt 25EC	5.60 (13.64)	4.33 (11.99)	2.50 (9.03)	4.14 (11.55)	95.40
2.	Antracol 70 WP	50.92 (45.50)	49.75 (44.83)	40.58 (39.55)	47.08 (43.30)	47.69
3.	Score 25 EC	41.00 (39.79)	34.50 (35.95)	14.92 (22.70)	30.14 (32.81)	66.51
4.	Natio75 WG	14.25 (22.16)	11.17 (19.49)	8.92 (17.35)	11.45 (19.67)	87.28
5.	Dithane M-45 75 WP	39.75 (39.06)	37.25 (37.59)	25.58 (30.34)	34.19 (35.66)	62.00
	Control					
	CD ($p=0.01$)	Fungicides			1.04	
		Concentration			0.80	
		Fungicide \times concentration			1.80	

Figure in parenthesis were arc sine transformed value



recorded and presented in Table 3.

It was evident from the data presented in Table 2 that all the fungicides were quite effective in inhibiting the mycelial growth of *Neovossia indica*. Among different fungicides tested, Tilt 25 EC was found to be the most effective with maximum average mycelial growth inhibition of 95.40% followed by Nativo 75 WG (87.28%), which were significantly outperforming Score 25 EC, Dithane M-45 75 WP and Antracol 70 WP. However, the minimum average mycelial inhibition of 47.69% was recorded in Antracol 70 WP, followed by Dithane M-45 75 WP (62%) and Score 25 EC (66.51%). Our results are in accordance with Kumar et al. (2014) who studied *in vitro* efficacy of thirteen fungicides against *Neovossia indica* and reported that Tilt 25EC has resulted in 94.41% inhibition in the mycelial growth. Nivedita et al. (2023) conducted *in vitro* experiment on the influence of fungicides against mycelial growth of *Neovossia indica*. They tested four fungicides (Bavistin, Vitavax, Tilt, Follicur) against radial growth of *Neovossia indica*. Results showed that Tilt 25 EC resulted in maximum mycelial growth inhibition (96.75%) at 200 ppm concentration. Rathore et al. (2024) evaluated the efficacy of eight fungicides and reported that Tilt 250 EC was found to be the most effective, significantly inhibiting the mycelial growth of *Neovossia indica*, showing zero average mycelial growth.

4. Conclusion

Among the four botanical extracts evaluated, *Datura* showed the highest average mycelial inhibition of 55.03%. Among the five evaluated fungicides, Tilt 25EC was found to be the most effective fungicide under *in vitro* condition with maximum average mycelial growth inhibition of 95.04%.

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6. References

- Anonymous, 2023. Department of Agriculture and Farmers Welfare. Available from: <https://agriwelfare.gov.in/>. Accessed on 18/09/2024.
- Bishnoi, S.K., Kumar, S., Singh, G.P., 2021. The re-emerging Karnal bunt disease of wheat and preparedness of the global wheat sector. *Current Science* 120, 1814–1817. <https://www.jstor.org/stable/27310483>.
- Daloz, A.S., Rydsaa, J.H., Hodnebrog, S.J., Oort, B.V., Mohr, C.W., Agrawal, M., Emberson, L., Stordal, F., Zhang, T., 2021. Direct and indirect impacts of climate change on wheat yield in the Indo-Gangetic plain in India. *Journal of Agriculture and Food Research* 4, 100132. <https://doi.org/10.1016/j.jafr.2021.100132>.
- Emebiri, L., Singh, P., Mui-Keng, T., Davila, F.G., He, X., Singh, R., 2019. Reaction of Australian durum, common wheat and triticale genotypes to Karnal bunt (*Tilletia indica*) infection under artificial inoculation in the field. *Crop Pasture Science* 70(2), 107–12. [10.1071/cp18235](https://doi.org/10.1071/cp18235).
- Geisslitz, S., Shewry, P., Brouns, F., America, A.H., Caio, G.P., Daly, M., Weegels, P.L., 2021. Wheat ATIs: Characteristics and role in human disease. *Frontiers in Nutrition* 8, 265. <https://doi.org/10.3389/fnut.2021.667370>
- Goswami, S.K., Singh, V., Chakdar, H., Choudhary, P., 2018. Harmful effects of fungicides-current status. *International Journal of Agriculture, Environment and Biotechnology* 11, 1011–1019. <https://www.researchgate.net/publication/324273873>.
- Gurjar, M.S., Aggarwal, R., Jogawat, A., Kulshreshtha, D., Sharma, S., Solanke, A.U., Dubey, H., Jain, R.K., 2019. De novo genome sequencing and secretome analysis of *Tilletia indica* inciting Karnal bunt of wheat provides pathogenesis related genes. *3 Biotech* 9(6), 219. doi: 10.1007/s13205-019-1743-3.
- Gupta, R.P., Singh A., 1983. Effect of certain plant extract and chemicals on teliospore germination of *Neovossia indica*. *Indian Journal of Mycology and Plant Pathology* 13, 116–117. <https://www.cabidigitallibrary.org/doi/full/10.5555/19851308052>.
- Kaur, J., Bala, R., Kaur, H., Pannu, P., Kumar, A., Bhardwaj, S.C., 2018. Current status of wheat diseases in Punjab. *Agricultural Research Journal* 55(1), 113–116. DOI No.: 10.5958/2395-146X.2018.00018.2.
- Khalid, A., Hameed, A., Tahir, M.F., 2023. Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification and function of seed storage proteins in bread making quality. *Frontiers in Nutrition* 10, 1053196. <https://doi.org/10.3389/fnut.2023.1053196>.
- Kumar, S., Singh, D., Pandey, V.K., Singh, S., 2014. *In vitro* evaluation of fungitoxicants and Phyto-extracts against *Neovossia indica* (Mitra) Mund. the causal agent of Karnal bunt of wheat. *International Journal of Plant Protection* 7(2), 448–452. DOI: 10.15740/HAS/IJPP/7.2/448-452.
- Kumar, S., Singroha, G., Singh, G.P., Sharma, P., 2021. Karnal bunt of wheat: etiology, breeding and integrated management. *Crop Protection* 139, 105376. <https://doi.org/10.1016/j.cropro.2020.105376>.
- Neeraj, G.S., Kumar, A., Ram, S., Kumar, V., 2017. Evaluation of nematicidal activity of ethanolic extracts of medicinal plants to *Meloidogyne incognita* (Kofoid and white) chitwood under lab conditions. *International Journal of Pure Applied Bioscience* 1, 827–831. <https://doi.org/10.18782/2320-7051.2525>.
- Nivedita, Mahajan, S., Kaur, H., Astha, Paswal, S., 2023. *In vitro* evaluation of fungicides and plant extracts against the mycelial growth of *Neovossia indica*. *The Pharma Innovation Journal* 12, 5017–5019. <https://www>.



- thepharmajournal.com/archives/2023/vol12issue3/PartBC/12-3-583-649.pdf.
- Manakkatt, H.M., Gurjar, M.S., Saharan, M.S., Aggarwal, R., 2024. Expression analysis of genes involved in teliospores germination of *Tilletia indica* inciting Karnal bunt of wheat. Molecular Biology Reports 51(1), 726. <https://link.springer.com/article/10.1007/s11033-024-09690-4>
- Muhammad, A., Muhammad, R., Muhammad, S., Aftab, B., Muhammad, I., 2013. Response of some commercial cultivars and advanced lines of wheat against Karnal bunt of wheat and its management through chemicals. International Journal of Plant Research 3(4), 47–51. doi:10.5923/j.plant.20130304.01.
- Rathore, T., Shashank, S., Pandey, K.V., Sharma, S., Kumar, J., Nandy, R., Anand, A., Singh, V., 2024. Bio-assay of fungicides against the *Neovossia indica* inciting Karnal bunt of Wheat. International Journal of Environmental and Climate Change 14(1), 496–501. DOI: 10.9734/IJECC/2024/v14i13861.
- Singh, J., Aggarwal, R., Gurjar, M.S., Sharma, S., Jain, S., Sharan M.S., 2020. Identification and expression analysis of pathogenicity related genes in *Tilletia indica* inciting Karnal bunt of wheat. Australasian Plant Pathology 10, 393–402. 10.1007/s13313-020-00711-x.
- Singh, S., Sehgal, D., Kumar, S., Arif, M., Vikram, P., 2020. GWAS revealed a novel resistance locus on chromosome 4D for the quarantine disease Karnal bunt in diverse wheat pre-breeding germplasm. Scientific Reports 10, 1–11. <https://www.nature.com/articles/s41598-020-62711-7>
- Vishwakarma, S.K., Singh, R., Khilari, K., Mishra, P., Singh, H., Yadav, M.K., 2023. Integrated disease management (IDM) modules against Karnal bunt (*Tilletia indica*) of wheat. International Journal of Environment and Climate Change 13(10), 2261–2267. DOI: 10.9734/ijecc/2023/v13i102889.
- Vincent, J.H., 1947. Distortion of fungal hyphae in the presence of certain inhibition. Nature 159(4051), 850. 10.1038/159850b0.
- Yadav, I.S., Singh, N., Wu, S., Raupp, J., Wilson, D.L., Rawat, N., Gill, B.S., Poland, J., Tiwari, V.K., 2023. Exploring genetic diversity of wild and related tetraploid wheat species *Triticum turgidum* and *Triticum timopheevii*. Journal of Advance Research 48, 47–60. <https://doi.org/10.1016/j.jare.2022.08.020>.

