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REACTION OF LINSEED (*LINUM USITATISSIMUM* L.) GERMPLASM FOR RESISTANCE TO *FUSARIUM* WILT CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *LINI*

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ABSTRACT:
Fusarium wilt, caused by *Fusarium oxysporum* f. *sp. lini*, is one of the most destructive soil-borne diseases limiting linseed (*Linum usitatissimum* L.) production worldwide. The pathogen persists in soil for prolonged periods and infects plants at different growth stages, resulting in severe yield losses and reduced crop productivity. The development and deployment of resistant cultivars remain the most economical, environmentally sustainable, and durable strategy for disease management. The present investigation was undertaken to evaluate twenty-five linseed germplasms for their resistance to *F. oxysporum* f. *sp. lini* under controlled and natural disease conditions using test-tube inoculation, artificially inoculated pot culture, and natural field screening. Germplasm accessions were collected from All India Coordinated Research Project on Linseed, Bihar Agricultural University, Sabour. Disease reactions were categorized as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible based on percent wilt incidence. Under artificial screening conditions, wilt incidence ranged from 0 to 100% and under natural field conditions from 5 to 98%, indicating significant genetic variability for resistance among the evaluated germplasms. Of all the entries screened by different methods, Neelam was found to be the most resistant. ST-4, ST-3, BRLS 106-1, BRLS 137, VKS 14-2, JRF-2, Parvati, Priyam, H-40 and Kartika also showed resistant reaction under artificial and field conditions. In contrast, BRLS 110-7, BRLS 109-5, BRLS 132-1, Pratap Alsi-2 and RLC 173 were highly susceptible indicating that these genotypes may not be useful for resistance but can be used as susceptible checks for future screening programs. The uniformity of resistance observed across different screening environments suggests that these germplasms are dependable as potential donor parents for resistance breeding. These findings provide useful genetic resources for developing resistant linseed cultivars to *Fusarium* wilt and contribute to sustainable disease management strategies to improve crop productivity in various agroecological settings.

KEYWORDS: *Linum usitatissimum*; *Fusarium oxysporum* f. *sp. lini*; *Fusarium* wilt; disease resistance; germplasm screening; resistance breeding.

1. INTRODUCTION

Linseed (*Linum usitatissimum* L.), also known as flax, is one of the oldest domesticated crops and is cultivated worldwide for its dual-purpose value as an oilseed and fibre crop. The seed is a good source of high-quality edible oil rich in α-linolenic acid (omega-3 fatty acid), proteins, lignans, dietary fiber, vitamins and minerals. It is an important raw material for food, nutraceutical, pharmaceutical and industrial applications. Linseed is mainly grown in India during the rabi season in Madhya Pradesh, Uttar Pradesh, Chhattisgarh, Bihar, Rajasthan, Maharashtra, Jharkhand and Odisha. Linseed is economically and nutritionally important but productivity of linseed is much lower than its genetic potential due to several biotic and abiotic constraints. *Fusarium* wilt is one of the most devastating diseases (Moyse et al., 2023; Shamsher Alam et al., 2022).

Fusarium wilt is a serious soil-borne disease of the plant's vascular system and is caused by *Fusarium oxysporum* f. sp. lini and can infect the plant from the seedling stage to maturity. The pathogen invades the root system and colonizes the vascular tissue resulting in yellowing, drooping, vascular browning, permanent wilting and eventually plant death. The fungus can remain in the soil for years in the form of chlamydospores and survive without a susceptible host, making it very difficult to eradicate. Disease severity is influenced by environmental conditions, pathogen virulence and host susceptibility (Augustyniak et al., 2024; Gordon, 2017) and severe epidemics result in substantial reductions in seed yield and oil quality under favourable conditions. Consequently, *Fusarium* wilt remains one of the principal biological constraints limiting sustainable linseed production (Gordon, 2017; Samsonova et al., 2021; Laurence et al., 2021).

Management of *Fusarium* wilt through chemical fungicides is often ineffective because the pathogen is soil-borne and survives deep within the soil profile, where fungicidal treatments provide only limited and temporary suppression. Likewise, cultural practices such as crop rotation and field sanitation contribute to disease reduction but seldom provide complete control owing to the long-term survival of the pathogen in soil. Therefore, the cultivation of resistant cultivars has emerged as the most economical, environmentally safe, and sustainable strategy for managing *Fusarium* wilt (Ekka et al., 2018; Moyse et al., 2023; Cloutier et al., 2024). Host resistance not only minimizes dependence on chemical pesticides but also provides stable disease control under diverse agro-climatic conditions while supporting integrated disease management programmes.

Considerable genetic variability for resistance to F. oxysporum f. sp. lini has been reported among linseed cultivars and germplasm collections. Previous work demonstrated a wide range of disease reaction from highly resistant to highly susceptible genotypes. Significant variation in resistance among linseed varieties under Indian conditions was first reported by Sharma et al. (1972). Later on, promising resistant germplasm suitable for breeding programs was identified by Kishore et al. (2011a), Kishore et al. (2011b), and Kumar et al. (2014). However, resistance observed in one screening environment may not always be stable since disease expression is influenced by inoculum load, environmental conditions and pathogen variability. Therefore, the reliable identification of resistant sources needs evaluation in several screening systems combining controlled artificial inoculation and natural field conditions. Recent molecular and genomic investigations have further demonstrated substantial genetic and pathogen variability associated with *Fusarium* wilt resistance in flax (Rozhmina et al., 2022; Gopala Nair et al., 2025; Logachev et al., 2024).

Uniform disease pressure can be achieved with artificial screening methods, such as test-tube and pot-culture inoculation techniques, and this can allow for the early identification of resistant genotypes in breeding programs. Pot-culture screening is an intermediate system, which more closely resembles natural infection. Test-tube screening allows an accurate evaluation of the seedling responses under controlled laboratory conditions. Resistance identified in artificial environments is next tested in the field under natural disease pressure for stability and practical usefulness (Kroes et al., 1998; Yadav et al., 2023). Combining complementary screening strategies improves the reliability of resistance assessment and reduces the probability of selecting genotypes that perform well in only one testing environment.

Many studies have reported resistant sources of linseed, but there is limited information on the comparative performance of diverse germplasm in different screening environments of eastern India. The identification of stable and consistent resistant germplasm is a necessary step to develop improved cultivars which are resistant to *Fusarium* wilt in different agroecological conditions. Such germplasm can also serve as valuable donor parents in resistance breeding programmes aimed at enhancing the durability of host resistance and improving linseed productivity.

Therefore, the present investigation was undertaken to evaluate twenty-five linseed germplasms against *Fusarium* oxysporum f. sp. lini using test-tube inoculation, artificially inoculated pot culture, and natural field screening, with the objective of identifying stable sources of resistance for future breeding and sustainable disease management.

2. MATERIALS AND METHODS

2.1 Plant Materials

Twenty-five linseed (Linum usitatissimum L.) germplasms were obtained from the All India Coordinated Research Project (AICRP) on Linseed, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India. The germplasm collection consisted of BRLS 109-5, BRLS 109-2, ST-4, ST-3, BRLS 106-1, BRLS 110-7, BRLS 130-4, BRLS 130-6, BRLS 132-1, BRLS 137, VKS 14-1, VKS 14-2, TL-99, Pratap Alsi-2, JRF-2, Parvati, RLC 173, Priyam, H-40, Dibya, SIJKO-37, Neelam, Neela, Polf-23, and Kartika. These germplasms were selected to assess the extent of genetic variability in resistance to *Fusarium* wilt under controlled and natural disease conditions.

2.2 Pathogen and Inoculum Preparation

A highly virulent isolate of *Fusarium* oxysporum f. sp. lini used in artificial inoculation experiments was obtained from the survey. The pathogen was cultured on sterilized sand-maize medium. The medium was prepared by mixing 95 parts of sterilized sand with 5 parts of maize-meal (w/w). The medium was sterilized prior to inoculation to eliminate contaminating microorganisms and then inoculated with actively growing fungal cultures under aseptic conditions. Appropriate incubation of the colonized substrate was followed by its use as the inoculum source for pot-culture screening. Sand-maize medium was employed for the uniform establishment of the pathogen and consistent disease pressure during artificial inoculation.

2.3 Screening under Test-Tube Conditions

Twenty-five germplasms were initially screened under controlled laboratory conditions using the test-tube inoculation technique. Surface-sterilized seeds were inoculated according to the standard protocol for the assessment of seedling resistance to F. oxysporum f. sp. lini. Seedlings were kept under suitable environmental conditions to allow the disease development. Wilt symptoms were observed after inoculation and disease incidence was calculated as the percentage of seedlings that showed wilting. The germplasms were classified

into five disease reaction categories based on the incidence of disease: resistant 0-10%, moderately resistant 11-20%, moderately susceptible 21-50%, susceptible 51-80% and highly susceptible >80%. This method enables a fast and reliable assessment of seedling resistance to uniform pathogen pressure.

2.4 Screening under Artificially Inoculated Pot Conditions

The selected germplasms were further evaluated under greenhouse pot-culture conditions using pathogen-infested soil. Colonized sand-maize inoculum was incorporated into sterilized potting medium at the rate of 50 g per pot. The potting mixture consisted of autoclaved soil and autoclaved cocopeat in a 2:1 (v/v) ratio to provide a uniform growth medium while minimizing interference from naturally occurring soil microorganisms. Each treatment was maintained in three replications under identical environmental conditions. Plants were observed periodically for symptom development, and final wilt incidence was recorded at the completion of the experiment. Pot-culture screening served as an intermediate evaluation system between laboratory assays and field testing by exposing plants to controlled yet relatively natural infection conditions.

2.5 Field Evaluation under Natural Disease Conditions

Field evaluation of all twenty-five germplasms was conducted during the rabi season of 2025–26 at the experimental farm of Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India, where natural incidence of *Fusarium* wilt occurs regularly. The germplasms were grown under recommended agronomic practices without artificial inoculation. Disease observations were recorded periodically throughout the crop growth period, and final wilt incidence was assessed at crop maturity. Field evaluation was undertaken to validate the resistance observed under artificial screening and to identify germplasms expressing stable resistance under natural environmental conditions.

2.6 Disease Assessment

Disease severity was assessed based on wilt incidence and expressed as percentage wilt incidence using the following equation:

Wilt incidence (%) = (Number of wilted plants / Total number of plants observed) × 100.

Based on wilt incidence, each germplasm was assigned to one of five disease reaction categories:

Wilt incidence (%)	**Disease reaction**
0–10	Resistant (R)
11–20	Moderately Resistant (MR)
21–50	Moderately Susceptible (MS)
51–80	Susceptible (S)
>80	Highly Susceptible (HS)

This classification enabled uniform comparison of germplasm performance across the three screening environments.

2.7 Statistical Analysis

Disease incidence data generated from the three screening methods were subjected to statistical analysis to determine the significance of differences among germplasms. The standard error of the mean (SEm ±) and critical difference (CD) at the 5% probability level were calculated following standard statistical procedures. The statistical analysis facilitated reliable comparison of disease responses and identification of significantly resistant and susceptible germplasms.

3. RESULTS AND DISCUSSION

3.1 Response of Linseed Germplasm under Test-Tube Screening

The disease response of the twenty-five linseed germplasms under the controlled test-tube conditions exhibited considerable variation. The occurrence of wilt ranged from 0 to 100 % indicating a high genetic variability for resistance against *Fusarium* oxysporum f. sp. lini. Three germplasms viz. ST-4, BRLS 106-1 and Neelam showed complete resistance with 0% incidence of wilt. Disease incidence of twenty percent was recorded in eleven germplasms and categorized as moderately resistant whereas, forty percent incidence was recorded in four germplasms and categorized as moderately susceptible. Three entries recorded 60-80% disease incidence and BRLS 110-7, VKS 14-1, Pratap Alsi-2 and RLC 173 recorded 100% wilt incidence indicating extreme susceptibility to the pathogen. Statistical analysis revealed highly significant differences among germplasms with SEm (±) 6.53 and CD (P = 0.05) 13.11 and confirmed that variation observed were real genetic differences and not due to random experimental error.

3.2 Response under Artificially Inoculated Pot Conditions

The variability among the tested germplasms was also confirmed by evaluation under artificially inoculated pot conditions. Wilt incidence ranged from 0 to 100 % indicating the range of resistance among individual genotypes to the pathogen under greenhouse conditions. Among the tested entries, Neelam was disease free (0% incidence of wilt), whereas ST-4, ST-3, BRLS 106-1, BRLS 137, VKS 14-2, JRF-2, Parvati, Priyam, H-40 and Kartika recorded only 10% incidence of wilt and were rated as resistant. Neela showed 20 per cent incidence of wilting and was classified as moderately resistant. BRLS 109-2, BRLS 130-4, Dibya and Polf-23 were moderately susceptible. BRLS 130-6, TL-99, RLC 173 and SIJKO-37 were susceptible. The highest disease incidence was noted in BRLS 110-7 (100%) while BRLS 109-5, BRLS 132-1, VKS 14-1 and Pratap Alsi-2 recorded 90% wilt incidence thus proving their highly susceptible nature. The statistical analysis showed a SEm (±) of 5.16 and a CD (P = 0.05) of 10.37. Germplasm was significantly different.

Screening in pot-culture provides an intermediate level of evaluation by combining controlled inoculation with conditions that more closely resemble the natural root environment. The stable resistance mechanisms of these germplasms as seen by the consistent resistance in Neelam, ST-4, BRLS 106-1 and several other germplasms under both laboratory and greenhouse conditions are not just due to escape from infection. Similar variability in host response has been reported in previous investigations, emphasizing that genetic diversity of linseed germplasm can be utilized effectively for resistance breeding. The continued susceptibility of BRLS 110-7 and other entries in the various screening systems provides additional justification for the use of the entries as susceptible checks in future pathological studies.

3.3 Performance of Germplasm under Natural Field Conditions

In natural field conditions, wilt incidence ranged from 5 to 98% indicating that the disease expression was still highly variable even under natural inoculum pressure. The lowest incidence of wilting was observed in Neelam (5%) followed by BRLS 106-1 (6%), ST-4 and ST-3 (7%), VKS 14-2, JRF-2, Priyam and Kartika (8%), BRLS 137 and H-40 (9%) and Parvati (10%). Under field conditions, eleven of these germplasms were rated resistant. Neela recorded 11% incidence of wilting and was classified as medium resistant. Five germplasms were moderately susceptible and VKS 14-1, TL-99 and SIJKO-37 were susceptible. The maximum per cent wilt incidence was noticed in BRLS 110-7 (98%), followed by Pratap Alsi-2 (88%), RLC 173 (86%), BRLS 132-1 (85%) and BRLS 109-5 (82%) and all were grouped under highly susceptible.

Field evaluation represents the ultimate assessment of resistance because it reflects the combined influence of host genotype, pathogen variability, and prevailing environmental conditions. The consistent performance of Neelam and several other resistant germplasms under natural disease pressure confirms the durability and practical usefulness of their resistance. Such stable field resistance is particularly valuable for breeding programmes because it is more likely to remain effective under farmers' field conditions. Conversely, the consistently high susceptibility of BRLS 110-7, BRLS 109-5, BRLS 132-1, Pratap Alsi-2, and RLC 173 suggests that these genotypes lack effective resistance mechanisms against the pathogen and may serve as susceptible standards for future screening experiments.

3.4 Comparative Evaluation of Screening Methods

Comparison of three screening methods showed high agreement for disease reactions of several germplasms. The germplasms like Neelam, ST-4, ST-3, BRLS 106-1, BRLS 137, VKS 14-2, JRF-2, Parvati, Priyam, H-40 and Kartika showed consistent resistant reactions in test-tube, pot-culture and field evaluations, indicating stable resistance irrespective of screening environment. Therefore, these germplasms are valuable genetic resources for incorporation in resistance breeding programs. However, BRLS 110-7, BRLS 109-5, BRLS 132-1, Pratap Alsi-2 and RLC 173 were invariably found to be highly susceptible under all the screening methods indicating stable susceptibility to F. oxysporum f. sp. lini.

The agreement among laboratory, greenhouse and field evaluations indicates that the combination of these complementary screening methods provides a reliable approach for identification of resistant germplasm. Laboratory assays permit rapid preliminary screening, greenhouse experiments confirm resistance under controlled infection, and field evaluation confirms resistance under natural disease pressure. This sequential selection improves the efficiency of selection and reduces the probability to carry forward unstable or environment dependent resistant genotypes.

3.5 Implications for Resistance Breeding

The differential disease reactions of the germplasms evaluated confirm the existence of considerable genetic diversity for *Fusarium* wilt resistance in the available linseed germplasm. These results are consistent with earlier reports of pathogenic variability of F. oxysporum f. sp. lini and differential host responses among flax genotypes (Maillot et al., 2023). Recent genomic studies have further demonstrated substantial variation among F. oxysporum f. sp. lini strains with different virulence levels (Krasnov et al., 2020; Dvorianinova et al., 2021; Logachev et al., 2024). Previous work by Thakur and Husain (1972) showed that multiple pathogenic races of the fungus exist and Kroes et al. (1999) showed that there is a wide variation in aggressiveness among pathogen isolates. More recent studies have also highlighted the need to validate resistant germplasm under both artificial

and natural disease conditions for reliable identification. Previous investigations have also demonstrated genetic polymorphism among fungal pathogens associated with flax diseases (Novakovskiy et al., 2020).

Neelam was the best source of resistance among the germplasms evaluated, as it consistently exhibited the lowest incidence of wilt in all the three screening methods. Similarly, ST-4, ST-3, BRLS 106-1, BRLS 137, VKS 14-2, JRF-2, Parvati, Priyam, H-40 and Kartika demonstrated stable resistance and can be utilized as valuable donor parents in developing *Fusarium* wilt-resistant cultivars. Inclusion of these resistant germplasms in breeding programs would be useful to reduce reliance on chemical control measures, improve disease management and enhance productivity and sustainability of linseed cultivation.

Table 1. Reaction of twenty-five linseed germplasms to *Fusarium* oxysporum f. sp. lini under test-tube, pot-culture, and field screening.

Sl. No.	Germplasm	Test tube DI (%)	Pot DI (%)	Field DI (%)	Field reaction
1	BRLS 109-5	80	90	82	HS
2	BRLS 109-2	20	40	32	MS
3	ST-4	0	10	7	R
4	ST-3	20	10	7	R
5	BRLS 106-1	0	10	6	R
6	BRLS 110-7	100	100	98	HS
7	BRLS 130-4	40	40	35	MS
8	BRLS 130-6	40	60	34	MS
9	BRLS 132-1	80	90	85	HS
10	BRLS 137	20	10	9	R
11	VKS 14-1	100	90	80	S
12	VKS 14-2	20	10	8	R
13	TL 99	40	60	63	S
14	Pratap Alsi-2	100	90	88	HS
15	JRF-2	20	10	8	R
16	Parvati	20	10	10	R
17	RLC 173	100	60	86	HS
18	Priyam	20	10	8	R
19	H-40	20	10	9	R
20	Dibya	20	40	38	MS
21	SIJKO-37	60	60	62	S
22	Neelam	0	0	5	R
23	Neela	20	20	11	MR
24	Polf-23	40	40	37	MS
25	Kartika	20	10	8	R

DI = disease incidence; R = Resistant; MR = Moderately Resistant; MS = Moderately Susceptible; S = Susceptible; HS = Highly Susceptible. Test-tube and pot-culture values are based on three replications.

Table 2. Distribution of linseed germplasms according to disease reaction under natural field conditions.

Disease reaction	Number	Germplasms
Resistant	11	ST-4, ST-3, BRLS 106-1, BRLS 137, VKS 14-2, JRF-2, Parvati, Priyam, H-40, Neelam, Kartika
Moderately resistant	1	Neela
Moderately susceptible	5	BRLS 109-2, BRLS 130-4, BRLS 130-6, Dibya, Polf-23
Susceptible	3	VKS 14-1, TL 99, SIJKO-37

Disease reaction	Number	Germplasms
Highly susceptible	5	BRLS 109-5, BRLS 110-7, BRLS 132-1, Pratap Alsi-2, RLC 173
Total	25	—

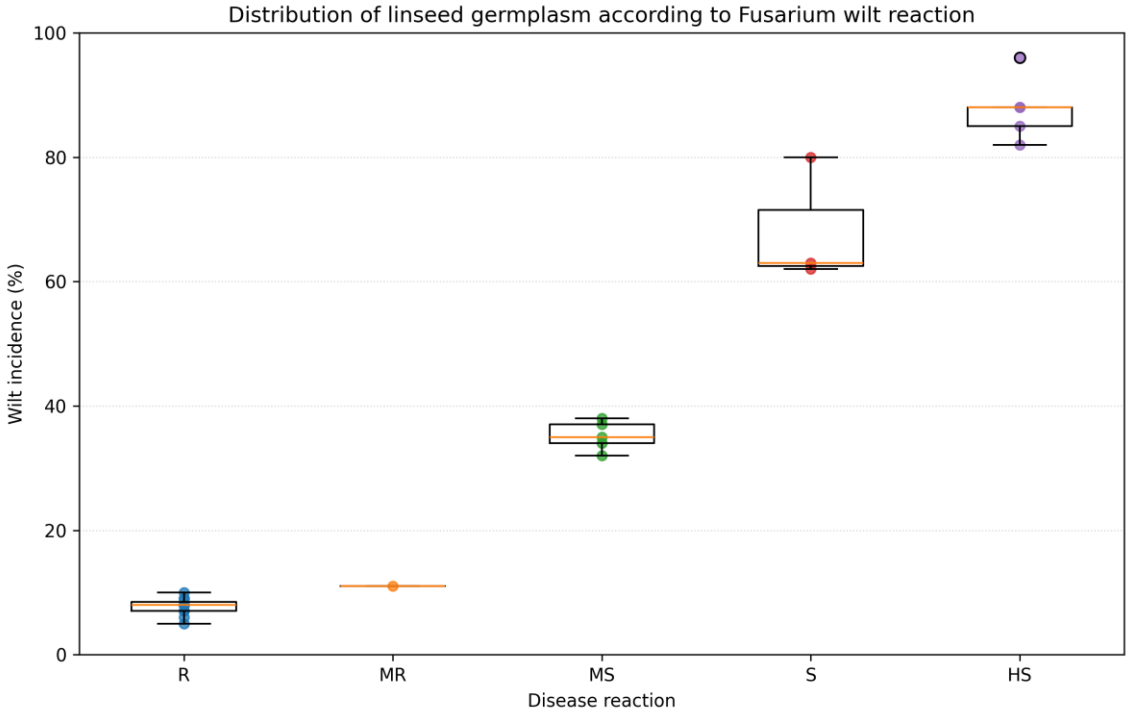

Figure 1. **Distribution of twenty-five linseed germplasms among five disease-reaction categories under natural field conditions.**

4. CONCLUSION

The present investigation demonstrated considerable genetic variability among twenty-five linseed (Linum usitatissimum L.) germplasms in their response to *Fusarium* wilt caused by *Fusarium* oxysporum f. sp. lini. Evaluation under test-tube inoculation, artificially inoculated pot culture, and natural field conditions revealed consistent differences in disease incidence, confirming the effectiveness of integrating multiple screening methods for reliable identification of resistant genotypes.

Among the evaluated germplasms, Neelam exhibited the highest level of resistance across all screening environments, while ST-4, ST-3, BRLS 106-1, BRLS 137, VKS 14-2, JRF-2, Parvati, Priyam, H-40, and Kartika also expressed stable resistance under both artificial and natural disease pressure. These germplasms constitute valuable sources of resistance and can be utilized as donor parents in breeding programmes aimed at developing *Fusarium* wilt-resistant linseed cultivars. Conversely, BRLS 109-5, BRLS 110-7, BRLS 132-1, Pratap Alsi-2, and RLC 173 consistently exhibited high susceptibility and may serve as susceptible checks in future pathological and host–pathogen interaction studies.

The identification of stable resistant germplasm provides an important foundation for sustainable management of *Fusarium* wilt and supports the development of improved cultivars with enhanced disease resistance. Future investigations should focus on multilocation validation of the resistant genotypes, evaluation against diverse pathogen isolates, and integration of molecular approaches to identify resistance-associated loci that can accelerate marker-assisted breeding for durable *Fusarium* wilt resistance in linseed. This combined strategy will strengthen resistance breeding programmes and contribute to improved productivity and long-term sustainability of linseed cultivation.

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Conflict of Interest
The authors declare that they have no conflict of interest regarding the publication of this manuscript.
Author Contributions
Abhinav Shubham: Investigation, data collection, data analysis, manuscript preparation.
Suresh Patil: Conceptualization, supervision, methodology, manuscript review and editing.
Ambati Lokeshwar Reddy: Technical guidance, data interpretation, manuscript review.
J.N. Srivastava : Technical guidance and manuscript review
Srinivasaraghavan A : Conceptualization, manuscript review and editing
A.K. Singh: Technical guidance and manuscript review

Data Availability Statement
The data generated and analyzed during the present study are included within the manuscript. Additional information may be made available by the corresponding author upon reasonable request.

DECLARATION OF GENERATIVE AI & AI-ASSISTED TECHNOLOGIES
During the preparation of this manuscript, the authors used Grammarly solely for grammar, spelling, sentence structuring, and readability improvements. Grammarly was not used to generate scientific content, arguments or data; all ideas, interpretations and conclusions are the authors' own. After using Grammarly, the authors carefully reviewed and edited all suggested changes and take full responsibility for the accuracy and integrity of the manuscript.

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