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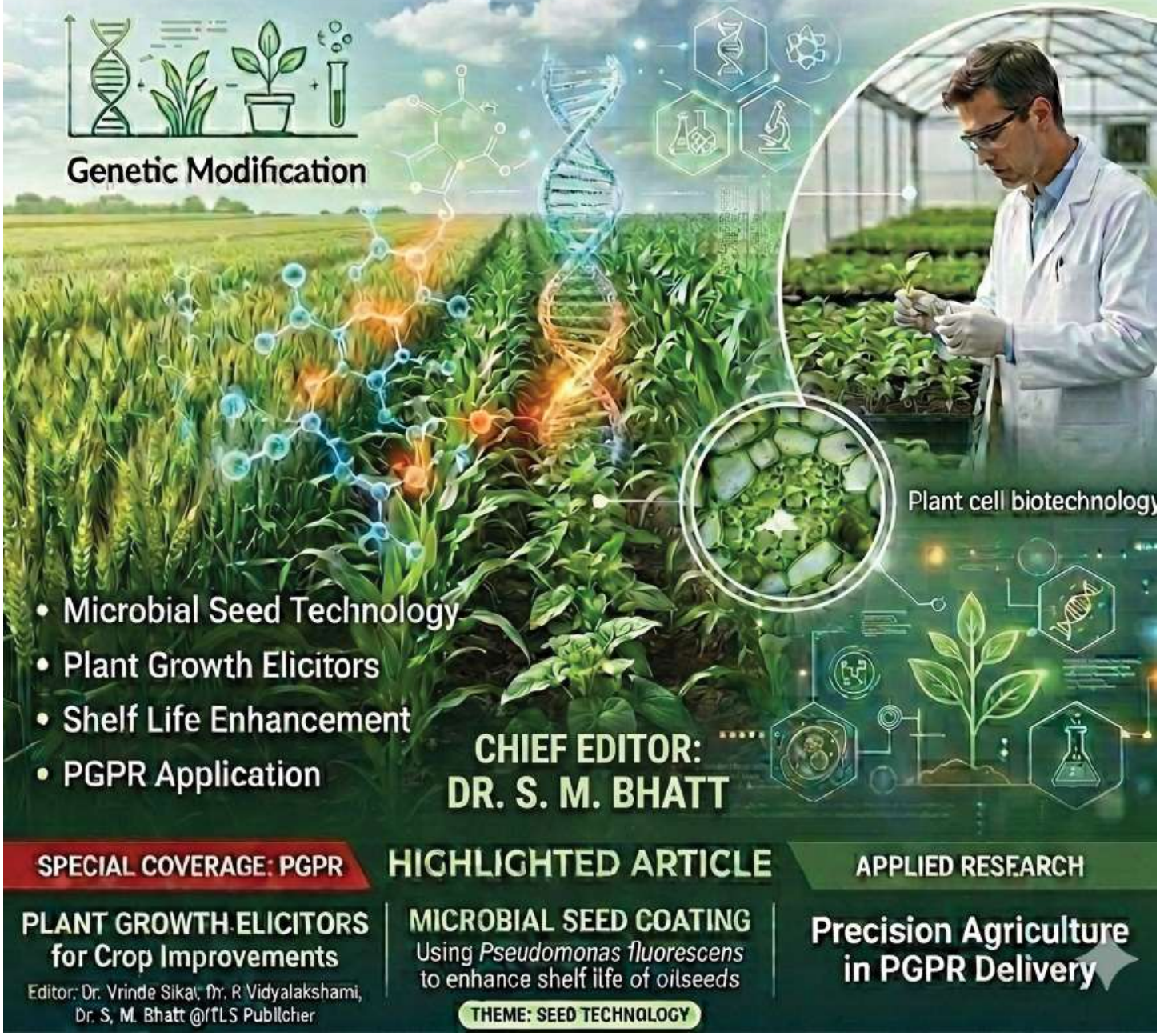

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HIGHLIGHTED ARTICLE

MICROBIAL SEED COATING
Using *Pseudomonas fluorescens* to enhance shelf life of oilseeds

THEME: SEED TECHNOLOGY

APPLIED RESEARCH

Precision Agriculture in PGPR Delivery

Original Research

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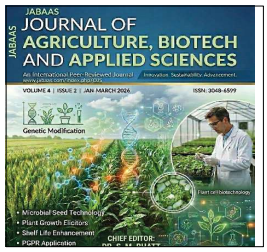

MICROBIAL SEED COATING USING PSEUDOMONAS FLUORESCENS TO ENHANCE SHELF LIFE OF OILSEEDS

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ABSTRACT

Fungal contamination is a major factor affecting the quality and storage life of oilseeds, particularly soybean, resulting in reduced germination, poor seed health, and economic losses. Although chemical pesticides are widely used for control, their long-term use raises concerns related to environmental safety and human health. Hence, the development of sustainable and eco-friendly alternatives has become essential. This study investigates the potential of *Pseudomonas fluorescens* as a biological seed coating agent to enhance the shelf life and quality of soybean seeds. The bacterium exhibits strong antagonistic activity against fungal pathogens through the production of antimicrobial compounds, siderophores, and lytic enzymes. It also promotes plant defense by inducing systemic resistance, thereby increasing the ability of plants to withstand infections. Seed treatment with *Pseudomonas fluorescens* helps in reducing fungal load, improving germination percentage, and enhancing seedling vigor. In addition, treated seeds show better resistance to storage fungi such as Aspergillus and Fusarium, leading to improved storability and quality.

The findings of this study highlight the effectiveness of microbial seed coating as a sustainable approach for managing seed-borne pathogens and reducing post-harvest losses. This method offers a promising alternative to chemical treatments and supports the adoption of eco-friendly practices in agriculture.

Keywords: : Biopesticide, Pseudomonas fluorescens, Seed coating, Soybean, Storage stability, Fungal pathogens, Sustainable agriculture, Biological control

Citation
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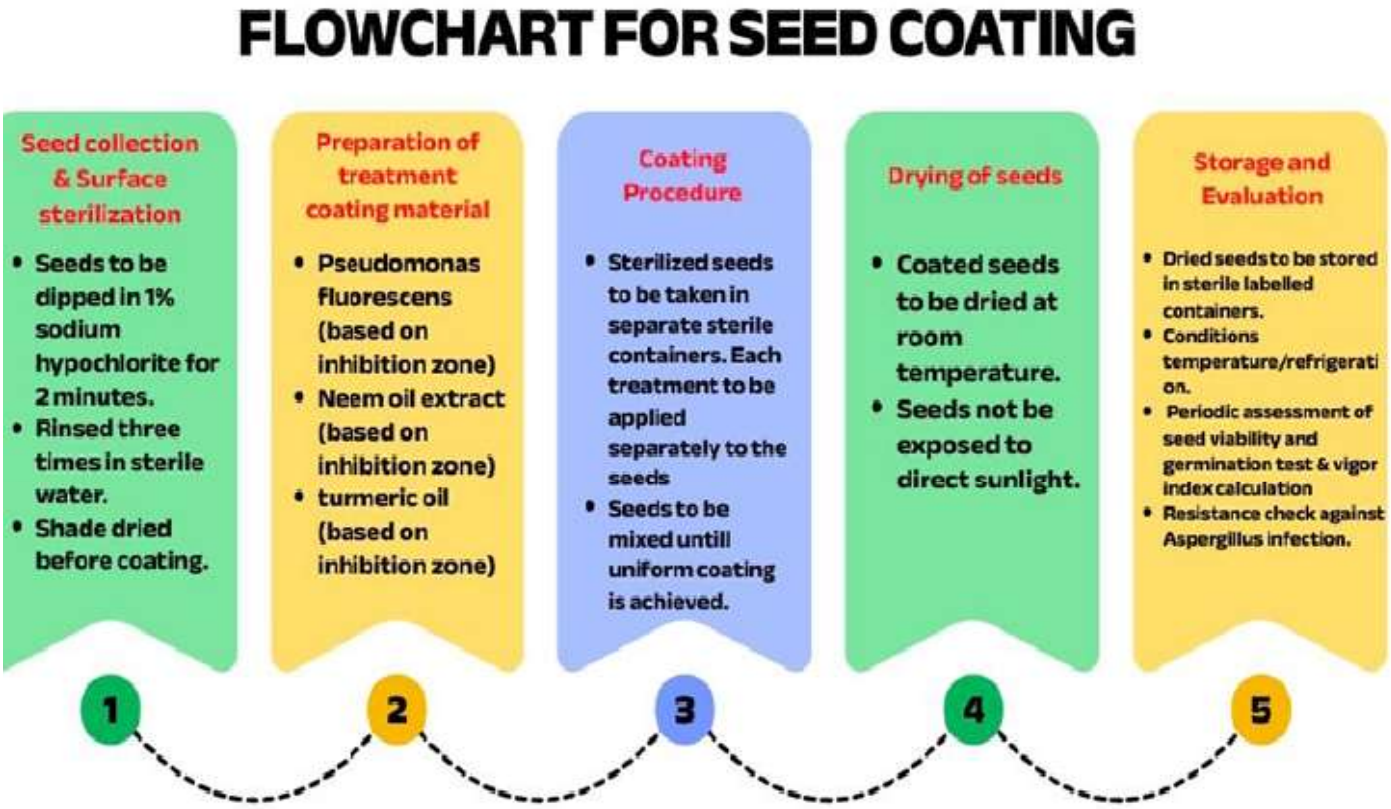

FLOWCHART FOR SEED COATING
Seed collection & Surface sterilization
Seeds to be dipped in 1% sodium hypochlorite for 2 minutes.
Rinsed three times in sterile water.
Shade dried before coating.
1
Preparation of treatment coating material
Pseudomonas fluorescens (based on inhibition zone)
Neem oil extract (based on inhibition zone)
turmeric oil (based on inhibition zone)
2
Coating Procedure
Sterilized seeds to be taken in separate sterile containers. Each treatment to be applied separately to the seeds
Seeds to be mixed untill uniform coating is achieved.
3
Drying of seeds
Coated seeds to be dried at room temperature.
Seeds not be exposed to direct sunlight.
4
Storage and Evaluation
Dried seeds to be stored in sterile labelled containers.
Conditions temperature/refrigeration.
Periodic assessment of seed viability and germination test & vigor index calculation
Resistance check against Aspergillus infection.
5

INTRODUCTION:

Oilseed crops like soyabean is valued for its high protein and oil content, which plays an important role in Nutritional value and Commercial value. But its productivity and storage quality are often affected by fungal spores that are plant pathogens and decline its storage shelf life.Such contaminations occur during cultivation and postharvest process. Fungi spores of *Fusarium, Aspergillus, and Rhizoctonia* are known to reduce seed viability which leads to poor germination, and also form harmful toxins, leading to significant economic losses. In recent years, there has been increasing interest in environmentally safe alternatives to chemical pesticides. Development of pesticide resistance in pathogens is also of great concern due to improper usage of chemical pesticides that are resulting in environmental contamination, and health risks. One such sustainable approach involves the use of beneficial microorganisms as biopesticides.

Pseudomonas fluorescens is a popular plant growth-promoting bacterium and also plays an important role in protecting crops against pathogens. The various mechanisms by which it acts involves the production of antimicrobial compounds, competition for nutrients, and secretion of enzymes that break down fungal cell walls. *Pseudomonas species* are also known to enhance the plant's natural defense system by induced systemic resistance, that make the plants to respond effectively to infections.

When applied as a seed coating agent, this bacterium establishes itself on the seed surface and in the rhizosphere after germination, thereby a protective barrier against harmful microorganisms is established. This reduces infection during storage and early growth stages. It also improves seedling vigor and uniform crop establishment.

Apart from disease control, *Pseudomonas fluorescens* also supports plant growth by enhancing nutrient uptake and producing growth-promoting substances. The use of such biological agents in seed treatment is gaining attention as a practical and eco-friendly strategy to improve both crop performance and storage life.

The main aim of this study is to assess the effectiveness of Pseudomonas fluorescens as a green seed coating agent in reducing fungal contamination, improving germination, and increasing the shelf life of soybean.

The innovation aspect of this study can be attributed to the use of both microbes and plants in coating seeds and testing the efficiency of these seeds in being stored effectively.

Seed shelf-life is an important factor in maintaining seed quality during storage. Seed-associated fungi including Aspergillus spp. and Fusarium spp. are major contributors to seed deterioration during storage, leading to reduced germination and vigor *(Haas & Défago, 2005)*. Biological control agents are increasingly used as alternatives to chemical treatments for protecting seeds. *Pseudomonas fluorescens,* a Gram-negative fluorescent bacterium, is widely recognized as a biocontrol organism that suppresses plant pathogens *(Weller, 2007)* through multiple mechanisms such as metabolite production and nutrient competition *(Raaijmakers et al., 2002).* Application of this bacterium as a seed coating material helps to extend shelf-life, protect against fungal infection, and improve seed germination and vigor.

2. REVIEW OF LITERATURE

Microbial seed coating involves covering seeds with a suspension of plant growth–promoting microorganisms (PGPM) plus binders/polymers, often as filmcoating, dressing, or pelleting. It is recognized as an efficient, lowdose delivery system that can improve germination, seedling establishment, yield, and tolerance to biotic and abiotic stress while reducing agrochemical use. *(O'Callaghan- et al.,2006),* Reviews emphasize that appropriate binders/fillers can extend microbial survival on the seed and are central to achieving adequate shelf life of both seeds and inoculants. *(Mitchener, B- et al., 2025)*

Biopriming is a related technique in which seeds are partially hydrated in a microbial suspension and then redried; it is frequently used with P. fluorescens and other PGPR for disease suppression and growth promotion *(Chin, J.-et al., 2022, , J., Patta, et al 2019)*, *P. fluorescens* is well established as a plant growth–promoting rhizobacterium and biocontrol agent, able to suppress diverse fungal pathogens, enhance nutrient uptake, and improve plant vigor and yield.

Beyond individual crops, broader reviews describe *P. fluorescens*as a **"prospective green antimicrobial"** that can enhance plant antioxidant status, immunity, and stress tolerance and serve as an alternative to synthetic pesticides. In oilseed rape *(Brassica napus)*, seed biopriming with *P. fluorescens* resulted in 10^6–10^8 CFU per seed, but during longterm dry stor-

age at 21 °C and very low humidity, bacterial viability declined rapidly, from ~10^9 CFU/seed to 10^4 after 4 weeks and 10^2 after 12 weeks. Nevertheless, maximum germination and early seedling growth were not negatively affected; an additive kimchi paste dramatically increased bacterial survival (~800 fold) during storage.

SAMPLE COLLECTION

To obtain potential isolates of *P. fluorescens*, different environmental samples that are naturally rich in microorganisms were chosen.

The sources included:

- **Fish tank wastewater** – collected from domestic aquaria containing accumulated organic waste.
- **Pond surface water** – taken from the upper layer of a stagnant pond.
- **Rice-soaked water –** prepared by soaking raw rice grains in sterile water for 24 hours and using the supernatant fluid.

Each sample was collected aseptically using sterile glass bottles (20–30 ml capacity). The bottles were tightly closed immediately after collection and properly labelled. No transport medium or preservative was used to avoid alteration of the natural microbial population. All collected samples were transported to the laboratory immediately and processed under aseptic conditions to preserve microbial viability *(Cappuccino & Sherman, 2014).*

ISOLATION OF PSEUDOMONAS FLUORESCENCE

The samples were collected from the Environments like fish tank water, pond water, and rice-soaked waterand screened for the presence of Pseudomonas fluorescence on Nutrient Agar – a Basal Medium *(Cappuccino & Sherman, 2014)*. All the samples were aseptically inoculated onto Nutrient Agar plates individually using a sterile loop and incubated at 37°C for 24–48 hours. Visible colonies appeared in 24 hrs of incubation with greenish yellow fluorescens.

Pigmented colonies were carefully picked and streaked onto King's B agar *(King et al., 1954),* a selective medium for *P. fluorescens* Sterile inoculated plates were incubated again at 37 °C for 24–48 hours. After incubation, colonies were observed under UV light *(King et al., 1954)* at 365 nm. The colonies were identified as *Pseudomonas fluorescens* stored for further examinations.

ANTIMICROBIAL EFFECT OF PSEUDOMONAS FLUORESCENCE

The antimicrobial effect of the isolated *P. fluorescens* was evaluated against common Aspergillus spp. a seed pathogen. *P. fluorescens* are known to inhibit the growth of plant pathogenic fungi. This bacterium produces several antimicrobial compounds such as hydrogen cyanide (HCN) and siderophores *(Raaijmakers et al., 2002),* A compound to suppress fungal growth by interfering with cell wall formation and membrane stability- phenazine, and 2,4-diacetylphloroglucinol (DAPG). for the properties, P. fluorescens is considered an effective biocontrol agent *(Compant et al., 2005)* and is often used in seed coating to protect seeds from fungal attack. Antifungal activity of Pseudomonas sps helps in extending the shelf life of seeds by preventing fungal contamination and maintaining seed quality during storage.

ANTI-FUNGAL EFFECT STUDIES

The antifungal activity of Pseudomonas fluorescens, neem oil, and turmeric extract was accessed against Aspergillus spp. Mueller–Hinton Agar (MHA) *(Bauer et al., 1966)* plates were used as assay medium and well diffusion method was employed. The fungal suspension was evenly spread over the surface of sterile MHA plates to obtain uniform growth. 6 mm diameter wells were made aseptically using a sterile well borer, and 100 micro L of each test sample was added into the marked wells. Three separate plates were prepared - one with a higher concentration of neem oil, another with a higher concentration of turmeric extract, and the third with a higher concentration of Pseudomonas fluorescens, while maintaining the other two agents at lower levels in each setup. A well containing Clotrimazole was maintained as the control. The plates were incubated at 28 ± 2°C for 48–72 hours, and the presence of a distinct inhibition zone surrounding the wells was considered as evidence of antifungal activity. Among the three treatments, the plate with a higher concentration of neem oil showed the widest inhibition zone *(Subapriya & Nagini, 2005),* followed by *Pseudomonas fluorescens,* while turmeric extract showed moderate activity *(Prasad & Agarwal, 2011)* compared to the control.

SEED COATING FORMULATION

Seed coating was carried out by using the effective concentrations derived from the antimicrobial assay. The zone of inhibition values obtained for Pseudomonas fluorescence, neem oil, and turmeric extract were taken as the reference for preparing the respective formulations.

Table 1 Selected agents

Plate No.	*Pseudomonas fluorescence* (µL)	Turmeric extract (µL)	Neem oil (µL)
Control	-	-	-
Plate-1	60	10	30
Plate-2	30	20	50
Plate-3	25	50	25

Seeds were surface sterilized and subsequently coated with the prepared formulations to ensure uniform coverage. The coating is to be done in such a way that the entire surface of the seed is covered uniformly. After coating, the seeds are to be shade-dried and stored under sterile conditions for further evaluation. *(Taylor et al., 2001).*

STORAGE STABILITY TEST

The coated seeds were stored under two conditions: room temperature and refrigeration. The experiment was conducted at regular intervals (0, 2, 4, 8, and 12 weeks) to evaluate storage stability. The following parameters were evaluated using the following criteria:

1. ***Viability of Pseudomonas fluorescens –*** Coated seeds are to be shaken in sterile water, diluted and plated on King's B agar. The number of colonies indicates the survival of the bacterium on the seeds.
2. ***Germination Percentage –*** A set of coated and control seeds is to be germinated on moist blotter paper, and the percentage of germination is to be recorded.
3. ***Resistance to Fungal Infection*** – Seeds are to be exposed to Aspergillus spores, and the level of infection is to be compared with control seeds.

The treatment that maintained higher bacterial viability, good germination, and lower fungal infection during storage is to be considered effective for extending seed shelf-life. *(Bennett, 2017).*

RESULTS &DISCUSSION

ISOLATION AND IDENTIFICATION OF PSEUDOMONAS FLUORESCENS

The bacterial isolate was success fully obtained using nutrient agar medium.

After incubation colonies showed a smooth texture with circular morphology and a pale-yellow pigmentation. Further confirmation of the isolate was performed using king's B agar medium *(King et al., 1954),* where colonies showed a distinct greenish-yellow fluorescence when exposed to ultraviolet light (365 nm).

The Green pigmented colonies of *Pseudomonas fluorescens* forming pyoverdine was identified and isolated *(Weller, 2007). Pseudomonas fluorescens* appeared as Gram-negative rod and are motile in microscopy.

ANTIMICROBIAL ACTIVITY AGAINST ASPERGILLUS SPP.

To evaluate the anti-fungal potential of the prepared bioagents using the agar well diffusion method, measured volumes of (25 µL, 50 µL, and 100 µL) the treatment mixture were added into marked wells. Clear zones of inhibition were observed around the wells and confirmed the antifungal efficiency of the treatment mixture against Aspergillus sps. *(Raaijmakers et al., 2002).* The diameter of the inhibition zones increased with increasing concentration, indicating a concentration-depending response. Among the treatments, neem oil demonstrated comparatively higher antifungal activity, followed by Pseudomonas fluorescens and turmeric extract *(Compant et al., 2005).*

EFFECT OF SEED COATING ON FUNGAL GROWTH

BIOAGENT-COATED SEEDS (NEEM + TURMERIC + PSEUDOMONAS)

On Day I, no detectable fungal growth was observed in seeds exposed to Aspergillus spp. (Infected Control) But by Day 3, initial fungal growth appeared on the surface of these seeds and on Day 8, active fungal colonization was observed, and germination was also reduced *(Haas & Défago, 2005).*

Surprisingly seeds coated with the bioagent formulation showed no fungal growth throughout the observation period. The coating remained uniform, and seeds also maintained its morphology even after 8 days without shrinking. This clearly showed the treatment effectively suppressed fungal growth while

Figure 1: Antifungal activity test against Aspergillus spp.

Figure 2: Antifungal activity test against Aspergillus spp.

EFFECT OF SEED COATING ON FUNGAL GROWTH

Figure 3a:
Day 1 – Initial seed condition

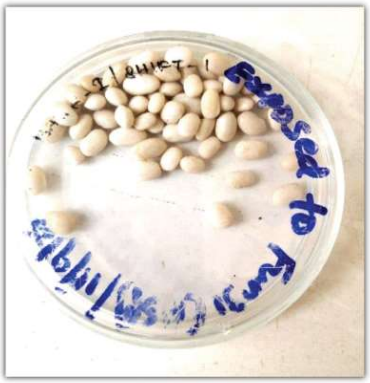


Figure 3b:
Day 3 – Early fungal growth visible

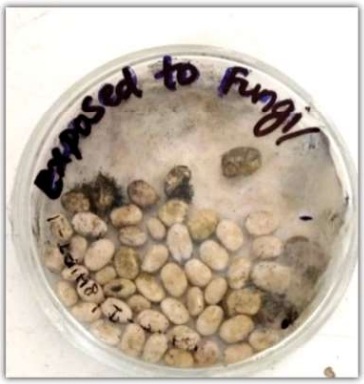


Figure 3c:
Day 8 – Heavy fungal growth and poor germination

BIOAGENT-COATED SEEDS (NEEM + TURMERIC + PSEUDOMONAS)

Figure 4a:
Day 1 – Initial seed condition visible

Figure 4b:
Day 3 – Coating uniform, no fungal growth

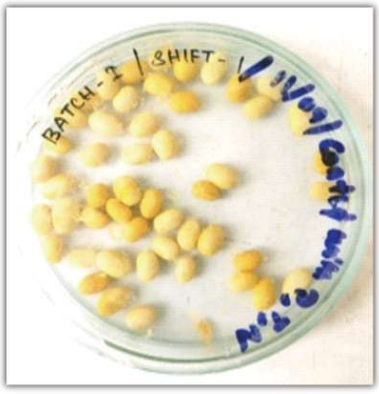


Figure 4c:
Day 8 Coating intact, seeds healthy

Figure 5a:
Day 1 – Initial seed condition

Figure 5b:
Day 3 – No visible changes

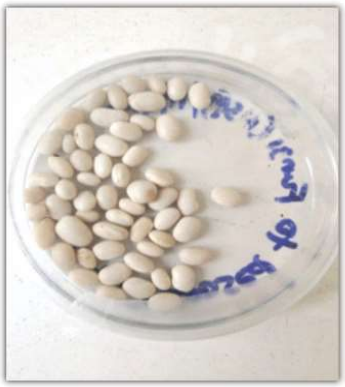


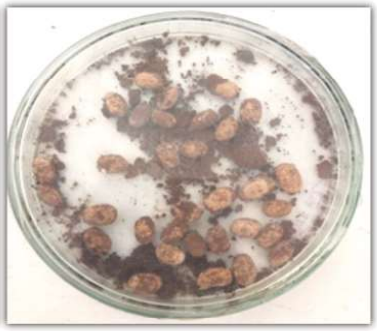
Figure 5c:
Day 8 – Light fungal contamination visible

RED SOIL-COATED SEEDS

Figure 6a:
Day 1 – Initial seed condition with red soil

Figure 6b:
Day 3 – Seeds healthy, no visible changes

Figure 6c:
Day 8 – Seeds healthy, fungal contamination and no



COMBINED TREATMENTS (BIOAGENT + RED SOIL)

Figure 7a:
Day 1 – Initial seed coating visible

Figure 7b:
Day 3 – Early germination visible

Figure 7c:
Day 8 – Seeds growing well with minimal fungal contamination

preserving seed viability throughout the observation period *(Weller, 2007; Rocha et al., 2019).*

UNTREATED SEEDS (CONTROL)

Untreateds seeds though initially had no significant changes but during the course of study developed (Day 8), slight fungal growth and also rate of germination significantly lower than the lower than treated seeds *(Haas & Défago, 2005).*

RED SOIL-COATED SEEDS

Seeds coated with red soil remained stable initially but showed slight fungal contamination by Day 8. No significant germination was observed, suggesting that

red soil alone has limited antifungal effectiveness.

COMBINED TREATMENTS (BIOAGENT + RED SOIL)

Neem-Dominant Combination: Early germination was observed in neem dominant bio mixture by Day 3 and healthy seedling growth by Day 8. Fungal contamination was significantly reduced in this lot. This combination exhibited comparatively better performance among the treatments. Pseudomonas-Dominant Combination showed no germination the initial stages, but by Day 8, all the seeds actively germinated and no fungal growth was observed in any of the seeds *(Rocha et al., 2019).*

EQUAL RATIO COMBINATION

When the mixture was made with equal proportion of all the 3 components Neem, Pseudomonas and Turmeric, the germination was fully inhibited even though there was a strong suppression of fungal growth even after 8 days (Total course of study). This shows the equal proportion combination strategy cannot be used for seed coating *(Paravar et al., 2023).*

Neem-Dominant Combination:

Figure 8a:
Day 1 – Initial seed coating visible

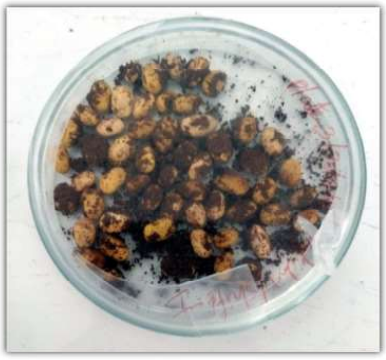


Figure 8a:
Day 1 – Initial seed coating visible

Figure 8a:
Day 1 – Initial seed coating visible

EQUAL RATIO COMBINATION
equal proportion of all the 3 components Neem, *Pseudomonas* and Turmeric,

Figure 9a:
Day 1 – Initial seed coating visible

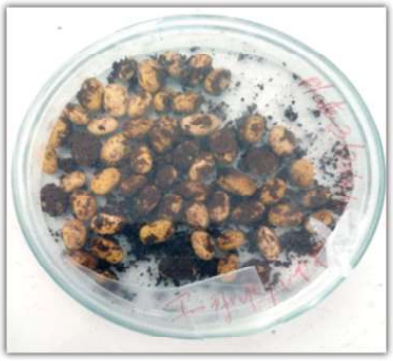


Figure 9b:
Day 3 – Coating uniform, no

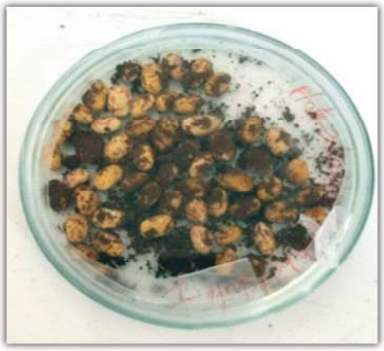
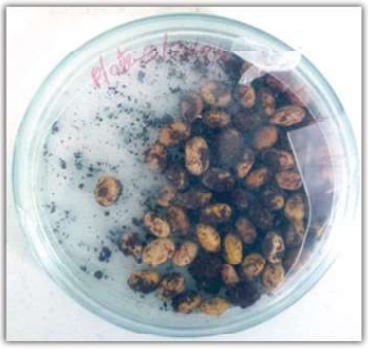


Figure 9c:
Day 8 – Seeds still not germinated



DISCUSSION:

It is clear from the results of the current study that the use of bio-based seed coating has an important role in regulating fungal contamination as well as enhancing the performance of the seeds during storage. The variations among different treatment used in this experiment demonstrate that each type plays an important role in inhibiting fungi and promoting germination of the seeds based on their characteristics and concentrations.

Of all the types of treatment that were used in this study, the most effective against fungi was neem oil. The high efficacy of neem oil could be because of the secondary metabolites found in it such as azadirachtin *(Subapriya & Nagini, 2005).*

The potential of *Pseudomonas fluorescens* as a biological seed treatment was high as well. It had an ability to inhibit the growth of fungi but stimulate seed germination and development. It is characterized by the production of several antimicrobial agents, including phenazines, hydrogen cyanide, and siderophores, which are responsible for the antagonism *(Raaijmakers et al., 2002).*

Furthermore, it competes with pathogens for nutrients and colonization niches on seeds' surface and thus prevents their growth. Additionally, its ability to colonize the rhizosphere promotes its effectiveness *(Weller, 2007).* Delayed germination, observed with Pseudomonas-dominated treatments, confirms the sustainability of its effect.

Low efficiency of antifungal activity of turmeric extracts may be explained by certain factors, including extraction method, concentration, and stability of active compounds (curcumin) *(Prasad & Aggarwal, 2011).* Despite exhibiting poor results when used independently, use of turmeric extract in combination treatments may result in additional advantages.

One of the important findings of this experiment includes the necessity to optimize formulation ratios. *Neem-based and Pseudomonas-based treatments demonstrated higher germination rates* and less fungal growth. In contrast, germination was fully inhibited by the combination treatment despite effective control over fungi growth. Thus, excessive concentrations of biologic agents can have an inhibitory effect on seeds. It should be noted that optimizing the ratio of ingredients allows obtaining high antifungal effect as well as proper germination.

Untreated samples demonstrated gradual fungal growth beginning with Day 3 and continued up to Day 8, together with low germination. This proves the role of storage fungi such as Aspergillus in reducing seed quality *(Haas & Défago, 2005).* Seed samples covered with red soil only were also ineffective in protecting fungi-free environment.

The results obtained from this research are in line with previous results on microbial seed coating. According to past literature, the process of seed coating with useful microbes is associated with improvement in seed germination, seedling vigor, and plant resilience to environmental stress *(Paravar et al., 2023).* Moreover, microbial seed coating is recognized as an effective method as it delivers beneficial microorganisms directly to the surface of seeds *(Rocha et al., 2019).*

Bioagents were proven to be helpful for retaining seed quality in storage as well as reducing susceptibility to fungal infection. The seeds treated in this experiment had no visible signs of shrinkage and damage in spite of exposure to adverse conditions. This indicates that bioagents are effective for preservation of seed quality during storage. Improved germination percentage among treated seeds proves that bioagents are harmless and safe for seed viability.

Moreover, some recent publications discuss a new technology of seed coating using green-synthesized nanoparticles. The latter is characterized by high activity against harmful pathogens due to better interaction with seeds. Therefore, green-synthesized nanoparticles have potential for usage in seed coating formulations. Use of microbial and botanical treatments together, as seen in this experiment, appears to be a promising strategy towards development of green seed coatings.

These can serve the purpose of immediate anti-fungal activity and also the purpose of long-term anti-fungal protection due to microbial activity. Nevertheless, the success of such bio-chemical mixtures highly depends on correct optimization of the ingredients used.

As a whole, the outcomes of this experiment indicate that bio-based seed coatings represent a viable strategy for replacing traditional chemical seed treatments. They not only help to get rid of fungi but also stimulate germination of seeds and growth of seedlings. Sustainable methods like these allow saving the chemicals, which have negative effects on the environment.

Another significant observation made in this study is the persistence of the microbial population on

TABLE 2: DAY-WISE GERMINATION AND TREATMENT RESPONSE OF COATED SEEDS

TREATMENT TYPE	DAY 1	DAY 3	DAY 8
Control (Untreated)	No visible change	No germination	Poor germination with fungal growth
Bioagent Coated (Neem + Turmeric + *Pseudomonas*)	No change	No fungal growth	Healthy seeds, no fungal contamination
Neem Dominant	No change	Early germination	Healthy growth, minimal fungal infection
Pseudomonas Dominant	No change	No germination	Complete germination, no fungal growth
Equal Ratio (All 3)	No change	No germination	No germination, fungal suppression observed
Red Soil Coated	No change	No visible change	Slight fungal contamination, no germination

the seed surface during storage periods. The efficiency of the bio-based seed coating largely relies on the capacity of the microbial populations to retain their viability through storage. It was observed that the microbial numbers decrease gradually during storage; however, treated seeds perform relatively better than untreated seeds. Thus, despite having a smaller number of beneficial microbes, the seeds may exhibit better results.

The method used for coating plays an essential role in making the treatment efficient. For uniform coating, it becomes necessary to have equal distribution of the bioagents onto the seed surfaces to ensure good antifungal action and seed performance. In addition, using an appropriate coating technique can enhance adhesion of the microorganisms on seeds *(O'Callaghan et al., 2006).*

Moreover, biological seed treatments like biopriming have been observed to boost the germination of seeds and seedling development. This method enhances the colonization and function of microorganisms, resulting in more effective plant establishment *(Chin et al., 2022; Patta et al., 2019).* From the findings presented above, it is clear that the application of plant and microbial inoculations can offer dual benefits in protection and growth, hence being suitable substitutes in agriculture.

CONCLUSION

In summary, this paper provides insights into the possible use of biological coating of seeds as a promising means of improving the quality and shelf-life of oilseed seeds. It is evident from the analysis carried out in the experiment that bioagents have a huge contribution in minimizing the occurrence of fungi and maintaining seed viability during storage.

Amongst the bioagents used in the study, **neem oil is found to perform better** compared to others in terms of controlling the growth of fungi and increasing the rate of seed germination. In addition to this, *Pseudomonas fluorescens* is found to contribute significantly towards the health of seeds and offer protective effects on them. Turmeric extract also shows limited benefits on its own, although it could possibly be used in appropriate combinations.

The study also brings into focus the ratio between bioagents, which plays a crucial role since some inappropriate ratios tend to impair the germination

process despite controlling fungal growth. In essence, the use of microbial and herbal agents provides a safe and environmental way of managing seeds.

The germination capability and disease resistance remained intact until the period between 8 to 12 weeks, while there was no growth and decay started right after one week.

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1. Competing Interests: The authors declare that they have no competing interests.

2. Availability of Data and Materials: This research article is based solely on publicly available research articles, books, and scientific reports. All referenced materials are properly cited within the manuscript. No new experimental data were generated or analyzed in this study. Readers can refer to the cited sources for further details.

3. Consent for Publication: yes

4. Ethics Approval and Consent to Participate: Not applicable.

5. Use of AI Tools: AI tools were utilized to assist in grammar refinement, content structuring, and enhancing readability. However, the intellectual content, critical analysis, and interpretation of the literature were independently developed by the authors. All sources used in the manuscript are appropriately cited to ensure academic integrity and originality.

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