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Alterations in seedling vigour in mungbean selected genotypes under pre-sowing yeast and chitosan as an elicitor seed treatment against *Macrophomina phaseolina* in India

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Abstract
Climate change, resource depletion, and biodiversity loss are all threatening our agriculture. A new agricultural revolution is required to improve crop yield while also ensuring food quality and safety in a sustainable manner. Seed priming with yeast and chitosan can help to ensure agriculture's long-term viability. Seed elicitor priming is a powerful technique that alters seed metabolism and signalling pathways, affecting not just germination and seedling establishment, but also the entire plant life cycle. Improved plant growth and development, higher productivity, and improved food nutritional quality are just a few of the benefits. Elicitor -priming affects biochemical pathways as well as the balance of reactive oxygen species and plant growth hormones, promoting stress and disease resistance while reducing pesticide and fertiliser use. The germination percentage, shoot height, and shoot weight were all used to assess seed vigour. The addition of yeast and chitosan as an elicitor had a modest positive effect on seed vigour, implying that pre-sowing treatments can have positive or negative effects on seed vigour depending on the dosage of treatments. More research is needed to establish their effects and the best seed priming dosage.

Keywords: *Macrophomina phaseolina*, elicitors/inducers, mungbean, pre-sowing seed treatment, vigour index

Introduction
Mungbean is the world's most important legume crop (*Vigna radiata* L. Wilzeck). Vigna belongs to the Papilinoidae subfamily and the Leguminoseae family. It is mostly grown in Asia, but it has recently spread to Africa and the Americas. Because of its high protein content, Vigna radiata is consumed in the form of sprouts and dry seeds. Mungbean is a valuable crop that is commonly grown in dry and semiarid areas due to its rapid growth and early maturity, as well as its ability to replenish soil fertility. The charcoal rot disease, caused by the soil-borne plant pathogen M. phaseolina, is wreaking havoc on this valuable crop (Fuhlbohm *et al*., 2013). This common fungal pathogen is found in the tropics and subtropics, where it infects over 500 plant species, including angiosperms and conifers (Dhingra and Sinclair, 1978) [8].
Although some chemical fungicides have been shown to be effective against M. phaseoli (Ilyas *et al*., 1975), no registered fungicide against this fungal pathogen is currently available on the market. There have been numerous reports in the past and recent literature claiming that plant extracts and soil amendments containing specific plant species can be effective in the management of plant diseases (Lewis and Papavizas, 1971; Javaid and Iqbal, 2014; Javaid and Rauf, 2015; Khurshid *et al*., 2016).
Plants are affected by *Macrophomina phaseolina* in the root, stem, branches, petiole, leaves, pods, and seeds. Furthermore, *Macrophomina phaseolina* seed infection ranges from 2.2-15.7 percent, resulting in a 10.8 percent reduction in grain yield and a 12.3 percent reduction in protein content in urdbean (Kaushik *et al*., 1987) [11]. In mature plants, *Macrophomina phaseolina* causes red to brown lesions on the roots and stems. As a result of the dark mycelia and black microscelerotia, plants became defoliated and wilted (Abawl and Pastor- Corrales, 1990) [1]. *Macrophomina phaseolina* is a heat-tolerant pathogen that thrives in temperatures ranging from 60 to 65 °C (Bega and Smith, 1962: Milhail and Acron, 1984) [4].
Increasing plant resistance has the potential to be useful in agricultural applications. The elicitor should be nontoxic, biodegradable, and cost effective for this application. Yeast meets all of these requirements and has been used as an elicitor of defence responses in cell cultures and whole plants in various studies.

In different plant cell cultures, yeast induced phytoalexin biosynthesis, expression and activity of the enzyme phenylalanine ammonia lyase, and accumulation of the oxylipins JA and 12-oxo-phytodienoic acid (OPDA) (Basse and Boller 1992; Blechert *et al*. 1995; Parchmann *et al*. 1997; Suzuki *et al*. 2005) [3, 5, 16, 6]. Less is known about yeast's effect on whole plants. Soybean treatment increased phytoalexin accumulation (Hahn and Albersheim 1978) [9]. The use of yeast cell-wall extracts on barley improved its resistance to powdery mildew (Reglinski *et al*. 1994) [18]. However, the mechanisms underlying this increased resistance remain unknown.

Chitosan is a naturally occurring substance found in the cell walls of fungi. In the host-parasite contact, chitosan generated by the fungus enters the plant cells and accumulates within the fungal cell. Chitosan's ability to start phytoalexin production, protect pea tissue against *F.solani f. sp. pisi*, and/or directly stop fungal development suggests it could play a key role in disease resistance (Manjunatha *et al*. 2009) [13].

Materials and Methods

The experiment was carried out on two gentoypes of mungbean, resistant and susceptible, namely Bireswar and Samrat, which were surface sterilised with 1.0 percent sodium hypochlorite and sown post seed treatment with the Preparation of yeast elicitor solution. To make the 0.3 percent Yeast Elicitor solution, yeast (Saccharomyces cerevisiae) was cultured in YEPDA broth (Yeast extract 1%, Peptone 2%, and Dextrose 2%) in a 250 mL Erlenmeyer flask containing all of the ingredients and then incubated on an orbital platform shaker at 300 °C and 140 rpm for 72 hours. After 72 hours of incubation, the broth was filtered, and the filtrates were collected and mixed with ethanol solution, which was equivalent to 0.3 percent, according to the key outlines provided by Cakir *et al*. 2009 [7]. By adding the required amount of distilled water to the stock solution, 0.02 percent, 0.05 percent, 0.1 percent yeast elicitor solutions were prepared. And also with the To make a 0.3 percent chitosan stock solution, 3 g dried chitosan was slowly dissolved in glacial acetic acid while being constantly stirred with a magnetic stirrer, then diluted with distilled water to a volume of 1000 mL, which was equivalent to 0.3 percent. By adding the needed amount of distilled water to the stock solution, 0.02 percent, 0.05 percent, 0.1 percent, and 0.2 percent chitosan solutions were created.

Control plants were grown and sprayed with distilled water. Following harvest, treated and untreated seeds, as well as infected seeds, were sent to the lab for analysis to determine seed vigour and, as a result, to recommend and extend pre-sowing seed treatment with elicitors to farmers in order to improve seed quality, which is linked to future growth and disease resistance dimensions. The seedling vigour of harvested green gram seeds was tested using the Petriplate method. Petri dishes were lined with two layers of germination paper, and 35 seeds were given control medium before being placed in a germinator at 25ºC for eight days, which is the final count period for mungbean. Continuous observation was performed every 24 hours from the time seeds with torn seed coats and extreme lengths of more than 2mm were judged to have germinated (Karaguzel *et al*., 2002). Ten random seedlings were observed in each Petri dish and their root and shoot lengths were measured to calculate seedling vigour. Seedling vigour is the cumulative effect of emerging seeds under a variety of biotic and abiotic conditions. Seedling vigour is a combination of several growth indices such as seedling length, fresh seedling weight, and seedling dry weight. While evaluating pre-sowing seed treatment on a variety of crops, a group of researchers observed several crops in various modes of observation (Neeraj *et al*. 2012; Torkal *et al*., 2015; Mohamadui *et al*., 2012; Rajesh *et al*., 2017) [15, 17].

Treatment specifics

T1: Induced seed treatment with pathogen inoculation
T2: Induced seed treatment without pathogen inoculation
T3: Pathogen inoculation with no seed treatment (water).
T4: No seed treatment (water), no pathogen inoculation.

Seed Germination Test

Three replicates of 25 seeds from each variety were treated with different elicitors at varying doses, and germination percentage was determined using the blotting paper method specified in the International Rules for Seed Testing (ISTA, 1993) [10]. The seeds were incubated in a Petri dish with moistened blotting paper. The percentage of germination was calculated after seven days using the formula:

1.Germination percentage = total number of seeds multiplied by the number of seeds germinated.

Seedling Vigour Index

Three replicates of 25 seeds from each variety were treated with different elicitors at various concentrations and stored in a Petri plate. After one week of incubation, the germination percentage and seedling vigour of seed samples kept at room temperature were measured in order to compute the vigour index using the Abdulbaki and Anderson (1976) [2] formula.

2.Vigour index = (mean root length + mean shoot length) × Germination percentage

Results and Discussion

The influence of elicitors on several seedling parameters was evaluated using the seedling vigour index in two mungbean genotypes: bireswar (resistant genotype) and Samrat (sensitive genotype) against Charcoal rot caused by *M. phaseolina.* Elicitors at varied concentrations reduced disease occurrence and demonstrated a substantial difference between various defense-related chemicals (Rayanoothala *et al*.,2021). Increases in the concentrations of the various elicitors were found to result in a considerable rise in defense-related chemicals in both pathogen and non-pathogen inoculation plants which was also my study of experiment which is not included in this article. Table 1,2,3 and 4 and Figure 1 and 2 demonstrate that, In both healthy and diseased mungbean seeds, treated mungbean seeds showed a greater vigour index in all the two genotypes, where seedling vigour index was determined after knowing the germination % and seedling length.

In the Bireswar mungbean genotype, the greatest seeding vigour index was observed at 0.02% concentration of chitosan elicitor (24.513) followed by 0.05% yeast treated mungbean seeds (24.366). Similarly, it showed a substantial increase in vigour index as compared to control plants, but had a greater vigour index than infected plants in the samrat variety. The effectiveness of 0.02% concentration of chitosan elicitor content, on the other hand, followed the same pattern as the other two genotypes. The graph indicated the efficiency of elicitors in all two genotypes, as well as between damaged and healthy seeds.

Table 1: Varietal reaction to Yeast and chitosan on Vigour index of mungbean (Bireswar)

Inducers	0.02%	0.05%	0.1%
Yeast	24.229	24.366	23.993
Chitosan	24.513	23.025	21.875
Yeast +P	23.733	23.872	23.492
Chitosan+P	24.023	22.503	21.325
Control		19.613	

- The above are the means of the three replications of the transformed values.

Table 2: SEm±, CD (*P*≤0.05), CV % Of inducers and their interaction with different concentrations (Bireswar)

	Inducers	Concentrations	I x C	Check vs Others
SEm±	34.51549	34.51549	34.51549	37.57277
CD (P≤0.05)	99.36677	99.36677	99.36677	108.1684
CV %	159.1223			

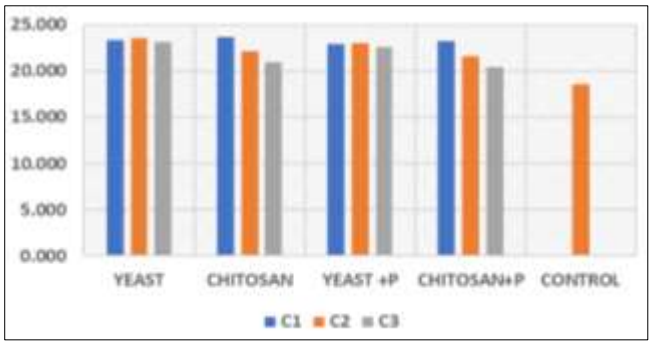

Fig 1: Graphical representation of impact of elicitors on seedling vigour in bireswar genotype of mungbean

Table 3: Varietal reaction to Yeast and chitosan on Vigour index of mungbean (Samrat)

Inducers	0.02%	0.05%	0.1%
Yeast	23.415	23.557	23.171
Chitosan	23.709	22.168	20.971
Yeast +p	22.902	23.046	22.652
Chitosan+p	23.202	21.625	20.396
Control		18.599	

- The above are the means of the three replications of the transformed values.

Table 4: SEm±, CD (P≤0.05), CV % Of inducers and their interaction with different concentrations (Samrat)

	Inducers	Concentrations	I x C	Check vs Others
SEm±	34.51549	34.51549	34.51549	37.57277
CD (P≤0.05)	99.36677	99.36677	99.36677	108.1684
CV %	355.5616			

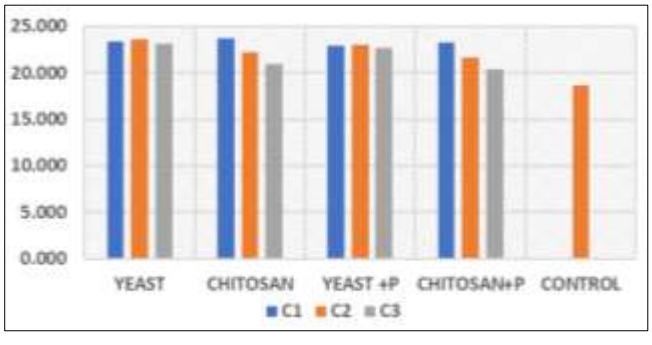

Fig 2: Graphical representation of impact of elicitors on seedling vigour in Samrat genotype of mungbean

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Conflict of interest
There are no conflict of interests to declare to publish this article

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