# The Effect of Different Seed Treatment Methods on Growth and Development of Mungbean


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## Abstract:

Mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*] is an important pulse crop cultivated mainly in Asia. One of the constraints to mungbean production is poor seedling emergence in the field. A review of literature was conducted to evaluate the effect of different seed treatment methods on growth and development of mungbean. Experiments were conducted both under controlled condition (glasshouse) as well as in the field at The World Vegetable Center, South Asia, Hyderabad during June-August 2016 to investigate the effects of different seed treatments on the growth of mungbean using the most popular variety, NM 94. The treatments consisted of seed priming, *Trichoderma viridae, Rhizobium*, neem oil, thiram, chlorpyriphos, and in different combinations. Data were collected on seedling height, days to flowering, number of root nodules per plant and on the incidence of root rot disease. In the glasshouse experiment, significant ($P < 0.001$) differences were observed between the treatments for seedling height, an indirect measure of plant vigour. However, no nodulation was recorded for the treatments, which highlights the importance of quality control in rhizobia production. In the field experiment, no significant difference was observed for the traits studied. All the treatments in the field showed good nodulation, ranging from 24 to 45 nodules per plant, which indicated the abundance of resident rhizobia in the field. Incidence of root rot (*Macrophomina phaseolina*) disease was observed in patches in the field, implying the susceptibility of NM 94 variety to the disease. New sources of resistance to the disease has to be identified so as to improve the popular variety.

## Background: World Vegetable Center

The World Vegetable Center, previously known as the Asian Vegetable Research and Development Center (AVRDC) is an international, non-profit organization. The World Vegetable Center has a mission to reduce poverty and malnutrition through improved production and consumption of vegetables. The organisation commenced working from 1971, with its headquarters in Taiwan and has expanded its operations to other parts of the world. The South Asia office is located in Hyderabad, India. The Center works on four thematic areas: Germplasm, Breeding, Production and Consumption. The Center's Genebank maintains the world's largest public vegetable germplasm collection with more than 61,000 accessions from 155 countries, including about 12,000 accessions of indigenous vegetables. The activities of the global legume breeding program is currently being coordinated from the South Asia office. Mungbean ([*Vigna radiata* (L.) R. Wilczek var. *radiata*] is one of the mandate crops of the World Vegetable Center.

## Literature Review:

### Introduction

Mungbean is an important pulse crop cultivated predominantly in South Asia, East Asia and South-East Asia and covers more than 6 million ha worldwide. India, with approximately 3 million ha, has the largest mungbean production area followed by China and Myanmar. The production in India amounts to 1.81 million t (Nair *et al.*, 2012). Poor crop establishment is a major constraint for mungbean production and high yields can be associated with early vigour. The average productivity of mungbean is low as several constraints are there which affects the productivity. Inclusion of quality seeds in cultivation leads to increase of 15-20% seed yield (Yadav *et al.,* 2014).

### Importance of seed treatment

Seed treatment is the use and application of biological and chemical agents that control or contain primary soil and seed borne infestation of insects and diseases which pose devastating consequences to crop production and improving crop safety leading to good establishment of healthy and vigorous plants resulting better yields. Seed treatment may increase the germination, ensure uniform seedling emergence, protect seeds or seedlings from early diseases and insect pests thereby improve the crop emergence and growth, improve plant population and thus increase productivity.

### Types of seed treatment

Seed treatment with different types like bio-agents, botanicals and chemicals may improve the seedling health at initial stage and encourage the vigour of the plants. Many researchers conducted experiments on the effect of different seed treatments on mungbean.

#### *Seed priming*

For achieving good crop establishment and to enhance the biological nitrogen fixation of crops seed priming is a technique in which germination processes begin but radicle emergence does not occur (Taylor *et al.*, 1998). Musa *et al.*, 2001 stated that seed priming hastens germination, enhances crop establishment and promotes seedling vigour. The process involves soaking the seed overnight (for about 8 hrs), surface drying them and sowing within the same day. Seed priming was found effective for legumes and the yield of legume crops were increased by priming seeds before sowing (Rashid *et al.*, 2004). Application of *rhizobium* with seed priming significantly increased nodulation and nitrogenase activity (Harris *et al.*, 2004). Umair *et al.*, 2011 conducted different kind of seed priming in mungbean and found that seed priming increased the seed yield of mungbean considerably and significantly increased nodulation, nitrogen fixation and nutrient uptake. Priming also reduced the incidence of stem and root diseases and increased nodulation by native rhizobia.

***Trichoderma spp.***

Biological control is a potential alternative to chemical fungicides. Trichoderma comes under mycoparasites which includes the most widely used biocontrol agent for seed borne, soil borne and other diseases. Hussain *et al.,* 1990 observed that *Trichoderma harzianum* was effective in controlling the infection of *Macrophomina phaseolina* in mungbean. *Trichoderma harzianum* was more effective in reducing pre and post emergence mortality caused by root rot and *Trichoderma viridae* is moderately effective to root pathogens (Kumari *et al.,* 2012). Gawade *et al.,* 2016 conducted an experiment in mungbean with different seed treatments. Among the different treatments they found that *Trichoderma viridae* combined with *Pseudomonas fluorescens* was effective in reducing the seed mycoflora which causes diseases. Kar and Sahu (2008) reported that *Pseudomonas fluorescence* and *Trichoderma viridae* is effective against diseases as well as increasing seed germination of mungbean and tomato.

***Rhizobium***

Harris *et al.,* 2004 reported that application of *rhizobium* along with seed priming significantly increased nodulation and nitrogenase activity. El-Gali (2012) reported that rhizobacteria increases the nodulation and also plays an important role in suppressing the root rot disease in bean. Yadav *et al.*, 2014 reported that seed treatment with *rhizobium* increased seed yield and harvest by nearly one and half times. The increased seed yield and harvest index were mainly owing to contribution of increased yield contributing traits like number of pods per plant, pod length, number of seeds per pod including seed weight.

***Neem oil***

Seed treatment with neem oil formulations and powdered neem will suppress the nematode population growth and increase the grain yield significantly (Vijayalakshmi and Majumdar, 1999). Kumar *et al.,* 2015 reported that Neem oil improves the emergence of seedlings significantly in combination with Thiram.

***Thiram***

Rao *et al.,* (2012) found that seed treatment with hydration and drying followed by dry dressing with Thiram @ 0.25% was very effective for crop establishment with vigorous seedlings, increased seed germination, improved field emergence index and increased seed yield. Kumari *et al.,* 2012 reported that Thiram @ 100ppm concentration effectively inhibit the mycelial growth of *Macrophomina phaseolina* by 98.30%. Shahid and Khan (2016) conducted seed treatment in mungbean with different fungicides and they found thiram to be more effective than mancozeb and vivatex. When seeds were treated with thiram before sowing, it enhanced the yield by 19.7%, increased the functional nodules by 29.6-36.3% and increased the chlorophyll content by 19.8-28.3%. It also reduced the pathogen by 33.3-54.1% in soil. Kumar *et al.*, 2015 reported that seed treatment with thiram followed by osmo-priming had increased germination significantly over control. Apart from fresh seed lot, old seed lots also responded well with thiram when combined with halopriming or polymer and the speed of emergence also increased significantly.

***Chlorpyriphos***

Seed treatment with chlorpyriphos provided good seed germination in the field, maintained optimum plant population and provided protection against termites and other soil insects (Singh *et al.*, 2007).

**Combinations of seed treatments**

Muthomi *et al.,* reported that combination of copper oxy chloride and rhizobia enhanced nodule formation and therefore biological nitrogen fixation in food legumes was increased. When fungicide was used in combination with *Rhizobia*, the disease incidence was significantly reduced and nodulation increased compared to when applied alone. Jayaraj and Ramabadran (1999) reported that there was significant reduction in incidence of root rot in black gram (*Vigna mungo* (L.) Hepper) when *Trichoderma* and *Rhizobia* were applied in combination compared to *Trichoderma* alone. The combination of bioagents such as *Rhizobium*+*Bacillus subtilis*+*Trichoderma viride* showed significant results compared to individual treatments of bioagents and fungicides and played a significant role in field emergence, days to 50% flowering, plant height, number of branches, number of pods/plant, seed index, seed yield per plant and high benefit cost ratio (Kanti *et al.*, 2013). Bagwan (2010) reported that neem oil enhanced the growth of *Trichoderma*.

## Experiment Report:

### Introduction

Mungbean ([*Vigna radiata* (L.) R. Wilczek var. *radiata*] is an important pulse crop in South, East and Southeast Asia. High in protein and iron, it can have a positive impact on many health related issues. It is grown on 6 million hectares worldwide mainly by smallholder farmers. Also known as moong, green gram, golden gram, chickasaw pea, and chop suey bean, the mungbean is an ancient crop in India. One of the major challenges in mungbean production is poor seedling emergence. Seed treatments may be able to improve the germination and plant vigour of mungbean.

### Objective

In this study our objective was to identify the effect of different seed treatments on the growth of mungbean, using the most popular variety, NM 94.

### Materials and Methods

An experiment on effect of different seed treatments on mungbean growth and development was conducted during *Kharif*, 2016 in both glasshouse and field of World Vegetable Center, Hyderabad using the popular mungbean variety, NM 94. For this experiment, seeds with different treatments were sown in a randomised block design (RBD) with three replications. Seeds were treated with fungicide first and dried for 30 minutes and later treated with insecticide. The treatments details are listed in Table 1. Analysis of variance was conducted for both glasshouse and field experiments using Statistical Analysis System (SAS) (SAS Institute, Cary, NC). Square root transformation was applied for percentage of root rot damage in the field experiment.

**Table 1: List of treatment details used in this experiment**

| Treatment number | Treatment details | |
|---|---|---|
| T1 | Priming | Seed soaked in distilled water for 5 hours and dried at room temperature for 12 hours |
| T2 | *Trichoderma* | @ 10 g / Kg |
| T3 | *Rhizobium* | @ 25 g / Kg |
| T4 | Neem oil | @ 5ml / Kg |
| T5 | Thiram (fungicide) | @ 3 g / Kg |
| T6 | Chlorpyriphos (insecticide) | @ 5ml / Kg |
| T7 | Priming +*Trichoderma* | |
| T8 | Priming + *Rhizobium* | |
| T9 | Priming+Neem oil | |
| T10 | Priming + Thiram | |
| T11 | Priming + Chlorpyriphos | |
| T12 | Priming + *Trichoderma* + *Rhizobium* | |
| T13 | Priming + *Trichoderma* + Neem oil | |
| T14 | Priming + *Rhizobium* + Neem oil | |
| T15 | Priming + *Trichoderma* + *Rhizobium* +Neem oil | |
| T16 | Priming + Thiram + Chlorpyriphos | |
| T17 | *Trichoderma* + *Rhizobium* | |
| T18 | *Trichoderma* + Neem oil | |
| T19 | *Trichoderma* + *Rhizobium* +Neem oil | |
| T20 | *Rhizobium* +Neem oil | |
| T21 | Thiram + Chlorpyriphos | |
| T22 | Control | Not treated / primed |

## Results

*Glasshouse experiment*

Analysis of variance (Table 2) revealed no significant difference for days to flowering between the treatments. However, significant differences ($P<0.001$) were recorded between the treatments for seedling height (10 DAS). No nodulation was observed in the treatments.

**Table 2: Analysis of variance (test of fixed effects) for different treatments for seedling height and days to flowering in mungbean plants grown in a glasshouse**

| | | F values | |
|---|---|---|---|
| Effect | DF | Seedling height | Days to flowering |
| Treatment | 21 | 1.06** | $0.68^{ns}$ |

$^{ns}$ Not significant , ** Highly significant

Multiple comparisons between the treatments for seedling height (10DAS) revealed that all the treatments except T8 (Priming + *rhizobium*) and T10 (Priming + Thiram) recorded significantly (P<0.001) higher plant height than the control (T22). All the treatments except T10, T16 and T22 resulted in significantly higher plant height than T8. Significantly (P<0.001) higher plant height was recorded in T7 in comparison to T10, T16 and T17. Both T9 and T11 treatments recorded significantly (P<0.001) higher values over T5, T10, T16 and T17. T12, T13 and T14 treatments showed significantly (P<0.001) higher values over T5 and T16. Significantly higher plant height (P<0.001) was observed in T15 over T5, T16 and T17. T18 recorded significantly (P<0.001) higher values over T5, T6, T16 and T17. The treatment T18 (*Trichoderma* + Neem oil) which recorded the highest plant height was significantly (P<0.001) on par with T1, T2, T3, T4, T7, T9, T11, T12, T13, T14, T15, T19, T20 and T21 (Fig. 1).

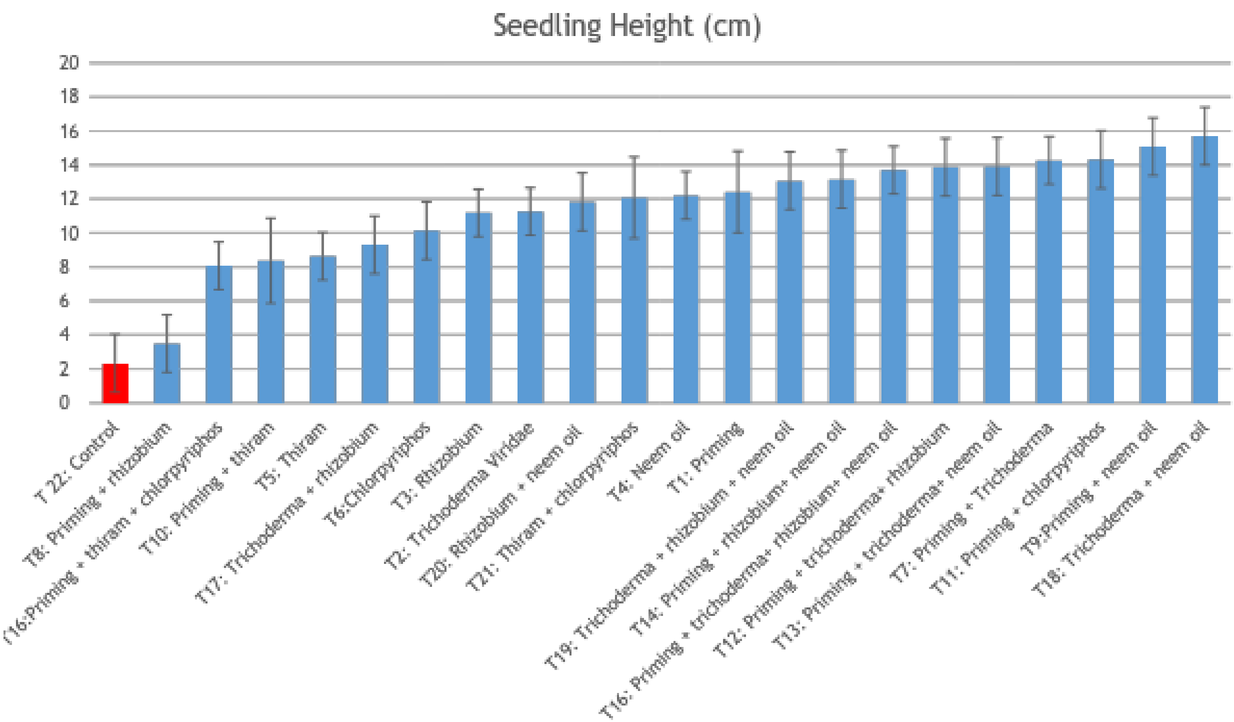


**Fig. 1: Comparison of different treatments for seedling height (10DAS)**

*Field experiment*

Analysis of variance (Table 3) indicated no significant difference between the different treatments for seedling height (10 DAS), days to flowering, root nodules per plant and percentage of root rot damage. The seedling height ranged from 9.04 to 10.26 cm and days to flowering from 29 to 32 days after sowing. Number of root nodules per plant ranged from 24 to 45 (Fig. 2) and percentage of root rot (*Macrophomina phaseolina*) damage ranged from and 0 to 3.9% (Fig. 3a &3b).All the root nodule samples examined showed pink colour, indicative of active nodulation.

**Table 3: Analysis of variance (test of fixed effects) of different treatments for seedling height, days to flowering, number of root nodules per plant and percentage of root damage**

| Effect | DF | F values | | | |
|---|---|---|---|---|---|
| | | Seedling height | Days to flowering | Number of root nodules per plant | % of root rot damage |
| Treatment | 21 | 1.06$^{ns}$ | 0.68$^{ns}$ | 0.94$^{ns}$ | 0.53$^{ns}$ |

$^{ns}$ Not significant


**Fig. 2. Root nodule formation observed in mungbean plants grown in the field.**

**Fig. 3a Root rot symptoms on mungbean plants observed in the field**

**Fig. 3b. Healthy mungbean root vs infected root**

*Morphological characteristics of* *M. phaseolina*

Plants showing dry root rot disease symptoms were uprooted from the field, brought to the laboratory in pre-sterilized polyethylene bags. Symptoms observed in the field were initially chlorosis of plants, followed by root and stem rots, which finally caused severe wilting and death of the plants. Roots of the diseased plants were cut in small pieces and surface sterilized with 0.8% sodium hypochlorite for 2 min. Pieces of sterilized disease tissue were plated on pre poured potato dextrose agar (PDA) medium in triplicates. Inoculated plates were incubated at 28± 2°C for 6-7 days and colonies were identified according to Dhingra and Sinclair (1978). Fungal pathogen was identified by its macroconidia morphology (size, shape of basal and apical cells), type of conidiophores (present or absent, arrangement), cultural characteristic as well as by using fungal keys. Culture was purified by single sclerotial isolation and maintained on PDA slants at 4°C for further study. Colonies were dark brown-greyish in colour initially whitish on the PDA (Potato dextrose Agar) medium (Fig. 4a). Abundant aerial mycelium is found to be produced in the culture plate with sclerotia imbedded within the hyphae or engrossed in the agar. Observations were made under the camera attached microscope. The mycelium of fungal was hyline, septate, pycnidia black, globose, homogenous in size, conidia hyaline, cylindrical, 1-celled (Fig. 4b).

Dhingra O. D. and Sinclair J. B. 1978. Biology and pathology of Macrophomina phaseolina. Imprensa Universitaria, Universidade Federal De Vicosa, Vicosa- 36.570 Minas Gerais-Brazil, p.166.

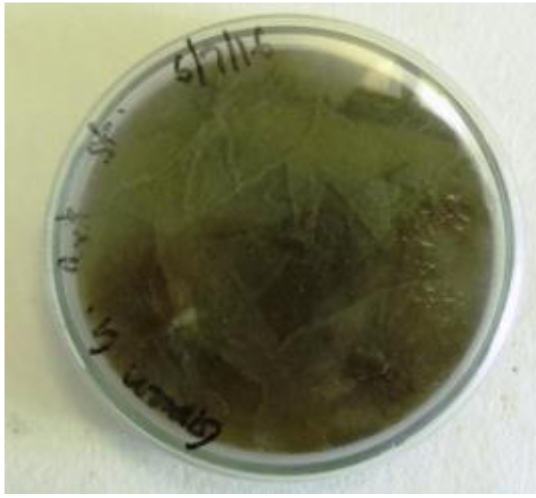

**Fig. 4a *Macrophomina phaseolina* on PDA plate**

**Fig. 4b. Pycnidia (macroconidia) of *M. phaseolina* with mycelia**

## Conclusion:

The absence of root nodules in the glasshouse experiment revealed that the *rhizobium* strain used was not effective. Hence quality control of rhizobia production is important. Difference in plant vigour observed in the glasshouse treatments needs further investigation. Prolific nodulation recorded in the field experiment showed the abundance of resident rhizobia in fields with history of mungbean cultivation. Incidence of dry root rot in the field experiment was observed. But was not uniform. The causal organism was confirmed as *Macrophomina phaseolina*. Also, we found that the most popular mungbean variety, NM 94 is susceptible to dry root rot. Hence the need to explore sources of resistance to the disease. Data will be recorded on traits such as pod yield per plant in both glasshouse as well as in the field, once the plants reach maturity.

## Personal Reflection:

Being raised on a farm with both of my parents having careers in agriculture, I have been completely surrounded by agriculture which instilled a passion for farming early in life. Participating in 4-H and FFA expanded my world in agriculture. I developed a love for raising livestock and learning in my agriculture classes in high school. My trips to the FFA Washington Leadership Conference and National FFA Conferences were a continued reminder to me of the world's food insecurity. At the same time with each advancing science class I completed, I became more deeply interested in the amazing world of science. I learned about the endless possibilities we have in improving agriculture with science.

My original research paper for the World Food Prize was on India and food wastage. As I started my research, it was interesting to me how many different areas such as spoilage and waste, policies and governance, plant science, climate volatility, education and so on could be improved upon to affect world hunger. After attending the Global Youth Institute in 2015, I began to look at the world with a different set of eyes. Prior to the World Food Prize, I had a passion and desire to help feed the world, but I did not know where to begin. Those special days in October helped clarify the problems and brainstorm solutions for the biggest impact. Dr. Borlaug's formula for progress was science, common sense, and plain old hard work. Dr. Borlaug's formula was true years ago and it's even truer today. I came home from that conference with a like mindedness of Dr. Borlaug who once said, "Food is the moral right to all born into this world". I believe that statement to be true.

Come spring when I received the news that I would be one the of 24 Borlaug-Ruan interns, I was not able to contain my excitement. It was the opportunity that I had always wished for and I could not believe it was becoming my reality. Soon my departure date came and after saying goodbye, I quickly realized how torn I was to be leaving my family and friends for the summer. I began to doubt if I was prepared for what was ahead. Signing up for the internship was taking a huge leap of faith.

In the early days of being in the World Vegetable Center office, I was very quiet. I lacked confidence where to begin, but by digging in and asking questions, I soon realized that other researchers were going to be great support. The bond we created in those short two months is a sweet memory that I was treasure forever. I always felt incredibly welcomed and loved.

During my internship this summer, I had experiences that I never expected. I originally thought I would only be introduced to Indian culture but from living on the ICRISAT campus, I met people from all over the world. Not only have I gained a special place in my heart for Indian culture but I was also able to learn about other cultures. From learning to dance the tango, writing my name in Chinese, and engaging in conversations about serious world issues and events, I was able to expand my knowledge and thoughts about the diverse world around me. I learned about what other cultures and people value compared to my home culture. I loved getting to hear other people's stories from all over the world. I quickly learned that everyone has something to share and just how much I can learn from others by listening to their stories. I loved gathering into our friends' rooms and enjoying a meal that we all prepared together. Spending time and laughing with these caring and passionate people was very special. I can really not stress enough the kindness I have found in the people I met. It was exciting to work around others who all shared a common goal of fighting world hunger.

As I thoroughly enjoyed the culture and the people that surrounded me, I would often remind myself that the work and research project I was doing mattered to feeding a hungry world. I appreciated the cultural experience I was having and the daily living conveniences of being on a modern campus, but I did not want to lose sight of the big picture; daily food needs of those less fortunate than me. It was a good motivator to pay attention to details in the work I was doing. I trust someday Indian farmers will see the results in their crops of my research on mungbean seed treatments.

This internship has changed me in several ways. It has increased my confidence and assertiveness. I feel more comfortable about my knowledge in science and working in a lab. It has helped me to be a better student as I begin my Food Science major at Iowa State University. I believe I have improved in how I value relationships. I also have a greater appreciation of the food supply and the freedoms we have in America. Traveling abroad allowed me to become comfortable with the uncomfortable. I was no longer nervous to be so far out of my comfort zone. By the end of the summer, experiencing and trying new things became a daily routine that gave me much enjoyment.

## My Future Plans:

While in school and through other internships, I hope to gain more experience working in a lab and understanding science. I was told at orientation in May that the travel bug would bite and this internship would cause me to want to travel. The travel bug really did bite, but part of my desire in travel is to gain more understanding of ways to improve food insecurity and experience different cultures. While I enjoyed my lab work this summer, I also have a desire to use my farm background to find ways to reduce crop waste once a crop is produced. My attendance at the 2016 World Food Prize continued to fuel my passion and thought processes toward working in world hunger efforts. I am currently working towards fundraising to purchase food packs for a recently built hospital in Guatemala where expecting mothers could benefit from better nutrition.

I am still amazed about everything I was able to learn and do in the 8 weeks of my 2016 summer. Nothing had fully prepared me for this internship, but the internship has definitely better prepared me for my future in fighting world hunger. I am simply grateful.

## Photos: World Vegetable Center

◀ Applying seed treatments

▼ Sowing mungbean seeds in glasshouse and field

Measuring seedling height in glasshouse ▶

## Photos: Experience


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