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Assessment of the Toxicity of Carbendazim on Seed Germination and Vegetative Growth of *Vigna mungo*

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Abstract

The present study was conducted to assess the toxicological effect of carbendazim on seed germination, seedlings survival, plants height and number of branches of *Vigna mungo* taking its variety IPU-13-01. The healthy seeds of *Vigna mungo* was treated with 0.125%, 0.250%, 0.375% and 0.500% concentrations of carbendazim before sowing in the experimental field in both M_1 and M_2 generation and result were recorded. The observation clearly showed negligible or no toxicological effect up to 0.250% treatment concentration. However higher than 0.250% concentration of carbendazim showed toxicological effect on germination as well as morphological characters taken for study. The 0.500% concentration showed even 12-13% reduction in height and 27% reduction in number of branches. Thus upto 0.250% concentration is safe for farmer use while more than 0.250% concentration has toxic effect on *Vigna mungo* which showed increasing trends of toxicity with increasing the concentration above 0.250%.

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Keywords

Vigna mungo; Carbendazim; seed germination; morphological characters; M_1 & M_2 Generation.

Introduction

Pulses are an important commodity crop category that provides high-quality proteins, generally three times more than cereals and are the cheapest way to treat protein deficiency in humans. The United Nations recognized the ability of pulses to influence future global food security, nutrition and environmental sustainability by naming 2016 as “International Year of Pulses”. Pulses are considered ‘Smart Foods’ because they are essential for the food basket (dal-roti, dal-chawal), due to good source of plant protein and help to prevent obesity and diabetes. Furthermore, pulses are highly water effective, can survive in dry regions and are beneficial to soil fertility by fixing nitrogen. Like other crops In India, black gram is the third most important pulse crop. Commonly known as "urd bean". Black gram (*Vigna mungo* (L.) is an important grain legume that provides a high-quality, easily digestible protein source. It is a member of the genus *Vigna* within the family Leguminosae. Thailand, Australia and other Asian and South Pacific nations grow it less frequently than the Indian subcontinent. Disease is the major constraint in economic crop production as they inflict heavy loses. Therefore In the agriculture field, majorly plant diseases are controlled by chemical products such as fungicide, insecticide, nematicide and bactericides etc. Though, the recent studies showed that an average of 35% of agricultural yield lost due to different fungal infections, despite extensive usage of fungicides.

Futhermore Biswas *et al.,* (2014) examined the effect of agrochemicals on the environment and human health. Problems including his study are cancer, neurological disorders, birth defects, reproductive and developmental abnormalities and mutagenicity. Tanweer (1982) observed that fungicide-treated seed improves seed health, plant stand and crop output by controlling seed-borne illness. Farmers employ agrochemicals to boost plant yields. However, several studies have shown that its use can cause major problems for humans, environment, water bodies, plants and soil quality. Along with this Shahid, M. *et al.,* (2018**)** reported that fungicides kitazin, hexaconazole and carbendazim—negatively affected pea growth, yield and physiology. Carbendazim was most toxic, severely reducing germination and biomass. Hexaconazole lowered pigments and nutrients, while kitazin increased stress biomarkers. Overall, fungicides induced oxidative damage, cellular injury and anatomical changes, emphasizing the need for careful, sustainable fungicide use in agriculture.

Furthermore Deshmukh *et al.,* (2020) found that dry seed treatment with a combination of carbendazim and mancozeb, theophonate methyl or carbendazim 2.5 gram Kg^{-1} seed is highly effective in the field to produce maximum seed germination, improved plant growth & yield. Along with this Buts *et al.,* (2013) reported that up to 0.50% concentration treated seeds, bavistin has a beneficial impact on the overall growth of the *Vigna* radiata plant. It negatively affects germination, the number of pods, the number of branches and the weight of the seed in the M_1 and M_2 generations at concentrations more than 0.50%. Moreover M.U. Ghurde *et al.,* (2021) studied the effect of bavistin on wheat physiology and growth. Chlorophyll content, root-shoot ratio, seed germination and seedling growth all decreased as concentrations increased (0.2-1%). Although fungicides control disease, high concentrations have phytotoxic effects that delayed photosynthesis and general plant growth, resulting in decreased growth and yield in *Triticum aestivum*.

Bakre D.S. *et al.,* (2017) also reported that Carbendazim increased chilli seed germination, growth, vigor and biomass up to 0.2%, but then fell to 0.4%. Copper oxychloride has inhibitory and phytotoxic effects at higher doses. The combined low-dose treatment (0.05%) increased biomass the most effective, indicating reciprocal benefits – and showing that effective combination can stimulate plant development better than individual applications.

Several researchers have reported the effects of various fungicides on various plants, focusing on a few characteristics. The current study aims to assess the toxicological effect of various concentration of carbendazim on seed germination and vegetative growth characteristics of *Vigna mungo* taking its varieties IPU-13-01 up to two generation i.e. M_1 and M_2 generations.

Materials and Methods

Plant Materials

We procured seeds of *Vigna mungo* (2n=22) variety IPU-13-01 from the Indian institute of pulse research, Kanpur. Equal sized, healthy seeds were chosen to receive Carbendazim treatment.

Chemical used

Carbendazim is systematic fungicides and its molecular weight is 191.2g/mol and chemical formula is $C_9H_9N_3O_2$. Just before being sown in the field, the seeds were first soaked in water for four hours. After that, 100 seeds were put in separate petridishes having concentrations of 0.125%, 0.250%, 0.375%, and 0.500% of carbendazim for two hours in the lab. The treated seeds were then sown in the experimental plots (field) under protection in lines with a distance of 10 cm between the plants and 30 cm between the lines.

The measure of germination was the presence of hypocotyls and cotyledons above the soil's surface. Procedures were put together for regular irrigation and weeding. Fertilizer and chemicals were not used. Before the beginning of March, the seeds were planted in the field and they were harvested between the last of May to second week of June. The morphological characteristics were studied in relation to the number of branches, plant height, seed germination & seedling survival. This generation is known as M_1.

Four sets of mature seeds obtained from M_1 generation plants treated with different concentrations were collected and stored separately. Again these obtained seeds of each sets were treated with corresponding concentration of carbendazim before sowing in the experimental fields and resulting crops is designated as M_2 generation.

Statistical analaysed

During the collection of morphological features in the M_1 and M_2 generations, the phenotypic variability was calculated. A standard statistical method is used to compile raw data. To get to a conclusion, we calculated the mean of the observed data and calculated the standard deviation.

Results and Discussion

The current study's findings are shown in tables 1 and 2, as well as Fig. 1 to 4 and expressed combined with the debate in a separate heading as under.

Effect of carbendazim on seed germination

The germination percentage were 97, 96, 95 & 93 in M_1 generation whereas it was 100, 100, 97 & 95 in M_2 generation under the treatment of 0.125%, 0.250%, 0.375% and 0.500% concentration of carbendazim respectively. The germination percentage was 97 and 100 in M_1 and M_2 generation respectively under control. (Table 1 and 2)

Thus, it is clear from the obtained results that, up to 0.250% treatment concentration, there is no significant effect on the germination percentage in M_1 and M_2 generation in *Vigna mungo.*

However, in M_1 and M_2 generations, the germination percentage decreases as the carbendazim treatment concentration increases over 0.250%.

In comparison to the control, this shows the harmful effect of carbendazim at treatment concentrations greater than 0.250% in *Vigna mungo* (tables 1 and 2).

The M_1 and M_2 generations show a maximum fall of 4.17% and 5.26%, respectively, in the 0.500% concentration treatment (fig.1).

Shahid, M. *et al.,* (2018**)** and Buts *et al.,* (2018) also observed the same effect in their studies. Gupta A. & Srivastava S. (2017) have also reported that Low concentrations of mancozeb (1, 3 and 6 mg) enhanced seed germination, seedling growth, vigor index, chlorophyll and phenolic content, indicating a stimulatory effect. In contrast, higher concentrations (9 and 12 mg) significantly inhibited germination and growth parameters, demonstrating phytotoxic effects.

Monika, & Kidwai, M. K. (2017) have also reported that Pesticides, specifically fungicides like Mancozeb, adversely affect the germination of mustard varieties RH-30 and Laxmi, resulting in reductions of 30-60% and 17-55% at doses of 1 mg.kg-^{1}, 2 mg.kg-^{1} and 4 mg.kg^{1}.While Laxmi demonstrates better overall plant length and vigor, RH-30 suffers from significant growth suppression, indicating that high levels of Mancozeb stress both varieties, with Laxmi exhibiting more resilience after seven days.

Effect of carbendazim on seed survival

The seed survival percentage were 96, 95,93 & 90 in M1 generation whereas it was 100,100,97 & 95 in M2 generation under the treatment of 0.125%, 0.250%, 0.375% & 0.500% concentration of carbendazim respectively. The germination percentage was 95 and 97 in M1 & M2 generation respectively under control. (Table 1 and 2)

As compared to the control, the greatest reductions were shown in the seeds treated with a 0.500% concentration treatment in both generations; however, in the M_1 and M_2 generations, the reduction started with a 0.375% concentration treatment.

At 0.500% treatment concentration, there is a decrease of 5.26% and 6.18%, in seedling survival in M_1 & M_2 generations respectively. (fig.2)

Hatamleh *et al.,* (2022) reported that high mancozeb concentrations significantly decreased tomato (*Solanum lycopersicum*) chlorophyll content, plant growth and seed germination. At higher dosages, the fungicide showed severe phytotoxic effects, including oxidative stress and membrane damage.

Effect of carbendazim on plant height

In M1 generation, average height of plant recorded are 47.14cm, 47.08cm, 45.84cm & 41.57cm in 0.125%, 0.250%, 0.375%&0.500% concentration treatment of carbendazim respectively in comparision to 47.36cm in control (table 1). In M_2 generation, the value for same criteria are 49.70cm, 48.88cm, 47.51cm & 43.02cm respectively in comparision to 49.76cm in control (table 2).

The average height of all treated plants decreased compared to the control in all treatment concentrations in the M_1 and M_2 generations, we observed that with increasing treatment concentration, the height of the plant is decreasing. In comparison to control the highest reduction in height is observed at 0.500% concentration treatment with a reduction of 12.22 & 13.58% in M_1 and M_2 generation respectively (fig. 3).

Table.1 Effect of carbendazim on seed germination and vegetative growth of Vigna mungo variety IPU-13-01 in M1 generation

Treatments (Carbendazim)	Germination out of 100 seeds in the field	Seedling Survival (%)	Height (cm)±SD	No. of branches per plant ±SD
0.125%	97	96	47.14±1.8	**8.98±2.0**
0.250%	96	95	47.08±5.3	**8.48±4.1**
0.375%	95	93	45.84±5.2	**7.92±2.5**
0.500%	93	90	41.57±2.1	**6.48±1.1**
Control	**97**	**95**	**47.36±3.5**	**8.91±1.8**

Table.2 Effect of Carbendazim on seed germination and vegetative growth of *Vigna mungo* Variety IPU-13-01 in M_2 generations.

Treatments (Carbendazim)	Germination out of 100 seeds in the field	Seedling Survival (%)	Height (cm) ±SD	No. of branches per plant ±SD
0.125%	100	97	49.70±2.92	**9.04 ±1.10**
0.250%	100	97	48.88±2.45	**8.85±0.94**
0.375%	97	95	47.51±2.53	**8.80 ±0.95**
0.500%	95	91	43.02±2.22	**6.50±0.77**
Control	**100**	**97**	**49.76±2.27**	**8.91±0.77**

Figure.1 Percentage variation in germination of Vigna mungo treated with Carbendazim in respect to control in M1 and M2 generation.

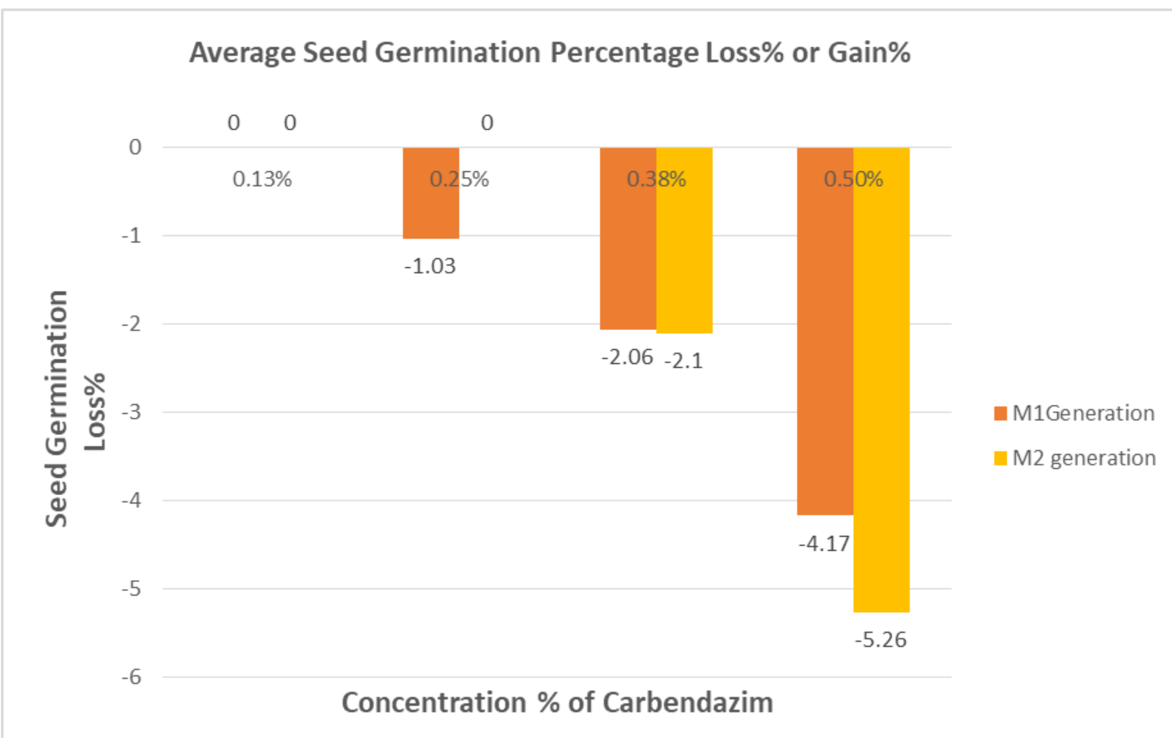


Figure.2 Percentage variation in seedling survival of *Vigna mungo* treated with Carbendazim in respect control in M1 and M2 generation**.**

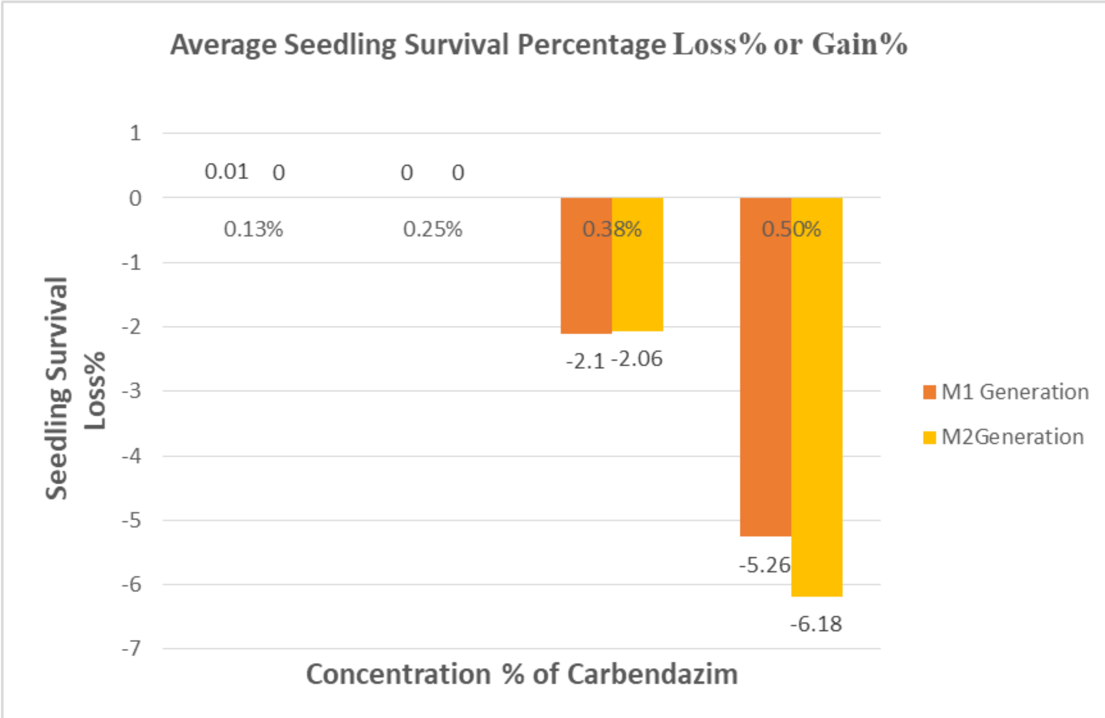


Figure.3 Percentage variation in height of *Vigna mungo* treated with Carbendazim in respect to control in M_1 and M_2 generation.

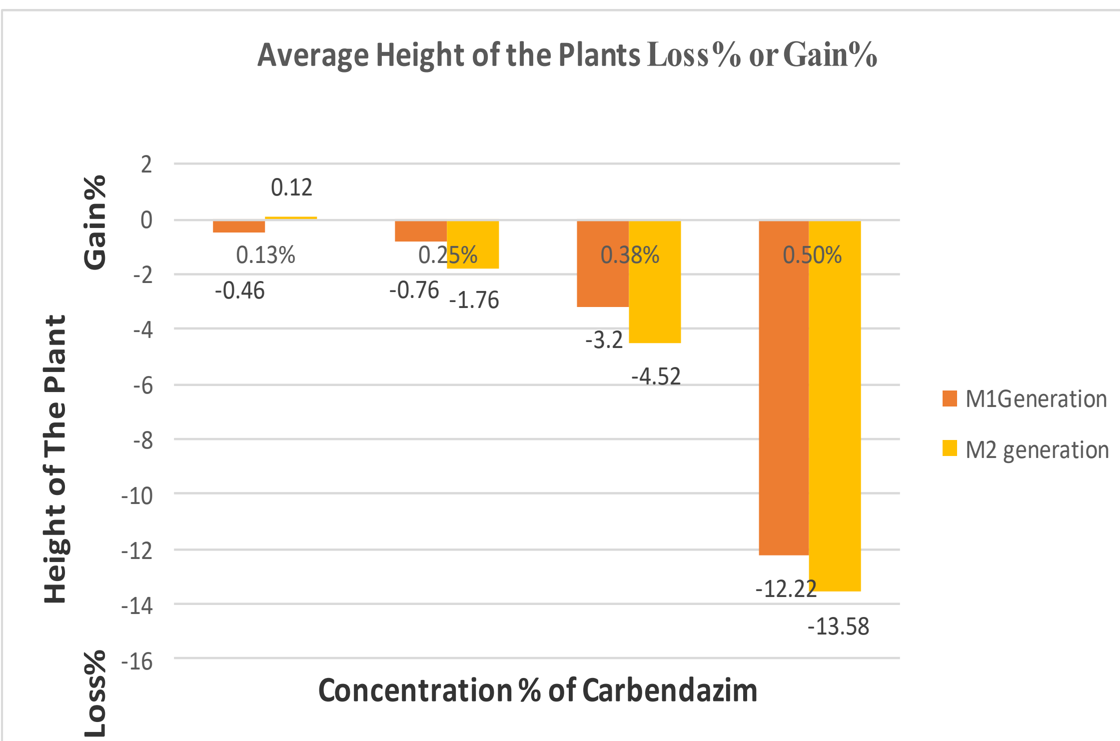
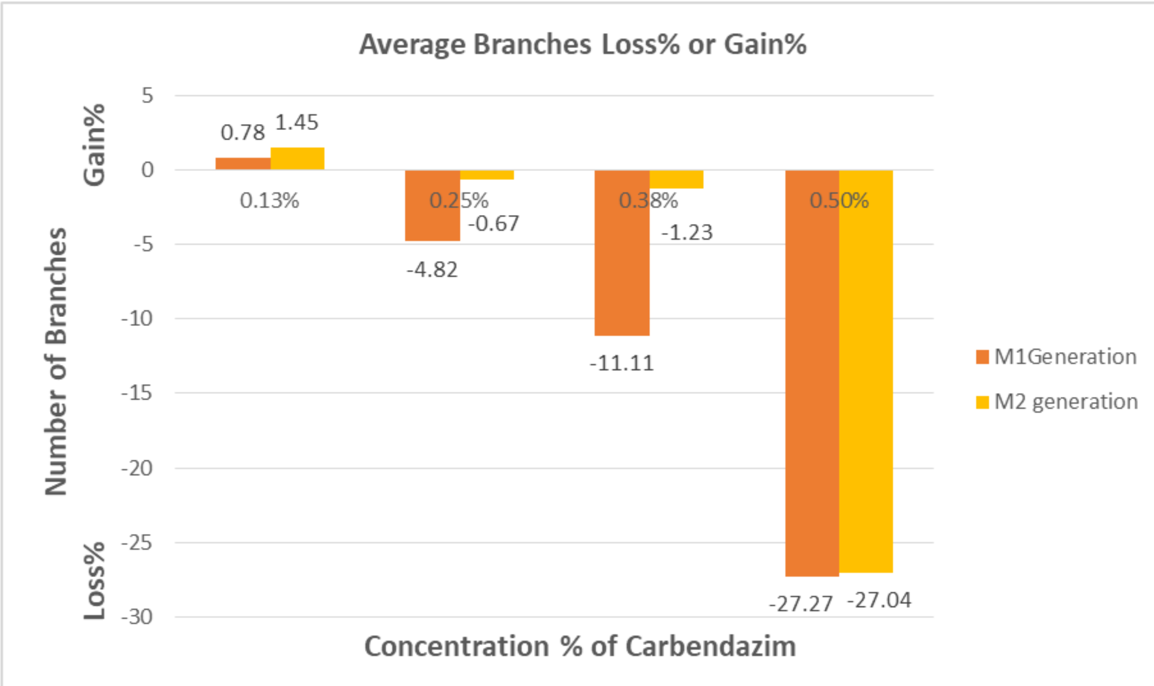


Figure.4 Percentage variation in number of branches of *Vigna mungo* treated with Carbendazim in respect to control in M_1 and M_2 generation.



Effect of carbendazim on number of branches per plant

The average number of branches in the M_1 generation are 8.98, 8.48, 7.92 and 6.48 for carbendazim treatment concentrations of 0.125%, 0.250%, 0.375% and 0.500% respectively. In M_2 generation, the average number of branches are 9.04, 8.85, 8.80 and 6.50 for plants treated with 0.125%, 0.250%, 0.375% and 0.500% concentrations, respectively. In control, it is 8.91 for both the M_1 and M_2 generations. (Table 1 and 2). Data shows that in both the M_1 and M_2 generations, the average number of branches decreases as the treatment concentration increases in both generations, the control group exhibited maximum branching. The branching process is negatively impacted by carbendazim, as observed by a decrease of 27.27% and 27.04% in the M_1 and M_2 generations respectively, among 0.500% concentration treated plants. The significant negative impact on branching process began with 0.375% concentration treatment of Carbendazim which increased with increasing treatment concentration (fig. 4)

Kumar S., & Buts A.K., (2025) Found that the toxicological effects of mancozeb were investigated during two generations on two varieties of *Pisum sativum*. Concentrations between 0.1% and 0.5% had a lack of impact up to 0.2%. At concentrations above 0.2%, there were negative impacts on growth, reproduction, and yield, which became less beneficial at higher levels. At 0.3%, however, stimulating effects such as increased branch number and seedling survival were evident.

In conclusion, we aimed to evaluate the harmful effects of a single dosage of carbendazim treating seeds before sowing on germination and morphological characteristics of *Vigna mungo* (urad bean) up to two generations in actual field condition. Based on observations and the discussion made above, we can conclude that urad beans can tolerate the harmful effects of carbendazim up to 0.250% concentration in terms of seed germination, plant survival and plant height. It is evident that the toxicity of carbendazim on seed germination and morphological characters of crop increases with increasing in the treatment concentration. Therefore, farmers should only use the Carbendazim to treat fungal infection in *Vigna mungo* taking its concentration upto 0.250%. Above this level, it will negatively impact on germination & other morphological characters which increases with increasing the treatment concentration. A awareness should be raised about the careless use of carbendazim due to its toxic effects, which may also have an impact on human health through bio-magnification.

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