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EFFECT OF ZIRAM ON SEED GERMINATION AND SEEDLING GROWTH IN MUNG BEAN (VIGNA RADIATA (L.) R. WILCZEK)

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Abstract

Seed dressing with fungicide is a conventional method used for the control of seed & soil borne pathogen that results in healthy plant development and increases the crop yield (Andreas et al., 2008). Although the seed treatments protect the seed &seedlings from pests & disease, it causes secondary effects on germination & growth (Wahengbam et al., 2013). Present paper showed the effect of fungiside
On seed germination and growth of plant. Fungisisde is used to kill the fungi but there are some addeverce effect.In this paper showed that effect of Zirram fungiside made by sygenta. For experiment we used mung beans ie,. *Vigna radiate* L.Different con of Ziram Showed different effect We measure the the hight of plumule and radical. Fungiside eighther incresease or decrease the hight of plumule or radical. Also we Count Protein conc,

Key words ; Ziram , fungiside

INTRODUCTION

Fungicides constitute one of the most effective and integrative method to control diseases against phyto-pathogenic fungus in agriculture. However, the toxicity and the pollution generated by fungicides cannot be neglected. The toxic effect of a given pesticide on seeds depends on its distribution, persistence, metabolism, its active form, and its concentration. Some pesticides interfere with the metabolic pathways of plants and some interfere specifically with the photosynthetic process (Anne-Noe¨lle *et al*, 2012). Shurtleff (1980) reported that crop seeds are vulnerable to a wide range of diseases that reduce yield and quality (vigour and germination). Consequently, numerous fungicides are produced into the market in order to control the causal pathogens. Taylor and Harman (1990)

revealed that fungicide seed treatment is considered as adequate means to improve the quality of the seed considerably and to increase plant growth and productivity. In addition, fungicides can also mitigate the internal and external seed or soil borne pathogens (Scot, 1989). Balie and Elward (1980) reported that besides helping to improve the physical properties of the seed, the chemical treatment of the seeds can reduce stress conditions and act as an efficient carrier of nutrients, fungicides and insecticides.

The great variety of known fungicides are classified into two main categories: contact and systemic. Contact fungicides, such as copper or sulfur have a preventive action by killing fungi as their spores germinate, before mycelia can grow and develop within plant tissues (Yuste and Gostincar, 1999). Systemic fungicides, known as curative or eradication fungicides, can also kill the fungus when mycelia have penetrated into the parenchyma, stopping the dispersal or infection within the plant (Yuste and Gostincar, 1999). Among systemic fungicides, benzimi-dazoles are a group of organic fungicides that are extensively used in agriculture along with the other classes of fungicides Seed dressing with fungicide is a conventional method used for the control of seed & soil borne pathogen that results in healthy plant development and increases the crop yield (Andreas et al., 2008). Although the seed treatments protect the seed &seedlings from pests & disease, it causes secondary effects on germination & growth (Wahengbam *et al*., 2013). Mancozeb, organic contact fungicide have preventive action by killing or inhibiting fungi or spores before the mycelia grow and develop within the plant tissues (Yuste and Gostinear, 1999). They undergo transformation to ethylene di isothiocyanate, which inactivates thiol groups of enzymes and metabolites in fungal cells. These compounds control broad range of fungi at relatively low application rates (Maria, 2012).

However, numerous researchers have reported on the adverse effects of fungicides on the germination and growth of the crop plants. Treating vegetable and crops seeds with fungicides will protect them against soil-borne fungi which could cause diseases, especially radicle-rot (Pimentel and Greiner, 1997). With the wide increase of the use of such chemicals, it was found that they have harmful effect on humans, animals, plants and microorganisms. Therefore, there was a crucial need to study about the toxicity of fungicides to plants.

Objectives of the Investigation

The aim of this study was to evaluate the effect of the fungicide, namely Ziram, on the germination and seedling growth parameters of mung bean seeds. The specific objectives of this study were to:

a) Examine the influence of Ziram, on the percent seed germination in mung bean seeds *in vitro,*

b) Examine its influence on the growth of plumule and radicle at 96 hours of germination,

To evaluate whether total proteins have any role to play in the observed increase or decrease in the seedling growth.Importance of Mung Bean

The mung bean is a major edible legume seed in Asia (India, South East-Asia and East Asia) and is also eaten in Southern Europe and in the Southern USA. The mature seeds provide an invaluable source of digestible protein for humans in places where meat is lacking or where people are mostly vegetarian. Mung beans are cooked fresh or dry. They can be eaten whole or made into flour, soups, porridge, snacks, bread, noodles and ice-cream. Split seeds can be transformed into dhal in the same way as black gram or lentils. Mung beans can be processed to make starch noodles (vermicelli, bean thread noodles, cellophane noodles) or soap. The immature pods and young leaves are eaten as a vegetable (Mogotsi, 2006).

Several mung bean products are useful for livestock feeding (Vaidya, 2001). They include, Mung beans, raw or processed, as well as split or weathered seeds. By-products of mung bean processing: mung bean bran (called chuni in India), which is the by-product of dehulling for making dhal, and the by-product of the manufacture of mung bean vermicelli. Mung bean is sometimes grown for fodder as hay, straw or silage (Mogotsi, 2006). It is particularly valued as early forage as it outcompetes other summer growing legumes such as mung bean or velvet bean in their early stages (Lambrides *et al*., 2006). The mung bean plant makes valuable green manure and can be used as a cover crop (Mogotsi, 2006).

Mung bean (*Vigna radiata* (L) Wilezek) is one of the most important pulse crops. It is grown in almost all parts of the country. Mung bean is an excellent source of high quality protein. It is consumed in different ways as dal, halwa snack & so many other preparations. Ascorbic acid (vitamin C) is synthesized in sprouted seeds of Mung bean. The amount of riboflavin & thiamin is also increased. Mung bean is a major leguminous crop. It has the capacity to fix atmospheric nitrogen-through symbiotic nitrogen fixation. It is also used as a green manure crop. It also provides an excellent green fodder to the animals. Being a short duration crop, it fits well in various multiple

& intercropping systems.

Fig. 1. The fungicide used as a source of Ziram in the experiments.

The Fungicide Used

The commercial fungicide employed was Ziram, a standard, relatively new product on the market. Ziram 27% SC (Trade name Cuman L (Fig. 1)), is a fungicide manufactured by Syngenta. It is an organic fungicide, which contains dithiocarbamates (Gupta, 2011). Dithiocarbamates (DTC) are organosulfur compounds represented by a general structure (R1R2)N-(C=S)-SX, where R can be substituted by an alkyl, alkylene, aryl, or similar other group, and X usually by a metal ion (Edwards, 1991; Kamrin, 1997). Thiram, Disulfiram, Ziram, and Ferbam are examples of some common DTC pesticides. Ziram is used to kill a number of fungi, by interfering with various enzymes involved in the

respiration process and inhibiting the spore germination and mycelium growth. The agricultural fungicide, Ziram is 100% safe to use with crop plants when used at recommended doses. Ziram is a dithiocarbamate fungicide and an ubiquitin proteasome system (UPS) inhibitor. Ziram Causes Dopaminergic Cell Damage by Inhibiting E1 Ligase of the Proteasome (Arthur et al, 2008).

Chemical Formula

Chemical formula of Ziram is $C_6H_{12}N_2S_4ZN$; Molecular weight is 305.83; Melting Point is 246°C and solubility in water is 65 mg/l; (Michael O'Malley, 2010)

Mode of action

As per the claims of manufacturer, Ziram inhibits spore germination & mycelium growth by interfering with various enzymes involved in the respiration process. This property of acting on many sites of the biochemical process ensures that the product is free from resistance problems & effective against many fungi.

Recommended Usage

Ziram 27% is used as pesticide on fruit crops like grapes, apples, almonds and peaches to control black rot, downy mildew, shot hole, brown rot and peach leaf curl. It is also used on on vegetables. It is the most stable of the metallic dithiocarbamates and said to be non- phytotoxic.

In the present investigation, we wish to investigate the effects of commonly used fungicide, Ziram, at higher concentrations, than the recommended doses, on seed germination, seedling growth and protein metabolism, on an amenable plant system, Mung bean (*Vigna radiata* (L.) R. Wilczek).

1. MATERIAL AND METHODS

Selection of Experimental Plant Material

Mung bean (*Vigna radiata* (L.) R. Wilczek), family Fabaceae, is used as experimental plant material to study the effect of Ziram on seed germination and seedling growth. It has been selected as experimental material for this investigation because of the fact that – a) It is widely cultivated in and around the work place, i.e., Bhende and its surrounding villages, b) well adapted to the local conditions, c) its seeds germinate easily in Petri plates lined with moist filter papers and 4) takes less time to germinate and easy to germinate in all seasons.

Systematic Position

Kingdom : Plantae Subkingdom : Angiosperm
Class : Dicotyledons Sub-class : Polypetaleae
Order : Rosales
Family : Fabaceae Genus : Vigna Species : radiata

Seeds of mung bean (*Vigna radiata* (L) Wilczek Var. Vaibhav) were procured from Mahatma Phule Agricultural University, Rahuri. They were selected for their uniformly in size, colour and weight. They were surface sterilized with 0.1% Mercuric chloride solution for 1 minute and thoroughly washed under running tap water. The seeds were presoaked in distilled water, overnight.

After 12 hours of imbibition, the seeds were taken out of the water and subjected to treatment with different concentrations of Ziram, viz., 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, for 2 hours. These were washed thoroughly under running tap water for 30 min, in order to remove the traces of pesticide at the end of the treatment. They were

Fig. 2. Morphology of Mung bean (Vigna radiata (L) Wilezek). A) Plant in vegetative stage; B) Plant with semi mature pods; C) seeds; and D) Germinated Seedlings.

(A) (B)

(C) (D)

rinsed twice with distilled water. Some seeds were not treated with Ziram, and they served as control. Both Ziram treated and untreated (control) seeds were allowed to germinate in heat sterilized Petri Plates of 9 cm diameter and 1.5 cm height, lined with moist filter papers (Whatman No. 1). Germination studies were conducted by keeping the Petri dishes in darkroom and daily count of germinated seeds was taken up to four days. Petri dishes were watered as required to replace evaporation losses.

Calculation of Percent Seed Germination

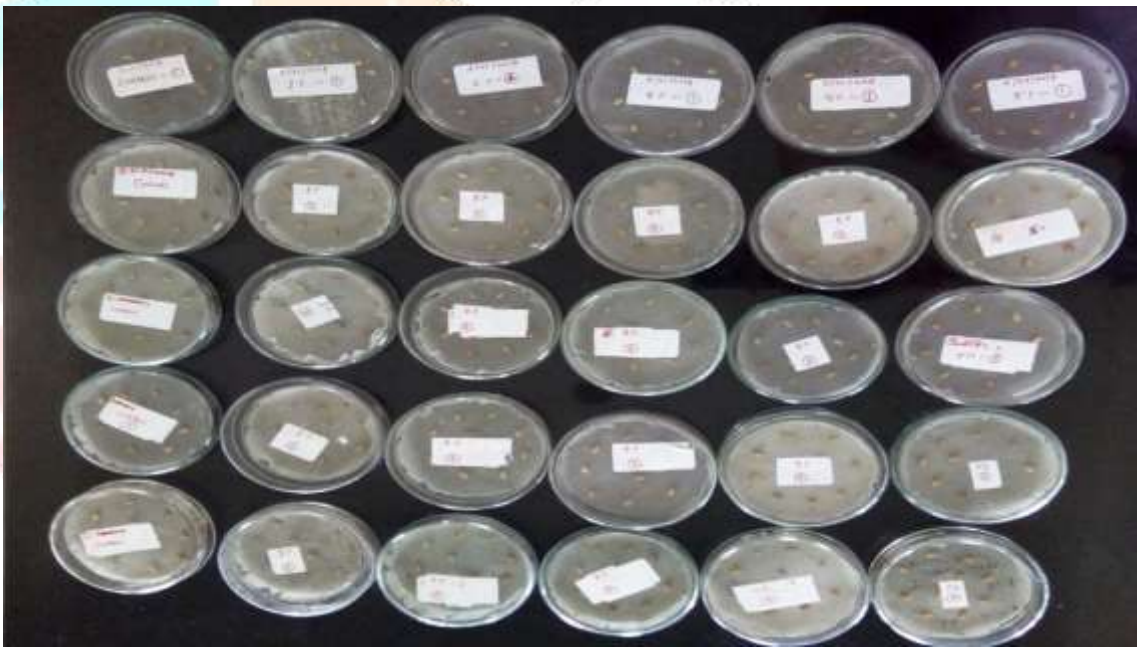
Percent germination was calculated from seeds kept for germination in Petri plates lined with moist filter papers, at room temperature (25 ± 2^{o}C), after 4 days (96 hours) of seed germination. Seeds that gave rise to both plumule and radicle were considered as germinated.

percent seed germination was calculated as follows…

$$\text{Percent seed germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds kept for germination}} \times 100$$

After 4 days (96 hours) of germination, length of radicle (embryonic root), length plumule (embryonic shoot) and total seedling height (length of radicle + length of plumule) were noted. Significant stages of germinated seedlings are photographed for reaching meaningful conclusions on the effect of ziram on Seed Germination and Seedling Growth in Mung Bean (*Vigna radiata* (L.) R. Wilczek).

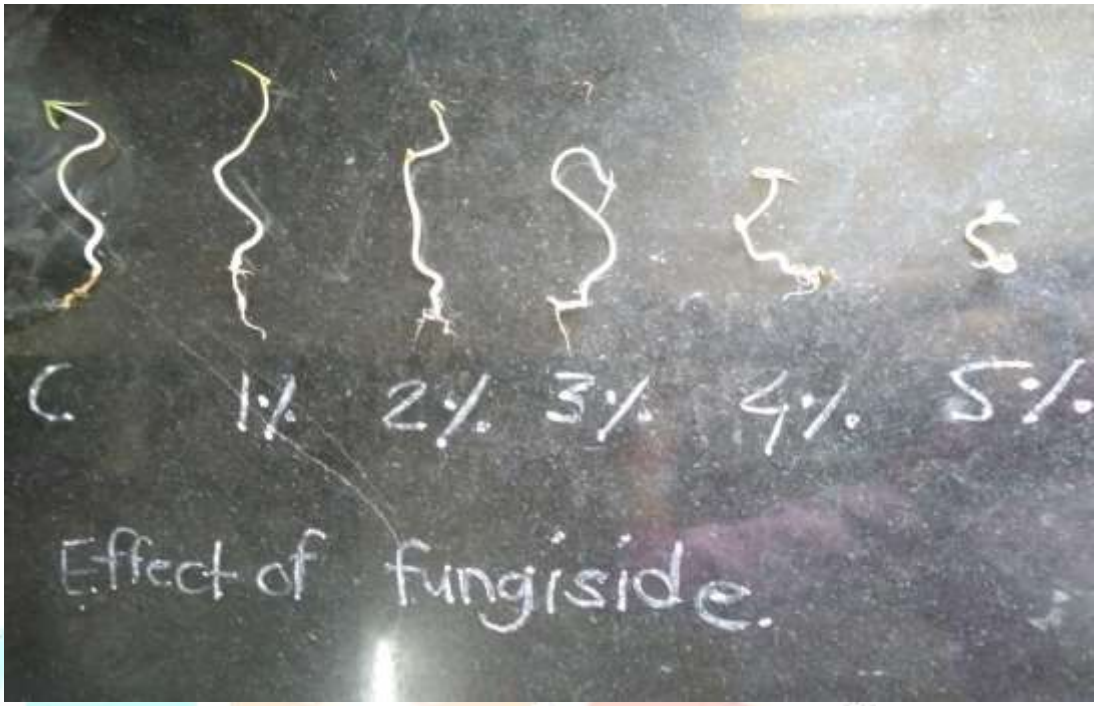
Fig.3. Experimental set up of mung bean seeds treated and untreated with Ziram for germination in Petri plates lined with moist filter papers.



Calculation of LD_{50}

LD_{50} dose (50% concentration of Lethal Dose) for the fungicide was determined on the basis of total number of seeds germinated after 96 hours of seed germination. The fungicide dose at which 50% seeds have died and only 50% seeds survived is adjudged as LD50 dose for the fungicide.

Fig. 4. Photograph showing total seedling length of control (C) and Ziram treated mung bean seedlings after 96 hours of germination.



Estimation of Total Proteins

The colorimetric method of Lowry et al. (1951) was followed to estimate the total protein present in radicle, plumule and total seedling. The plumule, radicle or total seedlings after 96 hours of seed germination were collected, cut into small pieces and pooled for sampling. From this, one gram plant material was taken, finely chopped, and homogenized in 10 ml cold, double distilled water using mortar and pestle. The homogenate was collected. A known volume (0.1 and 0.5 ml) from the homogenate was pipetted and mixed distilled water to make the final volume to 1 ml and used for estimation of total protein. To 1.0 ml of supernatant 5.0 ml of alkaline copper reagent was added and shaken well. After 10 minutes, 0.5 ml of 1N Folin-Ciocalteu Phenol reagent was added and immediately shaken well and kept undisturbed for 30 minutes. The optical density was read at 660 nm using Systronics visible spectrophotometer. Bovine Serum Albumin fraction V, (BSA) procured from Merck Chemical Company was used as standard.

Work Place

The experiments were performed in the laboratory of the Department of Botany at Jijamata College of Science and Arts, Dnyaneshwarnagar, Bhende, during the period September 2017 to February, 2008.

Statistical Analysis

The data were analyzed using Data Analysis Pack provided in Microsoft Excel, 2013. A sample size of 50 individual seeds were used in all treatments including control. Means were supported with standard error of means. Significance of means of individual treatments were compared with control means following Student's t – distribution, at probability level, $P \leq 0.05$.

2RESULTS AND DISCUSSION

Effect of Different Concentrations of Ziram on Percent Seed Germination

Percent seed germination and percent inhibition of mung bean seeds, treated with different concentrations of Ziram (for two hours at 25□2°C temperature) are shown in table-1. From the table it is evident that all concentrations of Ziram except 1.0% inhibited seed germination but to different extents.

Maximum percent of seed germination is observed in distilled water grown (control) and 1.0% Ziram treated seeds (Table 1.). Other concentrations has inhibited seed germination to different extents. Reduction in percent seed germination was dose dependent. There is a gradual reduction in percent seed germination along with an increase in the concentration of Ziram (Table 1 and Fig. 5). Near 50% inhibition (52.64% inhibition) of seed germination was observed in 3% concentration of Ziram. Hence this concentration was adjudged as LD_{50} for the mutagen.

The highest seed germination percentage of mung bean seeds were recorded as 102% when treated with 1.0% dose of Ziram and the lowest was 13.15% at 5% dose of the fungicide. In 1% treatment, the seed germination was increased up to 2% over control. (Table 1). This result is in conformity with the findings of Umesh and Sanjay (2012) who observed that benomyl, dithane M-45 and bavistin effectively increased the germination percentage of cowpea. In another study, De and Chaudhury (1999) reported that bavistin, mancozeb, M-45 and vitavax treated seeds of lentil enhanced the germination compared to control. These findings completely agree with the present observation.

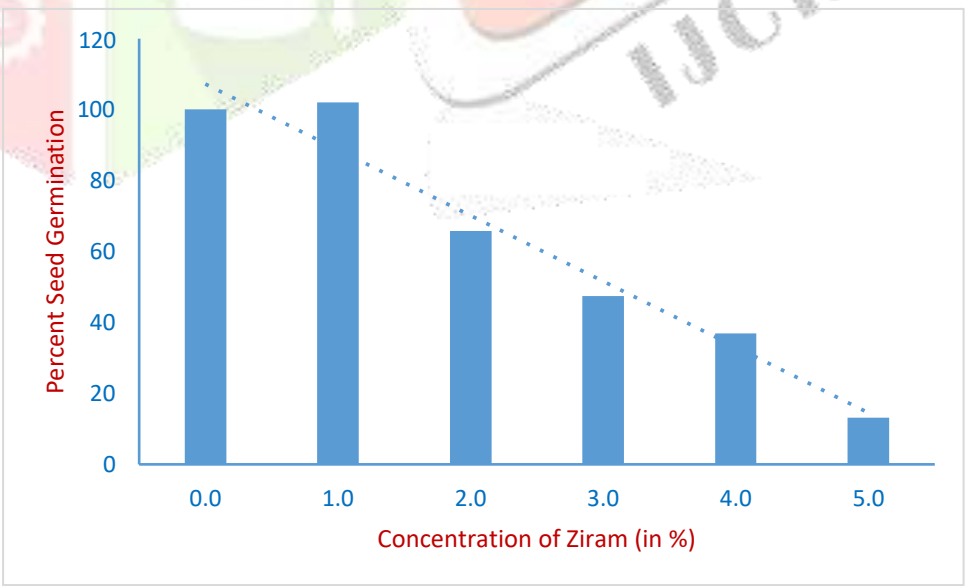
Saeidi and Mirik (2006) in their study on flax seed treated with Captan 0.2% and Carbendazim 0.15% they reported that seed germination was not significantly affected except for some seeds after long storage periods. But our experimental results clearly indicate that

higher concentrations of Ziram (2.0, 3.0, 4.0 and 5,0%) inhibited seed germination to significant extents.

Table-1. Effect of different concentrations of Ziram on percent seed germination and percent inhibition in *Vigna radiata* (after 96 hours of germination).

Concentration of Ziram (in %)	Percent Seed germination	Percent Promotion / Inhibition
0.0	100.0	00.00
1.0	102.0	02.00
2.0	65.78	-34.22
3.0	47.36	-52.64
4.0	36.84	-63.16
5.0	13.15	-86.85

Fig.5. Effect of different concentrations of Ziram on percent seed germination and percent inhibition in *Vigna radiata* (after 96 hours of germination).



Our experimental results fortify the earlier findings of Siddiqui and Uzzaman (2004) that low concentrations of fungicides enhanced germination of Maize (*Zea mays*) seeds. Study conducted under controlled environment by Sharma and Srivastava, (2005) showed that fungicide Bavistin enhanced germination of pea (*Pistum sativum*). In contrast, Siddiqui et al., (1999) reported the inhibition of seed germination and seedling growth in *Penesetium americanum* L. due to application of organophosphate pesticides.

Effect of Different Concentrations of Ziram on Growth of Radicle

Results obtained on effect of different concentrations of Ziram on Growth of Radicle is shown in table 2. Our experimental findings indicate that Ziram has promoted the growth of radicle at lower concentrations (1.0%) and inhibited the growth of radicle at higher concentrations (Fig. 6). The promotory effect seems to be marginal but the inhibitory effect is dose dependent. There is a decrease in the growth of radicle, along with an increase in the concentration of Ziram (Fig.2).

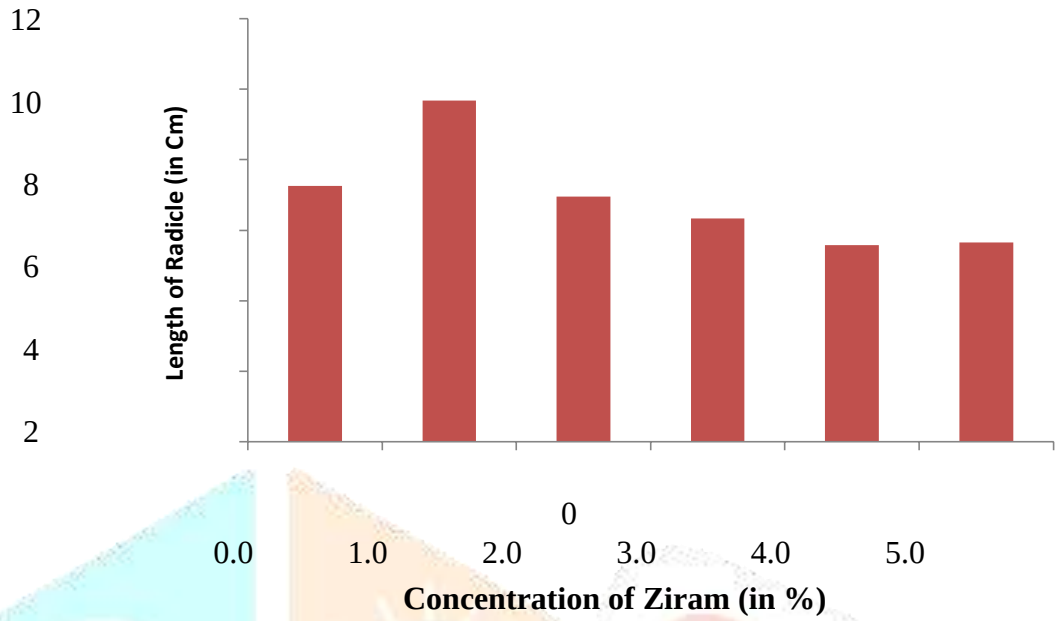
Table-2. Effect of different concentrations of Ziram on radicle growth in *Vigna radiata* (after 4 days of germination).

Concentration of Ziram (in %)	Radicle length (In cm)	% promotion / inhibition over control
Control	7.25 ± 2.99	0.00
1.00	9.68 ± 0.16*	33.51
2.00	6.96 ± 0.12*	-04.00
3.00	6.34 ± 0.09*	-12.55
4.00	5.58 ± 0.07*	-21.00
5.00	5.66 ± 0.13*	-21.00

Note: All readings are mean of 50 samples
± Standard Error (SE)
*Significant at P=0.05 level

So it was evident from the observations that the fungicide exerted a clear inhibitory effect on growth of the radicle of seedlings. The extent of inhibition was dose dependent. About 21.00% inhibition in radicle growth was observed with 5% Ziram after 4 days of germination. (Table-2).

Fig. 6. Effect of different concentrations of Ziram on radicle growth in *Vigna radiata* (after 4 days of germination).



Effect of Different Concentrations of Ziram on Growth of Plumule

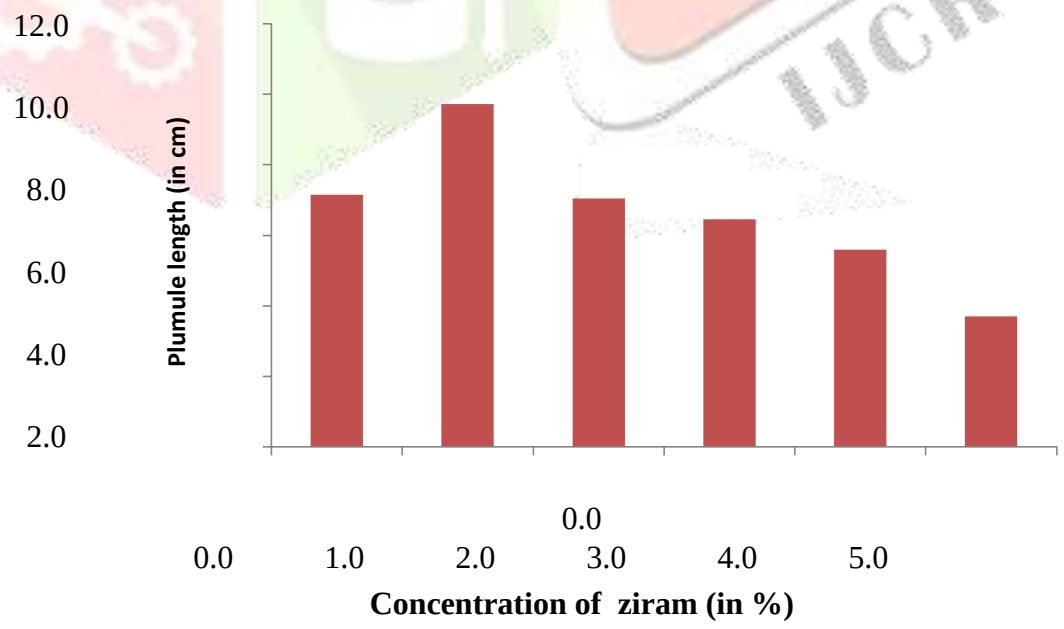
Results obtained on effect of different concentrations of Ziram on Growth of Plumule is shown in table 3 and Fig. 7. Seeds treated with various concentrations of Ziram showed a gradual decrease in plumule lengths (Table-3). 1% concentration of fungicide showed 35.94% increase in length of plumule as compared to those of control seedlings. Other concentrations inhibited the plume growth to different extents. The inhibitory effect was dose dependent (Fig. 7). The 2% concentration of the fungicide inhibited the embryonic shoot length by 1.40 percent, the 3% by 9.79%, the 4% by 21.82% and the 5% by 48.25%. Thus the rate of inhibition increased with the increase in the concentration of the fungicide.

Table-3. Effect of different concentrations of Ziram on plumule growth in *Vigna radiata* (after 4 days of germination).

Concentration of Ziram (in %)	Plumule length (in Cm)	% promotion / Inhibition over control
Control	7.15 ± 0.26	00.00
1.00	9.72 ± 0.14*	35.94
2.00	7.05 ± 0.12*	-01.40
3.00	6.45 ± 0.09*	-09.79
4.00	5.59 ± 0.07*	-21.82
5.00	3.70 ± 0.14*	-48.25

Note: All readings are mean of 50 samples
± Standard Error (SE)
*Significant at P=0.05 level

Fig.7. Effect of different concentrations of Ziram on plumule growth in *Vigna radiata* (after 4 days of germination).



Effect of Different Concentrations of Ziram on Total Seedling Growth

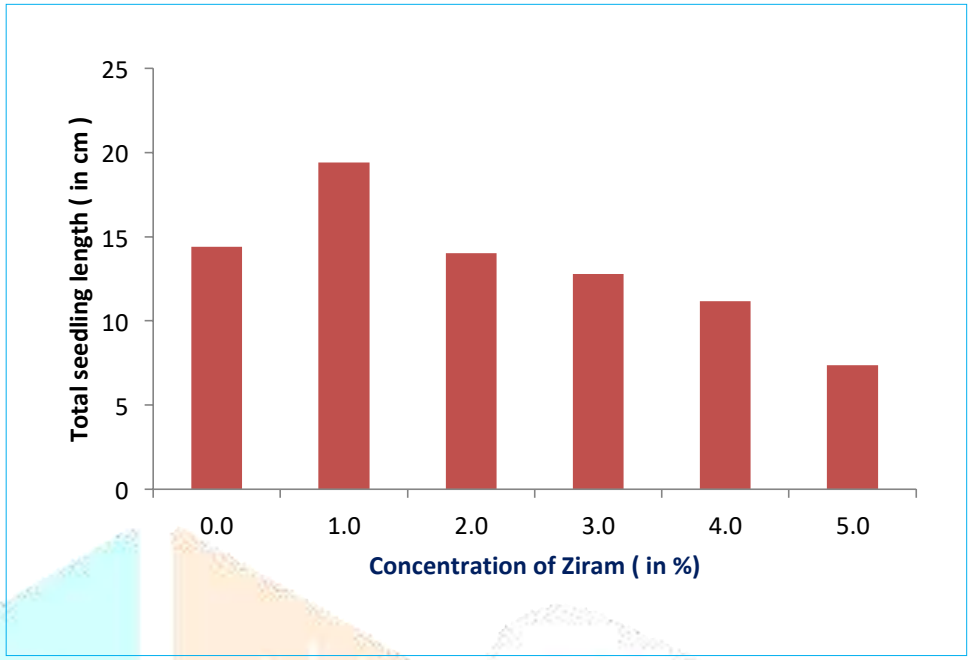
Data pertaining to effect of different concentrations of Ziram on the growth of total seedling in mung bean after 96 hours of seed germination, is embodied in table 4 and shown diagrammatically in Fig. 8. From the data it is evident, that Ziram at lower concentrations (1%) promoted growth of seedling and at higher concentrations (2% and above) inhibited the same. The seeds treated with 1.0% concentration of Ziram showed 34.72% increase in total seedling length as compared their control counterparts. The inhibitory effect was dose dependent. There was a direct relationship between the concentration of the fungicide and the inhibitory effect shown by it. Thus the concentrations of the fungicide, 2% and 4% showed an inhibition of 2.71% and 11.18% in seedling growth while higher concentrations viz., 4% and 5% showed 22.36% and 48.88% inhibition in total seedling growth respectively (Table. 4). Accordingly the total seedling length decreased from 14.40 Cm in control to 7.36 Cm in 5% Ziram treated seedlings.

Table-4. Effect of different concentrations of Ziram on total seedling length (growth) in *Vigna radiata* (after 4 days of germination).

Concentration of Ziram (in %)	Total Seedling Length (in Cm)	% promotion / reduction over control
Control	14.40 ± 0.54	00.00
1.00	19.40 ± 0.26*	34.72
2.00	14.01 ± 0.22*	-02.71
3.00	12.79 ± 0.14*	-11.18
4.00	11.18 ± 0.23*	-22.36
5.00	7.36 ± 0.27*	-48.88

Note: All readings are mean of 50 samples
± Standard Error (SE)
*Significant at P=0.05 level

Fig.8. Effect of different concentrations of Ziram on total seedling length (growth) in *Vigna radiata* (after 4 days of germination).



Ziram also inhibited the growth of radicle, plumule and overall seedling (Figs 6, 7 and 8) at higher concentrations, than the recommended one. Seeds treated with various concentrations of Ziram showed a gradual decrease in plumule, radicle and seedling lengths. It was observed that all the concentrations used had significant effect on the germination and growth of seeds/seedling (radicle and plumule).

There is a decrease in the growth rate, along with an increase in the concentration of Ziram. So it was evident from the observations that Ziram exerted a clear inhibitory effect on growth of the seedlings. The extent of inhibition was dose dependent. About 9.79% retardation of plumule growth and 12.55% retardation of radicle growth occurred with 3% Ziram after 4 days of germination. On the whole 11.18% retardation of overall seedling growth was recorded at -3.0% concentration of Ziram (Tables 2, 3 & 4).

Effect of Ziram on Total proteins

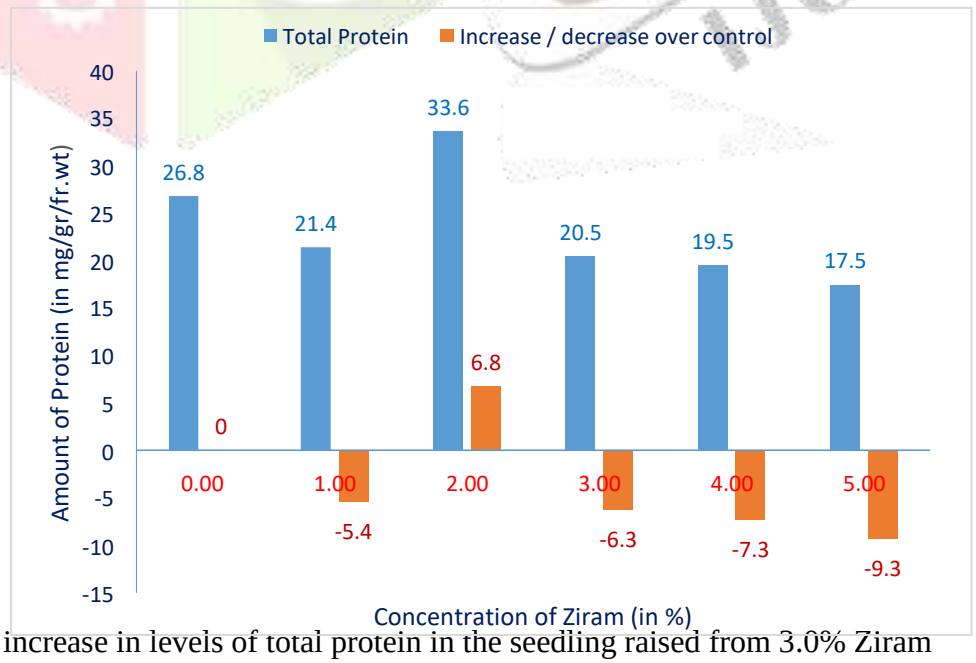
All concentrations of the fungicide, except 3.0%.used in the study, exhibited inhibitory effect on the total protein synthesis in the germinating seeds. Untreated (control) seeds of

mung bean after 96 hours of germination showed 26.8 mg protein/gr/fr.wt. All other seeds, treated with different concentrations of Ziram except 3.0% showed decrease in levels of total protein as compared to the control seedlings (Table 5).

Table.5. Effect of different concentrations of Ziram on total protein content in seedlings of mung bean after 96 hours of seed germination.

Conc. of Ziram (in %)	Amount of total protein (In mg/gr.fr.wt.)	Observed increase / decrease over control	% inhibition / promotion of protein synthesis over control
0.0	26.8	0.00	0.00
1.0	21.4	-5.4	-20.15
2.0	33.6	6.8	25.37
3.0	20.5	-6.3	-23.51
4.0	19.5	-7.3	-27.24
5.0	17.5	-9.3	-34.70

Fig 9. Effect of different concentrations of Ziram on total protein content in seedlings of mung bean after 96 hours of seed germination.



There was an increase in levels of total protein in the seedling raised from 3.0% Ziram

treated seeds. In this case the protein content has gone up by 25.37%. Maximum inhibition in total protein synthesis (or lowest levels of total protein content, 17.5 mg/gr/fr.wt) was observed in seedlings raised from 5.0% Ziram treated seeds (-9.3 mg/gr/fr.wt less as compared to control. Fig. 9).

In all other cases, the total protein content in the seedlings decreased as the concentration of the fungicide increased. Thus there is an inverse relation between the concentration of fungicide and total amount of proteins. This clearly indicates that the fungicide has clearly exerted an inhibitory effect on protein synthesis. This effect is dose dependent. The lowest amount of total protein content (34.70% inhibition) was observed in the treatments that used 5.0% concentration of the fungicide.

From the above experimental results, it is evident that the use of Ziram at higher than recommended doses cause serious detrimental effect on percent seed germination and seedling growth parameters (length of radicle, plumule and seedling height). The fungicide used in the study, has mostly exerted inhibitory effect on the percent seed germination, growth of radicle and plumule in *Vigna radiata*. The fungicide at 1.0% concentration has shown the growth promoting effect on the germination and growth of radicle and plumule. Similarly it also showed promotory effect on protein synthesis at 3.0 concentration of the fungicide.

There are reports to show that higher concentrations of pesticides have harmful effects on the various growth parameters of plants (Reyes, 1975; Foster et al., 1980).

Pablo *et al*., (2003) reported that many fungicides are phytotoxic and retard plant growth by reducing the rate of photosynthesis. Many findings indicate that some fungicides may affect the photosynthetic process. Some pesticides lead to alteration through stomatal closure, while others affect the structure and the functioning of chloroplasts. (Gomathinayagam *et al.* 2007). Bader and Abdel Basset (1999) showed that triforin type fungicides strongly inhibit electron transport reactions of chloroplast. Zamin *et al*., (1999) has reported that repeated use of fungicides, produces chlorosis in plants, which in turn leads to decrease in rate

of photosynthesis and decrease in cell division. This might be the reason for the observed inhibition of seeding growth in our studies.

The improvement in growth parameters may be because of its application suppressed and /or elimination of pathogenic population and the other factors. Growth stimulation may also be due to the increase in the growth promoting factors i.e., increase in cytokinin or gibberellins production etc. Earlier studies suggested that toxicant produced by pesticides application retarded the protein and Carbohydrate synthesis by inducing alteration in cytochrome oxidase activity, blocking alternative respiratory pathways (Siddiqui and Ahmed, 2000). In the present experiment with petridishes, it was observed that germination and growth of the mung bean seeds/seedlings showed inhibitory as well as growth promoting effect in various concentration of the fungicide used.

Our experimental results fortify the earlier findings of authors (Reyes, 1975; Foster et al., 1980, Elloit and Wensha, 2006), who reported that higher concentrations of fungicides have harmful effects on the various growth parameters of plants. It was observed that some concentrations are highly phytotoxic to plants. The effect of pesticides on germination of seeds and plant growth ultimately affect the plant health and productivity in the process. It also decreases the soil fertility status contributing to loss of productivity of the exposed crop plants at large.

Our experimental results strongly suggest that the use of pesticide containing Ziram cause serious detrimental effect on the seed germination and seedling growth, when used at higher concentrations (2.0% and above), higher than the recommended doses (0.125% - 0.25%). The pesticide used in the study, has exhibited inhibitory effect as well as growth promoting effect on the germination and seedling growth of radicle, plumule and total seedling growth in mung bean.

3. SUMMARY AND CONCLUSIONS

Seed dressing with fungicide is a conventional method used for the control of seed & soil borne pathogen that results in healthy plant development and increases the crop yield (Andreas et al., 2008). Although the seed treatments protect the seed &seedlings from pests & disease, it causes secondary effects on germination & growth (Wahengbam et al., 2013). Numerous researchers have reported on the adverse effects of fungicides on the germination and growth of the crop plants. Therefore, there was a crucial need to study about the toxicity of fungicides to plants. The present investigation was undertaken with an objective of evaluating the effect of commonly used fungicide, Ziram, on the rate of seed germination and seedling growth parameters viz., growth of plumule, radicle, total seedling length, on an amenable plant system, mung bean.

Mung bean (*Vigna radiata* (L.) R. Wilczek), family Fabaceae, is used as experimental plant material to study the effect of Ziram on seed germination and seedling growth. It has been selected as experimental material for this investigation because of the fact that – a) It is economically most important pulse crop, widely cultivated in and around the work place, i.e., Bhende and its surrounding villages, b) well adapted to the local conditions, c) its seeds germinate easily in Petri plates lined with moist filter papers and 4) takes less time to germinate and easy to germinate in all season.

Seeds of mung bean were procured from Mahatma Phule Agricultural University, Rahuri. They were selected for their uniformly in size, colour and weight. They were surface sterilized with 0.1% Mercuric chloride solution for 1 minute and thoroughly washed under running tap water. The seeds were presoaked in distilled water, overnight. Next day they were subjected to treatment with different concentrations viz., 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, of Ziram for 2 hours. These were washed thoroughly under running tap water for 30 min, in order

to remove the traces of fungicide at the end of the treatment. Some seeds, not treated with Ziram, were directly placed in Petri plates for germination and they served as control. Both Ziram treated and untreated (control) seeds were allowed to germinate in Petri Plates, lined with moist filter papers.

After four days (96 hours) of seed germination, parameters like percent of seed germination, length of radicle (embryonic root), length plumule (embryonic shoot) and total length of seedling (length of radicle + length of plumule) were noted. 1 gr. control and fungicide treated 96 hour old seedlings were analyzed for total protein content following the method of Lowry *et al*., (1951). LD_{50} dose for the fungicide was determined on the basis of total number of seeds germinated after 96 hours of seed germination. The fungicide dose at which 50% seeds have died and only 50% seeds survived is adjudged as LD50 dose for the fungicide.

The data obtained was analyzed using Data Analysis Pack provided in Microsoft Excel, 2013. A sample size of 50 individual seeds were used in all treatments including control. Means were supported with standard error of means. Significance of means of individual treatments were compared with control means following Student's t – distribution, at probability level, $P \geq 0.05$. All experiments were performed in the laboratory of the Department of Botany at Jijamata College of Science and Arts, Dnyaneshwarnagar, Bhende, during the period September 2017 to February, 2008.

Experimental results obtained indicate that the fungicide has exerted inhibitory influence on germination of seeds, except at lower (1%) concentration. Reduction in percent seed germination was dose dependent. There is a gradual reduction in percent seed germination along with an increase in the concentration of Ziram. Near 50% inhibition of seed germination was observed in 3% concentration of Ziram. Hence this concentration was adjudged as LD50 for the mutagen. Lower concentration (1%) of Ziram exerted a promotory effect on seed germination.

Ziram also inhibited the growth of radicle, plumule and overall seedling at higher concentrations, than the recommended one. Seeds treated with various concentrations of Ziram showed a gradual decrease in plumule, radicle and seedling lengths. There is a decrease in the growth rate, along with an increase in the concentration of Ziram. So it was evident from the observations that Ziram exerted a clear inhibitory effect on growth of the seedlings. The extent of inhibition was dose dependent.

Ziram also exerted a clear inhibitory effect on total protein content in the seedlings. This effect was dose dependent and increased as the concentration of the fungicide increased. Thus there is an inverse relation between the concentration of fungicide and amount of total proteins. This clearly indicates that the pesticide at the selected concentrations have clearly exerted an inhibitory effect on protein synthesis.

Our experimental results fortify the earlier findings of Siddiqui and Uzzaman (2004) and Srivastava, (2005) that low concentrations of fungicides enhance seed germination.

It has been observed that the use of Ziram at higher than recommended doses cause serious detrimental effect on the seed germination and seedling growth. The fungicide used in the study, have inhibitory effect as well as growth promoting effect on the germination and seedling growth of radicle and plumule of *Vigna radiata*. The fungicide at 1.0% concentration has shown the growth promoting effect on the germination and growth of radicle and plumule. The improvement in growth parameters may be because of its application suppressed and /or elimination of pathogenic population and the other factors. Growth stimulation may also be due to the increase in the growth promoting factors i.e increase in cytokinin or gibberellins production etc.

Our experimental results fortify the earlier findings of authors (Reyes, 1975; Foster et al., 1980, Elloit and Wensha, 2006), who reported that higher concentrations of fungicides have harmful effects on the various growth parameters of plants. It was observed that some

concentrations are highly phytotoxic to plants. The effect of pesticides on germination of seeds and plant growth ultimately affect the plant health and productivity in the process. It also decreases the soil fertility status contributing to loss of productivity of the exposed crop plants at large.

Our experimental results strongly suggest that the use of pesticide containing Ziram cause serious detrimental effect on the seed germination and seedling growth, when used at higher concentrations (2.0% and above), higher than the recommended doses (0.125% - 0.25%). The pesticide used in the study, has exerted inhibitory effect on protein synthesis. As proteins are required for regular growth and development in plants, inadequate synthesis of proteins in cells may be the ultimate reason for the observed inhibitory effect of the pesticide. In absence of required proteins, the growth of embryonic root, shoot and total seedling length in mung bean is inhibited. Further studies at molecular level are required to understand how Ziram inhibits protein synthesis in plants.

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