



## EFFECT OF CARBENDAZIM AND COPPER OXYCHLORIDE ON SEED GERMINATION, SEEDLING GROWTH, VIGOUR INDEX AND BIOMASS OF CHILLI (*CAPSICUM ANNUUM L.*)

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### ABSTRACT

Carbendazim and copper oxychloride are commonly used in control of many fungal diseases in agriculture. The study was undertaken to assess the effects of carbendazim and copper oxychloride on seed germination, seedling growth, vigour index and biomass of chilli. The study was carried out for 14 days after soaking the seeds in different concentration of carbendazim and copper oxychloride (0.05, 0.1, 0.2, and 0.4 %) with five replicates, a control (Untreated) was maintained. The carbendazim treatment results showed increasing trend in germination percentage, growth, vigour index and biomass of the seedlings. The germination parameters like root length, shoot length, vigour index increased at 0.2 % and at higher concentration i.e., 0.4% it declined. Copper oxychloride showed phytotoxic effect on chilli showing inhibitory effects on the selected parameters at 0.2% and 0.4%. The synergistic effect of carbendazim and copper oxychloride will be beneficial for the plant growth showing maximum biomass at 0.05% suggesting combine use of both fungicides will be more beneficial for the plant growth as growth promoter.

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### INTRODUCTION

In the agriculture field, majorly the plant diseases are controlled by chemical products such as fungicide, insecticide, nematicide, 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 (Baderet *et al.*,1999). Pesticide seed treatment prior sowing is considered most efficient method of controlling fungal infection in plants (Rotrekl *et al.*,2008). The absorbance of certain amount of pesticides by plants changes the plant metabolism (Koehle *et al.*,2002), which varies from crop to crop. The phenotypic changes are often marked by the biochemical changes in the seed reserves as well as the internal enzymes which are considered as important factors for the seedling growth, where the seed reserves get hydrolysed and an adjustment in the cell and organelle constituents, for example, proteins, lipids and starches takes place (Ashton *et al.*, 1972; Avinash *et al.*,2012). Chilli (*Capsicum annum L.*) is an important cash crop of India, which belongs to the family Solanaceae. The crop production is profoundly affected by various fungal diseases and is controlled by wide spectrum of fungicides. Carbendazim, the derivative of thiophanate and benomyl belong to the benzimidazole group of systemic fungicides.

Seed treatment with carbendazim 2g/kg is considered an efficient method to control of fungal disease (Rajanna, 2015). Copper oxychloride is another important fungicide 1.5g/Kg used in control of early and late fungal infections. Unplanned and repeated usage of fungicides alters the physiological responses and metabolic activities in plants causing phytotoxicity (Anitha *et al.*,2015). The earlier study has reported the chromosomal aberrations in somatic germ cells of pearl millet and sunflower by carbendazim (Harichand *et al.*,1991). Though copper is an essential micronutrient for the metabolic processes, the higher usage of copper based compounds can cause undesirable effects and has been ecologically harmful (Flemming *et al.*,1989). Therefore the present study was undertaken to assess the effect of carbendazim and copper oxychloride when used alone and in combination with different concentrations on chilli seed germination, seedling length, vigour index, fresh and dry weight.

### MATERIALS AND METHODS

#### Chemicals and seed material

The chilli seeds (variety sitara) were procured from Monsanto seed company, Bangalore. The commercial grade (50% pure) Carbendazim and Copper oxychloride were obtained from agriculture market Dharwad, Karnataka.

#### In-vitro phytotoxicity assessment by seed germination assay

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The seeds were surface sterilized with 0.5% sodium hypochlorite for ten minutes, followed with distilled water wash for few times. At different concentrations the (test solution) solution of carbendazim and copper oxychloride fungicides were prepared.

The concentrations of fungicides were selected based on their recommended doses for application by the agrochemicals. The doses were less than recommended (0.05%), the recommended dose (0.1%), higher than the recommended (0.2 and 0.4%) respectively. For the combined application the doses were (0.025% Carb + 0.025% Coc) (0.05% Carb + 0.05% Coc) (0.1% Carb + 0.1% Coc) (0.2% Carb + 0.2% Coc) prepared respectively.

The sterilised chilli seeds were soaked for 24 hours in different beakers containing the different concentration of test solution. For assessment of germination percentage the treated seeds were uniformly placed in a sterilized glass petri dishes containing sterilized cotton bed, moistened with 10 ml of test solution. For each concentration five replicates were maintained and untreated seeds were kept as control. For each concentration 10 seeds were used and the total no. of seeds was 50 (10 X 5). All the petri dishes were maintained under room temperature. The moisture content of the seeds was maintained by adding test solution and equal volume of water (10 ml). The control seeds were treated with distilled water.

The number of germinated seeds in each concentration was noted 6 days after sowing, and germination percentage was calculated. The germination percentage was calculated using the following formula,

$$\text{Germination (\%)} = \frac{\text{number of seed germinated}}{\text{Total number of seeds sown}} \times 100$$

#### ***Effect of carbendazim and copper oxychloride on Seedling length***

The root and shoot length of the germinated seedlings was measured after 14 days of treatment from each concentration and expressed in centimetre.

#### ***Effect of carbendazim and copper oxychloride on Vigour index***

The vigour index of germinated seedlings from each concentration was calculated by the formula suggested by Abdul- Baki and Anderson (1972)

$$\text{Seedling Vigour Index} = \text{germination (\%)} \times \text{Seedling length}$$

#### ***Effect of carbendazim and copper oxychloride on biomass***

The five seedlings from each treatment were taken to determine the fresh biomass of chilli seedlings, followed with drying the seedlings in oven at 80 °C for 24 hours for the dry weight analysis. The dry and fresh weight of the seedlings was noted in grams.

#### ***Statistical analysis***

The influences of Carbendazim and Copper oxychloride on chilli seedlings were expressed with a statistical analysis conducted with SPSS 20.0 (IBM) software. Five independent experiments were performed for each experimental condition, and the data were conveyed as mean. One-way analysis of variance was performed on multiple comparisons among treatment and control groups. Differences with P 0.05 were considered as statistically significant.

## **RESULTS AND DISCUSSION**

Farming is the main source for utilization of chemicals (Satake M *et al.*,1997). Seeds are thought to be as a reasonable host to keep up the pathogenic microorganisms even without the host. Treating such seeds with fungicides or bactericides will shield from the fungi, nematodes or different nuisances (Buss *et al.*,2001). Treating vegetable and harvests seeds with fungicides will ensure them against soil-borne organisms which could bring about sicknesses, particularly root-decay (Pimentel *et al.*,1997). With the wide increment of the utilization of such chemicals, it was found that they have harmful impact on human, creatures, plants and microorganisms. Along these lines, it is important to concentrate their danger to plants. The earlier studies have shown that systemic pesticides may hinder the development and advancement of plant (Ramadan *et al.*,1990).

The seeds treated with different concentrations of carbendazim and copper oxychloride alone and in combination have shown significant effect on the germination, seedling growth, vigour index and biomass, stated the dose dependent effect of the fungicides.

#### ***Impact on germination***

The utilisation of alone and combined use of carbendazim and copper oxychloride demonstrated a noteworthy increment in seed germination. The maximum seed germination was noted (86 and 84%) at 0.05% and 0.2% concentration of carbendazim and control showed 84% of germination. These results were similar to the earlier study where the carbendazim showed positive influence on the rice germination at 6 and 9mg of concentration (Anitha *et al.*, 2015). Carbendazim has also been reported for the highest growth promoting effects on the germination. This also may be because of the influence of this fungicide on the cytokine and gibberellin production (Amar *et al.*, 1973). Likewise carbendazim is also responsible for the chromosomal deviation in physical and germ cell of pearl millet and sunflower (Harichand *et al.*, 1991). In our study it was noted that at highest concentration that is 0.4% inhibited the germination percentage of chilli seeds (table 1), representing that, the usage of these fungicides above the recommended levels actuates both metabolic and biochemical changes that will influence the development and advancement of plants (Anitha *et al.*,2015).

Copper is a quite abundantly used heavy metal in the agriculture field in various forms, as a fungicide, bactericide, algicide etc. copper is also an essential micronutrient required for the plant growth in trace amounts (Kabata-Pendias and Pendias, 2001). It is also involved in physiological processes such as, seed germination, cell wall lignification, photosynthesis, protein and carbohydrate metabolism (Peralta *et al.*, 2000), disease resistance (Kabata-Pendias and Pendias, 2001). Copper oxychloride supported the early germination.

# Studies On Effect Of Carbendazim And Copper Oxychloride On Seed Germination, Seedling Growth, Vigour Index And Biomass Of Chilli (*Capsicum Annum L.*)

**Table 1** Effect of carbendazim on seed germination, root and shoot length, vigour index and biomass of chilli

Sl.No	Treatment	Germination of seeds in %	Root length (cm)	Shoot length (cm)	Vigour index	Fresh weight in (g)	Dry weight in (g)
1	Control	84	7.76±0.01	3.7±0.03	962.64	0.07±0.002	0.002±0.001
2	0.05% Carb	86	7.84±0.03	3.8±0.02	1001.04	0.08±0.0001	0.003±0.001
3	0.1% Carb	82	7.98±0.09	3.72±0.05	959.4	0.13±0.13	0.002±0.001
4	0.2% Carb	84	7.68±0.05	3.62±0.10	949.2	0.07±0.0009	0.034±0.001
5	0.4% Carb	70	6.52±0.08	2.96±0.08	663.6	0.06±0.001	0.002±0.01

Results are expressed as (n=5) Mean±SE and analysed using One-way ANOVA.

**Table 2** Effect of copper oxychloride on seed germination, root and shoot length, vigour index and biomass of chilli

Sl.No	Treatment	Germination of seeds in %	Root length (cm)	Shoot length (cm)	Vigour index	Fresh weight in (g)	Dry weight in (g)
1	Control	84	7.76±0.01	3.7±0.03	962.64	0.07±0.002	0.002±0.001
2	0.05% Coc	90	6.64±0.10	2.84±0.02	853.2	0.07±0.002	0.083±0.001
3	0.1% Coc	78	4.3±0.1	2.84±0.13	556.92	0.06±0.000	0.004±0.03
4	0.2% Coc	68	4.04±0.14	2.66±0.11	455.6	0.06±0.000	0.003±0.001
5	0.4% Coc	42	2.3±0.04	1.88±0.15	175.56	0.05±0.001	0.005±0.001

Results are expressed as (n=5) Mean±SE and analysed using One-way ANOVA

The highest germination was noted 90% at 0.05%, which was sequentially reduced with increased concentrations, to 42% (table 2). Our finding are in agreement with Jay *et al.*, (2011) where they reported the germination percentage of mung bean was not significantly affected with different levels of copper sulphate; and Morteza (2011) who found non-significant differences with different levels of copper sulphate on germination of *Agropyron elongatum*. In 2009, Liu *et al.*, reported that the lowest concentrations of copper was least harmful to the seeds germination, while with increasing concentrations of copper the germination of the seeds declined. The excess amount of copper can be phytotoxic and can induce several malformations (Pandey, 2008), and also inhibits the seed germination, plant growth. Induce chlorophyll degradation and also interfere in the photosystem activity (Balsberg Pahlsson, 1989; Fernandes and Henriques, 1991; Yruela *et al.*, 1996; Caspi *et al.*, 1999)

The combination of carbendazim and copper oxychloride also exerted the similar effects, showing maximum germination at 0.05 and 0.1% (table 3). The reduction in germination at high concentration was noted in case of both fungicides when they applied alone and in combination. In short the joint application of both fungicides reduced the phototoxic effect of copper in certain extent. The earlier study where the carbendazim and boron applied together showed increase in resistance against plant pathogens (Juan M Ruiz *et al.*, 1999).

Carbendazim is a derivative of benomyl and both possesses cytokine like properties (Skene *et al.*, 1972). In all three treatments compared to control the root length was affected with increase in concentration of fungicides. The highest root length was noted with carbendazim treatment (7.84 cm and 7.98 cm) at 0.05 and 0.1%. Least growth was noted (6.52cm) at 0.4% respectively in comparison with control (7.84 cm). The high concentration of copper lead to phytotoxicity causing the root length suppression, leaf chlorosis (Pandey *et al.*, 2008), in support of this work we found the similar results where the copper oxychloride adversely affected the root growth, where the highest growth was noted at 0.05% (6.64 cm) and lowest length was noted at 0.4% as (2.3 cm) at higher concentration (table 2). Only the carbendazim induced the root growth, with the combined treatment of both fungicides the highest length was noted at 0.05 % (6.82 cm) and lowest was noted (4.32 cm) which was much higher than the copper oxychloride treatment at 0.4% (table 1, 2, 3)

**Shoot length:** The data presented in table (1, 2, and 3) depicted the effect of both fungicides alone on shoot length. Some benzimidazoles have been reported for their phytotoxicity. For example, benomyl reduced the growth in cucumbers (Woo *et al.*, 1996), lettuce (Rouchaud *et al.*, 1985), and in loblolly pine and in other species (Stumpff *et al.*, 1991).

**Table 3** Effects of carbendazim and copper oxychloride on seedgermination root and shoot length, vigour index and biomass of chilli

Sl.No	Treatment	Germination of seeds in %	Root length (cm)	Shoot length (cm)	Vigour index	Fresh weight in (g)	Dry weight in (g)
1	Control	84	7.76±0.01	3.7±0.03	962.64	0.07±0.002	0.002±0.001
2	0.025% Carb+0.025% Coc	88	6.82±0.04	3.52±0.10	909.92	0.08±0.001	0.013±0.001
3	0.05% Carb+0.05% Coc	88	5.74±0.04	3.38±0.05	802.56	0.07±0.001	0.008±0.001
4	0.1% Carb+0.1% Coc	66	5.6±0.08	3.14±0.07	576.84	0.06±0.002	0.009±0.0005
5	0.2% Carb+0.2% Coc	48	4.32±0.10	2.28±0.16	316.8	0.01±0.001	0.004±0.003

Results are expressed as (n=5) Mean±SE and analysed using One-way ANOVA

## Seedling growth

**Root Length:** Carbendazim and copper oxychloride considerably affected the seedling growth (Fig.1, 2). Root growth was more adversely affected than shoot growth. The effect of carbendazim on root growth was statistically significant at different concentrations (Fig. 1. A).

Where in our study there was a significant increase in shoot length at 0.05, 0.1, and 0.2% of carbendazim treatment (3.82 cm and 3.7 cm). In support to Mihuta-Grimm (1990) study, we also noted the lowest length at 0.4% (2.96 cm), who reported the stunted growth and chlorosis with the high rate of benomyl application in tomato crop.



Fig. 1. Effects of carbendazim (A) and copper oxychloride (B) at different concentrations on chilli seedlings with respect to control



Fig. 2. Joint effect of carbendazim and copper oxychloride (C) at different concentrations on chilli seedlings with respect to control

Copper oxychloride showed inhibitory action in all concentrations, on chilli seedlings, with respect to control (3.7 cm). The highest shoot length was (2.84 cm) and lowest noted was (1.88 cm) (table 2) (Fig.1.B). These discoveries are in accordance with those of Jay *et al.* (2011) on mung bean cultivar and Dharam *et al.* (2007) on wheat who announced a lessening of plumule and radicle length with increment in copper sulphate concentration. The lessening in the shoot length could be because of excess collection of copper salt in the cell wall, which changes the metabolic activities and constrains the cell wall elasticity (Naseer, 2001). In our analysis combined treatment showed positive signs and supported the shoot growth at 0.05, 0.1, and 0.2% of concentration (table 3) (Fig. 2 C). Nevertheless the leaves, shoot, root responses to the environment stimuli (the fungicide application) show that the metabolic processes responses to the environmental or chemical stress and largely affect the plant growth and development (Pablo,*et al.*,2002).

#### Vigour index

Seed vigour testing is an important criterion to specify the specific property of a seed. Many factors like genetic constitution, environment and nutrition of mother plant, maturity at harvest, seed weight and size, mechanical integrity, deterioration and ageing and pathogens influences the seed vigour (Perry, 1984). Seed vigour analysis test mainly based on the principle that vigorous seeds grow at a faster rate than poor vigour seeds even under favourable environments. Vigorous seeds rapidly germinate, metabolize and establish in the field and the seed showing the higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973). In our study as compared to control in all the concentrations the vigour index was quite affected. Only with carbendazim 0.05, 0.1% treatment increased the vigour index as shown in (table 1). The copper oxychloride and combined treatments of carbendazim and copper oxychloride decreased the vigour index in all treatments (table 2, 3).

Our data supported the study carried by Habtamu Ashagre (2015), on phytotoxic effect of copper sulphate in haricot bean, where the shoot length, root length, and vigour index decreased significantly with increase in copper sulphate concentration.

#### Biomass

The seedling fresh and dry weight was noted. The resulted biomass shows the dose dependent effect of both fungicides. The carbendazim increased the dry weight in the low doses. The highest fungicide concentration remained toxic in all treatments, for all the selected parameters in chilli seedlings. The highest fresh biomass was noted (0.07 g) and dry biomass was noted as (0.002 g) for 0.05% concentration. Tripathi *et al.*,(1982) also observed the similar behaviour in carbendazim foliar application where the carbendazim increased the biomass production at  $20 \mu\text{g mL}^{-1}$  concentration. Further the experiment also illustrated the reduction in biomass as the carbendazim concentrations increased to  $100 \mu\text{g mL}^{-1}$  suggesting disorganisation of plasma membrane, increased efflux of ions, may become the reason for reduced biomass. Like this study, blitox, hexaconazole and tetraconazole applications also showed the highest inhibitory effect on the production of biomass in case of *Zea mays* (Wahengbam, *et al.*,2013). The copper oxy chloride also showed the similar effects on the seedling biomass. The seedling fresh weight and dry weight decreased significantly with increase in copper oxychloride concentration as compared to control (table 2). Dharam *et al.*, (2007) also reported that fresh weight, and dry weight of seedlings decreased with increasing copper concentration in wheat, Habtamu Ashagre (2015) has also reported the similar effects of copper sulphate on haricot bean. Finally the combined treatment of carbendazim and copper oxychloride increased the dry weight (table 3). The highest fresh weight was noted dry weight was 0.08g and dry weight was 0.013 at 0.05% concentration with respect to control. These results suggest the positive effect that adequate application of carbendazim and copper oxychloride is

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beneficial for the plant growth. The results supported beneficial effects of carbendazim and boron applied together in tobacco plants (Juan, *et al.*, 1999).

These results suggest that the application of carbendazim and copper oxychloride could reduce the recommended dose applications without decreasing its effectiveness. Thought the further research in a molecular level is required to prove this hypothesis.

## CONCLUSION

The study indicated that seed treatment with any fungicide at low concentration was beneficial for the healthy growth of the seedling. Therefore the 0.05, 0.1% of concentration was recommended or applicable. The study also showed the beneficial effect of combined use of two fungicides at lower concentrations, showing the positive effect on growth and biomass. Hence the careful screening and concentration selection of fungicide should be done very crucially before the field application.

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