



Research article

Effect of Mancozeb on Mustard (*Brassica juncea* L.): An *In-vitro* study

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Abstract: Pesticides are extensively used all over the globe for the control of pest population responsible for reduction in yield and among them fungicides are the specifically used to control fungal plant pathogens. An *In-vitro* plant study was conducted to assess the effect of Mancozeb on early growth parameters on two Mustard (*Brassica juncea*) varieties *i.e.* RH- 30 and Laxmi. Mancozeb (75% WP) is a widely applied contact fungicide for control of various diseases but several toxicity issues are associated with Mancozeb. Mustard is one of the most important oil yielding crops and is majorly used for various purposes such as medicinal uses, condiment and industrial uses. In the experiment three different doses of Mancozeb (75% WP) *i.e.* 1 mg.kg⁻¹ Half Recommended Dose (½RD), 2 mg.kg⁻¹ Recommended Dose (RD) and 4 mg.kg⁻¹ Double Recommended Dose (2RD) applied on RH-30 and Laxmi varieties. Results revealed that, RD and 2RD reduced the germination up to 30–60 % in variety RH-30 whereas in variety Laxmi reduced germination percentage is 17–55 % in comparison of control. The overall length of plants of variety Laxmi was found to be increased in comparison of control but in plants of variety RH-30 the results was just opposite. Overall results indicate that excess amount of Mancozeb induced stress in both mustard varieties which resulted in low germination percentage. Whereas after seven days, Laxmi resulted in better growth than RH-30 plants. Also, Seeding vigour was recorded higher in Laxmi than RH-30.

Keywords: Pesticides - Fungicides - Seeding Vigour - Mustard.

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INTRODUCTION

Mustard (*Brassica juncea* L.) is one of the most important oil crops of the world after soybean and groundnut (FAO 2012) and in India it is popularly cultivated as major oil seed crop (Meena *et al.* 2010). It is predominantly cultivated in the states of Rajasthan, Uttar Pradesh, Haryana, Madhya Pradesh and Gujarat which contribute 81.5% in terms of area and 87.5% in term of production and majorly used for various purposes such as medicinal uses, condiment, and industrial uses. Mustard is rich in phytonutrients, minerals, vitamins and antioxidants as well as constituents like sinalbin and sinigrin and serves as a functional food (Kumar & Andy 2012). According to Ayurveda, Mustard leaf is considered as a vegetable, while the seeds are used as a condiment and constitute the source of mustard oil. Mustard seed is considered as rich source of oil and protein. The seed has oil content as high as 46–48 % while seed meal has 43.6% protein (use of meal) and low in glucosinolate content. Also, seed residue used as an ingredient for cattle feed and poultry feed in India (Mandal *et al.* 2002, Manohar *et al.* 2009).

Some anti-nutritional factors present in rapeseed-mustard crops are known to be associated with some plant defence system and other important biological functions (Kumar *et al.* 2014).

Application of pesticide is strategy for effective management of pests and diseases and the productivity of crops depends on their effective control (Pretty & Bharucha 2015). Pesticides are extensively used all over the globe to control different pest population and among them fungicides are specially used for control fungal plant pathogens (Mancini & Romanazzi 2013). In the past few, non-target environmental impact and Excessive use of

fungicides have caused environmental pollution and development of fungicide resistance in plant pathogens which led to the search for alternative methods (Hollomon 2015). In recent years, crop suffers from many diseases out of them Alternaria blight [*Alternaria brassicae* (Berk.) Sacc. & *A. brassicicola* (Schw.)] is one of the serious and widely occurred diseases all over the country (Meena *et al.* 2011, Chaurasia & Bhajan 2015). Alternaria blight is reported to causes blight of leaf, pod, stem and seed abnormalities (Chattopadhyay *et al.* 2005). It is reported that Alternaria blight reduce upto 70% yield of mustard in India (Rathi *et al.* 2015).

Table 1. Physical and Chemical Properties of Mancozeb as described by (Harvest 2016).

Chemical Name	Manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt
Empirical Formula	$(C_4H_6MnN_2S_4)_x(Zn)_y$
Molecular Weight	541
Structural Formula	
Flashing point	138°C (150°C Dec.)
Solubility	6–20 mg.l ⁻¹ in water, practically insoluble in most organic solvents
Vapour pressure	Negligible
Acute oral LD50	5 g.kg ⁻¹ (rat)
Mancozeb formulation	75% WP

Mancozeb (75% WP) is a contact fungicide mainly used for control various diseases on wide variety of crops (Jankowski 2007, Mathivanan & Prabhavati 2007). Mancozeb [[1, 2-ethanediybis-[carbomodithio- ato]] (2-)] manganese, mixture with [[1, 2-etha-nediybis-[carbomodithioato]]-(2-)] zinc is a fungicide of the carbamate pesticide family. It is marketed by the trade names Dithane M45, Indofill, Manzeb, Nemispot, Manzane etc. It is applied on various crops including oil bearing plants and field crops against a wide spectrum of fungal diseases (Nirwan *et al.* 2016). The fungicide Mancozeb 75% WP [Indofil M-45] was used in the present study.

Table 2. Diseases controlled by fungicide Mancozeb on Mustard (*Brassica juncea* L.).

Diseases	Causal organism	References
Sclerotinia rot	<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	Biswas <i>et al.</i> (2007), Vishwanath & Kolte (1997)
White rust	<i>Albugo cruciferarum</i> (Gray) Kuntze	Bhatia & Gangopadhyay (2008)
Alternaria blight	<i>Alternaria brassicae</i> (Berk.) Sacc. and <i>A. brassicicola</i> (Schw) Wiltshire	Meena <i>et al.</i> (2011)
Powdery mildew	<i>Erysiphe cruciferarum</i> Opiz ex L. Junell	Biswas <i>et al.</i> (2007), Vishwanath & Kolte (1997)
Downy mildew complex	<i>Hyaloperonospora parasitica</i> (Pers.) Constant.	Biswas <i>et al.</i> (2007), Vishwanath & Kolte (1997)

Mancozeb is applied on various crops against a wide spectrum of fungal diseases (Hayes & Paiford 1995, Goswami & Ghosh 2012). Mancozeb has power to inhibit spore formation in pathogenic fungi thereby causing its death (Elsamen *et al.* 2015). Mancozeb is reported to be associated with several health hazards when applied in very high doses. The resulting symptoms are convulsions, slurred speech, confusion, slowed heartbeat etc. Mancozeb is reported to be an established carcinogen (FAO/WHO 1993, Vettorazzi *et al.* 1995, Liu *et al.* 2016). Mancozeb is reported to cause teratogenic, carcinogenic and goitrogenic effects on mammals. Ethylene thio urea (ETU), carbon disulphide (CS₂), thio urea etc are metabolites produced in degradation of Mancozeb reported to be responsible for inducing thyroid toxicity and neurotoxicity (Kackar *et al.* 1997, Domico *et al.* 2007, Miller *et al.* 2009). ETU is reported to cause ground water pollution due to its high solubility in water (Belpoggi *et al.* 2002, Srivastava & Singh 2013). The fungicide Mancozeb has a considerable deleterious impact on soil microflora, nitrification, ammonification, soil microbial biomass, carbon mineralization and soil enzymes which may result in harmful effects on nutrient uptake and plant growth. These findings suggest that the use of Mancozeb to control plant diseases in apple orchard soil requires simultaneous application of large quantities of nitrogen based fertilizers (Dias 2012).

There is very little information available with regard to the effect of fungicide on oil crops generally and on Mustard crop particularly. Therefore the present study was undertaken with a view to understand the effect of commonly used fungicide Mancozeb on initial plant growth and chlorophyll content of Mustard (*Brassica juncea* L.).

MATERIAL AND METHODS

The present study was conducted at the laboratory of Environment Toxicology and Microbiology, Department of Energy and Environmental Science of Chaudhary Devi Lal University, Sirsa. Soil sample was collected from the fertile field of village Umedpura of District Sirsa. In this field, wheat, cotton and mustard are mainly cultivated crops. The soil was processed in Laboratory, excess moisture was removed by drying the soil in shade and sieved properly to make it usable for pot trial study. The pots were prepared by 100 gm soil in each pot, and this study was triplicated for each treatment (Kidwai 2007). Fungicide Mancozeb 75% WP [Indofil M-45] was procured from local market of Sirsa. The three different doses of fungicide i.e. half recommended dose, recommended dose and double recommended dose were used in this study.

Table 3. Different doses of Mancozeb 75% WP with their application rate used for seed treatment.

Doses	Application rate (per kg seed)
Half recommended dose (½RD)	1 mg.kg ⁻¹
Recommended dose (RD)	2 mg.kg ⁻¹
Double recommended dose (2RD)	4 mg.kg ⁻¹

Seed of two popular mustard varieties *i.e.* RH-30 and Laxmi (procured from Krishi Vigyan Kendra, Sirsa) were surface sterilized with 0.1% HgCl₂ (mercuric chloride) for 5 min then washed thoroughly with distilled water (Hirve & Bafna 2013) and allowed to germinate in pots. Three sets in each dose were maintained along with the control for comparative study. On the seventh day, growth parameters and chlorophyll content were evaluated. Germination percentage was estimated as described by Rangwala *et al.* (2013),

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Root and shoot length of seedlings were recorded using the standard centimetre scale (Kabir *et al.* 2008, Rangwala *et al.* 2013). Seedling Vigour index was calculated applying formula suggested by Aery (2010),

$$\text{Vigour index} = \frac{\text{Germination \%}}{\text{Total length (root length + shoot length) in cm}}$$

Three plants of each treatment were weighed in order to determine the fresh weight (Kabir *et al.* 2008, Rangwala *et al.* 2013). Spectrophotometric estimation of the Chlorophyll 'a', Chlorophyll 'b' and total Chlorophyll of mustard crop was done by following the method as described by Aery (2010).

$$\text{Chlorophyll 'a' (mg.g}^{-1}\text{)} = 12.7A_{663} - 2.69A_{645} \times V / 1000 \times W$$

$$\text{Chlorophyll 'b' (mg.g}^{-1}\text{)} = 22.9 A_{645} - 4.68A_{663} \times V / 1000 \times W$$

$$\text{Total Chlorophyll (mg.g}^{-1}\text{)} = 27.8 A_{662} \times V / 1000 \times W$$

Where, V =Vol. of the extract in ml, A₆₄₅=Optical density measured at 645 nm, A₆₆₃=Optical density measured at 663 nm and A₆₆₂=Optical density measured at 662 nm.

Table 4. Codes for the Treatment used in the study.

Treatments (T)	Code
Control RH-30	TR ₁
Control Laxmi	TL ₁
Half Recommended Dose of Mancozeb (RH-30)	TR ₂
Half Recommended Dose of Mancozeb (Laxmi)	TL ₂
Recommended Dose of Mancozeb (RH-30)	TR ₃
Recommended Dose of Mancozeb (Laxmi)	TL ₃
Double Recommended Dose of Mancozeb (RH-30)	TR ₄
Double Recommended Dose of Mancozeb (Laxmi)	TL ₄

RESULTS AND DISCUSSION

Table 5. Variation in early growth parameter and chlorophyll content in Mustard varieties.

T	Germination %	Fresh weight (gm)	Total length (cm)	Seedling Vigour Index	Chl 'a' (mg.g ⁻¹)	Chl 'b' (mg.g ⁻¹)	Chl 'total' (mg.g ⁻¹)
TR ₁	80±0.2	0.82±.01	6.8±.005	544±.057	0.502±.0005	0.248±.0005	0.742±.0005
TR ₂	70±.25 (13)	0.81±.005 (1)	6.4±.01 (6)	448±.15 (18)	0.463±.001 (8)	0.228±.001 (8)	0.643±.002 (13)
TR ₃	60±.05 (25)	0.71±.01 (13)	6.1±.05 (10)	366±1 (33)	0.428±.001 (15)	0.201±.002 (19)	0.599±.003 (19)
TR ₄	50± 2 (38)	0.43±.22 (48)	3.1±.2 (54)	155±4 (72)	0.201±.001 (60)	0.083±.001 (67)	0.219±.11 (70)
TL ₁	90±2	0.89±.03	8.6±0.4	774±1	0.691±.01	0.535±.005	0.751±.001
TL ₂	80±.05 (0.11)	0.88±.03 (1)	8.5±.15 (1)	680±8 (12)	0.68±.01 (2)	0.531±.001 (1)	0.747±.002 (1)
TL ₃	70±.5 (22)	0.85±.01 (4)	7.1±.1 (17)	497±4 (36)	0.677±.002 (2)	0.527±.01 (1)	0.698 ±.01 (7)
TL ₄	60±1 (33)	0.61±.01 (31)	4.2± .1 (51)	252±1 (67)	0.311±.001 (55)	0.209±.05 (61)	0.354±.1 (53)

Note: * All the values are mean of triplicate value, figure in parenthesis indicates percent decrease in comparison of control.

The fungicide Mancozeb has highly influenced all the studied growth parameters of mustard seedlings. Germination percentage of Mancozeb fungicide treated seed was decreased with the increase in concentration of fungicide Mancozeb. Other studies also reported that more fungicide produces negative interference and abiotic stress in germination of seeds and decreased it drastically in the treated sets (Hori *et al.* 2007, Cataneo *et al.* 2010, Marini *et al.* 2011, Parween *et al.* 2016). The results of our present study were conflict to Benicio (2015) who reported that fungicides doses not produces interference in germination of seeds.

The results of present study demonstrated that shoot and root length was decreased with increasing concentration of fungicide. Petit *et al.* (2012), Anitha & Savitha (2013) and Shakir *et al.* (2016) were also reported that growth of plants was reduced after increasing the dose of Mancozeb fungicides. Results were in agreement with the work done by Windham & Windham (2004), Bensoltane *et al.* (2006) and Mohammed & Alrajh (2014) who have reported that Mancozeb is a systemic fungicides which are based on sterol biosynthesis inhibitor are closely related to plant growth regulators the use of which at higher than labeled rates shorten the internodes which may lead to slow-shoot and root growth.

Seedling vigour index was decreased at almost all the concentration of fungicide as compared to control in the present study. But the results of our present study were contrary to Morales *et al.* (2012) who observed higher vigour of rice seeds treated with Carboxin and Thiram fungicides.

In the study, results of mustard seedlings treated with Mancozeb indicate decrease in fresh weight with increasing concentration of fungicide. These results were in parallel with the findings of Avinash & Hoshmani (2012) that fresh weight of leaves of sorghum seeds treated with Carbendazim decrease with increase in concentration of fungicide.

Chlorophyll content were also reduced in the study as increased in concentration of Mancozeb fungicide and the results of our study were similar to the effect of tricyclazole on Maize seeds (Avinash 2012). According to Anitha & Savitha (2013), Petit *et al.* (2012) and Shakir *et al.* (2016) chlorophyll content were also reduced after increasing the dose of Mancozeb fungicides.

Majid *et al.* (2014), Singh & Kaur (2016) and Parween *et al.* (2016) reported oxidative stress caused by Mancozeb that affect overall plant growth as well as biochemical parameters especially chlorophyll content. It was also reported that higher dose of Mancozeb have negative effect on plant growth and activity of mycorrhizal symbiosis (Saleh 2006, Chaurasia 2014). According to Kackar *et al.* (1997) and Mirkovic *et al.* (2015) chronic oral feeding of Mancozeb has produced dose dependent toxicity and death of animal by structural and functional changes like significant increase in thyroid, body weight ratio and histopathological changes. Easton *et al.* (2001) reported that by the effect of Mancozeb fungicide novel proteins being induced and stress were increased. Some new technology like Nano and PEG applied for Mancozeb claimed much eco-friendly (Majumder *et al.* 2016).

CONCLUSION

From the present study it was concluded that the recommended dose of Mancozeb favours growth of seedlings but concentration higher than recommended dose can be unfavourable for proper growth of plants and chlorophyll content. Further field trial is in process to study the effect of Mancozeb involving other biochemical parameters. Explore the most promising opportunities to increase benefits and reduce health and environmental risk of fungicide use. We should educate and aware the farming community about using the proper quantity of fungicide and adopt eco-friendly practices for sustainable agriculture which is direly needed in the larger interest of planet earth.

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