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Effect of Mixed Commercial Formulations of Pesticides on the Germination of Mono and Dicotyledonous Crop Seeds

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Abstract: Commercial formulations of various pesticides of different classes find direct applications in agriculture. Farmers are encouraged to apply these pesticides to improve the crop production. We tried to study the effects of a few commercially available pesticides on the crop seed germination. Green gram (dicotyledonous crop) and wheat (monocotyledonous crop) seeds were considered for the study. Commercially available pesticides were mixed and spiked into the sterilised soil at various concentrations. Green gram seedling vigour index reduced from 249.79 in controls to 147.33 at 50 ppm while for wheat it came down from 204 in control to 32.96 at 50 ppm of each pesticide in themixture. Seed viability was recorded and found to be affected by the pesticide-mix. The roots were affected severely in both green gram as well as wheat. The average root lengths reduced from control to 50 ppm in both the cases. The number of secondary roots in green gram decreased from an average 6.87 in control to 0.18 in 50 ppm while the number of root fibres in wheat came down from an average 8.3 in control to 2.6 in 50 ppm pesticide mix. Average leaf length in green gram reduced from 1.57 cm in control to 1.23 cm in 50 ppm. The overall plant height (root + shoot) in the case of green gram and wheat were recorded 27.73 cm and 20.12 cm in controls respectively which decreased and reduced to 18.37 cm and 3.39 cm respectively at 50 ppm level of pesticides. There was a clear indication that the indiscriminate use of pesticides would lead to reduced produce even though the crop is protected from pests.
Keywords: Commercial Formulation, Green Gram, Pesticide, TTC, Vigour Index, Viability.

I. INTRODUCTION

Pesticides are used in agriculture at various stages of cultivation. The synthetic pesticides were developed based on the requirements of farmers to protect and improve the agriculture produce. The indiscriminate use of these chemicals made the residues to remain in soil and water. These residues affect the germination of new food crops leading to huge losses to the farmers without their knowledge. The harmful effect of pesticides on crops has been demonstrated by many researchers (Dalvi and Salunkhe, 1975; Kadam*et al.*, 1981; Mitra and Raghu, 1989; Zhang *et al.*, 2008; Cram and Vaartaja, 1955; Boutin*et al.*, 2000; Olszyk*et al.*, 2009; Bidlan*et al.*, 2004; Raju*etal*., 2017). Most of them used a single pesticide in pure form for their studies. Deepthi*et al.* (2005) worked out DDT toxicity studies on plants. Manonmani (2011) and Bidlan*et al.* (2004) have demonstrated the toxic effects of Tech. HCH on various crop seed germination while Raju*et al.* (2017) demonstrated the effect of two pure forms of Lindane and DDT in combinations on green gram germination. In this paper, we demonstrate the effects of amixture of commercial formulations of synthetic pesticides available in the market on the seed germination, the vigour index and the viability of the embryo which can be playing a role of asilent killeron agriculture produce.

II. MATERIALS AND METHODS

A. Pesticides

Chloropyriphos, profenofos+cypermethrin, □-methrin+triazophos, and □-cyhalothrin were procured from Hindustan Pulverising Mills Chemicals & Fertilizers Ltd, India. Dichlorvos and cypermethrin were purchased from Insecticide India Ltd and Bharat Insecticides Ltd. respectively.

B. Soil

The soil was collected from the gardens of Delhi Technological University, Delhi, India. The soil was alluvial soil and rich in organic matter and inorganic minerals.

C. Seeds
Seeds were purchased from the local market of Delhi, India.

D. Chemicals
Inorganic salts of analytical grade were obtained from standard manufacturers.

E. Pre-experimental arrangements
The soil was dried in the shade and sieved to get particles of size 20 µm diameters. This soil was sterilised by autoclaving thrice at 121 ^oC for 1h every alternate day. The autoclaved soil was incubated at ambient temperature for 18-24 h before taken for the next cycle.Plastic cups of diameter 10 cm and depth 6 cm were pricked with a needle on the sides and the bottom to facilitate aeration and to drain any extra buffer added during the experiment. The inside of these perforated cups was wiped with 70% ethanol.

F. Experiment
1) Seedling vigour and effect on various parts of the germinating plants: 100 g of the sterile soil was spiked with various amounts of the pesticide mix and filled in the sterile perforated plastic cups. Initially, around 80 g of the spiked soil was placed in the cups and wetted with a buffer of pH 7. Ten seeds per cup were placed and overlaid with remaining 20 g of the soil followed by wetting with the buffer. All the cups were incubated at room temperature with the relative humidity of 70-75%. The soil spiked with plain sterile water was used as the control. 10 cups of 10 seeds each (100 seeds), for each sampling and each concentration of the pesticide mix, were considered for proper statistical analysis. Sterile buffer (pH 7) was added every day to maintain the moisture content in the soil. Cups (samples) were drawn after a week's incubation for the analysis of root length, shoot length, the number of leaves, and the number of secondary roots.

2) Seed Viability in the presence of pesticides: Viability tests were conducted per Bidlan*et al.* (2004). Ten seeds were soaked in sterile buffer (pH 7) containing various concentrations of pesticides in sterile Petri plates. Replicates of 10 were used for the studies. The soaked seeds were taken out after 72 h exposure at room temperature, cut along the margin (in the case of the green gram) to expose the embryo. The seeds were placed in 0.1% of triphenyltetrazoliumchloride (TTC) solution and incubated at 37 ^oC for 24 h away from light. Seeds were removed from TTC, washed well in distilled water and placed in 10 mL 95% ethanol until the full pink colour (triphenylformazan) was extracted. This colour was read at 480 nm (Eppendorf basic spectrophotometer, Germany).

III. RESULTS AND DISCUSSION

A. Effect of pesticide mix on food crop seed germination
The germination was drastically affected by the increase in pesticide concentrations. The Fig. 1- Fig 8 gives direct visual evidence of toxicity of the pesticides towards seed germination and seedling development. The root length, shoot length, leaf length, and the number of secondary roots was measured, and the results are presented in Table 1. It is clear from the observations in Fig. 1- Fig 8 and Table 1 that the overall germination and development of the seedling are severely affected as we increased the pesticide concentrations. Cram and Vaartaja (1955) demonstrated the effect of eight pesticides on spruce and caragana seeds. They concluded chinosol, ceresan and manzate to be lethal while thirampreparates, white Stauffer N-244, and orthocide 75 were mildly toxic. Our observations show the percent germination ranged from 70% in 50 ppm to 78.4% in lower concentrations of pesticide mix. Surprisingly, the 10 ppm concentrations of pesticides gave an astounding 90% germination while control had 81.1% seeds that germinated. Even after the repetition, the 10 ppm germination percentage remained near 90 indicating the ability of this concentration in the soil to stimulate germination and remove the dormancy. Many research groups in the past have reported similar observations. Zhang *et al.* (2008) reported the use of ALA (5-aminolevulinic acid) and propyl-4-(2-(4, 6-dimethoxypyrimidin-2-yloxy) benzyl amino) benzoate together inhibited the root and shoot in *Brassica* but the lower concentrations improved the growth. Kadam*et al.* (1981) indicated the application of pesticides could lead to the quality change in nutrients of the food crops.

B. The Seedling Vigour
The seedling vigour index (V.I.) is an indicator of plant's health. In the present study with both green gram and wheat reflected the negative effects on the vigour indices of both these plant species with increasing pesticide concentrations. Table 2 gives the V.I. of the green gram at different concentrations of the pesticide- mix. At 50 ppm pesticides level, the V.I. was reduced by almost 41% in green gram while it was reduced by 83.8% in wheat (Table 3). Even though the germination percentage doesn't give much information as to what is happening to the overall health of the plant, V.I. reflects the abnormal condition by taking into account both root and shoot length along with the germination percentage.

C. Effect on Roots

The mean root lengths in the case of wheat decreased from 10.64 cm in control to 2.91 cm, 1.11 cm, 0.73 cm and 0.46 cm in 5 ppm, 10 ppm, 20 ppm and 50 ppm respectively (Fig. 7). The mean root lengths of green gram also reduced with increasing concentrations of the pesticides (Fig. 3 and Table 1). An interesting observation that can be drawn from Fig. 3 and Fig. 7 is that with increasing concentrations of pesticides, the root length shortens and is almost diminishes to the extent that it cannot support the growing seedling. The reduced root length cannot hold itself to the soil and there is every possibility for the plant to droop easily and uproot from the land. Earlier, Bidlan*et al.* (2004) showed that at lower concentrations there was anincrease in root lengths of green gram and radish that reduced drastically at higher concentrations of tech. HCH. Our personal observations with most of the tested food crops in thelab have very similar effects. While the root shrinks with increasing pesticide concentrations, the number of rootlets (secondary roots/ root fibres) increases initially (till 5 ppm). These rootlets are more rigid and short (Fig. 3 and Fig. 7).

D. Effect on leaves

The average leaf length in green gram also found to reduce from 1.57 cm in control plants to 1.23 cm in 50 ppm. The reduced size of leaves can later be responsible for the reduced yield/ produce thereby affecting the farmer economically and the world community with food scarcity. This is because small leaves will carry small amounts of chlorophyll that in turn result in the lower rate of photosynthesis, hence lower agriculture produce. All these factors need to be considered, and the unaccounted losses should be brought to the notice of farmers helping them make appropriate decisions about the use of pesticides marketed by much-hyped propaganda for crop protection and production.

E. Effect on the seed viability

Fig. 9 shows the effect of different concentrations of the pesticide mixtures on the viability of the green grams embryos after 72 h of exposure. The viability of the green gram embryos decreased with the increase in pesticide concentration. It was observed that the viability of wheat embryos increased within the first 24 h for all concentrations and then decreased with increasing pesticide concentration and exposure time (Fig. 10). The decrease in viability count can be attributed to the toxicity of pesticide molecules. The initial rise in viability might be due to the initial and quick stimulatory effect of the pesticides in all concentrations. Dalvi and Salunkhe (1975) advocated the use of plants as indicators in the form of a bioassay to assess the adverse effects of pesticides on crops. They also gave a warning signal almost 42 years ago that the toxicological implications of these pesticides in a long-term exposure can be dangerous not only to the plants but also to the consumers.

IV. CONCLUSION

It is evident from the present research that the commonly consumed legume and wheat, which forms the staple food for a large proportion of thepopulation, are adversely affected in presence of the commercially available pesticides in the market. The overall crop plant’s ability to synthesize food grains to its fullest potential is affected in the agricultural soil contaminated with the new generation of pesticides being made available in the market to the farmers. The inability of the roots to hold to the soil due to reduced length and rootlets can also put the crop at risk during heavy winds and rains. In general, even the effect can be devastating leading to biodiversity erosion. This report is one of the many to make the scientific fraternity, world community, Government policymakers and the farmers realise and decide the safe and better future for humankind and ecosystem at large.

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Table 1a: Average measurements of various parts of green gram in different concentrations of pesticide mixture

	MWPL	MSL	MRL	MLL	MSR
control	27.73	21.49	6.23	1.57	6.87
2ppm	27.82	22.2	5.62	1.49	8.75
5ppm	24.15	20.66	3.49	1.37	9.71
10ppm	25.25	21.21	4.04	1.34	1.75
20ppm	22.17	18.69	3.48	1.29	0.32
50ppm	18.37	15.23	3.13	1.23	0.18

MWPL= Mean whole seedling length; MSL= Mean Shoot Length; MRL= Mean Root Length; MLL= Mean Leaf length; SR= Number of secondary roots.

Table 1b: Average measurements of shoot and root length of Wheat seedling in different concentrations of pesticide mixture

	MSL*	MRL**
Control	10.18	10.64
5ppm	7.68	2.9
10ppm	5.15	1.11
20ppm	3.75	0.73
50ppm	2.94	0.86
*MSL=Mean Shoot Length **MRL= Mean Root Length		

Table 2: Seedling vigour index of green gram in different concentrations of pesticide mixture

control	249.788
2ppm	236.9955
5ppm	205.7312
10ppm	202.5
20ppm	194.3324
50ppm	147.3288

Vigour index= (average shoot length + average root length) x percent germination/10

Table 3: Seedling vigour index of wheat in different concentrations of pesticide mixture

Control	204.00
5ppm	88.91
10ppm	60.7
20ppm	44.33
50ppm	32.96

Vigour index= (average shoot length + average root length) x percent germination/10

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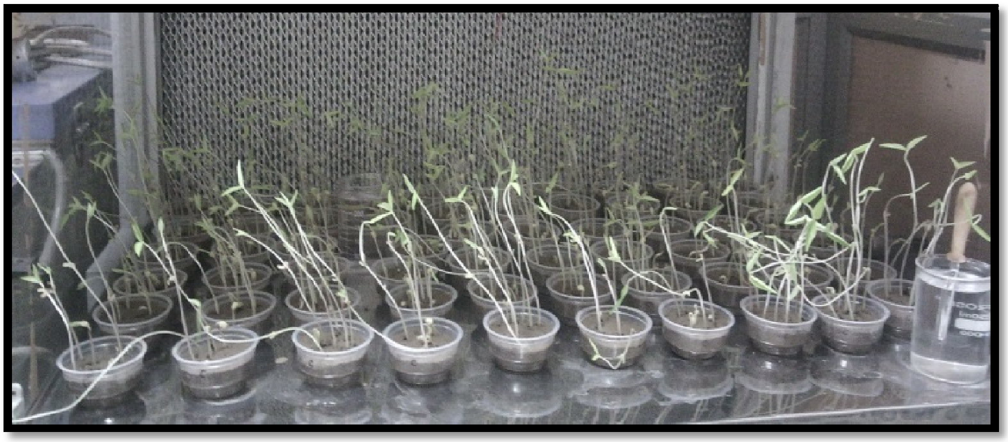

Fig. 1

Control

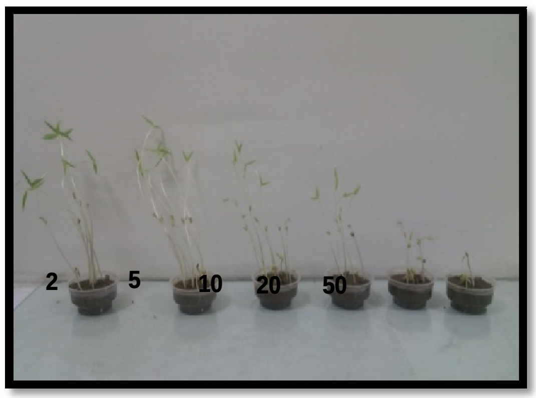

Fig. 2

Fig. 3

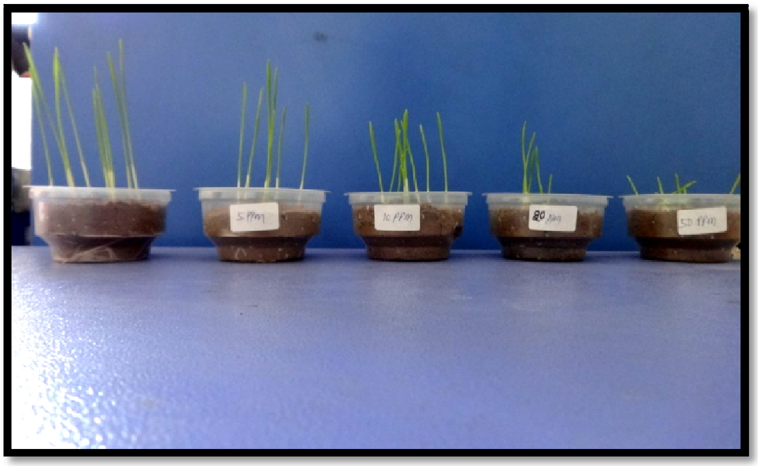

Fig. 4

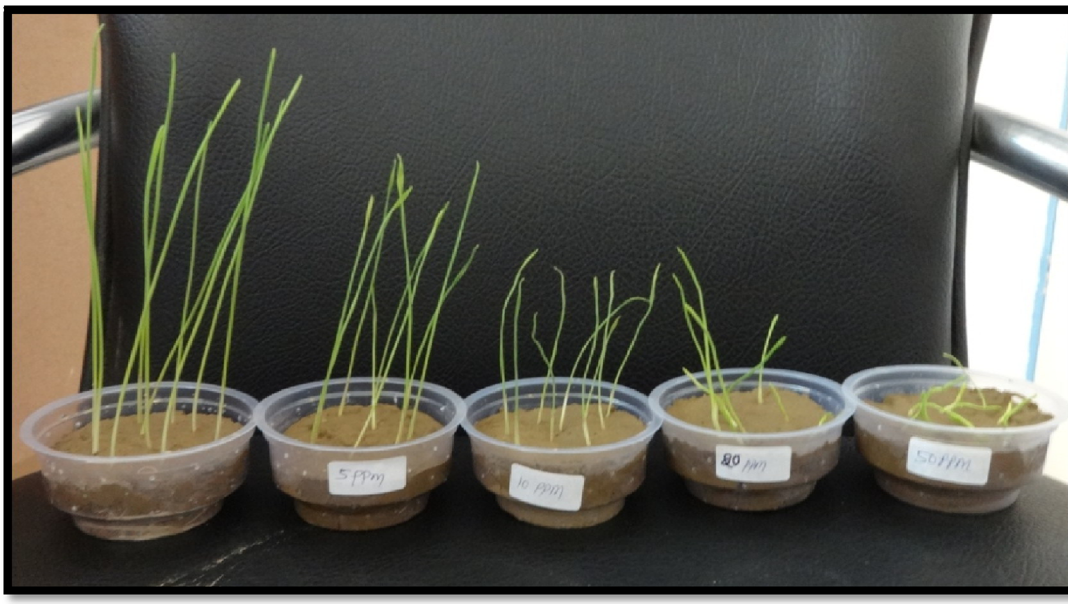

Fig. 5

Fig. 6

Fig. 7

Fig. 8

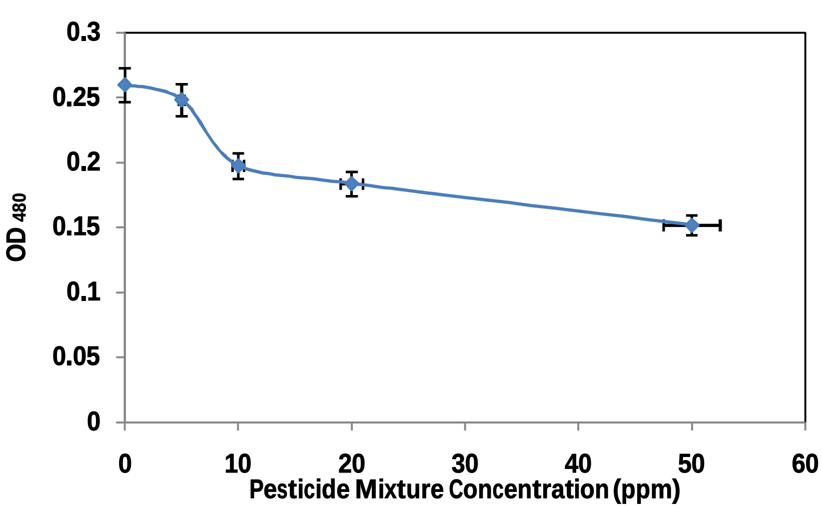

Fig. 9

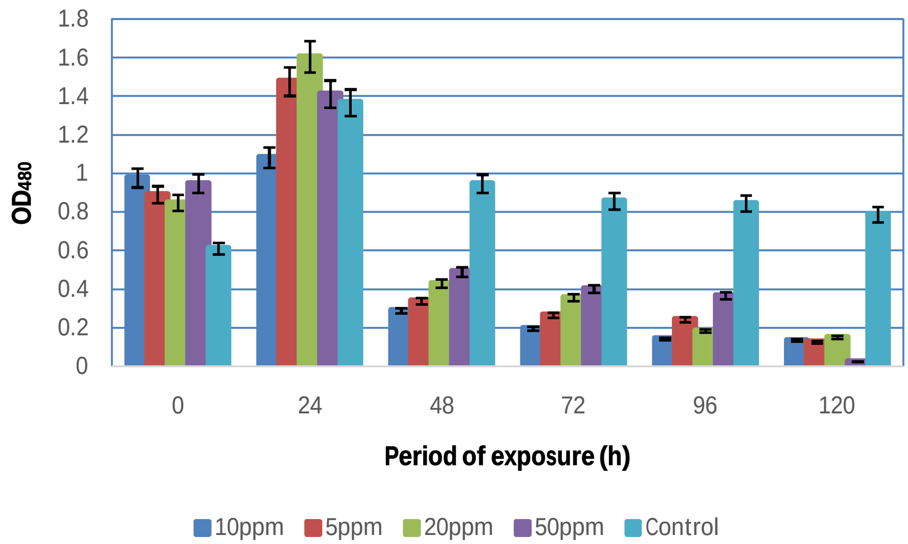

Fig. 10

LEGENDS TO FIGURES

Fig. 1: Green gram seedlings germinating

Fig. 2: Comparative heights of green gram seedlings at different concentrations (in ppm) of pesticides after 120 h of sowing.

Fig. 3: Comparative root development of seedlings in green gram at different concentrations of pesticides after 120 h of sowing. From bottom to top: Control, 2 ppm, 5 ppm, 10 ppm, 20 ppm and 50 ppm pesticide concentration effect on the roots.

Fig. 4: Comparative heights of wheat seedlings at different concentrations (in ppm) of pesticides after 60 h. The leftmost cup is the control.

Fig. 5: Comparative heights of wheat seedlings at different concentrations of pesticides after 120 h. The leftmost cup is the control.

Fig. 6: Comparative whole lengths of wheat seedlings at different concentrations of pesticides after 120 h. From left to right: seeds from Control, 5 ppm, 10 ppm, 20 ppm and 50 ppm of pesticide mix.

Fig. 7: Roots of wheat at different concentrations of pesticides after 120 h. The leftmost cup is the control. From left to right: seeds from Control, 5 ppm, 10 ppm, 20 ppm and 50 ppm of pesticide mix.

Fig. 8: Shoot lengths of wheat seedlings at different concentrations of pesticides after 120 h. From left to right:seeds from Control, 5 ppm, 10 ppm, 20 ppm and 50 ppm of pesticide mix.

Fig. 9: Viability of green gram embryos in different concentrations of pesticide mixture after 72 h exposure.

Fig. 10: Viability of wheat embryos after various periods of exposure to different concentrations of the pesticide mix.