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Response of African marigold (*Tagetes erecta* L.) to different concentrations of chlorpyrifos and microbial diversity in root rhizosphere

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ABSTRACT

Objective: To assess the response of African marigold (*Tagetes erecta* L.) to exposed different concentration of chlorpyrifos by evaluating morphology (root and shoot length), biomass (fresh weight and dry weight), photosynthetic pigments (chlorophyll a and b), protein and microbial diversity in root rhizosphere.

Methods: The study was carried out in pot culture and treated with various concentrations (0.5%, 1.0%, 2.0%, and 2.5%) as well as control treatments. The morphological, biomass, photosynthetic pigments, protein, and microbial diversity were analyzed on 30, 60, and 90 days.

Results: The obtained results revealed that the tested pesticide reduced the growth, biomass and photosynthetic pigment of African marigold when applied at higher concentration than the optimum dosage. But the lower dose the pesticide had some stimulatory effect of analyzed parameters. A similar effect of pesticide was observed on the microbial population of root rhizosphere that is decreased in microbial population was caused at higher doses. But it was increased at lower doses.

Conclusions: It can be concluded that pesticide above the certain dosage level adversely affect all the analyzed parameters at higher doses. The application of recommended doses should be discouraged. Further study is needed for the effect of pesticide use on microbial diversity, since these studies are carried out in a controlled pot experiment, including the current study. Thus, future study directed towards by studying the phytoremediation of these contaminated site with intraction of microbes.

1. Introduction

Pesticides have assumed great importance in today high yielding and intensive system as well as public health programme, around the world. In India pesticide production plant was started in 1952. At present, India is the second largest producer of pesticide in Asia after China and globally twelfth rank[1]. The 85% of pesticides were used in agriculture purpose while remaining 15% are used in other purpose[2,3]. Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate] is an organophosphorus insecticide extensively used to control pests on grain, fruit, cotton, nuts, vegetables crops, as well as lawns and ornamental plants. It is the fourth highest depleted pesticide in India[4]. For the past several

decades it has been broadly used and it produced great potential toxic effects on human health. Hence it requires great environmental concern[5]. Repeated and extensive use of pesticide affects growth of plant, animals. It accumulates as a residue in fruits and vegetables. It causes the loss of biodiversity and declined natural habitats[6]. The pesticide toxic effect in plant is commonly inhibited by the way of photosynthesis and mitochondrial electron transport[7]. The high concentration of imidacloprid is importantly declined the germination and seedling growth of rice[8]. In the experiment, the treatment of mancozeb was adversely affect plant morphology and anatomical traits of *Lens culinaris* L.[9]. The screening of pesticide tolerance plant is measured some phytotoxicity studies such as seed germination and seedling growth, which can be an effective tool for pesticide tolerant plant[10,11].

Rhizosphere defined as the narrow zone of soil surrounding plant roots that is specifically influenced by plant root activities and is in association with root hairs and plant produced materials[12], which contains various microbial community with bacteria being the most dominant members[13]. In soil ecosystem, microbes play an important role for maintaining soil fertility, nutrient cycling and degradation of organic matter[14,15]. The root rhizosphere

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microbial community also plays a vital role in soil quality through its involvement in biogeochemical and nutrient cycling, long-term soil sustainability, and resistance to perturbations[16]. Microbes maintain soil fertility as well as remove the soil contaminants like pesticide from soil[17]. In general the pesticide presumed that only affect target pests. Whereas, the pesticide adversely affects the microbial population[18]. The analysis of soil microbial properties, especially biomass and microbial diversity is good indicators of soil health[19,20]. The physiological and biochemical behavior of soil microbes are altered due to the pesticide interaction in soil[21]. The study revealed by Gundi *et al.*[22] the insecticide such as monocrotophos, quinalphos and cypermethrin affect microbial population, and adversely effect with the highest level[23]. The application of different pesticide alters the microbial community structure and function. However, the soil microbial parameters like enzyme activity and microbial community in chlorpyrifos treated soil, provide viable microorganism in chlorpyrifos treated soil[24]. The aim of the present study is to investigate the effect of chlorpyrifos on plant growth, photosynthetic pigments, protein and rhizosphere microflora in order to identify suitable plant or microbe combination to be used for phytoremediation of chlorpyrifos.

2. Materials and methods

2.1. Plant material and treatments

The experiments were conducted in the Botanical garden, Annamalai University, India. The seeds of African marigold (*Tagetes erecta* L.) were obtained from Tamilnadu Agricultural University, Coimbatore. The seeds were surface sterilized with 0.5% sodium hypochloride for 10 min and thoroughly washed with distilled water. The plastic pots (34 cm H × 34 cm B) were filled with mixture of sand, soil and manure in the ratio of 1:4:1 and kept under field conditions. Chlorpyrifos 20% EC (PYRICON) was purchased from local agro agencies, Chidambaram, Tamilnadu. Before the seed sowing the plastic pots were treated with different concentration like 0.5%, 1.0%, 2.0%, 2.5 % without treatments of pesticide considered as control.

2.2. Growth analysis

Samples were taken at an interval of 30, 60, 90 days after sowing (DAS). For the measurement of root and shoot length the seedling separated and their length was measured in millimeters (mm) with the help of measuring tape. To determine of fresh weight and dry weight, seedlings were separated into roots and shoots and were weighed. The fresh roots and shoots were kept in hot air oven at 80 °C for 24 h then the weight of sample was recorded using an electrical single pan balance and values were expressed in g⁻¹ plant.

2.3. Photosynthetic pigment estimation

Photosynthetic pigment (chlorophyll a and b) content estimation was done according to the method[25]. Five hundred mg of fresh leaf material was ground with a mortar and pestle with 10 mL of 80% acetone. The homogenate was centrifuged at 1000 r/min for 15 min. The supernatant was saved. The residue was re-extracted with 10 mL of 80% acetone. The supernatant solution read at 645 and 663 nm in a UV-Spectrophotometer (Hitachi). The chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were estimated and expressed in mg/g of fresh weight basis.

$$\text{Chlorophyll 'a'} = (0.0127) \times (\text{O.D } 663) - (0.00269) \times (\text{O.D } 645)$$

$$\text{Chlorophyll 'b'} = (0.0229) \times (\text{O.D } 645) - (0.00488) \times (\text{O.D } 663)$$

2.4. Protein content estimation

The protein content of samples was estimated by the method of Lowry *et al.*[26]. Five hundred mg of plant material was macerated in a pestle and mortar with 10 mL of 20% trichloroacetic acid. The homogenate was centrifuged for 15 min at 1000 r/min. The supernatant was discarded. To the pellet, 5 mL of 0.1 mol/L NaOH was added and centrifuged for 5 min. The supernatant was saved and made to 10 mL with 0.1 mol/L NaOH. This extract was used for the estimation of protein.

One mL of the extract was taken in a 10 mL test tube and 5 mL of reagent 'C' was added. The solution was thoroughly mixed and kept in darkness for 10 min. Later, 0.5 mL of Folin-Phenol reagent added and the mixture was kept in dark for 30 min. The absorbance sample was read at 660 nm. The protein content was expressed in mg/g fresh weight.

2.5. Total bacterial count

Soil samples were taken under plant rhizosphere zone. The number of colony forming unit (CFU) in selective media was determined by means of serial dilution technique and the spread plate method. One gram of soil was mixed with 9 mL of distilled water and mixed thoroughly. One milliliter from the solution was then mixed with 9 mL of distilled water to make 10⁻⁴ dilution of this solution and in same pattern dilution made up to 10⁻⁹ dilution. Viable counts for bacteria were determined using a nutrient agar medium containing (per liter of water) the following components; peptic digest of animal tissue, 5.000 g; beef extract, 1.500 g; sodium chloride, 5.000 g; yeast extract, 1.500 g; agar, 15.000 g and finally the pH were adjusted 7.4 ± 0.2.

2.6. Statistical analysis

All treatments were replicated three times. All presented data are expressed as mean ± SD. The statistical graphs were carried out by using Microsoft Offices Excel 2003.

3. Results

3.1. Effect of pesticide on root and shoot length

The response of root and shoot length in African marigold is shown in Figures 1 and 2. In case of root, the highest reduction in length was observed at 2.5% concentration where the root length was 2.9, 7.4 and 6.3 cm as compared to 6.7, 10.5 and 15.5 cm in control. At the lowest concentration 0.5%, almost all the treatments days the root length 7.5, 11.1 and 19.6 cm was improved when comparing with control. In case of shoot, the growth was adversely affected with increasing concentrations of pesticide (Figure 2). The most significant inhibition in shoot length of 17.3, 21.5 and 23.7 cm was observed when the plant at subjected to 2.5% of pesticide as compared to control (32.3, 37.3 and 49.7 cm) plant. The lowest concentrations 0.5% of the shoot length 35.3, 48.5 and 57.4 cm were significantly increased in all the treatments days. The effect pesticides on fresh and dry weight are depicted in Figures 3 and 4. A significant reduction in fresh weight 4.20, 4.54, 18.12 and 0.73, 1.52 and 4.31 mg/plant was observed at higher concentration of

pesticide 2.5% treatments as compared to control (12.81, 13.10, 30.12 and 2.08, 3.76 and 7.10 mg/plant) plant, respectively. At lower concentration 0.5% the fresh and dry weight was gradually increased to 13.99, 15.60, 33.76 and 2.33, 4.81 and 8.25 mg/plant.

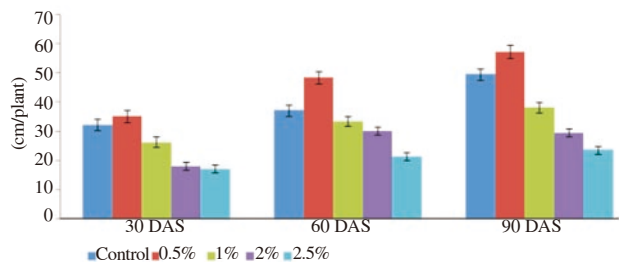


Figure 1. Effect of different concentrations of chlorpyrifos on shoot length.

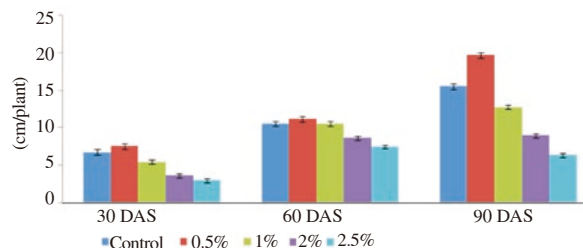


Figure 2. Effect of different concentrations of chlorpyrifos on root length.

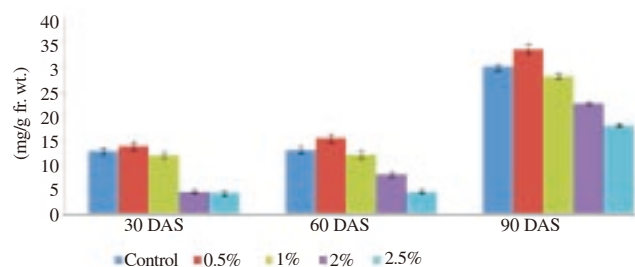


Figure 3. Effect of different concentrations of chlorpyrifos on fresh weight.

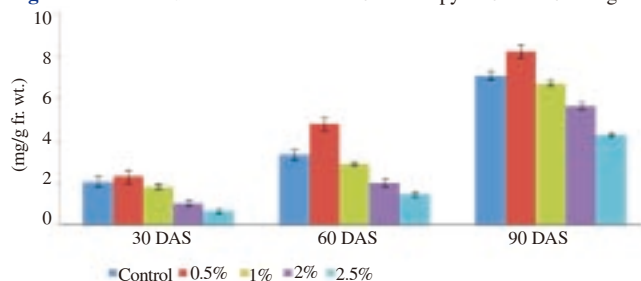


Figure 4. Effect of different concentrations of chlorpyrifos on dry weight.

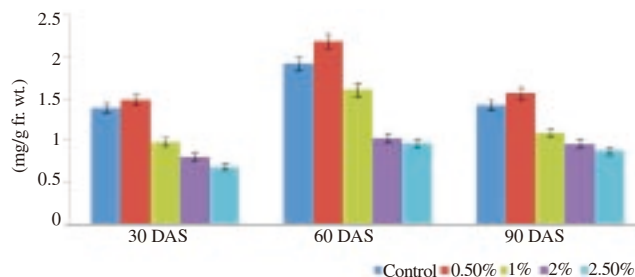


Figure 5. Effect of different concentrations of chlorpyrifos on chl a.

3.2. Effect of pesticide on photosynthetic pigments

The photosynthetic pigments such as chlorophyll a and b contents are shown in Figures 5 and 6. The lowest concentration 0.5%, a significant increase in fresh weight 1.49, 2.19, 1.57 and 1.38, 2.10

and 2.01 mg/g, respectively was observed with respective interval days as compared to control (1.39, 1.92, 1.43 and 1.11, 1.83 and 1.74 mg/g fresh weight) plant. A significant reduction in chlorophyll a and b (0.69, 0.97, 0.89 and 0.84, 0.99 and 0.97 mg/g fresh weight) was observed in higher concentration 2.5% of treatments.

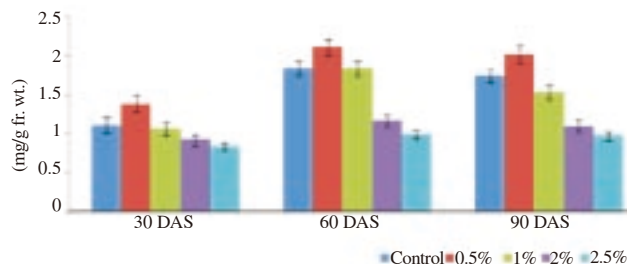


Figure 6. Effect of different concentrations of chlorpyrifos on chl b.

3.3. Effect of pesticide on protein content

The responses of pesticide on protein content are shown in Figure 7. The lowest concentration 0.5% of pesticide, the protein (2.01, 3.93, and 3.14 mg/g fresh weight) were significantly increased as compared to control (1.88, 3.61 and 2.84 mg/g fresh weight) plant. In case of the highest concentration 2.5% of pesticide, the protein content (0.96, 1.57, and 1.06 mg/g fresh weight) was negatively affected in all treatment days.

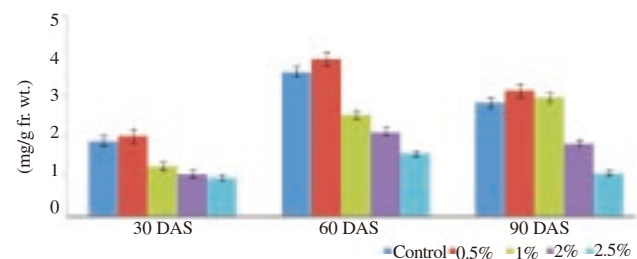


Figure 7. Effect of different concentrations of chlorpyrifos on protein.

Table 1

Effect of pesticide on bacterial population in rhizospheric soil.

Treatment concentrations	30 DAS ($\times 10^5$ CFU/g in soil)	60 DAS ($\times 10^5$ CFU/g in soil)	90 DAS ($\times 10^5$ CFU/g in soil)
Control	24.16 \pm 0.72	43.76 \pm 1.31	61.16 \pm 1.83
0.5%	29.65 \pm 0.88	50.13 \pm 1.50	64.74 \pm 1.94
1%	25.54 \pm 0.76	46.11 \pm 1.38	57.25 \pm 1.71
2%	20.15 \pm 0.60	39.37 \pm 1.18	45.71 \pm 1.37
2.5%	14.22 \pm 0.42	23.74 \pm 0.71	31.52 \pm 0.94

3.4. Effect of pesticide on bacterial population in root rhizosphere

Data in Table 1 show in bacterial population plant rhizosphere soil in different days. The highest population was observed in 0.5% concentration (29.65, 50.13 and 64.74 ($\times 10^5$ CFU/g in soil) with comparison control (24.16, 43.76, and 61.16 ($\times 10^5$ CFU/g in soil) respectively. The lowest population was observed in higher concentration (14.22, 23.74 and 31.52 ($\times 10^5$ CFU/g in soil) in respective treatments days.

4. Discussion

The present study showed that the lower concentration of chlorpyrifos (0.5%) significantly increased the growth parameters

such as root length and shoot length. Nevertheless, at higher concentration, the growth parameters such as shoot length and root length are remarkably reduced in all the growth phase under study. The low concentration of chlorpyrifos (0.3 mmol/L) enhanced the growth parameter such as plant height, biomass and photosynthetic pigments. Whereas, the magnitude decreases with increase in the concentration of treatment as compared with control[27]. Other researchers revealed that the application of chitosan, in the morphological parameters like plant height, biomass is increased up to 25 ppm concentration over the control plant of okra[28]. The various concentrations of profenofos when subjected to *Vigna radiata* L., the fresh and dry weights were found to increase up to 0.02% treatments[29]. The reduction of root and shoot length with exposure of high concentration of dimethoate, which might be accumulation of dimethoate in root as it was directly contacted with pesticide[30]. The authors concluded that the reduction of biomass in wheat plant was affected by affecting the seedling growth, development of shoot and root axis production[31]. Similar results were reported that the lower doses enhanced the growth of soybean but higher values of pesticide declined the growth of soybean[32]. The increase of plant growth performance in low dose of dimethoate might be, the increase of cell membrane permeability of root cell, making enhancement of nutrient influx into the root cell, and their subsequently transport to leaf and shoot[33].

The reduction of meristematic growth and development by inhibiting the hydroxyl phenyl dehydrogenase plays important function in meristematic growth[34]. Our results are in close conformity with the parameter, such a morphology found to be increased at recommended dose level. However they said higher dose caused the toxic effect in tomato[35]. The presence of pesticide residue in soil and deficiency of nutrient affects the uptake of micronutrient in plant, and ultimately it reflects the abnormality of plant growth parameters[36].

4.1. Photosynthetic pigments and protein

In the present study, maximum photosynthetic pigments and protein content are observed in lower dose of chlorpyrifos treatments and later declined sharply with increase of concentration of treatments. The changes of photosynthetic pigment in plants are usually used as a tool for the assessment of stressful conditions[37]. The reduction of net photosynthetic rate is declined when nine different pesticides are exposed in cucumber (*Cucumis sativus* L.)[38]. Further studies suggested that the growth, photosynthesis and chlorophyll synthesis are stimulated at low doses 0.02 and 0.2 mg/dm³ in *Scenedesmus quadricauda* tissues, while higher dose significantly suppressed these parameters[39]. The reduction of chlorophyll-a, b and a+b content in duckweed is connected with phytotoxic effect of (Roundup ultra 360 SL) glyphosate-based herbicide[40]. The higher dose of deltamethrin concentration affected chlorophyll a more than chlorophyll b. The most negative effect is determined in 0.1 ppm than the other concentration[41]. The reduction of photosynthetic pigment, which might be the imidacloprid, can inhibit by the gene concerned for photosynthesis and chlorophyll-protein complexes[42]. In the study, the application of captan, the recommended dosage 2.5 g/L increased the chlorophyll a and b than the higher concentrations and control treatments[43]. The result of earlier studies proved that the chlorophyll content was gradually increased up to 0.1% concentration both pesticide of DDT and Bordeaux[44]. They suggest

that 0.1% is optimum dosage for plant growth. Recently it has been reported that the lowest concentration of glyphosate influences the increase of chlorophyll content[45]. Whereas at higher concentration is negatively destroyed the chlorophyll of tested algal species. Other studies recorded that the optimum dosage of chlorpyrifos exhibits the increase of chlorophyll content. In case of higher dosage is negatively affected in cockscomb plant[46].

The pesticide toxicity delayed protein and carbohydrate synthesis by altering cytochrome oxidase activity and blocking alternative respiratory pathways[47]. The protein content is decreased in *Anabaena variabilis* with the treatment of herbicide of thiocarbamate[48]. In addition, the protein formation in maize seedling is decreased as the metribuzin causes the shortage of ammonia[49]. During the stress condition, plant might be induced the specific changes in protein synthesis to protect it from stress[50]. The imidacloprid treatments had significantly decreased protein content of rice seedling. The maximum protein (40%) content was declined with the highest concentration of treatments 0.015% with pesticide as compared to control[51]. The protein content was increased up to 250 + 250 ppm level in both combined pesticide of endosulfan and kitazin. However, the protein in brinjal is declined negatively above the concentrations[52]. The reduction of protein with the treatment of herbicide is altered by affecting metabolism of cell including synthesis of nucleic acid, enzyme and other functional protein[53]. Reduction of protein under the herbicide treatment is targeted by the protein synthesis associated enzyme[54]. Recent study concluded that the application of pesticide and fungicide negatively affect the total protein with increase the concentration than the control plant of *Capsicum annuum* L.[55].

4.2. Total microbial count

Microbial population in soil and rhizosphere of pesticide treated soil – they provide some critical information such as biogeochemical process, control of pathogen and also render service to humanity[56]. The bacterial population was determined in terms of CFU using viable plate count methods. When a soil is treated with chlorpyrifos, the bacterial population was increased than the control treatment[57]. In contrast, that the increasing concentration of herbicide is declined the bacterial count even soil treated with recommended doses[58]. Bacterial population is marginally increased when adding carbofuran or butachlor in paddy soil. The increase of the concentration of pesticide gradually inhibited the microbial population[59]. The increase of soil microbial biomass when addition of pesticide might be due the microbes are breakdown of the pesticide product afterwards were used as a source of energy for the surviving microorganism[60]. The increase of some microbial population might be the microbes that can tolerate and grow in presence of pesticide. Once the microorganism tolerated, it becomes degrading the chemical compound and increasing their microbial population and enzymatic activities of soil[61]. In contrast, the two pesticides of cypermethrin and monocrotophos had only lowest effect in soil microbes[62]. Further studies suggested that the bacterial population was significantly induced with application of carbofuran in agricultural soil[63]. The physicochemical soil properties such as pH, temperature, moisture content and organic matter content also influenced the heterogeneous microbial population in rhizospheric soil[64]. Moreover, insecticide treated soil increased the total number of culturable bacteria[65]. According to interpretation, the

decrease of CFU counts with the higher dosage of fenitrothion was likely, to be toxic effect caused by the intermediate metabolite of fenitrothion[66].

Response of African marigold to chlorpyrifos stress was determined by studying growth parameters, photosynthetic pigment (chlorophyll a and b) and protein. It can be concluded that pesticides above the certain dosage level adversely affect the growth of African marigold. At higher doses, all other studied parameters are caused toxic effect. The application of chlorpyrifos above the recommended dose should be discouraged. The study indicates that chlorpyrifos caused negative effect on bacterial population. While optimal dosage of chlorpyrifos could restore the bacterial community faster as compared control treatments. It is notable analyzed that pesticide at higher dosage negatively affects the parameter. Further study is needed for the effect of pesticide use on microbial diversity, since these studies are carried out in a controlled pot experiment, including the current study. Thus, future study directed towards by studying the phytoremediation of these contaminated site with interaction of microbes.

Conflict of interest statement

We declare that we have no conflict of interest.

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