

Original Research Article

doi: <https://doi.org/10.20546/ijcrbp.2018.504.009>

Assessment of Phytoremediation Potential of Cockscomb (*Celosia cristata* L.) in Chlorpyrifos Polluted Soil

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Article Info	ABSTRACT
Date of Acceptance: 12 March 2018 Date of Publication: 06 April 2018	
Keywords	
Amino acid Chlorpyrifos Cockscomb Pesticides Protein content	In modern agriculture, the rapid use of pesticide and agrochemical were preserved and restore soil fertility. It is important to remediate polluted soils in order to prevent the further contamination of the soil. The aim of this study to assess the phytoremediation potential of cockscomb (<i>Celosia cristata</i> L.) in chlorpyrifos polluted soil. In an experiment, the garden soil was spiked with four different concentrations of chlorpyrifos (0.5, 1.0, 2, and 2.5%). The treatment without chlorpyrifos spiked in to the soil represented as the control. The plant samples were collected periodically at 30, 60, and 90 days for biochemical analysis. At end of 90th day plant sample were collected Gas Chromatography Mass Spectrum studies (GC-MS). The low level (0.5%) of chlorpyrifos exhibit that enhance the protein content of tested plant, as compared to control plant. However, at higher concentrations (2.5%) of chlorpyrifos were decreased the protein content. The amino acid and proline were elicited with the increasing concentration of chlorpyrifos as compared to control. Maximum increases in amino acid and proline was noted at the highest concentration (2.5%) of pesticide treatment. The GC-MS study showed that disappearance of chlorpyrifos by cockscomb (<i>Celosia cristata</i> L.) may be assigned to the uptake potential of these plants. However, further studies are needed to better understand the chlorpyrifos impact in cockscomb (<i>Celosia cristata</i> L.) and potential mechanism took up to improve the phytoremediation of chlorpyrifos.

Introduction

Modern agriculture, which needs high crop productivity due to the increasing food necessity of

a growing world population, requires intense uses of fertilizers and agrochemicals to preserve and restore soil's fertility and protect crops from pests (Bhandari, 2014). The indiscriminate and unskillful

use of pesticides affects the growth of plant and animal, increases pest resistant to pesticides, accumulates residue in fruits and vegetables, causes biodiversity losses, and declines natural habitats (Baig et al., 2012). Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) is widely used and broad-spectrum organophosphate insecticide. It was introduced in 1965 by the Dow chemical company for widespread use as a foliar insecticide for important crops such as rice, cereals, cotton, tobacco, fruits, vegetable crops, pasture, ornamental horticulture plants, and soil applications to control termites (Mallick et al., 1999; Wang et al., 2004). Chlorpyrifos is an environmental concern because it is moderately toxic and used excessively posing a high contamination risk to soil and groundwater. Chlorpyrifos can enter surface waters via several routes, including run-off, leaching erosion, tail waters with lesser inputs via drift of sprays, and deposition from the atmosphere (Giesy et al., 2014). It has been suggested the ability of chlorpyrifos, and other organophosphorus pesticides to accumulate in living tissues may create a potential risk to humans and other organisms (Wang et al., 2005).

It is important to remediate polluted soils in order to prevent the further contamination of the soil. Among all the available technology, phytoremediation technology has more attention due to its cost effectiveness and working simpleness. Moreover, the technology is entirely based on growing plants and their ability to uptake, transform, or degrade the pollutants from various environmental media (Passatore et al., 2014; Bell et al., 2014). In order to be effective in remediating polluted sites, plants should be able to reach the pollutants. Moreover, the ideal plant for the phytoremediation should have a rapid growth, high biomass development, and be competitive to other plants (Pilon-Smits, 2005). The advantage of phytoremediation is natural process of plants. It takes place including water and uptake of chemical, metabolism within the plant, and release of exudates which leads to loss of contaminants (McCutcheon and Schnoor, 2003; Deepali et al., 2009). Although phytoremediation has been widely

tested for clean-up of various organic and inorganic pollutants, only few plant species such as *Eichhornia crassipes* (Anudechakul et al., 2015), populus and salix (Lee et al., 2012), *Lolium multiflorum* (Ahmad et al., 2012) are so far reported for the phytoremediation of chlorpyrifos. Nevertheless, some study reported only the remediation of chlorpyrifos associated with the plant and microbes (Yang et al., 2005; Yu et al., 2006). It remains to be seen whether plants are able to take up and transform pesticide without the involvement of microbes. Therefore, we conducted this study to investigate the potential of using cockscomb (*Celosia cristata* L.) as tools for the remediation of chlorpyrifos contaminated soil.

Materials and methods

Experimental setup and treatments

The experiments were conducted in the Botanical garden, Department of Botany, Annamalai University, Tamil Nadu, India. The seeds of cockscomb (*Celosia cristata* L.) were procured from Tamil Nadu 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 authorized Agro Agencies, Chidambaram, Tamil Nadu. The garden soil was spiked with four different concentrations of chlorpyrifos (0.5, 1.0, 2.0, and 2.5%). The treatment without chlorpyrifos spiked in to the soil represented as the control. The plant samples were collected periodically at 30, 60, and 90 days for biochemical analysis. At end of 90th day plant sample were collected Gas Chromatography Mass Spectrum studies (GC-MS).

Biochemical analyses

The fresh plant leaves were instantly used for the estimation of protein, amino acid and proline content. The protein content in the leaves was

estimated by method of Lowry et al. (1951), similarly, amino acids content (Moore and Stein, 1948) and proline (Bates et al., 1973).

Determination of protein content

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 1 000 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.

Determination of amino acid content

Five hundred mg of plant material was taken in a pestle and mortar and homogenized with 10 ml of 80 percentage boiling ethanol. The extract was centrifuged at 800 rpm for 15 min and the supernatant was made up to 10 ml 80 percentage ethanol and used for the estimation of amino acid. One ml of ethanol extract was taken in a 25 ml test tube and neutralized with 0.1 N sodium hydroxide using methyl red indicator. To which 1 ml ninhydrin reagent was added and the content were kept in a boiling water bath for 20 min then 5 ml of diluting reagent was added, cooled and diluted to 25 ml with distilled water. The absorbance was read at 70 nm in a UV-spectrophotometer. The amino acid content was expressed in mg g⁻¹ fresh weight.

Determination of proline content

The plant material was homogenized in 3 percentage aqueous sulfosalicylic acid and the homogenate was centrifuged at 8,000 rpm. The supernatant was used for the estimation of proline

content. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100 °C for one hour. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene and absorbance was read at 520 nm. The proline content was expressed in mg/g fresh weight.

Extraction and analysis of chlorpyrifos residue in plant sample

The 25 g of the entire plant were sliced in small pieces and homogenized with acetonitrile in a blender. The 100 ml of acetonitrile was added and blended for 2-3 min at moderate to high speed. The homogenate was filtered by passing through the glass wool. The mixture was filtered as before and combined the filtrates. The pesticide residue were quantified and analyzed by GC-MS.

GC-MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite 5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min), followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C, and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments were from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GCMS NIST (2008) library.

Statistical analysis

All treatments were replicated three times. All presented data are expressed as mean ± standard deviation (SD) of the mean. The statistical graphs

were carried out by using Microsoft Office Excel (2003).

Results

Effects of chlorpyrifos on protein content

The protein content of cockscomb grown in five different chlorpyrifos treatments are presented in Fig. 1. As it is expected, that the low level (0.5%) of chlorpyrifos exhibit that enhance the protein content of tested plant, as compared to control

plant. However, at higher concentrations (2.5%) of chlorpyrifos were decreased the protein content.

Effects of chlorpyrifos on amino acid and proline contents

As shown in Figs. 2 and 3, cockscomb plants exhibited that the amino acid and proline were elicited with the increasing concentration of chlorpyrifos as compared to control. Maximum increases in amino acid and proline was noted at the highest concentration (2.5%) of pesticide treatment.

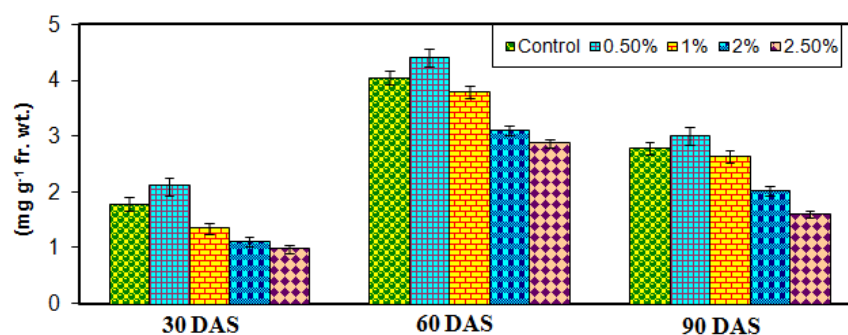


Fig. 1: Effect of pesticide on protein content (Mean $\pm$ SD n = 3).

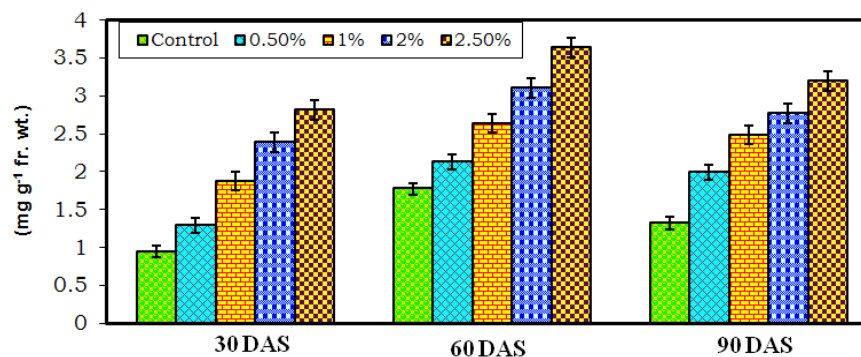


Fig. 2: Effect of pesticide on amino acid content (Mean $\pm$ SD (n = 3)).

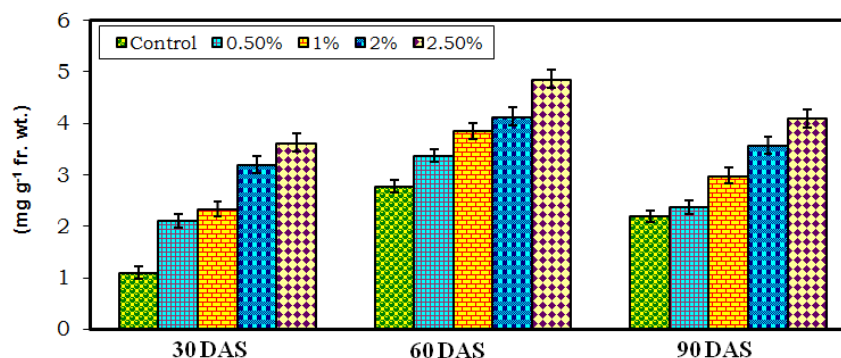


Fig. 3: Effect of pesticide on proline content (Mean $\pm$ SD (n = 3)).

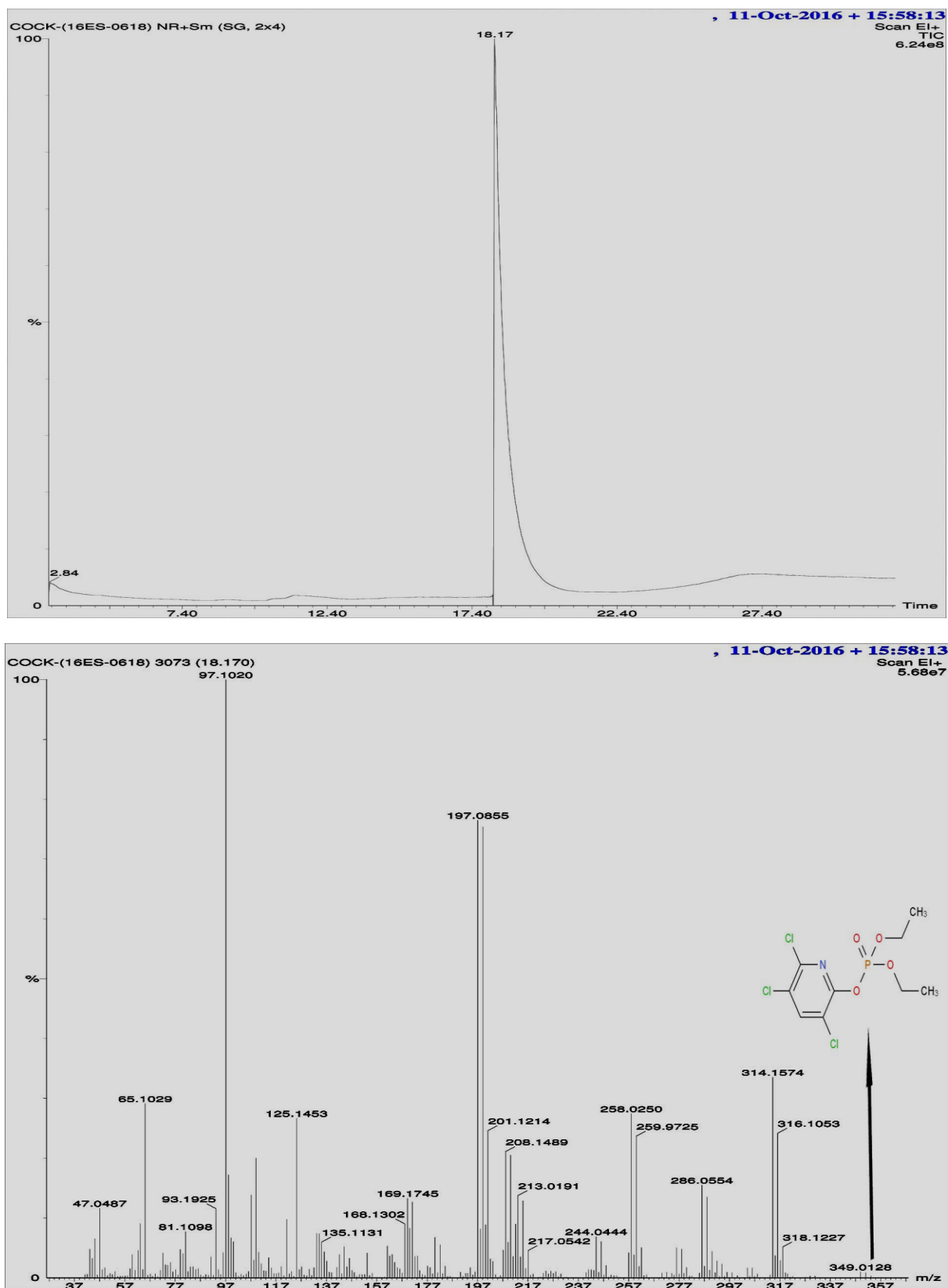


Fig. 4: GC-MS spectrum of the pesticide chlorpyrifos in cockscomb (*Celosia cristata* L.)

Phytoaccumulation of chlorpyrifos by cockscomb (*Celosia cristata* L.)

Cockscomb (*Celosia cristata* L.) grown in 2.5 % pesticide chlorpyrifos treated plant was harvested after 90 days and the plant extract was subjected to GC-MS analysis. The number of spectral peaks was found to be in this GC-MS analysis. The peaks showed the presence of chlorpyrifos at R. T. of 18.17 minutes and molecular weight 350.59 g/mol. The comparison was made with standard library of MS spectrum GCMS NIST (2008) library. Mass spectral database confirmed the matching of molecular weight 350.59 g/mol of chlorpyrifos intensity at R. T. 18.17 minutes of the sample to standard spectra of chlorpyrifos. The mass spectra obtained in case showed that the chlorpyrifos was degraded to some small metabolites. This indicated that chlorpyrifos is probably completely metabolized by cockscomb into smaller intermediates. The details of the report are displayed in the Fig. 4.

Discussion

Effect of pesticide on protein content

In the present study, maximum protein content was observed in lower dose of chlorpyrifos treatment as compare to control. However, the protein content was declined with increasing concentrations. Our results are closely confirmatory with the increase of protein at low concentration of pesticide might be pesticide stimulate synthesis of stress retarding protein (Kumar et al., 2008). In *Chlorella vulgaris* and *Spirulina platensis* the protein content was gradually increased with the treatment of 2, 4-D up to 9.103 mM, thereafter and the protein content declined at the concentration of 9.102 mM (Saygideger and Okay, 2008). The pesticide of bavistin and monocrotophos are shown the soluble protein was decreased, as the increase of pesticide was reported by Kamble and Sabale (1999). However, suggested that pesticide toxicity delayed protein and carbohydrate synthesis by altering cytochrome oxidase activity and blocking alternative respiratory pathways (Siddiqui and

Ahmed, 2000). Moreover, the protein content was decreased in *Anabaena variabilis* with the treatment of herbicide of thiocarbamate (Battah et al., 2001). In the study, Soliman and Ghoneam (2004) indicated that the changes of protein content as a result of treatment of pesticide and herbicide application. The degradation of protein might be the enhanced activity of proteases or autophagy to degrade oxidized protein under stress condition (Xiong et al., 2007).

The decrease of protein formation in maize seedling due to the herbicide that metribuzin cause the shortage of ammonia and subsequently decrease the protein formation (Nemat Alla et al., 2008). The reduction of protein in maize seedling might be herbicides are disturbance of an enzyme involved metabolic pathways such as cell metabolism, nucleic acid synthesis and enzymes this supported by many reports (Hasaneen et al., 2009; Rosenbom et al., 2010; Hala and Ibrahim, 2011). During the stress condition, plant might be inducing the specific changes in protein synthesis to protect from stress (Salama et al., 2011). The result indicates that the both two herbicide (2, 4-D and isoproturon) are reduce protein content in *Triticum aestivum* L. as the increasing concentrations. The reduction of protein content might be reduced synthesis and possibly due to damage of root or vascular tissues (Kumar, 2012). The recent study exposed that protein content declined with the higher level of 1, 2, 3-thiazole compounds (AL-Quraan et al., 2015). Further studies suggested that the protein in *Ocillatoria limnetica* with the treatment of glyphosate, might be the cellular activities was adversely affected by glyphosate treatment (Salman et al., 2016).

Effect of pesticide on amino acid content

In the experimental result, the response of different concentrations (0.5%, 1%, 2%, and 2.5%) chlorpyrifos amino acid content exhibited that increased with increasing concentrations. Amino acid contents play an important role as a physiological indicator while, plant growing of environmental stress condition (Bao et al., 2012).

The study reported that insecticide was significantly increased the free amino acid content in rice plant (Yuan et al., 2001). The amino acid content in loose silky-bent (*Apera spica-venti* L.) over the application of prosulfocarb with increasing concentration (Hjorth et al., 2006). The some amino acid like asparagines and proline are playing an important role under stress condition (Lea et al., 2007).

Further, study depicted that under the treatment of triazophos in rice plant, the free amino acid content was significantly increased at different days (Ge et al., 2013). Recent study found that amino acid content was increased in *Cicer arietinum* L. with the foliar application fungicide (tops-in-M) both seedling and fruiting stage (Hameed et al., 2015).

Effect of pesticide on proline content

Lower dose of chlorpyrifos has significant effect on proline content of *Celosia cristata* L. an increased with adding of pesticide treatments. The maximum enhancement was observed in 2.5% concentration. Proline are well known studied that, when the plants are under stress condition such as drought, salinity and other environmental condition it accumulate high level (Ashraf and Foolad, 2007; Kovacik et al., 2009). The proline might be function as a signaling molecule that can regulate mitochondrial function and influence gene expression in the nucleus and thereby increase plant ability to tolerate under stress condition (Szabados and Savoure, 2010). On other hand the proline most common compatible osmolytes when plant under stress condition (Mafakheri et al., 2010). Plant has ability to protect from different environmental stress by accumulating of proline (Hossain et al., 2014).

The proline acts as an osmoprotectant and serves to stabilization of enzyme and membrane structure under stress condition (Bandurska, 2001). The accumulation of proline in *Dactylis glomerata* L. might be the osmotic adjustment with the treatment of phenylureas linuron and xometuron (Duran-Serantes et al., 2002). Moreover, the increase of

proline content in rice plant with the treatment of 1, 2, 4-trichlorobenzene (Du et al., 2006). In previous studies, the accumulation of proline after the treatment of thiocarbamate herbicide triallate and phenylurea herbicide diuron has been reported in various crops such as *Pisum sativum*, *Phaseolus vulgaris*, *Vicia faba* and *Glycine max*, respectively (Fayez and Kristen, 1996; Fayez, 2000). In addition to that, the treatment of mancozeb and profenofos increased the proline content with the increased concentration of both pesticide and fungicide on *Anabaena* sp. and *Scenedesmus* sp. were noted (Chaurasia, 2014). Recent study revealed that proline content was increased in tomato plants with the increasing concentration of thiamathoxan concentration (Yildiztekin et al., 2015).

Phytoaccumulation of chlorpyrifos by cockscomb (*Celosia cristata* L.)

As shown in Fig. 4, the plant grown in 2.5 % concentration of chlorpyrifos. The results shown in the figure demonstrated that the disappearance of chlorpyrifos by cockscomb (*Celosia cristata* L.) may be assigned to the uptake potential of the plant. It has been reported that that plant can actively accumulate trace-organic contaminants. So plant would play an important role in advanced treatment of chlorpyrifos (Tront and Saunders, 2006). However, Turgut (2005) proposed that the uptake and translocation of organic compounds depends on their hydrophobicity, solubility, polarity and molecular weight of compound. Once pesticides enter into the plants, it distributed from the roots principally into leaves through diffusion if volatile (Karthikeyan et al., 2004). A significant accumulation of pyrene in roots of *Bruguiera gymnorhiza*, regarding 3.5% from an initial pyrene concentration of 3 mg/kg in soil (Ke et al., 2003). The transport of natural contaminations including plant take-up and digestion are principle critical process in phytoremediation (Henderson et al., 2007). It has been accounted for that a significant amount of chlorpyrifos was aggregated in plant tissue and roots were more amassed than the shoot of poplar and willow plants (Pugliese et al., 2004).

Study reported that the pathway and the intermediate byproducts of chlorpyrifos radio degradation in acetonitrile (Mori et al., 2006). The major products have been identified as the desulphuration product, chlorpyrifos-oxon and the hydrolysis product, 3, 5, 6-trichloro-2-pyridinol (TCP). The uptake of chlorpyrifos by the emergent plant *Juncus effusus*, followed by rapid loss with background levels in plant leaves after 32-day exposure was reported by Lytle and Lytle (2002). However, the plant's uptake efficiency of these compounds is determined by many soil and plant characteristics (Hussain et al., 2009). It has been reported that roots were significantly accumulating these compounds because of their direct contact with the toxic chemicals, then the compounds were transported to aboveground organs (Azmat et al., 2009). Recently, the study by Wang et al. (2016) reported that *Acorus calamus* accumulated higher concentrations of chlorpyrifos in both shoots and roots. In the experiment, Romeh and Hendawi (2013) found that the significant amount of chlorpyrifos was accumulated in roots, than the shoots in *Plantago major*.

In addition to that, chlorpyrifos can removed and degraded more rapidly in an aquatic system, with the aid of plant and microbes interactions (Anudechakul et al., 2015). Moreover, the study reported that hygrophytes brought to an important function in removing chlorpyrifos considering the solution culture experiment in sterile condition and nature environment (Wang et al., 2010). Be that as it may, interestingly, the biomass of both *Pistia stratiotes* and *Lemna minor* were accounted for to increment within the sight of an underlying chlorpyrifos level of 0.1 and 0.5 mg/L, yet a higher measurement of 1.0 mg/L chlorpyrifos was poisonous for both plant species and caused a decline in the plant biomass (Prasertsup and Ariyakanon, 2011). The chlorpyrifos evacuation proficiency of *Eichhornia crassipes* in this examination was high and like that detailed for *Acorus gramineus*, where the evacuation efficiencies of the organophosphorus pesticides diazinon, fenitrothion, malathion and parathion

were 89%, 89%, 93% and 90%, individually (Chuluun et al., 2009). Moreover, *Canna indica* Linn. contributed 74% in debasement of triazophos, assumed the vital part in the remediation procedure, and demonstrated the capability of phyto-remediation of TAP from polluted water (Cheng et al., 2007).

Conclusion

The assessment of phytoremediation potential of cockscomb (*Celosia cristata* L.) in chlorpyrifos polluted soil was determined by studying biochemical parameters such as protein, amino acid, proline and phytoaccumulation study. It can conclude that the protein content was enhanced at lowest level of pesticide treatment. At higher level, the pesticide caused negative effect in protein content. On other hand, the amino acid and proline content were increased with increasing concentration of pesticide. The GC-MS study showed that disappearance of chlorpyrifos by cockscomb (*Celosia cristata* L.) may be assigned to the uptake potential of these plants. However, further studies are needed to better understand the chlorpyrifos impact in cockscomb (*Celosia cristata* L.) and potential mechanism took up to improve the phytoremediation of chlorpyrifos.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

The authors thanks to University Grants Commission, Government of India for providing fellowship (UGC-SAP-BSR) research fellowship during this study.

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How to cite this article:

Santhoshkumar, M., Baskaran, L., Mahakavi, T., Bakiyaraj, R., 2018. Assessment of phytoremediation potential of cockscomb (*Celosia cristata* L.) in chlorpyrifos polluted soil. Int. J. Curr. Res. Biosci. Plant Biol. 5(4), 60-71. doi: <https://doi.org/10.20546/ijcrbp.2018.504.009>