BEHAVIOR OF PESTICIDE RESIDUES EXTRACTED FROM DIFFERENT COTTON FIBERS DETERMINED BY THE INTERACTION WITH GREEN ALGAE

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Abstract

A method based on the monitoring of changes in the oxygen level caused by the interaction of residual analytes and the green algae, Scenedesmus (chlorophyta), has been performed in this study, for the detection of pesticides and hazardous compounds. The advantages of being photoautotroph, morphologically simple and having relatively simple reproductive system makes algae a suitable criterion for the determination of intervening predators affecting their normal life cycle. Algae Growth Analyzer equipped with miniature sensitive oxygen electrode, a light source and cover to model light and dark phases was used enabling to follow the lifecycle of algae producing oxygen. Three different varities of cotton namely Giza 86 from Egypt, MNH 93 from Pakistan and cotton from India of the crop 2010/2011 were analyzed. One of each cotton samples was the classical conventional cotton and the other was organic cotton produced without utilizing the pesticides. Cryogenic homogenization was carried out for sample pretreatment and Ultrasound assisted extraction (USE) was used with the solvent, Acetonitrile, for each of the samples, respectively. The method shows reasonable results and can successfully be utilized for the detection of residual pesticides on different types of cotton and especially to compare the classical conventional and organic cotton in terms of their cytotoxicity.

Introduction

Cotton is the most important natural textile fiber in the world, used to produce apparel, home furnishings and industrial products (P J Wakelyn, 2007). Cotton today provides almost 38% of the world textile consumption, second only to polyester, which recently took the lead (Myers, 1999). Cotton production is highly technical and difficult because of pest pressures and environment, e.g. drought, temperature and soil nutritional conditions. The total area dedicated to cotton production accounts approximately 2.4% of arable land globally and cotton accounts for an estimated 16% of the world's pesticide consumption (Grose, 2009). Pesticides are widely used for the control of weeds, diseases, and pests all over the world, mainly since after Second World War, and at present, around 2.5 million tons of pesticides are used annually and the number of registered active substances is higher than 500. Humans can be exposed to pesticides by direct or indirect means. Direct or primary exposure normally occurs during the application of these compounds and indirect or secondary exposure can take place through the environment or the ingestion of food (Turiel, 2008).

This is why development of natural biological methods of insect control was initiated. Cotton grown without the use of insect control was initiated. Cotton grown without the use of any synthetically compounded chemicals (i.e. pesticides, fertilizers, defoliants, etc.) is considered as ''organic'' cotton. It is produced under a system of production and processing that seeks to maintain soil fertility and the ecological environment of the crop (Hearle, 2002). Pesticides are toxic compounds that may cause adverse effects on the human and the environment. Benzoylureas, carbamates, organophosphorous compounds, pyrethroids, sulfonylureas and triazines are the most important groups (Alder, 2006).

As the pesticide residue is a potentially serious hazard to human health, the control and detection of pesticide residue plays a very important role in minimizing risk. Many methods have been developed in the last few years for the detection of pesticides. The most widely used methods are gas chromatography (GC), high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), immune assay and fluorescence. However, these techniques, which are time consuming, expensive and require highly trained personnel, are available only in sophisticated laboratories (Mulchandani, 1999).

Assessment of human exposure to pesticides and other toxicants through biological monitoring offers one means to evaluate the magnitude of the potential health risk of these chemicals (MA J, 2002). Algae occupy an important position as the primary producers in aquatic ecosystems and they are the basis of many aquatic food chains. For this

reason, they are used in environmental studies for assessing the relative toxicity of various chemicals and waste discharges (Sanchez-Fortun, 2008).

The term algae refers to both macroalgae and a highly diversified group of microorganisms known as microalgae. The number of algal species has been estimated to be one to ten million, and most of them are microalgae (Laura, 2006). Algae are eukaryotic and predominantly aquatic, photosynthetic organisms. They range in size from the tiny flagellate micromonas that is 1 micro metre (0.000039 inch) in diameter to giant kelps that reach 60 meters (200 feet) in length (Rogers, 2011). No easily definable classification system acceptable to all exists for algae because taxonomy is under constant and rapid revision at all levels following every day new genetic and ultra structural evidence. Prokaryotic members of this assemblage are grouped into two divisions: Cyanophyta and Prochlorophyta, whereas eukaryotic members are grouped into nine divisions: Glaucophyta, Rhodophyta, Heterokontophyta, Haptophyta, Cryptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta, and Chlorophyta (Laura, 2006).

Single celled microalgae are among the most productive autotrophic organisms in nature due to their high photosynthetic efficiencies and the lack of heterotrophic tissues (Z. Perrine, 2012).The green pigment chlorophyll (which exists in three forms: chlorophyll a, b and c) is present in most photosynthetic organisms and provides an indirect measure of algal biomass (Chapman, 1996). Even though all algae species combined represent only 0.5% of total global biomass by weight, algae produce about 66% of the net global production of oxygen on earth – more than all the forests and fields (Edwards, 2008).

Algae possess a number of distinct physical and ecological features and their ability to proliferate over a wide range of environmental conditions reflects their diversity (Rogers, 2011; Edwards, 2008).

The action of toxic substances on algae is therefore not only important for the organisms themselves, but also for the other links of the food chains (M. D. Ferrando, 1996). Algal toxicity tests and Life-cycle toxicity tests are increasingly being used in bioassay test batteries and it has been observed in several studies that for a large variety of chemical substance algal tests are relatively sensitive bioassay tools (Jianyi Ma, 2002; Kevin, 1993).

Algal growth rate has been considered as a more meaningful and consistent parameter than total cell number or biomass as expressed, for instance, by cell volume. Moreover, toxicity data based on growth rate were found to provide greater reproducibility and, therefore, it has been proposed to use this approach in order to compare test results from different laboratories (Nyholm, 1985). Thus, inhibition of photosynthetic performance could also be used as a tool to evaluate the presence of pollutants (Sanchez-Fortun, 2008).

Keeping in mind the above mentioned factors, the goal of the present work was to develop a new method for the detection of pesticides and hazardous compounds based on the monitoring of changes in the oxygen level caused by the interaction of residual analytes and the green algae, Scenedesmus (chlorophyta).

Materials and Methods

Three samples of Egyptian cotton Giza 86 (G86), Pakistani cotton MNH 93 and Indian Cotton were collected from the cultivation season 2010/2011. Both varieties have classical conventional cotton and organic cotton. HPLC grade Acetonitrile solvent was used for the extraction procedure. Green Algae of the family Scenedesmaceae and Genus SCENEDESMUS was arranged by Bvt technologies, Czech Republic.

The determination of pesticides in samples at low concentrations is always a challenge. The main aim of any extraction process is the isolation of analytes of interest from the selected sample by using an appropriate extracting phase. The development of an appropriate sample preparation procedure involving extraction, enrichment, and cleanup steps becomes mandatory to obtain a final extract concentrated on target analytes. It is always necessary to carry out some pretreatments to get a homogeneous and representative subsample.

Cryogenic Homogenization

All samples of classical conventional cotton and organic cotton were arranged inside of a pre-chilled Teflon mill in the form of pallets which contains a concentric Teflon ring and Teflon puck in liquid nitrogen surrounding.

Each sample was milled for approximately 10 minutes with an interval of 2 min for grinding and 1 min for cooling. After the milling the resulting powder was sampled, cleaned and stored for analysis. Fig 1 shows the different steps for the cryogenic homogenization. Once the entire sample was homogenized and blended, the powder was sampled, cleaned and stored for analysis.

(a) (b) (c)

Figure 1. (a) Raw cotton (b) Freezer Mill (c) Homogenized sample

Ultra Sound Assisted Extraction (USE)

Ultra sound extraction method was used for the extraction from all of the above mentioned six samples. A total of 1.0 gm homogenized sample was transferred to the flask along with 15 ml of the solvent Acetonitrile. The flask was placed in the extraction apparatus Sonorex at a controlled temperature of 60 ^{O}C (Fig.2). Samples were extracted for 30 minutes. The extracts were then filtered and stored for further analysis.

Figure 2. Ultra sonic Extraction and Cotton samples

Algae Growth Analyzer (AGA)

Algae Growth Analyzer is universal device enabling to follow the lifecycle of algae or other biological objects producing oxygen. The device bears light source, exchangeable color filters, sensitive oxygen electrode and cover to model dark phase as shown in the Figure 3. It is controlled by Bioanalyzer potentiostat that allows user to program light and dark phases, measure and evaluate the oxygen electrode response. The device provides faster analogy of DIN 863 toxicity test that takes about 1 hour. Initially calibration of the device is done with 1 gm Na_2SO_4 and 5 ml Distilled water to consume all the oxygen inside the glass cell repeatedly for three times. Then it is washed with distilled water for three times.

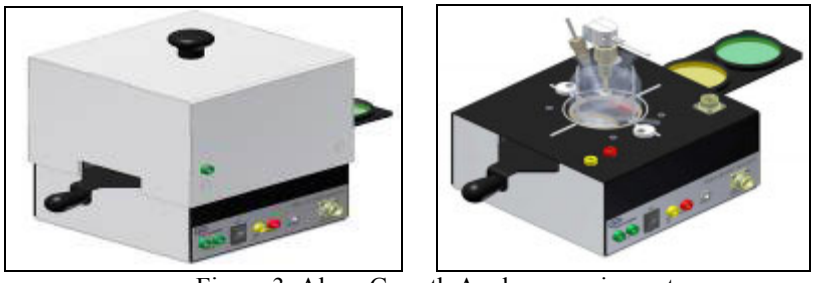

Figure 3. Algae Growth Analyzer equipment

As all the resulted extracts were extracted by the solvent, acetonitrile, so as to ignore the impact of solvent in the communication of analytes with algae, this solvent was evaporated completely at room temperature. 2 ml of all these extracts were put in petri dishes, separately, the solvent was evaporated and then the pure extracts were treated directly with 5 ml algae samples in petri dishes. We allowed them to cultivate for one hour and then the samples were tested in Algae Growth Analyzer.

Results and Discussion

All the above mentioned extracts were analyzed by AGA for duration of 30 minutes each. With the help of miniature Oxygen electrode, we have obtained the oxygen production activity of the algae in presence of the extracts by recording the oxygen produced in medium.

The results of Giza Cotton from Egypt were shown in Fig.4. There are the differences in the oxygen production but in each case the addition of extract increases the production of oxygen. Where as if we compare the classical and organic cotton, the stimulating agents in classical cotton are more and this is the reason of increase of oxygen production.

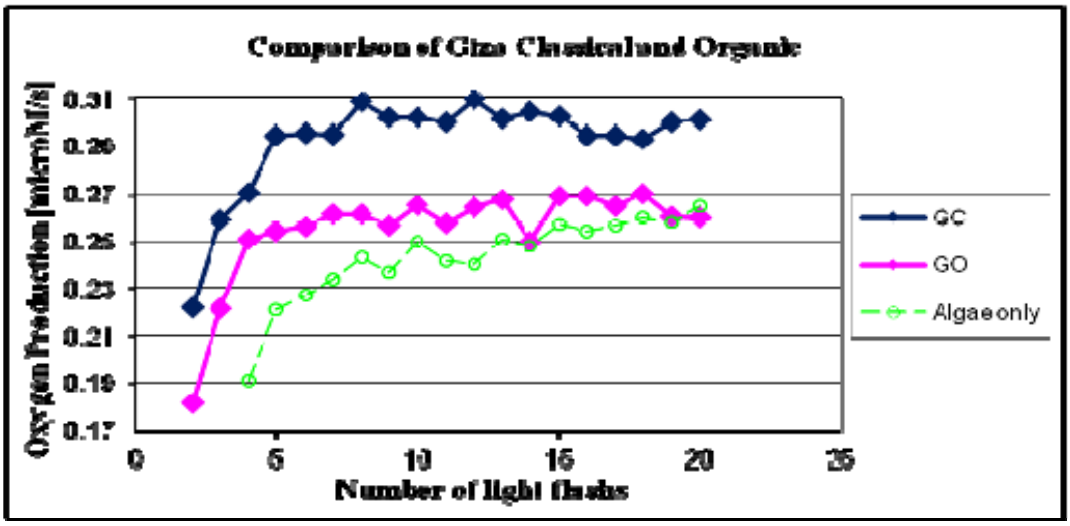

Figure 4. Comparison of Giza Classical and Organic Cotton

The results in Fig.5 contain the Pakistani classical and organic cotton. In this case there is also a difference in the production of oxygen. However comparing the classical and organic cotton, the stimulating agents in organic cotton are more and this is the cause of their high effect.

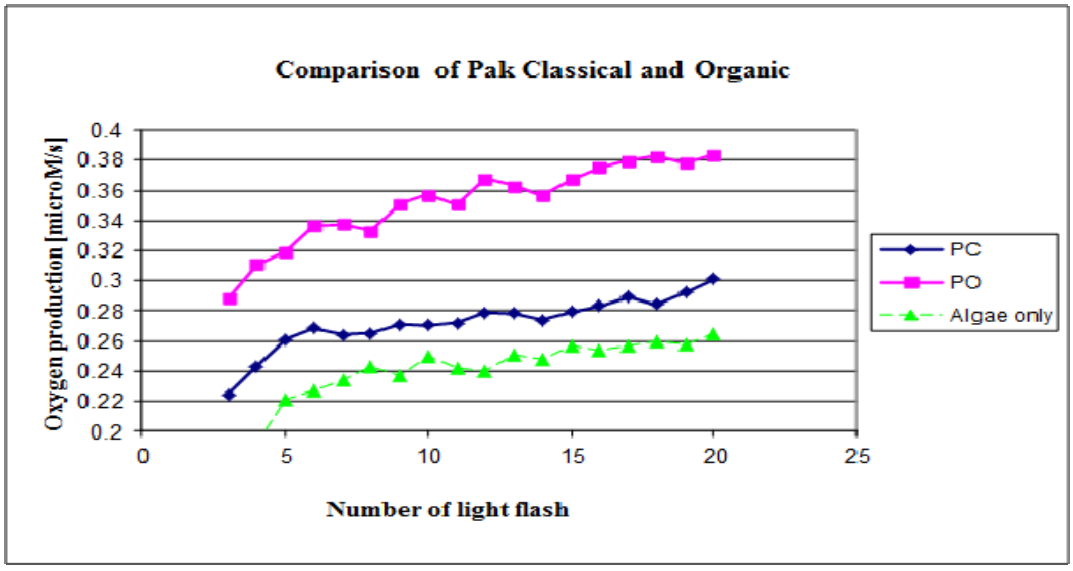

Figure 5. Comparison of Pak Classical and Organic Cotton

The results of Indian cotton are shown in the Fig.6. It is quite visible that there is a significant difference in the oxygen production. Classical cotton shows higher effect in this case. Organic cotton extracts in this case may have some contaminants and pollutants which hinder in the streamline of oxygen production by the algae.

In case of Giza and Indian cotton, the classical cotton shows the stimulating effect on photosynthetic activity of the algae where as in the case of Pakistani cotton this effect is caused by the organic cotton. However algal species vary widely in their response to toxic chemicals and differential sensitivity of green algae to the compounds has been observed in some reports (MA J, 2002; Jianyi Ma, 2002).

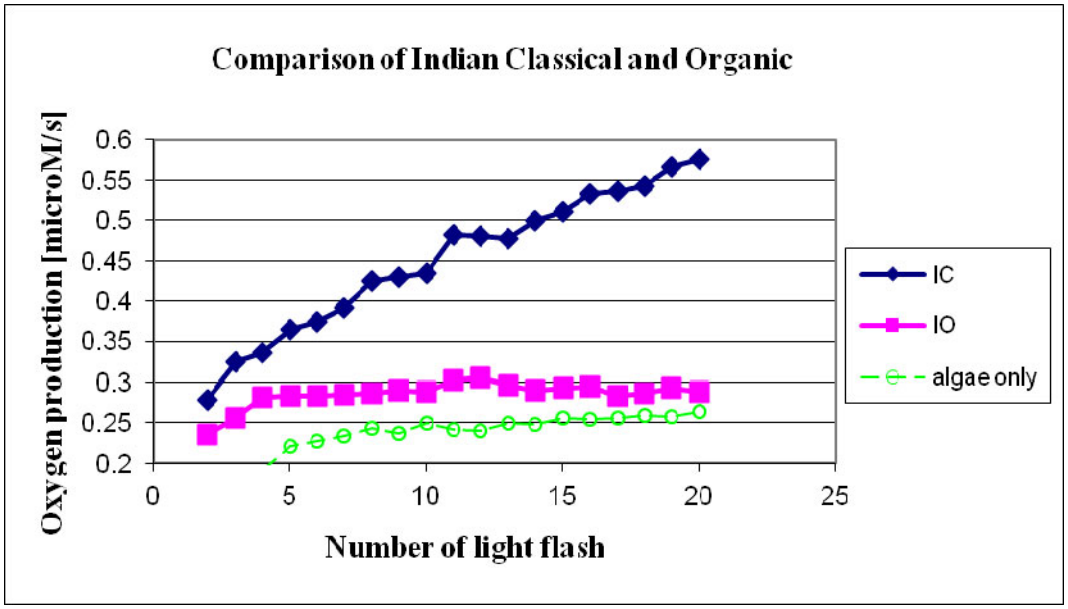

Figure 6. Comparison of Indian Classical and Organic Cotton

Conclusion

This study is based on the development of a new method for the detection of pesticides and hazardous compounds based on the monitoring of changes in the oxygen level caused by the interaction of residual analytes and the green

algae, Scenedesmus (chlorophyta). The results obtained with this laboratory algal test indicate a reasonable interaction of the analytes and the photosynthetic activity of the algae. Clearer picture of this interaction may be observed by prolonging these tests. Further research must be needed to verify the usefulness of the method presented here for the screening of pesticides on some more varieties of cotton of different regions.

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