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Image processing based technique for classification of fish quality after cypermethrine exposure

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ABSTRACT

The quality of fish is primarily dependent on its handling, processing, storage, exposure to contaminants and on climatic variability. Fishes nurtured at fresh and contaminated water exhibit marked differences in quality. Among different contaminants, pesticide is reported as a predominant non-specific menace to fish health and quality. Detection and identification of pesticide residues in fish is a challenging task and requires costly sophisticated instruments. This paper proposes an image processing based non-destructive technique for identifying quality differences between pesticide treated and untreated (control) fish. To evaluate the quality variability, rohu *(Labeorohita)* fishes were treated with mild dose of cypermethrin for seven days and bio-accumulation status was recorded through GC–MS at post-harvested condition followed by imaging at two days interval. Gill tissue was selected as focal tissue for image processing which was segmented and different features were extracted in wavelet domain using Haar filter. Features were selected up to the third level of decomposition in wavelet domain and analysed for discriminatory features. The discriminatory variations in the different features of images were related to the difference between pesticide treated and untreated fish using strategic image processing techniques. Supervised classification was performed on the extracted features using support vector machine (SVM) classifier. The experimental results indicate that the proposed method is efficient for identification of pesticide treated and untreated fish from the features of the images. The accuracy of identification is high and the computation time is faster enough to make this method efficient as a real time application.

1. Introduction

Aquaculture has a tradition of about 4000 years and is recorded as the fastest growing food segment along with field crops and livestock sector (FAO corporate Document Repository) (Troell et al., 2014). A number of aquaculture practices are used world-wide in three types of environment (freshwater, brackish water, and marine) for a great variety of culture organisms where fish is considered as a prime cultivable commodity and source of quality fat, easy digestible animal protein and minerals. At par with its productivity enhancement, deterioration in fish quality has raised overall concern in recent past (Fatima et al., 2014). This is happening due to contaminated-aquatic system, higher pathogenic infestation and changes in water quality as a consequence of climate change. Among different aquatic pollutants, Pesticides pose a major menace and creep into aquatic bodies (Sarkar, Chatterjee, Adhikari, & Ayyappan, 2005) as a natural outflow from agriculture field, effluents from pesticide manufacturing industries or mosquito control programmes which remain the utmost challenge (Adhikari, Sarkar, Chatterjee, Mahapatra, & Ayyappan, 2004) for fish quality.

Residual presence of pesticides has been reported in different fish varieties from aquatic sources (Akhtar et al., 2014). These pesticides alter normal physiology and health status of fish and cumulatively change the overall quality and its marketability. Multiple reports of compromised quality are surfaced from the post-harvested fishes as surveyed at markets and distribution channels (Hossain, Rahman, Hassan, & Nowsad, 2013). Determination of pesticide impact on fish quality is highly required and for that assessment, detection of pesticide residue in fish is necessary. But this is a cumbersome process, as it necessitates sophisticated and costly instruments like LC-MS and GC–MS which are often non-accessible to common fish loving community and other important stakeholders. Most studies on the effects of pesticides

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exposure are confined to reporting biochemical and physiological changes in fish and very little attention has been paid to compare the real effects like variations in quality (Fırat et al., 2011 Sep).

Among different group of pesticides, cypermethrin is a synthetic pyrethroid and widely used to eradicate multiple crop pests and is applied in fish ponds to control *Argulus* sp (Singh, Singh, & Yadav, 2010).

Gill is the red coloured respiratory tissue of fish. Usually fish gill colour, its odour pattern, or eye colour is used to resolve the freshness issue of a sample (Macagnano et al., 2005). Among that, gill colour pattern is popular and practiced as a quality indicator by fishermen and customers. Rohu (*Labeorohita*) is one of the most popular, commercially successful Indian major carps (Das et al., 2005). Hence, selecting rohu gill to evaluate pesticide infected changes in fish quality was considered as an accep model.

Image analysis is a non-hazardous, non-destructive tool for evaluating photographic data and its color and texture variations and imaging tool scan be an important method to validate the quality of fish (Menesatti, Costa, & Aguzzi, 2010). Some research has already been conducted in food processing sectors using these image processing. Feng Wang et al. (Wang et al., 2013) deciphered a regression based technique one fish eye for detecting its freshness. Fairuz Muhamad et al. (Muhamad, Hashim, Jarmin, Ahmad, 2009) described a fuzzy logic based technique to classify fish freshness. Dubey et al. (Dubey et al., 2013) expressed his opinion on sum and difference histogram texture based features for detection of defects in fruits.

The main contribution of this paper is an efficient image processing based method to predict the quality and presence of pesticide in the fish from the wavelet domain features of the segmented tissue of the gills. Image captured at regular time intervals has been studied and discriminatory features have been strategically extracted for classification between pesticide treated and untreated fish. A comprehensive statistical and image processing techniques has been applied to explore these feature variations in segmented gill pattern to develop viable tool for predicting the presence of pesticide residue in fish.

Another contribution of the paper lies in the accurate segmentation of the gill tissues from the fish image. The features of this segmented tissues is used for classification between pesticide treated and untreated fish by using SVM (Support Vector Machine) classifier. Features of the gills have most prominent discriminatory variation in image coefficients which provides an accurate and efficient framework for classification. The proposed method achieves 95% to 100% sensitivity when the SVM is applied with polynomial kernel with order 1 and 2. The proposed method for classification is precise, simple and computationally efficient making it suitable for real time application.

The highlight of this work is that a strategic relationship is established for discriminatory image coefficients with the quality and classification of the fish that can act as indicative parameter for presence of pesticides as a viable image processing non-destructive diagnostic tool for quality check and control.

The remaining paper is divided into 3 sections: Section 2 describes the materials and methods used for the experiment. It gives a brief description of the treatment process of the fish. Section 3 explains the K-Means Clustering algorithm which is used for the automatic segmentation of the gills and discusses the identification process by using analysis of features in wavelet domain and classification using SVM and also consist experimental results and Section 4 reveals comprehensive discussion of the different parameters used in the study and a comparative noble outcome that will be deciphered in conclusion.

2. Material and methods

2.1. Experimental design

The live *Labeorohita* (Rohu) were sampled from National Institute of Abiotic Stress Management (NIASM), Baramati, Pune, Maharashtra fish farm. The collected fishes were acclimatized in aquarium (100 L volume) for 7 days prior to the experiment. The average weight and length of the fishes were 90.40 ± 1.20 g and 21.60 ± 0.50 cm respectively. The pond water were free from any pathogenic infestation and toxic residues as were measured through routine microbiology and toxicity detection protocols, prior to the initiation of the experiments. The fishes were treated with one experimental (1 ppb) dose of cypermethrin and control, in triplicate for seven days and after that, fishes from treatment as well as control were killed in chilled water to avoid rigor mortise. Fishes were thereafter preserved in thermocol boxes with a fish to ice ratio of 1:2. Images of fish gill were captured using a digital camera (NIKON D90) on the first day and then at two days interval period, till sixth day. At the end of the experiments, gill, skin and tail were dissected out from cypermethrin treated and untreated fish for analysis of its residues and preserved at -20^{O}C.

2.2. Sample extraction for cypermethrin residue analysis

Already developed and validated method was used for sample extraction (Chatterjee et al., 2015). Solvent volume and weight of salts were changed as per currently available samples amount from original method of 5 g sample. For different body parts of the fish viz., gills (2 g), skin (1.5 g) and tail (1 g), variable amounts of sample were taken. In brief, the sample preparation involved extraction with 10 mL of acidified acetonitrile (1% acetic acid) in presence of 2 mL of hexane, 6 g of magnesium sulphate (MgSO4) and 1.5 g of sodium acetate. A 2 mL portion of the acetonitrile (middle layer) extract was kept at −20 °C for 20 min (to freeze out the fat contents from the extract) and cleaned with 100 mg of $CaCl_2$ and 150 mg of MgSO4. The supernatant was again cleaned by dispersive solid phase extraction with a combination of 50 mgPSA (primary secondary amine) + 50 mg florisil + and 150 mg C18. The supernatant was filtered through a PTFE membrane filter and analyzed by GC–MS/MS (Chatterjee et al., 2016).

2.3. Instrumental detail

A gas chromatograph (GC) attached to a triple quadrupole mass spectrometer (GC: 7890A, MS: 7000B, Agilent Technologies, Palo Alto, USA) was used. The system is controlled using Mass Hunter software (version B.05.00.412). The analytical separation was performed using a DB-5MS (30 m × 0.25 mm, 0.25 μm) capillary column (Agilent Technologies). The carrier gas (Helium) flow was set at a constant rate of 1 mL/min. The oven temperature program was set at initial temperature of 100 °C (1 min hold), ramped to 200 °C at 50 °C/minute (0 min hold), then at 10 °C/min up to 310 °C (3 min hold) resulting in a total run time of 17 min. The transfer line temperature was maintained at 285 °C.

The multi-mode inlets (MMI) were operated in the split less mode and 2 μL of sample was injected into a gooseneck liner (78.5 mm × 6.5 mm, 4 mm). Split less program was set from the initial temperature of 80 °C (0.1 min hold), raised to 325 °C at 450 °C/min (5 min hold) followed by 10 °C/min up to 250 °C (0 min hold). The purge flow to split vent was maintained at 30 mL/min, at a pressure of 7.414 psi at 2 min after injection. The mass spectrometer was operated in MS/MS mode with acquisition starting at 4.4 min. Multiple reaction monitoring parameters for cypermethrin was 162.9 > 90.8 (Collision energy = 5) applied as the quantifier and 162.9 > 127.0 (CE = 15) as qualifier ions. Electron impact ionization (EI+) was achieved at 70 eV and the ion source temperature was set at 280 °C.

2.4. Image acquisition of fish samples for image processing

Samples were illuminated using four fluorescent lamps (length