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Monitoring of Disease Residues in Pomegranate

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Abstract: This paper is an attempt to automatically grade the disease on the Pomegranate plant leaves. This innovative technique would be a boon to many and would have a lot of advantages over the traditional method of grading. There has been a sea change in the mindset and the effort put down by the agricultural industry by adapting to the current trends & technologies. One such example is the use of Information and Communication Technology (ICT) in agriculture which eventually contributes to Precision Agriculture. Presently, plant pathologists follow a tedious technique that mainly relies on naked eye prediction and a disease scoring scale to grade the disease. Manual grading is not only time consuming but also does not give precise results. Hence the current paper proposes an image processing methodology to deal with one of the main issues of plant pathology i.e. disease grading. The results are proved to be accurate and satisfactory in contrast to manual grading and hopefully take a strong leap forward in establishing itself in the market as one of the most efficient and effective process. The proposed system is also an efficient module that identifies the Bacterial Blight disease on pomegranate plant. At first, the captured images are processed for enhancement. Then image segmentation is carried out to get target regions (disease spots) on the leaves and fruits. Later, if the diseased spot on leaf is bordered by yellow margin then it is said that leaf is infected by bacterial blight otherwise not. Similarly when black spots are targeted on fruits, they are checked for whether a crack is passing through these spots. If cracks are passing through the spots then the disease identified would be Bacterial blight. Based on these two characteristics bacterial blight on pomegranate can be appropriately identified.

Keywords: Percent Infection, Bacterial Blight, K-means clustering, Morphology, colour image segmentation, Precision agriculture.

1. INTRODUCTION

Sole area that serves the food needs of the entire human race is the Agriculture sector. Research in agriculture is aimed towards increase of productivity and food quality at reduced expenditure and with increased profit [1]. In the past few years new trends have emerged in the agricultural sector. Due to the manifestation and developments in the fields of sensor networks, robotics, GPS technology, communication systems etc, precision agriculture started emerging [2]. Precision agriculture concentrates on providing the means for observing, assessing and Sole area that serves the food needs of the entire human race is the Agriculture sector. Research in agriculture is aimed towards increase of productivity and food quality at reduced expenditure and with increased profit [1]. In the past few years new trends have emerged in the agricultural sector. Due to the manifestation and developments in the fields of sensor networks, robotics, GPS technology, communication systems etc, precision agriculture started emerging [2].

Precision agriculture concentrates on providing the means for observing, assessing and controlling agricultural practices. It also takes into account the pre- and post-production aspects of agricultural enterprises. The objectives of precision agriculture are profit maximization, agricultural input rationalization and environmental damage reduction, by adjusting the agricultural practices to the site demands. The challenge of the precision approach is to equip the farmer with adequate and affordable information and control technology.

Plant disease is one of the crucial causes that reduces Quantity and degrades quality of the agricultural products. Disease is impairment to the normal state of the plant that modifies or interrupts its vital functions such as photosynthesis, transpiration, pollination, fertilization, germination etc. The emergence of plant diseases has become more common now days, as factors such as climate and environmental conditions are more unsettled than ever [2]. Plant diseases are usually caused by fungi, bacteria and viruses. Also there are other diseases which are caused by adverse environmental conditions. There are numerous characteristics and behaviors of such plant diseases in which many of them are merely distinguishable. The ability of disease diagnosis in earlier stage is an important task. Hence an intelligent decision support system for Prevention and Control of plant diseases is needed. This system uses some high-tech and practical technology to appropriately detect and diagnose the plant diseases. Technological advancement is gradually finding its applications in the field of agriculture [3]. The information and communication technology (ICT) application is going to be implemented as a solution in improving the status of the agricultural sector [4]. The idea of integrating ICT with agriculture sector motivates the development of an automated system for pomegranate disease classification and its grading.

Pomegranate (Punica granatum), so called "fruit of paradise" is one of the major fruit crops of arid region. It is Popular in Eastern as well as Western parts of the world. The fruit is grown for its attractive, juicy, sweet-acidic and fully luscious grains called 'Arils' [6]. The fruits are

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mainly used for dessert purposes. In India it is cultivated over the area of about 63,000 ha, and its production is about 5 lakh tons/annum. Important varieties cultivated are Ganesh, Dholka, Seedless (Bedana), Bhagwa, Araktha.

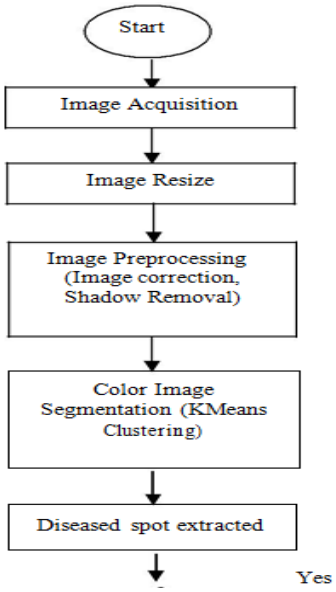
Figure1 shows three varieties of pomegranate fruit.

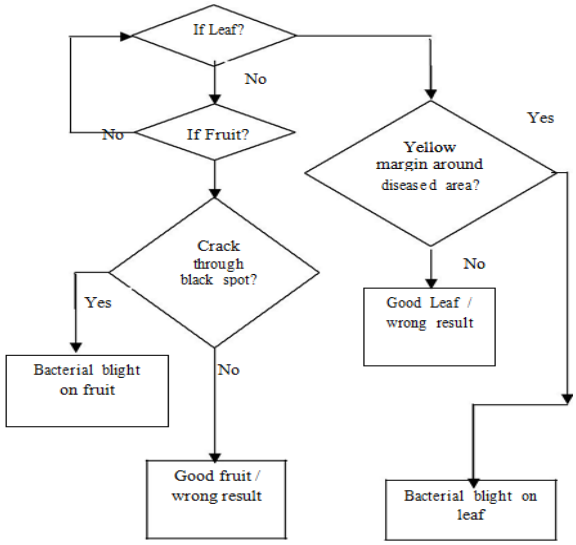
Based on size and colour, pomegranate fruits are graded as follows:

Super size- in which fruits are free from spots and individual fruit weight is more than750grams.
King size –in which fruits are attractive and individual fruit weight, is 500-700 grams.
Queen size- in which fruits are attractive, red and individual fruit weight is 400-500 grams.
Prince size- in which fruits are attractive, red and individual fruit weight is 300-400 grams.

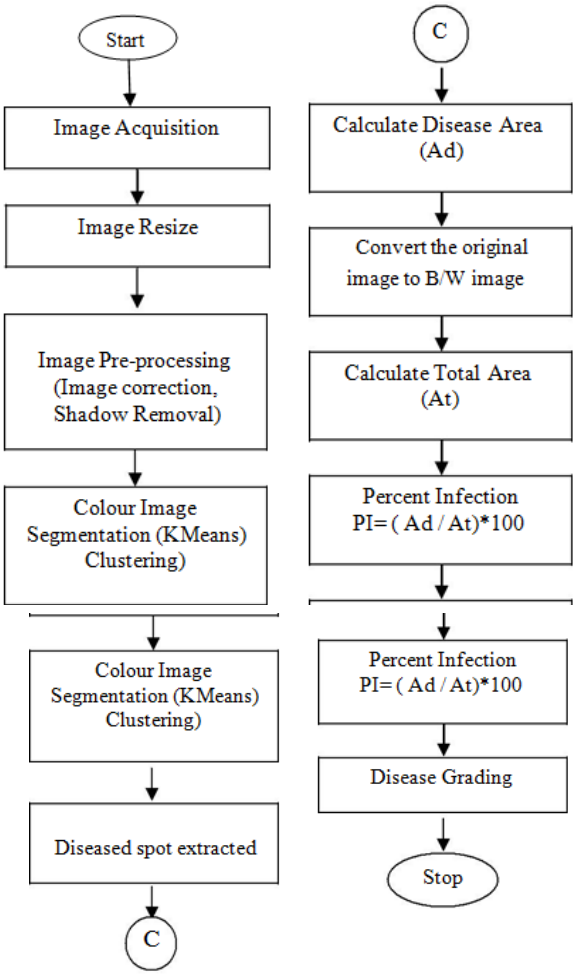
Unfortunately there are no organized marketing systems for pomegranate. Usually farmers dispose their produce to contractors who will later transport too far off markets. There is scope for exporting Indian pomegranates to Bangladesh, Bahrain, Canada, Germany, United Kingdom, Japan, Kuwait, Sri Lanka, Omen, Pakistan, Qatar, Saudi Arabia, Singapore, Switzerland, U.A.E. and U.S.A.

Diseases and insect pests are the major problems that threaten pomegranate cultivation. These require careful diagnosis and timely handling to protect the crops from heavy losses [6]. In pomegranate plant, diseases can be found in various parts such as fruit, stem and leaves. Major diseases that affect pomegranate fruit are bacterial blight (Xanthomonas axonopodis PV punicae), antracnose (Colletotrichum gloeosporoides) and wilt complex (ceratocystis fimbriata).





Botanical Name: Punica granatum (Lythraceae)



2 EXTRACTION PROCEDURE

The fruit samples were cut into small pieces (≈ 1 cm^2) and crushed in a blender as per the procedure described in chapter 4.1. A portion of 15 g sample from 1 kg of the blended pomegranate fruits was taken in a 50 mL centrifuge tube and the pesticide residues were extracted

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using 10 mL ethyl acetate and 10 g anhydrous sodium sulphate. The mixture was homogenized at 15000 rpm for 2 min. and centrifuged at 3000 rpm for 5 min. For GC-MS analysis an aliquot of 1 mL was cleaned using dispersive solid phase extraction (d-SPE) with 25 mg primary secondary amine (PSA) and 5 mg graphitized carbon black (GCB) previously weighed in eppendorf tube. The eppendorf tube was vortexed thoroughly and centrifuged at 10000 rpm for 5 min. The supernatant was filtered through 0.2 µm membrane filter and analysed by GC-MS. For LC-MS/MS analysis an aliquot of 5 mL was drawn into 15 mL centrifuge tube to which 50 mg PSA and 50 mg C_{18} was added. A portion of 4 mL of cleaned extract was transferred into 10 mL test tube and 0.2 mL of 10% diethylene glycol (in methanol) was added to it. The mixture was vortexed thoroughly and evaporated upto dryness at 35°C under gentle flow of nitrogen. The residues were dissolved in 1 mL methanol and 0.1% acetic acid (in water) and mixed thoroughly by vortexing. This solution was then centrifuged at 10000 rpm for 5 min. and filtered through 0.2 µm membrane filter and analysed by LC-MS/MS.
The extraction and determination of the residues in juice and anardana samples were carried using methods described in chapter 3.2 and 3.3.

3. DETERMINATION OF THE RESIDUES

The analysis of GC amenable pesticides was carried using the method described in chapter 3.1 whereas for LC amenable pesticides following method was used. For LC-MS/MS analysis a Perkin-Elmer 200 series HPLC system equipped with series 200 vaccum degasser, series 200 auto sampler, series 200 pump, and series 200 column oven was used for analysis. A portion of 20 µL of the sample extract was injected on Princeton SPHER C_{18} (150 mm × 4.6 mm ×5 µm) column (Princeton, NJ, USA) which was maintained at 30°C at the mobile phase flow rate of 1.2 mL min^{-1}. The mobile phase was composed of (A) methanol/water (20:80, v/v) with 5 mM ammonium formate and (B) methanol/water (90:10, v/v) with 5 mM ammonium formate; gradient 0 - 1.0 min/90% A, 1 - 2 min/ 90% - 0% A, 2 - 16min/0%A, 16 - 17 min/ 0% - 90% A, 17 - 23 min 90% A. Prior to use, the solvents were filtered through 0.22 µ filter with applied vacuum.

The triple quadrupole mass spectrometer was equipped with TurboIon Spray Interface (ESI). The instrument was operated in positive ion electrospray in the multiple reaction monitoring (MRM) mode. The specificMS/MS parameters are given in table 5.5.1. The MS parameters included ion spray voltage of 5500 V; nebulizer gas 30 psi; curtain gas 20 psi; heater gas 60 psi and the ion source temperature of 500°C.

Selection and tuning of the transitions as well as analyte dependent parameters, DP (declustering potential), CE (collision energy) and CXP (cell exit potential) were performed by direct infusion of individual pesticide solution in 10 mM ammonium formate in methanol/water (1:1, v/v) at a concentration of 1 mg L^{-1}. A dwell time of 50 ms per transition was used.

4. METHOD VALIDATION

a. Linearity
The calibration curves for all the compounds in pure solvent and individual matrix were obtained by plotting the peak area against the concentration of the corresponding calibration standards at five calibration levels ranging between 10 -250 ng mL^{-1}.

b. Sensitivity
Limit of detection (LOD) was determined by considering a signal to noise ratio (S/N) of 3 with reference to the background noise obtained from sample, whereas, the limit of quantification (LOQ) was determined by considering S/N of 10.

c. Recovery and repeatability
The recovery experiments were carried out on fresh untreated fruits by fortifying the samples (15 g) in six replicates with pesticide mixture separately at four concentration levels, i.e. 10, 25, 50 and 100 ng g^{-1} and extracting by the method described above. The quantification of recovery samples was done using the calibration obtained from matrix matched standards.

5. RESULTS AND DISCUSSION

5.1 Method validation

The LC-MS/MS chromatogram obtained is as shown in figure 5.5.1**.**

Linearity of the calibration curve was established for all the pesticides with correlation coefficient (r^2) >0.99 (Table 5.5.2) for solvent as well as matrix standards. The LOQ of all the compounds (Table 5.5.2) were lower than the harmonized MRL of the European Union. The recovery of all the compounds was found to be 70 - 120% with the RSD below 20% (Table 5.5.2).

5.2 Monitoring of the samples

The above said methods were applied for monitoring of pesticide residues from 176 fresh pomegranate samples collected from the pomegranate growing area in Maharashtra. The table 5.5.3 shows the details of sample collection and details of nonconformance. Solapur, Sangli, Nashik and Pune were the major pomegranate growing areas in Maharashtra, hence most of the samples were collected from these places. Table 5.5.3 shows that 63 samples out of total 176 samples analysed contained no detectable residues. Further it was observed that out of 176 samples, the residues were found in 115 samples out of which 80 samples contained the residues below their respective MRLs and in 35 samples violating their respective MRLs leading to the nonconformance for the export to EU. Table 5.5.3 indicates that during 2007 - 08 only 2 samples out of 65 samples (3% of the collected samples) collected were non-conforming for the export to EU, while during 2008 - 09 the number increased to 34 samples (29.5%) out of 114 samples. This might be due to the increased infestation of the pests and diseases in that year. From table 5.5.4 it was observed that as many as 27 pesticides were detected in pomegranate fruits. From the

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result of current study it could be concluded that the nonconformity of the samples increased due to fungicides which include chemicals like carbendazim, hexaconazole, difenconazole, flusilazole, chlorthalonil, thiophenate methyl, iprobenphos and metalaxyl. This might be due to their uncontrolled application for the control of various diseases including bacterial blight on pomegranate orchards. The other pesticides include organophosphates, few neonicotinoids and other classes of compounds from organochlorine and pyrethroid group of pesticides. No residues of the banned pesticides in India were found in any of the samples analysed.

Out of the 25 pomegranate samples collected from local retailers, the residues of carbendazim were found in 8 samples. The other samples were free from the residues of pesticides. The juice and anardana samples were free from the residues of any pesticides.

6. CONCLUSION

The established and validated methods were applied for the monitoring of pesticide residues in 176 pomegranate orchards from 2007 to 2009. The results of the current study concluded that the residues of pesticides were above their respective MRLs in 36 samples out of the total analyzed samples. The results showed that farmers have used organophosphate, neonicotinoid, triazole, benzimidazole group of pesticides for the control of pests and diseases on pomegranate. Out of the 25 fresh fruit samples collected from market, the residues of carbendazim were found in 8 samples. It was indicated that there was indiscriminate use of fungicides to control diseases on the pomegranate. Thus, there is urgent need for developing the preharvest intervals for these pesticides in order to minimize indiscriminate use of such pesticides and to improve the internal quality and to enhance the overall export of the pomegranates. This can be achieved by adopting Good Agriculture Practices (GAP) for orchard management. The juice and anardana samples were free from any residues.

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