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Research Article

# Taxonomic Divergence of Medically Important and Toxigenic *Aspergillus minisclerotigenes* from *Aspergillus flavus*

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**Abstract** | Molds produce noxious mycotoxins and cause more than 30% yield losses. The aflatoxins producer *Aspergillus minisclerotigenes* and *Aspergillus flavus* are morphologically similar species that belong to the *Aspergillus* section *Flavi*. *A. minisclerotigenes* and *A. flavus* were isolated from soybean and okra seeds, respectively. The isolated species were first identified morphologically. ITS1–5.8S–ITS4 primers sequence and amplification of ISSR nucleotide sequences using three primers [P01 $(AGAG)_4$ G, P02 $(GTG)_5$, and P03 $(GACA)_4$] confirmed that *A. minisclerotigenes* and *A. flavus* are two genetically distinct strains. Furthermore, both strains were qualitatively analyzed for aflatoxins (AFB1 and AFB2) production by thin-layer chromatography (TLC). A polyphasic strategy as adopted for the current study is a reliable and reproducible means to differentiate *A. minisclerotigenes* from *A. flavus*, indeed essential in interpretations of taxonomic and nomenclature of *A. flavus* group that may allow prior diagnosis and selection of effectual antifungal agents.

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**Keywords** | Mold, Extrolites, Section *Flavi*, ITS, Toxigenicity

## 1. Introduction

Various toxigenic strains of *Aspergillus* section *Flavi* produce lethal aflatoxins (G1, G2, B1 and B2) in agricultural commodities (Ismaiel and Papenbrock, 2015) and are a frequent cause of infections in humans and animals (Elad and Segal, 2018). The section *Flavi* included 33 species, and the species relationship within the section is still unclear. The classical means for the identification of these species still primarily depend on cultural and morphological traits. However, it is often tricky to differentiate these species because the phenotypic differences are not divergent and are easily ostentatious by the surroundings and are also mystified by the high degree of intra- and interspecies variations (Lee *et al.*, 2004). Among different species within section *Flavi*, *A. minisclerotigenes* exhibited a close phylogenetic relationship with *A. flavus*.

*A. flavus* is an extremely competitive cosmopolitan, notorious plant pathogen with wide host range, which has been initially described two centuries ago (Link, 1809). *A. flvaus* produces only produce B type, but there are also reports indicated the production of G type aflatoxins toxin as well (Frisvad *et al.*, 2019). *A. minisclerotigenes* has been described 10 years back (Pildain *et al.*, 2008), and is present in Central, East and Southern Africa and Australia (Probst *et al.*, 2014). It can grow on many substrates like maize, almond, groundnut and spices and produce both B and G aflatoxins (Makhlouf *et al.*, 2019).

For food safety purposes, correct species identification is of high importance and by using a polyphasic

strategy based on the combination of phenotypic and genotypic characteristics may contribute to the differentiation of toxigenic *Aspergillus* species within Flavus group. The current study was aimed to employ a polyphasic strategy that included phenotypic as well as genomic criteria (based on ITS and ISSR analysis) to discriminate the *A. minisclerotigenes* from *A. flavus*.

## 2. Materials and Methods

### *2.1 Isolation and identification*

Soybean (*Glycine* max) and okra (*Abelmoschus esculentus*) seeds from storage house, Lahore Pakistan during 2014, were found contaminated by morphologically similar molds. These seeds after surface sterilization with Clorox for one minute thoroughly washed with distilled water and incubated on moist blotter paper for 5 days at 27 °C. The grown spores were transferred to Malt Extract Agar (MEA) and Czapek Dox Agar (CZA) media and incubated for 3-4 days at 30 °C. The pure cultures were used for pathogen identification using macroscopic and microscopic features (Pildain *et al.*, 2008).

### *2.2 Extrolite analysis*

Isolated pathogens were preliminary characterized for their aflatoxigenicity based on emission of blue or green fluorescence after UV light excitation at 365 nm after growth on coconut cream agar (CCA) medium (Lin and Dianese, 1976).

A portion of CCA medium (6-7 cm) without fungal mycelium was cut and put into the 250 mL of Erlenmeyer flask filled with 50 mL of chloroform, incubated at 27 °C in shaking incubator at 200 rpm for 3 hours. Chloroform contents were filtered (Whatman No. 1) and separated into separate bottles. Extracts were allowed to dry at 35 °C for 5 days and dissolved into 2 mL of commercial methanol and aflatoxins of different isolates were saved at 4°C for qualitative analysis of aflatoxins by thin–layer chromatography (Guezlane-Tebibel *et al.*, 2013).

Both strains were analyzed by spotting crude extract (55 µL) of aflatoxins along with the standard of AFBs (AFB1 and AFB2). The TLC plates used were coated with silica gel 60 F254 on aluminum sheet, 20 x 20 cm. TLC plates were developed in chloroform and acetone (90:10, v/v) solvent system (Reddy *et al.*, 2004). The mobile phase was allowed to run 3/4 of the TLC plate. The plates were dried in the dark and then observed under UV light at 365 nm and samples spots were compared with standard aflatoxins spotted on the same plate.

### *2.3 Genetic analysis*

Method of Weigand *et al.* (1993) was used for the isolation of genomic DNA from fungal species. Using genomic DNA as a template, ITS1/ITS4 [ITS1 forward (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 reverse primer (3′-TCC TCC GCT TAT TGA TAT GC-5′)] regions of the genome were amplified (White *et al.*, 1990). The amplified fragments were separated in 1% agarose gel by electrophoresis. PCR products were purified by using a PCR purification kit (Enzynomics) and the fragments were sequenced in both orientations from Macrogen, Korea by using ITS forward and reverse primers. Three primers P01, P02 and P03 were used for ISSR amplification (Table 1) and the amplified PCR products were separated by gel electrophoresis and analyzed.

**Table 1: ISSR primers to amplify fungal DNA.**

| Primer name | Primer sequence |
|---|---|
| P1 | 5′- AGA GAG AGA GAG AGA GG -3′ |
| P2 | 5′- GAG AGA GAG AGA GAG AT -3′ |
| P3 | 5′- GAG AGA GAG AGA GAG AC -3′ |

## 3. Results and Discussion

Two post-harvest fungal strains of *A. flavus* group named *A. minisclerotigenes* and *A. flavus* were subjected to a polyphasic approach for authentic identification.

### *3.1 Morphological characterization*

The colonies of *A. miniseclerotegenious* were dull green to greyish green in color and yellow at reverse on MEA (Figure 1a and c Am), 50–65 mm in diameter without zonation and displayed sclerotia production, while colonies on CZA attained a diameter of 30-40 mm and sclerotia were present (Figure 1b and d Am). Uni and biseriate conidial heads bearing long conidiophores (0.9-1.2 mm) and globose vesicles (25-40 µm). The size of metulae and phialides were 5-8 µm with 8-12 µm, respectively, while globose conidia (3.5-5 µm diameter) were pale green or olive green and smooth-walled to echinulate (Figure 1e-f).

*A. flavus* colonies were 50-60 mm in diameter (without zonation) and exhibited sclerotia production on MEA (Figure 1a and c Af). On CZA medium, fungal colonies were slow-growing, attained diameter

of a 30-40 mm (without zonation), having sclerotia, that were heavily produced in the center of each colony (Figure 1b and d Af). Conidial heads were typically radiate, splitting into several poorly defined columns. Subglobose to globose (25-45 µm) vesicles were hyaline, while both metulae and phialides were present. Metulae with 6.5-10 × 3-4.5 µm dimensions completely covered vesicle surface, however, phialides were 8-12 × 3-5 µm in size. Subglobose to globose (3.5-4.5 µm) Conidia were pale green and conspicuously echinulate (Figure 1h-j).

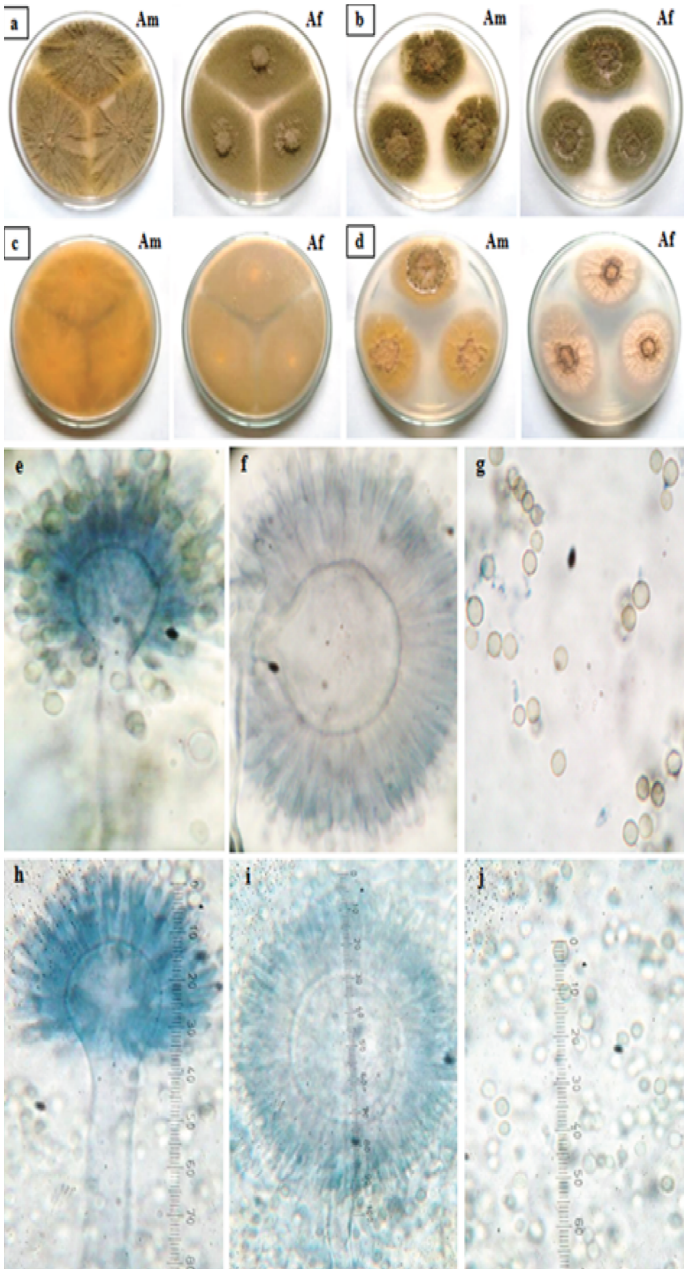


**Figure 1: Comparison of colonies grown on MEA front and reverse (a and c) and on CZ (b and d). Microscopic study of *A. minisclerotigenes* (e-g) and *A. flavus* (h-j) showing seriation (uniseriate and biseriate) and conidial attachment. Am: *A. minisclerotigenes*; Af: *A. flavus*.**

A vial of a pure culture of *A. miniseclerotegenious* (FCBP-1353) and *A. flavus* (FCBP-0529) were deposited in the First Fungal Culture Bank of Pakistan.

### *3.2 Aflatoxins production*

The culturing of both strains on CCA medium revealed that both *Aspergillus* species were capable of producing aflatoxins AFBs (Figure 2). Aflatoxins analysis on TLC also confirmed that *A. minisclerotigenes* (FCBP-1353) and *A. flavus* (FCBP-0529) were toxinogenic with consistent mycotoxigenic profile. Both were produced AFBs (AFB1 and AFB2) and showed clear bands on the TLC plate under UV light (Sultan and Magan, 2010) (Figure 3).

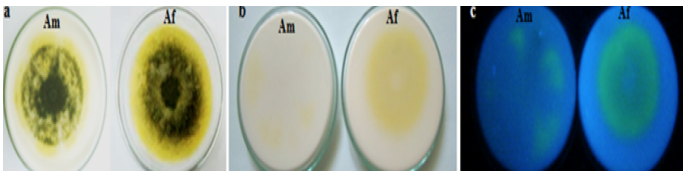


**Figure 2: Comparative screening of aflatoxin production by *A. minisclerotigenes* and *A. flavus* grown on CCA. a: colony from front side; b: reverse colony; c: reverse colony under UV light. Am: *A. minisclerotigenes*; Af: *A. flavus*.**

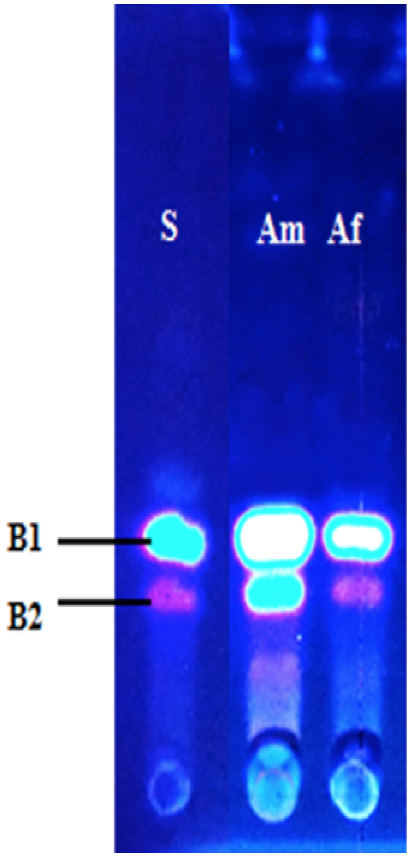


**Figure 3: Aflatoxins production on TLC. S: AFBs Standard, Am: *A. minisclerotigenes* and Af: *A. flavus*.**

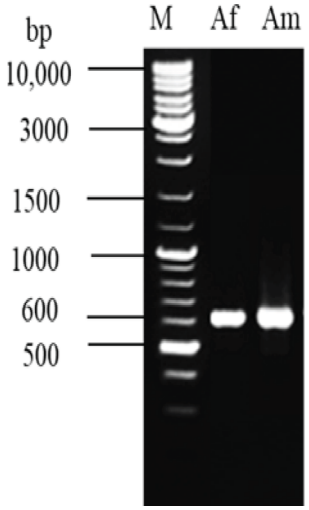


**Figure 4: Amplified ITS region of strains, M=1kb DNA marker; Af: *A. flavusus* and Am: *A. minisclerotigenes.***

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FCBP1365    1   TCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGG  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. mini.  100   TCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGG  159

FCBP1353   61   AGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTA  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. mini.  160   AGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTA  219

FCBP1353  121   AAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. mini.  220   AAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT  279

FCBP1353  181   AACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCC  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. mini.  280   AACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCC  339

FCBP1353  241   CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGT  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. mini.  340   CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGT  399

FCBP1353 301    GTGTTGGGTCGTCGTCCCCTCTCCggggggACGGGCCCCAAAGGCAGCGGCGGCACCGC  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. mini   400   GTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGC  459

FCBP1353  361   GTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGC   414
                ||||||||||||||||||||||||||||||||||||||||||||||||||||
A. mini.  460   GTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGC   513
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**Figure 5: ITS sequence alignment of *A. minisclerotigenes.***

The BLAST results revealed 100% identity of *A. minisclerotigenes* FCBP1353 to the 8 strains including G5 (KF841549.1), E76 (JX456215.1), E74 (JX456193.1), E44 (JX292091.1), E21 (JX292090.1), CS5 (JF412778.1), NRRL 29002 (JF412775.1), CS2 (JF412776.1) and some other *A. minisclerotigenes* strains.

### *3.3 Genetic analysis*

The obtained nucleotide sequence of PCR product of both species were sent for DNA sequencing and identified as 551 bp of ITS region of *A. minisclerotigenes* and 536 bp of *A. flavus* (Figure 4). The ITS sequence of *A. minisclerotigenes* and blast results in Figure 5 also showed 100% identity to the 8 strains of *A. minisclerotigenes* available in GenBank including G5 (KF841549.1), E76 (JX456215.1), E74 (JX456193.1), E44 (JX292091.1), E21 (JX292090.1), CS5 (JF412778.1), NRRL 29002 (JF412775.1), CS2 (JF412776.1) and some other *A. minisclerotigenes* strains. Likewise, *A. flavus* (FCBP–0529) blast analysis showed 100% identity with more than 25 strains including KJ473711.1, KJ013417.1, KF753952.1,KF656712.1,KF723010.1,KJ123911.1, GU172440.1, GU076485.1, KF031021.1 and some other *A. flavus* in GenBank (Figure 6). The nucleotide sequence of *A. minisclerotigenes* (FCBP-1353) *A. flavus* (FCBP-0529) were deposited to GenBank under the accession no. KJ564033 and KJ999747, respectively. The uniformity of ITS fragment size in several fungal groups builds nucleotide sequencing of ITS fragments obligatory to expose interspecific, and in some cases, also intraspecific variation (Hinrikson *et al.*, 2005; Inglis and Tigano, 2006). The ITS region was very functional in resolving taxonomic difficulties in many fungal genera as verified by Driver *et al.* (2000) and Inglis and Tigano (2006). Hinrikson *et al.* (2005), revealed that the small variation in band size probably made ITS an unreliable parameter for separating *Aspergillus* species. Unlike ITS, ISSR profile has significant importance as an assisting tool for identification, genetic diversity analysis and differentiation among strains (Batista *et al.*, 2008; Zhang *et al.*, 2013). ISSR analysis has also been shown usefulness in population genetics, epidemiological surveys and ecological studies of *A. flavus* (Batista *et al.*, 2008). Amplification of ISSR with three primers confirmed (Figure 4) genetic differences between *A. minisclerotigenes* and *A. flavus* (Hatti *et al.*, 2010).

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FCBP0529    1   ACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGC  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. flavus[  59   ACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGC  118

FCBP0529   61   CGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTA  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. flavus  119   CGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTA  178

FCBP0529  121   GTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGT  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. flavus  179   GTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGT  238

FCBP0529  181   TCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGT  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. flavus  239   TCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGT  298

FCBP0529  241   GAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTC  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. flavus  299   GAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTC  358

FCBP0529  301   CGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCgg  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. flavus  359   CGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGG  418

FCBP0529  361   ggggggACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTT  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. flavus  419   GGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTT  478

FCBP0529  421   TGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGG  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A. flavus  479   TGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGG  538

FCBP0529  481   TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT   524
                ||||||||||||||||||||||||||||||||||||||||||||
A. flavus  539   TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT   582
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**Figure 6: ITS sequence alignment of *Aspergillus flavus.***

The BLAST results revealed 100% identity of *A. flavus* (FCBP0529) to the more than 25 strains including S19 (KJ473711.1),BC-212(KJ013417.1),LPSC1183(KF753952.1), PTN13 (KF656712.1), KVCET2 (KF723010.1), G49 (KJ123911.1), UPM A8 (GU172440.1), A2 (GU076485.1), KAR-8 (KF433946.1) and J8M-40 (JN226905.1), PW2961 (KF562204.1), PW2953 (KF562196.1), MDU-5 (KC914096.1), JP44MY8 (KF031021.1) and some other *A. flavus* strains.

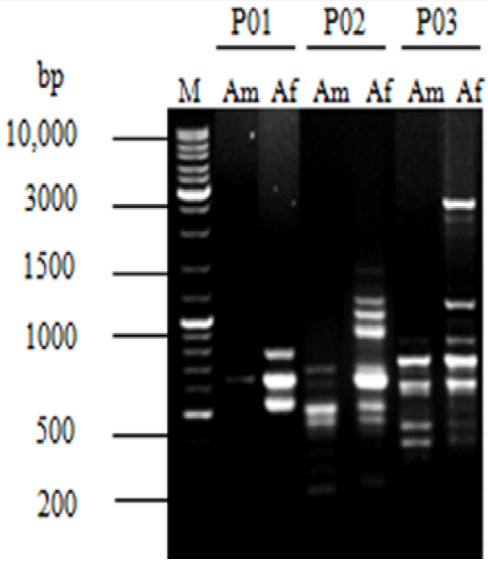


**Figure 7: DNA banding profile of PCR-ISSR amplification product. M: DNA marker; Am: *A. minisclerotigenes* and Af: *A. flavus*.**

## 4. Conclusions

In the current study, high relatedness between two medically important strains of *A. flavus* group concluded that the process of differentiating them needs an under-species classification accomplished by a number of different tactics including morphological basis, amplified ITS fragment, ISSR molecular markers, which is actually a supplementary tool for genetic characterization and could be useful in distinguishing between strongly correlated species or strains.

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## Author's Contribution

**Amna Shoaib**: Supervised research and wrote the manuscript.
**Zoia Arshad Awan**: Performed experiments and collect the data.
**Naureen Akhtar**: Supervised research and wrote the manuscript

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Research Article

# Renal Dysfunction in Ischemic Stroke Subjects

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**Abstract** | To determine whether decreased kidney function is a risk-factor in first-ever ischemic stroke. The study was conducted from Jan, 2013 to Jan, 2014 in Services and Jinnah Hospital Lahore, Pakistan. A total of 150 subjects were included in this study, divided into ischemic stroke group (n=100) and control (n=50). Kidney function estimation was done using serum creatinine and blood urea along with eGFR calculation by MDRD equation. Kidney dysfunction was defined as eGFR of <60 ml/min/1.73m$^2$. Statistical analysis was donedone by using SPSS. The serum creatinine of ischemic stroke patients was found to be 2.41 mg/dl compared to 0.93mg/dl in controls. The blood urea in ischemic stroke group was 67.3 mg/dl in contrast to 34.6 mg/dl in controls. The eGFR in ischemic stroke patients was calculated to be 54.62ml/min/1.73m$^2$, compared to 85.90 ml/min/1.73m$^2$ for controls. Prevalence of eGFR <60 ml/min/1.73m$^2$ in patients with stroke was 63%, significantly higher ($p<0.05$) than in controls. Moderate to severe reduction of eGFR in patients with ischemic stroke indicated renal impairment and kidney dysfunction. The risk of first ever ischemic stroke increases with low eGFR.

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**Keywords** | Ischemic stroke, Kidney dysfunction, eGFR, Serum creatinine, Blood urea

## 1. Introduction

Stroke has been known to the third most common cause of death in the world, after cardiovascular diseases and all types of cancers (Banerjee et al., 2006). 15 million people suffer from stroke worldwide annually. Among these, 5 million die and 5 million suffer from a persistent disability resulting a huge burden on families and communities and only 5 million-people attain optimal recovery (Roger et al., 2012). About 80% strokes are ischemic stroke. Ischemic stroke is defined as severe disturbance of the blood to the specific parts of brain i.e. cerebellum, brain stem or spinal cord in a focal area leading to infarction. Ischemic stroke results in bland ischemia (non-hemorrhagic ischemia) and infarction in a typically vascular distribution. The vascular distribution is often very helpful in differentiating stroke from tumor or demyelination (Wityk et al., 2007).

The non-modifiable risk factors of stroke are age, gender, ethnicity and genetics. Whereas, cardiovascular diseases, hypertension, diabetes, hypercholesterolemia, smoking, alcohol consumption, drug users and inactive lifestyles are potentially modifiable risk factors for ischemic stroke (Carter et al., 2007).

The worldwide epidemic of chronic kidney disease (CKD) will result in a more kidney dysfunction affected individuals over the next decade (Coresh et al., 2007), doubling the number of end-stage renal disease patients (Golssteen et al., 2001) and a

subsequent increased morbidity and mortality rate in CKD complications. Increased mortality in elderly, hypertensive and myocardial infarction or stroke suffered patients has been found associated with elevated serum creatinine. Wannamethee et al., 1997 investigated the relationship between blood creatinine concentration and the risk of major ischemic heart disease, stroke events and all-causes of mortality in a general population of middle-aged men. Creatinine concentration was found to be correlated with a significant increase in stroke in both normal and hypertensive men.

Renal dysfunction has been suggested as risk factor and prognostic factors in cerebrovascular diseases. Regarding the association of renal dysfunction with stroke subtypes, conflicting results have been observed (Bos et al., 2007; Nakayama et al., 2007). The aim of the present study was to investigate the association of renal function with first-ever ischemic stroke patients.

## 2. Materials and Methods

### *2.1 Research design*

The study was carried in different hospitals specifically Services hospital and Jinnah hospital, Lahore. The study period extended from January 2013 to January 2014. A total of 150 subjects were included in this study which were divided as control group (n=50) and ischemic stroke group (n=100). The control group was selected after examination by the physician and they were found healthy. They were included for comparison with ischemic stroke subjects.

Subjects with confirmed clinical diagnosis of stroke by physician were included and they were brought to the hospital within 48 hours. It had been made sure that the patients were first-ever ischemic stroke and did not have a previous stroke history. The data was collected with the help of questionnaire regarding the age, diabetes, hyperlipidemia, high blood pressure, heart diseases, personal and family history of stroke, obesity, smoking, and a sedentary lifestyle. The ethicsal permission was granted by the university board of Lahore College for Women University Lahore and by the ethical committee of the hospitals. After collection of the blood the serum was separated by centrifugation.

### *2.2 Calculation of eGFR*

GFR was calculated using the 4-variable Modification of Diet in Renal Disease (MDRD) formula. This formula in the form of equations was developed in 1999 for the estimation of eGFR by routine measurement of serum creatinine, along with the readily available demographic variables age, gender and race (Levey et al., 1999).

For creatinine in mg/dl:

$$eGFR = 186 \times Serum\ Creatinine^{-1.154} \times Age^{-0.203} \times (0.742\ if\ female) \times (1.212\ if\ black).$$

According to the National Kidney Foundation definition, CKD is a kidney damage reflected by an estimated GFR of <60mL/min/1.73 $m^2$ of body surface area. CKD was further classified into moderate reduction of GFR of 45 to 60 and severe reduction of <45 mL/min/1.73 $m^2$. This further categorization is a Modification of National Kidney Foundation classification scheme chosen based on prior studies in patients with cardiovascular disease. Higher values i.e. >60 were not further categorized because the MDRD equation have substantial errors for GFR estimates in the normal – high range (Stevens et al., 2007; Brosius et al., 2006; Rule et al.,2004).

### *2.3 Statistical analysis*

The data was then analyzed statistically using statistical software package SPSS version 13.0 for windows. The comparison of clinical characteristics and renal parameters between the ischemic stroke and control groups was performed with Students T- test.

## 3. Results and Discussion

It was observed that there were 48% females and 52% males in the studied groups.

The average age of ischemic stroke subjects was 62.3 ± 1.48 yrs. and that of controls was 60.6 ± 1.79 yrs. with non-significant difference between the two groups ($p \geq 0.05$). The average calculated BMI of ischemic stroke and control subjects was 26.4 ± 0.32 kg/$m^2$ and 26.36 ± 0.45 kg/m2 respectively with non-significant difference between the groups ($p \geq 0.05$).

Diabetes was frequent in 43% of the subjects. The frequency of Irregular heart beat due to hypertension in ischemic stroke subjects was 38%, whereas, other heart diseases or problems found in the subjects was 22%. 56% of the subjects took excessive oily or high

cholesterol food on regular basis. 32% of the subjects with ischemic stroke smoked as well. About 71% of the subjects had sedentary life style, while 29% did walk or other moderate activity for at least 30 minutes a day or at least 3 hours a week.

The demographic and clinical parameters of both groups were presented in Table 1. As compared with controls, subjects with ischemic stroke had a lower eGFR and were older. The prevalence of an eGFR (<60 ml/min/1.73m$^2$) in patients with stroke was 63% significantly higher than in controls.

The subjects was further categorized into 3 categories on the basis of CKD and its severity i.e. stage 1 (including patients with normal GFR), stage 2 (patients with moderate reduction of GFR) and stage 3 (patients with severe reduction of GFR). Baseline clinical features of these groups estimated by MDRD equation were shown in Table 2. On the basis of the formula of renal function estimation 16% of ischemic stroke subjects had moderate reduction of eGFR ranging within 45 to 60 ml/min/1.73m$^2$ and 45% of the patients had severe reduction of eGFR that was <45 ml/min/1.73m$^2$. The rest of 39% ischemic stroke patients had eGFR >60 ml/min/1.73m$^2$. The differences of age and gender were also seen in the three groups. Subjects with extremely low GFR were older and were more likely to be women (Tables 1 and 2).

In our study, subjects were from single ethnicity and from the same area. It was observed that there was a relationship between kidney dysfunction and future risk of ischemic stroke and it has been confirmed that subjects with ischemic stroke have reduced kidney function or greater prevalence of CKD when compared to controls. In other studies, the relationship between ischemic and hemorrhagic types of stroke has been carried out but no study has been carried out with ischemic stroke. This study was carried out on homogenous sample (ischemic group) to reduce confounding factors.

Our central finding is that, low eGFR and the presence of CKD is a strong predictor of first-ever stroke. The correlation between renal function and stroke has previously been noted in a study in UK[7], whereas high normal serum creatinine levels or low eGFR were a risk factor for stroke in general population. Renal function as assessed by blood urea showed an association similar to that observed with creatinine, with significantly elevated blood urea concentration in ischemic stroke patients when compared to controls. A slightly weaker association between blood urea and risk of stroke was observed by Wannamethee et al., 1997.

**Table 1: Clinical characteristics of ischemic stroke and control subjects.**

| Sr. no. | Mean | Groups | | P value |
|---|---|---|---|---|
| | | Ischemic stroke | Controls | |
| 1 | Age (yrs) | 62.3 ± 1.48 | 52.6 ± 1.79 | 0.11** |
| 2 | Serum Creatinine (mg/dl) | 2.41 ± 0.244 | 0.93 ± 0.28 | 0.001** |
| 3 | Blood Urea (mg/dl) | 67.3 ± 5.77 | 34.6 ± 1.25 | 0.001** |
| 4 | eGFR (ml/min/1.73m$^2$) | 54.62 ± 4.55 | 85.90 ± 4.34 | 0.001** |
| 5 | eGFR <60 ml/min (%) | 63% | 6% | 0.001** |

GFR, glomerular filtration rate; **p≤0.001--- Highly significant.

**Table 2: Mean renal functional parameters in different stages.**

| Characteristics | Stage 1 (>60) | Stage 2 (45 – 60) | Stage 3 (<45) | P-value |
|---|---|---|---|---|
| Total Percentage (%) | 39% | 16% | 45% | |
| Age (yrs) | 60.97 ± 2.14 | 59.3 ± 5.37 | 64.62 ± 1.97 | 0.361 |
| Female (%) | 43.6% (17) | 31.25% (5) | 57.8% (26) | |
| Male (%) | 56.4% (22) | 68.75% (11) | 24.2% (19) | |
| Serum Creatinine (mg/dl) | 0.88±0.045 | 1.36±0.05 | 4.10±0.42 | 0.00** |
| Blood Urea (mg/dl) | 40.21±4.17 | 51.25±7.19 | 96.49±10.54 | 0.001** |
| eGFR (ml/min/1.73m$^2$) | 95.22±7.36 | 53.19±1.51 | 19.95±1.6 | 0.001** |

**p ≤ 0.001 --- Highly significant.

Glomerular filtration rate is of central importance for measuring renal function. Serum creatinine concentration is mainly determinant of the glomerular filtration rate and is used as an index of renal function (Waller et al., 1991). However, inference of renal dysfunction from the serum creatinine level is complicated by the differing rates of creatinine production among individuals, as muscle mass vary. This is why; women and the elderly people often have low serum creatinine levels (Maaravi et al., 2007; Froissart et al., 2005). There are substantial errors for GFR estimation by MDRD in the normal high range[11], and creatinine-based estimations are not reliable with particularly low creatinine generation.

There are number of evidences regarding the correlation between renal dysfunction and cerebrovascular morbidity (Bos et al., 2007; Nakayama et al., 2007) CKD is also found to be associated with increased risk of ischemic stroke (Koren-Morag et al., 2006).

Mechanisms under investigation showed that under the impact of renal dysfunction risk of cerebro-cardiovascular diseases increases. The continual increase in cerebrovascular risk with increased GFR was associated with decrease renal function, oxidative stress, inflammation and conditions that promote clotting (Johnson et al., 2007; McCollough et al., 2007; Soriano et al., 2007; Valkonen et al., 2001). Which leads to atherosclerosis and endothelial dysfunction.

We identified a significant prevalence of kidney dysfunction in patients presenting early to the hospital with ischemic stroke (<24 h) by using the eGFR. A study conducted by Mc Walter *et al*, also reported similar results (Mc Walter et al., 2002). In our study the higher frequency of renal dysfunction may be because our 80% of subjects were hypertensive.

In this study, patient with an eGFR of <45 ml/min were most significantly associated with stroke. This is in contrast to most other studies in which mild reduction of eGFR of 45 – 60 ml/min was associated significantly with stroke (Losito et al., 2011; Tsagalis et al., 2008). In these studies the eGFR distribution was normal with large number of patients having normal to mild lowering GFR. In our study, on the other hand, a sharp lowering of GFR was observed in many patients.

The cause and effect relationship between kidney dysfunction and ischemic stroke is vague until now. In this study low eGFR (estimated glomerular filtration rate) is correlated with an increased risk of future ischemic stroke. This result is in consistent with the study conducted in America (USRDS Annual Data Report, 2009). A relationship of mild to severe renal disease to long-term mortality in persons with self-reporting stroke has also been found recently (Ani et al., 2010). Our population differs from this as method of enrolment, hospital setting and type of stroke analyzed. Also, unlike other studies it was not a long-term follow-up study.

The clinical implication of this study indicates that people suffering from kidney dysfunction may be at high risk for future ischemic stroke. Patients with early stages of kidney dysfunction need close surveillance.

Our study has several probable limitations. First, the study is hospital based, so stroke patients treated at home were not included. Secondly, although the use of the MDRD equation is a quite reliable mean of estimating GFR, as has been previously used in many clinical trials but it tends to overestimate GFR in high levels of renal function and is also affected by age. Finally, another limitation of our study was the absence of follow-up or mortality data; therefore, we were not able to interpret the effect of renal dysfunction on mortality. Despite these limitations, our study has strong basis to reinforces the belief that there is a strong correlation between renal impairment and first-ever ischemic stroke and that renal function proves an important independent risk factor for first symptomatic stroke events.

## Conclusion

In this study, kidney function found to be significantly associated with ischemic stroke. A reduced eGFR showed renal dysfunction in few ischemic stroke patients (16%) while a severely reduced eGFR was observed in 45% patients. This suggests that estimated GFR associated to the other known prognostic factors as kidney dysfunction or CKD was an independent risk factor for ischemic stroke.

## Author's Contribution

SS: Conceived idea and designed the project &writing

of Manuscript
FM: Analysis & writing of manuscript
TF: Analyzed the results
SN: helping in experimental work & writing manuscript
RT: data collection and did experimental work.

*Conflict of interest*

The authors have declared no conflict of interest.

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Research Article

# Economic Losses Due to Trypanosomiasis of Camels in Balochistan

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**Abstract** | The present study was designed to evaluate the present status of Trypanosomiasis in Province Balochistan of Pakistan. The two districts Musa Khail and Jhal Magsi with thick populations of camels and have different climate and geographical distribution were selected. The questionnaire was developed for survey of Camel farmers and information regarding the age of respondent, experience, type of community, feeding/watering pattern of camels, prevailing camel diseases in the area, treatment facilities, traditional remedies used by them against various diseases in camels and economic losses were collected. A total of one thousand and forty (n=1040) camel owners/respondents from three groups viz settled, transhumants and nomads were interviewed in Districts, Musa Khail and Jhal Magsi during the year 2011. The direct as well as indirect economic losses due to camel Trypanosomiasis based on the prevalence of Trypanosomiasis, mortality rate, abortion and perceptions of the respondents were recorded. The camel dies due to Trypanosomiasis in direct visible losses and invisible losses include reduced fertility, meat loss, low quality of hide, loss of draught power and traction force and change in herd. While, indirect losses include additional costs of drugs, veterinarian fee, preventive medicine and quarantine. Moreover, the present study demonstrates that the respondents above the age of 50 years were more experienced in disease diagnosis and use of traditional veterinary practices.

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## 1. Introduction

Balochistan is area-wise largest and southwestern province of Pakistan. It has an area of 3, 48,000 Sq km, which is about 43 percent of the total area of Pakistan. The climate of the province is arid and semi-arid (Nagy et al., 1989). The province has been divided into various ecological Zones i.e. Tropical Thorn forest, Sub Tropical Broad leaves Evergreen forest, Dry Temperate Forest and Arid Desert. Furthermore, only four percent of the area is cultivable land while rest is comprised of arid grazing lands, rangeland mountain forests, barren rocky mountain, and deserts (Nagy et al., 1989).

The traditional livestock production system in Balochistan is broadly categorized into settled, transhumant and nomadic. The local ecological conditions make it necessary for livestock owners to migrate in search of grazing areas. The available data demonstrates that migratory livestock constitutes about 90% of the total livestock population in Balochistan Khan et al. (2018) and Khan et al. (2018). Furthermore, 60% of the migratory livestock is transhumant while remaining 30% is nomadic livestock production system (Jasra et al., 2001). However, the animals raised on farm are either for household consumption or for sale near religious festivals, when the prices are high. This supplementary

livestock production accounts for major portion of household income and helps to improve farm productivity.

The camel is the most efficient domesticated animal of Balochistan which is used for work, transport, and production of milk and meat. Camel belongs to family *Camelidae*, order Artiodactyla (even toed ungulates) and suborder Tylopoda (pad-footed) comprises two species: *dromedaries* and *bacrtianu. Dromedaries* the dromedary one humped or Arabian species, while *bacrtianus* or two humped camel. The name dromedary for the one humped camel is derived from the Greek word 'dromeus' a runner, or 'dromas' running. This name originally designated only the swift Arabian camels renowned for their fleetness of foot. Later, it applied to all one humped camels. The Bacterian camel was named after the area of Bacteriana in Central Asia. The habitat of dromedary is Northern Africa, Sudan, Ethiopia and Northern Kenya the near East and West Central Asia. Dromedaries were originally domesticated in Central and Southern Arabia (Zenuer, 1963) and were lately dispersed to North Africa and eastwards to the deserts and semi deserts of the Middle East. The Roman used camels in many parts of their empire thus accounting for the presence of camels in parts of Europe and Asia (Ripinsky, 1983). The bactrainus camel occupies the colder areas of Southern Russia, Mangolia, East central Asia and china (Wilson, 1984).

Camel possess certain physiological features that enables him to thrive in extremely arid conditions (Shwartz, 1992) and necessitate to explore his more useful traits which may guarantee the survival of camel as a domestic animal. Like cattle, about 12% of the total body water is contained in the alimentary tract of fully hydrated camel. However, the camels have well adopted water conservation system which facilitates its survival in desert conditions and reduced water availability. The water conservation is controlled through a large number of endocrine cells in the epithelium of fore stomach and highly efficient renal mechanism. Water is continuously re-circulated through blood from the duodenum and colon into the fore stomach. Furthermore, the physiological system of camel is well adapted to high temperature and blood volume is maintained by partial water diversion from skin to other body tissues and organs.

*Trypanosomiasis* (Surra) is the highly damaging infectious disease of camels which is widespread throughout the camel rearing areas. The causative agent of camel Trypanosomiasis is *Trypanosoma evansi* which belongs to subgenus Trypanozoon. The protozoan trypanosome is monomorphic, slender and motile with an average 25 µ lengths and 1.5 µ widths. The body of trypanosome is pointed at both ends, nucleus is centrally located and a well-developed undulating membrane and flagellum are present. *Trypanosoma evansi* was discovered in infected camels and equids from District Dara Ismail Khan, Khyber Pakhtoonkhwa by Griffith Evans in 1880 (Indrakamhang, 1998). Much wider range of hosts like Bactrian camel and dromedaries, cattle, buffalo, horses and pigs are available for trypanosome in Asia.

*Trypanosomiasis* is transmitted from infected camels to healthy camels through the biting of various species of haemtophagous flies like *Tabanus, Stomoxys, Lyperosia* and *Haematobia*. *Trypanosoma evansi* lacks the genes necessary for mitochondrial development and is therefore unable to undergo growth and differentiation in the insect vector. The widespread of Trypanosomiasis (surra) in camels poses a major constraints and economic loses to camel productivity in different parts of the world (Elamin et al., 1999). Available information by different authors on the prevalence of surra caused by *Trypanosoma evansi* in many countries of the world as reported, are: Losos, 1980, Nigeria (27 percent), Chad (30 percent), Dia et al., 1997 Mauritania (24 percent), Pacholek et al., 2001, Niger (29 percent), Njiru et al., 2001, Kenya (28 percent), Ethiopia (21 percent), Jordan (33 percent), Pathak et al., 1999, India (22 percent) Elamin et al., 1999, Sudan (33 percent), Zarif-Fard et al., 2011. Prevalence of trypanosomiasis in province of Punjab, was recorded 4 percent while in other cities of Punjab province, such as Lahore 16.60 percent, Gujranwala 18.2 percent, Sargodha 8.8 percent, Faisal Abad 4 percent and Okara 5.5 percent (Shehzad et al., 2012). While in province of Sind Pakistan13.72 percent (Shah et al., 2004) and in other cities of Sind province such as Hyderabad 2.5, Mirpur Khas 7.5 percent Umerkot 12.5 percent, Badin 15 percent, Thatta 22.5 percent and in Larkana 7.5 percent (Bhutto et al., 2010).

The social and economic importance of Crop diseases is well-documented in the literature and had a profound and well-recognized effect on human welfare and migration patterns throughout history (Apple, 1978). However, economic impact

of livestock disease has still not been thoroughly study. The possible explanation of little knowledge about the economic impact of livestock is the longer period of even more than one year for completion of one round of livestock system, typical wealth value and/or transportation of livestock from one place to another. Furthermore, the income generation through livestock is distributed into a wide range of outputs such as milk, meat, draught/traction power, dung (for fertilizer, fuel or buildings), hides, wool, fiber and animals. Moreover, these outputs may be brought into personal use or sold to generate wealth. In many cultures, the proprietorship of livestock is essential in terms of social status and livestock owners increase the number of animals owned. Furthermore, livestock productivity and reinvestment are considered as a safer investment option in countries with long-lasting inflation problems. These terms of animal productivity and reinvestment can be applied to animals, individual farm enterprises, production systems or entire industries.

Efficiency of the livestock production system is defined as the rate of output (e.g. milk, meat, traction, and manure) divided by the rate of input (e.g. livestock, feed, labor, medicines). Moreover, the animal diseases in a given production system are known to cause reduced productivity. There are various mechanisms by which disease affects animal productivity. The reduced productivity caused by diseases can be directly witnessed by farmers in the form of death of affected animals, low milk production, loss of body condition, low weight gain, loss of body weight ultimately reduces the draught/traction force of the animal and dehydration effects on meat and hide quality. However, there may also be invisible losses such as decreased levels of fertility result in 'calves not born', which in turn alters the herd structure. In some cases herd structure is modified which limit the capacity of the farmer to maintain and improve the herd through selection. The other indirect losses caused by animal diseases may include treatment cost, vaccination and quarantine cost and denial to access better markets because of the presence of. Moreover, the cost of losing an animal because of disease is taken as the market value of that animal.

The value of animal is also lowered if it is sold after death (salvage value) or slaughtered earlier because of sickness. Nevertheless, slaughtering sick animals will reduce the mortality rates and increase off take, which may appear to increase output. Moreover, the disease has a negative effect on the performance of the survived animals in terms compromised fertility, late puberty, low production of milk and meat, decreased draught and traction power and loss of body condition. Keeping in view, the economic losses caused by animal diseases to the livestock and ultimately to national per capita income it is needed to establish and standardize the serological technique for efficient diagnosis and control of *Trypanosomiasis* and to save camel as a valuable livestock asset of Pakistan. Hence, the objective of the current study to record the economic losses due to camel Trypanosomiasis a case study of Musa Khel and Jhal Magsi. Moreover, the rest of the study is organized on the following way. The upcoming section offers methodology of the study while the subsequent section three and fourth proposed results and discussions and then finally conclusion.

## 2. Materials and Methods

The present study was the designed for assessment of camel Trypanosomiasis in two ecological zones i.e. medium upland cold climate (Musa Khel) and low land hot climate (Jhal Magsi) of Baluchistan, to investigate the economic losses due to camel Trypanosomiasis in Musa Khel and Jhal Magsi. The field study was conducted in districts of Musa Khail and Jhal Magsi during year 2011. A total 1040 camel owners were interviewed from both districts during the study period. The questionnaire was especially designed for the current study which includes questions regarding age of respondent, experience, type of community, feeding/watering pattern, prevailing camel diseases, treatment facilities, traditional remedies used against various diseases and economic losses in the area due to camel diseases. The questionnaire was tested in selected camel farmers to remove the ambiguities. Initially all of the animals were clinically examined for any apparent sign of Trypanosomiasis infection. The common signs of infection include high temperature, anemia, depression, dullness, emaciation, edema, abortion, nervous signs, circling movement, trembling, unusual aggressiveness and aimless movements.

The economic losses caused by camel Trypanosomiasis were determined on the basis of farmer's response, mortality and prevalence of the disease in the study area. The estimation of economic losses is presented in Figure 1.

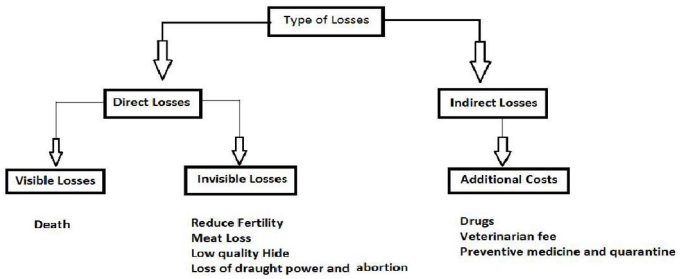


**Figure 1: Economic losses due to trypanosomiasis.**

### *2.1 Statistical analysis*

The data was analyzed using computer software program "SPSS version 17" for calculation of chi square test, mean and standard Error and ANOVA. The Blood parameters for means value were analyzed using means significance difference Duncan's Multiple range test (DMRt) through Costat-2003, Co-Hort, version 6.303 software.

## 3. Results and Discussion

Economic losses were estimated by the prevalence rate of disease, mortality and abortion in the study area. The direct and indirect economic losses caused by camel Trypanosomiasis were investigated in the present study. The direct losses include visible and invisible losses. The mortality rate is the index of visible direct economic loss while the invisible direct economic losses include reduced fertility, meat Loss, low quality of hide, loss of draught power and traction force, change in herd and abortion. However, the indirect economic losses include expenditure of drugs, veterinarian fee and preventive medicine / quarantine.

**Table 1: Approximate direct economic losses due to Trypanosomiasis in Camel in District Musa Khail during 2011.**

| S.No | ARHC (PKR) | Average are of diseased camel (PKR) | Average difference/ losses (PKR) | Annual losses (PKR Millon) |
|---|---|---|---|---|
| Live camel (Diseased) | 100,000 | 40,000 | 60,000*12^ | 5.7 |
| Dead camel | | | | 108 |
| Meat | 87,500** | 78,750*** | 8750*95 | 0.83 |
| Hide | 1000 | 700 | 650*95 | 61750 |
| Abortion | | | 12000*9^^ | 0.10 |
| Total | | | | 8.5 |

**Note:** *(Rs. 350/- per Kg Average 250 kg/animal), ***(10% reduced carcase weight). **Remarks:** ^*prevalence rate (95 out of 800) camels, ^^ 9 cases of abortion were recorded during 2011. Whereas (ARHC), Average rate of Healthy Camel, ARHC), Average are of Diseased Camel.

The average flock size comprised of 17 camels (range 15-20) and estimated mortality rate caused by Trypanosomiasis was 2 to 5 percent. The average mortality caused by Trypanosomiasis was Rs:1.8 million in district Musa Khail (Table 1) and Rs:2.5 million in district Jhal Magsi (Table 3).

**Table 2: Approximate in-direct economic losses due to Trypanosomiasis in Camels in district Musa Khail during 2011.**

| Expenditure | Rate (PKR) | Total (PKR) |
|---|---|---|
| Veterinarian fee @ Rs: 500/- per day for three days | 1500X95* | 142500 |
| Cost of medicine per day @ Rs: 800/- for three days | 2400X95 | 228000 |
| Cost of preventive medicine pour on for flys @ Rs: 3000/- per litre for 100 camels | 3000X8* | 24000 |
| Total | 3900 | 24000 |

According to farmers/butchers perception, the prices of camels infected with Trypanosomiasis reduced from 60 to 75 percent per animal, 10 percent for meat, 50 percent for hide. In addition, the estimated indirect economic losses were about Rs:12000/- per animal. In district Musa Khail, the economic loss caused by trypanosomiasis were Rs: 5.7 million diseased animal, 0.83 million meat losses, Rs:61750/- hide and of Rs:0.10 million due to abortion (Table 1). However, the economic losses caused by trypanomiasis in district Jhal Magsi include Rs:8.22 million diseased animals, Rs:11.9 million meat, Rs:89050 hide and Rs:0.14 million due to abortion (Table 3).

**Table 3: Approximate direct economic losses due to Trypanosomiasis in Camel in district Jhal Magsi during 2011.**

| S. No. | ARHC | ARDC | ADL | AL (Million) |
|---|---|---|---|---|
| Live camel (Diseased) | 100000 | 40000 | 60000X137* | 8.22 |
| Dead camel | | | | 2.5 |
| Meat | 87500^^ | 78750^ | 8750X137** | 11.9 |
| Hide | 1000 | 700 | 650X137** | 89050 |
| Abortion | | | 12000X12** | 0.144 |

**Note:** (ARHC) Average Rate of Healthy Camel (PKR), (ARDC) Average Rate of Diseased Camel (PKR), (ADL) Average Difference/losses (PKR), (AL)Annual Losses Jhal Magsi (PKR, Milliom) ^(10% reducded carcase weight), kg/animal) ^^(Rs. 350/- per Kg Average 250, *prevalence rate (137 out of 800) camels, **25 camels were reported during 211, ***12 cases of abortion were recorded during 2011.

In district Musa Khail, expenditure of drugs used for diseased animal were Rs:142500/-, veterinarian fee Rs:228000/- and Preventive medicine and quarantine Rs:24000/- (Table 2) while in district Jhal Magsi drugs used for diseased animal cost Rs:205500/-, veterinarian fee Rs:328800/- and Preventive medicine and quarantine Rs:24000/- (Table 4).

**Table 4: Approximate in-direct economic losses due to Trypanosomiasis in Camels in district Jhal Magsi during 2011.**

| Expenditure | Rate (PKR) | Total (PKR) |
|---|---|---|
| Veterinarian fee @ Rs: 500/- per Day for three days | 1500X137* | 205500 |
| Cost of medicine per day @ Rs: 800/- for three days | 2400X137* | 328800 |
| Cost of preventive medicine pour on for flys @ Rs:3000/- per litre for 100 camels | 3000X137* | 24000 |
| Per litre for 100 camels | 3900 | 558300 |

The economic losses has estimated by the number of camels were studied i.e. 800 90 camels in each district. The average flock size comprised of 17 camels (ranged 15-20). Previously, average 18 animals per herd were observed in 620 herds from Ethopia, Africa. In present study, the economic losses caused by camel Trypanosomiasis include the direct economic losses due to 2-4 percent mortality (Rs:4.30 million), indirect economic losses comprised of diseased animals Rs:13.92 million, hide Rs:0.15 million, meat losses Rs:12.73 million, abortion Rs:0.252 million, veterinarian fee Rs:0.348 million, medication cost Rs:0.556 million and preventive medication Rs:48000 rupees. In a previous study, economic losses due to Theileriosis in cattles were estimated to be Rs:3.39 million three districts Rawalpindi, Lahore and Multan, Punjab, Pakistan. In another study, the average live and carcass weights of healthy camel were reported 400 and 211 kg respectively (Kurtu, 2004). However, in Kenya, the estimated annual incidence rate was 15 percent and 6.9 percent and mortality rate was 9.9 percent and 5.2 percent due to trypanosomosis in adult and young camels (Mochabo et al., 2005).

The ethno-veterinary practices have been previously reported from urban and peri-urban area of Faisalabad, Pakistan Balochistan, Pakistan (Kakar, 2005), Kenya (Bett et al., 2009) and Al-Showak and Al-Obeid areas of Sudan. The participatory epidemiological techniques were used for the estimation of relative incidence and impact on livelihoods of livestock diseases amongst nomadic pastoralists.

The camel farmers/herders at two study areas i.e., Musakhail and Jhal Maghsi adopted three type of camel rearing systems i.e., Settled, Transhumant and Nomads. Same practices were also observed in four geographically distinct areas in Sudan (Salim et al., 2011) and in Balochistan, Pakistan (Kakar, 2005). The higher setteled and nomads population in Musa khail might be due to owner's own agricultural land and movement of camel flock from Afghanistan, while higher population of transhumant at Jhal Maghsi might be due to low agricultural land. Same study was conducted by in Chad.

The present study showed that settled, transhumants and nomads herders had access to camel treatment facilities from both private veterinary clinics (~42 percent) and government hospitals (33 percent). However, only 25.38 percent population was using the traditional methods for treatment of trypanosomiasis. A similar study conducted in Nigeria reported that 78.9 percent camel herders were using traditional methods of treatment (Chafe et al., 2008). In another study, it was reported that majority of the camel owners (90%) provided health care to their animals using mainly allopathic drugs, traditional healers and medicinal DFF the most common clinical signs of camel Trypanosomiasis observed in the present study included anemia, hyperthermia, edema, dullness and emaciation. A previous study conducted in Sudan also reported the history of intermittent fever, emaciation, oedema, and poor body condition, which were significantly correlated with positive serological status in CATT and trypanosome DNA detection through PCR (Kurtu et al., 2004). Furthermore, in another study showed signs of inappetence, lethargy, going down in condition, urticarial swellings, edema of pads and occasional shivering in effected camels.

## Conclusion

This study was developed to evaluate the current status of Trypanosomiasis in Balochistan, Pakistan. The two districts Musa Khail and Jhal Magsi with thick populations of camels and have different climate and geographical distribution were selected. The settled and transhumants communities had easy access to government hospitals and private veterinary clinics while nomads mostly rely on the use of traditional

veterinary practices. The most common prevailing diseases of camel according to respondents were pneumonia, indigestion, parasitic infestation, mange, lameness, Trypanosomiasis, vector fly and nervous disorders. The most common clinical signs of camel Trypanosomias is hyperthermia, anemia, depression, dullness, emaciation, edema (in dependent parts of body), abortion, nervous signs, circling movements, trembling, unusual aggressiveness and aimless running were recorded. A total of one thousand and forty (n=1040) camel owners/respondents from three groups viz settled, transhumants and nomads were interviewed in Districts, Musa khail and Jhal Magsi during the year 2011. The direct and indirect economic losses according to the prevalence of trypanosomiasis are recognized as mortality, abortions and a camel trypanosomiasis. Furthermore, the current study exhibits that the respondents above the age of 50 years were more experienced in disease diagnosis and use of traditional veterinary practices.

## Authors Contribution

Dr. Ihsanullah Kakar and Dr. Sarwar Khan have designed, analysed, and organised the manuscript. While Khalid Khan and Sajjad helped in estimation, data collection and edited of the manuscript.

*Conflict of interest*

The authors have declared no conflict of interest.

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Research Article

# Impact of Different Integrated Pest Management Modules on Pest Infestation, Pesticide Residue and Yield in Mango Fruits

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**Abstract** | Integrated pest management (IPM) relies on a merger of rational practices and always considered as an effective and environment-friendly approach to managing pests. The present study was conducted at different locations in the Multan District, Punjab Province, Pakistan for two consecutive years 2016-17 to devised four IPM modules (PRMM-1, PRMM-2, PRMM-3, and PRMM-4) for mango to evaluate their impact on insect pests, pesticide residues and yield in mangoes. The modules were based on a combination of different IPM tactics to suppress the pest population with no or minimal use of insecticides. Each module was applied in an area of 0.405 ha of a mango orchard. Fruit samples collected from all modules were subjected to analytical analysis using QuECHERS for sample preparation followed by quantification with GC-ECD. Recoveries of the samples analyzed were ranged from 78-98%. Results revealed that 75.00% samples of pesticide residues mitigation in PRMM-4 followed by PRMM-3 (66.66%), PRMM-2 (33.33%) and PRMM-1 (25.00%). Similarly, the highest number of samples (41.66%) from PRMM-4 exceeded maximum residual limit (MRL) values of the Codex Alimentarius Commission while the lowest (16.66%) were observed in PRMM-1. In addition, all the modules showed a significant difference in pest population reduction of *Bactrocera* spp., *Drosicha mangiferae* and *Idioscopus clypealis*. Although, pesticide contamination was higher in PRMM-1, however PRMM-2 was found best module when compared in terms of pest population reduction (90.79%), average yield and cost-benefit ratio (1:63.28). Conclusively, the pesticide residues can be minimized by applying different control measures with proper integration.

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**Keywords** | Mango, Integrated pest management, Module comparison, Pest population, Pesticide residues

## 1. Introduction

A number of insect pests, fruit flies (*Bactrocera* spp.), mealy bug (*Drosicha mangiferae*) and hopper (*Idioscopus clypealis*) cause both qualitative losses in mango (Peña et al., 2002; Nault et al., 2003). Pesticides are used to reduce yield losses and considered as an economical, labor-saving and efficient contrivance of pest management (Damalas and Eleftherohorinos, 2011). Chlorpyrifos, lambda-cyhalothrin, profenophos, deltamethrin, bifenthrin (Gulzar et al., 2015), neonicotinoids and carbamates are recommended for the management of pests causing damage in mango orchards (Aslam et al., 2004). These pesticides or their residues in mango are potential candidates for health hazards in the

indigenous population of the country (Farooq et al., 2019). Residues of cypermethrin, cyfluthrin, methyl parathion, dieldrin, monocrotophos, and methamidophos have been detected in different varieties of mango (Shah et al., 2007; Khan et al., 2009). Pesticide residue monitoring and risk evaluations are options and imperious steps to muddle through the scenario and mitigate the health risks associated with pesticide use (Handa et al., 1999; Anwar et al., 2011). Different techniques have been used recently for this work (Vidal et al., 2002; Tao et al., 2009). These techniques performed and excellent job in the detection and separation of pesticide residues from fruits and other matrices (Amvrazi and Tsiropoulos, 2009). Similarly, extraction solvents used for these studies also important because these chemicals provide high polarity and high recovery of pesticide residues (Knežević and Serdar, 2009; Wang et al., 2012).

IPM has been experimentally proven to be significantly more effective than conventional methods of pest control such as biological, cultural and chemical alone (Pedigo, 1996) and attributes the least risks while generating higher outputs with the least expenses (Clercq et al., 2011). Considerate and applicable use of economic decisions is significant in crop production while dealing with the pest populations to increase the yield, whereas minimizing the management cost in terms of resources and environmental safety (Baker et al., 2002; Tang and Cheke, 2008). IPM is a long term approach which combines highly compatible pest management tactics (Hassan and Bakshi, 2005; Khan et al., 2010) such as cultural (Charles et al., 2000), physical (Atta et al., 2019a), biological (Atta et al., 2019b; Pickett et al., 2010; Rizwan et al., 2019a; 2019b) and also rational chemical control (Pilgrim et al., 2010) to reach an endurable economic levels of pest populations (Tang and Cheke, 2008). It also attributes the least risks while generating higher outputs with the least expenses (Wright et al., 2005; Clercq et al., 2011). The economic decision levels are the major components of any cost-effective IPM program (van Lenteren and Woets, 1988; van Lenteren, 2000). Compatible pest management measures must be integrated to make IPM programs more effective (Grasman et al., 2001). Therefore, the present field study was performed to investigate the pesticide residues in mango fruits and the integration of different techniques to mitigate pesticide residues and their impact on cost-effective mango production.

## 2. Materials and Methods

### *2.1 Pesticide residue mitigation modules for mango*

Five mango orchards, having commercial mango variety "Chounsa" (heavily infested with *D. mangiferae*, *Bactrocera* spp. and *I. clypealis*) were selected in the Multan district, Pakistan. Four IPM based pesticide residue mitigation modules (PRMM-1, PRMM-2, PRMM-3 and PRMM-4) were designed in comparison to control (Table 1). The IPM module practices were performed on a year-round basis in randomized completely block design (RCBD) with three replications. Conventionally grown mango orchard was also maintained (PRMM-4) with all the regular practices by the farmer while no chemical was applied in control. The pest population was determined from the marked unit area (0.46 m above the ground) on the trunk of the trees for *D. mangiferae*. The number of larvae in the fruits and the number of adults in the traps were recorded for *Bactrocera* spp., while the number of nymphs and adults per inflorescence or pest per sweep were observed for *I. clypealis*. The data was recorded for the year 2016-17. The impact of all modules was observed through pest population reduction at different intervals. Percent population reduction (PPR) over control was calculated using the following formula (Farooq et al., 2019).

$$PPR = \frac{M_2 - M_1}{M_1} \times 100$$

Where;
$M_1$= Average population in treatment; $M_2$ = Average population in control.

### *2.2 Pesticide residue analysis*

A random sampling of mango fruits was performed to obtain 1000g of the sample from each block. Packed and marked fruit samples were transported in ice coolers to the laboratory for analysis (Cook, 2002). Samples were homogenized and only edible parts of the fruits were used for analysis. Samples were stored at -40°C, if the analysis was not performed immediately (Chowdhury et al., 2013).

All solvents and reagents were of HPLC grade such as anhydrous magnesium sulphate ($MgSO_4$), acetonitrile (MeCN), primary secondary amines (PSA) and anhydrous sodium acetate (NaAc) were used for sample preparation. Insecticide reference standards were purchased from SIGMA-

ALDRICH®. Insecticides such as lambda-cyhalothrin, cypermethrin, indoxacarb, imidacloprid, pyriproxyfen, acetamiprid, buprofezine, and chlorpyrifos were analyzed (Qin et al., 2015). The purity of all pesticide standards and other chemicals was not less than 98% (Bakırcı et al., 2014).

For extraction and clean up, QUeCHERS (AOAC) method was followed (Zhao et al., 2007). The 100 µl of internal standard was added in accordance with the target pesticides then 6g of $MgSO_4$ and 1.05g NaOac were added in the homogenized sample in 15 ml vial and shaken with hand for at least a minute. The vial with mixture was centrifuged at 5000 rpm for 5 mins, 1.05 ml of supernatant was taken in the vial containing 2 ml of dispersive SPE (primary and secondary amides and $MgSO_4$). The mixture was shaken with the hand and centrifuge for 5 mins at 10×1000 rpm. A supernatant into a lid vial was left for the overnight centrifuge to dry and 100 µl of acetonitrile was added and vortex to re-suspend the supernatant. The sample was placed in a centrifuge for 1 min to separate any possible solids and was transferred into LC vials for analysis (Anastassiades et al., 2003; Martínez-del-Río et al., 2013; Rejczak and Tuzimski, 2015). Extraction and clean-up were performed using the QuECHERS AOAC method and kits were purchased from Agilent Technologies® for mango (5982-5755+5982-5058) where the first number is the part number for Extraction kit while the latter is kit number for clean-up kit.

Gas Chromatograph equipped with mass spectrometry (MS) was used under specific operational conditions (temperature, flow rate) for optimum behavior and quantitative recoveries (Tao et al., 2009). Residues were identified on the basis of their respective retention times while quantification on the basis of respective peak areas was reported on the basis of sample weight (mg $kg^{-1}$). All spikes and method blank samples were processed through the analytical method (Zhao et al., 2007). Quantification was based on an external standard calculation using the peak area.

For the determination of recovery, precision and detection limits, pesticide-free samples (blank samples) were used (Lehotay, 2007) conducted by analyzing apple matrices as representative matrices for fruits. Samples were augmented five times at 0.05, 0.1 and 0.50 mg $kg^{-1}$. The detection limit was derived from the analysis of 10 independent sample blanks fortified at the lowest concentration for acceptable recoveries (between 78% and 98%) and precision (RSD) lower than 20% (Bakırcı and Hışıl, 2011).

### *2.3 Cost-Benefit Ratio*

Cost of all the inputs (fungicides, insecticides, fertilizers, and irrigation), cost of farm mechanization, packaging, transportation, and labor was estimated. The total revenue was calculated from the yield and market price of the product for all PRMMs. All the values were put into the following formula (Lu et al., 1999) to calculate the cost-benefit ratio (CBR).

$$CBR = \frac{PV_b}{PV_c}$$

Where;

$PV_b$= All cost received including benefits; $PV_c$= All cost spent.

## 3. Results and Discussion

All the modules showed a significant difference in the pest population reduction. PRMM-2 showed maximum population reduction for *D. mangiferae* (91.30%) followed by PRMM-1 (86.27%), PRMM-3 (77.55%) and PRMM-4 (74.46%). Similarly, the maximum population reduction of *Bactrocera* spp. was observed in PRMM-2 (92.85%) followed by PRMM-1 (81.25%), PRMM-3 (76.47%) and PRMM-4 (73.33%). The analysis revealed a non-significant difference between PRMM-1 and PRMM-2 for population reduction of *I. clypealis* and caused 83.33% and 88.23% reduction in the population of *I. clypealis*, respectively. Similarly, PRMM-3 and PRMM-4 showed 73.33% and 68.75% population reduction of *I. clypealis*, respectively in comparison to control (Table 2).

Overall, maximum pest reduction was observed in PRMM-2 (90.79%) in which different control tactics (cultural, mechanical, and attract and kill methods) including pesticides were integrated. While the minimum pest population reduction was observed in PRMM-4 in which farmer practices (pesticides) were applied to manage the pests. PRMM-2 performed 18.61% better than the chemical method in terms of pest population reduction, while in PRMM-1 (cultural + mechanical + attract and kill methods) pest reduction was 11.43% higher over chemical control method which is in common practice by most of the

farmer community in the country (Table 2).

**Table 1: Application of different pest control strategies in pesticide residue mitigation modules in mango orchard.**

| Module | Insect pest control strategy | | | |
|---|---|---|---|---|
| | **Cultural** | **Mechanical** | **Attractants and kill** | **Chemical** |
| PRMM-1 | 1. Plastic sheets of 1.54 m width × length, mounds of plant debris up to 0.46 m high on plastic sheets and without plastic sheets at four directions at 1.85-2.75 m away from the tree, to collect egg carrying female *D. mangiferae*.<br>2. Removal of fallen fruits from the field to stop emergence and re-infestation. | Bands of plastic sheets and 4 cm grease applied at the height of 0.46 to 0.62 on the trunk. Bands were applied on the smooth surface of mud and wet FYM (1:1) to collect egg-laying female *D. mangiferae*. | 1. GF-120 (0.5 L/acre with 4.5 L of water) solution was applied to each second tree and others were skipped. In repeat applications, skipped trees were applied.<br>2. Methyl eugenol + Spinosad (6-8 drops of M.E and 3-4 drops of Spinosad on pluck of cotton and placed in a trap) 6 traps/ acre, traps refreshed at 12-15 days interval. | No application |
| PRMM-2 | -do- | -do- | -do- | Tracer® (Spinosad) at the rate of 10 ml/acre with 100 liters of water. |
| PRMM-3 | No application | No application | Methyl eugenol + Spinosad (6-8 drops of M.E and 3-4 drops of Spinosad on the pluck of cotton and placed in a trap) 6 traps/ acre, traps refreshed at 12-15 days interval. | 1. Confidore® 20% SL (Imidacloprid)200 ml + 100 L water/ acre<br>2. Jatar® 10% EC (Bifenthrin) 20 ml + 100 L water/ acre<br>3. Mospilan® 20 SP (Acetameprid) 150 gm + 100 L water/ acre |
| PRMM-4 | No application | No application | No application | 1. Confidore® 20% SL (Imidacloprid)200 ml + 100 L water/acre<br>2. Jatar® 10% EC (Bifenthrin) 20 ml + 100 L water/ acre<br>3. Diptrex® 80% WP (Trichlorofon) 250 g + 100 L water/acre<br>4. Mospilan® 20 SP (Acetameprid) 150 gm + 100 L water/acre |
| Control | No application | No application | No application | No application |

**Table 2: Percent reduction of pest population in pesticide residue mitigation modules in mango orchard.**

| Pest | IPM Module | | | | |
|---|---|---|---|---|---|
| | PRMM-1 | PRMM-2 | PRMM-3 | PRMM-4 | Control |
| *Drosicha mangiferae* | 86.27 | 91.30 | 77.55 | 74.46 | 0 |
| *Idioscopus clypealis* | 83.33 | 88.23 | 73.33 | 68.75 | 0 |
| *Bactrocera spp.* | 81.25 | 92.85 | 76.47 | 73.33 | 0 |
| Overall Reduction (%) | 83.61 | 90.79 | 75.78 | 72.18 | 0 |

**Table 3: Concentration of Pesticide residues quantified in Mango samples collected from different Modules (Maximum-Minimum).**

| Pesticide | MRLs | PRMM-1 | PRMM-2 | PRMM-3 | PRMM-4 | Control | Recoveries ± SD | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Fortification level (mg/kg$^{-1}$) | | |
| | | | | | | | 0.05 | 0.10 | 0.50 |
| Lambda cyhalothrin | 0.2 | 0.2037-0.0050 | 0.2425-0.0105 | 0.2675-0.0035 | 0.2679-0.0090 | 0.0023-ND | 91 ± 0.52 | 94 ± 0.36 | 90 ± 0.56 |
| Cypermethrin | 0.7 | 0.0706-0.0069 | 0.7622-0.0095 | 0.8264-0.0143 | 0.7953-0.0154 | 0.0203-0.0145 | 90 ± 0.63 | 95 ± 0.17 | 91 ± 0.54 |
| Indoxacarb | 0.02 | ND-ND | ND-ND | 0.0775-0.0196 | 0.0698-0.0052 | ND-ND | 96 ± 0.12 | 94 ± 0.71 | 85 ± 0.52 |
| Imidacloprid | 0.2 | 0.3434-0.0097 | 0.2325-0.0018 | 0.3376-0.0155 | 0.3630-0.0257 | 0.0109-ND | 89 ± 0.10 | 91 ± 0.33 | 92 ± 0.15 |
| Pyriproxyfen | 0.5 | ND-ND | ND-ND | 0.0406-0.0090 | 0.5864-0.0056 | ND-ND | 90 ± 0.56 | 93 ± 0.31 | 95 ± 0.28 |
| Acetamiprid | 0.01 | 0.1596-0.0063 | 0.0140-0.0052 | 0.0250-0.0068 | 0.0315-0.0047 | 0.0145-ND | 94 ± 0.43 | 97 ± 0.27 | 89 ± 0.26 |
| Buprofezine | 0.9 | 0.9152-0.0064 | 0.9193-0.0368 | 0.9391-0.0491 | 0.9650-0.0016 | ND-ND | 95 ± 0.49 | 93 ± 0.64 | 88 ± 0.51 |
| Chlorpyrifos | 0.05 | 0.0534-0.0031 | 0.0622-0.0054 | 0.0620-0.0066 | 0.0570-0.0035 | 0.0096-0.0075 | 96 ± 0.35 | 94 ± 0.09 | 95 ± 0.26 |

**Table 4: Cost-benefit for different pesticide residue mitigation modules in mango fruits.**

| Module | Yield (Kg/Acre) | Marketable yield (Kg/Acre) | Gross return (USD) | Increased yield over control (Kg/acre$^{-1}$) | Value of increased yield over control (USD) | Management cost (USD) | Net profit (USD) | CBR |
|---|---|---|---|---|---|---|---|---|
| PRMM-1 | 18341 | 14880 | 6645.29 | 6160 | 2231.88 | 40.94 | 2191.85 | 53.51 |
| PRMM-2 | 19106 | 16287 | 6922.46 | 7567 | 2741.67 | 42.66 | 2699.93 | 63.28 |
| PRMM-3 | 17280 | 13473 | 6260.87 | 4753 | 1722.10 | 55.08 | 1667.03 | 30.27 |
| PRMM-4 | 16723 | 11945 | 6059.06 | 3225 | 1168.48 | 52.18 | 1116.30 | 21.40 |
| Control | 14545 | 8720 | 5269.93 | - | - | - | - | - |

* 1 USD = 138 PKR (Pakistani Rupees)

The pesticide residue values were compared with MRL values of the Codex Alimentarius Commission. The fruit samples collected from PRMM-4 treated orchard were found highly contaminated (75.00%) with 41.66% samples exceeded MRL, while pesticide contamination in PRMM-1 treated orchard was 25.00% and 8.33% samples were above MRL. Therefore, PRMM-1 samples were the least contaminated with pesticides. PRMM-2 and PRMM-3 showed 33.33% and 66.66% pesticide contamination, respectively. The PRMM-3 was second-most contaminated plot with 33.33% samples exceeding residual limits while PRMM-2 ranked third with 16.66% samples above MRL. The comparison with PRMM-4 (pesticide-treated plot), it was observed that in PRMM-2 was 41.67% less contaminated while PRMM-1 was 50.00% less contaminated with pesticide residues. In addition, the control treatment showed 8.33% contaminated samples with pesticide residues while no sample surpassed the limits. So there was very significant difference in contamination and residual limits in IPM and conventionally grown mango (Table 3; Figure 1). Recoveries for the pesticides analyzed was ranged from 89-96% at 0.05 mg $kg^{-1}$, 93-97% at 0.10 mg $kg^{-1}$ and 85-95% at 0.50 mg $kg^{-1}$ concentration levels with RSD less than 20% (Table 3).

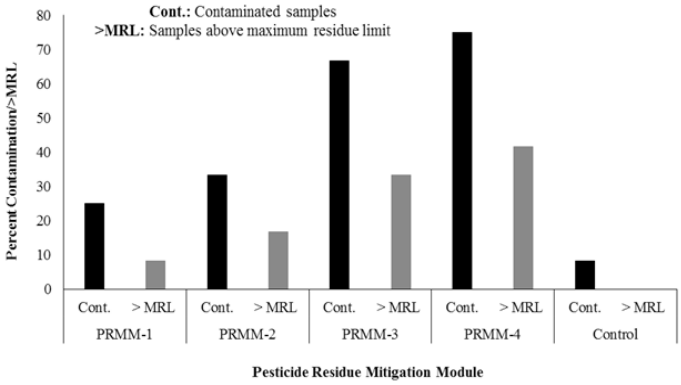


**Figure 1: Comparison of Pesticide residues in Mango samples collected from pesticide residue mitigation modules in mango orchard.**

The application cost of all the inputs (insecticides, fungicides, fertilizers, irrigation, farm mechanization, packaging, transportation, and labor) was calculated for all modules. As the modules were compared for the cost of pest management, therefore, only costs of insecticides and IPM (sanitation, sticky band, slippery band, attract and kill tactics such as methyl eugenol combined with Spinosad and GF-120) were used to determine the CBR for all evaluated modules. The results of CBR indicated that PRMM-2 resulted in 1:64.69 followed by that of PRMM-1 (1:54.75), PRMM-3 (1:30.27) and PRMM-4 (1:21.40) (Table 4).

In this experiment, the mango was grown under four different PRMM. In the present study, the integration of soft insecticides in IPM caused 92.66% reduction in the population of *Bactrocera* spp. This shows that the combinations of multiple control tactics are more reliable for effective pest management in mango. The use of methyl eugenol and protein hydrolysate achieved 83.00% reduction in *Bactrocera* spp. population (Ndiaye et al., 2008). It has been reported the reduction in infestation in *Bactrocera* spp. was observed with cultural (90%), MAT (100%), BAT (60%), cover spray of insecticides (50%) and integration of MAT with cultural method (100%) (Patel at el., 2005). Similar results were observed when methyl eugenol and GF-120 were integrated with spinosad in PRMM-2 (90.79%). A combination of MAT, sanitation (as cultural practice) and methyl eugenol was used to suppress the population of *Bactrocera* spp. in mango (Verghese et al., 2006). The integration of these three control measures resulted in 95.00% reduction in the population of *Bactrocera* spp. as compared to control (67.00% infestation). These results are slightly different from the results of the present study where a combination of sanitation, mechanical, GF-120, methyl eugenol, and cover spray caused 90.79% reduction in the pest population. Differences in the results are attributed to the fact that these modules were implicated to suppress the population of three major pests of mango in comparison to the other studies where a single pest was target. Maximum control of *I. clypealis* was obtained with three applications of thiamethoxam, spinosad, and carbaryl at different rates of application but the yield of the individual tree was not more than 125.36 kg per tree (Kumari et al., 2014) while in case of the present study, maximum yield with PRMM-2 (non-chemical methods) was 516 kg per tree. The only difference between the two approaches was the use of chemicals for the suppression of *I. clypealis* alone (Kumari et al., 2014) while in the present study the objective was to manage all major insect-pests of mango. The minimum rate of return in mango and cashew was 100% using biological and chemical control (George et al., 2013).

The samples from the modules showed a significant

difference in the residual concentrations and number of contaminated samples. Chlorpyrifos showed maximum contamination percentage in PRMM-4 with 42.00% samples above MRL, while the maximum percentage sample concentration above residual limits was 17.00% in PRMM-2. Similar results were reported from 40 pesticides including chlorpyrifos (Sumitra et al., 2006). Likewise, vegetable samples from IPM and non-IPM origin exhibited that 20% samples with IPM origin were found contaminated with pesticide residues in comparison of 47% sample contamination with non-IPM origin (Kumari et al., 2012). These results are slightly different from the present study where percentage of contaminated samples was higher but it is in agreement of the statement that agricultural produce with IPM origin is safer in terms of residues. The deviation in the results may be attributed to the fact that results of fruit samples and vegetable samples are being compared.

The comparative results from IPM and non-IPM orchards revealed that only a few samples from non-IPM grown orchard possessed a concentration of cypermethrin (Singh et al., 2009). During the analysis of 150 peach samples for residues, results showed that no sample from IPM orchard exceeded the MRL values while 7% sample conventional orchard were quantified with a concentration above acceptable limits (Tsakiris et al., 2004). These results clearly indicated the pesticide residues mitigation potential of IPM while the difference in percentage of the contaminated samples may be subjected to the target commodity. A comparative analysis of different models (conventional, IPM and organic) described that 82% conventional samples were quantified with residues, 49% samples with IPM origin were contaminated while 23% samples from organic sources were determined with pesticide residues (Baker et al., 2002). A similar trend was observed in the current study that showed pesticide contamination of 75.00% for conventional, 33.33% for IPM and 8.33% for organic samples.

## Conclusions

The findings of the present study revealed that mango is contaminated with residues of a variety of pesticides collected from different locations in Multan the District, Pakistan. Mitigation of these pesticide residues is possible by minimizing the use of pesticides in the production of mango using nonchemical methods for pest population suppression. A more extensive study is needed for other fruits and vegetables to assess the scenario of pesticide residues and IPM modules needed to be devised and tested to mitigate residues.

## List of Abbreviations

IPM: Integrated pest management; QuECHERS: Quick Easy Cheap Effective Rugged Safe; GC-ECD: Gas Chromatography with Electron Capture Detector; PRMM: Pesticide Residue Mitigation Module; MRL: Maximum Residual Limit; PPR: Percent Population Reduction; $M_1$: Average Population in Treatment; $M_2$: Average Population in Control; HPLC: High Performance Liquid Chromatography; $MgSO_4$: Magnesium Sulphate; MeCN: Acetonitrile; PSA: Primary Secondary Amines; NaAc: Sodium Acetate; AOAC: Association of Official Analytical Chemists; NaOac: Sodium Acetate; SPE: Solid Phase Extraction; MS: Mass Spectrometry; RSD: Relative Standard Deviation; CBR: Cost-Benefit Ratio; $PV_b$: All cost received including benefits; $PV_c$: All cost spent; MAT: Male Annihilation Technology; BAT: Male Annihilation Technology; Cont.: Contaminated samples; ND: Not Detected.

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### *Conflict of interest*

The authors declare that there is no conflict of interest regarding the publication of this article.

## Author's Contribution

MAF designed and conducted the experiment, collected and analyzed the data, and wrote the manuscript. MJA helped in apprehending the idea of this research, designing the layout of the experiment and improving the write-up, format, and language of this manuscript. MDG, BA and AN contributed in data sets for analysis, reviewed the final manuscript

and made the format of this manuscript according to the format of this journal. This final manuscript was ultimately perused, scrutinized and approved for final submission by all the authors.

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Research Article

# Insecticidal Influence of Thiamethoxam and Imidacloprid against *Trogoderma granarium* (Coleoptera: Dermestidae)

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**Abstract** | The khapra beetle is categorized as notorious species, an extended standing species around the world and at the same time it is perceived among the world's 100 most essential intrusive species. Thiamethoxam and imidacloprid are Neonicotinoids with a minute toxicity and they have no teratogenic effects on grains, therefore both are used as cereals protectant against stored grain pests world widely. The recent study was planned to evaluate the efficacy of thiamethoxam and imidacloprid for the control of *Trogoderma granarium* under laboratory conditions. The insecticides were tested at four different concentrations (0.25, 0.5, 1 and 2ppm). Mortality of insects was documented after 24, 48 and 72 hours. Abbot's formula was used to determine the corrected mortality. At 2ppm concentration thiamethoxam and imidacloprid provided 87.57% and 78.59% mean mortality of khapra larvae on treated filter paper after 72 hours respectively. On treated wheat, thiamethoxam provided 82.61% while imidacloprid gave 78.18% mean mortality of khapra larvae at 2ppm after 72 hours. Mortality was dose and exposure dependent. Results demonstrated that thiamethoxam was more lethal as compared to imidacloprid for concern insects.

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## 1. Introduction

The khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae), referred as cabinet beetle and it is notorious around the world for causing damages in stored products and cereals. It is economically important for causing massive damage in stored cereal products and heat of grains provide it a suitable environment. It has voracious feeding habit, but its larvae can live on very low moisture content of grains and can bear starvation period up to three years (Perez-Mendoza et al., 2003). The cabinet beetle is categorized as an extended standing species around the globe, at the same time it perceived as significant intrusive member between hundred worst pest around the globe (Myers and Hagstrum, 2012). It is originated from Indian subcontinent in the 1800's, then from here it spread to more than 40 Asian countries. This beetle transfer to other countries like Europe, Africa and Middle East through shipping of products (Day and White, 2016).

Damaging of stored grains is a serious issue all over the world. Stored grain insects cause 9-20% losses in developed and developing countries, respectively (Philips and Thorne, 2010). Due to insufficient facilities, insect pests cause huge distortion during storage and transporting processes. For the protection

of cereals, proper and accurate technologies are required. In traditional storage process, some pesticides or insecticides are added in powdered form for preventing the grains from pests' activities (Amruta et al., 2015). Thiamethoxam is considered as a chemical and 2nd generation neonicotinoid which act as pesticide for the management of insects and pests (Tomlin, 2003). It shows great water solubility and low in-stability (United States Environmental Protection Agency, 2015). It is enlisted as more effective insecticide in more than one hundred and thirty countries like Russia, Europe, Canada, India, Brazil, USA and Australia (Hilton et al., 2015). It is widely used and formulated for management at the stage of seed sowing, growing up stages and control of stored pests (Rajashekar et al., 2010).

Imidacloprid is broadly used for controlling of few chewing and many sucking insects and acts as stomach, contact and neurotoxic poison (Schmuck et al., 2003). It is nicotinic acetylcholine receptor which is nicotine in nature and acts on the nervous system of insects. Its activity caused blockage in neurons, unconsciousness, lack of coordination, reduced feeding activity, desensitization, can't stabilize their body temperature which ultimately lead them to death. There is no antidot due to which the effects of this insecticides can't be controlled (Kagabu, 2004).

## 2. Materials and Methods

The present research was designed to illustrate the influence of thiamethoxam and imidacloprid on khapra beetle under laboratory conditions of Faisalabad, Punjab, Pakistan.

### *2.1 Experimental layout*

**Rearing of test insects:** The insects were collected from damage areas like storage godowns, grain market and bins of Faisalabad district. Insects were separated from damaged grains with the help of sieve. The population used in the tests were reared under the laboratory conditions. The cultures initially collected from different damaged areas of grain market and released in jars. The *T. granarium* culture was nurtured individually on wheat kernels, with 13% moisture content and 30±2°C temperature at 65±2 relative humidity. The wheat was consumed as foodstuff and nurturing media for test insects. Adults were released in jars and jars were tightly enclosed by muslin cloth by help of elastic bands, to prevent them from outflow. After 3 days, those beetles were sieve out from the culture and it was noted that the wheat had freshly laid eggs and that wheat was put again in the same jars and then placed them in incubator for providing optimum environment for proper growth at 30±2°C and 65±5% RH, to get homogeneous progeny which was used in different bioassays.

### *2.2 Bioassay*

The insecticides were tested on both filter paper and wheat kernels. Experiment was performed in incubator set at 30±2° and 65±5% R.H. Formulations of thiamethoxam and imidacloprid were tested at the doses of 0.25, 0.5, 1 and 2ppm which were prepared with distilled water along with control. Then each dose was applied on filter paper and wheat by using micro pipette separately. Each concentration was sprayed on 20g of fresh sterilized wheat and leave it to dry well to evaporate the moisture. The petri dishes (9cm diameter) were used with treated filter paper and wheat respectively. Twenty insects were introduced individually in petri dishes. The petri dishes were covered with tape to prevent the insects from escaping. After the exposure of individuals with treated insecticides, the mortality rate was noted after 24, 48 and 72 hours. The collected figures were resulted through statistics.

### *2.3 Statistical analysis*

The mortality data of insects was documented after 24, 48 and 72 hours. The percent mortality was corrected by using Abbott's formula (Abbott, 1987). The data was analyzed statistically using statistical software 8.0 and 8.1 (Lewicki and Hill, 2006). ANOVA techniques were applied to determine the corrected mortality data, whether the effects of treatment vary significantly. Tuckey HSD test at α 5% was used for comparing the means of comparison (Tuckey, 1949).

## 3. Results and Discussion

### *3.1 Mortality of Trogoderma granarium larvae on treated filter paper*

The research was documented to check the insecticidal effects of thiamethoxam and imidacloprid on khapra beetle. The experiments were carried out under the layout of CRD. Each treatment was repeated three times including control test. Main effects and related interactions for mortality of khapra larvae are presented in Table 1. All main effects (time, insecticide and concentration) and their interaction

effect showed the significant results concerning mortality of khapra larvae.

**Table 1: ANOVA of main effects and related interactions for mortality of khapra larvae on filter paper (error df = 48).**

| Homogenous larvae | | | |
|---|---|---|---|
| **Source** | **Df** | **F** | **P** |
| Time | 2 | 1572.32 | 0.000000 |
| Insecticide | 1 | 593.37 | 0.000000 |
| Concentration | 3 | 786.68 | 0.000000 |
| Time × Insec. | 2 | 2.32 | 0.108822 |
| Time × Conc. | 6 | 1.55 | 0.183974 |
| Insec. × Conc. | 3 | 0.73 | 0.539301 |
| Time × Insec. ×Conc. | 6 | 4.58 | 0.000950 |

Table 2 presented that thiamethoxam provided 27.73% mortality after 24 hours of application at 0.25 ppm concentration. Mean percentage mortality vary significantly from each other. 58.60% was the maximum recorded mortality after 24 hours of application. Similarly, mortality was increased significantly after 48 hours of exposure, provided 43.19% least mortality while the highest observed mortality was 70.66% at the dose rate of 0.25 and 2 ppm respectively. After the application period of 72 hours the maximum noted mortality was 87.57% at the highest dose rate of 2ppm. Lowest observed mortality was 58.60% at low dose rate that is mentioned above. On the other hand, mortality by imidacloprid at different conc. levels were significant. Imidacloprid showed 43.04% maximum mortality after 24 hours at high dose rate followed by 16.18% mortality at low dose. After 72 hours of exposure the maximum mortality was 78.59% whereas the lowest mean mortality was 50.87% (Table 2). Conc. showed the highly significant results about the larval mortality. Results exhibited that rise in mortality values was due to increase in doses of pesticides.

**Table 2: Mean percentage mortality (±SE) of *Trogoderma granarium* larvae on treated filter paper for 24, 48 and 72 hours with four dose rates of thiamethoxam and imidacloprid.**

| | | Exposure hours | | | | |
|---|---|---|---|---|---|---|
| | | **24** | **48** | **72** | | |
| **Insecticide** | **Dose (ppm)** | | | | **F** | **P** |
| Thiamethoxam | 0.25 | 27.73 ± 0.96 cf | 43.19 ± 1.158 be | 58.60 ± 0.94 ad | 225.41 | >0.01 |
| | 0.5 | 33.75 ± 1.23 ce | 55.05 ± 1.44 bd | 67.26 ± 1.34 ac | 159.00 | >0.01 |
| | 1 | 48.52 ± 0.96 cb | 65.07 ± 1.07 bb | 76.47 ± 1.49 ab | 136.96 | >0.01 |
| | 2 | 57.80 ± 1.23 ca | 70.66 ± 0.24 ab | 87.57 ± 0.86 aa | 287.29 | >0.01 |
| Imidacloprid | 0.25 | 16.18 ± 1.20 cg | 32.82 ± 0.90 bf | 50.87 ± 0.57 ac | 249.01 | >0.01 |
| | 0.5 | 27.98 ± 1.51 cef | 40.92 ± 0.95 be | 57.40 ± 1.16 ad | 143.79 | >0.01 |
| | 1 | 37.54 ± 0.84 ccd | 50.87 ± 0.94 bd | 68.81 ± 1.03 ac | 277.00 | >0.01 |
| | 2 | 43.04 ± 1.51 bcc | 60.31 ± 0.42 bc | 78.59 ± 1.31 ab | 225.78 | >0.01 |
| F | | 4.78 | 2.57 | 2.15 | | |
| P | | 0.01 | 0.09 | 0.13 | | |

Means followed by the same letter(s) within each row are not significantly different (df: 2, 23, Tuckey HSD test at P < 0.05). Within each column, means are followed by the same letter(s) are not significantly different (df: 7, 71, Tuckey HSD test at P < 0.05).

**Table 3: ANOVA of main effects and related interactions for mortality of khapra larvae on treated wheat kernels (error df = 48).**

| Homogenous larvae | | | |
|---|---|---|---|
| **Source** | **Df** | **F** | **P** |
| Time | 2 | 1898.61 | 0.000000 |
| Insecticide | 1 | 713.07 | 0.000000 |
| Concentration | 3 | 1319.77 | 0.000000 |
| Time × Insec. | 2 | 20.06 | 0.000000 |
| Time × Conc. | 6 | 3.15 | 0.010866 |
| Insec. × Conc. | 3 | 6.19 | 0.001218 |
| Time × Insec. ×Conc. | 6 | 5.15 | 0.000372 |

### *3.2 Mortality of T. granarium on treated wheat*

Main effects and related interactions for mortality of khapra larvae are presented in Table 3. All main effects (time, insecticide, concentration) and their interaction showed the significant results about mortality of khapra larvae.

Results revealed that after 24 hrs the highest mortality was 57.98% followed by the insecticide imidacloprid 49.66%. Similarly, after 48hrs thiamethoxam exhibited 74.44% whereas, imidacloprid provided 64.48% mortality. The result of mortality after 72hrs of application exhibited that thiamethoxam gave

**Table 4: Mean percentage mortality (±SE) of *Trogoderma granarium* larvae on treated wheat grains for 24, 48 and 72 hours with four dose rates of thiamethoxam and imidacloprid.**

| | | Exposure hours | | | | |
|---|---|---|---|---|---|---|
| | | 24 | 48 | 72 | | |
| **Insecticide** | **Dose (ppm)** | | | | **F** | **P** |
| Thiamethoxam | 0.25 | 35.08 ± 0.90 cd | 46.10 ± 0.89 bd | 52.43 ± 0.82 ae | 100.55 | >0.01 |
| | 0.5 | 41.35 ± 0.62 cc | 56.74 ± 0.18 bc | 65.79 ± 0.87 ad | 386.96 | >0.01 |
| | 1 | 48.87 ± 0.94 bc | 63.81 ± 1.02 bb | 72.33 ± 0.87 ac | 155.44 | >0.01 |
| | 2 | 57.98 ± 0.65 ac | 74.44 ± 0.74 ab | 82.61 ± 1.08 aa | 217.93 | >0.01 |
| Imidacloprid | 0.25 | 18.63 ± 0.87 cf | 36.03 ± 0.75 be | 46.82 ± 0.80 af | 305.88 | >0.01 |
| | 0.5 | 30.97 ± 0.93 ce | 46.60 ± 0.71 dd | 56.02 ± 1.12 ae | 181.05 | >0.01 |
| | 1 | 37.65 ± 0.36 ccd | 58.18 ± 1.00 bc | 66.64 ± 0.63 ad | 434.98 | >0.01 |
| | 2 | 49.66 ± 0.87 bc | 64.48 ± 0.62 bb | 78.18 ± 0.74 ab | 356.62 | >0.01 |
| F | | 9.47 | 3.98 | 3.48 | | |
| P | | 0.00 | 0.02 | 0.04 | | |

Means followed by the same letter(s) within each row are not significantly different (df: 2, 23, Tuckey HSD test at P < 0.05). Within each column, means are followed by the same letter(s) are not significantly different (df: 7, 71, Tuckey HSD test at P < 0.05).

maximum mortality 82.61% and 78.18% was lowest in case of imidacloprid at high dose rate (Table 4). The results respond that mortality rate increased significantly due to rise in dose rate and exposure period.

Thiamethoxam and imidacloprid are broadly utilized for seed coat treatment against various insect pests (Khan et al., 2015). The insecticide usage is effective for enhancing agriculture production, control of pests, diseases, human interruption to stored products and effects of environmental conditions during storage process (Pynenburg et al., 2011; Castro et al., 2009; Elbert et al., 2008; Sirchio and Sutton, 2007; Kagabu, 2004; Schmuck et al., 2003). Maienfisch et al. (2001) examined thiamethoxam as a commercial neonicotinoid pesticide. It worked via make bond with nicotinic acetylchlonic receptors and showed outstanding systematic features for management of commercially significant pests. It was introduced for treatments of seeds in horticulture crops, for soil and foliar applications throughout the world.

The highest 87.57% larval mean percent mortality was observed at highest conc. (2ppm) followed by 72.33% at 1ppm, 65.97% at 0.5ppm and 52.43% mortality was noted at 0.25ppm in case of thiamethoxam. Imidacloprid gave 78.18% at 2ppm, 66.64 at 1ppm, 56.02 at 0.5ppm and 46.82% mortality at 0.25 ppm on treated filter paper. In case of thiamethoxam, maximum recorded mortality on treated wheat was 82.61% followed by 72.33%, 65.79% and 52.43% at dose which mentioned above. On the other hand, imidacloprid provided 78.18% at 2ppm followed by 66.64% at 1ppm, 56.02% at 0.5ppm and 46.82% mortality at 0.25ppm. In both experiments, thiamethoxam gave the highest mortality than imidacloprid. Filter paper larval mortality of *T. granarium* was highest than wheat mortality.

In this study the other investigated feature was the effect of exposure period on the larval mortality of khapra beetle. The mortality attained for thiamethoxam after application period of 72hrs was maximum against khapra beetle. The interaction effect between insecticides and concentrations were also investigated that presented the highly significant results up to 72hrs of application period. The results of this study were similar with Athanassiou et al. (2015) which concluded that increase in conc. and exposure period increased the mortality of *T. granarium*. Results were significant when different concentrations were applied on test insects. Mortality of test insect was increased by increasing different concentrations of insecticides. In our experiment thiamethoxam provided the high larval mortality of *T. granarium*. Sur and Stork (2003) explained the role of imidacloprid as a protectant in plants. It was uptake, metabolized and translocated by plants. It applied on seeds during harvesting stage.

## Conclusion

From these results we concluded that thiamethoxam and imidacloprid have the significant results against

the control of *T. granarium* but thiamethoxam is more lethal than imidacloprid and can be used for the complete control of *T. granarium*.

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## Author's Contribution

This work was carried out in collaboration among all authors. Authors HR and NAK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AL, WB, and KR managed the analyses of the study. Authors HR and NAK managed the literature searches. All authors read and approved the final manuscript.

*Conflict of interest*

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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Research Article

# Biology of Diamondback Moth, *Plutella xylostella* (Lepidoptera: Plutellidae) of Cauliflower under Laboratory Conditions

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**Abstract** | Diamondback moth, *Plutella xylostella* is the serious and cosmopolitan pest for crops and vegetables especially cauliflower all over the world. The larvae feed on foliage and cause severe damage. Present study was carried out under laboratory conditions to elicit information about every stage like egg, larva, pupa and adult of *Plutella xylostella* on natural diet. The study revealed that incubation period of eggs varies from 3.2-4.3 hours. There are four larval instars of *Plutella xylostella*. The first, second, third and fourth instar larva survived for 3-5,3,1-4 and 2-3 days respectively while pupal and prepupal period lasted for 5-3 days, respectively. Adults lived for 4 to 5 days and life period under laboratory condition varies from 13 to 23 days.

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## 1. Introduction

Cauliflower (*Brassica oleracea*) is an important winter vegetable and known as the main source of income for the farmers in the world and especially in Pakistan (FAO, 2008). The vegetables belonging to family Brassicaceae have medicinal value having glucosinolates and anticancer substances used for the treatment of cancer (Cohen et al., 2000; Lampe and Peterson, 2002; Keck and Finley, 2004). It is an important component of human diet (Shelton, 2004) in temperate and tropical regions of the world. There are various limiting factors including biotic and abiotic factor but the insect pests are the major reason for limiting cauliflower production (Nyambo and Pekke, 1995) also the quality of crop is affected during severe infestation of pests (Sánchez and Vergara, 2014). Insect pests and diseases which attack on cauliflower are army worm, aphids, cabbage worms, flea beetles and club root and black rot, respectively (Nyambo and Pekke, 1995).

Among the insect pests, Diamondback moth, *Plutella xylostella* (Linn.) (Lepidoptera: Plutellidae) is the serious, cosmopolitan pest of cauliflower and caused about 90% damage globally (Verkerk and Wright, 1996; Sarfraz et al., 2006; Tufail et al., 2008; Sandström et al., 2011; Karlsson et al., 2013; Furlong et al., 2013). This pest being the main cause of low production (Talekar and Shelton, 1993; Sarfraz et al., 2006) is distributed throughout the world especially in those places where crucifers are cultivated (Shelton, 2001).

Majority of crops like radish, cabbage, broccoli, turnip, cauliflower and many others are the hosts of Diamondback moth (DBM) (Talekar and Shelton,

1993). When host is unavailable cruciferous weeds are also attacked by this pest. The host's leaves, seed pods, flowers, buds and outer layer of stem is attacked by the pest (Oke, 2008). During earlier stages of crops, larvae of DBM mine the crops and later stages feed on the leaves. Irregular patches appear on the leaves due to severe attack of larvae and 62 to 78 % leaves are consumed by a single larva (Gangurde and Wankhede, 2009). Whole leaf is fed by larvae except veins, last instars are voracious feeders than earlier instars (first three) giving the leaves a sieve-like appearance. Plant growth is stunted due to excessive feeding of larvae of DBM which attack from seedling to harvesting stage resulting in the reduction of quantity as well as quality of crops (Gangurde and Wankhede, 2009). Last three stages feed on the plant surface and are mostly found inside the leaves. The developmental time depend upon the abiotic factors like temperature and humidity. The rate of development is faster in warm and slower in cool conditions. Generations of DBM overlap and all stages are present in the field during warm temperatures (Zhu et al., 2018).

First time DBM was originated from North America in 1854 (Saravaiya and Patel, 2005). DBM has been considered as a periodic migrating pest and became an important pest for farmers all over the world (Sandström et al., 2011; Karlsson et al., 2013). The majority of farmers plough down their fields due to severe losses caused by DBM. In tropical and subtropical regions of the world, more than 10-21 generations are reported in a year (Oke et al., 2010) while these are 4 - 20 in temperate regions (Vickers et al., 2004). There are 7-14 generations of this pest in Pakistan and India (Talekar and Shelton, 1993; Vickers et al., 2004).

This insect is an important pest of all crucifers globally especially in southern parts of Pakistan (Abro et al., 1992). Economic threshold level of DBM is 0.05/plant (Verkerk et al., 1957). It has been reported that pest cause about 100% yield losses (Abro et al., 1994) while 90% losses in Pakistan (Verkerk and Wright, 1996).

Due to importance of this pest, the current study was carried out to determine the biological cycle of DBM under laboratory conditions.

## 2. Material and Methods

### *2.1 Rearing of diamondback moth*

The biological study of DBM was carried out under laboratory condition at 26 ± 5°C and 60% temperature and relative humidity, respectively, in MNS-University of Agriculture, Multan, Pakistan during 2019. The adults and fourth instar larvae were collected from nearby cultivated cauliflower fields and released with cauliflower leaves and cotton swab with 10% sugar solution into plastic jars, top of which was covered with muslin cloth for aeration and further egg laying.

After egg laying, eggs were collected from jars with the help of forceps and camel hair brush and shifted into petri dishes for biological records. For this purpose, 30 petri dishes were used and one egg was kept in each petri dish. After hatching, new and fleshy leaves were provided to larvae on daily basis for pupation. Newly emerged adults were counted and shifted into new jars for further reproduction. The culture was multiplied and maintained for three generations. All the information such as egg to larva and pupa to adult of pest was recorded during the whole study.

## 3. Result and Discussion

### *3.1 Biology of DBM*

During the experiment all four stages of the DBM were observed *i.e.*, egg, larva, pupa and adult. Quality and quantity of cruciferous crops reduce due to severe attack and feeding of first instar larvae of *P. xylostella* from seedling to harvesting of crops (Gowri and Manimegalai, 2016). The wing span (length and width) of each female and male was different from each other. It has been observed that female wing span was larger than males.

The descriptions of the recorded stages have been provided below.

#### *3.1.1 Adult*

Adults were grayish brown in color with distinct antennae. These were small, slender and 6 mm long. There was no difference in length between both sexes (male and female). Adult male and female longevity was 9.0±0.69 and 13.0±0.73 days, respectively (Åsman et al., 2001). The moths were weak fliers and active at both, night and dusk. The wings length of female was larger than male. The mating mostly occurs during

dusk. After mating as host available female laid eggs on the same day of mating (Åsman et al., 2001). The similar results had been reported by earlier studies (Gowri and Manimegalai, 2017).

### *3.1.2 Egg*

Within few hours of emergence, mating takes place which continued about 1-2.5 hours. Males mate more than two times while females only once in their life cycle (Wang et al., 2005). Eggs were yellow and pale green in color. During the study it has been observed that female laid about 200-210 eggs on the lower surface of leaves, for protection from wind and rain (Talekar and Shelton, 1993). The similar findings had been observed by other scientists (Justus et al., 2000) whereas our findings are dissimilar with the findings of Yamada and Kawasaki (1983). Female lays eggs up to10 days after emergence while reported by Yamada (1978) that female lays egg up to 5 days after emergence, which is dissimilar to our findings. The eggs were laid on the groove surface of the leaves not on the smooth surface of plants (Silva and Furlong, 2012). Eggs can be seen with the help of hand lens (CABI, 2015). The female of diamondback moth laid eggs singly or in small group near the midrib or on the lower surface of leaves or container wall surface. The incubation period of eggs varied from 3.2-4.3 days with average of 3.5± 0.54 days as represented in Table 1, while incubation period observed by other scientists was 2 days (Gowri and Manimegalai, 2017), 3 to 4 days (Gangurde and Wankhede, 2009), 3.33 ± 0.42 days (Dhaduk, 2007) and 3.0 to 5.25 days (Ramegowda et al., 2006) as mentioned in Table 1. Variations in incubation period were due to various factors like food and climate change.

**Table 1: Incubation period of eggs.**

| Number of eggs observed | Incubation period (Days) | | |
|---|---|---|---|
| | Min. | Max. | Ave. ± SD |
| 30 | 3.2 | 4.3 | 3.51± 0.54 |

### *3.1.3 Larva*

There were four larval instars of DBM. The newly emerged larva was very small in size. The head of newly hatched larvae was pale brown while fully grown caterpillar was light green and 10 mm long in length. The length and width of head capsule was 1.46 mm and 1.55 mm. The newly hatched larvae made very small holes on the plants leaves (Nirmala and Desh, 1995) like mines. The first instar larvae were leaf mining and difficult to see due to small in size. The first instar larvae were small about 0.05-0.1 cm in length and after 5-6 days change into second instar as shown in Table 2. The similar results had been reported by other scientists (Sharma et al., 1999; Kumar et al., 1999; Dhaduk, 2007). The sparse short erect hairs are also present on the whole body of the larval instars with small white patches. The five pairs of prolegs are also present in the newly hatched larvae. The second instars are very active and larger as compared to first and change into third instar after 3-4 days. The body and head capsule color of the larva were yellowish green and light brown, respectively and the width and length of head capsule was 3.12 mm and 2.65 mm. The second instar takes 3-4 days for its development into third instar larvae which are light yellow in color. The third instar feed more vigorously than first and second instar and after 4-5 days change into fourth instar. Head capsule of the larvae was 4.56 mm and 4.32 mm in length and breadth, respectively as shown in Table 3. There were similar habits of feeding in third and second instars but stop the feeding during pre-pupal stage. The fourth instar larvae were dark green in color, length and width of head capsule of the larvae was 4.99 mm and 4.98 mm. Our findings were similar with earlier studies (Capinera, 2000).

**Table 2: Life cycle of diamondback moth, *Plutella xylostella* (Linn) on cauliflower under Laboratory condition.**

| Objects | Laboratory condition (26±5 C° and 60% R.H) |
|---|---|
| | Life cycle (Days) |
| 1st instar | 5.65 ± 0.62 |
| 2nd instar | 3.57 ± 0.74 |
| 3rd instar | 4.45 ± 0.65 |
| 4th instar | 3.78 ± 0.96 |
| Total | 15.89 (12-17) |
| Pupal period (days) | 4.77 ± 1.13 |
| Pre-oviposition period | 0.50 ± 0.24 |
| Oviposition period | 17.30 ± 0.81 |
| Post-oviposition period | 4.12 ± 1.12 |
| **Adult longevity (days)** | |
| Male | 11.0 ± 0.88 |
| Female | 13.0 ± 0.92 |

### *3.1.4 Pre pupa and pupa*

There were two inactive phases of this pest i.e., pupa and prepupa. During the pre-pupal stage, larva showed sluggish movement and reduce the feeding which last for about 1 to 3 days with an average of

1.45 ± 0.65 days. At last, pre-pupa changed into pupal stage which last for 1 to 3 days (Stapathi, 1990; Kandoria et al., 1994; Kapadia and Koshiya, 1999; Gowri and Manimegalai, 2017) as describe in Table 4. Our current findings were similar with other studies (Ahmad et al., 2008; Ahmad et al., 2011).

**Table 3: Average of head capsules length and width of different larval instars.**

| Stages | Head capsule | |
|---|---|---|
| | Length (mm) | Width (mm) |
| First instar | 1.46 | 1.55 |
| Second instar | 3.12 | 2.65 |
| Third instar | 4.56 | 4.32 |
| Fourth instar | 4.99 | 4.98 |

**Table 4: Pre-pupal and pupal period of DBM.**

| Number of larva | Pupal period | | | Pre-pupal period | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Ave. ± SD | Min. | Max. | Ave. ± SD |
| 30 | 3.50 | 5.50 | 5.05 ± 0.56 | 1.00 | 2.50 | 1.75 ± 0.65 |

## Conclusion

*P. xylostella* is a serious threat to successful prophecy of cruciferous vegetables. knowledge of the biology of Diamondback moth effects the host plant quality and helps in the management of this insect.

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## Author's Contributions

MR planned, conducted the experiment, recorded the data and wrote the manuscript. UNU provide technical guidance and reviewed the manuscript. ZR helped in data recording. GM and SHMB helped in data analysis.

*Conflict of interest*

Authors have no conflict of interest.

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Research Article

# Feeding Potential of *Chyroperla carnea* on *Myzus persicae* (Sulzer) under Laboratory Conditions

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**Abstract** | *Chrysoperla carnea* is a major predator of soft bodies insects like aphid, white fly and thrips. The feeding potential of *Chrysoperla carnea* larvae on different nymphal instars of *Myzus persicae* was investigated in ambient laboratory conditions at University of Agriculture, Faisalabad. *Chrysoperla carnea* was found very active and consumed all stages of aphid. The predation rate of *Chrysoperla carnea* was increased with increase in larval instars (1st to 3rd). Third instar of *Chrysoperla carnea* was very voracious feeder and fed large number of aphid instars nymphs (1st, 2nd and 3rd). The larval predatory potential was 413.9±0.07 aphid per larvae. The current study results revealed that *Chrysoperla carnea* has great potential for biological control of aphid.

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## 1. Introduction

Biological control is playing key role in insect pests management from last two decades. The biological agents (predators) belong to insect orders like Coleoptera, Diptera, Himeptera, Neuroptera and Hymenoptera that are used exclusively for pest management and feed on larval as well as adult stages. Man is exploiting, commercially rearing these predators and released to the agricultural fields for better efficiency.

Several studies have been conducted on different regions of the world related to biology of *Chrysoperla carnea* (Eraky and Nasser, 1993; El-Hag and Zaitoon, 1996). The biological agents such as *C. carnea* and many others have been released in filed for pest control especially aphid species. Neuropteran predators, *Chrysoperla carnea* is a polyphagous and major predator of soft bodies insects such as aphid, thrips, mealy bug, mites, whitefly and many other orthropods (Carrillo and Elanov, 2004; Shalaby et al., 2008; Yuksel and Goemen, 1992; Singh and Manoj, 2000; Venkatesan et al., 2002, 2000; Zaki and Gesraha, 2001). Adults of *C. carnea* feed on flower nectar, pollen and honey dew excretion during aphid sucking cell sap (Saminathan and Baskaran, 1999; Kareim, 1998). Genus *Chrysoperla* belongs to family, Chrysopidae and order Neuroptera contains many important species of predatory insects of which the green lacewing, *Chrysoperla carnea* (Stephens) is one of them.

*C. carnea* is now commonly reared in laboratories and used extensively all over the world (Liu and Chen, 2001; Balasubramani and Swamiappan, 1994;

Tauber et al., 2000). It has significant potential for commercialization and use against a variety of crop pests in combination with other insect pest management tactics (Atakan, 2000; Sengonca et al., 1995; Daane et al., 1996; Legaspi et al., 1996). Therefore, the current work was undertaken to check the feeding potential of larvae to prey like aphids.

## 2. Materials and Methods

### *2.1 Study area*

An experimental study was conducted to check the feeding potential and developmental period of *C. carnea* larvae fed on *Myzus persicae* during 2018 under laboratory conditions at 26±5C° temperature and 60% relative humidity (RH) in University of Agriculture, Faisalabad.

### *2.2 Culture maintenance of green peach aphid in green houses conditions*

Under the glass house conditions, the culture of green peach aphid (*Myzus persicae*) was sustained on okra plants. For aphid culture maintenance the seeds of okra variety sabaz pari were sown, under glass houses. Aphids were collected from nearby okra fields and released on sowing okra plants inside the glasshouse. The aphid population was multiplied freely and the colony was established.

### *2.3 Mass rearing of chrysoperla carnea under laboratory*

For mass rearing purpose, adults of *C. carnea* were collected from nearby cabbage fields. The collected adults of *C. carnea* were reared on artificial diet such as yeast + sugar + honey + distilled water in ratio of 8:4: 2:1. Adults was reared in a rectangular cage with 5cm thick and transparent plastic sheet. A black granulated paper was inserted inside the cage as substrate for oviposition. On daily basis, eggs were collected with the help of razer and placed into plastic jars for hatching.

Newly hatched 1st instars larvae of *C. carnea* were collected from the culture with help of camel hair brush and released into plastic containers containing the counted number of 1st and 2nd instar of *M. persicae* nymphs while 2nd and 3rd instars of *C. carnea* were fed with mixed instars of nymphs. First instar of *C. carnea* was provided 15 green peach aphid nymphs. The number of aphids for *C. carnea* feeding were increase gradually (20, 25, 30…60 and 65 respectively) each day, till the larvae reached to pupal stage. Feeding potential was observed after every 24 hours.

### *2.4 Statistically analysis*

The mean data were statistically analyzed using one-way ANOVA and standard error tests.

## 3. Results and Discussion

The various insect pests such as sucking and chewing attack on the different crops parts from sowing to harvesting. Among them, aphid species are important pest of various crops such as cabbage, cotton and potato etc. (Sattar and Abro, 2011).

Green lace wing is known as aphid lions with golden eyes and widely distributed in all agricultural habitats. Green lace wing, *C. carnea* is consumed soft bodies insects such as all stages of aphid (Hoffmann and Frodsham, 1993). Aphid is the major prey of *C. carnea* (Balakrishnan et al., 2005; Chakraborty and Korat, 2010).

The study revealed that the predatory performance of *C. Carnea* enhanced with the growth of grub. As the grub grew from first instar to third instar, the feeding capacity improved in all the species of aphids used as prey (Saminathan et al., 2003; Jagadish and Jayaramaiah, 2004; Krishnamoorthy and Mani, 1982; Megahed et al., 1984). Rabinder et al. (2008) reported that aphid is the major prey of *C. carnea.* The current study was resulted that all three larval instars of *C. carnea* were good predator of aphids. The results indicate that 3rd instar larvae were more voracious than the first two instars. The mean feeding potential of 1st, 2nd and 3rd instars were 60.80±1.816, 110.09±5.98 and 237.20±19.511 aphids, respectively. The similar findings have been reported by earlier studies (Khan et al., 2013). Feeding Potential of *C. carnea* larval instars on *Myzus persicae* was given in Figure 1.

The first, second and third larval instars of *C. carnea* were fed an average of 11.48, 79.52 and 83.00 aphids respectively (Singh and Manojkumar, 2000) while (Singh and Hamid, 1998) reported that the *C. carnea* consumed an average of 21.68, 76.92 and 160.92 cabbage aphids in its first, second and third instar larva, respectively. The similar findings have been observed by other researchers (Rana and Srivastava, 1998).

Another study was carried out in 2010, to check the effect of different temperatures on the consumption capacity of *C. carnea* on four aphid species such as *Aphis craccivora* Koch, *Aphis gossypii* Glov, *Myzus persicae* Sulz and *Lipaphis erysimi* Kalt (Renu and Pathak, 2010). The study revealed that capacity of feeding varies with respect to different temperatures. The environmental conditions like temperature and relative humidity (RH) were played key role in feeding behavior. The feeding capacity of *C. carnea* was increased with increase and decrease in temperature, prey density and relative humidity (RH) respectively.

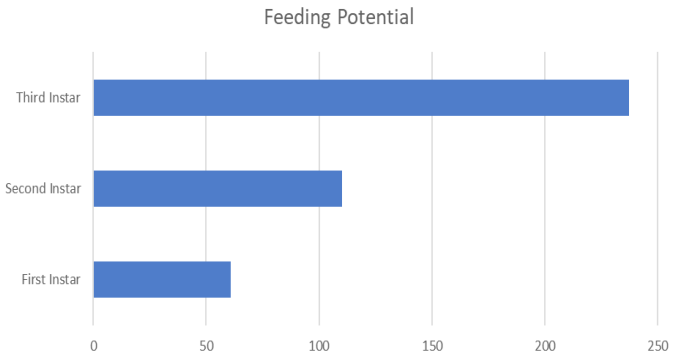


**Figure 1: Feeding potential of *C. carnea* larval instars on *Myzus persicae*.**

## Conclusions and Recommendations

The rearing of green lacewing, *Chrysoperla carnea* (Stephen) is prove an effective strategy for management of many pests such as whitefly, aphids, thrips, coccids, mites, mealy bugs, lepidopteran eggs and a variety of other slow or non-moving soft-bodied arthropods.

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## Author's Contribution

MR wrote the manuscript, GM and MF conducted the study, MWS, MAR and SU critically reviewed the manuscript.

*Conflict of interest*

Authors declared no conflict of interest.

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Research Article

# Allelopathic Effect of Weed Species on Germination and Seedling Traits of Wheat Varieties

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**Abstract** | Wheat productivity and quality is significantly impaired by weeds' infection which compete for water, nutrients and sunlight. This study was conducted to determine the allelopathic effect of weed species on germination and seedling traits of wheat (*Triticum aestivum* L.). Wheat varieties Amber and TJ83 were subjected to powder of *Chenopodium album*, *Convolvulus arvensis* and *Avena fatua* under three different treatments i.e. 25, 50 and 75g. The effect of these weed powders on seed germination (%), shoot length (cm), root length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), root dry weight (g), and seed vigor index of test species was investigated under laboratory conditions. The powder of weed plants produced significantly ($p<0.05$) harmful outcomes on all growth parameters of wheat varieties as compared to the control treatment. The maximum seed germination (82.16a %), shoot length (27.70 cm), root length (14.90 cm), shoot fresh weight (2.19 g), root fresh weight (1.16 g), shoot dry weight (0.54 g), root dry weight (0.27 g), and seed vigor index (3483.5) were recorded in variety Amber under the control (where no allelopathic weed powder was applied). The minimum seed germination (28.16%) was observed in variety TJ83 under the treatment of *Avena fatua* powder (@ 75g $kg^{-1}$ of soil). Shoot length and root length of the studied wheat varieties were also affected in inverse proportion to the concentration of allelopathic weed powder. Shortest shoots (14.46 cm) and roots (3.20 cm) were seen in variety TJ83 at the highest concentration of *Avena fatua* powder (75g $kg^{-1}$ soil). The minimum shoot fresh weight (0.13 g), root fresh weight (0.10 g), shoot dry weight (0.04 g), and root dry weight (0.03 g) were also noticed in variety TJ83 under the highest treatment of *Avena fatua* powder (75g $kg^{-1}$ soil). Similarly, minimum seed vigor index (496.9) was also seen in same variety under the maximum treatment of *Avena fatua*. In respect of the above findings it was concluded that the powder from weed species reduced germination and subsequent plant growth of wheat which hints towards importance of apposite measures within due time against weed species to harvest better crop yield.

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**Keywords** | Wheat, Weeds, *Avena fatua, Chenopodium album, Convolvulus arvensis*

## 1. Introduction

Wheat (*Triticum aestivum* L.) crop is deeply rooted in human culture and civilization and plays a great role in the global economy as well as food security. It is a major grain crop of Pakistan and a staple food for billions of people world over (Shewry, 2009). It is used to make flour for leavened,

flat and steamed breads and most of the baked foods; and for fermentation to make beer and alcohol. In Pakistan, about 60 % of the daily diet of a common man is covered by wheat while average utilization per person is about 125 kg per year (Mengal et al., 2015). Wheat is among the cheapest sources of food that provides good amount of calories and protein in the normal human eating routine Kumar et al. (2011).

Pakistan Economic Survey reports clarify that wheat share 9.1 % in agriculture sector, and 1.7 % in overall GDP of Pakistan (GOP, 2018). Wheat was cultivated on an area of about 8,734 thousand hectares during the season 2017-18. The total production of the crop was recorded to be 25.492 million tonnes as compared to 26.674 million tonnes in 2016-17, highlighting a decline of 4.4 percent. The main reasons for decline and variation in total production include: Delayed harvesting of kharif crops and consequently late planting of wheat, unavailability of improved inputs e.g. seed, inefficient fertilizer use, weed infestation, shortage of irrigation water, drought, terminal heat stress, and soil degradation (Ibrahim et al., 2013).

Wheat productivity and quality is significantly impaired by weeds' infection which compete for water, nutrients and sunlight. Weeds are responsible to cause 17-25 % losses in wheat annually due to their competitive and allelopathic nature (Jabeen et al., 2013). Allelochemicals are harmful to crop plants resulting in reduced and delayed germination and decline in seedling growth. Re-plantation problems, poor crop stand and direct interference by certain weeds have been attributed in the part of allelochemicals (Abbas et al., 2014). A large number of allelochemicals, which are released by weed plants, have inhibitory effects towards the crops (Jabeen et al., 2013). It is reported that allelochemicals which are liberated by many plants from leaves, stem, roots, fruit and seeds as residues, exudates and leachates interfere with the growth of other plants (Asgharipour and Armin, 2010).

In Sindh province, about thirty different weed species have been identified in wheat, of which 12 to 16 weed species cause losses up to economic threshold level (Jabeen et al., 2013). Wild oat (*Avena fatua*) is very competitive with wheat and it is reported that 10 wild oat plants $m^{-2}$ can damage wheat production by 20 % (Khan et al., 2012). Moreover, field bind (*Convolvulus arvensis*) weed is also said to be as one of the most harmful weeds in the world which causes 20-70 % losses towards crops yields and gives rise to certain issues during harvesting as well (Peterson et al., 2002). Further, *C. arvensis* reduces the wheat germination by 14 % and yield by 80 % (Yarnia, 2010). Likewise, Lambs quarters (*Chenopodium album*) is also one of the most common weeds of temperate areas (Jhade et al., 2009). It can liberate allelochemicals into the soil; in addition to showing inhibitory influences on the development and growth of surrounding plants (Abdul et al., 2012). It has been proposed that these weeds contain secondary metabolites which interfere the growth of neighboring plants (Dhole et al., 2011).

The present study was designed to evaluate the effect of various allelopathic weed powders on wheat germinability and related traits. Various levels of three common weed species was investigated on two wheat varieties. Hence, this study gives an insight into inhibitory effects of weeds at different concentrations and the role genetic architecture of the varieties could play against the same.

## 2. Materials and Methods

This experiment was performed at Weed Science and Allelopathy Laboratory, Department of Agronomy, Sindh Agriculture University, Tandojam, during the year 2018. Allelopathic effects of three different weed species were investigated on germination and seedling traits of wheat (*Triticum aestivum* L.). Whole mature weed plants of lamb's quarters (*Chenopodium album*), fieldbind weed (*Convolvulus arvensis*) and wild oat (*Avena fatua*) were up rooted randomly from Agriculture Research institute (ARI), Tandojam. The collected weeds were washed, dried and then ground. After grinding, the weeds were weighed in various ratios of 25, 50 and 75g. The powder of each weed treatment was mixed thoroughly with 1000 g soil (sandy loam) and then sufficient quantity of water was added to all the containers. Healthy seeds of both wheat varieties were sterilized with 3% sodium hypochlorite solution and then thoroughly washed with the sterile distilled water several times. Thirty healthy seeds of both wheat varieties were sown under each treatment (control, 25, 50 and 75g) and kept in room temperature. The number of seeds germinated were counted after 7 days of treatment.

**Table 1: Effect of allelopathic weed powders on wheat.**

| Allelopathic weed powders | Seed germination (%) | Shoot length (cm) | Root length (cm) | Shoot fresh weight (g) | Root fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) | Seed vigor index |
|---|---|---|---|---|---|---|---|---|
| | **V I: Amber** | | | | | | | |
| $W_{1=}$ Control (untreated) | 82.16a | 27.70 a | 14.90 a | 2.19 a | 1.16 a | 0.54 a | 0.27 a | 3483.5 a |
| $W_{2=}$ *Chenopodium album* powder: 25g $kg^{-1}$ soil | 77.80ab | 25.43 b | 13.73 b | 1.97 ab | 0.76 c | 0.38 c | 0.25 ab | 3019.2 b |
| $W_{3=}$ *Chenopodium album* powder: 50g $kg^{-1}$ soil | 64.23c | 22.46 d | 8.90 e | 1.62 c | 0.62 d | 0.30 de | 0.23 bc | 2014.5 e |
| $W_{4=}$ *Chenopodium album* powder: 75g $kg^{-1}$ soil | 44.76e | 19.10 gh | 6.60 gh | 0.58 f | 0.49 f | 0.24 fg | 0.11 f | 1151.5 h |
| $W_{5=}$ *Convolvulus arvensis* powder: 25g $kg^{-1}$ soil | 63.16c | 25.16 b | 12.10 c | 1.60 cd | 0.43 g | 0.30 e | 0.12 f | 2352.5 d |
| $W_{6=}$ *Convolvulus arvensis* powder: 50g $kg^{-1}$ soil | 55.30d | 19.43 g | 8.40 ef | 1.12 e | 0.37 h | 0.18 ij | 0.10 fg | 1538.2 g |
| $W_{7=}$ *Convolvulus arvensis* powder: 75g $kg^{-1}$ soil | 44.46e | 18.36 h | 5.40 j | 0.32 fghi | 0.26 ij | 0.15 jk | 0.07 hi | 1056.2 h |
| $W_{8=}$ *Avena fatua* powder: 25g $kg^{-1}$ soil | 55.66d | 20.53 ef | 7.10 g | 0.53 fg | 0.29 i | 0.11 lm | 0.22 bcd | 1536.8 g |
| $W_{9=}$ *Avena fatua* powder: 50g $kg^{-1}$ soil | 46.00e | 18.36 h | 5.26 j | 0.39 fghi | 0.19 k | 0.08 mno | 0.07 hij | 1214.3 h |
| $W_{10=}$ *Avena fatua* powder: 75g $kg^{-1}$ soil | 37.93g | 15.80 i | 4.20 k | 0.15 hi | 0.15 lm | 0.07 no | 0.04 jk | 758.4 ij |
| | **V II: TJ-83** | | | | | | | |
| $W_{1=}$ Control (untreated) | 75.03b | 25.66 b | 13.26 b | 1.69 bc | 1.11 b | 0.48 b | 0.21 de | 2657.0 c |
| $W_{2=}$ *Chenopodium album* powder: 25g $kg^{-1}$ soil | 65.46c | 24.20 c | 12.10 c | 1.61 c | 0.66 d | 0.34 d | 0.20 de | 2397.7 cd |
| $W_{3=}$ *Chenopodium album* powder: 50g $kg^{-1}$ soil | 55.00d | 21.23e | 8.06 f | 1.25 de | 0.56 e | 0.28 ef | 0.19 e | 1632.2 fg |
| $W_{4=}$ *Chenopodium album* powder: 75g $kg^{-1}$ soil | 43.13ef | 16.70 i | 5.73 ij | 0.48 fgh | 0.39 h | 0.20 hi | 0.10 fg | 982.7 hi |
| $W_{5=}$ *Convolvulus arvensis* powder: 25g $kg^{-1}$ soil | 56.53d | 20.76 e | 10.73 d | 1.47 cd | 0.35 h | 0.23 gh | 0.10 fg | 1829.4 ef |
| $W_{6=}$ *Convolvulus arvensis* powder: 50g $kg^{-1}$ soil | 46.83e | 16.70 i | 7.00 g | 1.02 e | 0.28 ij | 0.16 ijk | 0.08 gh | 1107.9 h |
| $W_{7=}$ *Convolvulus arvensis* powder: 75g $kg^{-1}$ soil | 38.30fg | 14.63 j | 3.93 k | 0.23 ghi | 0.18 kl | 0.13 kl | 0.04 k | 1171.1 h |
| $W_{8=}$ *Avena fatua* powder: 25g $kg^{-1}$ soil | 45.63e | 19.63 fg | 6.06 hi | 0.43 fghi | 0.24 j | 0.10 lmn | 0.22 cd | 1171.1h |
| $W_{9=}$ *Avena fatua* powder: 50g $kg^{-1}$ soil | 35.93g | 16.56 i | 4.26 k | 0.29 fghi | 0.14 mn | 0.07 no | 0.05 ijk | 747.0ij |
| $W_{10=}$ *Avena fatua* powder: 75g $kg^{-1}$ soil | 28.16h | 14.46 j | 3.20 l | 0.13 i | 0.10 n | 0.04 o | 0.03 k | 496.9 j |
| LSD | 4.91 | 0.93 | 0.66 | | 0.04 | 0.03 | 0.02 | 284.75 |
| SDE | 2.42 | 0.46 | 0.32 | 0.17 | 0.02 | 0.01 | 0.01 | 140.66 |

## 3. Results and Discussion

The study revealed that allelopathic weed powders from all the weed plants under consideration viz. lamb's quarters (*Chenopodium album*), fieldbind weed (*Convolvulus arvensis*) and wild oat (*Avena fatua*) caused harmful and statistically significant effects on all parameters of the wheat growth as compared to the control (Supplementary Material Table 1-2). Seed Germination (%) is one of the most important indications for number of seedlings expected to ultimately grow. The results of seed germination (%)

of wheat varieties, as effected by various allelopathic weed powders, are shown in Table 1. The statistical analysis indicated that the seed germination (%) of wheat varieties were significantly ($p<0.05$) influenced by allelopathic weed powders. The maximum seed germination (82.16a %), was recorded in Amber variety and in the control where no allelopathic weed powder was applied in the soil. Whereas, minimum seed germination (28.16%) was observed in variety TJ83 under the treatment of *Avena fatua* powder at 75g $kg^{-1}$ of soil. These results were in agreement to the report of Jabeen et al. (2013) which showed that allelopathic weed powders decreased the germination of wheat. Nouri et al. (2012) also reported that exposing the seeds of any plant species to allelochemicals cause drastic decline in seed germination.

Shoot length and root length of the studied wheat varieties were also affected in inverse proportion to the concentration of allelopathic weed powder. The maximum shoot length (27.70 cm) and root length (14.90 cm) were recorded in variety Amber in control, while the shortest shoots (14.46cm) and roots (3.20 cm) were observed in variety TJ83 at the highest concentration of *Avena fatua* powder (75g $kg^{-1}$ soil). Jabeen et al. (2011) observed that on interaction with allelopathic *Asphodelus tenuifolius* powder, height of the wheat seedlings was reduced.

The maximum shoot fresh weight (2.19 g), root fresh weight (1.16 g), shoot dry weight (0.54 g), and root dry weight (0.27 g) was observed in variety Amber under the treatment of control, followed by the treatment having 25g $kg^{-1}$ of *Chenopodium album* powder. The minimum shoot fresh weight (0.13 g), root fresh weight (0.10 g), shoot dry weight (0.04 g), and root dry weight (0.03 g) was found in variety TJ83 under the highest treatment of *Avena fatua* powder (75g $kg^{-1}$ soil). According to Shaukat et al. (2002) the fresh and dry weight of plant species reduces with the increasing quantity of weed material they are exposed to. The conclusions of Ullah et al. (2010) and Hadi et al. (2013) are also similar to our results which proposed that the growth and yield of plant species decrease when the inhibitory effect of weed material is enhanced.

Table 1 Two varieties of wheat *viz.* Amber and TJ-83 were subjected to various concentrations of *Chenopodium album, Convolvulus arvensis* and *Avena fatua* powder. All of the wheat traits were seen to be significantly affected by the allelopathic characters of the weeds.

The maximum seed vigor index (3483.5) was observed in variety Amber under the control, whereas minimum seed vigor index (496.9) was noticed in variety TJ83 under the highest treatment of *Avena fatua* powder (75g $kg^{-1}$ soil). Likewise, Tanveer et al. (2010) and Katoch et al. (2012) have reported that residue of allelopathic weeds in soil have inhibitory effects on the emergence percentage, mean emergence, and the time of emergence index of wheat.

The results of this experiment were in agreement to earlier reports which proposed that alligator weeds produce strong allelopathic effects on field crops including wheat, eggplant and grape (Liu et al., 2007; Zhang et al., 2009). A number of studies have shown that residues from several allelopathic weed species release allelochemicals into the soil, thus affecting the performance of associated and next-season crop plants (Shaukat et al., 2003; Singh et al., 2003). The presence of phenolic compounds such as caffeic acid, chlorogenic acid, 4-hydroxy-3-methoxybenzoic acid, ferulic acid, mcoumaric acid, p-coumaric acid, gallic acid, syringic acid, and vanilic acid in the weeds play critical a role in inhibiting the seed germination and growth of the wheat (Inderjit and Weiner, 2001). Channappagoudar et al. (2005) also stated that phenolic compounds are major phytotoxins which cause inhibition in seed germination and early seedling development, as observed in this study.

## Conclusions and Recommendations

Powder of all the three weed species caused harmful effects on wheat germination and growth. It was concluded that higher amount i.e. 75g powder of three weed species lamb's quarters (*Chenopodium album*), fieldbind weed (*Convolvulus arvensis*) and wild oat (*Avena fatua*) produced allelochemicals which decreased germination and consequent wheat plant growth significantly. Overall, the variety Amber was seen to be more resistant to such damage by the weed species.

## Author's Contribution

**Pushpa, Farheen Deeba Soomro, and Muhammad Tahir Khan:** Wrote the manuscript.
**Nighat Seema Soomro, Shahla Karim Baloch, and**

**Mehmooda Buriro:** Supervised the research work. Aijaz Ahmed Soomro: did statistical analysis of the data.

**Qamar Uddin Jogi, and Muhammad Nawaz Kandhro:** Critically reviewed the article and provided technical inputs into the research work.

*Supplementary material*

There is supplementary material associated with this article. Please view it at: http://dx.doi.org/10.17582/journal.jis/2019/5.2.100.105

*Conflict of interest*

The authors have declared no conflict of interest.

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Research Article

# Lemon Juice and Microwave Assisted Modification of Date Seed Husk for Arsenic Biosorption

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**Abstract** | Arsenic poisoning and its removal from drinking water has become a serious issue now a days. For arsenic removal batch studies were conducted using low cost adsorbent (raw date seeds husk and lemon juice microwave activated date seeds husk) by taking 25ppm/50ml initial concentration of $NaAsO_2$. Best removal (90%) was achieved for Lemon juice microwave activated date seed husk LMDS (time= 30mins, agitation speed= 150 rpm, pH= 5, adsorbent dose= 0.1 g). While 85% removal efficiency was observed for Raw date seed husk RDS (time= 45mins, agitation speed=250 rpm, pH= 4 and adsorbent dose= 0.5 g). The adsorption isotherms (Freundlich and Langmuir) and adsorption dynamic kinetic studies were also conducted. The comparison between the FTIR, EDX and SEM of RDS and LMDS revealed that with lemon juice microwave activation of adsorbent there were activation of more active sites which in turn increased the removal efficiency.

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**Keywords** | Biosorption, RDS, LMDS, Natural modification, SEM, EDX, Batch studies

## 1. Introduction

Arsenic toxicity is now recognized as a global health problem associated with almost more than 21 countries (Smith et al., 2000). In nature arsenic exists in the form of ores (powdery, amorphous and crystalline). Different anthropogenic and natural sources (mining and smelting processes, pesticide use and coal combustion) are responsible for water contamination by arsenic (Raj et al., 2013). Arsenic in environment (groundwater and in turn in drinking water) is not only creating the other health problems (respiratory, renal, neurological, cardiovascular, mutagenic etc.) but also has a prominent role in creating various types of cancer (Hall, 2002; Roy et al., 2013; Saha et al.,1999; Asif and Chen, 2017) in several developing regions (Brouwer et al., 2007). Ground water arsenic is present in the form of As V (arsenate) and As III (arsenite) (Kumari et al., 2005; Wasiuddin et al., 2002; Amin et al., 2006; Roy et al., 2013).

For arsenic remediation various techniques involving adsorption were considered (Sharma and Bhattacharya, 2017). Various adsorbents used for arsenic removal are DE-4 resin (Qureshi and Sahabuddin, 2012), red mud (*Bauxsol*), a waste from aluminum manufacturing (Genc et al., 2003), iron coated brown seaweed (*Sargassum muticum*) (Vieira et al., 2017), Ceria ($CeO_2$) coated powdered activated carbon (Sawana et al., 2017), modified fungal biomass of *Aspergillus niger* coated with iron oxide (Pokhrel and Viraraghavan, 2006), sand was coated with ferric chloride and used as filtering media (Devi et al., 2014), limestone-based material is effective for

reducing arsenic (Davis et al. , 2018) and silica based chitosan beads (Malwal and Packirismy, 2017).

These conventional methods are reported to be noneconomical, less efficient and not safe environmentally (Eccles, 1999; Barakat, 2011; Azimi et al., 2016). A best and alternative method for arsenic remediation (Kratochvil and Volesky, 1998; Asif and Chen, 2017) is biosorption which utilizes natural, dead biomass (plants, agricultural wastes) or microorganisms (Roy et al., 2017; Ozer et al., 1998; Dimme et al., 2017; Amin et al., 2017; Sidhu et al., 2014; Javanbakht et al., 2014). These natural materials are excessively available having low or no cost and also have high decontamination ability. Biosorbents for removal of arsenic can be used in activated or in natural form (activated cashew shells, millet stalks, seed powder (Raj et al., 2013), *Azadirachta indica* bark powder (Roy et al., 2017), rice husk (Asif and Chen, 2017) and biochars derived from rice husk (Agrafioti et al., 2014).

The metal decontamination ability is attributed due to the presence (Basso et al., 2002; Chen et al., 2010), activation and modification of various groups (-COOH, OH, $NH_2$, $SO_4^{-2}$, $PO_4^{-3}$) (Deng et al., 2003). These groups when introduced or activated can enhance the adsorption power of adsorbent (Oliveira et al., 2009). Metal entrapment by these natural residues is due to metal substrate interactions by various processes (Srivastava and Anil, 2016). So far biosorbents can be activated by using different chemicals like $FeCl_3$, $ZnCl_2$ (Liu et al., 2016), $H_2O_2$, $H_2SO_4$, NaOH (Shwantes et al., 2016), urea (Rehman et al., 2013) and thiourea (Salman et al., 2014).

Various biosorbents were successfully being used for removing heavy metals due to their different up take capacities (qmax values) Table 1. Present study was designed to check the effectiveness of *Phoenix dactylifera* seeds (date seeds) belonging to family *Arecaceae,* an agricultural waste in removing arsenic ions from aqueous solution. There are many areas in Sindh and Baluchistan which are producing high quality dates of different varieties. Their seeds can be reused in different ways. In present study two types of biosorbents i.e., raw date seeds husk (RDS) and microwave assisted lemon juice modified date seed husk (LMDS) were used for the first time as a new biosorbent and its modification with lemon juice is totally ecofriendly instead of using various chemicals as modifiers or activators.

**Table 1: Comparison of sorption capacities of different biosorbents for the removal of Heavy metals.**

| Biosorbent | Heavy metal | $q_{max}$ mg/g | Reference |
|---|---|---|---|
| Oil palm shell | Pb (II) | 3.39 | (Chong *et al.*, 2013) |
| Oil palm shell | Cu (II) | 1.75 | (Chong *et al.*, 2013) |
| Palm shell | Hg (II) | 83.33 | (Ismaiel *et al.*, 2013) |
| Corn straw | Cd (II) | 38.91 | (Chi *et al.*, 2017) |
| Corn straw | Pb (II) | 28.99 | (Chi *et al.*, 2017) |
| Raw date seeds | As (III) | 1.3332 | Present study |
| Lemon juice microwave activated date seeds | As (III) | 1.4828 | Present Study |

By adding lemon juice there is the introduction of additional -COOH groups on adsorbent surface (date seed husk) and with microwave radiation these groups are much more activated as $COO^-$ thus providing more adsorbent sites for arsenic ions removal.

The capability of RDS and LMDS for arsenic ions removal was checked by studying isothermal, kinetic and batch studies.

## 2. Materials and Methods

Shaker (yellow line), Atomic Absorption spectrometer, PH meter, Microwave oven (2,450 MHz), Midac FTIR 2000 spectrometer (406-7800$cm^{-1}$), EDX, SEM, 0.1M NaOH, 0.1M HCL (MERCK), Sodium Arsenite salt ($NaAsO_2$) and lemon juice.

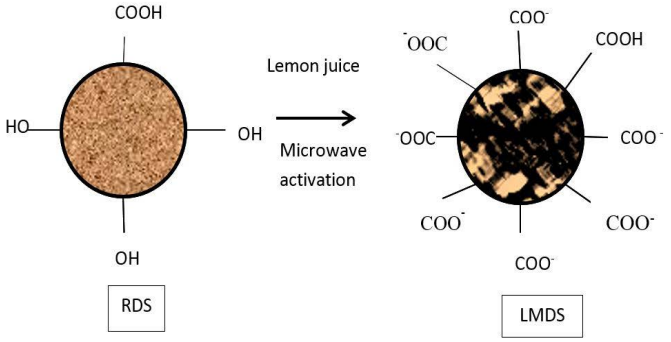
### *2.1 Preparation of solution*

Stock solution of 1M Sodium Arsenite salt ($NaAsO_2$) was prepared. From this stock solution 25 ppm/50 ml volume of solution was prepared and mixed into the activated as well as non-activated date seed husk.

### *2.2 Preparation of biosorbent*

**Preparation of raw date seed husk:** Date seeds (*Phoenix Dactylifera)* an easily available raw material were purchased from seed bank of Lahore. These were then washed, dried, grinded and sieved (60 ASTM). This was raw date seed husk (RDS).

**Preparation of modified Date Seed husk:** 60g of the preserved raw date seed husk (RDS) and lemon juice (1:1) was mixed fairly. This was then subjected to microwave oven for 25 min. to get lemon juice microwave activated date seed husk (LMDS) Scheme 1.




**Scheme 1: Surface modification of RDS with lemon juice and microwave radiation.**

## 3. Results and Discussion

**FTIR Analysis:** FTIR spectra of raw date seed husk (RDS) and lemon juice microwave activated date seed husk (LMDS) Figure 1 showing various functional groups on the surface of RDS and LMDS. LMDS showed the presence of additional peaks at 1746.57 $cm^{-1}$ (C=O Stretching), 1458.21$cm^{-1}$ (C-H Stretching), 1373.34 $cm^{-1}$(O-H Bending), 1151.52 $cm^{-1}$(C-O Stretching), 1010.72 $cm^{-1}$ (C-N Stretching) and 721.39 $cm^{-1}$ (C=C Bending). RDS showed different peaks in the region of 1616.38 $cm^{-1}$ (C=C Stretching), 1445.67 $cm^{-1}$(C-H Bending), 1249.89 $cm^{-1}$(C-O Stretching), 1154.42 $cm^{-1}$ (C-O Stretching), 1036.76$cm^{-1}$(S=O Stretching), 869.91 cm-1(C=C Bending), 816.87$cm^{-1}$(C=C Bending). There was also shift in peaks of RDS from 2923.17 $cm^{-1}$ to 2922.21 $cm^{-1}$, 1743.68$cm^{-1}$ to 1746.57 $cm^{-1}$, 1445.67 $cm^{-1}$ to 1458.21 $cm^{-1}$, 1154.42 $cm^{-1}$ to 1151.52 $cm^{-1}$ and 1036.76 $cm^{-1}$to 1010.72 $cm^{-1}$ after modification with lemon juice and microwave activation. Thus the presence of these groups were involved in biosorption of As (III) by *Phoenix Dactylifera*. LMDS had more active sites and had more functional groups indicating the involvement of these groups in biosorption of As (III).

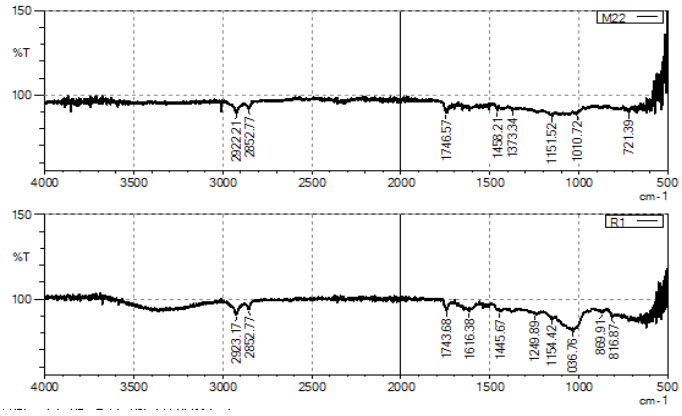



**Figure 1: Overlay FTIR spectra of RDS and LMDS.**

**SEM Analysis:** It was noticed that the surface of RDS was rough and consisted of particles of various shapes and sizes. Particles were large in size providing small surface area Figure 2a. After lemon juice microwave activation surface of LMDS had become irregular and wavy and had holes which were unevenly spread on the small size particles of LMDS Figure 2b. After activation of sample with lemon juice surface morphology was changed and increases in surface area consequently giving higher sorption capacity of the sorbent.

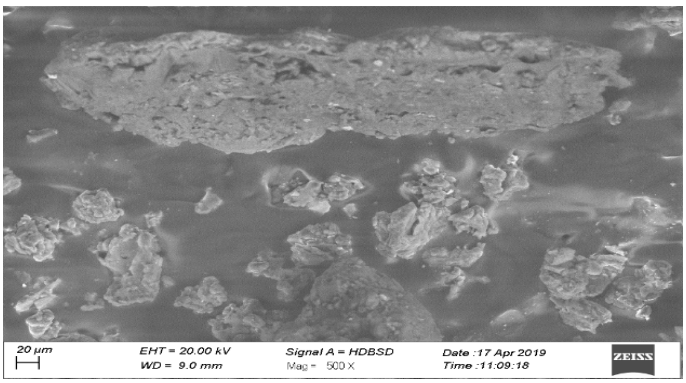



**Figure 2a: SEM image of RDS.**

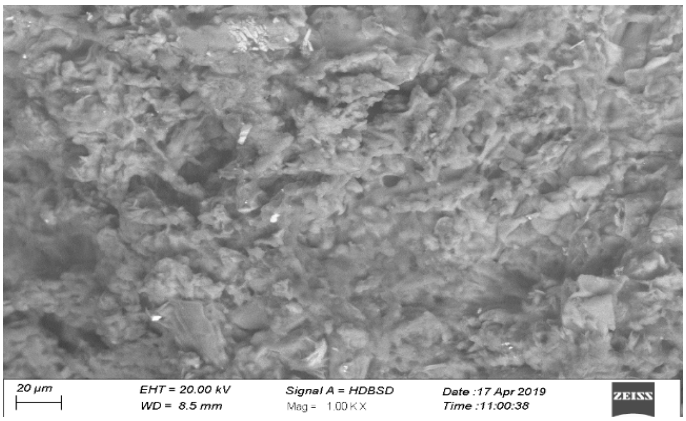



**Figure 2b: SEM image of LMDS.**

**EDX Analysis:** EDX image of RDS Figure 3a shows its elemental composition as having Ca, K, Na and no arsenic but for LMDS an additional prominent peak is observed due to retention of arsenic on the surface of biosorbent Figure 3b.

### *3.1 Batch studies*

**Effect of shaking speed:** It was observed that adsorption of Sodium Arsenite ($NaAsO_2$) (25ppm /50ml) had increased with the increased shaking speed (50 – 400 rpm) for both RDS and LMDS. At 250 rpm for RDS adsorption reaches a maximum of 60.50 % and at 150 rpm for LMDS maximum

adsorption was 90.25 % Figure 4a. Moderate speed provided more active sites as compared to low speed and further increase in speed decreased the contact time resulting in desorption.

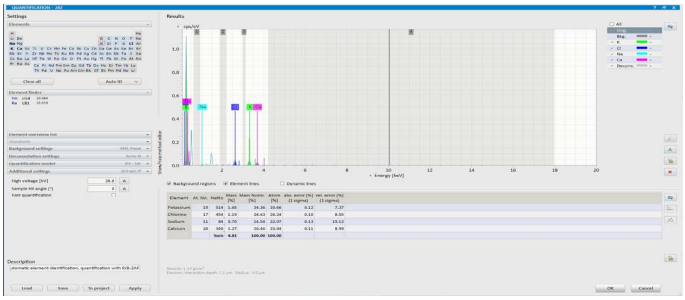


**Figure 3a: EDX image of LMDS without arsenic.**

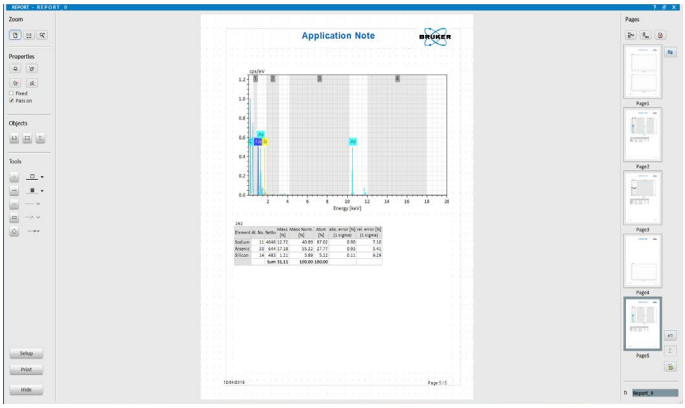



**Figure 3b: EDX image of LMDS with arsenic.**

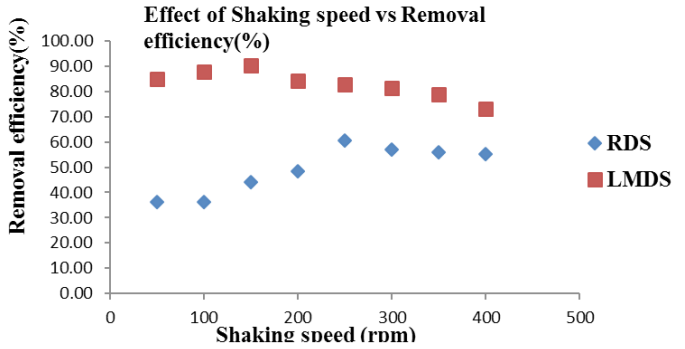



**Figure 4a: Effect of shaking speed for Arsenic removal onto RDS and LMDS. Adsorbent dose= 0.3 g, Solution concentration (25 ppm / 50 mL), Contact time= 30 minutes.**

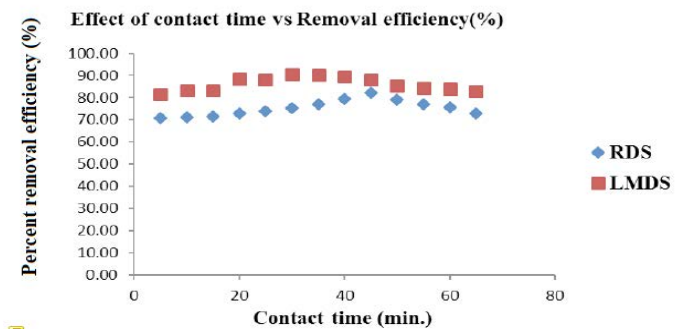



**Figure 4b: Effect of contact time for Arsenic removal onto RDS and LMDS. Adsorbent dose= 0.3 g, Shaking speed (RDS, LMDS= 250 rpm, 150 rpm), Solution concentration (25 ppm / 50 mL).**

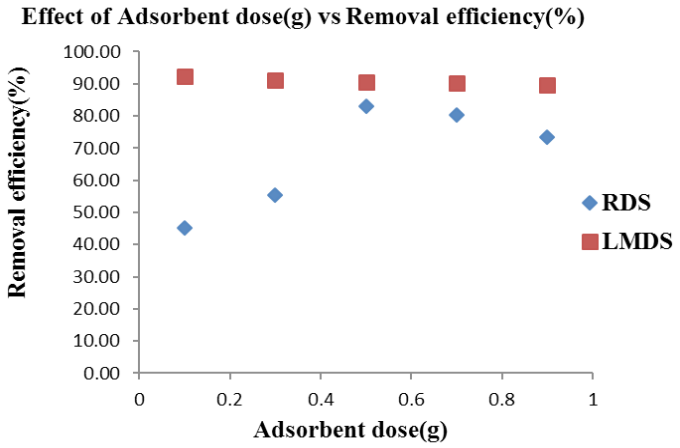



**Figure 4c: Effect of adsorbent dose (g) on Arsenic removal onto RDS and LMDS. Shaking speed (RDS, LMDS= 250rpm, 150 rpm), Solution concentration (25 ppm / 50 mL), Time= 30 minutes.**

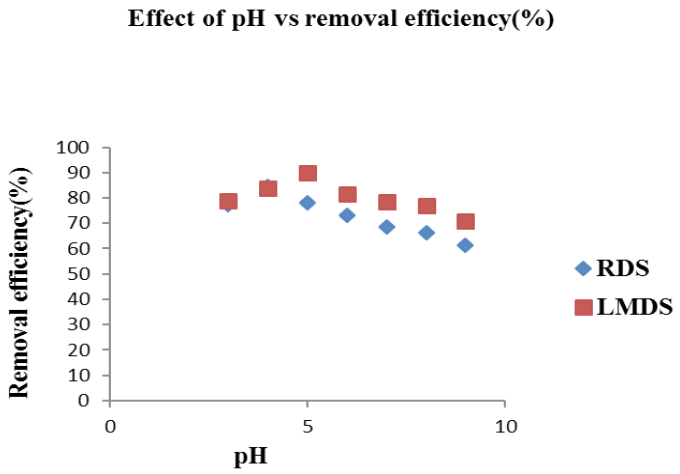



**Figure 4d: Effect of pH on lead removal onto RDS and LMDS. Adsorbent dose (RDS, LMDS= 0.3g), Shaking speed (RDS, LMDS= 250 rpm, 150 rpm), Solution concentration (25 ppm / 50m L), Time=30 minutes.**

**Effect of contact time:** Removing capacity (%) of RDS and LMDS was increased with the increase in their time of contact Figure 4b. Maximum removal efficiency (%) of RDS was 82.25 % at 45 minutes contact time whereas LMDS had 90.59 % removal efficiency at 30 minutes contact time. Therefore, the 30 minutes contact time was chosen for further batch adsorption experiment.

**Effect of adsorbent dose:** Adsorption behaviour of RDS and LMDS was studied by keeping the adsorbent amount from 0.1g–0.9g Figure 4c.

Maximum adsorption occurred when RDS is 0.5 g and LMDS was only 0.1 g increase in adsorption was due to the increased sites and then decrease was due to their aggregation and decreased surface area.

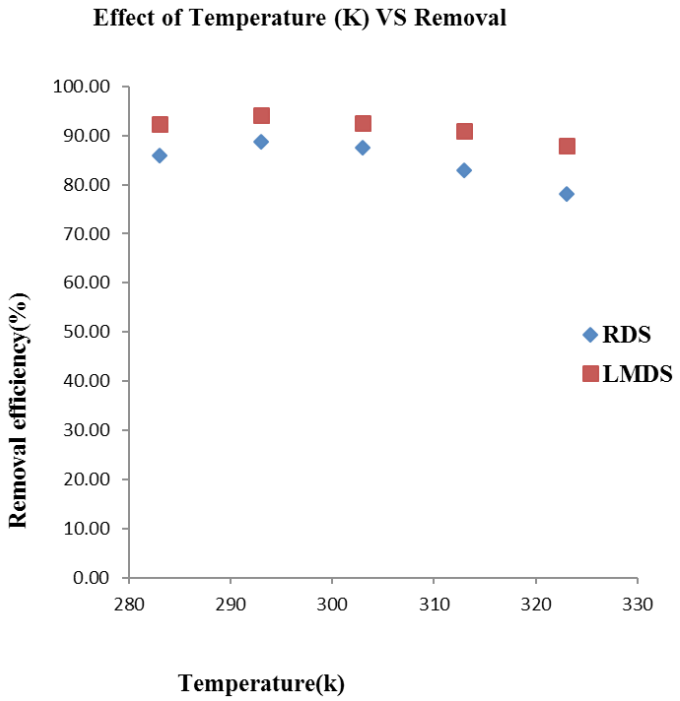



**Figure 4e: Effect of temperature (K) on Arsenic removal onto RDS LMDS. Adsorbent dose = 0.3g, Shaking speed (RDS, LMDS = 250 rpm, 150 rpm), Solution concentration (25 ppm / 50m L), Time=30 minutes.**

**Effect of pH:** The pH (3-9) also effected the adsorption capacity of RDS and LMDS due to the presence of different forms of carboxylic group on biosorbent surface at different pH. $As^{+3}$ adsorption was low at pH 3 because at pH 3 the dominating specie was –COOH and it then increases and reaches the highest at pH 5 where $–COO^-$ was mainly present on adsorbent surface. For RDS $As^{+3}$ maximum removal efficiency (%) was at pH 4 (84.625 %) and for LMDS at pH 5 (89.75 %) Figure 4d. At still higher pH (6.0) adsorption decreases due to the precipitation of $As^{+3}$as insoluble metal hydroxides. The mechanism of $As^{+3}$biosorption is:

$$3DS\text{-}OH + As^{+3} \longrightarrow (DSO)_3\,As^{+3} + 3H^{+}\ (RDS)$$

$$DSLM^{-3} + As^{+3} \longrightarrow DSLM\text{-}As\ (LMDS)$$

**Temperature dependence:** Temperature is an important parameter to study extent of adsorption. Experiment were conducted in the temperature range 283.0 K - 333.0 K in which 293.0 K was the suitable temperature for both the sorption of $As^{+3}$ using RDS (90.16 %) and LMDS 93.53 % Figure 4e. Above 293.0 K adsorption decreases due to very high movement of $As^{+3}$and decrease in interaction between adsorbent surface and adsorbate. The value of ΔG for RDS and LMDS showed that the reaction is spontaneous and its greater value for LMDS than RDS means that adsorption was more favorable with LMDS Figure 5.

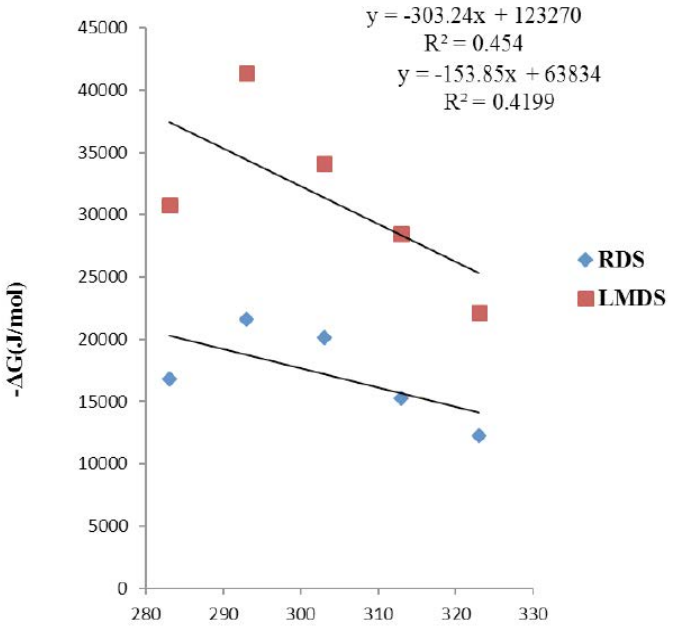



**Figure 5: Thermodynamic study of RDS , LMDS.**

### *3.2 Isothermal study*

**Langmuir Isotherm:** $q_{max}$ values for RDS and LMDS were 1.3332 mg $g^{-1}$ and 1.4828 mg $g^{-1}$ Table 2. Greater $q_{max}$ for LMDS had more active sites as compared with RDS. $R_L$ values of LMDS and RDS were 0.0237 and 0.0335 respectively which indicated that data is not according Langmuir Figure 6a.

**Table 2: Isothermal parameters for Arsenic sorption onto LMDS and RDS.**

| Adsorbent | Langmuir | | Freundlich | |
|---|---|---|---|---|
| LMDS | $q_{max(mg/g)}$ | 1.4828 | n | 0.094 |
| | b(L/g) | 1.6503 | $K_F$ | 2.7064 |
| | $R^2$ | 0.7581 | $R^2$ | 0.9245 |
| | $R_L$ | 0.0237 | 1/n | 10.638 |
| RDS | $q_{max(mg/g)}$ | 1.3332 | n | 0.2135 |
| | b(L/g) | 1.1527 | $K_F$ | 2.605 |
| | $R^2$ | 0.7855 | $R^2$ | 0.9226 |
| | $R_L$ | 0.0335 | 1/n | 4.683 |

**Freundlich isotherm:** Magnitude of $K_F$ and n (Freundlich constant) indicated high adsorptive

capability of *Phoenix Dactylifera*. The value of $K_F$ for LMDS and RDS were 2.7064 and 2.605 respectively Table 2. The value of $R^2$ for LMDS and RDS were 0.9245 and 0.9226 respectively which is a good fit of Frendulich isotherm Figure 6b.

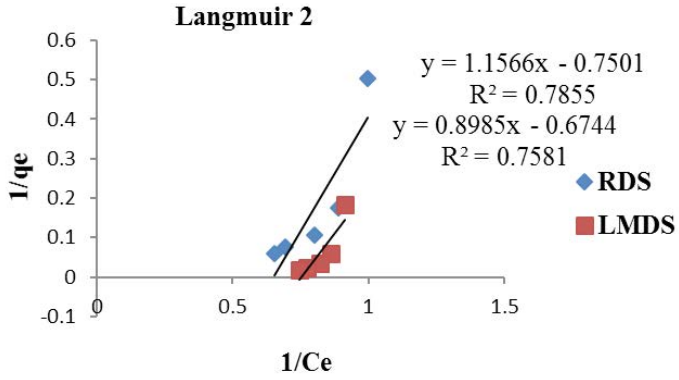



**Figure 6a: Langmuir 2 Isotherm for RDS, LMDS.**

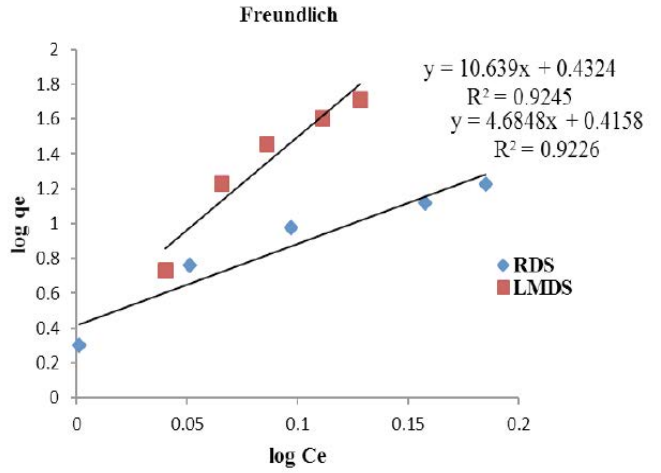



**Figure 6b: Freundlich Isotherm for RDS, LMDS.**

**Table 3: Kinetic Study for Arsenic sorption onto LMDS and RDS.**

| **Pseudo first order kinetic Model** | | | | |
|---|---|---|---|---|
| Adsorbent | $q_e$ (exp) | $K_1$ | qe (cal) | $R^2$ |
| RDS | 11.79 | 0.00007 | 2.582 | 0.0425 |
| LMDS | 12.4896 | 0.0002 | 2.6072 | 0.3419 |
| **Pseudo second-order kinetic model** | | | | |
| Adsorbent | qe (exp) | $K_2$ | qe (cal) | $R^2$ |
| RDS | 1.92864 | 0.2151 | 0.216 | 0.9936 |
| LMDS | 3.6049 | 0.2151 | 0.4381 | 0.9966 |

**Kinetics studies:** *Phoenix Dactylifera* kinetics studies were not according to pseudo first order reaction but is according to second order Table 3 as the experimental values of $K_1$ and $q_e$ were not according to calculated values and the $R^2$ (correlation coefficients) were low Figure 7a. Whereas $k_2$ (pseudo second order rate constant) and qe values agreed with experimental data and $R^2$ values is a good fit for pseudo second order reaction Figure 7b.

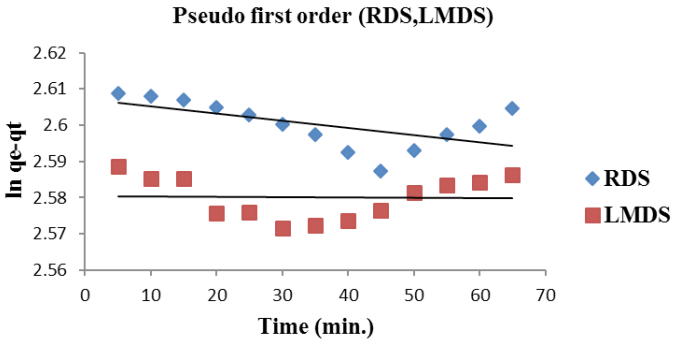



**Figure 7a: Pseudo first order kinetics for (RDS, LMDS).**

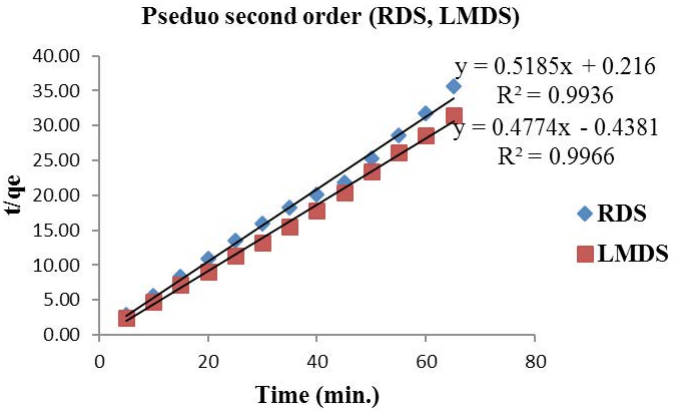



**Figure 7b: Pseudo second-order kinetics for (RDS, LMDS).**

## Conclusions and Recommendations

On the basis of the result it can be concluded that Date seed (*Phoenix Dactylifera*) could be successfully used for As (III) removal from aqueous solutions. The results obtained suggest that LMDS is a good adsorbent as compared to RDS because it had more active sites. Experimental data fitted very well to the Freundlich adsorption isotherm models. FTIR spectra identified different functional group present in the *Phoenix Dactylifera*. Process adopted is simple and economically valueable. It could be used for sorption of different other heavy metals like Cd, Pb etc. and also for different dyes such as methylene blue etc. from water. Activation by lemon juice was very effective, low cost and at the end of reaction sludge or by products were not formed. This method is easily applicable in agricultural country where a lot of agricultural waste is present and lemon juice is also abundantly available. This method can be applied on industrial scale to remove contaminants from waste water.

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## Authors Contribution

All this work was planned and supervised throughout by Dr. Tahira Moeen Khan. Iram Riaz and Shahida Hameed helped in sample preparation and statistical data. Prof. Dr. Bushra Khan helped in the analyzing of data.

### *Conflict of interest*

The authors have declared no conflict of interest.

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Journal of Innovative Sciences

Research Article

# Suitability of Different Rootstocks to Overcome the Reduction of Size Problem in the Feuter's Early (*Citrus reticulata*) Mandarin

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**Abstract** | Performance of different exotic citrus root stock was studied at Citrus Research Institute Sargodha from 2013-18. The trail was conducted according to RCB design with three replications. The Five Treatments four exotic and one local root stock ($T_0$ Rough lemon, $T_1$ Troyer citrange, $T_2$ Cox mandarin, $T_3$ C-35, $T_4$ Carrizo citrange) were budded with Feutrells early in 2013. The statistical analysis of vegetative data from trial site shown good compatibility in term of scion/root stock ratio. Maximum plant height attained in $T_0$(Rough lemon) with max. canopy volume. All the exotic root stocks are compatible with Feutrell's early. Objectively maximum fruit weight, fruit size, juice percentage and yield obtained in $T_1$ Troyer citrange and $T_2$ Cox mandarin as compared to local root stock rough lemon. Results depict that exotic citrus root stock Troyer citrange and Cox mandarin performed well with Feutrell's early in the local climatic conditions of Sargodha Punjab, can be used to overcome the smaller size of Feutrells early along with added edge of better results in various other parameters like tree yield, fruit weight, juice percentages etc.

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**Keywords** | Exotic root stocks, Plant height, Canopy volume, Rough lemon, Troyer citrange, Cox Mandarin, fruit size, Scion/stock ratio

## 1. Introduction

All citrus varieties are very popular due to their nutritional value and thrust quenching properties. Pakistan citrus industry is well dominated by mandarin group. More than 95% of the citrus is produced in the Punjab among which only Kinnow mandarin contributes 70% of total produce (Niaz et al., 2004). There is dire need to improve and diversify the Pakistan citrus industry by using different root stock and to exploit the potential of commercial cultivars. Feutrell's early an early second largest maturing mandarin variety of citrus in terms of production at Punjab after Kinnow. It represents mandarin group in the market in November. Feutrells early has a problem of size, as it is observed that with period of time its fruit size is on the decreasing trend. Role of rootstocks in affecting the various fruit characteristics is authenticated in the citrus industry. It is believed that more than 20 plant characteristics can be affected by rootstocks like plant health, Physico-chemical composition and including fruit size, fruit weight, yield and other quality parameters (Castle et al., 1995). Lima (1992) explained budded/grafted on Rangpur lime were vigorous in vegetative growth as compare to Rough lemon. Niaz et al. (1994) reveal that Kinnow and Feutrell's early budded with have vigorous growth but it is more susceptible to Phytophthora disease. Most of the citrus commercial varieties are propagated by asexual propagation using budding or grafting methods. Most of the citrus crop experts are convinced about the importance of the root stock for good crop production. Root stock significantly affects the canopy volume and

functioning such as photosynthesis (Richardson et al., 2003). Root stock have difference in adoption to soils type and root dispersion manner, mycorrhiza dependence and this lead to difference leaf mineral concentration or in the leaves of grafted cultivars budded/grafted on them and finally affects vegetative and reproductive physiology of fruit (Basal, 2009). The leaf nutrient composition of scion cultivars was significantly affected by different root stocks (Jaskani et al., 2016). Pestana et al. (2005) reported that root stock show significant difference in Iron absorption.

The main factor that limits the growth of citrus plant include Citrus Tristeza Virus (CTV) and Phytophthora spp., which are present in all orchards in Pakistan. Some abiotic stresses such as quick decline, root rot salinity and flooding, also reduces citrus growth in certain areas. Currently used citrus root stock in Punjab is rough lemon and which is susceptible to these problems. Thus, attempt have been made to solve abiotic issues by changing root stock.

Choice of root stock is among the most important decision a grower makes and implication for yield and quality are enormous. Drivers of root stock adoption are wide ranging with most important being tolerance to Citrus Tristeza virus, Phytophthora, Nematodes and salts, but water use efficiency and drought tolerance are increasingly are important to achieve better performance. Although the metabolic functions in a grafted plant are divided between two plant fraction, it is well known that root stock greatly influence variety behavior as it ensures provision of mineral and water for total plant. Campeanu et al. (2009) suggested that for better quality fruit, mineral nutrient contents of scion cultivar should be taken in consideration. Various studies have shown that tree growth, flower development, yield and fruit quality of scion cultivar of mandarin (Smith et al., 2004).

The work overviews the response in term of plant growth, fruit quality and yield parameters of Feutrell'early grafted on different root stocks under agro ecological conditions of Punjab Pakistan.

## 2. Materials and Methods

Mature fruits from rough lemon rootstock were collected from the citrus foundation block at Citrus Research Institute Sargodha in August 2010. Seeds were extracted and sown in the nursery beds. The seedling were transplanted in nursery area in September 2011. Rough lemon and four other rootstock seedlings namely Troyer, Cox mandarin, C-35 and Carrizo Citrange were imported from Australia under ASLP project were budded employing T-budding method with Feutrell's' early in October 2012. The prepared uniform plants were planted at square No. 15 at Citrus Research Institute Sargodha in September 2013. The plants were arranged in randomized complete block design (RCBD), with three replication and one plant per treatment. The plants were irrigated every 5-7 days interval received 200gm nitrogen 200gm phosphorus and 200gm potash and 10 kg farm yard manure first two year, then during third and fourth year 1 kg Urea 1kg phosphorus and 12 kg FYM yearly. The initial readings of scion girth, stock girth, stem diameter and canopy volumes were measured by measuring tape. The height of the plant was measured in meter from ground level to tip of the plant by measuring Rod. The spread of the tree was recorded by measuring maximum spread in north-south and east-west direction in meters with the help of measuring Rod calculating mean spread of the plant. The canopy volume of selected plants were computing using formula (Albrigo et al., 1975).

$$PScv = \frac{\pi D12}{4}\left[2\left\langle\frac{Ht - Hc}{3}\right\rangle + \langle Hc - Hs\rangle\right]$$

PScv= Canopy Volume($m^3$); Ht= Overall canopy height above ground level(m); D1= Canopy diameter parallel to the row(m); Hc= Height to the point of maximum canopy diameter(m); Hs=Height from ground to canopy skirt(m); H= height of plant.

$$R = \frac{Sum\ of\ E - W\ and\ S - N\ direction\ (m)}{4}$$

E-W= East-West; N-S= North-South.

The TSS was measured by digital Refractometer and the percentage of acidity was determined by dilute juice against 0.1 sodium hydroxide by using phenolphthalein indicator (AOAC, 1985).

Formula: 1 ml of 0.1 N NaOH = 0.0064 g of citric acid was employed.

The layout of the orchard was designed according to complete block design (CRBD) with three replication and each replication with one plant. Five average

growth data was statistically analyzed by using statistix 8.1 software. Difference among the means were tested by LSD.

## 3. Results and Discussion

### *3.1 Scion/Stock ratio*

Five years average data of scion and stock ratio shows that all the five root stock has good compatibility with Feutrell'early.

### *3.2 Plant height (m)*

Feutrell'early budded on different root stock attained maximum plant height on Cox mandarin root stock followed by Troyer citrange and Rough lemon (Figure 1A) average of plant height for consecutive five years. These three root stocks are significantly different. Dubey et al. (2016) observed highest plant height in rough lemon followed by Troyer and no significant difference which is contrary to present study. The remaining root stock has lower plant height. Dwarfing effect was observed in C-35 root stock as its character.

### *3.3 Canopy volume ($m^3$)*

Figure 1A shows that max. canopy volume attained in Feutrell's early budded with Rough lemon followed by Cox mandarin and Troyer. These three are significantly different. The same behavior was observed by Dubey et al. (2016) in which canopy volume was found maximum followed by Cox mandarin and Troyer but difference was not significant.

### *3.4 Fruit weight (g)*

Data regarding influence of rootstock on fruit weight (Figure 1B) reveal that Feutrell' early budded with Troyer produced heaviest fruit followed by Carrizo and Cox mandarin. Both have no significant difference. The lowest fruit weight was recorded in C-35. Al-Hosni et al. (2011) found heavy fruit weight in Hamlin budded with Troyer in Oman.

### *3.5 Fruit size (dia, mm)*

Maximum fruit size in term of diameter was registered in Troyer budded with Feutrell's early (Figure 1B). Statistically Feutrell,s early fruit size remained at par with respect to Musambi budded on Troyer Citrange. and Cox mandarin which are significantly different to remaining three root stock whom have no significant difference. However larger fruits earn more than smaller ones in the market. Fruit size in term of diameter ranged from 50mm to 80mm. The same has been observed in the literature suh as Lucena-Cavalcante et al. (2006); Al-Hosni et al. (2011).

### *3.6 Juice (%age)*

Highest juice percentage was recorded in fruits of Feutrell,s early budded on Troyer. Juice percentage from all the five root stock was statistically at par and no significant difference observed (Figure 1B).

More juicy fruit is not only better accepted in the juice market but also in the fresh market. All the root stock under trial had juice percentage above the minimum accepted for most citrus varieties for consumption as fresh fruit (35%).

### *3.7 Peel weight, peel thickness, rag percentage and seed weight*

These three parameters were not significantly affected by the different root stock. Although more peel contents could be a disadvantage when the ratio between peel and pulp decreases mean less edible portion. Literally the relation between peel thickness and root stock has been reported and confirmed such as Sharma et al. (2004) and Al-Jaleel et al. (2005). Large number of seeds in the pulp of the fruit is negative character. Fruit harvested from all root stock contain almost equal no of seed having average weight 14-15g.

### *3.8 Total soluble solids, acidity and TSS/Acid ratio*

Total soluble solids in all four root stock were significantly different from Rough lemon local root stock (Figure 1C). Fruit harvested from Feutrell's early budded on Troyer root stock have the highest TSS contents as compared to other exotic root stock but all the four exotic root stocks found at par statistically. Lowest TSS contents were recorded in Rough lemon root stock budded Feutrell's early fruits. Feutrell's early fruits on Rough lemon were found more acidic and less acidic in Cox mandarin were registered. Sweetness is important quality parameter for fruits; it is actually considered the sum of sucrose glucose and fructose contents, which are the indication of ripeness (Gomes et al., 2002). Sweetness of the fruit is judged on the basis of sugar to acid ratio. For pleasant fruit taste this ratio is very important. Fruits collected from Cox mandarin budded root stock had the highest sugar to acid ratio followed by Troyer. Lowest ratio was observed in fruits of scion on Rough lemon which mean poor quality fruit. This could be the drawback of the Rough lemon root stock, in spite of the fact that widely adopted root stock in Punjab Pakistan

due to its positive growth characters.

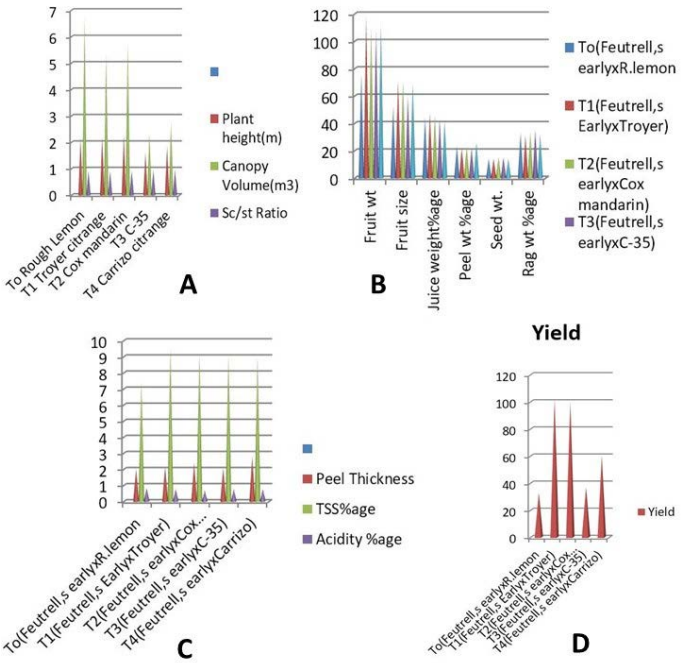


**Figure 1: A: Maximum canopy volume attained in Feutrell's early budded with Rough lemon followed by Cox mandarin and Troyer; B: Maximum fruit size in term of diameter was registered in Troyer budded with Feutrell's early; C: Total soluble solids in all four root stock were significantly different from Rough lemon local root stock; D: Troyer citrange and Cox mandarin exceeded all the other root stocks followed by Carrizo.**

(yield fruit tree$^{-1}$). The statistical analysis of the data indicates highly significant difference amongst root stocks; Troyer citrange and Cox mandarin exceeded all the other root stocks followed by Carrizo (Figure 1D).

## Conclusions and Recommendations

Present study conclude that better fruit size could be attained in Feutrell's' early budded with Troyer Citrange and Cox mandarin without limiting any other qualitative and quantitative character. These root stock can replace Rough lemon root stock which have certain problem of serious nature. Furthermore, studies are required to prove the bearing length of the plants.

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## Authors Contribution

All authors have equally contributed to this research work.

## Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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