ISSN Print: 2518-4261
ISSN Online: 2518-427X
Vol. 54(3), September 2017

PROCEEDINGS
OF THE PAKISTAN ACADEMY OF SCIENCES:
B. Life and Environmental Sciences

PAKISTAN ACADEMY OF SCIENCES
ISLAMABAD, PAKISTAN

PAKISTAN ACADEMY OF SCIENCES

Founded 1953

Proceedings of the Pakistan Academy of Sciences, published since 1964, is quarterly journal of the Academy. It publishes original research papers and reviews in basic and applied sciences. All papers are peer reviewed. Authors are not required to be Fellows or Members of the Academy, or citizens of Pakistan.

Annual Subscription: **Pakistan:** Institutions, Rupees 2000/- ; Individuals, Rupees 1000/-
Other Countries: US$ 100.00 (includes air-lifted overseas delivery)

HEC Recognized, Category X; PM&DC Recognized

Published by **Pakistan Academy of Sciences**, 3 Constitution Avenue, G-5/2, Islamabad, Pakistan
Tel: 92-5 1-920 7140 & 921 5478; **Fax:** 92-51-920 6770; **Website: www.paspk.org**

Printed at **PanGraphics (Pvt) Ltd.,** No. 1, I & T Centre, G-7/l, Islamabad, Pakistan
Tel: 92-51-220 2272, 220 2449 Fax: 92-51-220 2450 E-mail: pangraph@gmail.com

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 149–163 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Geospatial Analysis of Landslide Susceptibility and Zonation in Shahpur Valley, Eastern Hindu Kush using Frequency Ratio Model

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Abstract: This study dealt with geospatial analysis of Landslide Susceptibility (LS) and resultant zonation in Shahpur valley, eastern Hindu Kush (HK) using Frequency Ratio Model (FRM). Geologically, HK region constitutes the youngest mountain system. In the study area, landslide is a recurrently occurring natural event. Every year, landslides incur significant property and human losses. The extent of damages is expected to multiply in future due to overgrazing, deforestation, increase in population and infrastructural expansion over the fragile slopes. In the HK region, Shahpur valley was selected as the test area to apply FRM using geospatial technique and explore various factors for determining LS. Initially, a reconnaissance field survey was conducted for preparation of landslide inventory map. SPOT5 pan sharpened image of 2.5 m was used to map various sizes of activated landslides and its subsequent locations were verified in the field. The selected LS factors including surface geology, slope gradient, proximity to fault lines, land use, slope aspect, proximity to roads and proximity to stream/river were used. The relationship between landslide and determining factors were spatially analyzed using FRM. As a result, the Frequency Ratio Score (FRS) was calculated for each factor. Based on cumulative FRS, landslide Susceptibility Indices (LSI) were developed and classified into very high, high, moderate, low and very low LS zones. The central part of the valley was found to be highly susceptible to landslide hazard as both natural and anthropogenic factors were prevalent in this region. Finally, the LS zones were validated by the success rate curve approach.

Keywords: Landslide, frequency ratio model, geospatial, landslide susceptibility, Hindu Kush

1. INTRODUCTION

This paper deals with the geospatial analysis of Landslide Susceptibility (LS) and to develop zones of low to high susceptibility in Shahpur valley, Hindu Kush (HK) using Frequency Ratio Model (FRM). Globally, the frequency and trend of landslide events are on rise [1, 2]. Landslide is considered as one of the devastating disasters in term of damages and human casualties [3]. Every year hundreds of people are affected by landslides and it also put tremendous pressure on individuals, community and national economy [4]. The Hindu Kush Karakorum Himalayan (HKKH) region is highly susceptible to landslide occurrences due to its geological structure, topography, climate and growing human interventions [5-7]. In HKKH region, it is estimated that on average every year property loss of US$ one billion occur due to extreme natural events, out of which over 30% is attributed to landslides [8-10]. In the HKKH region, during 1990 to 2005, an increasing trends of landslide events has been recorded [9] and it was predicted that it will further escalate in future due to the growing human interventions over the fragile slopes and an increase in extreme weather events [11].

In HKKH region, landslide is a recurrently occurring natural phenomenon [5, 12]. The ever

Received, February 2017; Accepted, August 2017
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increasing population has forced the dwellers to potentially fragile slopes for habitation, terraced farming and infrastructural development [13]. In addition to population growth, decrease in forest cover and rapid expansion in infrastructural development over the unstable slopes have further aggravated the landslide risk [10, 14]. Parallel to human intensifying factors, the immature geology, wide range of diurnal and seasonal temperature and precipitation have categorized the Hindu Kush region as the worst landslide affected area [10, 15]. It is hard to predict occurrences of landslides; however, it can be assessed through its history and potential causative factors for determining susceptibility zones.

Scientific community has worked on LS and its zonation in different part of the world [16, 17]. Landslide is controlled by perceptible contributing factors, which can be interpreted through field survey and satellite image interpretation [9]. LS aims to foresee, where the slope failures has potential to activate [18]. Topography, geomorphological factors and removal of vegetation cover are considered as the most important landslide triggering factors [17]. Landslide Susceptibility Zonation (LSZ) classifies the target area into homogeneous zones according to probability of landslide occurrence [19, 20].

Now-a-days, geospatial technology has been widely used as an effective tool for LS analysis and resultant susceptibility zonation [21]. The scope and application of geospatial techniques have been boost-up due to its spatio-statistical capabilities

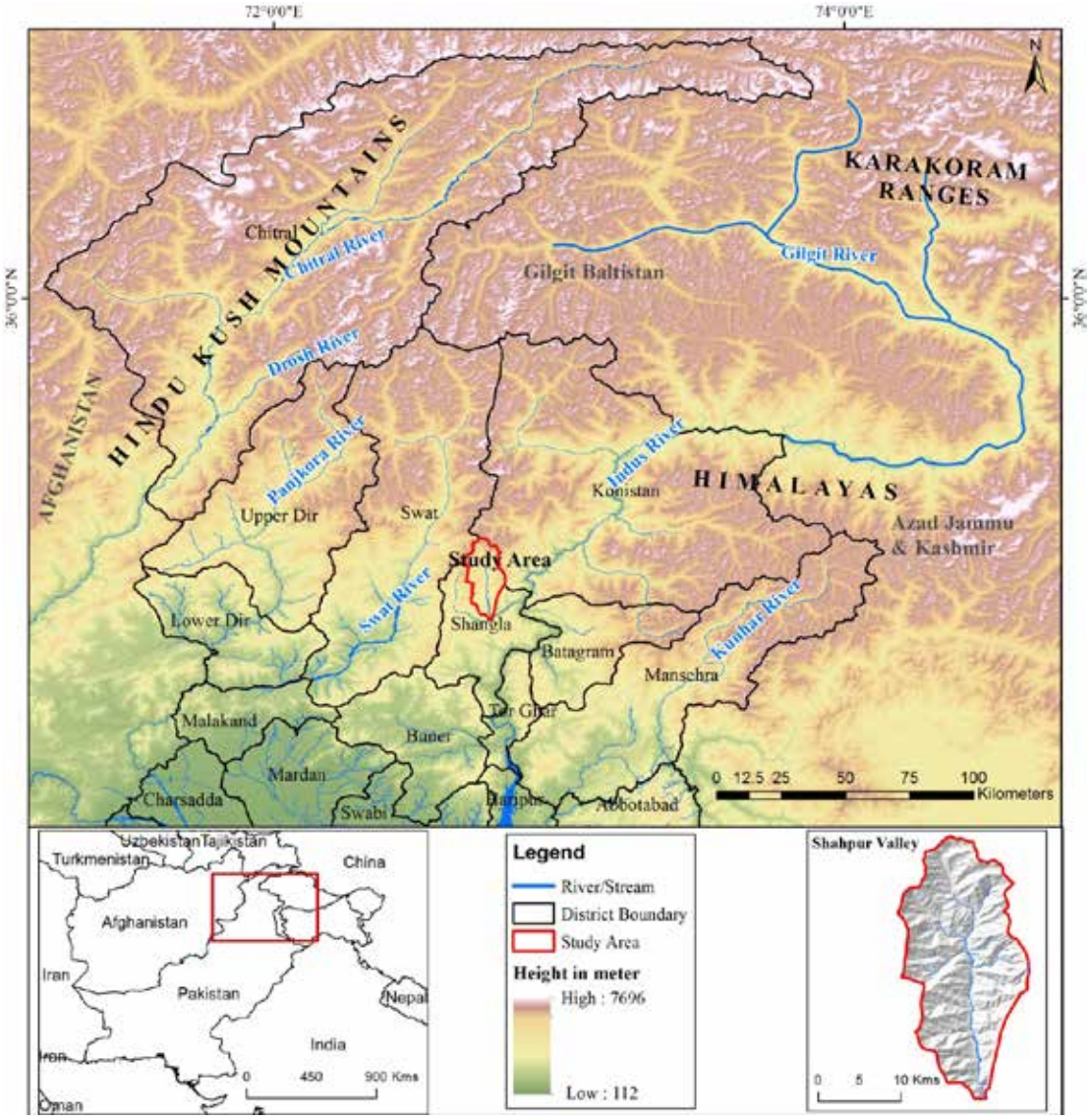

Fig. 1. Location of Shahpur valley in Hindu Kush Mountain system.

and potentials of handling large dataset [22, 23]. LSZ plays an important role in land use planning to minimize the potential impacts of future landslides [24]. Frequency Ratio Model (FRM) is an empirical quantitative approach for LSZ [25]. It provides spatial correlation between location of past landslides and associated triggering factors [17, 26].

Shahpur valley is located in the offshoot of eastern Hindu Kush Mountain [27]. Geographically, Shahpur valley stretches between latitudes 34° 52′ 31″ to 35° 9′ 35″ North and longitude 72° 40′ 10″ to 72° 48′ 44″ East (Fig. 1). Total area of Shahpur valley is approximately 259 km^2. Climatically, the study area falls in humid to sub-humid zone and receives ample amount of rainfall from summer monsoon, whereas in winter the higher altitude receive snowfall [28]. The climate of the area remains mild to warm in summer and cool/ cold in winter. The average annual rainfall varies from 1200mm to 1600 mm. The surface terrain has great variation that ranges from 879 m in the south to over 4,400 m in the north (Fig. 2). In the upper reaches, the slopes are steep, whereas the lower part of the valley has comparatively gentle slopes. Shahpur valley has immature lithology and dominated by unconsolidated material. In order to achieve this goal, three objectives were set-up. To assess the impact of selected causative factors on landslide and to analyze the significant of Frequency Ratio Model in landslide susceptibility zonation. And finally to draw the landslide susceptibility zonation map. In the study area, landslide is a recurrently occurring phenomenon and usually it incur heavy property and human losses.

2. MATERIALS AND METHODS

In order to achieve the study objectives, LSZ using FRM has been applied in GIS environment. To test the FRM, Shahpur valley was selected for detailed insight analysis and to grasp the governing landslide susceptibility factors, which frequently trigger the slope failures. Many factors are involved in slope failure processes and LS assessment in an inter-disciplinary context. LSZ is largely depends on the accuracy of data, selection of appropriate parameters, analytical techniques and the methodology used to process the acquired data.

In the present research, attempt has been made to develop LSZ that ranges from low to very high susceptibility in Shahpur valley. Initially, the inventory of past landslides was acquired and their causative factors were explored from the landslide victims and local population. In this regard, a spatial data layer of past/activated landslide events/sites were identified and digitized on SPOT Satellite image of 2013. For preparing landslide inventory, a detailed field investigation was also carried out to verify the site and situation of activated landslide. To get the opinion and indigenous knowledge about the causative factors that initiate landslides, a Focus Group Discussions (FGDs) were also conducted with the key stakeholders in the study region.

In this study, surface geology, proximity to stream, land use, slope aspect, slope gradient, proximity to fault line and proximity road network were selected as determining factors, which eventually activate a potentially unstable slope. The data pertaining to landslide triggering factors were acquired from relevant government line departments, community and non-governmental organizations. For surface geology and tectonics, the Geological map of North Pakistan was used [29, 30]. The spatial database for administrative boundaries and settlement was extracted from topographic sheets (scale 1:50,000) of Survey of Pakistan. SPOT Satellite image having 2.5 m spatial resolution was acquired from SUPARCO. Spatial features of road network were acquired from the office of Communication and Works Department, Peshawar. In order to get, land use spatial database, supervised classification was applied on satellite image. Similarly, ASTERGDEM 30 m has been used for extracting digital terrain, slope aspect, slope angle and hydrology of Shahpur valley. Parallel to this, a detailed field survey was also conducted for ground verification.

ArcGIS has been used for preparing landslide inventory and generation of thematic layers of selected parameters. The basic assumption in LSZ was that the factors which influence the occurrence of landslide events in the past would be the same to trigger new landslides in future. Following this assumption, a relationship was determined between

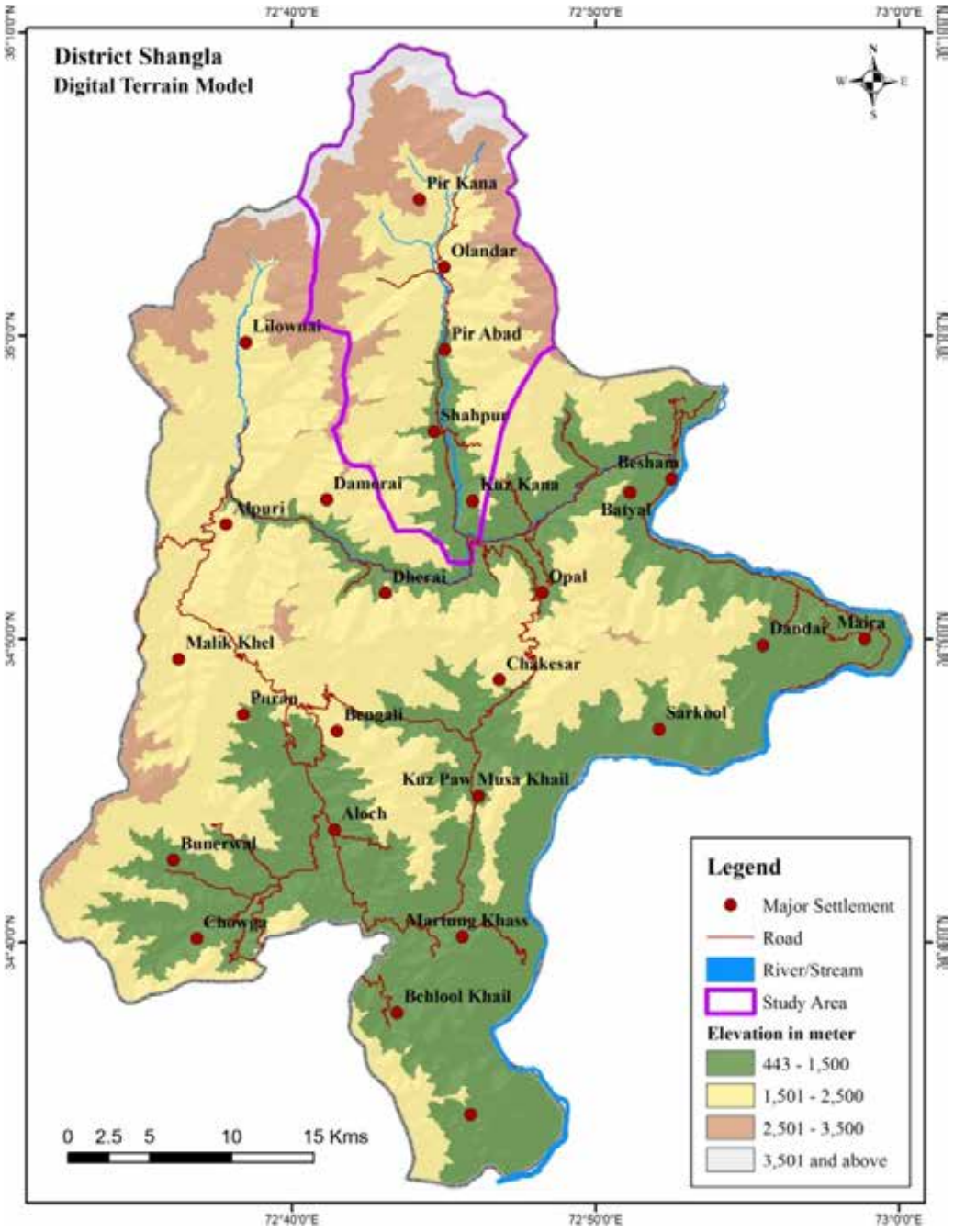

Fig. 2. Location of Shahpur valley and the surface terrain as extracted from ASTER GDEM.

causative factors and spatial distribution of activated landslides. Such relationship was quantified using Frequency Ratio Model (FRM) as applied by many researchers [21, 25, 31, 32]. In FRM, a statistical value was calculated for each class of a factor map using Eq. 1

$$FR = \frac{N_{pix(Si)} / N_{pix(Ni)}}{\sum N_{pix(Si)} / \sum N_{pix(Ni)}} \quad \text{Eq. 1}$$

Where $N_{pix\ (Ni)}$ is the total number of pixels in the entire study area having class i, $N_{pix\ (Si)}$ is the number of pixels containing class i, $\sum N_{pix\ (Si)}$ is the total number of pixels containing landslide in the study area, $\sum N_{pix\ (Ni)}$ is the total pixels in the study area.

The FR value was calculated for all the classes of the selected factors including surface geology, proximity to thrust and fault line, land use, slope gradient, slope aspect, proximity to roads

and proximity to stream. In a FR scale, a score greater than 1 (one) indicates strong and positive relationship between landslide occurrences and the concerned class of the factor map and high landslide susceptibility, whereas a score less than 1 (one) is an indication of negative relationship and low susceptibility to landslide. The FR value for each class of a factor map was obtained from the relationship between all landslide locations and each class of a factor map. The Landslide Susceptibility Index (LSI) was calculated by summation of each factor map using value of FR. As a result, LSZ map was developed based on LSI. Finally, the LSZ map was validated at high confidence level.

3. RESULTS AND DISCUSSION

Landslides are important processes on hill slopes and a devastating extreme event in many parts of the world [33, 34]. It has wide-range of impacts on people and economy of the mountainous community. The impact of landslide is hard to assess as it is some time occurs in association with other hazards, which act as landslide triggering factors. Frequency Ratio Model has already been tested by many researchers for LS analysis in different part of the world [26, 35-37]. Landslide is a natural process and cannot be eliminated but the risk can be minimized. In this paper the FRM was applied for susceptibility zonation, which indicates the areas prone to landslides by integrating the triggering factors of landslides with the inventory of spatial distribution of past landslides. LSZ has been extensively applied in the past two decades and it has helped in reducing landslides [37].

3.1. Inventory of Landslides in Shahpur Valley

The landslide inventory map shows spatial distribution of landslides (Fig. 3), which is the preliminary step for a reliable predictive LSZ. It is hard to visit and map every landslide on the ground due to time and resource constraint. It is therefore, in this study all the activated landslides were demarcated, interpreted and plotted (Fig. 3) on multi-spectral SPOT satellite image of April 2013 with a spatial resolution of 2.5 m. Removing uncertainty in image interpretation and periodic field visits were conducted for ground-truthing and maximum precision. These landslides were then rasterized with pixels of 10x10 m to calculate the number of pixels holding the activated landslides in different classes of each factor map for calculation of landslide frequency ratio.

3.2. Surface Geology and Frequency Ratio

The geology and tectonics of the HKKH region is marked by the collision of Indian plate with the Eurasian plate [38]. This collision occurred due to the northward subduction of Tethys ocean floor under the Eurasian plate, which initiated in the Cretaceous to Mio-Pliocene and still continue at a rate of 4.5 mm/year [38]. The Indus suture zone marks the boundary of collision between the Eurasian and Indian plate.

In Shahpur valley, LS has a close relationship with surface geology, its formation and rock type. It is fact that lithological formation and tectonics significantly influence the slope instability, as it has profound impact on the strength and permeability of rocks and soil. In Shahpur valley, the surface geology and tectonics of this region mapped after Baig (1990) where the dominant formations includes Alluvium, Alpurai Group, Besham Group, Darwaza Sar Potassic Granite Gneiss (DSPGG), Greenschist Melange, Jabrai Granite Gneiss, Jijal Ultramafic, Karora Group and Manglaur formation (Fig. 4a). Most of these lithological zones were complex of different rock types. Analyzing the relationship of past landslides distribution and lithological units through FRM, it was found that Darwaza Sar Potassic Granite Gneiss (DSPGG) and alluvium has highest tendency towards LS followed by Greenschist Melange (Table 1).

3.3. Proximity to Thrust/Fault and Frequency Ratio

Several authors have pointed out the strong relationship of thrust and fault lines with landslide occurrences [39, 40]. Proximity to fault line has become standard practice for assessing LS [41]. It is clear from the literature that slope failure increases in proximity to tectonic structures. Fault lines are usually associated with fractured zones. Presence of thrust and fault zones at high degree slope presents favorable conditions for landslide occurrence.

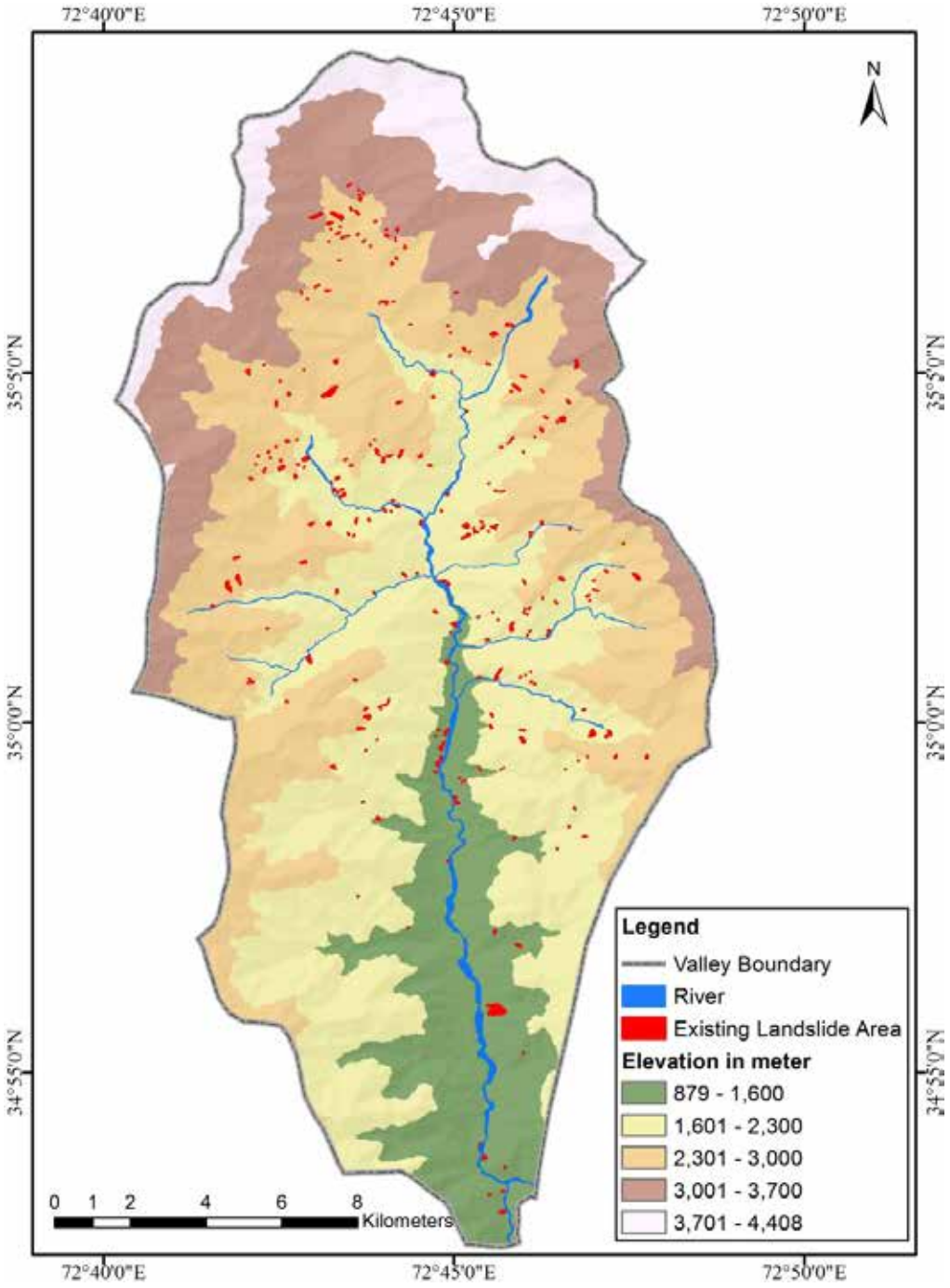

Fig. 3. Shahpur valley, spatial distribution of activated landslides.

In Shahpur valley, there is a complex tectonic setup and considered as one of the determining factor in slope failure processes. The thrust and fault line map has been produced from geological map of Besham area after Baig [29]. The Main Mantle Thrust (MMT) and Makhad Thrust (MT) were found passing through the study area (Fig. 4b). Puran fault, Alpurai fault, Chakesar fault and Karshut fault lines also passes through Shahpur valley and it has profound impacts on slope failure. Applying spatial techniques, on the thrust and fault lines Euclidean distance of 500, 1000, 1500 and more than 1500 meter were generated from thrust and fault line in GIS environment. In this process an attempt has been made to explore the impact of proximity to thrust and fault line on landsliding. It was found from the analysis that these tectonic structures have significant impact on landslide

Table 1. Shahpur valley, frequency ratio values of the landslide conditioning parameters.

Class	$N_{pix\ (Si)}$	%age of $N_{pix\ (Si)}$	$N_{pix\ (Ni)}$	%age of $N_{pix\ (Ni)}$	Frequency Ratio
Surface Geology					
Alluvium	1500	18.53	290137	11.20	1.66
Greenschist Melange	806	9.96	165892	6.40	1.56
Jabrai Granite Gneiss	903	11.16	497979	19.22	0.58
Alpuraicalc-mica-garnet schist	990	12.23	235014	9.07	1.35
Karora Group	967	11.95	501955	19.37	0.62
Besham Group	1437	17.76	441986	17.06	1.04
Manglaur Formation	1219	15.06	378895	14.62	1.03
Darwaza Sar Potassic Granite Gneiss	271	3.35	43693	1.69	1.99
Jijal Ultramafics	0	0.00	35939	1.39	0.00
Fault Line Buffer (m)					
0 – 250	4018	444.96	897661	180.26	2.47
251 – 500	2325	257.48	796194	159.89	1.61
501 – 1000	760	84.16	591759	118.83	0.71
> 1000	990	109.63	1894091	380.36	0.29
Slope Gradient					
$0\text{-}5^0$	91	1.12	67672	2.61	0.43
$6\text{-}15^0$	514	6.35	261202	10.09	0.63
$16\text{-}30^0$	2138	26.42	668428	25.81	1.02
$31\text{-}45^0$	4847	59.89	1365626	52.73	1.14
$> 46^0$	503	6.22	226881	8.76	0.71
Slope Aspect					
Flat	1	0.01	1003	0.04	0.32
North	503	6.22	214528	8.28	0.75
Northeast	531	6.56	284345	10.98	0.60
East	1444	17.84	387747	14.97	1.19
Southeast	881	10.89	395235	15.26	0.71
South	1775	21.93	366716	14.16	1.55
Southwest	1135	14.02	356711	13.77	1.02
West	819	10.12	317177	12.25	0.83
Northwest	1004	12.41	266347	10.28	1.21
Land Cover					
Range Land	2762	34.13	846981	32.71	1.04
Forest	2621	32.39	1035399	39.98	0.81
Glacier and Snow	108	1.33	111001	4.29	0.31
Agriculture Land	2100	25.95	416605	16.09	1.61
Settlement	48	0.59	37492	1.45	0.41
Barren Land	87	1.08	87813	3.39	0.32
Water bodies	367	4.53	54210	2.09	2.17
Road Buffer (m)					
0-100	769	9.50	134060	3.21	2.96
101-200	541	6.68	110469	2.64	2.53
201-300	591	7.30	100728	2.41	3.03
301-400	141	1.74	94590	2.26	0.77
> 400	6051	74.77	3739858	89.48	0.84
Stream Buffer (m)					
0-100	1918	23.70	294929	7.06	3.36
101-200	1555	19.21	266588	6.38	3.01
201-300	1021	12.62	257173	6.15	2.05
301-400	799	9.87	250374	5.99	1.65
401-500	395	4.88	241482	5.78	0.84
>500	2405	29.72	2869159	68.65	0.43

occurrence. The investigation further indicates that number of landslides is high near the thrust/ fault line and it decreases as one move away from the thrust and fault lines (Table 1).

3.4. Slope Gradient and Frequency Ratio

Physiography has greater impact on the human activity of local population and distribution of natural resources. The behavior of landslide has close association with the slope gradient [42] and recognized as a controlling factor in slope failure [7]. Slope gradient is directly proportional to slope instability and landslide density increases with increase in slope gradient [43]. It is, therefore, the frequency and landslide occurrence increases with increasing slope gradient. During the field survey, it was found that there is high concentration of landslide occurrence along the streams and roads, where abrupt change in slope gradient and lateral cutting is dominant factors.

In Shahpur valley, slope gradient map was produced from AsterGDEM 30 m resolution in GIS (Fig. 4c). The resultant map has been classified in different groups based on degree of slopes. The analysis reveals that with increasing slope gradient, the rate of landslide occurrences enhances and thus up to 45 degree slope there is a gradual increase in the value of frequency ratio as shown in Table 1.

3.5. Slope Aspect and Frequency Ratio

In the study region, slope aspect has been generated from ASTER GDEM 30m resolution in GIS environment. Slope aspect is an important factor which influences landslide occurrences and has been used by many researcher in LS mapping [44]. Literature reveals that slope aspect does not have direct impacts on landslide activation, but it indirectly stimulates landslide initiation. It is mainly depend upon the duration and intensity of sunlight on slopes, amount of precipitation receives and moisture retaining capacity, where all the factors have strong correlation with vegetation cover and landslide [3]. Similarly, rain-induced landslide is common phenomena in the HKKH region [43]. In Shahpur valley, the slope aspect has been displayed as flat, northward, northeast, east, southeast, south, southwest, west and northwest facing slopes (Fig. 4d). In the Hindu Kush region, the south facing slopes have high sunlight exposition and receive ample precipitation from monsoon in summer. It is therefore, the south facing slopes have high frequency ratio and thus have high tendency to landslide occurrence (Table 1).

3.6. Land Use/ Land Cover (LULC) and Frequency Ratio

LULC has an immense impact on slope stability [43] and it is vegetation cover that reduces the potentials of soil erosion and thus minimizes the chances of slope failure. The roots bind the soil and keep the slope stable [12]. However, the barren slopes have more exposure to geomorphic agents and led to erosion and slope instability. The LULC of Shahpur valley is extracted from multispectral satellite image of SPOT of April 2013 having 2.5m spatial resolution. In Shahpur valley, the major land cover were forest, agricultural land, rangeland, settlements, glacier/snow and water bodies (Fig. 5a).

In Shahpur valley, while analyzing the impact of land use/land cover on LS, it was found that waterbodies has the highest score of frequency ratio followed by agricultural land and rangeland (Table 1). The area covered by rivers and streams has been kept in class waterbodies. It has a profound impact on landsliding due to its lateral erosion, which in turn make the slope susceptible. In Shahpur valley, agricultural activities are mostly carried out on terraces of mountain slopes. The third highest frequency ratio exists in rangeland category (Table 1) due to consistent overgrazing and deforestation.

3.7. Proximity to Road and Frequency Ratio

The construction of roads involve mostly improper excavation and cutting of slopes which disturb the slope and increases the slope instability. Landslide expert consider roads as a major contributor in deforestation process [45] and in turn expedite the process of weathering and mass wasting. In Shahpur valley, proximity to road has been used as a determining parameter for slope instability. On road network data, multiple ring buffers of 0-100, 101-200, 201-300 and 301-400 m were applied to find out the impact of road proximity to landslide

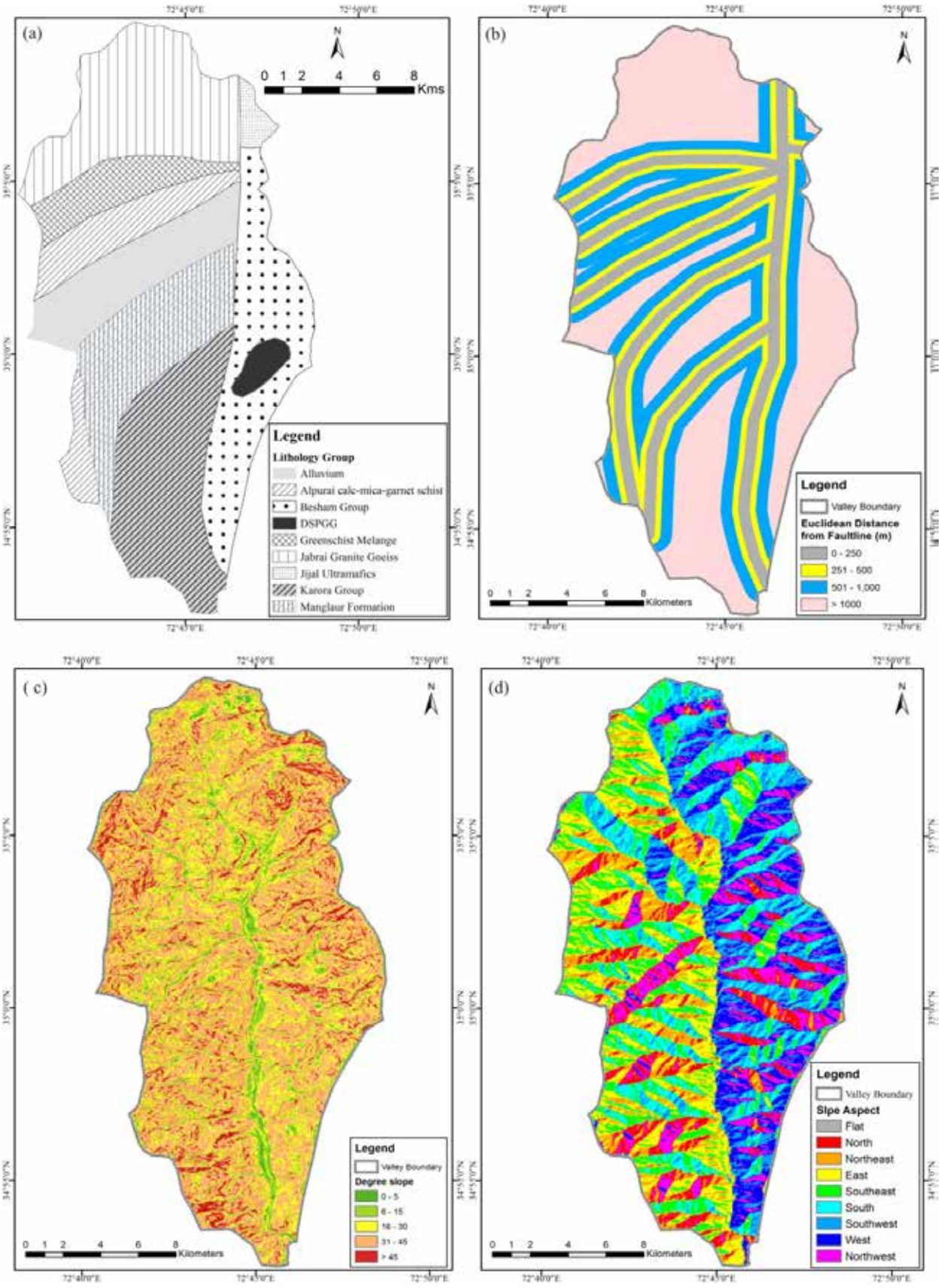

Fig. 4. Shahpur valley: (a) Surface geology; (b) Proximity to fault line; (c) Slope gradient; (d) Slope aspect.

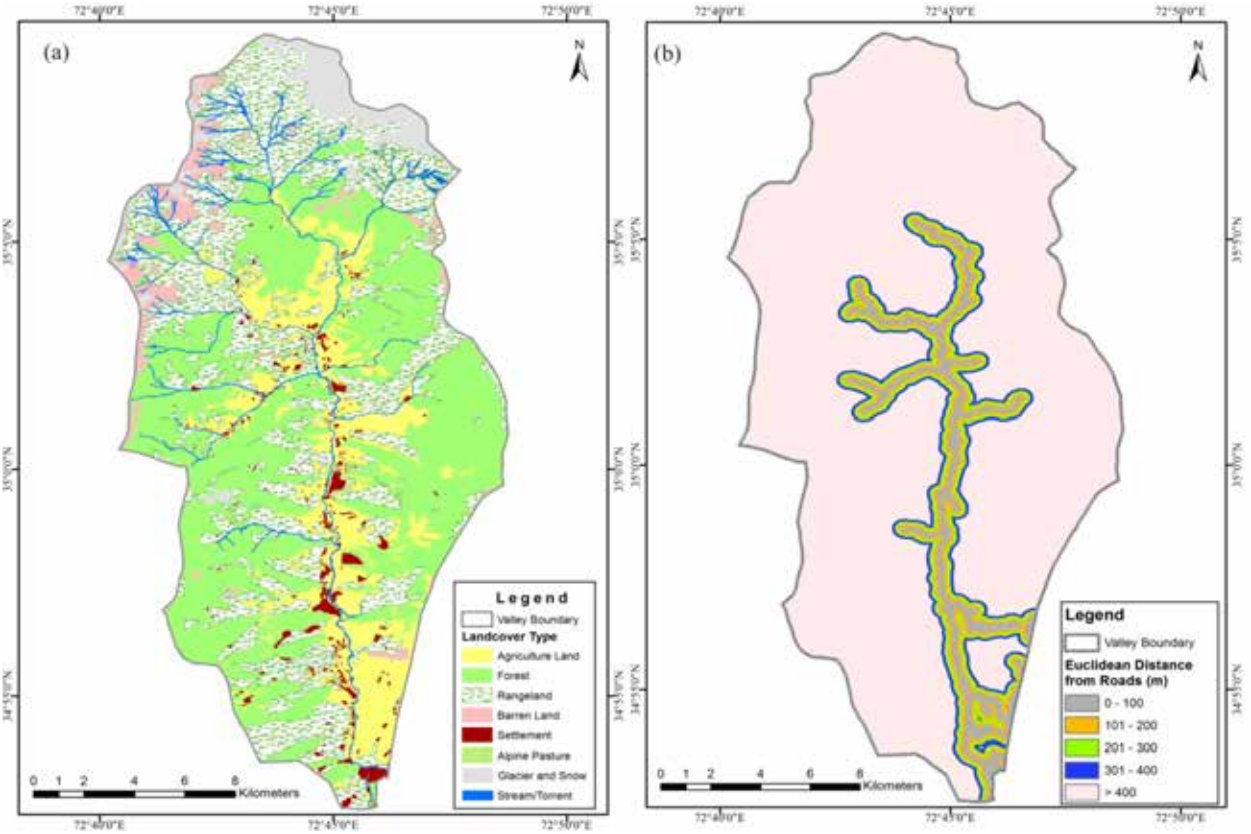

Fig. 5. Shahpur valley: (a) Land use/ land cover; (b) Proximity to road.

occurrences (Fig. 5b; Table 1). The analysis revealed that LS and road proximity has a close relationship. Landslide frequency ratio was found higher near the road up to 300 m and beyond 300 m there is decrease in frequency ratio score (Table 1). This means that slope near the road are more susceptible to landslide hazard and more stable as one move away from roads.

3.8. Proximity to River/Stream and Frequency Ratio

In order to analyze the impact of river/stream on landslide hazard, a statistical model frequency ratio was applied. Euclidean distance from river/ streams was taken as 0-100, 101-200, 201-300, 301-400, 401-500 and plus 500 m to analyze the impact of river/streams on landslide hazard (Fig. 6). While analyzing the relationship of distance from the stream and landslide occurrences, it was found that near the streams the frequency ratio was found higher indicating high LS. The highest frequency was found in the region of 100 m distance (3.36) followed by 100 to 200 m distance having 3.01 frequency ratio (Table 1). The analysis revealed that in class less than 401 m, the frequency ratio is greater than 1 indicating that the region has high susceptibility to landslide occurrences. Whereas the region with over 400 m distance from river/ stream, the frequency ratio is < 1 signify that the susceptibility of landslide occurrence is low (Table 1).

3.9. FRM and Landslide Susceptibility Zonation

Landslide hazard is the most common threat to the lives and property of the people of Shahpur valley. Hence, it is important to identify and map landslide prone areas for safer community. LSZ is one of the pre-requisite for developmental planning in mountainous areas and it is the process that divides the area into different classes according to their level of susceptibility based on certain parameters. All the thematic layers were integrated in GIS environment to generate the LSZ map of Shahpur valley. To minimize subjectivity, quantitative approach was applied for preparation of thematic maps. The objective of this approach was to

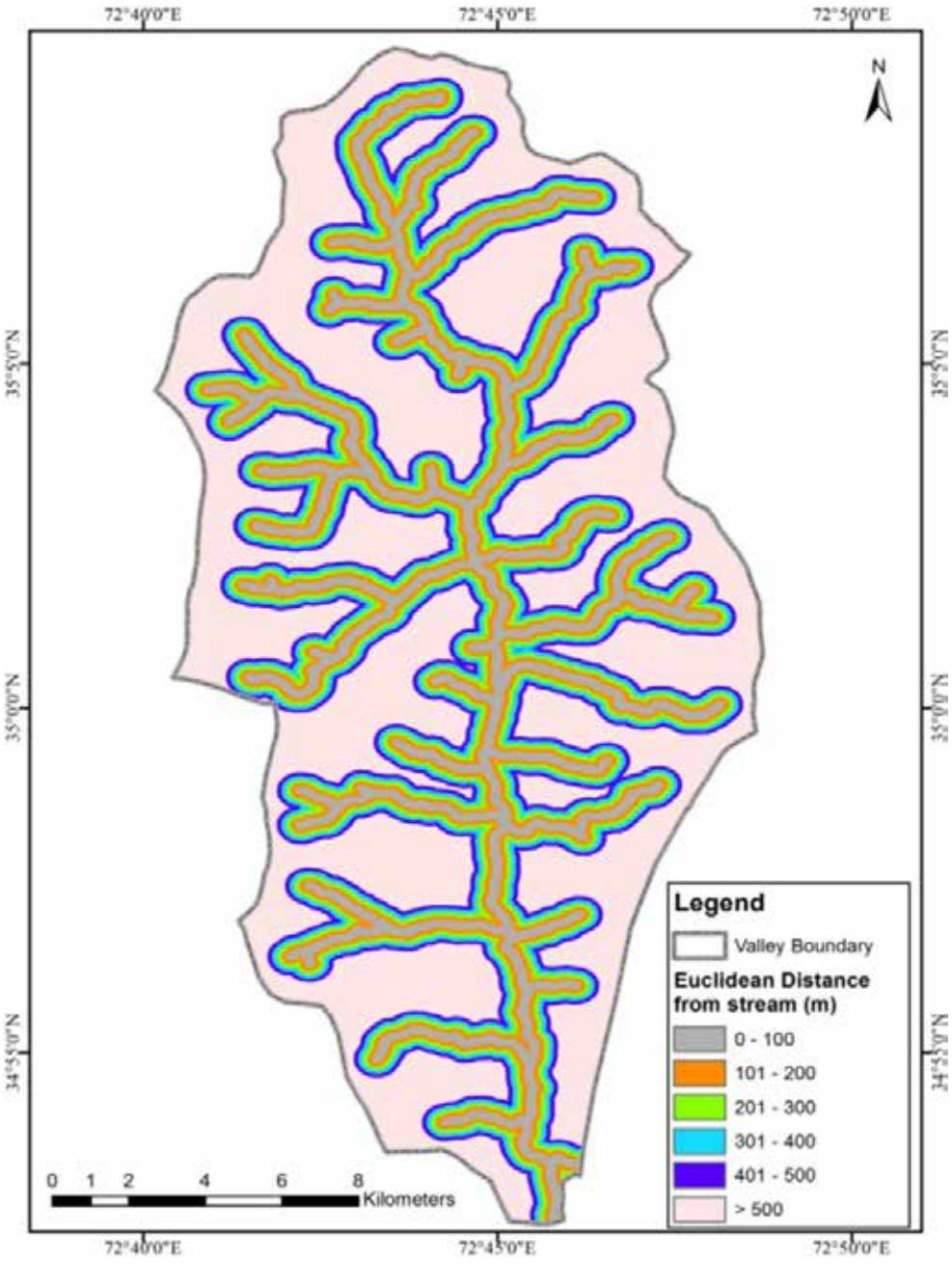

Fig. 6. Shahpur valley, proximity to river/ stream.

quantifying the relative importance of each sub-factor theme in an integrated factor map. The LSI was calculated by a summation of each factor map using frequency ratio value (Eq. 2):

$$LSI = \sum Fr \qquad \text{Eq. 2}$$

While using the LSI, all the values range from 2.99 to 15.14. Higher the value of LSI, greater would be the likelihood of landslide occurrences and vice versa. Based on LSI value, the Shahpur valley was divided into five zones of very low, low, moderate, high and very high susceptibility regions (Fig. 7). The analysis revealed that out of total study area, 2.7% area falls in very high landslide susceptible zone and 9.9% in high LSZ. It clearly indicates that approximately 13% of Shahpur valley lies in highly susceptible zone and may cause enormous damages in future if proper mitigation strategies were not taken in-time. In Shahpur valley, the past landslide events at high confidence level follow the

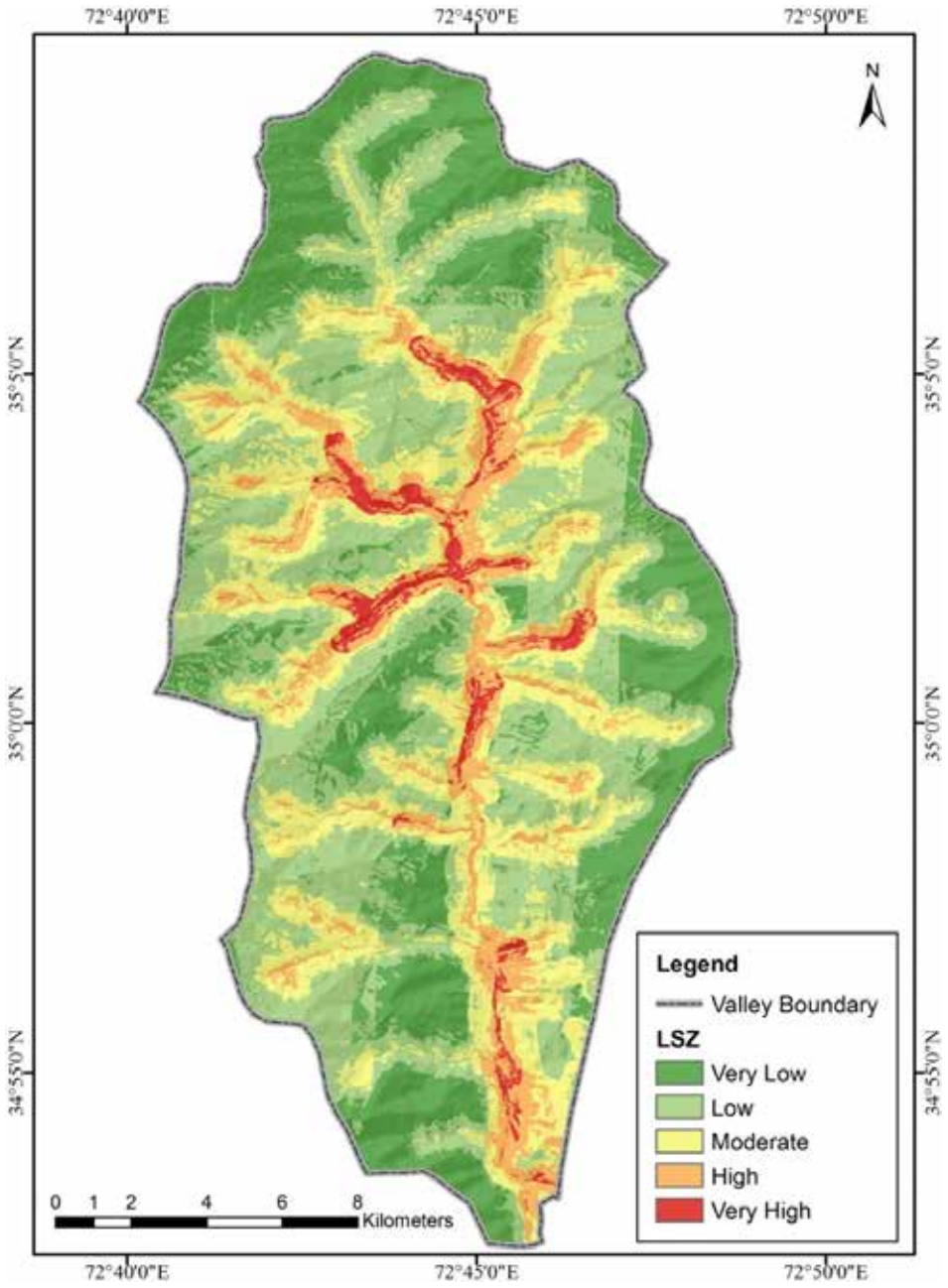

Fig. 7. Shahpur valley, landslide susceptibility zones.

predicted LS zones (Fig. 7). The analysis further verifies that in 2010, a heavy landslide event *Olandar* has incurred 60 deaths, also falls in very high susceptibility zone.

3.10. Validation of Landslide Susceptibility Zonation

In Shahpur valley, the LSZ was produced using FRM and the same was validated by comparing it with already activated landslide map. The success rate curve was calculated to evaluate the accuracy of FRM for selected contributing factors to landslide occurrences. For success rate curve calculation, the LSI values is divided into 100 equal classes sorted in descending order ranging from highly susceptible to very low susceptible classes. This was also overlaid with the existing landslide area layer and the area under already activated landslides falling in each susceptible class was calculated through spatial statistical tool in ArcGIS. The cumulative percentage was calculated and success rate curve

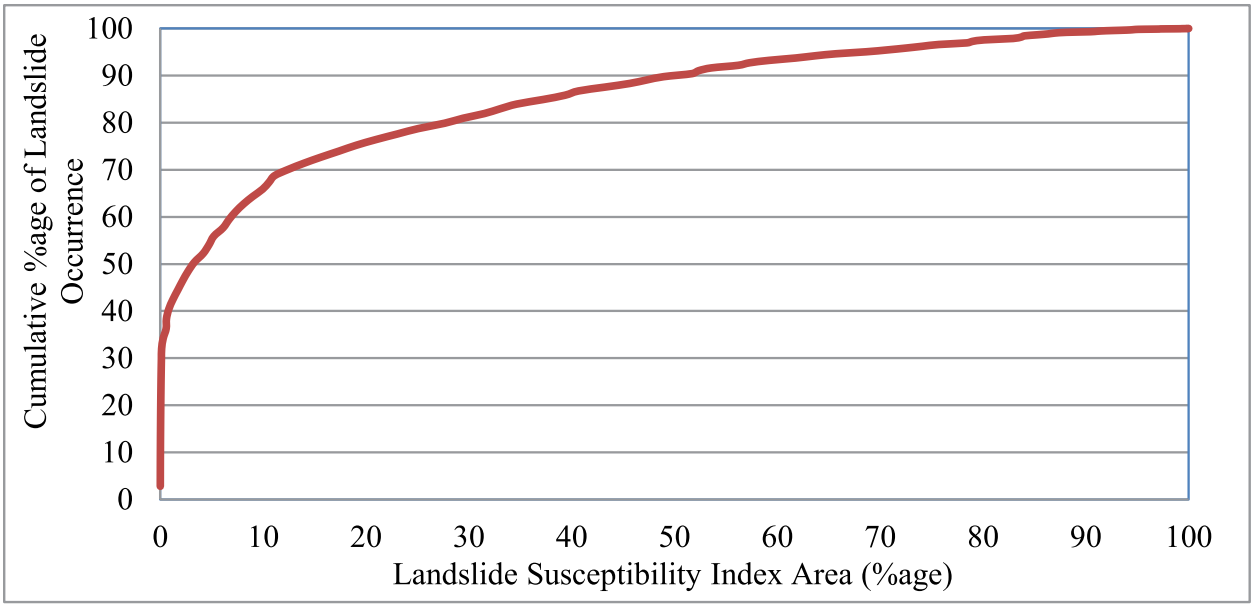

Fig. 8. Shahpur valley, landslide susceptibility success rate curve.

was built by plotting LSI on the x-axis and the cumulative percentage of landslides occurrence on the y-axis (Fig. 8). The success rate curve has steep curve which indicates significant result of FRM and productive results of landslide susceptibility zonation map. The final LSZ map was validated through success rate curve and the prediction accuracy was found as 91.11%. In Shahpur valley, the success rate curve validates satisfactory results of FRM (Fig. 8).

4. CONCLUSIONS

In Shahpur valley, Frequency Ratio Model was applied using susceptibility factors for identification and development of landslide susceptible zonation map. Initially, the detailed inventory of past landslide events was identified and plotted on multi-spectral SPOT satellite image of April 2013. In order to apply FRM in GIS environment, standard parameters of geology, thrust/fault line, land use/ land cover, slope aspect, slope gradient, proximity to river and roads were selected for susceptibility analysis. The analysis revealed that considering geology, Darwaza Sar Potassic Granite Gneiss, Alluvium and Greenschist Melange have the highest frequency ratio and thus highly susceptible to landslide occurrence.

While analyzing the impact of tectonic structure, it was also found that frequency ratio was also found high near the fault lines. Similarly, the impact of slope gradient on landslide susceptibility was found maximum at slope that ranges from 31 to 45 degree. Likewise, it was found that south and east facing slopes have high frequency ratio and thus have high susceptibility of landslides. It is because of its high sunlight exposure and the same it also receives plenty of precipitation. In addition to this, the impact of river bank erosion also joins hand in escalating the landslide susceptibility. In order to get landslide susceptible zonation map, frequency ratio of all the sub-classes of the selected factors were sum-up through weighted overly technique in GIS environment and the resultant LSI was calculated. The final LSZ map was validated through success rate curve and the prediction accuracy was found 91.11%.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 165–175 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Effect of Antioxidants on Storage Quality of Apple Sucrose Bars

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Abstract: An experiment was conducted to study the comparative effect of citric acid and ascorbic acid as antioxidants and variations in concentration of sugar (20, 30 and 35 °brix) on over-all quality of apple bars. Potassium meta-bisulphite and pectin were added as preservatives and binding agent, respectively. Quality of apple bars was evaluated within 3 months of storage, at 15-day intervals, in consideration of physicochemical and sensory aspects. In the bar samples, decrease was observed in water activity (0.69-0.64), moisture content (17.3-15.0 %), non-reducing sugars (4.12-3.92 %), pH (3.64-3.43), and ascorbic acid (3.11-0.61 mg/100 g). Increasing trends were noted in reducing sugars (17.28-17.31 %), titratable acidity (1.24-1.47%) total solids (83.26-87.38 %) and total soluble solids (63.17-68.46 °Brix) within 30 days of storage. Also, the apple bars exhibited noticeable changes in color (8.50-6.73), texture (8.50- 6.58), taste (8.50-6.51) and overall acceptability (8.00-5.68) during 90 days of storage. The bars prepared with 35° Brix and 0.1% citric acid exhibited comparatively higher stability in terms of physicochemical and sensory traits.

Keywords: Apple bar, sucrose, antioxidants, physico-chemical properties, sensory properties

1. INTRODUCTION

Apple is an important fruit and is produced mainly in central and south-western Asia. In Pakistan, it is nurtured in northern hilly areas of Khyber Pakhtunkhwa, Punjab and Baluchistan [1]. Apple is normally consumed as fresh fruit or as an ingredient in a variety of food products. Fruit bar is an intermediate moisture food (IMF) product having soft pliable texture, high moisture content (11-67% on dry weight basis) with minimum water activity of 0.60 sufficient to hold down enzymatic and microbial activities during storage at room temperature [2-5]. Fruit bar is developed by mixing proper amount of sugar, pectin, acid and color to fruit pulp, and then drying to the desired intermediate moisture content. These bars are like dried raisins having a chewy texture and are considered a natural source of dietary fiber [6]. In Pakistan, apple bars are commonly prepared in hilly areas of Gilgit-Baltistan with addition of sucrose which impart it extreme sweet taste and dark brown colour, probably due to the process of caramalization.

Sucrose, known as table sugar, is an organic compound of white color, which is odorless and crystalline with a sweet taste [7]. Earlier, sugar and preservatives were added in fresh mango and banana purees and slices to enhance their shelf-life and to minimize deterioration by using proper packaging and storage condition [8]. Application of flavoring agent citrate can extend shelf-life by preventing phenolase oxidase enzymatic reactions in sliced apple. Citric acid and ascorbic acids are found to be more useful [9-10]. Previously, it was noted that the addition of citric acid at a level of 0.6 % can improve the color, flavor and overall acceptability of the bars [11]. However, pectin in fruits act as a structural constituent but its proper integration with acids and sugar has to be maintained because it provide high ductile strength to leather [6, 12].

Food antioxidants also possess scavenging

Received, May 2016; Accepted, August 2017
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properties for free radicals. Previously it has been conferred that various plant extracts namely ascorbates, ascorbic acids, tocopherols, carotenoids, and phenolic compounds lessen the rancidity and discoloration of food products [13-14]. Citric acid is a phenolase oxidase chelating agent, and the inhibition of polyphenol oxidase (PPO) was attributed to the chelating action [15]. Citric acid application to the sliced apple can prevent browning and thus extends their life span but the combination of citric and ascorbic acids were proved more effective in maintaining the overall quality of IMF products [9-10]. This study was undertaken with the objective to develop apple bars with extended shelf life by the incorporation of sucrose and antioxidants at various levels. The study also aimed to investigate the effect of these additives on the physicochemical and sensory properties of apple bars during the storage period. Additionally, it offers opportunity to combat the post-harvest losses of apple fruits, consequently aids in the improvement of the farmer's economy.

2. MATERIALS AND METHODS

This research was carried out in the Food Processing Laboratory of Department of Food Science and Technology, The University of Agriculture, Peshawar. Apple and sugar were procured from the local market in Peshawar city for preparing apple bars. The needed chemicals were provided by the laboratory.

2.1 Preparation of Apple Bars

Sound and healthy apple fruits were rinsed carefully with tap water to remove dust and dirt particles and chemical residues to minimize the microbial load. All the fruits were peeled and cut into slices with the help of a stainless steel knife. Pulp was extracted by using pulping machine and bars were prepared as per mentioned in Table 1. The total soluble solids (TSS) of all the samples were modified with the addition of sucrose in proper amount and then the samples were acidified with addition of citric (CA) and ascorbic acid (AA) with certain modifications in previously conducted research work [16]. These prepared samples were wrapped in transparent polyethylene plastic bags and were stored at room temperature 25-35 ˚C for the period of three months (April-June) and studied for physicochemical and sensory attributes within an interval of 15 days.

2.2 Physicochemical Analysis

All apple bars samples were examined for physicochemical properties including pH, TSS, moisture content, water activity (aw), titratable acidity, ascorbic acid, total solids (TS), reducing sugars and non-reducing sugars by using standard methods of AOAC [17].

2.3 Sensory Analysis

Sensory analysis of apple bars was carried out by using the 9 point hedonic scale (1-9) of Larmond [18]. Panels of 10 judges were selected on the basis of experience in sensory analysis. The sensory properties including color, taste, texture and overall acceptability were examined by taking the mean values of the panelist scores.

2.4 Statistical Analysis

The data regarding all storage intervals and

Table 1. Plan of the study.

Treatment	Apple pulp	Sucrose (˚Brix)	Pectin (g/kg)	Antioxidant (%)	KMS (g/kg)
AB_0	500 mL	13	0	0	0
AB_1	500 mL	20	2	0.1 CA[1]	0.1
AB_2	500 mL	30	2	0.1 CA	0.1
AB_3	500 mL	35	2	0.1 CA	0.1
AB_4	500 mL	20	2	0.1 AA[2]	0.1
AB_5	500 mL	30	2	0.1 AA	0.1
AB_6	500 mL	35	2	0.1 AA	0.1

[1]CA= Citric acid
[2]AA= Ascorbic acid

treatments were statistically analyzed by CRD 2 factorial as recommended by Gomez and Gomez [19] and the means were separated by LSD test at 5% probability level as defined by Steel and Torrie [20].

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Analysis

In this study the effect of added antioxidants on water activity, ascorbic acid, percent acidity, moisture content, pH, total solids, total soluble solids, reducing sugar and non-reducing sugar contents of apple bars were analyzed during storage period of three months.

3.1.1 Water Activity (a_w)

The water activity of the entire sample analyzed at an interval of 15 days during storage. Initially, the a_w of all the apple sucrose bar samples was in the range of 0.67-0.70, which decreased from 0.66 to 0.61 during 90 days of storage period (P<0.05). Decreases in a_w of all the samples might be due to the free water binding capacity of sucrose, acids and pectin while a_w level around 0.60 is considered safe for microbial proliferation in apple fruit bar [2-3]. Similar decrease was observed in a_w of pawpaw and guava fruit leather from 0.64 to 0.61 during storage [22]. Higher stability, in a_w was noted in AB_6 (0.68) in comparison with its counterparts. While mean values for storage intervals showed decrease in a_w from 0.69 to 0.64 within 90 days of storage (Table 2). Similarly, a_w of apple- black current fruit leather also decreased to 0.60 during storage interval [21].

3.1.2 Ascorbic Acid

The apple sucrose bars samples were analyzed for

Table 2. Effect of treatment and storage period on water activity (aw) of apple bars.

Treatment	Storage duration (days)							
	0	15	30	45	60	75	90	Mean
AB_0	0.67	0.67	0.66	0.64	0.63	0.62	0.61	0.65 a*
AB_1	0.68	0.68	0.67	0.65	0.64	0.63	0.62	0.66 b
AB_2	0.70	0.68	0.67	0.67	0.66	0.65	0.64	0.67 de
AB_3	0.68	0.68	0.67	0.66	0.66	0.65	0.65	0.67 d
AB_4	0.69	0.68	0.67	0.66	0.65	0.64	0.63	0.66 c
AB_5	0.69	0.68	0.68	0.67	0.66	0.65	0.65	0.67 e
AB_6	0.70	0.69	0.69	0.68	0.67	0.66	0.66	0.68 f
Mean	0.69g*	0.72f	0.67e	0.66d	0.65c	0.64b	0.64a	

*Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05)

Table 3. Effect of treatment and storage period on ascorbic acid (mg/100 g) of apple bars.

Treatment	Storage duration (days)								Decrease
	0	15	30	45	60	75	90	Mean	(%)
AB_0	2.66	1.76	1.16	0.56	0.16	0.05	0.01	0.91 a*	99.62
AB_1	2.66	2.16	1.66	1.46	1.26	1.16	0.76	1.59 e	71.42
AB_2	2.73	2.06	1.56	1.36	1.16	1.06	0.46	1.49 c	83.15
AB_3	2.66	2.56	2.56	2.46	1.76	1.56	1.46	2.14 f	45.11
AB_4	3.86	1.46	0.86	0.36	0.07	0.06	0.03	0.96 b	99.22
AB_5	3.66	1.86	1.36	1.26	1.06	0.96	0.36	1.51 d	90.16
AB_6	3.56	3.16	2.96	2.26	1.46	1.26	1.16	2.26 g	67.41
Mean	3.11g	2.14f	1.73e	1.41d	0.99c	0.87b	0.61a		

Mean values within a column or a row followed by different letters are significantly (P<0.05) different from each other.

Table 4. Effect of treatment and storage period on % acidity of apple bars.

Treatment	Storage duration (days)								Increase (%)
	0	15	30	45	60	75	90	Mean	
AB_0	1.20	1.30	1.38	1.44	1.49	1.52	1.59	1.42 f*	32.5
AB_1	1.21	1.27	1.31	1.34	1.37	1.41	1.44	1.34 b	19.00
AB_2	1.33	1.37	1.40	1.43	1.46	1.48	1.52	1.43 g	14.28
AB_3	1.30	1.34	1.36	1.39	1.42	1.45	1. 48	1.39 d	13.84
AB_4	1.24	1.27	1.32	1.36	1.39	1.43	1.47	1.35 c	18.54
AB_5	1.30	1.32	1.37	1.40	1.44	1.47	1.49	1.40 e	14.61
AB_6	1.10	1.14	1.18	1.22	1.25	1.28	1.31	1.21 a	19.09
Mean	1.24 a*	1.29 b	1.33 c	1.37 d	1.40 e	1.43 f	1.47 g		

*Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05).

Table 5. Effect of treatment and storage period on moisture (%) of apple bars.

Treatment	Storage duration (days)								Decrease (%)
	0	15	30	45	60	75	90	Mean	
AB_0	16.5	16.20	15.47	15.12	14.51	14.14	13.90	15.12 a*	15.70
AB_1	16.95	16.84	16.42	16.14	15.97	15.48	15.21	16.14 c	10.20
AB_2	16.98	16.79	16.77	15.92	15.81	15.76	15.61	16.23 c	8.06
AB_3	16.90	16.88	16.79	16.76	15.90	15.82	15.76	16.40 d	6.74
AB_4	17.91	16.68	16.46	15.87	15.75	15.54	15.02	16.17 c	16.1
AB_5	16.97	16.74	16.26	15.84	15.48	15.12	14.96	15.91 b	11.8
AB_6	16.96	16.76	16.38	15.46	15.23	15.08	14.82	15.81 b	12.6
Mean	17.03 g*	16.70 f	16.36 e	15.87 d	15.52 c	15.28 b	15.04 a		

*Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05).

Table 6. Effect of treatment and storage period on pH of apple bars.

Treatment	Storage duration (days)								Decrease (%)
	0	15	30	45	60	75	90	Mean	
AB_0	3.43	3.40	3.37	3.34	3.31	3.28	3.25	3.34 b*	5.24
AB_1	3.25	3.21	3.17	3.13	3.06	3.05	3.01	3.13 a	7.38
AB_2	3.83	3.80	3.77	3.74	3.71	3.68	3.65	3.74 f	4.69
AB_3	3.95	3.93	3.91	3.87	3.86	3.85	3.83	3.88 g	3.03
AB_4	3.75	3.70	3.65	3.60	3.55	3.50	3.45	3.60 e	8.00
AB_5	3.55	3.51	3.47	3.43	3.36	3.35	3.31	3.43 c	6.76
AB_6	3.67	3.64	3.61	3.58	3.55	3.52	3.46	3.58 d	5.72
Mean	3.64 g*	3.60 f	3.57 e	3.48 d	3.48 c	3.46 b	3.43 a		

*Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05).

ascorbic acid content initially the ascorbic acid for treatments from AB_0 to AB_6 was 2.66 to 3.56 mg/100 gm which significantly (P<0.05) decreased to 0.01 and 1.16 mg/100 g during the entire storage period. Maximum stability in ascorbic acid content was observed in AB_3 (1.46 mg/100 g). While mean values for storage intervals showed decrease in ascorbic acid content from 3.11 to 0.61 mg/100g during three months of storage (Table 3). The losses in the ascorbic acid content might be due to high temperature provided in apple bar preparation, variation in storage temperature and oxidation of ascorbic acid to dehydro-ascorbic acid [26]. Previously, reduction in vitamin C content of guava (176.27 to 104.87 mg/100 g) and pawpaw (83.33 to 74.70 mg/g) fruits leather were observed during storage [24-25]. Similarly, reduction in ascorbic acid content due to oxidation was noted from 1.7 to 0.8% in IMF food product [27].

3.1.3 Acidity

The samples were tested for percent acidity at 15 days interval during storage and the initial values for the treatments AB_0 to AB_6 were 1.20 and 1.10%, respectively, which significantly (P<0.05) increased finally to 1.59 and 1.31% during 90 days of storage period. Similar increase in percent acidity up to 1.11% and 1.66% was noted in apple fruit bar during 60 and 90 days of storage [6]. Table 4 shows that higher increase in acidity was observed in AB_2 (1.43%) followed by AB_0 (1.42%) however, stability in acidity was observed in AB_6 (1.21%) followed by AB_1 (1.34%) during 90 days of storage. Increase in acidity of all the samples might be due to the addition of citric and ascorbic acid and also due to break down of sugar into acids during dehydration and storage. Similarly, percent acidity increased during storage from 0.42 to 0.48% in guava and 0.37 to 0.44% in mango leather [24-28].

3.1.4 Moisture Content

The moisture content of the apple sucrose bars declined during storage. The initial moisture content of the entire sample from AB_0 to AB_6 was 16.5 and 16.96%, which significantly (P<0.05) decreased to 13.90, 15.76% after 90 days of storage time. Maximum mean values of moisture content were noticed in AB_3 (16.40%) followed by AB_2 (16.23%), while the lowest mean values were observed in AB_0 (15.12%) followed by AB_6 (15.81%) in table 5. While mean values for storage intervals showed decrease in moisture content from 17.03 to 15.04% during 90 days. Decrease in moisture content is responsible for lower a_w of apple bar and it may be attributed to the water binding capacity of sucrose, pectin and also due to rise in environmental and room temperature at the onset of summer season. Similarly, reduction in moisture content of pear from (12.13 to 7.97%) and durian (15.82 to 14.36 %) fruit leathers was noticed during storage [29-30].

3.1.5 pH

The pH of the samples decreased during storage. Initially, the pH value with treatments AB_0 to AB_6 were 3.4 and 3.67, which declined to 3.25 and 3.46 (P<0.05) during three months of storage time. The highest mean value for pH was observed in AB_3 (3.88) followed by AB_2 (3.74) and AB_4 (3.60), while lowest mean value for pH was observed in AB_1 (3.13) and AB_0. While mean values for storage intervals showed decrease in pH from 3.64 to 3.43 throughout 90 days of room storage conditions (Table 6). Decrease in pH is always due to rise in acidity, while in apple bar samples decrease in pH might be due to the addition of citric acid and ascorbic acid. Previously, decline in pH from 3.80 to 3.60 was observed in mango and pine-apple fruits during storage [31-32].

3.1.6 Total Solids

The apple sucrose bars samples were analyzed at 15 days of interval for total solids. Initial total solids (TS) value of apple bar with treatments AB_0 to AB_6 were 82.90 and 84.14 which significant (P<0.05) increased to 83.08% and 88.27% within 3 months of storage at room temperature. Mean total solids for all the storage intervals increased from 83.26 to 87.38% (Table 7). Increase in TS may be due to the presence of fiber content and addition of pectin in apple bar preparation. Previous study showed that total solids increased from 69.66 to 70.77 in fruit bar during storage [23].

Table 7. Effect of treatment and storage period on total solid (%) of apple bars.

Treatment	Storage duration (days)								Increase (%)
	0	15	30	45	60	75	90	Mean	
AB_0	82.90	82.93	82.96	82.99	83.02	83.04	83.08	82.99 a*	0.21
AB_1	82.76	82.98	83.84	84.97	86.19	87.28	87.46	85.07 b	5.67
AB_2	83.13	83.84	84.67	87.37	87.62	87.72	88.95	86.18e	6.54
AB_3	84.31	85.93	86.06	85.30	86.87	86.95	88.39	86.21f	4.61
AB_4	82.53	83.43	83.97	84.72	86.02	87.02	87.59	85.04 b	6.13
AB_5	83.04	84.29	85.00	85.60	85.86	86.86	88.27	85.56 c	6.29
AB_6	84.14	84.25	85.01	86.03	87.26	87.87	88.25	86.11 d	4.65
Mean	83.26 a*	83.95 b	84.50 c	85.28 d	86.12 e	86.67 f	87.38 g		

*Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05).

Table 8. Effect of treatment and storage period on TSS (°brix) of apple bars.

Treatment	Storage duration (days)								Increase (%)
	0	15	30	45	60	75	90	Mean	
AB_0	20.03	24.66	28.03	30.03	35.03	40.03	48.03	32.26 a*	139.8
AB_1	69.76	69.86	70.11	70.13	70.36	70.46	76.63	71.04 e	9.84
AB_2	70.13	70.13	70.16	70.23	70.26	70.36	70.46	70.24 c	0.47
AB_3	71.23	71.23	71.23	71.26	71.36	71.46	71.56	71.33 g	0.46
AB_4	69.73	69.76	69.86	70.13	70.26	70.36	70.46	70.08 b	1.04
AB_5	70.16	70.23	70.26	70.26	70.46	70.56	70.66	70.37 d	0.71
AB_6	71.13	71.13	71.23	71.26	71.43	71.46	71.56	71.31 f	0.60
Mean	63.17 A*	63.86 b	64.41 c	64.75 d	65.60 e	66.39 f	68.46 g		

*Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05).

Table 9. Effect of treatment and storage period on reducing sugar (%) of apple bars.

Treatment	Storage duration (days)								Increase (%)
	0	15	30	45	60	75	90	Mean	
AB_0	3.277	3.967	3.967	3.967	3.977	3.977	3.987	3.874a*	17.8
AB_1	18.56	18.56	18.56	18.58	18.58	18.58	18.58	18.57c	0.10
AB_2	19.77	19.78	19.82	19.86	19.88	19.91	19.93	19.85d	0.80
AB_3	20.34	20.34	20.34	20.35	20.35	20.35	20.35	20.35g	0.04
AB_4	18.01	18.03	18.05	18.08	18.12	18.15	18.16	18.22b	0.82
AB_5	19.77	19.92	19.94	19.96	19.97	19.98	19.94	19.92e	0.85
AB_6	20.26	20.26	20.27	20.27	20.28	20.29	20.27	20.27f	0.04
Mean	17.28b*	17.26a	17.28b	17.29c	17.31d	17.31de	17.31de		

*Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05).

3.1.7 Total Soluble Solids

The initial readings for TSS from AB_0 to AB_6 were 20.03 and 71.13º Brix, which significantly (P<0.05) increased up to 48.03 and 71.56° Brix during storage period. Highest mean values for treatment were noted in AB_3 (71.33° Brix) followed by AB_6 (71.31° Brix), while the lowest mean values were noted in AB_0 (32.26° Brix) followed by AB_2 (70.25° Brix). The mean values of the storage interval increased from 63.17 to 68.46° Brix during storage (Table 8). Steady increase in TSS may be attributed to the addition of sucrose which was converted into glucose and fructose and also due to the loss of moisture content, which aided in increasing the shelf life of apple fruit bars [33]. Previous research work showed that TSS of IMF products including fruits jam, jellies, marmalade and leather minimally increases during storage, which stabilized the shelf life of these products [34-35].

3.1.8 Reducing Sugars

The apple sucrose bars samples were tested for reducing sugars at 15 days of interval. Initially the reducing sugar value for the sample AB_0 (3.27%) to AB_6 (20.26%) were recorded, which statistically (P<0.05) increased up to (20.27%) during storage period. Maximum mean values for treatment were noticed in AB_3 (20.35%) followed by AB_6 (20.27%). While mean values for storage interval showed increase in reducing sugar from 17.28 to 17.33% in (Table 9). Reducing sugar of all the apple bars might be increased due to conversion of polysaccharides and disaccharides to monosaccharaides. Previously researchers showed that reducing sugar content of strawberry jam and grape fruit-apple marmalade increased at par with apple sucrose bar during 3 months of storage at room temperature [34-35].

Table 10. Effect of treatment and storage period on non-reducing sugar (%) of apple bars.

Treatment	Storage duration (days)								Decrease (%)
	0	15	30	45	60	75	90	Mean	
AB_0	2.34	2.31	2.28	2.26	2.23	2.22	2.18	2.26 a*	6.83
AB_1	2.37	2.34	2.26	2.28	2.23	2.21	2.18	2.26 a	8.01
AB_2	4.48	4.46	4.43	4.41	4.38	4.34	4.33	4.41 c	3.34
AB_3	6.41	6.36	6.35	6.31	6.26	6.28	6.26	6.32 d	2.34
AB_4	2.41	2.36	2.36	2.33	2.26	2.28	2.25	2.32 b	6.63
AB_5	4.48	4.46	4.45	4.42	4.38	4.02	4.34	4.36 c	3.12
AB_6	6.38	6.36	6.34	6.31	6.28	6.26	6.23	6.30 d	2.35
Mean	4.12 e*	4.09 de	4.07 de	4.04 cd	4.00 bc	3.98 ab	3.92 a		

**Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05).

Table 11. Effect of treatment and storage period on color (using the 1–9 point hedonic scale of Larmond [18]) of apple bars.

Treatment	Storage duration (days)								Decrease (%)
	0	15	30	45	60	75	90	Mean	
AB_0	8.50	6.56	6.23	5.73	5.23	4.83	4.53	5.94 a*	46.70
AB_1	8.50	8.23	7.86	7.86	7.63	7.33	6.83	7.74 b	19.64
AB_2	8.50	8.43	8.23	8.03	8.86	7.83	7.53	8.07 g	11.41
AB_3	8.50	8.43	8.13	7.83	7.73	7.23	7.03	7.84 d	17.29
AB_4	8.50	8.43	8.03	7.83	7.73	7.43	6.86	7.81 c	19.29
AB_5	8.50	8.33	8.13	7.83	7.83	7.53	7.13	7.91 e	16.11
AB_6	8.50	8.43	8.16	7.86	7.86	7.66	7.23	7.95 f	14.94
Mean	8.50 g*	7.86 d	7.95 f	7.66 e	7.45 c	7.12 b	6.73 a		

*Mean values within a column or a row followed by different letters are significantly different from each other (P<0.05).

3.1.9 Non-Reducing Sugars

The non-reducing sugar of apple bars decreased during storage. Initial values for the treatments from AB_0 to AB_6 were 6.38% which statistically *(P<0.05)* decreased to 2.18 and 6.23% during 90 days of storage. Higher mean values for treatments were observed in AB_3 (6.32) followed by AB_6 (6.30) and AB_2 (4.41) %. While mean values of storage intervals showed decrease in non-reducing sugar from 4.12 to 3.92% as shown in table 10. Earlier, decrease in non-reducing sugar of apple fruit bar samples were noted which might be due to the modification or conversion of starch and other insoluble carbohydrates into sugar [36]. Similarly, many researchers observed decreasing trend in reducing sugars of IMF food products including guava slices, strawberry jam and in grape fruit apple blended marmalade [33-35].

3.2 Sensory Evaluation

The apple bars were evaluated at 15-day intervals during storage period, for sensory analysis such as color, texture, taste and overall acceptability.

3.2.1 Color

The samples of the apple sucrose bars observed for color at 15 days interval during storage at room temperature. It was noticed in sensory evaluation studies that the score for the characteristic reddish brown color of all the apple bar samples significantly ($P<0.05$) decreased from during three months of storage. Maximum mean values for the color of treatments (Table 11) was obtained by AB_2 (8.07) followed by AB_6 (7.95), while the minimum mean values were observed in AB_0 (5.94) followed by AB_2 (7.74) and the mean values for storage interval

Table 12. Effect of treatment and storage period on texture (using the 1–9 point hedonic scale of Larmond [18]) of apple bars.

Treatment	Storage duration (days)								Decrease (%)
	0	15	30	45	60	75	90	Mean	
AB_0	8.50	5.63	5.13	4.73	4.63	4.43	4.13	5.31 a*	51.41
AB_1	8.50	8.43	8.13	7.63	7.23	6.86	6.63	7.63 c	22.00
AB_2	8.50	8.43	8.33	8.23	8.03	7.73	7.53	8.11 g	11.41
AB_3	8.50	8.47	8.33	8.03	7.73	7.33	6.86	6.75 b	19.29
AB_4	8.50	8.43	8.23	7.73	7.33	7.03	6.73	7.71 d	20.82
AB_5	8.50	8.43	8.33	8.23	7.83	7.43	7.03	7.96 e	17.29
AB_6	8.50	8.43	8.43	8.26	7.86	7.46	7.16	8.00 f	15.76
Mean	8.50 g*	8.03 f	7.84 e	7.54 d	7.23 c	6.89 b	6.58 a		

*Mean values within a column or a row followed by different letters are significantly different from each other ($P<0.05$).

Table 13. Effect of treatment and storage period on taste (using the 1–9 point hedonic scale of Larmond [18]) of apple bar.

Treatment	Storage duration (days)								Decrease (%)
	0	15	30	45	60	75	90	Mean	
AB_0	8.50	5.06	4.73	4.53	4.23	3.86	3.53	4.92 a*	58.47
AB_1	8.50	8.43	8.13	7.73	7.33	6.86	6.63	8.02 d	22.00
AB_2	8.50	8.43	8.43	8.13	7.83	7.53	7.33	8.00 c	13.76
AB_3	8.50	8.46	8.23	7.86	7.73	7.26	6.86	8.12 f	19.29
AB_4	8.50	8.43	8.13	7.76	7.43	7.03	6.73	7.71 b	20.82
AB_5	8.50	8.43	8.43	8.13	7.83	7.63	7.23	8.02 d	14.94
AB_6	8.50	8.43	8.41	8.20	7.86	7.73	7.26	8.05 e	14.58
Mean	8.50 g*	7.95 f	7.78 e	7.47 d	7.17 c	6.84 b	6.51 a		

*Mean values within a column or a row followed by different letters are significantly different from each other ($P<0.05$).

also showed decrease in color from 8.50 to 6.73. The slight conversion in typical apple sucrose bar might be due to the activation of Maillard browning and oxidation of ascorbic acid into dehydroascorbic acid. Similarly, decrease in color score of apple and guava leather was observed from 6.00 to 5.00 and 7.10 to 6.16 during storage [5, 24].

3.2.2 Texture

Study showed significant effect of treatments and storage interval on texture of apple sucrose bars during storage period. It was noted that mean score of judges for texture of apple sucrose bar decreased significantly ($P<0.05$) from AB_0 8.50 to AB_6 to 4.13, 6.63, 7.53, 6.86, 6.73, 7.03 and 7.16 within 3 months of storage intervals. Highest mean value for texture was observed in AB_2 (8.11) followed by AB_6 (8.00) and the lowest mean values were recorded in AB_0 (5.31) followed by AB_3 (6.75). Concurrently, the mean values for texture of apple bar during 90 days of storage decreased from 8.50 to 6.58 (Table 12). Several ways can be used to note the texture of fruit leather but human taste buds are more complex in evaluating the texture of fruit bars in comparison with penetrometer which normally measures only one aspect of texture [29, 37].

3.2.3 Taste

As it is shown in table 13 that maximum mean values for taste among all the treatments were obtained by AB_3 (8.12) followed by AB_6 (8.05) and AB_1 (8.02) and the minimum mean values were observed in AB_0 (4.92) followed by AB_4 (7.71) (Table 13). Consequently, mean values for storage decreased from 8.50 to 6.51, respectively. Changes in taste of apple sucrose fruit leather might be due to variation in the amount of sugar and acids which require optimization [24] but the sweetness and acid ratio also depends upon type of fruit and may vary during storage [25].

3.2.4 Overall Acceptability

The overall acceptability score based on others sensory characteristics and it is evident from the sensory analysis related to color, flavor and taste that mean scores for over all acceptability of apple bar also significantly *($P<0.05$)* decreased from AB_0 to AB_6 during 3 months of storage period. The highest mean values for treatments were found in AB_6 (6.95) followed by AB_5 (6.91) and AB_2 (6.90), and the lowermost mean values were observed in AB_0 (4.82) followed by AB_1 (6.61) and AB_4 (2.32), while the mean values for storage interval showed decrease in overall acceptability from 8.00 to 5.68 (Table 14). Decreasing trend in overall acceptability of fruit bar might be influenced by the addition of acid, sucrose, conversion of color, consistency, storage time period and fluctuation in temperature [38].

4. CONCLUSIONS

The apple bars prepared with addition of citric acid and ascorbic acid along with pectin exhibited relatively higher shelf life on the basis of physicochemical analysis. Addition of ascorbic

Table 14. Effect of treatment and storage period on overall acceptability (using the 1–9 point hedonic scale of Larmond [18]) of apple bars.

Treatment	Storage duration (days)								Decrease (%)
	0	15	30	45	60	75	90	Mean	
AB_0	8.00	6.76	4.55	4.01	3.86	3.52	3.05	4.82 a*	61.87
AB_1	8.00	7.26	6.86	6.46	6.16	5.86	5.66	6.61 b	29.25
AB_2	8.00	7.16	6.86	6.66	6.76	6.46	6.36	6.90 e	20.5
AB_3	8.00	7.26	7.06	6.86	6.53	6.16	6.06	6.85 d	24.25
AB_4	8.00	7.06	6.86	6.66	6.46	6.16	5.96	6.74 c	25.5
AB_5	8.00	7.16	6.96	6.86	6.66	6.46	6.26	6.91 f	21.75
AB_6	8.00	7.36	7.16	6.66	6.66	6.46	6.36	6.95 g	20.5
Mean	8.00 g*	7.15 f	6.62 e	6.31 d	6.16 c	5.87 b	5.68 a		

*Mean values within a column or a row followed by different letters are significantly different from each other ($P<0.05$).

acid enhanced availability of vitamin C in the bars. Further, specific red brown colour of apple bars persisted during 3-month storage period and addition of acids minimized sweetness of the bars, by imparting mild sourness, compared with non-acidified bars, which resulted in higher acceptability of the product.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 177–182 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Nutritional and Microbial Quality of Mango-based Cereal Flakes Stored at Different Temperatures

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Abstract*:* Malnutrition in young children is a serious problem in Pakistan. In a recently carried out nutritional survey in the country, more than 44% of children, under five years, have been observed to be stunted and underweight. Because of their palatable taste and convenience of intake, snacks are a popular food item amongst children, but such food products may lack essential nutritional and hygiene quality. In order to address child malnutrition by catering nutritious and hygienic food, an instant food product was developed using cereals (i.e. wheat, suji, corn and rice) and mango pulp, blended in various proportions, to form cereal flakes for preparing a likable snack for children consumption. The ingredients (i.e., cereal paste, mango pulp, salt and ghee) were mixed, cooked and passed through a homogenizer to obtain a uniform mix. The mixture was cooked on a drum dryer (at steam pressure of 60 lb./sq. inch) to obtain long thin sheets, which were later broken manually into small pieces. The pieces were dried at 50 °C for an hour in a dehydrator to reduce their moisture content to about 2 percent and then were packed in high density polyethylene zipper bags. The rice-mango flakes were found to be highly acceptable for sensory attributes. They were stored for 180 days at two different temperatures, i.e., 25 °C and 37 °C, in a controlled temperature cabinet and the changes in nutritional and microbiological attributes were monitored at 30-day intervals. It was observed that moisture, protein, crude fat and carotene content in the flakes decreased significantly with increase in storage period (P<0.05) while total plate count remained within the permissible range at both temperatures till 150 days of storage. However, significant changes in total plate count were observed beyond 150 days. Total coliform, yeast and mold content could not be detected till 180 days of storage time at both temperatures. Thus, the mango-based cereal flakes remained nutritionally and microbiologically acceptable up to 150 days of storage both at 25 °C and 37 °C.

Keywords: Mango cereal flakes, nutritional and microbial quality, drum dryer, storage temperature

1. INTRODUCTION

Malnutrition is a global issue and dietary surveys have revealed that the masses in developing countries like Pakistan are under fed, primarily because of poor quality and quantity of their food. In a recent nutritional survey carried out in Pakistan, it has been reported that more than 44% of the children of school going age are stunted, underweight and malnourished.

Mango is a major fruit produced in Pakistan. It is one of the few fruits which can be utilized at all stages of its maturity. This fruit is used as a dessert, as a table fruit between meals and also is processed for preparing a lot of food products. Mango is not only quite nutritious but also is a popular fruit in Pakistan due to its excellent flavor, attractive aroma and therapeutic value. It is also utilized for processing into various types of food products, but only a very small fraction of mangoes produced in the world, i.e., 0.22% of the total produce, are utilized for food processing [1].

Cereals are staple foods in most countries of the world, including Pakistan, but contain inadequate content in certain minerals and vitamins to fulfill human nutrition needs. However, mangoes are an economical source of vitamins and minerals essential for human nutrition. For instance, mango fruit contains on an average of 4800 IU of vitamin

Received, October 2016; Accepted, September 2017
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A and 40 mg of vitamin C per 100 g edible portion [2, 3]. Therefore, the need for preparing a nutritious food product to address malnutrition in school going children cannot be over emphasized. The blend of cereals with mango fruit can ensure highly nutritious diet, which can also have a great market potential due to its peculiar taste. Snacks like *Slanties* and *Kurkury's* are very popular among children of school going age. Cereal flakes having fruit base, like mango, can be used as nutritional alternative for preparing commercial snacks. Many studies have been reported on preparation and storage of fruit-based cereal flakes [4, 5, 6]. These flakes will possess a pleasant mango flavor and can be used with milk as a breakfast food, like corn flakes; the mango-based cereal flakes being sweet and crisp, will be highly suitable as a baby food as well. They can also be used in ice cream as a thickener and flavoring material. Hence, in consideration of the above mentioned aspects, an instant snack food recipe was developed containing 50% cereals and 20% mango pulp in the product mix. This food product could supply the consumer with a large fraction of daily requirements of vitamins, minerals and protein. The main objectives of this study were to prepare tasty and nutritious cereal flakes having natural mango flavor and to explore its shelf life at 25 °C and 37 °C.

2. MATERIALS AND METHODS

Mango pulp for these experiments was procured from the Noor Industries, Faisalabad. Cereals, fractions and other ingredients and additives were procured from the local market in Islamabad, Pakistan.

2.1 Product Recipe

At first step, calcium carbonate and sodium bicarbonate were added in the mango pulp to adjust its pH to 5.0. Cereal pastes were prepared by cooking cereal source (wheat flour/ *suji*/ com starch / rice) with water (three times weight of cereal source) at temperature of 75-80 °C. Cereal pastes obtained from four different sources were added in the mango pulp according to recipe Table 1. The pulp-cereal mixture was continuously heated at temperature of 75-80°C for half an hour. Sugar and other ingredients (salt, *ghee*) were then mixed with cooked mass. The mixture was passed through homogenizer to get a uniform mix. The mix was formed into dough. The dough was cooked on double drum drier at steam pressure of 60 Ib./sq. inch. Long thin sheets thus obtained were broken manually into small pieces using a specific mold. The pieces were dried at 50°C for about 45 min in a dehydrator in order to reduce the moisture content to about 2 percent and then packed in high density polyethylene (HDPE) zipper bags**.**

2.2 Analytical Work and Shelf Life Studies

Mango cereal flakes which were ranked highest in organoleptic evaluation were further subjected to shelf life studies. The flakes were packed in polyethylene bags, sealed and kept at two different temperatures 25 °C and 37 °C in a controlled temperature cabinet for 180 days. Two samples from each lot were examined at monthly intervals for moisture, crude fiber, protein, crude fat, β-carotene and ash according to the respective methods described in AOAC [7]. Iron was determined by atomic absorption spectroscopy (using Varian, Model 220 FS, Australia).

2.3 Sensory Evaluation

The product was periodically evaluated by a panel of ten trained judges for color, taste, flavor, texture

Table 1. Recipes of the developed cereal flakes.

Ingredient (%)	Type of Cereal Flakes			
	Mango-Wheat (*Maida*)	Mango-Corn Starch	Mango-*Suji*	Mango-Rice
Mango pulp	20	20	20	20
Pre-cooked cereal paste	50	50	50	50
Sugar	20	20	20	20
Salt	1	1	1	1
Ghee	9	9	9	9

and overall acceptability on 9- point hedonic scale according to Larmond [8].

2.4 Bacteriological Status

The product was examined microbiologically for total viable bacterial count, total coliform, yeast and mold. Total plate count was determined by using plate count agar, yeast and mold was carried out in potato dextrose agar and coliform on Lauryl Tryptose Broth [9].

2.5 Statistical Analysis

Completely randomized design was used for data analysis and means were compared by Duncan Multiple Range Test for significance as described by Steel et al. [10] using Statisca Software version 8.1. Standard deviation was used to evaluate the dispersion from the mean.

3. RESULTS AND DISCUSSION

Mango pulp and cereal sources (rice, wheat corn starch, Semolina) were analyzed for proximate composition, iron and carotene contents. The results are presented in Table 2. Mango pulp was mixed with different cereals i.e. wheat flour (*maida*), *suji*, com starch, and rice. All the four products of mango cereal flakes, i.e., mango *maida* flakes, mango *suji* flakes, mango corn starch flakes and mango rice flakes were organoleptically evaluated by the panel of ten judges and mean score of these judges for color, flavor, taste, texture and overall acceptability were recorded. The data is presented in Table 3. It shows that sample D (mango with rice) was liked by most of the judges and got total score of 40.45 with overall mean of 8.09. The samples A, B and C were equally liked by judges, however, Mango rice flakes was considered best among all the products. The flakes prepared from mango pulp and rice were packed in HDPE Zippered bag. These samples were kept at two different temperatures of 25 °C and 37 °C for 180 days to assess effect of temperatures on the quality and shelf life of mango rice flakes. Proximate analysis of mango rice flakes are presented in Table 2. Storage stability of mango rice flakes packed in HDPE zippered bag was evaluated periodically for nutritional and microbial

Table 2. Nutritional composition of mango pulp and rice (means of three replications, with standard deviation).

	Proximate Composition	Mango pulp	Rice	*Suji (Semolina)*	*Maida (Patent Flour)*	Corn Starch
1	Moisture (%)	83.50± 0.23	12.00± 0.06	11.23 ± 0.04	13.56± 0.06	10.78± 0.03
2	Ash (%)	0.50± 0.03	1.19± 0.05	0.23 ± 0.02	0.45± 0.01	0.23± 0.025
3	Protein (%)	0.50± 0.02	7.50±0.03	6.56 ± 0.04	10.78± 0.02	7.4± 0.027
4	Crude fiber (%)	0.32± 0.03	0.61±0.02	0.76± 0.03	1.24± 0.04	0.8± 0.024
4	Crude fat (%)	0.10±0.01	0.82± 0.03	0.54± 0.01	1.18± 0.07	0.56± 0.03
5	Total carbohydrates (%)	15.08± 0.05	77.88± 0.10	80.68 ± 0.09	72.79± 0.06	80.23± 0.08
	Other Parameters:					
1	β-Carotene (mg/100g)	5.50± 0.03	-	-	-	-
2	Iron (mg/100g)	4.10±0.04	3.10± 0.02	1.00± 0.035	1.30± 0.04	0.70± 0.003

Table 3. Organoleptic evaluation of freshly prepared flakes from mango cereal (Mean score by 10 judges).

Type of Cereal Flakes	Appearance (9)	Texture (9)	Color (9)	Taste (9)	Flavor (9)	Total Score (45)
A. *Maida*	4.40 d*	4.00 d	4.75 d	5.00 d	6.00 c	24.15 d
B. Corn starch	5.50 c	4.60 c	5.15 c	5.30 c	6.90 b	27.45 c
C. *Suji* (Samolina)	5.60 b	6.00 b	5.39 b	5.75 b	6.95 b	29.69 b
D. Rice	7.80 a	7.80 a	7.90 a	8.55 a	8.40a	40.45 a

*Means within a column followed by the same letter have non-significant difference (P<0.05)

Table 4. Effect of storage time on proximate analysis and microbial status of mango rice flakes (means of three replications).

S. No.	Parameter	0 day		30 days		60 days		90 days		120 days		150 days		180 days	
		25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
1	Moisture (%)	2.21 ±0.03	2.21 ±0.02	3.77 ±0.02	3.71 ±0.03	4.39 ±0.02	4.32 ±0.03	5.40 ±0.04	5.00 ±0.036	5.61 ±0.035	5.30 ±0.038	5.69 ±0.03	5.60 ±0.04	6.41 ±0.04	6.19 ±0.03
2	Protein (%)	8.00 ±0.032	8.00 ±0.033	8.00 ±0.031	7.90 ±0.028	7.92 ±0.034	7.78 ±0.032	7.50 ±0.029	7.00 ±0.035	7.62 ±0.03	7.00 ±0.029	7.20 ±0.025	6.87 ±0.038	6.81 ±0.024	6.39 ±0.031
3	Crude fat (%)	0.92 ±0.014	0.92 ±0.01	0.92 ±0.015	0.90 ±0.012	0.90 ±0.018	0.87 ±0.010	0.88 ±0.012	0.84 ±0.013	0.86 ±0.014	0.82 ±0.012	0.82 ±0.012	0.80 ±0.011	0.81 ±0.011	0.79 ±0.012
4	Carotene (mg/l00 g)	4.00 ±0.027	4.00 ±0.028	3.70 ±0.032	3.20 ±0.027	3.10 ±0.029	2.70 ±0.026	2.70 ±0.028	1.50 ±0.026	2.10 ±0.024	1.30 ±0.025	1.89 ±0.023	0.69 ±0.023	1.55 ±0.026	0.32 ±0.02
5	Overall acceptability (out of 9.00 as total score)	8.24 ±0.02	8.24 ±0.02	8.19 ±0.02	7.97 ±0.02	8.04 ±0.02	7.85 ±0.02	7.94 ±0.02	7.70 ±0.02	7.86 ±0.02	7.53 ±0.02	7.77 ±0.02	7.38 ±0.02	7.69 ±0.02	7.23 ±0.02
6	Total plate count (CFU/g)	Nil	Nil	Nil	5	9	15	18	20	25	30	55	65	95	130
7	Coliform bacteria	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
8	Yeast and mold	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 5. Effect of storage temperature on proximate analysis and microbial status of mango rice flakes (means of three replications).

S. No.	Parameter	25°C							37°C						
		0	30	60	90	120	150	180	0	30	60	90	120	150	180
1	Moisture (%)	2.21 ±0.03	3.77 ±0.02	4.39 ±0.02	5.40 ±0.04	5.61 ±0.035	5.69 ±0.03	6.41 ±0.04	2.21 ±0.02	3.71 ±0.03	4.32 ±0.03	5.00 ±0.036	5.30 ±0.038	5.60 ±0.04	6.19 ±0.03
2	Protein (%)	8.00 ±0.032	8.00 ±0.031	7.92 ±0.034	7.50 ±0.029	7.62 ±0.03	7.20 ±0.025	6.81 ±0.024	8.00 ±0.033	7.90 ±0.028	7.78 ±0.032	7.00 ±0.035	7.00 ±0.029	6.87 ±0.038	6.39 ±0.031
3	Crude fat (%)	0.92 ±0.014	0.92 ±0.015	0.90 ±0.018	0.88 ±0.012	0.86 ±0.014	0.82 ±0.012	0.81 ±0.011	0.92 ±0.01	0.90 ±0.012	0.87 ±0.010	0.84 ±0.013	0.82 ±0.012	0.80 ±0.011	0.79 ±0.012
4	Carotene (mg/l00g)	4.00 ±0.027	3.70 ±0.032	3.10 ±0.029	2.70 ±0.028	2.10 ±0.024	1.89 ±0.023	1.55 ±0.026	4.00 ±0.028	3.20 ±0.027	2.70 ±0.026	1.50 ±0.026	1.30 ±0.025	0.69 ±0.023	0.32 ±0.02
5	Overall acceptability (out of 9.00 as total score)	8.24 ±0.02	8.19 ±0.02	8.04 ±0.02	7.94 ±0.02	7.86 ±0.02	7.77 ±0.02	7.69 ±0.02	8.24 ±0.02	7.97 ±0.02	7.85 ±0.02	7.70 ±0.02	7.53 ±0.02	7.38 ±0.02	7.23 ±0.02
6	Total plate count (CFU/g)	Nil	Nil	9	18	25	55	95	Nil	5	15	20	30	65	130
7	Coliform bacteria	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
8	Yeast and mold	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

attributes at two different temperatures Results are presented at Table 4 and Table 5.

3.1 Moisture

At 0 day, moisture content of mango cereal flakes was 2.21% at 25 °C and 37 °C. The moisture content of mango rice flakes increased gradually during storage in both the samples; after 180 days it increased to 6.41% and 6.19%, respectively. This increase may be attributed to the absorption of water by the product from atmosphere during storage because of slight permeable nature of polyethylene film. The increase in moisture may also be the result of browning taken place during the storage. These results were in agreement with those of Girdhari et al. [11] and Muzanila et al. [12] on mango and cassava cereal flakes, respectively.

3.2 Crude Fat

The fat contents in samples kept at 25 °C remained unchanged, whereas in samples kept at 37 °C decreased from 0.92 to 0.79% till completion of the experiment. Decrease in fat content could be attributed to the decomposition of fat to fatty acid as a result of lipolysis process which can occur at higher temperature [13]. The fat can also be oxidized by oxygen and other pro-oxidants with proportionately higher rate at increased temperatures [14].

3.3 β-Carotene

It is apparent from the results that the carotene content decreased in both samples but the effect was more pronounced in sample that was kept at 37 °C. In fact carotenes are readily oxidized by various pro-oxidants. Its breakdown rate increases rapidly with increase in storage time and temperature. Carotene content was also affected by light. Due to such factors the amount of carotene decreased in both the samples but with higher rate in the sample stored at 37 °C [5].

3.4 Protein

Protein content stored at 25°C decreased from 8.0% to 6.81%. In sample stored at 37 °C, the amount of protein decreased from 8.0% to 6.39% (Table 4). Storage temperature and time period significantly affected the changes in nitrogenous components. These changes in protein content of mango cereal flakes could be attributed to reactivation of proteases during storage [15] and also could be due to processing method and chemical interaction [16].

3.5 Organoleptic Evaluation

The freshly prepared product had highest acceptability of all mentioned characters by scoring 8.24 points as overall acceptability. The acceptability score gradually reduced to 7.69 and 7.23 till the end of the storage period at 25 °C and 37 °C, respectively. Resulting products of Millard's reaction might be responsible for adverse changes in sensory qualities during extended storage [17].

3.6 Microbiological Studies

Microbiological examination of mango rice flakes for total plate count, coliform bacteria, yeast and mold have been carried out at for 150 days applying plate count agar, *Lauryl tryptose* broth and potato dextrose agar techniques. The results are presented in Table 4 and 5.

Total bacterial count in both the samples stored at 25 °C and 37 °C was increased from nil at zero day to 95 and 130 CFU/g, respectively. Coliform bacteria, yeast and mold could not be detected in mango rice flakes stored at both the temperatures. However, a greater change in total plate count is observed at 25 °C than at 37 °C. This might be due to favorable temperature offered to the micro-organism [18]. It further proved that processing was carried out under hygienic conditions. The increase of bacterial growth may be due to the mishandling during packing or the quality of packing material. Hence it is concluded that the product i.e. mango based cereal flakes packed in HDPE zippered bags remained acceptable till storage period of 150 days at 25 °C and 37 °C.

4. CONCLUSIONS

The study concluded that nutritious mango-based rice flakes packed in HDPE-zippered bags can be stored best for 150 days at 25°C, rather than at 37°C. Under these storage conditions, the product retains its nutritional and organoleptic quality and could be a nutritious and tasty snack for school age children.

5. ACKNOWLEDGMENTS

The financial support from RADP sub-project "Provision of accredited lab testing services to comply WTO requirements in domestic and international trade" is acknowledged. Also, we are obliged to our fellow scientists, Mr. Muhammad Naeem Safdar and Mr. Nouman Rashid Siddiqui, for reviewing the manuscript.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 183–190 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Mixed Model of Additive Main Effects and Multiplicative Interaction for Stability Analysis of Cassava

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Abstract: The research objective was to analyze Genotype × Environment Interaction (GEI) using AMMI mixed model with Restricted Maximum Likelihood (REML) method both with and without coefficient of coancestry matrix (***A*** matrix) assuming residual error variance across environments were homogeneous and heterogeneous. Multilocation trials were conducted at five districts of East Java Province, Indonesia, from November 2010 to August 2011. The results showed that no PCs values that significantly different from AMMI mixed model analysis, both without and with ***A*** matrix, assuming homogeneous error variance across environments. While the result of AMMI mixed model analysis, both with and without ***A*** matrix, assuming heterogeneous residual error variance across environments had the same interpretation. The most stable genotype that located closest to the origin of biplot was genotype G13 (CMM 02033-1). The yield potential of G13 was not high (close to average). Four genotypes namely G4 (Adira 4), G6 (CMM 03036-7), G7 (CMM 03036-5), and G15 (CMM 02048-6) were the most unstable genotypes. Environment S4 (Malang) had the smallest interactions effect, while environments with the greatest interaction effect were S3 (Probolinggo) and S1 (Kediri), because these environments had a long vector.

Keywords: Altidute, AMMI, cassava genotype, mixed model, stability analysis

1. INTRODUCTION

Cassava is able to adapt to various environmental conditions, but usually the adaptability of each variety is narrow and large, and it indicated the influence of genotype and environment interaction [1]. GEI (genotype × environment interactions) cause limitations in selecting the superior genotypes, thus reducing benefits of the average analysis and conclusions become invalid [2].

Multilocation trials are necessary to: (i) compare the appearance of genotype, i.e., genotype appearance in general (in many environments) and genotype appearance at specific environment; (ii) estimate GEI component to measure the heritability and its impact on the selection; (iii) selecting location of testing and determining the environment in a broader scope; (iv) identify genotypes with specific adaptation, as well as determining the purpose of breeding [3].

A wide statistical method has been developed to determine the genotype x environment interaction. The most common method used is the combined analysis of variance. Then developed the technique of regression, univariate parameter stability (parametric and nonparametric stability), analysis of qualitative/crossover interaction, and

Received, December 2016; Accepted, August 2017
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multivariate analysis (cluster analysis, principal component analysis, factor analysis, Additive Main effects and Multiplicative Interaction (AMMI), and GGE biplot. The techniques of analysis developed in accordance with the development of multilocation trial data obtained. AMMI and GGE biplot get a lot of attention because of advantages in data interpretation of genotype x environment interactions compared to methods developed previously.

In fact, the data obtained from multilocation trials are often unbalanced, the variance across environments is not homogeneous, and there is the possibility of coefficient of coancestry among genotype used. AMMI is fixed model analyses with all the factors i.e environment, genotype, and their interaction are fixed. In its development, these factors can be random, so that mixed model is developed to analyze the genotype x environment interactions.

Model selection for GEI analysis is based on data obtained, i.e., by the presence or absence of heterogeneity of variance among environment, the data is balanced or unbalanced, the presence or absence of coefficient of coancestry between genotype, and so on. It is necessary to be conducted to get the best interpretation of the results based on data obtained from multilocation trials.

The research objective was analysis of GEI using AMMI mixed model with REML (Restricted Maximum Likelihood) method both with and without coefficient of coancestry matrix (***A*** matrix) assuming residual error variance across environments were homogeneous and heterogeneous.

2. MATERIALS AND METHODS

2.1 Implementation of Research

The study was conducted at five locations: Kediri (80 m ASL), Ponorogo (800 m ASL), Probolinggo (40 m ASL), Malang (530 m ASL), and Mojokerto (25 m ASL), from November 2010 until August 2011. Experiments were conducted at each location using a randomized complete block design with three replications. Genetic materials of research were 15 cassava genotypes, consist of 11 clones and four superior cassava cultivars as control involving Adira 4, UJ 5, Malang 4, and Malang 6.

Cassava was planted in a plot size of 5 m × 5 m with a spacing of 100 cm × 80 cm. Cassava cuttings about 20 cm long are planted with the vertically position of cuttings. Fertilization was given twice, at 1 month after planting with a dose of 100 kg ha^{-1} Urea + 100 kg ha^{-1} SP36 + 100 kg ha^{-1} KCl, and at 3 months after planting with 100 kg ha^{-1} Urea. Weeding was performed twice, at 1 month and 3 months after planting. The activities to improve the ridge were carried out before fertilization. Removal shoots with leaves two best buds performed at 2 months after planting. Harvest was conducted at 10 months. The character that observed was fresh tuber yield.

2.2 Statistical Methods

Linear mixed model equation used was $\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \boldsymbol{\varepsilon}$ (Equation 1). Because of genotype, environment, and interactions were random, then linear mixed model equation became $\mathbf{y} = \mathbf{X\beta} + \mathbf{Z}_g\mathbf{u}_g + \mathbf{Z}_e\mathbf{u}_e + \mathbf{Z}_{ge}\mathbf{u}_{ge} + \boldsymbol{\varepsilon}$ (Equation 2) with $\mathbf{y}$ = vector of parameters observed, $\boldsymbol{\beta}$ = a scalar of μ, $\mathbf{u}_g$ = vector $n\times1$ of random effect of genotype, $\mathbf{u}_e$ = vector $g\times1$ of random effects of location, $\mathbf{u}_{ge}$ = vector $ge\times1$ of random effects of genotype x environment interaction, $\mathbf{X}$ = column vector whose elements are 1, $\mathbf{Z}_g$ = incidence matrix $(n\times e)$ which connects $\mathbf{y}$ to $\mathbf{u}_g$, $\mathbf{Z}_e$ = incidence matrix $(n\times e)$ connecting $\mathbf{y}$ to $\mathbf{u}_e$, $\mathbf{Z}_{ge}$ = incidence matrix $(n\times ge)$ which connects $\mathbf{y}$ to $\mathbf{u}_{ge}$, $\boldsymbol{\varepsilon}$ = vector of random error. Random vectors $\mathbf{u}$ and $\boldsymbol{\varepsilon}$ are assumed normal distribution and independent with zero mean [4, 5].

The combined analysis of variance was conducted using REML method based on two assumptions, namely homogeneous residual error variance across locations ($\sigma^2_1 = \sigma^2_2 = \sigma^2_3 = \sigma^2_4 = \sigma^2_5$) and heterogeneous residual error variance across locations ($\sigma^2_1 \neq \sigma^2_2 \neq \sigma^2_3 \neq \sigma^2_4 \neq \sigma^2_5$) [6, 7].

Data were analyzed using the SAS program i.e proc mixed for REML analysis without and with matrix A, proc IML for AMMI analysis (to obtain PC1 and PC2 score), proc inbred to obtain coefficient of coancestry among genotypes (***A***

matrix). $\mathbf{U_{ge}}$ value obtained from Equation 2 is used for the singular value decomposition and partition of AMMI analysis. Singular value decomposition of $\mathbf{u_{ge}}$ can be written $u_{ge} = \sum_{k=1}^{t} u_{ik}\lambda_k v_{jk}$, followed by the partition of singular value with formula $u_{ge} = \sum_{k=1}^{t} (u_{ik}\lambda_k^{1/2})(\lambda_k^{1/2} v_{jk})$, where $u_{ik}\lambda_k^{1/2}$ is PC score for genotype g_i in the k^{th} axis and $\lambda_k^{1/2} v_{jk}$ is PC score for environment e_j in the k^{th} axis.

3. RESULTS AND DISCUSSION

3.1 Tuber Yield

Average of tuber yield across environments were significantly different, with S1 (Kediri) had the highest yield mean of 54.84 t ha^{-1} and S2 (Ponorogo) had the lowest tuber yield of 7.79 t ha^{-1} (Table 1), so that S1 can be considered as the most productive environment and S2 was the least productive environment. Tuber yield in S2 (Ponorogo) was very low, it was may associated with altitude of experiment location i.e above 800 m ASL. Acording to reference [8] states that cassava tuber yield was decreased in the highlands, which is caused by a decrease in the average of photosynthesis ability when cassava is cultivated in colder areas such as the highlands of the tropical and lowlands of sub-tropical. Cassava growth is slower in tropical highlands than in the lowlands; thus, it takes a longer period to obtain higher yields. Tropical lowlands have higher temperatures and strongly associated with plant growth and photosynthesis mean higher [9]. Growth and productivity of cassava require maximum temperature of 25 °C, high radiation and humidity, as well as adequate rainfall during the growing [10].

The mean value of 15 genotypes yield were tested in five environments was ranged from 23.95 t ha^{-1} (genotype G11) up to 37.79 t ha^{-1} (control varieties G3). Genotype G8 had yield mean of 37.52 t ha^{-1}, which was not significantly different from the control varieties G3, and higher than the other control varieties G1, G2, and G4. The yield mean in this study were higher than the results of [11] which tested 21 cassava genotypes in five environments

Table 1. Fresh tuber yield of cassava clones in each environment.

Code	Genotype	S1[a]	S2	S3	S4	S5	Genotype mean
G1	UJ5	43.69	5.81	28.37	34.11	14.72	25.22 fg*
G2	Malang 6	62.83	9.64	37.12	37.81	15.43	32.58 bcd
G3	Malang 4	67.76	8.08	36.69	52.39	24.51	37.79 a
G4	Adira 4	56.79	8.23	43.58	29.29	18.97	31.51 cde
G5	CMM 03025-43	53.08	6.33	23.82	31.17	19.41	26.91 efg
G6	CMM 03036-7	67.26	9.83	26.27	33.70	21.57	31.52 cde
G7	CMM 03036-5	52.85	8.35	37.22	23.19	23.11	29.93 cdef
G8	CMM 03038-7	65.86	10.99	42.21	44.76	20.85	37.52 ab
G9	CMM 03094-12	46.26	3.79	25.95	39.06	11.02	24.40 g
G10	CMM 03094-4	58.58	9.18	29.74	52.00	23.18	34.55 abc
G11	CMM 03095-5	44.29	3.89	27.73	28.45	13.86	23.95 g
G12	CMM 02040-1	48.25	9.24	25.41	37.86	17.74	28.13 defg
G13	CMM 02033-1	53.52	8.13	30.70	40.51	13.11	29.49 def
G14	CMM 02035-3	60.22	4.72	26.34	27.35	13.80	24.16 g
G15	CMM 02048-6	41.40	10.66	23.90	31.17	22.88	26.69efg
Environment mean		58.54 p	7.79 t	31.00 r	37.08 q	18.28 s	29.59

[a]S1 = Kediri; S2 = Ponorogo; S3 = Probolinggo; S4 = Malang; S5 = Mojokerto

*Mean values followed by different letters differ significantly ($P < 0.05$)

Table 2. The AMMI analysis result based on the REML method without and with A matrix assuming homogeneous error variance acrosss environments.

Eigen value (λ)			AMMI sum of square			Probability of H_0		Persentage	
	Without *A*	With *A*		Without *A*	With *A*	Without *A*	With *A*	Without *A*	With *A*
λ_1	12.23	12.58	PC1	448.54^{ns}	474.65^{ns}	0.9201	0.8979	37.72	37.60
λ_2	12.09	12.26	PC2	438.21^{ns}	450.96^{ns}	0.8610	0.8454	36.85	35.72
λ_3	7.93	8.13	PC3	188.67	198.53	0.9906	0.9880	15.87	15.73
λ_4	5.84	6.46	PC4	102.46	125.03	0.9977	0.9943	8.62	9.90
λ_5	1.94	2.10	PC5	11.25	13.27	1.0000	1.0000	0.95	1.05
Sum				189.13	262.44			100.00	100.00

H_0 : PCs were not affected ($PC_i = 0$) ns = not significant in Wald test PC = Principal Component

for two seasons with the yield ranges between 7.0 t ha^{-1} to 17.9 t ha^{-1} and lower than the reported by [12], who tested nine cassava genotypes in three locations with the range of yield between 26.4 t ha^{-1} to 49.7 t ha^{-1} with the average of 37.8 t ha^{-1}.

3.2. AMMI Mixed Model Technique without and with *A* Matrix Assuming Homogeneous Error Variance Across Environments

AMMI mixed model analysis without ***A*** matrix assuming homogeneous error variance across environments showed that PC1 and PC2 scores explained 37.72 % and 36.85 % of GE sum of square, respectively, and together its explained 74.57 % of the GE interaction variation (Table 2). But there are no PCs scores that significantly different, it was likely due to the small value of the AMMI sum of squares for each PC in this method compared with the Least Square method [13].

AMMI mixed model analysis without ***A*** matrix has not been reported on cassava, but it has been done in peanut [14] that assuming genotype as fixed effect and environment as random effect, in wheat [15] with different assumptions, namely genotype as a random effect and the environment as a fixed effect. The use of AMMI mixed models without ***A*** matrix provides better interpretation of genotype × environment interaction [14].

Singular value decomposition on AMMI analysis of mixed models using ***A*** matrix result PC1 and PC2 scores with the cumulative proportion of 73.32 % (Table 2). Although the cumulative proportion of PC1 and PC2 were 73.32 %, but the PCs scores was not significantly different. As AMMI, mixed model technique without ***A*** matrix, using the ***A*** matrix sum of squares AMMI also decreased, so that the PC values obtained no significantly different.

3.3 AMMI Mixed Model Technique without and with *A* Matrix Assuming Heterogeneous Error Variance Across Environments

AMMI mixed models analysis without ***A*** matrix assuming heterogeneous error variance across environment had proportion of PC1 and PC2 49.07 % and 24.47 % of the sum of squares of interaction, respectively. PC1 and PC2 scores explained 73.54 % of GE interaction. There were three PC values that significantly different on AMMI mixed model analysis without ***A*** matrix assuming heterogeneous error variance across environment (Table 3). This was in contrast to the results of the analysis using the assumption of homogeneous variance across environment where no PC scores were significantly different. If using ***A*** matrix assuming heterogeneous error variance acrosss environment showed that singular value decomposition result PC1 and PC2 scores 47.79 % and 25.13 %, respectively, with a cumulative proportion of 72.92 %. In this method, three PC scores were also significantly different ($P < 0.01$) (Table 3).

Biplot AMMI1 both without and with ***A*** matrix assuming heterogeneous residual error variance across environments showed that genotype G11, G13, and G14 had the lowest of PC1 score among the other genotypes, so that those genotypes were most stable compared with other genotypes, but it has low yield potential (below the mean). Genotype G4 and G15 had the largest PC1 score, so

Table 3. The AMMI analysis result based on REML method without and with A matrix assuming heterogeneous error variance across environments.

Eigen value (λ)				AMMI sum of square		Probability of H_0		Percentage	
	Without A	With A		Without A	With A	Without A	With A	Without A	With A
λ_1	12.24	12.55	PC1	449.58**	472.46**	0.00000	0.00000	49.07	47.79
λ_2	8.64	9.10	PC2	224.19**	248.45**	0.00035	0.00008	24.47	25.13
λ_3	7.52	7.87	PC3	169.58**	186.00**	0.00271	0.00100	18.51	18.82
λ_4	4.28	4.57	PC4	54.90	62.73	0.44519	0.32420	5.99	6.35
λ_5	2.45	2.51	PC5	17.94	18.93	0.93241	0.91913	1.96	1.92
Sum				916.19	988.57			100.0	100.0

H_0, PCs were not affected ($PC_i = 0$) **, Significantly different in Wald test 1 %

Table 4. The eigenvectors of genotypes and environments based on AMMI technique with the REML method without and with ***A*** matrix assuming heterogeneous residual error variance across environmants.

Code	Genotype	Fresh tuber yield (t ha^{-1})	Without *A*		With *A*	
			PC1	PC2	PC1	PC2
G1	UJ5	25.22	-0.23	-0.67	-0.23	-0.62
G2	Malang 6	32.58	1.08	0.43	1.09	0.50
G3	Malang 4	37.79	0.67	0.81	0.72	0.65
G4	Adira 4	31.51	1.69	-1.00	1.73	-1.03
G5	CMM 03025-43	26.91	-0.82	0.46	-0.84	0.43
G6	CMM 03036-7	31.52	-0.54	1.57	-0.51	1.51
G7	CMM 03036-5	29.93	0.42	-1.05	0.51	-1.24
G8	CMM 03038-7	37.52	1.32	0.22	1.32	0.24
G9	CMM 03094-12	24.40	-0.20	-0.28	-0.23	-0.17
G10	CMM 03094-4	34.55	-0.49	0.51	-0.51	0.49
G11	CMM 03095-5	23.95	-0.07	-0.64	-0.12	-0.56
G12	CMM 02040-1	28.13	-1.04	-0.11	-1.06	-0.08
G13	CMM 02033-1	29.49	0.09	0.06	0.04	0.19
G14	CMM 02035-3	24.16	0.16	0.93	0.13	1.07
G15	CMM 02048-6	26.69	-1.86	-0.95	-1.83	-1.08
S1	Kediri	54.84	1.14	2.50	1.23	2.39
S2	Ponorogo	7.79	-1.27	-0.31	-1.14	-0.42
S3	Probolinggo	31.00	2.89	-1.30	3.00	-1.39
S4	Malang	37.08	-0.19	0.50	-0.19	0.48
S5	Mojokerto	18.28	-0.98	-0.61	-0.83	-1.02

categorized unstable. The environment that had the smallest interaction effect was S4 followed by S5, S1, S2, and S3. S4 was environment with smallest interaction effect with the second rank of potential yield (37.08 t ha^{-1}), while S3 was environment with the greatest interaction effect with the potential yield 31.00 t ha^{-1} (Table 4, Fig. 1, Fig. 2).

Biplot AMMI2 either without or with matrix ***A*** assuming heterogeneous error variance across environment also had the same interpretation. The Genotype having highest level of stability was

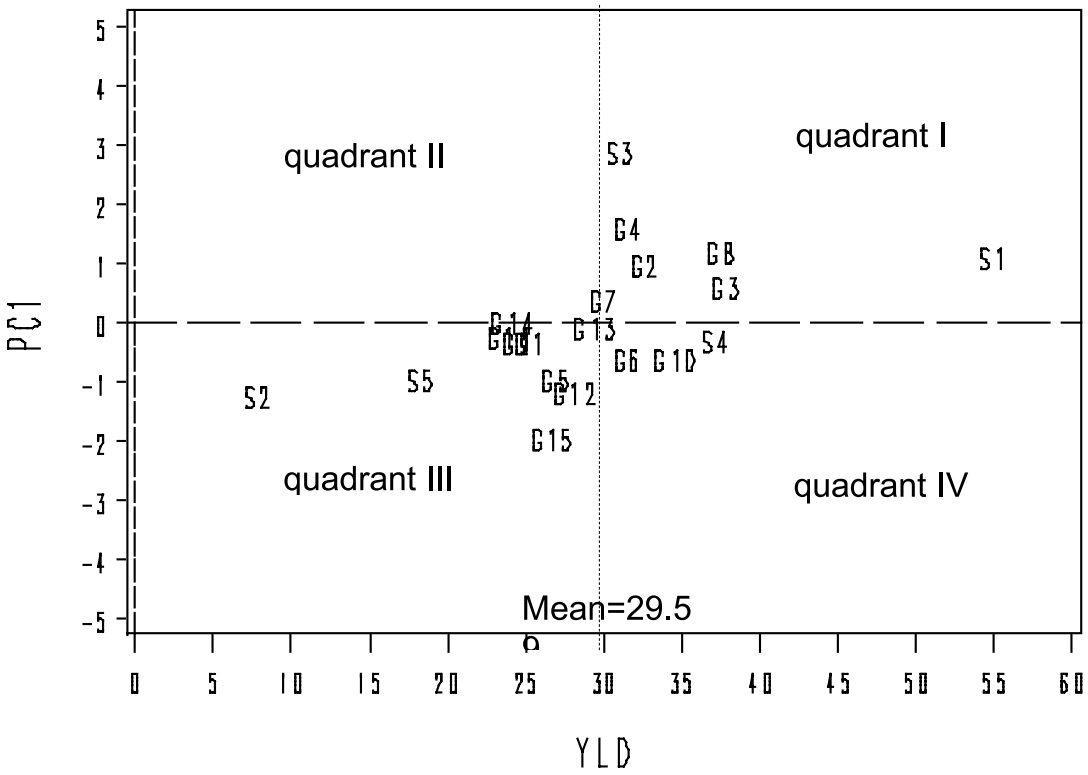

Fig. 1. AMMI1 biplot based on REML method without ***A*** matrix assuming heterogeneous error variance acrosss environments.

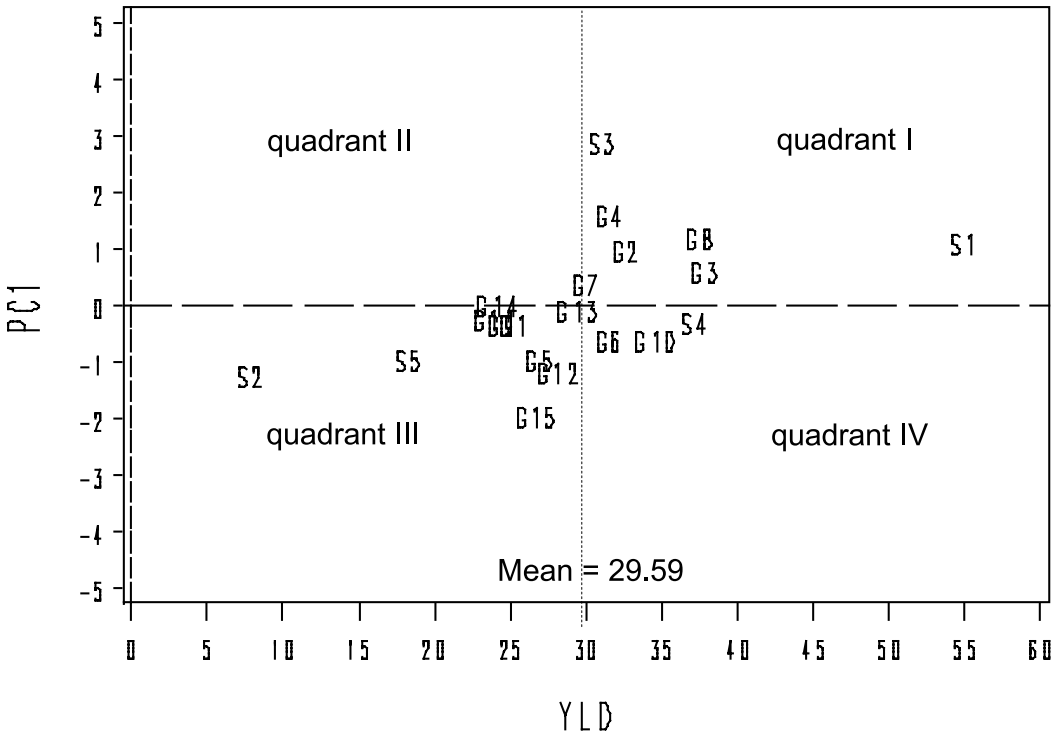

Fig. 2. AMMI1 biplot based on REML method with ***A*** matrix assuming heterogeneous error variance acrosss environments.

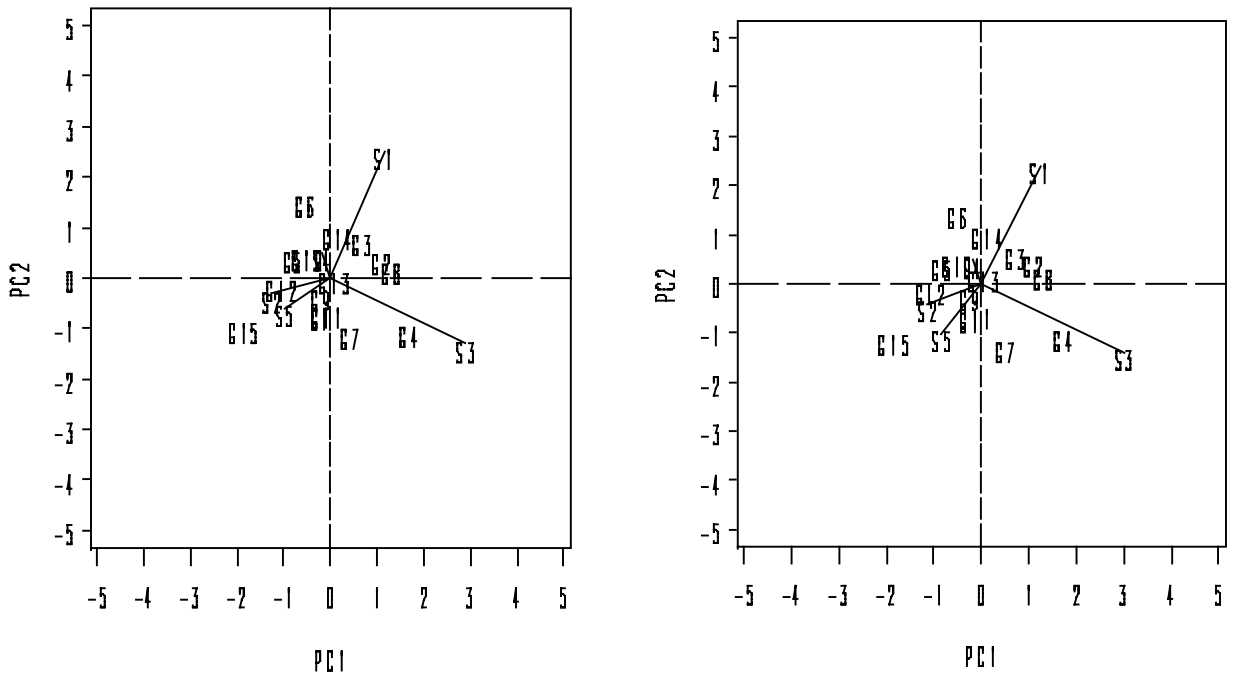

Fig. 3. AMMI2 biplot based on REML method without (left) and with ***A*** matrix (right) assuming heterogeneous error variance acrosss environments.

genotype G13 because it was located closest to the biplot origin, but the potential results were not too high (slightly below the yield mean). Genotype G4, G6, G7, and G15 were the most unstable genotype because of its distance from the biplot origin than other genotypes. G10 was specific adapted genotypes in environments S4, G4 control varieties specific adapted to the S3, G12 was specific adapted to S2 (Fig. 3).

In both biplot AMMI2 (Fig. 3), it appears that the environment which had the smallest interaction effect compared with other environmental was S4, meaning that the yield potential of genotypes tested were not influenced by environmental factors in the environment S4. The environment that had highest interaction effect were S3 and S1, as it had long environmental vectors.

Scores PC1 and PC2 from AMMI analysis based on REML method without or with ***A*** matrix assuming homogeneous residual error variance across environments explained 74.57 % and 73.32 % of GE interactions variation, respectively, but no PCs scores were significant different. Using heterogeneous residual error variance assumptions, PC1 and PC2 explained 73.54 % of GE interactions variation (mixed model AMMI without ***A*** matrix) and 72.92 % (mixed model AMMI with ***A*** matrix). The variation described declined compared with the Least Square method [13]. It can be visually seen on AMMI2 biplot obtained tends to closest to the biplot origin (0.0) compared with the Least Square method. This was in line with those reported by Sa'diyah et al. [16] that AMMI2 biplot based on mixed model AMMI was closest to the biplot origin. The two first PC scores on the mixed model AMMI obtained by reference [16] amounted to 44.49 %, less than the results of this study.

4. CONCLUSIONS

There were no PC scores that significantly different on AMMI analysis method based on REML method without and with ***A*** matrix assuming homogeneous error variance across environment. The results of AMMI analysis method based on REML method without and with *A* matrix assuming heterogeneous error variance across environment had the same interpretation. The most stable genotype was genotype G13 (CMM 02033-1) because it is located closest to the biplot origin, but the yield potential was not too high (close to the mean), whereas genotype G4 (Adira 4), G6 (CMM 03036-7), G7

(CMM 03036-5), and G15 (CMM 02048-6) were the most unstable genotype. Environmental S4 had smallest interaction effect, while environment with largest interaction effect were S3 (Probolinggo) and S1 (Kediri), because S3 and S1 had long environmental vectors.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 191–199 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Yield Stability of Groundnut Cultivars in *Ralstonia* Wilt Endemic Areas in Indonesia

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Abstract: Bacterial wilt caused by *Ralstonia solanacearum* (Smith) is an important production constraint production of groundnut (*Arachis hypogaea* L.) in some countries of Asia including Indonesia. Seventeen wilt resistant lines, including 11 breeding lines, developed from the germplasm obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), five improved cultivars, and a susceptible check cultivar (i.e., MLGG 0627) were tested for their pod yield and stability of resistance to bacterial wilt in five bacterial wilt endemic areas in Indonesia. The plant wilt intensity across all locations was high on the susceptible check cultivar, indicating severe incidence of the disease. Among the improved cultivars, only Gajah exhibited resistance to the disease and its resistance was stable across locations, whereas the other five improved cultivars were susceptible to the disease. Eight out of the 11 breeding lines were highly resistant to this bacterial wilt, comparable or even higher than Gajah's resistant level. All the resistant gentotypes produced average pod yield of 2.23 t ha^{-1}, ranging from 1.01 to 3.28 t ha^{-1}, which was higher compared to pod yield of the susceptible lines. Only two breeding lines (i.e., ChiIc-8 and LPTr-12) exhibited high yield potential (i.e., >3.0 t ha^{-1}). Average pod yield of susceptible genotypes ranged from 0.09 to 2.5 t ha^{-1} (mean, 0.87 t ha^{-1}).

Keywords: *Arachis hypogaea* L., bacterial wilt, germplasm, high yield, resistance

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important legume crop in Indonesia. However, its production is much less than required to meet the national requirement. Therefore, Indonesia is constrained to import about 242,800 t groundnut annualy [1]. The national average productivity of groundnut in Indonesia is quite low, *i.e.*, 1.66 t ha^{-1}, compared with yield potential of the improved cultivars which can produce up to >4 t ha^{-1}.

Bacterial wilt, caused by *Ralstonia solanacearum* (Smith), is an important production constraint for groundnut over large areas in some countries of Asia, including China, Indonesia, and Vietnam [2]. In Indonesia, the bacterial wilt disease has long been existed on groundnut planting areas. It was reported that since 1920 all soil in Java has been contaminated by the bacterium [3]. In a survey conducted in 1990, high disease intensity was found in groundnut crop in West Sumatra, Lampung, West Java, Central Java, East Java, Bali and South Sulawesi [4]. These areas contribute almost 70 % of the total groundnut production in Indonesia [5]. Yield loss caused by the disease ranges between 15 % to 35 % for resistant varieties and 60 % to 100 % for susceptible varieties [6, 7] when planted in high disease intensity areas.

Farmers in wilt endemic areas, still plant old varieties which were released around 1950, such as Macan, Jepara, Gajah and Kidang. The lower level of resistance to wilt is the main reason of farmers in the area for not planting new varieties. Breeding for wilt resistance has attempted to address the disease problem and many resistant

Received, January 2017; Accepted, August 2017
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cultivars have been developed. However, the source of resistance used in the breeding is limited. During 1950 to 2013, as many as 39 high yielding varieties of groundnut were released, 26 of them were declared as bacterial wilt resistant. Of those 26 high yielding bacterial wilt resistant varieties, 20 were derived from Schwarz 21 resistant variety, either directly or indirectly. Schwarz 21, a bacterial wilt resistant variety, was the first bacterial wilt resistant variety released in 1925 in Indonesia [8]. However, resistance expressions of those high yielding varieties were not on the expected level when planted in wilt endemic areas. For the reason, broadening the genetic base for wilt resistance and adaptation to the environments in diseased areas should be a priority.

Bacterial wilt has the potential to spread into new areas as the disease can spread through seed (*seed borne*), though the rate is low, 4 % to 8 % [9], through irrigation water [10–12], and the presence of disease as latent infection in resistant varieties [13]. Although many high yielding resistant varieties are available, the bacterial wilt remained a serious problem in most of the groundnut production centers in Indonesia. *Ralstonia* wilt is recently reported from areas that are not formerly reported as endemic areas, *i.e*., Malang, Probolinggo, Pasuruan, Tuban, and Borneo [14]. Allegedly there has been a decrease in the resistance of the old high yielding varieties [15], whereas the level of resistance of new high yielding varieties is lower than the existing local varieties [16]. The high yielding varieties, i.e., Komodo and Biawak, grown in Malang wilted up to 80 %. Domba and Singa varieties showed wilt incidence up to 60 % when grown in Banjarnegara. These studies suggested that resistance to *Ralstonia* bacterial wilt is critical in adoption of new groundnut varieties in Indonesia.

Therefore, a breeding program was initiated to develop new groundnut varieties with bacterial wilt resistance and high yield potential by employing new sources of wilt resistant, i.e., Turangga-s, Local Pati-s, and ICGV 93370. The new wilt resistant germplasm, obtained from an extensive screening, were crossed with high yielding genotypes. Genetic analysis in these three new resistant lines showed that resistance governed by a few genes with additive effect and narrow-and broad-sense heritabilities was high, suggested that employing pedigree breeding should be succesful in developing resistant lines [17]. Development of the segregating populations (F2 to F5) was conducted in endemic areas of Banjarnegara, Central Java. The susceptible genotypes were completely wilted. Selected resistant lines were further tested for their yield and a number of promising resistant lines have been obtained. The aim of the present study was to evaluate the resistance stability and pod yield of these promising lines against *Ralstonia* wilt in endemic areas.

2. MATERIALS AND METHODS

2.1 Planting Material

Field experiments were conducted during dry season of 2013 at five locations in Java, Indonesia which are known to be endemic areas for the bacterial wilt, *i.e*., Tayu, Ngetuk, Blingoh, Tulakan, and Wonogiri. Seventeen groundnut genotypes, including 11 breeding lines developed from the ICRISAT germplasm, five improved cultivars (i.e., Gajah, Bison, Kancil, Hypoma 1, and Tuban), and a susceptible check (i.e., cv. MLGG 0627) were tested. These varieties exhibited high yield potential, ranging from 2.4 t ha^{-1} and 3.7 t ha^{-1}, and thus have farmers' preference. Pre-planting bacterial enumeration indicated that *Ralstonia* population in the soils at experimental locations in Tayu and Ngetuk were quite high (2.1×10^6 cfu and 2.6×10^6 cfu g^{-1}, respectively), whereas bacterial population were high in Blingoh, Tulakan, and Wonogiri (i.e., 0.75 cfu $\times 10^6$ cfu g^{-1}, 1.36×10^6 cfu g^{-1}, and 1.88 x 10^6 cfu g^{-1}, respectively).

2.2 Experimental Design

The experiment at each location was arranged in a randomized block design, repeated three times. Each genotype was planted in a plot of 2.4 m × 5 m, plant spacing was 40 cm × 10 cm. Basal fertilizers, i.e., Phonska (N, P and K) @ 300 kg ha^{-1} and SP36 @ 100 kg ha^{-1}, were applied entirely at planting time.

2.3 Data Collection

Wilt disease was observed at weekly intervals after

planting, until harvesting time. At the end of the field trial, the percentage of wilted plants were counted to assess bacterial wilt incidence. Bacterial wilt reactions of the test genotypes were categorized as: (i) resistant (≤15 % wilted plants); (ii) moderately susceptible (>15 % to 25 %); (iii) moderately susceptible (> 25 % to 35 %); (iv) susceptible (> 35 %) [18]. Plants were harvested after 90 days. Fully matured pods were separated from plants, sundried, cleaned and weighed in grams, and then the dry pod yield per plot (12 m²) were converted to t ha^{-1}. Seed sample for 100 seed weight measurement was taken randomly from plot yield. Plant height, branch number $plant^{-1}$, and number pods $plant^{-1}$ were measured on 10 plant samples $plot^{-1}$.

2.4 Data Analysis

Analysis of variance was conducted for yield and wilt incidence data in each location. Pooled analysis of variance across five locations was performed on the two characters. In case of significant genotype x envionment interaction, further analysis for yield data was done to asses genotype's stability. Stability analysis parameters were estimated following Eberhart and Russell model [19] based on a linear model as Eq. 1:

$$Yij = Ui + Bi + Ij + dij,\ i = 1, 2, \ldots g \quad (1)$$

Where Yij = yield average of line i at test site j, Ui = overall mean, Bi = slope of response of lines on locations, Ij = location index, dij = deviation of the regression ith lines on jth location. A genotype with high mean seed yield, regression coefficient (b_i) close to unity and deviation from regression (s^2_{di}) near to zero was defined as a stable cultivar [19]. Simple correlation coefficients was estimated to determine the relationship between yield and wilt incidence and yield related traits.

3. RESULTS AND DISCUSSION

Reaction differences to bacterial wilt were evident among genotypes and locations, either on days to visible symptoms and disease incidence. First symptoms of bacterial wilt were observed one week after planting. Disease symptoms initially occurred on a few leaves, and then the plants die suddenly while the leaves were still green. The presence of dark brown color in xylem and the flow of bacteria mass from incised stems in the water is a diagnostic characteristic of this disease [20]. In resistant genotypes wilt symptoms stopped after 3 wk to 4 wk. The highest wilt incidence was recorded in Blingoh-Jepara, followed by Tulakan, Tayu, Wonogiri, and Ngetuk (Table 1). Average wilt incidence was not in line with the level of bacterial population in soil prior to planting.

The wilt incidence on susceptible check (MLGA0627) was high (53.23 % to 82.33 %) with an average of 68.14 %. Whereas the wilt incidence of Gajah and the 11 promising lines ranged from 0.33 % to 11.99 %, those wilt incidence levels were belong to resistant classification (Table 1). All high yielding varieties except Gajah were found susceptible with wilt incidence ranging from 40.73 % to 50.21 %. All the genotypes, except Bison in Tayu and Hypoma 1 in Wonogiri, were reacted consistantly to the bacterial wilt across the test sites (Table 1). The eleven breeding lines were consistantly resistant across the five sites, indicated their wilt resistant stability. Average bacterial wilt incidence of the resistant lines across locations was less than 5 % (Table 1). Bacterial wilt in groundnut is caused by *R. solanacearum* race 1 biovar 1, 3 and 4. Ralstonia biovar 1 which can infects groundnut is only found in the USA, whereas biovar 3 and 4 were reported to infect groundnut in Asia and Africa. Biovar 3 is dominant in Asia [20], and biovar 3 is more virulen compared to biovar 1 or 4 [6]. Isolates obtained from groundnut in Indonesia and China were mostly belongs to biovar 3 [4, 6], biovar 4 was only found in Manokwari, Papua province [6]. This report suggested that Ralstonia which existed in the five test sites was most likely belong to the same biovar, and this is explained by the stability of the genotypes's reactions against the bacterium.

Negative correlation was found between dry pod yield and wilt incidence (Table 2). High wilt incidence was correlated with relatively larger grain size and higher number of pods (Table 2). As wilt incidence increased the plant harvest and yield also decreased although pod number and seed mass per plant increased. This increase was likely attributed to a reduction in competition from adjacent peanut plants for water, nutrients, and light [21]. However, the increase of pod number and seed size were not

Table 1. The wilt incidence and reaction in groundnut genotypes at five test sites during dry season (DS) of 2013.

S. No.	Genotype	Blingoh		Ngetuk		Tayu		Tulakan		Wonogiri	
		WI[1] (%)	R[2]	WI (%)	R	WI (%)	R	WI (%)	R	WI (%)	R
1	ChiIc -1	8.97	R	2.68	R	1.24	R	3.05	R	3.37	R
2	ChiIc -3	3.82	R	0.60	R	2.32	R	3.51	R	0.84	R
3	ChiIc -8	5.18	R	2.75	R	1.57	R	1.80	R	3.22	R
4	LPTR -10	4.90	R	1.20	R	1.04	R	3.24	R	2.53	R
5	LPTR-12	2.83	R	1.82	R	1.63	R	0.67	R	1.08	R
6	ChiLP 14	2.61	R	4.92	R	1.71	R	3.20	R	2.10	R
7	LPTr -21	2.15	R	1.12	R	1.44	R	1.06	R	0.68	R
8	IcLP-24	8.21	R	1.28	R	1.35	R	0.53	R	0.33	R
9	IcLP -25	8.68	R	2.62	R	1.56	R	4.91	R	1.91	R
10	IcLP-27	6.10	R	0.89	R	3.37	R	3.57	R	0.47	R
11	Chico-s	7.80	R	2.36	R	1.57	R	2.53	R	0.91	R
12	Bison	54.67	S	26.67	S	28.89	MS	58.32	S	35.11	S
13	Hypoma 1	64.71	S	37.99	S	49.10	S	59.00	S	15.64	MR
14	Kancil	43.79	S	36.90	S	45.33	S	59.20	S	33.08	S
15	Tuban	63.18	S	33.68	S	40.11	S	67.71	S	46.40	S
16	MLGA0627	82.33	S	53.23	S	64.29	S	77.32	S	63.55	S
17	Gajah	11.19	R	0.75	R	1.46	R	0.80	R	0.37	R
	Average	22.42		12.44		14.59		20.61		12.45	

[1] WI : Wilt Incidence; [2]Classified according to Machmud and Rais [18]

Table 2. Correlations coefficients (r-values) between agronomic traits and *Ralstonia* wilt incidence in groundnut genotype tested at five endemic bacterial wilt locations during dry season of 2013.

Characters	Dry pod yield (t ha^{-1})	Wilt incidence (%)	100 seed weight (g)	Plant height (cm)	Branches plant[1]
Wilt incidence (%)	-0.835**				
100 seed weight (g)	-0.316**	0.404**			
Plant height (cm)	0.232*	-0.328**	-0.041ns		
Branch no./plant	0.067ns	0.144ns	-0.161ns	-0.330**	
No. pods/plant	-0.113ns	0.252*	-0.092ns	-0.517**	0.395**

[1] * and **= significant at $P = 0.05$ and $P = 0.01$ respectively; ns = non-significant

able to compensate plant harvest decrease due to susceptible reaction to the wilt which lead to the lower yield of the susceptible genotypes compared to the yield of the resistant ones.

Mean yield of the tested genotypes at all field locations is presented in Table 3. Average yield in each location representing environment productivity. The most productive environment on this test, also indicated by I_j value [19], is Tayu, followed by Wonogiri, Ngetuk, Tulakan and Blingoh (Table 5). Productivity of the environment was related to the genotypes'average wilt incidence at the respective location as indicated by b negative correlation between dry pod yield and wilt incidence (Table 2). The highest average pod yield across the locations obtained by line LPTR-12 with average dry pod yield 2.58 t ha^{-1}, while the highest pod yield was achieved by ChiIc-8 at Tayu location with dry pod yield reached 3.28 t ha^{-1}. ChiIc-8 derived from single cross between Chico and ICGV 93370, both of the parents were introduced from ICRISAT, India, a non wilt endemic area. Most of groundnut

Table 3. Dry pod yield of groundnut genotypes in adaptation trials at five wilt endemic locations, DS 2013.

S. No.	Genotype	Dry pod yield (t ha^{-1})					Mean	Min	Max
		Blingoh	Ngetuk	Tayu	Tulakan	Wonogiri			
1	ChiIc -1	1.31	2.15	2.95	2.30	2.42	2.22	1.31	2.95
2	ChiIc -3	1.41	1.97	2.54	2.32	2.70	2.19	1.41	2.70
3	ChiIc -8	1.51	1.99	3.28	2.40	2.44	2.33	1.51	3.28
4	LPTR -10	1.60	2.15	2.79	2.22	2.46	2.24	1.60	2.79
5	LPTR-12	1.67	2.57	3.11	2.64	2.92	2.58	1.67	3.11
6	ChiLP 14	1.38	1.48	2.48	2.18	2.62	2.03	1.38	2.62
7	LPTr -21	1.53	2.40	2.83	2.11	2.72	2.32	1.53	2.83
8	IcLP-24	1.56	2.39	2.96	2.19	2.78	2.38	1.56	2.96
9	IcLP -25	1.52	1.80	2.66	2.03	2.49	2.10	1.52	2.66
10	IcLP-27	1.85	1.99	2.80	2.44	2.74	2.36	1.85	2.80
11	Chico-s	1.53	1.94	2.60	1.01	2.20	1.86	1.01	2.60
12	Bison	0.57	1.05	2.04	0.65	2.50	1.36	0.57	2.50
13	Gajah	1.55	2.09	2.72	1.92	2.63	2.18	1.55	2.72
14	Hypoma 1	0.34	0.91	1.05	0.67	1.48	0.89	0.34	1.48
15	Kancil	0.85	0.86	1.24	0.41	1.06	0.88	0.41	1.24
16	Tuban	0.79	0.82	1.29	0.48	0.92	0.86	0.48	1.29
17	MLGA0627	0.11	0.28	0.56	0.09	0.67	0.34	0.09	0.67
	Mean	1.24	1.70	2.35	1.65	2.22			
	Ij	-0.59	-0.13	0.52	-0.18	0.39			

Table 4. Mean squares of pooled analysis of variance of pod yield and wilt incidence of 17 groundnut genotypes evaluated at five locations.

Source of variation	df	Mean square	
		Dry pods (t ha^{-1})	Wilt incidence (%)
Genotype (G)	16	7.00**	1.397**
Location (E)	4	10.44**	0.242**
G x E	64	0.21**	0.016**
Error	168	0.06	0.005

**= significant at $P = 0.01$

bacterial wilt resistance sources are of Chinese or Indonesian origin [22]. This result suggested that the wilt resistance could be found in genotypes that were introduced from wilt non-endemic area, and a chance to get genotypes resistant to wilt disease and high pod yield at once. Generally, resistant cultivars have lower yield potentials due to the presence of latent infection [23].

Pooled variance analysis revealed significant location (environment) effects for dry pod yield and wilt disease incidence. Similarly, there were significant genotype and genotype x location (G x E) interaction effects for the two characters (Table 4). Existence of G × E interaction on agronomic traits in crops, including groundnut, have been widely reported [24–27], likewise, the G by E interaction on the incidence of wilt disease [28, 29]. The G by E interaction indicated a diferential response among the tested genotypes across the five locations which cause changes in relative ranking

Table 5. Relative ranking of pod yield of 17 genotypes at five locations, DS 2013.

S. No.	Genotype	Rank No.					Average rank no.
		Blingoh	Ngetuk	Tayu	Tulakan	Wonogiri	
1	ChiIc -1	12	5	4	5	12	7.6
2	ChiIc -3	10	9	11	4	5	7.8
3	ChiIc -8	9	8	1	3	11	6.4
4	LPTR -10	3	4	7	6	10	6.0
5	LPTR-12	2	1	2	1	1	1.4
6	ChiLP 14	11	12	12	8	7	10.0
7	LPTr -21	6	2	5	9	4	5.2
8	IcLP-24	4	3	3	7	2	3.8
9	IcLP -25	8	11	9	10	9	9.4
10	IcLP-27	1	7	6	2	3	3.8
11	Chico-s	7	10	10	12	13	10.4
12	Bison	15	13	13	14	8	12.6
13	Gajah	5	6	8	11	6	7.2
14	Hypoma 1	16	14	16	13	14	14.6
15	Kancil	13	15	15	16	15	14.8
16	Tuban	14	16	14	15	16	15.0
17	MLGA0627	17	17	17	17	17	17.0

Table 6. Stability parameters for 17 groundnut genotypes estimated by Eberhart and Russel model

S. No.	Genotype	Average pod yield (t ha^{-1})	Pod yield range (t ha^{-1})	Regression coefficient (b_i)	Regression deviation (s^2_{di})
1	ChiIc -1	2.22	1.31 to 2.95	1.20ns	0.050ns
2	ChiIc -3	2.19	1.41 to 2.70	1.03ns	0.037ns
3	ChiIc -8	2.33	1.51 to 3.28	1.29ns	0.095ns
4	LPTR -10	2.24	1.60 to 2.79	0.93ns	-0.004ns
5	LPTR-12	2.58	1.67 to 3.11	1.15ns	0.030ns
6	ChiLP 14	2.03	1.38 to 2.62	1.09ns	0.083ns
7	LPTr -21	2.32	1.53 to 2.83	1.12ns	0.001ns
8	IcLP-24	2.38	1.56 to 2.96	1.19ns	-0.007ns
9	IcLP -25	2.10	1.52 to 2.66	1.02ns	-0.009ns
10	IcLP-27	2.36	1.85 to 2.80	0.88ns	0.018ns
11	Chico-s	1.86	1.01 to 2.60	1.05ns	0.183ns
12	Bison	1.36	0.57 to 2.50	1.76**	0.136ns
13	Gajah	2.18	1.55 to 2.72	1.08ns	-0.020ns
14	Hypoma 1	0.89	0.34 to 1.48	0.82ns	0.034ns
15	Kancil	0.88	0.41 to 1.24	0.44**	0.052ns
16	Tuban	0.86	0.48 to 1.29	0.44**	0.037ns
17	MLGA0627	0.34	0.09 to 0.67	0.53**	-0.007ns

*= significant at $P = 0.01$, ns = non-significant

of the tested genotypes. Pod yield of line no. 1, ChiIc-1, rank fifth in Ngetuk and Tulakan, but it turn to rank twelfth in Blingoh and Wonogiri (Table 5). Likewise for other genotypes, their relative yield rank changes over locations. Such genotypes' response variation may be due to inoculum pressure, pathogen virulence, environment, and interactions between host and those factors that affected genotypes' resistance level and yield. The varied reactions of some accessions were also found on ICGs 5272, 5273, and 5276 when they evaluated in Indonesia and China [30]. Changes in ranking complicate genotypes's evaluation and make it difficult to select the best genotype over the locations. Stability analysis developed by Eberhart and Russel [19] can be employed to evaluate genotypes' performance across locations when genotype by environement interaction exist and can be used as a tool to select the best genotype.

Stability refers to the behavior of a crop to varying environments. Different approaches to assessing stability were used [31], one of them is regression approach suggested by Eberhart and Russel [19]. Eberhart and Russel [19] used regression coefficient (b_i) and the deviation of the regression (s^2_{di}) as stability parameter. A genotype is stable if it has a regression coefficient (b_i) of unity and the deviation of the regression (s^2_{di}) equal to zero. Genotypes that have regression coefficient (b_i) > 1 will adapt well to the productive environments and genotype with the regression coefficient (b_i) < 1 will adapt well in marginal environments. Regression coefficient values (b_i) ranged from 0.44 to 1.76 and the amount of deviation from regression (s^2_d) ranged from -0.001 to 0.183 (Table 6). All the genotypes have a coefficient equal to unity, except Bison, Kancil, Tuban, and MLGA 0627; while the deviation of the regression is not significant for all the genotypes. These results suggested that all the promising lines, except Chico-s, classified as ideal cultivars, i.e., stable and have high average dry pod yield (> 2 t ha^{-1}) (Table 6). Characteristics which are recommended for increasing productivity of groundnut planted under endemic areas which is accounted for almost 70 % of the total groundnut production in Indonesia. Among the improved varieties, only Gajah belongs to that criterion. Bison belongs to below average stability, which means that the variety gave high yield in only productive environments. In this case, productive environment means low wilt disease incidence. Improved varieties were susceptible to the disease and relatively unstable (Table 6). The results were in agreement with previouse reports [16] that wilt resistance level of most groundnut improved varieties were lower compared to that of local varieties when planted in endemic areas.

4. CONCLUSIONS

High incidence of bacterial wilt disease, varying from 53.2 % to 82.3 % on the susceptible genotypes, verified that the trial locations were wilt endemic. Some breeding lines exhibited resistant to bacterial wilt disease consistently across the endemic areas. Eight of the 11 tested lines exhibited comparable or even better resistance compared with cv. Gajah, which is the most resistant improved cultivar of groundnut. The wilt disease intensity on the breeding lines ranged from 1.2 % to 2.9 %. Stable genotypes are characterized by bacterial wilt disease resistance and high yielding. Thus, the level of resistance to bacterial wilt disease contributes greatly in high pod yield when planted in the bacterial wilt endemic areas. Average pod yield of resistant genotypes ranged from 1.86 t ha^{-1} to 2.58 t ha^{-1}, while that of susceptible genotypes ranged from 0.34 t ha^{-1} to 1.56 t ha^{-1}. Only two breeding lines (i.e., ChiIc-8 and LPTr-12) exhibited high yield potential, i.e., > 3.0 t ha^{-1}); thus, these two lines are classified as stable and high yielding.

5. ACKNOWLEDGEMENTS

Authors thank Paidi for help in preparing the breeding material and ICRISAT for providing germplasm material. This work was financially supported by Indonesian government.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 201–206 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Shelf-life Determination of Fish *Koya* using Critical Moisture Content Approach

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Abstract: The objective of this study was to determine shelf-life of fish *koya* prepared from Snakehead Murrel [*Channa striata* Bloch, 1793)] and tempeh powder and packed in metalized plastic. Moisture adsorption isotherm of fish *koya* at 30 °C was determined by static gravimetric method over a wide range of water activity, *i.e*., from 0.113 to 0.843. The obtained moisture adsorption isotherm curve was expressed by employing Guggenheim-Anderson-de Boer equation. Accelerated shelf-life testing (ASLT) with critical moisture content approach was used to predict shelf-life of fish *koya*. The critical moisture content obtained by caking fish *koya* was 7.6 g H_2O 100 g^{-1} solids. *Koya* stored at 30 °C with 75 %, 80 %, and 85 % of relative humidity (RH) had shelf-life of 234 d, 203 d, and 180 d, respectively.

Keywords: Accelerated shelf-life testing, critical moisture content, fish *koya*

1. INTRODUCTION

Koya is a savory powder used as a topping on food. *Koya* powder is usually added to Indonesian traditional foods such as *soto* and noodles in East Java, especially in *Soto* Lamongan. *Koya* is made by mixing the softened fried garlic along prawn crackers. Regina et al. [1] conducted a research study about *koya* based in soy flour and different kinds of fish, such as catfish, tilapia, tuna, and mackerel. The results indicated that the fish *koya* had a moisture content of 13.10 % to 21.21 %, ash content of 5.54 % to 5.99 %, protein content of 27.13 % to 29.83 %, lipid content of 15.55 % to 21.76 %, and carbohydrate content of 30.28 % to 31.92 %.

In this research, fish *koya* was made from Snakehead Murrel (*Channa striata* Bloch, 1793) with addition of tempeh powder. Snakehead Murrel is wild freshwaters fish, which can be found in all parts of Indonesia. Snakehead Murrel in Indonesia is known as "gabus fish". Several studies on snakehead fish had been reported. Sugito & Ari [2] conducted a research on the addition of snakehead fish flesh and chilling applications in gluten *pempek,* while Sari et al. [3] reported that 15 % addition of snakehead fish flour gave the best texture of the biscuit.

Tempeh is a traditional food in Indonesia which is made from soybean grains fermentation. Bavia et al. [4] reported that the steps of tempeh processing are dehulling, cooking, inoculation, and is fermented by fungus like *Rhizopus oligosporus*. Tempeh from soy cultivar BRS 216 has high protein content (51.99 %), isoflavone (123 mg 100 g^{-1}), aglycone (49.00 mg 100 g^{-1} on average), and phytic acid (1.00 g 100 g^{-1}). The nutrient content of tempeh has benefits for human health, such as protection against chronic diseases. The addition of tempeh will increase the protein content in fish *koya.*

Fish *koya* is a hygroscopic dried food product and can be easily damaged by moisture absorption from the environment. The main physical damage is caking which can change the solubility, increase

Received, February 2017; Accepted, September 2017
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lipid oxidation and enzyme activity, alternate taste, and crispiness, and lower sensory qualities and shelf-life [5]. Knowledge about moisture sorption isotherm is needed to determine the quality, stability, and shelf-life of the product, especially in dried food [6].

Moisture sorption isotherm is a curve that describes the relationship between water activity (a_w) and moisture content [7]. This curve describes the ability of food to absorb moisture from the surrounding air and vice versa. Research on moisture sorption isotherms of food products has been widely conducted [7–10].

According to Labuza [11], shelf-life is the length of time a product is able to meet the expected quality of consumers, so it's very important to study. Ellis [12] reported that the shelf-life of product can be determined by two methods, *i.e.* Extended Storage Studies (ESS) and the Accelerated Shelf Life Testing (ASLT). The ASLT method can be done with two approaches, Arrhenius approach and critical moisture content approach. Arrhenius approach is used for food products which damaged due to chemical reactions triggered by storage temperature. The critical moisture content approach is used for food products which damaged due to moisture absorption [11]. The aim of this study was to determine the shelf-life of fish *koya* based on Snakehead Murrel [*Channa striata* (Bloch, 1793)] and tempeh powder using ASLT with critical moisture content approach.

2. MATERIALS AND METHODS

2.1 Fish Grinding

Snakehead Murrel was obtained from Cengklik reservoir, Boyolali, Central Java, Indonesia. Fresh fish was eviscerated and washed thoroughly then steamed for 10 min. After that, the fish was separated from its bone and ground. Then, the ground fish was stored in a refrigerator prior to analysis.

2.2 Tempeh Powder Production

The tempeh was produced in a home industry in Babad, Manang, Sukoharjo, Indonesia. The fresh tempeh was cut into 0.5 cm, then blanched at 80 °C to 90 °C for 10 min. After that, the pieces of tempeh were dried with cabinet dryer at 70 °C for 6 h to 7 h. The size was reduced to 60 mesh. The tempeh powder was then stored at room temperature in sealed jars equipped with silica gel.

2.3 Fish *Koya* Production

Based on the method by Regina et al. [1], *koya* seasoning consists of onion *(Allium cepa* var. *ascalonicum* (L) Back), garlic (*Allium sativum* L.), walnut (*Aleurites moluccana* (L.) Willd*)* and coriander (*Coriandrum sativum* L). The ingredients were mixed with thick coconut milk, ginger, galangal *(Alpinia galangal* (L). Willd.), crushed leaves of lemongrass *(Cymbopogon citratus* (DC.) Stapf, 1906), bay leaf *(Syzygium polyanthum* (Wight) Walp.), lime leaves *(Citrus hystrix* DC.*)*, brown sugar and salt then the mixture was boiled. After that, ground fish was inserted and stirred until dry. Once dry, tempeh powder was mixed in the mixture until the color became brown. Comparison between minced fish meat and tempeh powder was 3:2. The *koya* powder was placed on metalized plastic and then transferred into a jar equipped with silica gel, and was stored at room temperature.

2.4 Determination of Moisture Content (M_i)

The moisture content was determined using the thermogravimetric method [13]. Initial moisture content is expressed as g H_2O 100 g^{-1} solids.

2.5 Determination of Critical Water Content (M_c)

The critical moisture content was determined by storing unpacking fish *koya* at room temperature (30°C) in RH 75 % to 80 %. During storage, the sensory evaluation was conducted daily towards *koya* caking. The assay was conducted until the powder underwent caking. Scale ratings of the sensory test were 1 to 7, conducted by 25 panelists. Score 1 indicated that *koya* strongly formed lumps/ caking, while the score of 7 indicated that *koya* did not form lumps. The moisture content was analyzed using thermogravimetry methods [13] periodically and expressed in g H_2O per 100 g solids. Curve relationship between moisture content and *koya* coagulation score was made from experimental data. The *koya* can be assumed to form lumps/cake if the score was 3 (rather caking). By using the relationship curve between moisture content and

koya coagulation score, the moisture content of fish *koya* when reached score 3 can be determined. This moisture content was called the critical moisture content.

2.6 Determination of Moisture Adsorption Isotherms

Determination of moisture adsorption isotherms using thermogravimetric static methods [6] and adsorption isotherm curve was carried out at 30 °C. To obtain the different relative humidity (RH) 11.3 % to 84.3 %, saturated salt solution was used, ie: LiCl (11.3 %), KCH_3CO_2 (22.5 %), $MgCl_2$ (32.8 %), K_2CO_3 (43.2 %), Mg $(NO_3)_2$ (52.9 %), $NaNO_2$ (65.4 %), NaCl (75.3 %), and KCl (84.3 %). In equilibrium conditions, water activity (a_w) expressed as equilibrium relative humidity (ERH) divided by 100. Moisture adsorption isotherm curve was expressed in GAB (Guggenheim Anderson de Boer) model equations:

$$\frac{M}{Mo} = \frac{K.c.a_W}{(1 - K.a_W)(1 - K.a_W + c.K.a_W)} \quad (1)$$

with M for the moisture content, M_o for the monolayer moisture content, a_w for water activity, c and k were constants for GAB equation [6]. The value of K, C and M_o was determined by Bizot [14] method, *i.e.*:

(i) Modification of GAB equation becomes:

$$\frac{a_W}{M} = \frac{(1 - K.a_W)(1 - K.a_W + C.K.a_W)}{K.C.M_o} \quad (2)$$

(ii)Rearrangements of GAB equation:

$$\frac{a_W}{M} = \frac{1}{K.C.M_o} + \frac{(C-2)}{C.M_o} + \frac{K}{C.M_o}(1-C)a_W^2 \quad (3)$$

$$\frac{a_W}{M} = a_1 + a_2.a_W + a_3.a_W^2$$

with

$$a_1 = \frac{1}{K.C.M_o};\ a_2 = \frac{(C-2)}{C.M_o};\ a_3 = \frac{K}{C.M_o}(1-C) \quad (4)$$

(iii) The value of M_o, K, and C were determined as a function of the coefficients (a_1, a_2 and a_3), in order to obtain:

$$K = \frac{-a_2 \pm \sqrt{a_2^2 - 4.a_1.a_3}}{2.a_1} \quad (5)$$

$$C = 2 + \frac{a_2}{a_1.K} \quad (6)$$

$$M_0 = \frac{1}{a_1.K.C} \quad (7)$$

2.7 Determination of Packaging Water Vapor Permeability

Metalized plastic used as the packaging material was obtained from a local market in Surakarta, Central Java, Indonesia. Determination of packaging vapor permeability was performed using ASTM F1249-01 procedure with Mocon Permatran-W 3/31 at 38.7 °C. The value of Water Vapor Transmission Rate (WVTR) was obtained. This following equation is used to determine the packaging vapor:

$$k / x = \frac{WVTR}{P_{out}} \quad (8)$$

with k/x is the packaging permeability (g H_2O / day m^2 mm Hg), and P_{out} was the water vapor pressure at storage temperature × RH (mm Hg).

2.8 Determination of *Koya* Shelf-Life

The *Koya* fish shelf-life was determined by the ASLT critical water content approach [6] using the following equation:

$$ln\left(\frac{M_e - M_i}{M_e - M_c}\right) = \left(\frac{k}{x}\right)\left(\frac{A}{W_s}\right)\left(\frac{Po}{b}\right)\theta \quad (9)$$

with M_e for the equilibrium moisture content (the moisture content level when the product is in equilibrium with the external RH) (g H_2O per 100 g solids); M_i for the initial moisture content (g H_2O per 100 g solids); M_c for the critical moisture content (g H_2O per 100 g solids); k/x for the moisture permeability through packaging material (g H_2O $(m^2$ days mm Hg$)^{-1}$); A for the packaging area (m^2); W_s for weight of dry food solids (g); P_0 for the water vapor pressure at the storage temperature (mmHg); b for the slope of the linearized isotherm portion (*i.e.,* from M_i to M_c); Θ for the estimated shelf-life (days).

Koya shelf-life was determined at 30 °C in three storages of RH, *i.e.,* 75 %, 80 % and 85 %. Shelf-life expressed in days. The size of packaging was 10 cm × 10 cm with 25 g of *koya* per each pack.

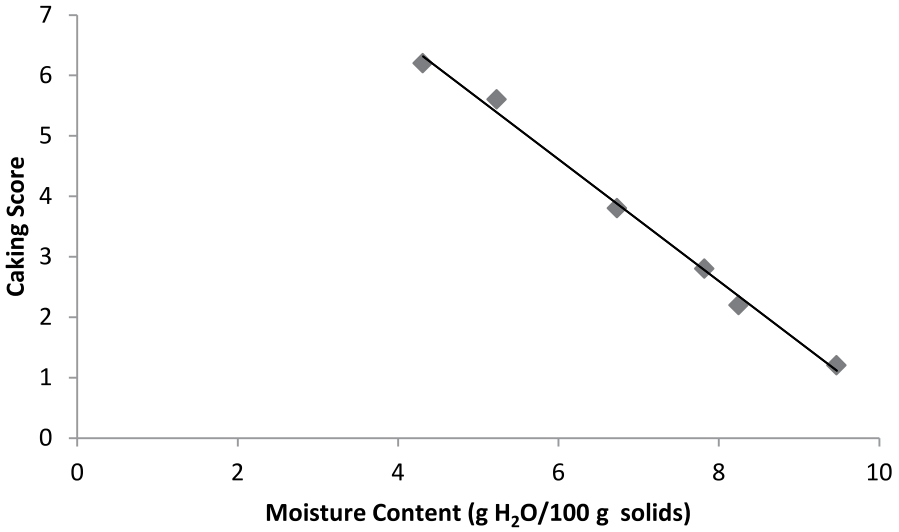

Fig. 1. Relationship between moisture content and caking score.

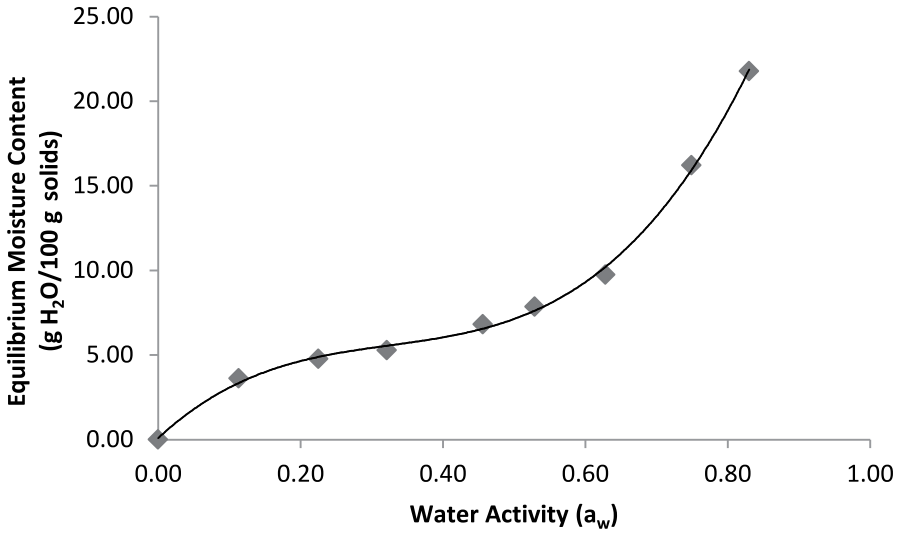

Fig. 2. Moisture adsorption isotherm of Koya at 30 °C.

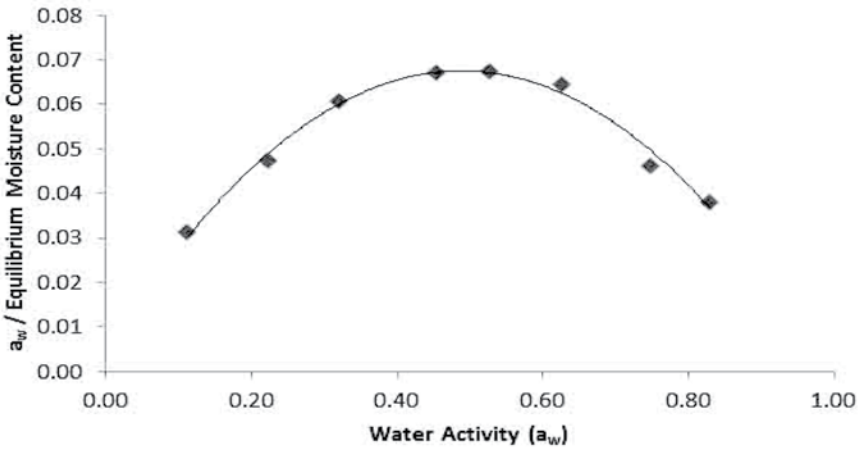

Fig. 3. Relationship between water activity and (aw/EMC),

Table 1. Calculation parameters for *Koya* shelf-life,

Parameter	Relative humidity		
	75 %	**80 %**	**85 %**
M_i (g H_2O per 100 g solids)	4.31	4.31	4.31
M_c (g H_2O per 100 g solids)	7.60	7.60	7.60
M_e (g H_2O per 100 g solids)	9.63	10.12	10.60
k/x (g H_2O (m^2 d mm Hg^{-1})	0.015	0.015	0.015
A (m^2)	0.02	0.02	0.02
P_0 (mm Hg)	31.82	31.82	31.82
B	0.097	0.097	0.097

3. RESULTS AND DISCUSSION

3.1 Initial Moisture Content and Critical Moisture Content

Initial moisture content (M_i) is one of the most important parameters. Initial moisture content of *koya* was 4.31 g H_2O per 100 g solids. The weight of packed *koya* was 25 g, so the amount of *koya* in each pack was 23.92 g (W_s).

The critical moisture content (M_c) was important to discover the consumer acceptance limits of *koya*. The critical moisture content is determined by the caking quality attributes. Fig. 1 indicated the relationship curve between moisture content and caking score, resulting in equation: caking score = -1.0077 (moisture content) + 10 655 (R^2 = 0.995).

The critical moisture content was determined when the *koya* caking score was 3. From the equation, the *koya* moisture content was 7.6 g H_2O per 100 g solids (*Mc*) while the caking score was 3.

3.2 Moisture Adsorption Isotherm

Water vapor adsorption pattern of *koya* made from Snakehead Murrel and tempeh powder was performed by storing *koya* at different levels of water activity (a_w) using eight types of saturated salt at 30 °C. During storage, the water from saturated salt will evaporate and be absorbed by *koya* or vice versa. The process will continue until the moisture content reached equilibrium with the constant weight of fish *koya*.

The relationship curve between *koya* equilibrium moisture content and a_w showed in Fig. 2 indicates that *koya* moisture adsorption isotherm curve was sigmoid shaped (like letter S). Labuza [6] stated that dry food and cereals have a sigmoid shape of moisture adsorption isotherm curve. Sigmoid shape occurred due to differential water attachment in food. *Koya* is one of the dried food product so the moisture adsorption isotherm curve is sigmoid. In the *koya* sigmoid shaped curve, there were two arches, first at a_w 0.2 and the second at a_w 0.6. Two arches of the curve indicated the physical-chemical properties change of the water binding in the material.

Koya moisture adsorption isotherm curve was expressed by employing Guggenheim-Anderson-de Boer (GAB) equation. To determine the constants C, K, and M_0 in the GAB equation, data relationship between water activity (a_w) and a_w/M_C (Fig. 3) were required. From these curves, an equation was generated, *i.e.*, $y = -0.2639x^2 + 0.2577x + 0.0046$. From the equation, the value of K, C and M_0 were obtained, *i.e.,* 0.943, 61.408, 3.754, respectively. The GAB equation for fish *koya* moisture adsorption isotherm as follows:

$$\frac{M}{3.754} = \frac{57.91a_w}{(1 + 56.024a_w - 53.72a_w^2)} \quad (10)$$

GAB equation produced good precision for a material which had sigmoid shaped curve [15]. Several studies also reported that the GAB equation was the right model for tapioca [16], potatoes [17], as well as dried strawberry [18].

3.3 Fish *Koya* Shelf-life

Based on Labuza equation about shelf-life, there were some parameters that determine the shelf-life by critical moisture content approach. *Koya* initial moisture content (Mi), critical moisture content (Mc), and solids of product (Ws) had been

determined in the earlier discussion. The results of packaging moisture vapor permeability indicated that metalized plastic as packaging material had a permeability (k/x) of 0.015 g H_2O (m^2 d mm Hg)$^{-1}$. The size of packaging was 10 cm × 10 cm and the surface area (A) was 0.02 m^2. Saturated moisture vapor pressure at 30 °C according to the saturated moisture vapor table was 31.82 mmHg. The equilibrium moisture content (Me) and slope (b) were determined using a moisture adsorption isotherm curve. The parameters used to determine the shelf-life showed in Table 1. Furthermore, those parameters were entered into the shelf-life equation. The *koya* shelf-life based on critical moisture content approach was determined at 30 °C with RH of 75 %, 80 % and 85 % and the results showed that the shelf-life was 234 d, 203 d and 180 d, respectively.

4. CONCLUSIONS

The deterioration of fish *koya* due to its caking started at moisture content of 7.60 g H_2O 100 g^{-1} solids. The shelf-life of fish *koya* packed in metalized plastic and stored at 30 °C was reduced with increase in relative humidity (RH); the shelf-life was 234 d at 75 % RH; 203 d at 80 % RH; and 180 d at 85 % RH.

5. ACKNOWLEDGEMENTS

This research was financially supported by The Grant Scheme Center of Excellence of the Sebelas Maret University, Indonesia.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 207–217 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Determination of Critical Level of Brassinosteroid (24-epibrassinoloid) for Heat-tolerance in Okra (*Abelmoschus esculentus* L.)

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Abstract: This study aimed at determining suitable dose of 24-epibrassinoloid (24-EBL) for improving morphological, physiological, and photochemical efficiency of PS II (Fv/Fm) characteristics in thermo-tolerant (*Sabaz pari* and *Green wonder*) and thermo-sensitive (MF-03 and Click-5769) genotypes of okra (*Abelmoschus esculentus* L.). The study was performed in a growth chamber at 40 °C by applying different levels of 24-EBL (i.e., 0 μM (control treatment), 0.25 μM, 0.50 μM, 0.75 μM, 1.00 μM, 1.25 μM and 1.50 μM). Foliar application of 24-EBL improved root and shoot growth, plant fresh and dry weight, leaf area, photosynthesis rate, chlorophyll content and photochemical efficiency of PS II in thermo-tolerant as well as of thermo-sensitive okra cultivars as compared to control plants. However, increase in the above stated characteristics was greater in thermo-tolerant okra genotypes as compared to thermos-sensitive genotypes, at all tested levels of 24-EBL. Greater reduction in electrolyte leakage in both thermo-sensitive and thermo-tolerant okra genotypes was observed with 1.50 μM 24-EBL. Considering increase in plant growth and biomass, leaf area, chlorophyll content, photosynthesis rate, photochemical efficiency of PS II and reduction in electrolyte leakage, both in thermos-tolerant and thermo-sensitive okra genotypes, it was concluded that 1.50 μM 24-EBL was the most suited level of brassinosteroid to improve the thermo-tolerance potential of okra genotypes.

Keywords: Okra, brassinosteroid, physiological, Fv/Fm, electrolyte leakage

1. INTRODUCTION

Plants are static in nature and they pass through different type of environmental (biotic and abiotic) stress during their growth life cycle [1]. These environmental stresses create bad effects on growth, development and production of plants. Nature has blessed the plants with different growth controlling hormones (gibberellins, auxins, cytokinins, abscisic acid, salicylic acid, ethylene, brassinosteroids, jasmonates, etc.), osmolytes (glycine betain, proline, free amino acids etc.) and antioxidants (peroxidase, super oxide dismutase, catalase, ascorbate peroxidase etc.) to mitigate the adverse effect environmental stresses [2-4]. The activation of these hormones may vary in plants based upon the nature and duration of environmental stress [5-6]. The hazardous effects of external environmental stress in plants can also be reduced through the foliar application of natural or synthetic plant growth hormones [7-10], osmolytes [11-13], antioxidants [14], etc.

Brassinosteroid can be extracted from flowers of brassica (*Brassica compestris*) plants and can be foliar applied to promote growth in plants under environments stress conditions [15-20]. Okra (*Abelmoschus esculentus* L.) is a famous crop of tropical and subtropical areas, which is classified in annual, often cross pollinated vegetable crops with

Received, March 2017; Accepted, August 2017
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11,480,000 hectares of cultivation and 7,896,300 tons of production around the globe [21-22]. Due to sudden environmental temperature fluctuations and increase in global temperature growth and production of crop plants has become adversely affected. Therefore, the present study was proposed to investigate the effect of foliar application of brassinosteroid on growth of thermo-tolerant and thermo-sensitive okra cultivars and to find a suitable level of brassinosteroid for better growth of okra which could be used in further study of reproductive study of okra plants under high temperature stress conditions.

2. MATERIALS AND METHODS

The present study was conducted in stress physiology lab, Department of Horticulture, University College of Agriculture, University of Sargodha, Punjab, Pakistan. Seeds of selected okra (*Abelmoschus esculentus* L. Moench) genotypes were collected from the Ayyub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Seeds were disinfected with 5% sodium hypochlorite solution for 15 min, followed by repeated washing with double distilled water and then these seeds were sown in plastic pots filled with peat moss (Sia Pindstrup Ltd., Talsi, Latvia). Three seeds per pot were sown in 4 inches plastic pots with perforated bottom. After seed's emergence, plant density per pot was adjusted to one by removing unhealthy and less vigorous seedling plants. Half strength Hoagland solution was drenched in pots as nutrition source. The nutrient solution was applied after every 10 d interval by adding 30 mLL^{-1} of distilled water and the plants were irrigated as per requirement by observing the moisture of rooting media. Growth chamber was adjusted to 25/23 °C day/night temperature with RH 75%, light intensity 550 µmol m^{-1} s^{-1} from fluorescent tubes and photoperiod 11.5 h. After the emergence of 5-6 true leaf temperature of growth chamber was raised to 40/28 °C while keeping all the other environmental conditions same as for 25/23 °C. Fifteen days after the induction of high temperature stress (40/28 °C) different levels of 24-epibrassinoloid i.e., 0 μM (control), 0.25 μM, 0.50 μM, 0.75 μM, 1 μM, 1.25 μM and 1.50 μM were foliar applied to identify the best suited level of 24-EBL for mitigation of adverse effects of heat stress and induction of thermos-tolerance in tested okra plants.

2.1 Growth Attributes

One week after foliar application of 24-EBL, plants from each replication were gently uprooted and gently washed with distilled water to remove growth media particles and then blotted with filter paper to remove surface water of leaves and shoots and then fresh weight of plants was measured through digital balance. The plants were oven dried (Memmert-110, Schawabach, Germany) at 72 °C for 48 h to measure dry weight per plants. Root length and shoot length was measured meter rod. The leaf area was calculated using leaf area meter (Ll-3100; LI-COR Inc., Lincoln, NE, USA).

Three young fully developed and healthy leaves per plant were selected and placed individually in the chamber of a portable Infrared Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, UK) for the measurement of net photosynthesis rate (Pn). Chlorophyll contents were determined by Chlorophyll content meter (Model CL-01, Hansatech Instruments, UK).

2.3 Photochemical Efficiency of PS II

Photochemical efficiency of PS II (Fv/Fm) was measured using a portable fluorometer (FMS2, Hansatech Ltd., Kings Lynn, Norfolk, England). Leaves were dark acclimated for 30 min and measurements of maximum yield of the photosystem II photochemical reactions (Fv/Fm) and quantum yield of the photosystem II electron transport (UPSII) were carried out according to the method of Yu et al. [23].

2.4 Electrolyte Leakage

For measurement of electrolyte leakage method of Shi et al. [24] was used with some modifications. The ten leaf discs (1cm in diameter) were placed in 50 ml autoclave vials, rinsed three times with 20 ml of distilled water to remove the electrolytes released during leaf disc excision. Vials were then filled with 20 ml of distilled water and were incubated at room temperature on shaker at 100 rpm for 24

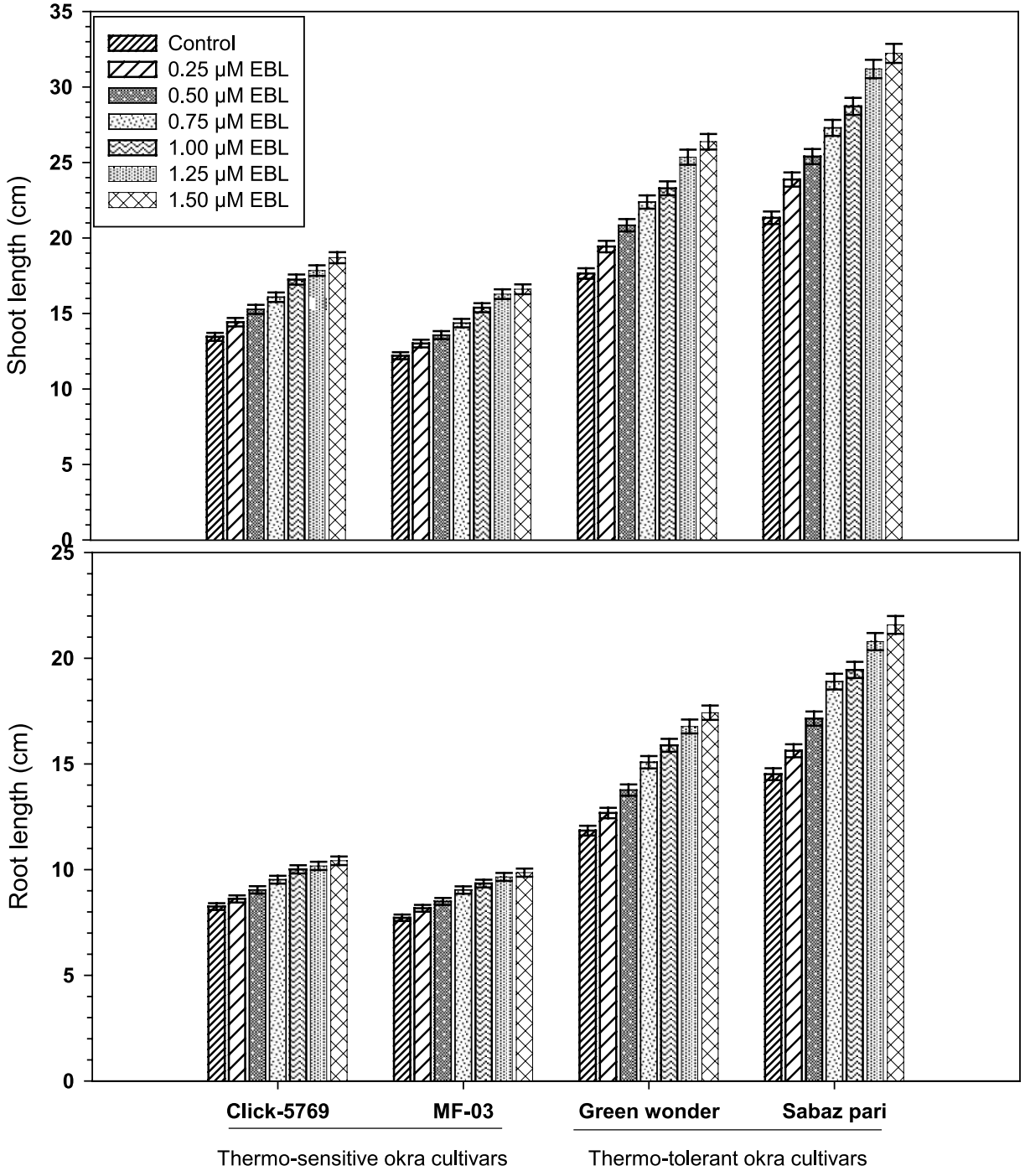

Fig. 1. Effect of 24-Epibrassinoloid application on root and shoot length of thermo-tolerant and thermo-sensitive okra genotypes.

hours. Electrical conductivity (EC_1) of the shaking solution was determined at the end of incubation period. After the measurement of EC_1, tubes were autoclaved at 120 ˚C for 15 mints and the electrical conductivity (EC_2) was measured followed by their cooling at room temperature. Electrolyte leakage was calculated as percentage of EC_1/EC_2.

2.5 Statistical Design and Analysis

The experiment was laid out under Completely Randomize Design (CRD) with five replications. There were five pots per replicate. The data were analysed by standard statistical procedures as described by Gomez and Gomez [25]. Tukey HSD test was used to evaluate the significance of

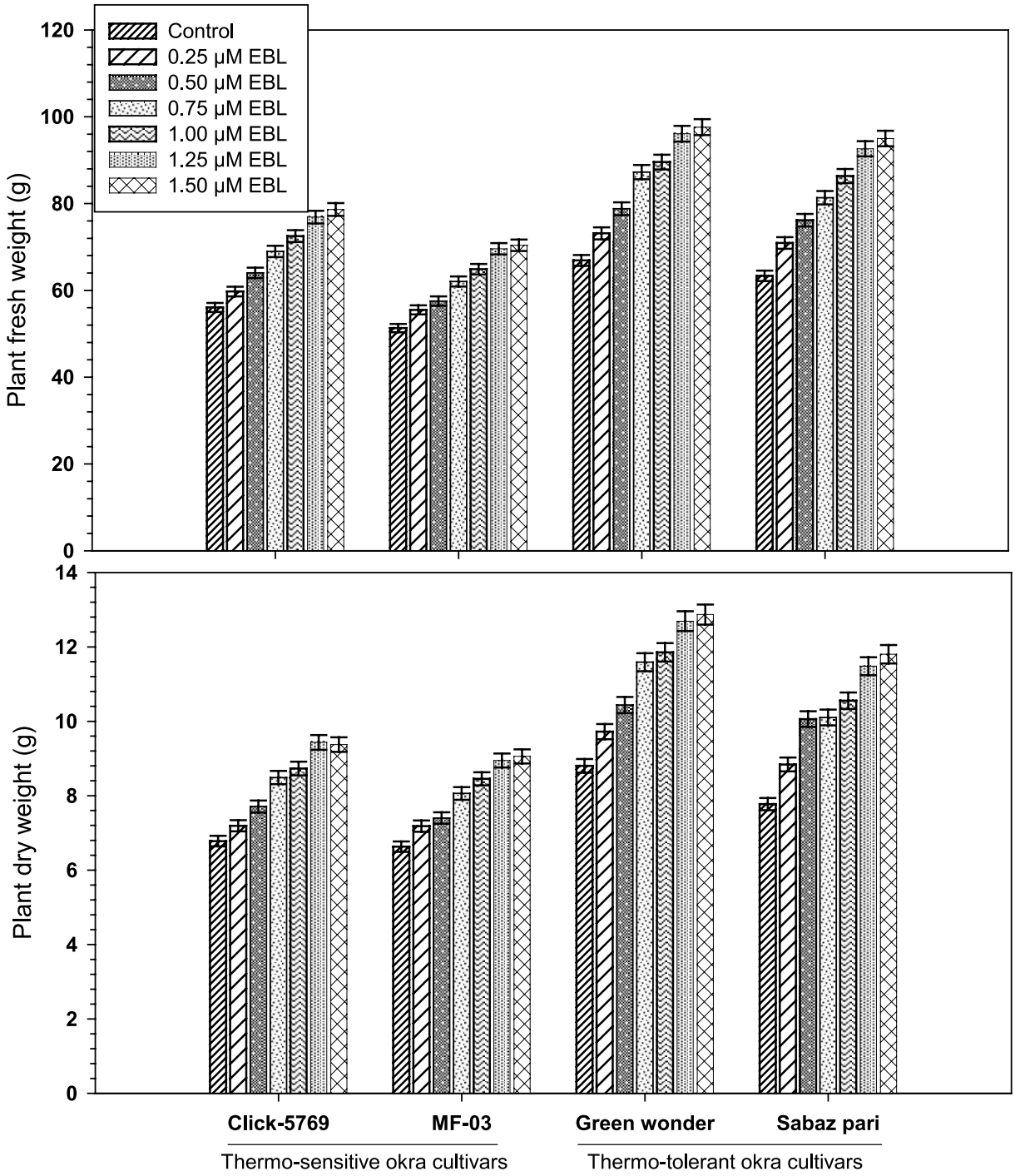

Fig. 2. Effect of 24-Epibrassinoloid application on fresh and dry weight of thermo-tolerant and thermo-sensitive okra genotypes.

differences between the treatments at $P < 0.05$ (n = 5). Data were analyzed using statistical package STATISTIX 8.1.

3. RESULTS

The data regarding thermo-tolerant and thermo-sensitive okra genotypes showed an increase in plants growth through the foliar application of 24-epibrassinoloid at 40 °C. The thermo-tolerant okra cultivar *Sabaz pari* showed greater increase in root and shoot length 48.67 % and 51.68 %, respectively. Thermo-sensitive okra genotype click-5769 and MF-03 showed least increase in root

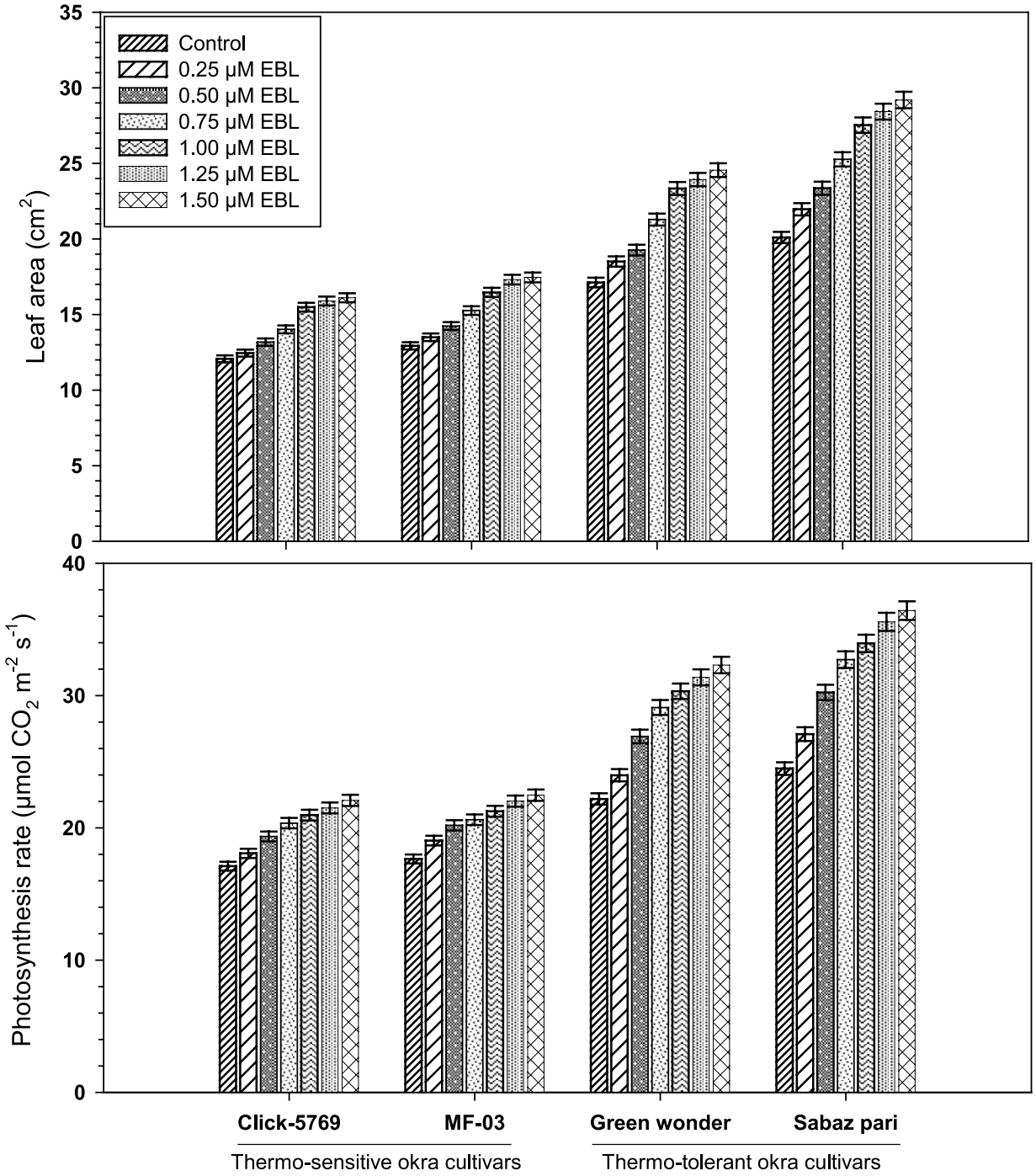

Fig. 3. Effect of 24-Epibrassinoloid application on leaf area and photosynthesis efficiency of thermo-tolerant and thermo-sensitive okra genotypes.

and shoot length 26.28% and 36.19% respectively wit foliar application of 1.5 *µM* 24-epibrassinoloid as compared to control (Fig. 1a, 1b). Foliar application of 24-epibrassinoloid enhanced the fresh and dry weigh of thermo-tolerant and thermo-sensitive okra plants but a greater increase in fresh and dry weigh 49.97% and 51.80% respectively was observed in thermo-tolerant okra genotypes *Sabaz pari*. The least increase in plant fresh weight 26.16 % was observed in thermo-sensitive genotype MF-03 while, plant dry weigh was observed lower 25.61 % in Click-5769 at 1.5 *µM* foliar application of 24-epibrassinoloid as compared to control (Fig. 2a, 2b).

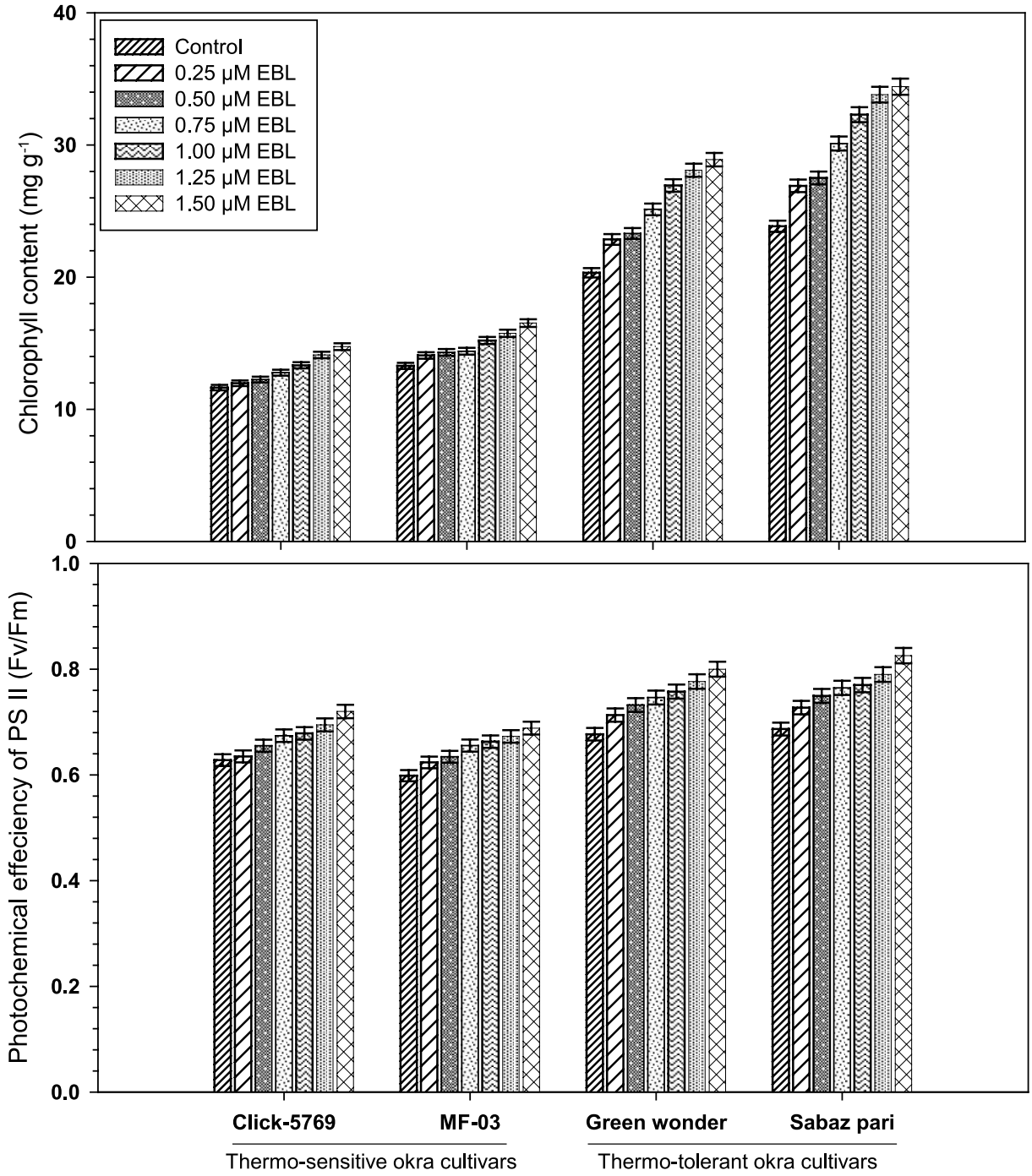

Fig. 4. Effect of 24-Epibrassinoloid application on chlorophyll content and photochemical efficiency of PS II (Fv/Fm) of thermo-tolerant and thermo-sensitive okra genotypes.

Leaf area and photosynthesis rate was observed higher in thermo-tolerant okra genotype *Sabaz pari* 43.41 % and 48.79 % respectively, whereas it was increased least 33.43 % and 27.31 % in thermo-sensitive okra genotypes click-5769 and MF-03 respectively, as compared to control plants (Fig. 3).

Chlorophyll contents and photochemical efficiency of PS II was increased in both thermo-tolerant and thermo-sensitive okra genotypes with increasing the concentration of foliar 24-epibrassinoloid. In comparison with control the greater increase in chlorophyll contents and photochemical efficiency of PS II 44.26 % and 40.18 % respectively was observed in thermo-tolerant

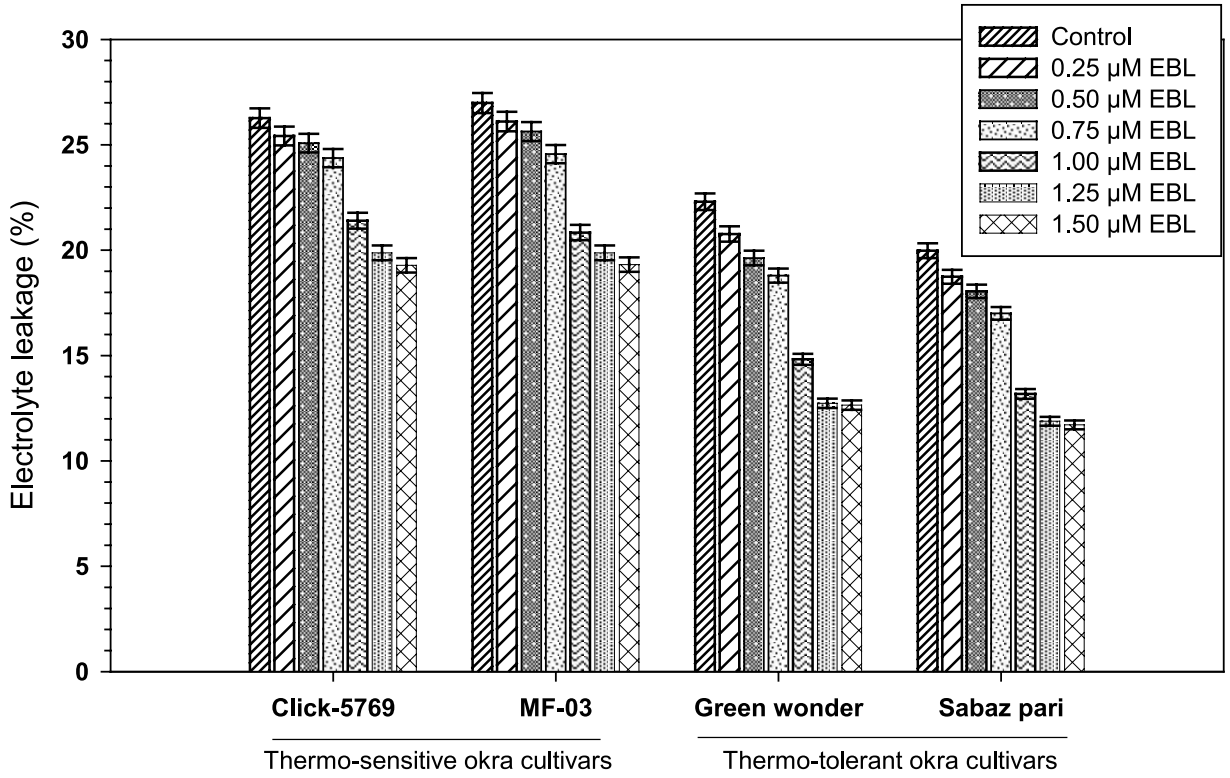

Fig. 5. Effect of 24-Epibrassinoloid application on electrolyte leakage of thermos-tolerant and thermos-sensitive okra genotypes.

okra genotype *Sabaz pari*. The least increase in chlorophyll contents and photochemical efficiency of PS II 24.33 % and 29.35 % respectively, was observed in thermo-sensitive MF-03 and click-5769 respectively (Fig. 4a, 4b).

In case of electrolyte leakage thermo-sensitive okra genotypes showed less reduction i.e. 26.60 % in electrolyte leakage. Thermo-tolerant green wonder showed higher reduction i.e. 43.28 % in electrolyte leakage in response to foliar application of 1.5 μM foliar application of 24-epibrassinoloid and compared with control (Fig. 5).

4. DISCUSSION

Plants growth regulators (PGR) can promote and inhibit the growth of plants depending upon the concentration of PGR used on plants. For example, auxin is a plant growth promoter at its lower concentration but it can be a weedicide when applied with a higher concentration on plants [26]. In our study, different levels of 24-epibrassinoloid (24-EBL) were tested to find a super optimal dose of 24-EBL for the growth of thermo-tolerant and thermo-sensitive okra genotypes screened from a preliminary experiment (data not shown). The results showed that under the varying concentrations of foliar applied 24-EBL plant growth was promoted in both thermo-tolerant and thermo-sensitive okra genotypes. The greater increase in growth parameters like root and shoot length, plant fresh and dry weigh and leaf area was observed in thermo-tolerant genotypes as compared to thermo-sensitive okra cultivars. This increase in okra plants growth was observed maximum under at 1.25 μM and 1.5 μM concentration of 24-EBL. The thermos-tolerant okra genotypes showed greater increase in plant growth due to their better adoptability under high temperature conditions as compared to thermo-sensitive okra genotypes, and high ability to absorb 24-EBL for improving their secondary metabolic activities which help them to grow better under high temperature conditions. El-Bassiony et al. [27] found that foliar application of 25 and 50 ppm of brassinosteroid increase the growth in snap bean plant under high temperature stress

conditions. Similarly, Kumar et al. [28] reported that foliar application of 24-EBL promote growth and antioxidant enzyme activities in mustard (*Brassica juncea*) at seedling stage, and the foliar application of 24-EBL can increase plant's tolerance under heat-induced oxidative damage.

Thermal or high temperature stress affect the rate of photosynthesis rate and chlorophyll contents in plants that effect their growth and reduces productivity of plant [29-30] through the production of reactive oxygen species the cause oxidative damage in plants [31-32]. In response of stress, plants modify their morphological, physiological and metabolic activities through producing compatible solutes, maintaining cell turgor by osmotic adjustment, and regulating the antioxidant system to re-establish the cellular redox balance and homeostasis [33-34]. One of the change in plant physiological activities under high temperature stress conditions is that they reduce their photosynthesis activities to coop the adverse effects of thermal stress [35-36], similarly, Yang et al. [37] reported reduction in photosynthesis and chlorophyll contents occur in plants under high temperature conditions, whereas thermos-tolerant cultivars shows less reduction in photosynthesis rate, chlorophyll contents, and stomatal conductance under high temperature stress, and exhibit more plant growth than thermo-sensitive plant genotypes. In our study, reduction of photosynthesis rate was observed higher in thermo-sensitive okra cultivars as compared to thermo-tolerant cultivars due to their less ability to withstand under high temperature conditions. However, the foliar application of 24-EBL improved the photosynthesis rate and chlorophyll contents in both thermo-tolerant and thermo-sensitive okra genotypes, but thermo-tolerant okra cultivars responded more better than thermo-sensitive genotypes at all levels of 24-EBL application. Whereas, foliar application of 1.5 μM of 24-EBL was observed more effective than other levels of 24-EBL. Which may be due to greater production of antioxidant enzymes, osmolytes (not studies in this experiment) and suppressing the effect of ROS activity in okra plants.

Thermal stress causes cell membrane injury in plants due to lipid per oxidation and denaturation of proteins structure [38] that may cause reduction in plants growth, early wilting and death of plants under high temperature conditions [39]. The application of hormones and PGR can cause a reduction of adverse effect of stress in plants by modulating there metabolic activities under stressed conditions [40-41]. In our study, 1.5 μM foliar application of 24-EBL reduced the cell membrane injury more in thermo-tolerant okra genotypes as compared to thermo-sensitive genotype. Hasanuzzaman et al. [41] reported that exogenous application of plant growth promoting substances help to reduce the adverse effect of heat stress on plants through their growth promoting and antioxidant abilities. Similar results had also been reported by Kumar et al. [28], Miura et al. [42], Balal et al. [43] in chickpea, Arabidopsis and cucumber respectively. Wise et al. [44] and Yin et al. [45] reported that photochemical efficiency of PS II become reduced under plant's stress conditions, whereas, foliar application of PGR can improve the chlorophyll florescence in plants [46]. In our study, photochemical efficiency of PS II was observed more under the foliar application of 1.5 μM 24-EBL in thermo-tolerant okra genotypes as compared to thermo-sensitive genotypes under the same level of 24-EBL foliar application. On the basis of above information, it can be concluded that foliar application of 1.5 μM 24-EBL can promote the growth of okra plants by promoting growth, photosynthesis, chlorophyll contents, photochemical efficiency of PS II and reducing the electrolyte leakage under high temperature stress conditions.

5. CONCLUSIONS

Under high temperature conditions foliar spray of 24-EBL improved growth of both thermo-tolerant (cv. *Sabaz pari* and Green wonder) as well as thermo-sensitive (cv. MF-03 and Click-5769) okra genotypes. With foliar application of 1.5 μM 24-EBL increase in plant growth and physiological traits of thermo-tolerant genotypes was greater compared with thermo-sensitive genotypes. Therefore, under high temperature conditions, foliar sparys of 1.5 μM 24-EBL may be applied to enhance thermo-tolerance in okra crop.

6. ACKNOWLEDGEMENTS

The authors acknowledge the Higher Education Commission of Pakistan for financial assistance for this investigation through video project No. 20-2315/ NRPU/R&D/HEC/12.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 219–235 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Cardioprotective Effect of Mango and Kinnow Peel Extracts on Doxorubicin-induced Cardiotoxicity in Albino Rats

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Abstract: Different dose levels of mango (*Mangifera indica* L.) and kinnow (*Citrus reticulate* L.) peel extracts were administered to rats for 60 days and myocardial infarction was induced by administering doxorubicin (DOX) injection 2.5 mg/kg body weight (BW) intraperitoneal in six equal doses on alternate days from 50th to 60th day. Cardiac biomarkers lactate dehydrogenase (LDH), aspartate transaminase (AST), creatine kinase-MB fraction (CKMB), creatine phosphokinase (CPK), lipid profile (triglycerides, cholesterol, high density lipoprotein (HDL)) and low density lipoprotein (LDL), renal function activities (blood urea nitrogen, creatinine, uric acid) in serum and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) in heart tissues were estimated. Histopathological studies were carried out for cardiac tissues of the studied rat groups. Mango and kinnow peel polyphenolic extracts at medium dose (i.e., 150 mg/kg BW) and high dose (i.e., 300 mg/kg BW) exhibited significant protection against doxorubicin induced myocardial infarction. Maximum cardioprotective activity was exhibited by groups pre-treated with 300 mg/kg of peel extracts, especially mango peel extracts, which maintained the membrane integrity of myocardial tissues, lowered the DOX-induced hyperlipidemia, nephrotoxicity and significantly restored the activity of cardiac endogenous antioxidant enzymes. Histopathological studies of cardiac tissues verifield the cardioprotective activity of both peels extracts at medium and high dose levels. Thus, mango and kinnow peel extracts have cardioprotective potential at medium and high dose levels.

Keywords: Polyphenols, peel extracts, cardioprotective activity, doxorubicin, histopathology

1. INTRODUCTION

Oxidative stress is the state that develops due to excessive production of free radicals and reactive oxygen species (ROS) in the body and is responsible for the pathogenesis of several chronic diseases such as atherosclerosis, diabetes mellitus, cardiovascular disease, cancer and neurodegenerative disorders [1-4]. Cardiovascular diseases are the leading cause of mortality in advanced and industrialized countries as well as increasing at alarming rate in developing countries. Coronary heart disease is one of the serious cardiovascular disorder results in high morbidity and mortality rate [5, 6]. Polyphenols are the plants antioxidants present especially in fruits and vegetables which impart a significant protective role on human health.

It has been reported that regular consumption of plant-derived foods containing polyphenols may limit the risk of coronary heart disease due to their antioxidant activity against free radicals and ROS [7, 8]. Cardiovascular health may be improved by dietary polyphenols through the regulation of platelet reactivity which have significant role in myocardial infarction venous thromboembolism.

Received, July 2017; Accepted, August 2017
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Decrease in platelet reactivity by polyphenols reduces the probability of blood clotting. Flavonoids such as quercetin, myricetin and kaempferol restrict platelet aggregation [9, 10].

Mango (*Mangifera indica* L.) is one of the popular tropical fruit whose peel constitutes about 15-20% of the mango fruit weight and is a rich source of cardioprotective polyphenols even higher than mango pulp [11]. Kinnow mandarin (*Citrus reticulate* L.) is the leading citrus fruit crop of Pakistan. Kinnow mandarin peel is about 35-40% of the fruit weight and is the major waste component after processing. Citrus peel is the rich source of phenolic compounds especially flavones, isoflavones, flavonones, flavonols and anthocyanidins [12]. Orange peels possess flavonones (naringenin and hesperitin), carotenoids, ascorbic acid which altogether contribute to protection against cardiovascular disease [13]. Pretreatments of rats with hesperidin lead to cardiac tissue protection from cardiotoxic effects of doxorubicin and thus hesperidin may be considered as cardio-protective agent [14].

Cardioprotective activity of plant polyphenols was assessed in animal models through chemically induced myocardial infarction. Doxorubicin or Adriamycin is an anthracycline drug used for the treatment of various malignancies such as solid tumors, lymphoma and leukemia [15]. However, its clinical use is now restricted due to dose-dependent cardiotoxicity leading to acute and chronic heart failure [16, 17]. Doxorubicin-induced cardiotoxicity has been mediated through various mechanisms including reactive oxygen species formation, mitochondrial DNA damage, cardiomycyte apoptosis, myofibrillar degeneration, inhibition of DNA and protein synthesis [18-20]. Injection of doxorubicin/adriamycin to animals such as rats leads to various morphological and metabolic disorders in cardiac tissues of experimental animals similar to human cardiomyopathy [21].

Keeping in view the above mentioned facts, the current study was designed to evaluate the cardioprotective activities of mango and kinnow peel polyphenolic extracts on doxorubicin induced cardiotoxicity in albino rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fruits of mango, Chaunsa variety, and kinnow mandarin were procured from the fruit market in Islamabad and taken to the Food Science and Product Development Institute (FSPDI) research laboratory, National Agricultural Research Center (NARC), Islamabad. Fruits were thoroughly washed under tap water. Fruit peels were removed, cut into small pieces, oven-dried at 50 °C for 48 hours, ground to fine powder in sample mill and packed in air-tight polyethylene zip bags.

2.2 Extraction of Polyphenols

Ultrasound-assisted extraction technique was employed for polyphenols extraction from mango and kinnow peel powders according to procedure depicted by Bimakr et al. [22] with minor variation. Peel powders samples were extracted with solvent 80% ethanol, sample to sovent ratio 1:20, at extraction temperature and time 45 °C and 60 minutes respectively in a sonicator set at 35 kHz frequency. Peel extracts were filtered, centrifuged, solvent evaporated by vacuum evaporator and microfiltered through 0.45 µm cellulose membrane filter, collected in amber glass bottles and refrigerated stored.

The total polyphenol content of mango and kinnow mandarin peel extracts was measured by the Folin-Ciocalteau method as described by Singleton et al. [23] and the absorbance was measured at 765 nm with UV-VIS Spectrophotometer (Agilent 8453, USA). Individual phenolic compounds in mango and kinnow peel extracts were quantified with high performance liquid chromatography (HPLC) according to the method described by Salvador et al. [24] with slight modifications. The analyses were conducted at a flow rate of 1 mL/min with the UV detector set at 280 nm for phenolic acids and 370 nm for flavonoids and sample injection volume 20 µL.

2.3 Experimental Conditions for Animals

Sprague Dawley strain albino rats of either sex weighing between 190-210 g were selected for the biological studies carried out at animal house,

National Institute of Health (NIH), Islamabad. Animals were housed in polypropylene cages with 12 hours light/dark cycle under environmental conditions of 25 ± 3 °C, relative humidity 50 ± 10% and had free access to feed and water *ad libitum*. The study protocol was approved by the institutional animal ethics committee, University of Sargodha, Pakistan.

2.4 Drugs and Chemicals

Doxorubicin (DOX) (Adriablastina, Pfizer) injections were purchased from a local pharmacy in Islamabad. Commercially available kits (DiaSys Diagnostic Systems GmbH, Germany) were used to estimate cardiac enzymes in serum, lipid profile and renal function activities. Other chemicals employed were of analytical grade.

2.5 Experimental Design

Animals were kept for one week acclimatization period and then randomly divided into 8 groups of 6 animals per group.

Group I: Negative control or normal control without any intervention; albino rats received standard feed and distilled water of for 60 days.

Group II: Positive control or DOX control group; rats received standard feed, distilled water for 60 days and DOX injection was administered 2.5 mg/kg body weight (BW) intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group III: Preventive group A; albino rats were pretreated with 75 mg/kg BW mango peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group IV: Preventive group B; albino rats were pretreated with 150 mg/kg BW mango peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group V: Preventive group C; albino rats were pretreated with 300 mg/kg BW mango peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group VI: Preventive group D; rats were pretreated with 75 mg/kg BW kinnow peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group VII: Preventive group E; rats were pretreated with 150 mg/kg BW kinnow peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group VIII: Preventive group F; albino rats were pretreated with 300 mg/kg BW kinnow peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

2.6 Biochemical Assessment

After 48 hours of last doxorubicin injection dose, the animals were anaesthetized with chloroform and blood was collected by cardiac puncture in blood collection tubes. Serum was separated by centrifugation at 4000 rpm for 10 min and used for biochemical studies [25].

2.7 Estimation of Cardiac Enzymes in Serum

Serum was analyzed for various enzyme biomarkers related to myocardial infarction like lactate dehydrogenase (LDH), aspartate transaminase (AST), creatine kinase-MB fraction (CKMB), creatine phosphokinase (CPK) according to procedures described by Thomas [26] and Rosalki [27] with commercially available kits (DiaSys Diagnostic Systems GmbH, Germany) by using Microlab Chemistry Analyzer (300 1x, Merck).

2.8 Evaluation of Lipids and Lipoprotein Profile

Serum triglycerides [28], total cholesterol [29], high density lipoprotein (HDL) [30] and low density lipoprotein (LDL) [31] were evaluated by using Microlab Chemistry Analyzer with commercially available kits (DiaSys Diagnostic Systems GmbH, Germany).

2.9 Estimation of Renal Function Profile

Blood urea nitrogen (BUN), creatinine and uric acid were analyzed in accordance with the methods described by First [32] with commercially available kits (DiaSys Diagnostic Systems GmbH, Germany) by using Microlab Chemistry Analyzer (300 1x, Merck).

2.10 Assessment of Antioxidant Enzymes in Heart Tissues

After blood collection by cardiac puncture, the albino rats were slaughtered and heart tissues were removed, washed with ice-cold saline and dried with filter paper. Heart tissues were diced and homogenized in 0.05 M ice-cold phosphate buffer. Homogenate was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was collected and utilized for the determination of antioxidant enzymes.

2.10.1 Superoxide Dismutase (SOD) Assay

Superoxide Dismutase activity was analyzed according to method described by Kono [33]. SOD activity was determined by observing the rate of inhibition of NBT reduction by superoxide radicals generated by the auto-oxidation of hydroxylamine hydrochloride. Briefly, 1.3 mL of EDTA solution (0.1 mM EDTA containing 50 mM sodium carbonate, pH 10.0) was mixed with 0.5 mL NBT (90 µM) and 0.1 mL Triton-X (0.6%). Then 0.1 mL hydroxylamine hydrochloride (20 mM, pH 6.0) was added and the rate of NBT reduction was observed for one minute at 560 nm. Then 0.1 mL enzyme sample was added to cuvette and the enzyme activity was calculated. SOD activity was expressed as units per mg protein change in optical density per minute where one unit of enzyme is the SOD amount required to inhibit 50% rate of reaction.

2.10.2 Catalase (CAT) Assay

Catalase activity was determined according to the method of Aebi [34] by using 0.1 mL enzyme sample, 1 mL hydrogen peroxide (2 mM) and phosphate buffer (0.01 M, 1 mL, pH 7.0). Catalase activity was analyzed by measuring the decrease in absorbance at 240 nm for 3 min by UV-Vis spectrophotometer and expressed as units per mg protein.

2.10.3 Glutathione Peroxidase (GPx) Assay

Glutathione peroxidase was estimated by the method described by Rotruck et al. [35]. Briefly, the assay mixture consisted of 0.5 mL phosphate buffer(0.4 M, pH 7.0), 0.1 mL sodium azide (10 mM), 0.2 mL reduced glutathione (4 mM), 0.2 mL enzyme tissue(supernatant) and 0.1 mL hydrogen peroxide (0.2 mM). The contents were incubated at 37°C for 10 min, the reaction was stopped by adding 0.5 mL 10 % trichloroacetic acid and centrifuged at 3000 rpm for 5 min. The glutathione in the supernatant was quantified by using 0.5 mL Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid "DTNB" in 50 mL phosphate buffer, pH 7.6) and absorbance was noted after 5 min at 412 nm by UV-Vis spectrophotometer and expressed as µg of GSH consumed/min/mg of protein.

2.11 Histopathological Studies

Heart tissues were fixed in 10 % formalin and embedded in paraffin. Paraffin blocks were prepared, cut at 5µm thickness, mounted on glass slides, deparaffinized and stained with hematoxlin and eosin for histopathological studies through light microscope [36]. The histopathological parameters to be studied were necrosis, loss of myofibril, cytoplasmic vacuole formation, edema, mitochondrial swelling and leukocyte infiltration.

3. RESULTS

Solvent ethanol categorized under GRAS (Generally Recognized as Safe) at 80% concentration level was employed for the extraction of polyphenols from mango and kinnow peels. Maximum polyphenols were extracted in mango peels (67.58± 0.21 mg GAE/g of extract) as compared to kinnow peel extracts (29.75 ± 0.23 mg GAE/g of extract) as evident in Table 1.

Peel extracts phenolic compounds identified and quantified through HPLC included phenolic acids, i.e., gallic acid, chlorogenic acid, ferulic acid, coumaric acid, caffeic acid and flavonoids catechin, epicatechin, hesperidin, naringenin, quercetin, kaempferol, mangiferin, myrecetin and rutin according to retention time and their peaks

Table 1. Total polyphenol content of mango and kinnow peels extracts.

Peel Extract	mg GAE/g extract
Mango peel	67.58±0.21a*
Kinnow peel	29.75±0.23b

**Values are mean ± standard error of triplicate analyses. Different letters denote a significant difference at P<0.05*

Table 2. HPLC quantification of polyphenols in mango and kinnow peel extracts.

Phenolic compound	Mango Peel Extract (µg/g)	Kinnow Peel Extract (µg/g)
Gallic acid	91.00±0.67[a*]	54.13±1.12[b*]
Chlorogenic acid	28.90±0.44[a]	20.52±0.82[b]
Ferulic acid	115.65±2.25[a]	65.21±1.16[b]
Coumaric acid	188.97±4.13[a]	27.29±0.44[b]
Caffeic acid	N.D.	2.43±0.30[a]
Catechins	67.41±1.28[a]	49.46±1.03[b]
Epicatechins	152.13±1.48[a]	18.62±0.54[b]
Magniferin	112.18±1.54[a]	N.D.
Hesperidin	N.D.	92.94±1.23[a]
Naringenin	N.D.	N.D.
Quercetin	30.46±0.60[a]	23.71±0.50[b]
Myrecetin	11.08±0.42[a]	N.D.
Rutin	80.24±2.29[a]	N.D.
Kaempferol	42.56±0.62[a]	16.85±0.41[b]
Total	**920.58+5.60[a]**	**371.16±6.79[b]**

All values are means of three replications
** Means followed by same letter do not differ significantly (P < 0.05)*
N.D. Not detected

spectral characteristics against those of standards. Mangiferin, rutin and myrectin were identified only in mango peel extracts while caffeic acid, hesperidin and naringenin were detected only in kinnow peel extracts (Table 2). Results revealed that mango and kinnow peel extracts phenolics varied considerably as function of solvent concentration level. Mango peel extracts showed comparatively higher quantity of phenolic compounds than kinnow peel extracts. Among the phenolic compounds, coumaric acid was the most abundant phenolic acid in mango peel extracts whereas ferulic acid and hesperidin were the most abundant in kinnow mandarin peel extracts. Gallic acid, catechin and epicatechin were the other phenolic compounds present in high concentration.

Bulk extraction of polyphenols was carried out from mango and kinnow peels by employing 80% ethanol for the prepation of polyphenolic extract doses to albino rats in order to evaluate cardioprotective activities of polyphenolic extracts.

3.1 Effect of Mango and Kinnow Peel Extracts on Serum Cardiac Markers

Serum cardiac markers (LDH, CK-MB, CPK and AST) in albino rats of each group were presented in Fig. 3. DOX administration to albino rats significantly increased serum cardiac marker. LSD-test revealed significant difference between serum enzyme levels of control and DOX group for all cardiac markers.

As regards LDH (Fig. 1A), doxorubicin administration significantly elevated the serum LDH level (342.33 ± 11.97 IU/L) in DOX group rats as compared to normal control group (182.83±13.05 IU/L). Pre-treatment with peel extracts significantly minimize the effect of doxorubicin administration on serum cardiac biomarkers level in different treatment groups. However, the changes were more pronounced at low dose of peel extracts especially kinnow peel extracts (304.67 ± 6.22 IU/L) and were non-significantly different from DOX group. Rats group pre-treated with high dose of mango peel extract had minimum elevation of doxorubicin induced LDH level (209.17 ± 5.51 IU/L) and were non-significantly different from control group. In case of CK-MB (Fig. 1B), significant increase in enzyme level of DOX group (52.50 ± 4.39 IU/L) was observed as compared to control (19.18 ± 3.83 IU/L). For pre-treatment groups, highest and lowest elevation in CK-MB level in serum were observed in kinnow peel extract low dose (46.50± 4.16 IU/L) and mango peel extract high dose (28.33± 2.13 IU/L) respectively. Cardiac biomarker CPK level in serum ranged from137.33±11.72 IU/L (control group) to 281.50±8.78 IU/L (DOX group) (Fig. 1C). Pre-treatment with mango peel extract high dose resulted in maximum protective effect due to minimum increase in CPK level (171.67±6.98 IU/L) as compared to other pre-treatment groups which might be due to activity of mango peel

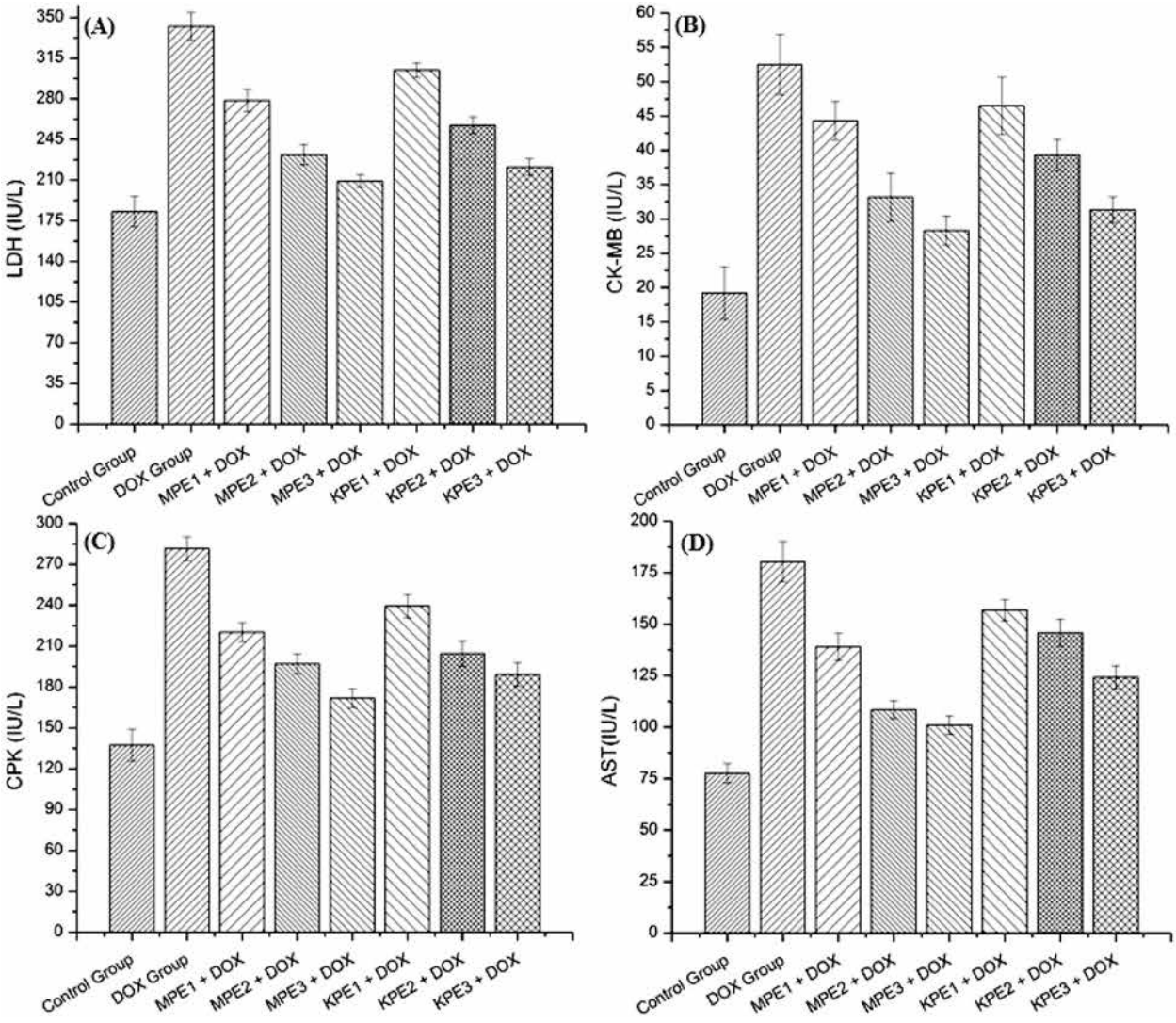

Fig. 1. Cardioprotective effect of mango and kinnow peel extracts on serum cardiac markers in doxorubicin induced cardiotoxicity in rats. LDH: Lactate dehydrogenase; CKMB: Creatine kinase-MB fraction; CPK: Creatine phosphokinase; AST: Aspartate transaminase; DOX: Doxorubicin; MPE1: Mango peel extract (75 mg/kg body weight); MPE2: Mango peel extract (150 mg/kg body weight); MPE3: Mango peel extract (300 mg/kg body weight); KPE1: Kinnow peel extract (75 mg/kg body weight); KPE2: Kinnow peel extract (150 mg/kg body weight); KPE3: Kinnow peel extract (300 mg/kg body weight).

extract on maintaining the membrane integrity thus restricting the enzymes leakage. As regards AST enzymes (Fig. 1D), doxorubicin administration significantly elevated the AST level in DOX group (180.33±9.81 IU/L) than control group (77.67±4.74 IU/L). Pre-treatment with peel extracts especially at medium and high doses efficiently hindered the AST secretions from cardiac tissues into the blood, therefore decreased doxorubicin induced AST levels.

3.2 Effect of Mango and Kinnow Peel Extracts on Serum Lipid Profile

Serum lipid profile (cholesterol, triglycerides, HDL, LDL) of different rat groups studied showed significant variations between normal and Dox-treated group for all parameters (Fig. 2). As regards cholesterol level (Fig. 2A), a significant elevation was observed in serum cholesterol level (115.33 ± 5.46 mg/dL) in DOX group as compared to control group without intervention (61.17 ±

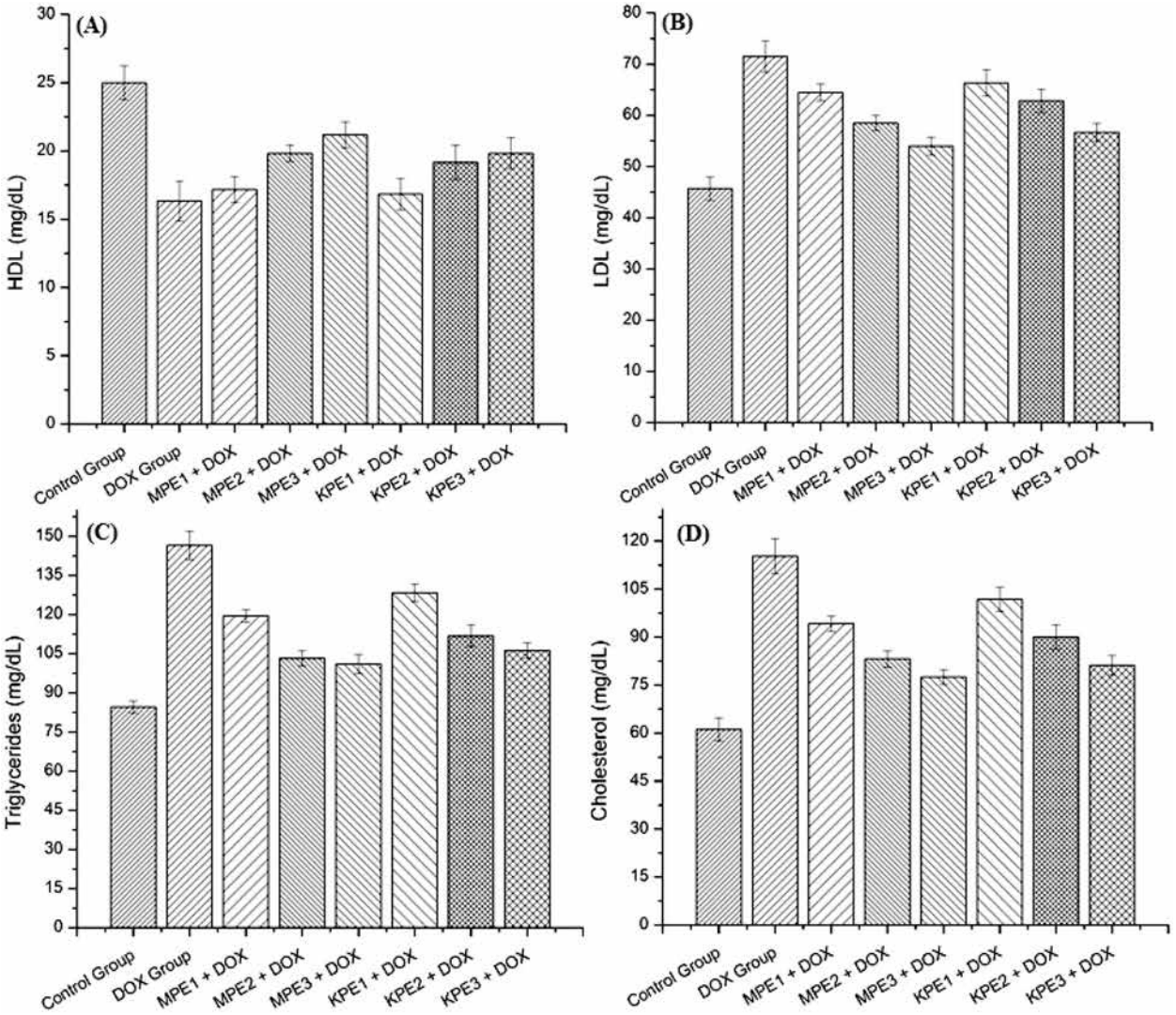

Fig. 2. Cardioprotective effect of mango and kinnow peel extracts on serum lipid profile in doxorubicin-induced cardiotoxicity in rats. HDL: High density lipoprotein; LDL: Low density lipoprotein; DOX: Doxorubicin; MPE1: Mango peel extract (75 mg/kg body weight); MPE2: Mango peel extract (150 mg/kg body weight); MPE3: Mango peel extract (300 mg/kg body weight); KPE1: Kinnow peel extract (75 mg/kg body weight); KPE2: Kinnow peel extract (150 mg/kg body weight); KPE3: Kinnow peel extract (300 mg/kg body weight).

3.57 mg/dL). Pre-treatment with peel extracts significantly minimize the effect of doxorubicin administration on serum cholesterol level in different treatment groups. Preventive effect of peel extracts against doxorubicin induced cholesterol level was pronounced at medium and higher doses. Maximum preventive effect (77.50 ± 2.29 mg/dL) was exhibited by mango peel extract high dose (300 mg/kg) while minimum preventive effect (101.83 ± 3.74 mg/dL) was observed in kinnow peel extract low dose (75 mg/kg) and was non-significant to DOX group. Thus the results showed that peel extracts especially at high dose levels were effective against DOX-induced hyperlipidemia.

In case of triglycerides (Fig. 2B), a significant increase (146.50 ± 5.46 mg/dL) was recorded in the level of serum triglycerides in rats of DOX group than normal control group (84.50 ± 2.36 mg/dL). Pre-treatment with mango and kinnow peel extracts at different dose levels significantly minimize the effect of doxorubicin administration on serum triglycerides concentration. However, preventive effect of peel extracts low dose was least as compared to medium and high dose levels. Highest preventive effect (103.17 ±2.91 mg/dL)

was exhibited by mango peel extracts medium dose i.e. 75 mg/kg and was non-significantly different to dose 300 mg/kg of mango peel extract (101.00 ± 3.66 mg/dL).

During the current study, administration of doxorubicin elevated serum LDL level in DOX treated group (71.50 ± 3.06 mg/dL) as compared to control group (45.67 ± 2.28 mg/dL) (Fig. 2C). There was an elevation in the LDL mobilization from the blood into myocardial membranes, leading to abnormally high deposition of cholesterol in the myocardium. Pre-treatment of rats with peel extracts medium and high dose significantly reduced the level of LDL. Maximum preventive activity (54.00 ± 1.73 mg/dL) was determined in mango peel extracts high dose (300 mg/kg) while minimum preventive activity (66.33 ± 2.55 mg/dL) was recorded in kinnow peel low dose (75 mg/kg) that was non-significantly different from DOX group.

A significant decline in HDL level (Fig. 2D) was estimated in doxorubicin treated group (16.33±1.45 mg/dL) than control group (25.00 ± 1.24 mg/dL). Pre-treatment of rats with peel extracts elevated the HDL level but the increase was non-significant except for 300 mg/kg dose level of mango peel extract (21.17 ± 0.95 mg/dL).

3.3 Effect of Mango and Kinnow Peel Extracts on Serum Renal Function Activities

Renal function activities parameters studied in albino rats include BUN, creatinine and uric acid. DOX administration to albino rats significantly increased their renal function activities parameters and induced kidney disorders. As evident by LSD-test, there were significant differences between control and DOX treated groups (Fig. 3). Pre-treatment with different doses of peel extracts decreased the DOX-induced renal disorders in the dose-dependent manner. As regards BUN level (Fig. 3A), a significant increase was observed in p DOX treated group (48.00 ± 3.24 mg/dL) as compared to control group without intervention (17.50 ± 1.73 mg/dL). Pre-treatment with peel extracts significantly minimize the effect of doxorubicin administration on blood urea level in different treatment groups. Preventive effect of peel extracts against doxorubicin induced elevated BUN level was pronounced at medium and higher doses. Maximum preventive effect (31.00 ± 2.14 mg/dL) was exhibited by mango peel extract high dose (300 mg/kg) while minimum preventive effect (42.33 ± 2.42 mg/dL) was observed in mango peel extract low dose (75 mg/kg) and was non-significant to DOX group. Thus the results showed that peel extracts especially at high dose levels were effective against DOX-induced BUN elevated levels.

Data regarding creatinine level revealed significant elevation in DOX group (1.328 ± 0.038 mg/dL) than normal control group (0.715 ± 0.035 mg/dL) (Fig. 3B). Preventive effect of peel extracts against doxorubicin induced elevated ctreatinine level was maximum (0.982 ± 0.027 mg/dL) at mango peel extract high dose (300 mg/kg) while low dose levels (75 mg/kg) peel extracts though prevented the creatinine elevation but the prevention was non-significant with the least preventive effect (1.220 ± 0.025 mg/dL) for low dose mango peel extract.

In case of serum uric acid (Fig. 3C), a significant increase was recorded in DOX group (8.75 ± 0.045 mg/dL) as compared to control group without intervention (3.59 ± 0.036 mg/dL). Pre-treatment with peel extracts significantly minimize the effect of doxorubicin administration on creatinine level in different treatment groups. Preventive effect of peel extracts against doxorubicin induced elevated creatinine level was pronounced at medium and higher doses. Low dose mango and kinnow peel extracts were non-significant to each other but were significantly different from other peel extracts. Maximum preventive effect (6.47 ± 0.076 mg/dL) was exhibited by mango peel extract high dose (300 mg/kg) while minimum preventive effect (8.16±0.045 mg/dL) was observed in kinnow peel extract low dose (75 mg/kg).

3.4 Effect of Mango and Kinnow Peel Extracts on Antioxidant Enzymes in Heart Tissues

During the study, DOX administration to albino rats significantly decreased the myocardial antioxidants superoxide SOD, CAT and GPx. Pre-treatment with peel extracts significantly minimize the effect of DOX administration on cardiac antioxidant level in different treatment groups (Fig. 4). As regards

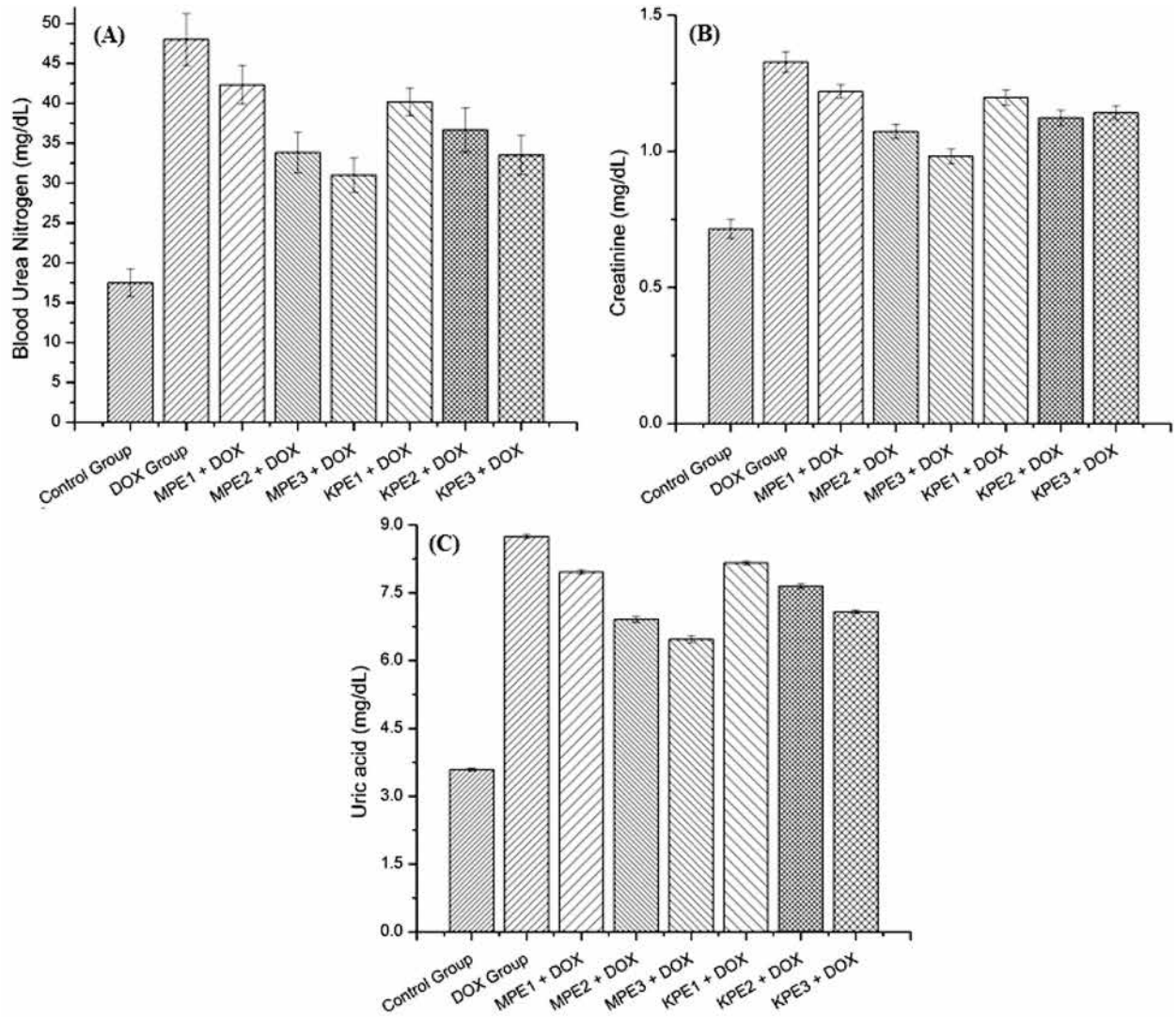

Fig. 3. Effect of mango and kinnow peel extracts on serum renal function parameters in doxorubicin-induced cardiotoxicity in rats. DOX: Doxorubicin,; MPE1: Mango peel extract (75 mg/kg body weight); MPE2: Mango peel extract (150 mg/kg body weight); MPE3: Mango peel extract (300 mg/kg body weight); KPE1: Kinnow peel extract (75 mg/kg body weight); KPE2: Kinnow peel extract (150 mg/kg body weight); KPE3: Kinnow peel extract (300 mg/kg body weight).

SOD activity (Fig. 4A), a significant decrease was observed in DOX group (3.13 ± 0.47units/mg protein) as compared to normal control group (6.95 ± 0.61 units/mg protein). The decreased activity of SOD may be due to accumulation of superoxide anions in the myocardial tissues which are detrimental for myocardium. Pre-treatment with peel extracts followed by DOX administration significantly minimize the effect of doxorubicin induced cardiotoxicity on cardiac SOD level in different treatment groups. Maximum preventive effect (5.15 ± 0.66 units/mg protein) was exhibited by mango peel extract high dose (300 mg/kg) whereas minimum preventive effect (3.40 ± 0.35 units/mg protein) was observed in kinnow mandarin peel extract low dose (75 mg/kg) and was non-significantly different to DOX group as well as mango peel low dose.

Endogenous enzyme catalase converts hydrogen peroxide (H_2O_2) into water and oxygen. Data regarding catalase activity (Fig. 4B) revealed significant decline in DOX group (19.78 ± 1.37 units/mg protein) than normal group (33.42 ± 0.84 units/mg protein). The decline in catalase

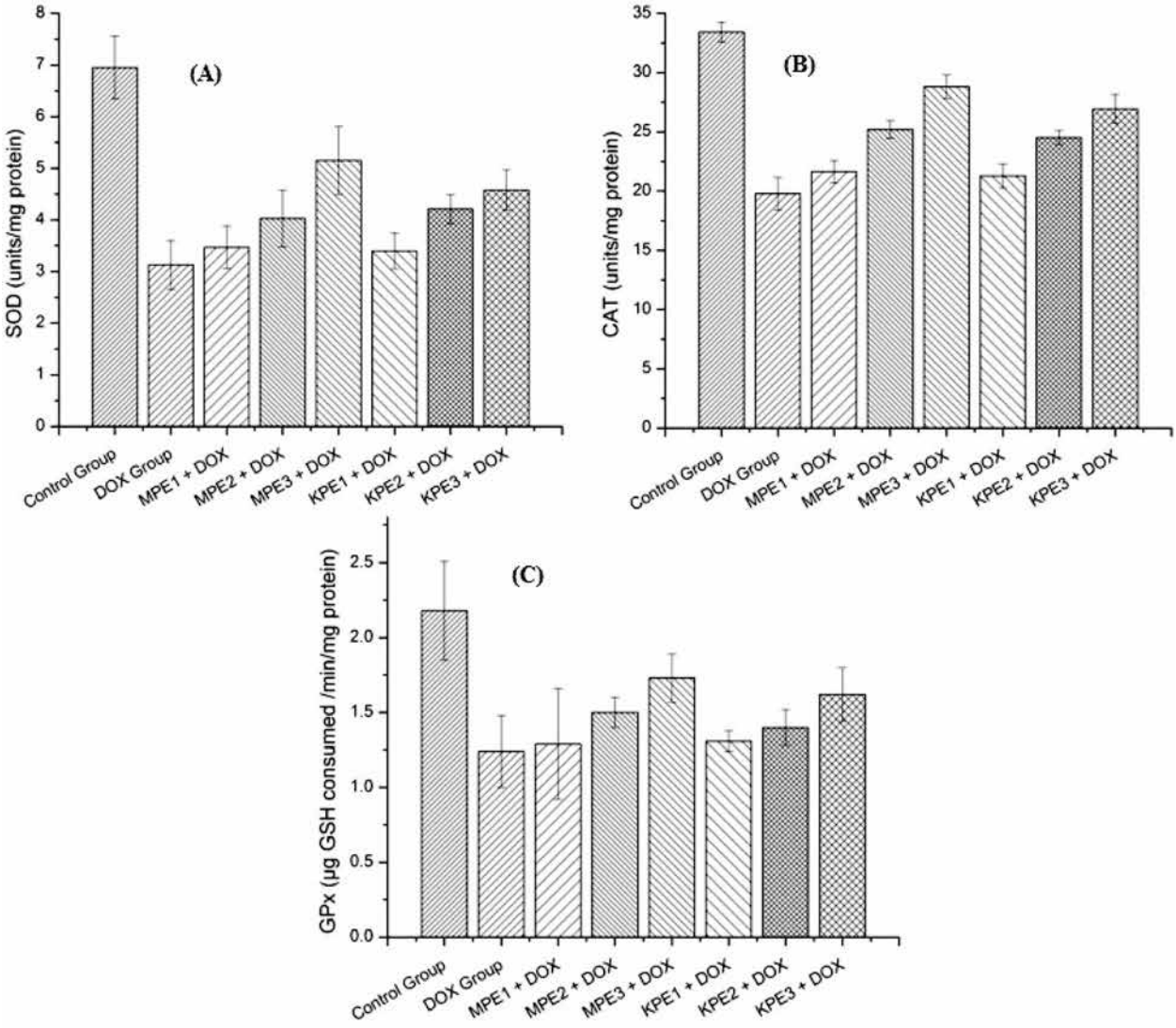

Fig. 4. Effect of different doses of mango and kinnow peel extracts on cardiac tissues antioxidant enzymes in doxorubicin-induced cardiotoxicity in rats. SOD: Superoxide Dismutase; CAT: Catalase; GPx: Glutathione peroxidase; DOX: Doxorubicin; MPE1: Mango peel extract (75 mg/kg body weight); MPE2: Mango peel extract (150 mg/kg body weight); MPE3: Mango peel extract (300 mg/kg body weight); KPE1: Kinnow peel extract (75 mg/kg body weight); KPE2: Kinnow peel extract (150 mg/kg body weight); KPE3: Kinnow peel extract (300 mg/kg body weight).

activity might be due to inactivation of superoxide dismutase by excessive superoxide anions leading to further inactivation of hydrogen peroxide scavenging catalase enzyme. Preventive effect of peel extracts against DOX induced decline in catalase level was maximum (28.82 ± 1.00 units/mg protein) at 300 mg/kg dose level of mango peel extract whereas kinnow peel extract low dose, i.e.,75 mg/kg exhibited lowest preventive effect (21.27 ± 1.01 units/mg protein).

Glutathione peroxidase reduces hydrogen peroxide, peroxides as well as peroxynitrite and thus plays a significant role in neutralizing the oxidative stress. DOX administration to albino rats significantly decreased the GPx activity in DOX treated group (1.24 ± 0.24µg GSH consumed /min/mg protein) as compared to normal group (2.18 ± 0.33 µg GSH consumed /min/mg protein) (Fig. 4C). Pre-treatment of albino rats with different doses of mango and kinnow peel extracts especially medium and high dose levels exhibited elevation in the GPx activity. Highest preventive effect (1.73 ± 0.16 µg GSH consumed /min/mg protein) was observed in groups pretreated with 300 mg/kg dose of mango

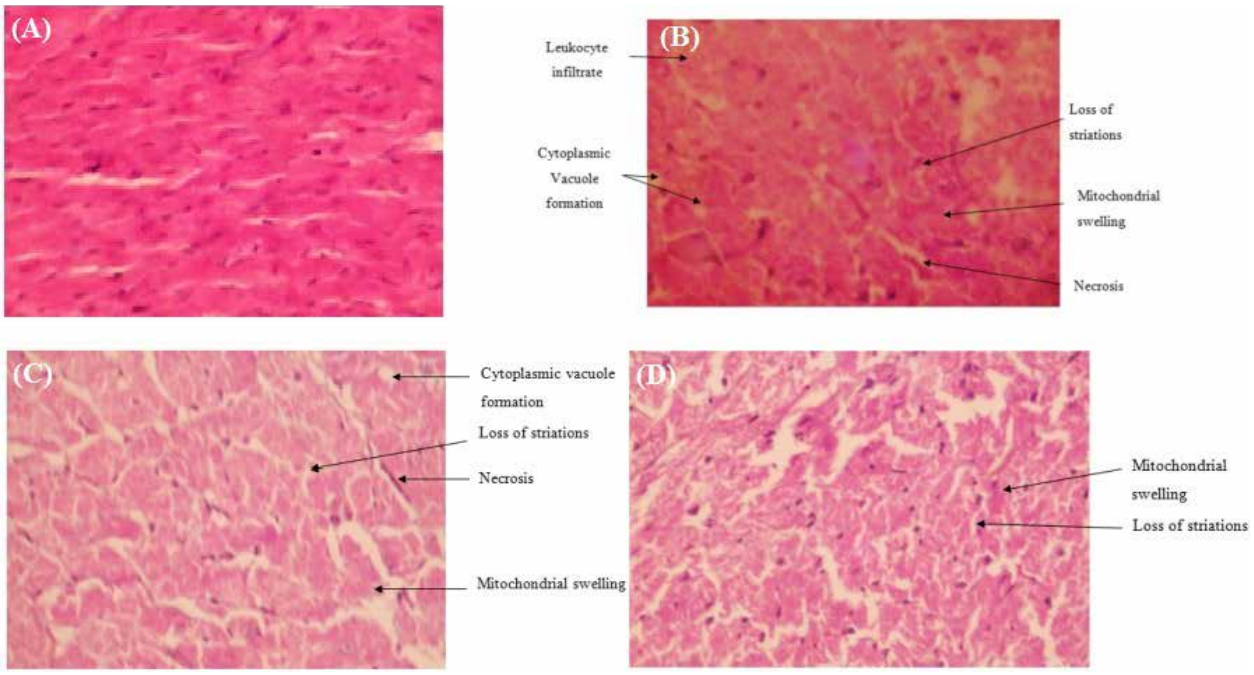

Fig. 5. (A) Photomicrograph of normal control without intervention rat heart tissue showing normal myocardium. **(B)** Photomicrograph of DOX group rat heart tissue showing marked degeneration as loss of striation, mitochondrial swelling, leukocyte infiltrate, cytoplasmic vacuole formation, necrosis and edema. **(C)** Photomicrograph of rat heart tissue pretreated with mango peel extract (75 mg/kg B.W.) + DOX treated rat heart tissue showing moderate to severe changes as mitochondrial swelling, loss of striations, cytoplasmic vacuole formation and necrosis. **(D)** Photomicrograph of rat heart tissue pretreated with mango peel extract (150 mg/kg B.W.) + DOX treated rat heart tissue showing moderate changes as mitochondrial swelling and loss of striations.

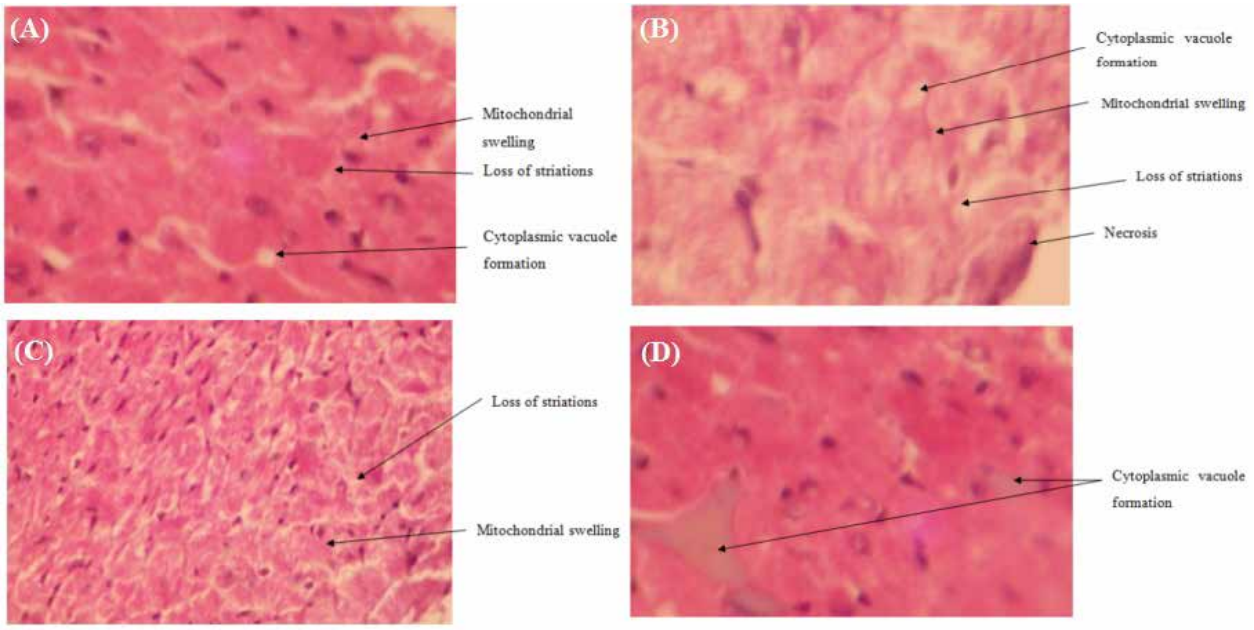

Fig. 6. (A) Photomicrograph of rat heart tissue pretreated with mango peel extract (300 mg/kg B.W.) + DOX treated rat heart tissue showing slight change as cytoplasmic vacuole formation. **(B)** Photomicrograph of rat heart tissue pretreated with kinnow peel extract (75 mg/kg B.W.) + DOX treated rat heart tissue showing severe changes as cytoplasmic vacuole formation, mitochondrial swelling, loss of striation, onset of necrosis and edema. **(C)** Photomicrograph of rat heart tissue pretreated with kinnow peel extract (150 mg/kg B.W.) + DOX treated rat heart tissue showing moderate changes as mitochondrial swelling loss of striations. **(D)** Photomicrograph of rat heart tissue pretreated with kinnow peel extract (300 mg/kg B.W.) + DOX treated rat heart tissue showing slight change as cytoplasmic vacuole formation.

peel extract which was non-significant to rats group pre-treated with 300 mg/kg dose of kinnow peel extract (1.62 ± 0.18 µg GSH consumed /min/mg protein but was significantly different from DOX group.

3.5 Effect of Mango and Kinnow Peel Extracts on Cardiac Histopathology

Histopathological evaluation of cardiac tissue on negative control or normal control group showed a normal mycordium with well-preserved cytoplasm, myocardial fibers of uniform configurations, no inflammatory cell infiltrates and regular morphology of myocardial cell membrane (Fig. 5A). Marked degeneration and cardiomyopathy occurred in myocardium of positive control or DOX administered group as evident by mitochondrial swelling, cytoplasmic vacuole formation, loss of striation, leukocyte infiltration and edema (Fig. 5B). Pretreatment with mango peel extract 75 mg/kg + DOX group showed moderate to severe changes in myocardium such as swelling of mitochondria, cytoplasmic vacuole formation, loss of striations and necrosis (Fig. 5C). Rats group pretreated with 150 mg/kg mango peel polyphenolic extract for 60 days + DOX administration indicated moderate changes in myocardium like mitochondrial swelling, loss of striation (Fig. 5D). However, rats group pretreated with 300 mg/kg mango peel phenolic extract for 60 days + DOX resulted in least changes in myocardium such as cytoplasmic vacuole formation and inhibited DOX induced cardiac damage (Fig. 6A). As regards cardiac histopathology of albino rats pretreated with kinnow mandarin peel extract, 75 mg/kg kinnow peel extract dose for 60 days + DOX was ineffective against doxorubicin induced cardiotoxicity and exhibited severe changes in myocardium similar to DOX group like cytoplasmic vacuole formation, mitochondrial swelling, loss of striation, edema and necrosis (Fig. 6B). Pretreatment with 150 mg/kg kinnow peel extract + DOX resulted in moderate changes to myocardium of rats such as loss of striation and mitochondrial swelling (Fig. 6C). Albino rats group pretreated with 300 mg/kg kinnow mandarin phenolic extract for 60 days + DOX showed slight changes in myocardium like cytoplasmic vacuole formation (Fig. 6D).

4. DISCUSSION

During the current study, the cardioprotective activities of mango and kinnow peel polyphenolic extracts were evaluated. Serum cardiac markers such as LDH, CK-MB, CPK and AST are the significant indicators of deviation in normal cardiac activity. Lactate dehydrogenase (LDH) is the enzyme expressed widely in body tissues especially in cardiac muscles that catalyzes the interconversion of pyruvate and lactate. Since LDH is released during tissue damage, it is considered as biomarker of cardiac injury. Another enzyme creatine phosphokinase is significant for muscle functions, generally present in cardiac muscles which are released into bloodstream after cardiac injury. CK-MB is another enzyme specifically found in heart muscles and considered as biomarker of myocardial infarction. Aspartate transminase (AST) catalyzes the amino groups intermolecular transfer and thus considered as a vital enzyme in metabolic reactions. Significantly higher concentrations of AST are present in heart and liver tissues. Upon injury to heart or liver, these enzymes are released into blood. Elevated concentration of AST is an important diagnostic test for myocardial infarction. During the study, DOX group exhibited significant elevation in the levels of cardiac marker enzymes which indicated severe myocardial damage. Low dose of peel extracts had non-significant effect on serum cardiac markers. Pre-treatment with medium (150 mg/kg BW) and high dose (300 mg/kg BW) of polyphenolic peel extracts exhibited cardioprotective activity as evident by restricted cardiac enzyme concentration in serum. Maximum cardioprotective activity was exhibited by groups pre-treated with high dose (300 mg/kg) of peel extracts especially mango peel extracts that maintained the membrane integrity of myocardial tissues. The cardioprotective potential of mango and kinnow mandarin peel extracts might be attributed to the presence of antioxidant phenolic compounds with free radical scavenging activity. Prabhu et al. [37] investigated the cardioprotective effect of mango xanthone mangiferin in rats and concluded that mangiferin protected the experimental myocardial infarction due to its antioxidant, free radical scavenging, immunomodulatory, antilipidperoxidative and

cardiotonic characteristics. Results were comparable with the investigations of Abdel-Raheem and Abdel-Ghany [14] who reported that hesperidin, a citrus bioflavonoid protected cardiac tissues against cardiotoxic effects of doxorubicin owing to its free radical scavenging activities.

Lipids play a significant role in the pathogenesis of cardiovascular disease, not only by inducing hyperlipidemia and subsequent development of atherosclerosis but also by modifying the cellular membrane structure, composition and stability. A high concentration of lipids in blood accelerates atherosclerosis and is considered as major risk factor in myocardial infarction [38]. Doxorubicin administration to albino rats significantly elevated the lipids level in blood and induced hyperlipidemia. Pre-treatment with different doses of peel extracts reduced the DOX-induced hyperlipidemia in the dose-dependent manner. Experimental hyperglyceridemia in doxorubicin administered rats might be due to decline in the lipoprotein lipase activity in the myocardium leading to lesser uptake of triglycerides from the blood circulation [39]. Low density lipoprotein (LDL) is a kind of lipoprotein that carries triglycerides and cholesterol from the liver to the exterior tissues and facilitates the movement of cholesterol and fat within the blood stream as well as modulates cholesterol synthesis. They are often considered bad cholesterol due to the fact that their elevated levels may pose serious cardiovascular disorders. High density lipoprotein (HDL) is a type of lipoproteins that remove lipids such as cholesterol, triglycerides and phospholipids from the cells, from atheroma within arteries and transport it back to liver for re-utilization or excretion and thus considered as good cholesterol. The HDL- cholesterol protective role may be ascribed to its antioxidant, antithrombotic characteristics as well as its role in reverse cholesterol transport [40]. The increase in the level of total cholesterol, triglycerides, LDLs and decrease in HDLs in the doxorubicin group revealed that doxorubicin interfered with the biosynthesis of lipids. Pre-treatment of mango and kinnow peel extracts at medium (150 mg/kg B.W.) and high dose (300 mg/kg) levels indicated a decline in serum lipid profile with concurrently elevation in HDLs. Therefore, peel extracts especially mango peel polyphenolic extracts showed a strong lipid lowering chemotherapeutic agent. Lipid lowering activity of peel extracts might be due to restriction of cholesterol biosynthesis, enhanced fecal bile acid secretion, catabolism of LDL cholesterol and enhanced uptake of LDL from blood by the liver [41]. Flavonoids may assist in uptake of oxidatively modified LDL by scavenging mechanism [42]. The findings of current study exhibiting doxorubicin induced hyperlipidemia and were in line with the earlier investigations [38, 43].

The BUN is a measure of nitrogen concentration in the blood that originates from urea and is considered as renal function marker, though not as significant marker as creatinine due to the fact that external factors such as dehydration and diet influenced blood urea levels. Serum creatinine is a byproduct of muscle metabolism, removed from the blood by the kidneys and since it is excreted unaltered by the kidneys, it is considered as the vital renal health indicator. Creatinine blood level elevates in case of malfunctioning of kidneys or deficient filtration in the kidneys. Uric acid is the breakdown product of purine nucleotides, elevated concentrations in the blood may lead to gout, kidney stones, hypertension, obesity and other medical disorders. Due to low excretion by the kidneys, serum uric acid concentration may be elevated and high concentration of uric acid in blood above normal is known as hyperuricemia which is also considered as risk factor for cardiovascular disorders [44, 45]. During the study, elevated levels of BUN, creatinine and uric acid in DOX or positive control group indicated that DOX interfered with the functioning of renal organs. Similar nephrotoxicity induced by DOX administration in albino rats was earlier reported by Shafik et al. [46]. Pre-treatment of mango and kinnow peel extracts especially at high dose (300 mg/kg) levels exhibited a reduction in serum renal parameters and ameliorated the nephrotoxicity which might be due to extracts phenolic compounds with free radical scavenging activity.

In biological systems free radicals are generated frequently but nature has provided within cells the reducing mechanism that neutralizes free radicals. Oxidative stress is the condition induced due to change in normal redox state which is ameliorated by

the endogenous enzymes like superoxide dismutase, glutathione peroxidase and catalase. Superoxide dismutase converts reactive oxygen species into hydrogen peroxide (H_2O_2) which is then converted into H_2O by catalase or glutathione peroxidase. During Ischemia, the endogenous antioxidant system is destroyed and hydrogen peroxide is converted into hydroxyl radical [41]. During the current study, endogenous antioxidant enzymes SOD, CAT and GPx activities were significantly lower in doxorubicin administered group which might be attributed to reduce availability of their substrates. These enzymes play a significant role in alleviating the ROS induced mycordial injury and constitute the first line of defense against oxidative injury. Generation of highly reactive free radicals restricted the antioxidant enzymes activities [46]. Restriction in antioxidant enzymes activities may result in elevated production of free radicals and hydrogen peroxide which ultimately forms hydroxyl radical(OH•) leading to number of detrimental reactions [41]. Pre-treatment with mango and kinnow peel extracts especially at 300 mg/kg dose level inhibited the doxorubicin-induced cardiotoxicity which might be due to peel extracts polyphenols with antioxidant activity. Low dose of peel extracts had non-significant effect on cardiac antioxidant enzymes. Mango peel extracts exhibited relatively higher cardioprotective activity due to prevention of antioxidant enzymes activity depletion in the rat myocardium. The elevation in heart tissues antioxidant enzymes might be due to cellular adaptive mechanism that leads to more synthesis of these antioxidant enzymes as well as ascribed to free radical scavenging activity of phenolic compounds present in the extracts [47]. Results were in line with the earlier investigations of Abdel-Raheem and Abdel-Ghany [14] who reported that polyphenol flavonoid hesperidin enhanced the superoxide dismutase activity and glutathione levels in cardiac tissues and attenuated the doxorubicin induced cardiotoxicity. Similarly, Bhupati et al. [25] reported *Vitis vinifera* (black grapes) significantly elevated the cardiac antioxidant enzymes SOD, CAT and GSH in doxorubicin-induced oxidative stress in rats due to free radical scavenging activity of polyphenols and flavonoids present in the extracts.

Histopathology studies of different groups cardiac tissues exhibited that peel extract dose levels 150 mg/kg and 300 mg/kg provided cardio-protection to albino rats myocardium against DOX induced cardiotoxicity. However, pretreatment with peel extracts 300 mg/kg BW for 60 days exhibited maximum cardio-protection as evident by mild histopathological changes as compared to DOX group cardiac histopathology. Mango peel extract exhibited comparatively higher cardioprotective activity than kinnow mandarin peel extract. The higher cardioprotective activity of mango peel extracts revealed by histopathological investigations might be due to free radical scavenging and protective activity of phenolic compound mangiferin present in mango peel extracts. The histopathological changes observed in the myocardium of doxorubicin administered rat group were similar to those earlier reported [48] Results were in agreement with the findings of Prabhu et al. [37] that mangiferin, a xanthone polyphenol pretreatment with 10mg/100g BW for 28 days revealed maximum protection evident by least histopathological changes as compared to isoproterenol induced myocardial infarction in rats. Similarly, Abdel-Raheem and Abdel-Ghany [14] observed that pretreatment with hesperidin flavonoid 200 mg/kg to albino rats protected the myocardium against DOX induced cardiotoxicity revealed by normal myocardium with no inflammatory cells infiltration alleviated the edema and blood vessels congestion. Likewise, phenolic compound gallic acid exhibited the cardioprotective properties by alleviating the myocardial damage to myocardial tissues induced by DOX [49].

5. CONCLUSIONS

Results of the current study indicated the cardioprotective effect of mango and kinnow peel polyphenolic extracts. Pretreatment of albino rats with mango and kinnow peel polyphenolic extracts significantly reduced the DOX-induced damage, i.e., elevation in cardiac enzymes, lipids, renal function parameters and decrease in myocardial enzymes as well as ameliorated the myocardial injury. Mango peel extracts exhibited comparatively more cardioprotective activity than kinnow peel extracts.

6. ACKNOWLEDGEMENTS

The research work was supported by the Research for Agricultural Development Program (RADP) of Pakistan Agricultural Research Council, Islamabad, Pakistan.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 237–257 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Influence of Housing Quality on Public Health in Lahore, Pakistan

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Abstract: This investigation examined the relationships between housing quality indicators and incidence of health issues among household members in district Lahore, the second largest city of Pakistan after Karachi. The primary data for this study were collected through a population-based survey by using a self-administered questionnaire. Face to face interviews with household members were conducted to acquire the required information about 600 randomly selected houses. Housing quality was assessed by employing physical, social, biological and chemical indicators for housing conditions. Health issues were assessed from the reporting about specific, general and psychological health problems. Pearson's chi square test was performed to determine relationships between housing quality indicators and health issues. Spatial interpolation techniques were applied using ArcMap 10.3 to highlight spatial variation of the reported housing quality indicators and health issues in Lahore district. Results of this study indicated that the residents are suffering from multiple diseases which are caused due to poor housing conditions. More than two-third of the studied households suffered with more than five problems relating to housing conditions. Thus, results of this investigation revealed cause-effect relationships between housing conditions and health issues. The health of the community can be improved with better housing conditions. Therefore, urgent initiative and actions by the concerned authorities are warranted to for improvement of housing quality to minimize the health issues.

Keywords: Housing quality, health issues, correlation, GIS, spatial analysis

1. INTRODUCTION

Pakistan is one of the most rapidly urbanizing countries of South Asia. More than 50% of Pakistan total urban population lives in 8 major cities [1]. According to Pakistan Demographic Profile [2], rate of urbanization in Pakistan is 2.68% during 2010 to 2014. This growing urbanization has a remarkable effect on housing and health issues in Pakistan. Good housing quality is essential for healthy life. Generally, the housing is acknowledged as one of the most vital necessities of healthy human life [3]. Good quality of housing affects occurrence of good health and poor housing is an indicator of poor health of people [4]. Housing is not just a place of shelter but it is more than that embracing all of social facilities and functions that are responsible to a worthy living [5]. Housing enhances the whole well-being and desires of the residents [6].

Housing quality in general denotes to the levels or grades of adequacy of residence unit and it is related to instant inhabited atmosphere, comprising the design and infrastructure of housing materials used in building, the quantity of interior and exterior space affecting the dwelling housing services and provision of services [7]. Standards of housing quality are frequently cast-off as measures or norms which are appropriate in authorized circumstances in which some questions appear as to the tolerability of structure, comparative to governing laws within the industry of house building. Thus, the definition of housing quality holds many features which comprise the physical state of the building and additional facilities that mark living in a specific extent. The features of housing contained by any neighborhood should be such that gratifies least health values and good living [8]. Housing quality is a more multifaceted perception with wider social and economic meaning. The situation accounts for both quantitative and qualitative magnitudes of housing units, their direct surrounds and requirements of the residents. The measureable dimension of housing

Received, January 2017; Accepted, August 2017
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quality denotes mainly detached structural, social and economic elements of housing outcomes which may be measured resulted from the presentation of the housing segment. These factors comprise deliberations such as value, magnitude, occupancy, economic influences, environmental impressions and structural customs of housing standards. Additionally, the qualitative measurement is considerably more general and tough to measure. It signifies the apparent significances and standards of issues, for example, comfort or quality of life that come up with the different types of dwelling, lifestyles, and the preferences and expectations of the inhabitants. Due to the high local and provincial differences in the quantitative and qualitative magnitudes of housing conditions it is not conceivable to describe one consistent set of standards and gauges that relate correspondingly to all zones at all periods [9, 10].

Fertig and Reingold [11] also investigated the relationship of housing with health. They proved through study that low level of housing and living in an unhealthy environment is a dangerous factor effecting public health. There are a number of other studies who assessed straight relationship between health issues and poor housing quality relating to housing condition, infrastructure, overcrowding and services including inadequate water supply, sanitation, electricity infrastructure and house waste management [11-20]. Gilbertson et al. [21] detected a noteworthy association between conditions of housing and mental and physical health of an individual. Poor-housing quality is health vulnerable to its residents. Mostly, it may cause infectious diseases such as tuberculosis, respiratory diseases, and skin-infections. It is also connected with depression, deficiency of vitamin D, anxiety, obesity, diabetes and cardiovascular diseases [22]. Different aspects of a house have different health issues, major of them are ventilation, lighting, disease vector and overcrowding. Proper ventilation is very important for those homes which have indoor cooking setup [23]. Use of wood and coal for burning is the variable of high air pollution. Houses with poor ventilation are a major cause of respiratory diseases, specifically, tuberculosis, bronchitis and asthma [24]. Satisfactory access of sun light is also essential for good houses capable to be live. Hepatitis A, visual problems and depression are mostly caused by low level of exposure to sun [25]. So, it is very important for every house to have windows. If the houses are not being kept neat and clean then they are infested by disease vectors like bugs, termites, cockroaches, mosquitoes, and moulds [26]. Density of residents and overcrowding in homes is a cause of ill-health because it makes disease transmission from one person to another very easy [27]. Lack of private space may cause anxiety among a family [28]. Overcrowding is correlated to levels of socioeconomic condition and it is a much elaborated determinant of low quality housing [29]. The increasing rate of urbanization and rapidly growing overall population in developing countries has created tremendous pressures on housing market to meet housing needs. The failure of housing marketplace to construct new and affordable housing has created problems for people to live below poverty level and in overcrowded dwellings [7].

For years, housing-quality has been recognized as a major impact source of resident's health but still to date there are major gaps in research on health-based housing assessment especially in Pakistan. In all developed countries quality of housing assessment surveys are taken on large scales. The major contribution of decent housing to public health has been recognized in laws of England for more than a century [30]. But in Pakistan there are no such initiatives or policies have been made by government, who can define and support healthy living. In Pakistan, it is highly needed to conduct researches on housing quality and related health issues. Assessment of quality of housing is necessary to calculate needs which are very basic to public, socioeconomic levels and planning of public health centers in cities of Pakistan. The Planning Commission of Pakistan estimates that by 2030, at least 50 percent of the Pakistani population will live in its cities. This increasing population burden will greatly influence the availability of quality-houses in major cities of Pakistan.

Pakistan's demographic profile [2] indicates that total population of Lahore is 7.566 million, of which 65% live in only 10 percent of the city area. In Punjab, 22 % of the urban population lives just in Lahore [31]. Urban growth rate of Lahore is 4.3 per

annum [1]. Lahore is one of the eight major cities of Pakistan which are highly influenced by the impact of urbanization. Due to rapid urbanization, mishandling of housing schemes, poverty and insufficient housing policies, people of Lahore are bound to live in unhealthy houses. Urban Unit [32] estimated that 40% of the population in Lahore is inhibited in slums with poor municipal and housing services. Population of the city is increasing tremendously. Migratory trends explain an increase in density towards some specific areas of Lahore [1]. Lahore is one of those modern cities of Pakistan which are experiencing outbreaks of many infectious diseases. Public health condition is very poor in Lahore and it varies from localities of poor quality-housing to good quality-housing. According to a disease pattern compiled from Punjab health departments of District Health Information System (DHIS), Secondary Health Care (SHC) and Primary Health Care (PHC) reports on the basis of regular mechanism studies and surveys, 2,971,178 cases of respiratory diseases, 100,204 cases of gastrointestinal disease, 31,163 cases of communicable diseases, 20,093 cases of cardiovascular diseases, 9,018 cases of skin diseases, 1,849 cases of psychiatric diseases, 14,315 cases of eye diseases and 2,615 cases of injuries have been reported in Punjab Province [33].

Population explosion and urbanization have a very bad impact on parameters of good-housing. People are compelled to live in houses having insufficient quality parameters in urban areas. Therefore, there is a great need of effective housing policies to sort out these problems and to fill the gaps which are created by shortage of housing supply. Particularly, access to housing of reasonably minimum quality must be promoted for residents living below the poverty line (Meng and Hall, 2006). With this rapid rate of increase in urbanization, population growth, decline in housing quality and vulnerability to public health there is a need to spatially assess housing quality and rate of its impact on health. Therefore, this study focuses the health-based housing quality by showing relationship of various poor housing parameters/indicators and health issues. Spatial maps are significant tools to assess spatial distribution of any data. They provide an overall view of variation in selected parameters which can be useful in planning, management and designing necessary policies. Geographical Information System (GIS) techniques were used to map variation into occurrence of disease and to analyze the spatial patterns of housing quality in the city. These techniques can be linked to each other to show the spatial pattern of housing-quality and related health issues in map view. The present study has two variables, housing quality (independent variable) and health issues (dependent variable). Results indicate that he poor housing conditions are causing ill health among the residents. However, the general public health can be improved with improved housing conditions. The study would be helpful to give awareness and set policies for healthy housing at public level.

2. MATERIALS AND METHODS

2.1 Study Area

Lahore is a metropolitan city of Pakistan and is famous for its rich historical background. Population wise, it is the 2nd largest city of Pakistan, 5th largest city in South Asia, and 30th largest city in the world [33]. Lahore is capital of the most populous province in Pakistan, the Punjab, and is a hub of educational, cultural and economic activities. This city is Lahore is situated on the left bank of River Ravi at 31° 15' N - 31° 42' N and 74° 01' E - 74° 39' E. Lahore is divided into nine Towns {i.e., Ravi Town (RT), Shalamar Town (ST), Wahga Town (WT), Aziz Bhatti Town (ABT), Data Gunj Bukhsh Town (DGBT), Gulberg Town (GT), Samanabad Town (ST), Iqbal Town (IT), Nishtar Town (NT), and Cantonment (Lahore Cantt (LC)} and also is an administrative division. Every Town in the district comprises of a cluster of Union Councils (Fig. 1). According to the Three Years Rolling Plan 2010-2013 of District Lahore, total population of Lahore is approximately 8,200,000 and its total area is 2,014 sq. km. Annual growth rate of population is 5.6%, population density is 8,200 persons per sq. km and rural and urban population is 2,076,000 (25.4%) and 6,114,000 (74.6%), respectively [33].

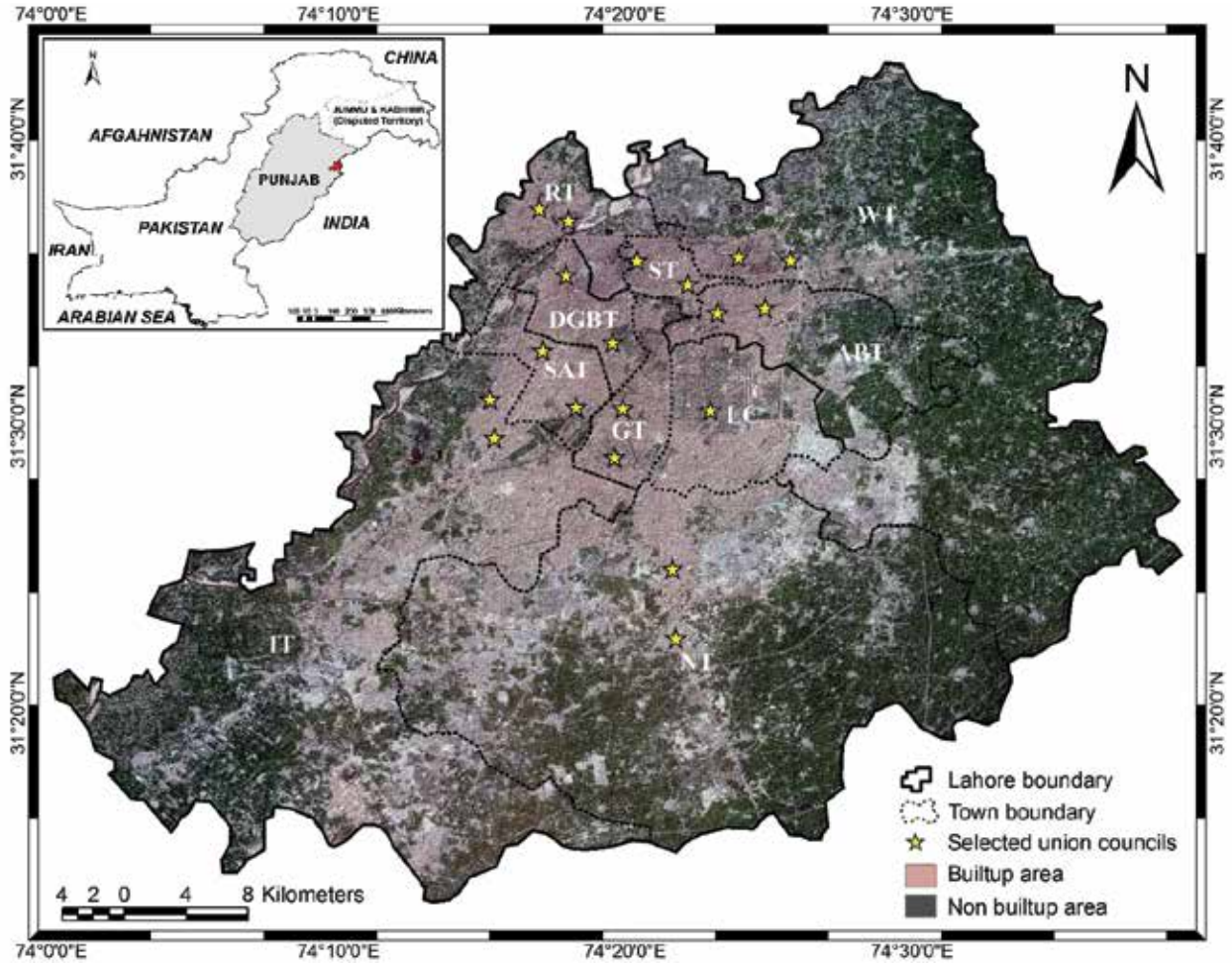

Fig. 1 Location of study area and the surrounding region (as inset). The built-up and non-built-up areas and location of study sites for data collection (yellow stars) are shown. The white coloured letters indicate: RT, Ravi Town; ST, Shalimar Town; WT, Wahga Town; ABT, Aziz Bhatti Town; DGBT, Data Gunj Bukhsh Town; GT, Gulberg Town; ST, Samanabad Town; IT, Iqbal Town; NT, Nishtar Town; and LC, Lahore Cantt.

2.2 Study Design and Data Collection

Face to face interviews and questionnaire responses from the household members were used to acquire the required information of 600 randomly selected houses. One person from each house responded to the questionnaire and the interview. Also, the same person apprised about health status of all family members. Housing quality was gauged using physical, social, biological and chemical indicators of the housing conditions. Health issues were assessed from the feedback about specific, general physical and psychological health issues. Analysis of the data were performed by using SPSS (Statistical Package for Social Sciences), Microsoft Excel and GIS (Geographical Information System). Pearson's correlation and chi square test was performed to analyze the relationship between housing quality and health issues. Spatial interpolation techniques were applied using Arc GIS 10.3 to highlight spatial variation of reported housing quality indicators and health issues in Lahore. Primary data was collected using multistage sampling techniques of normal distribution. 600 questionnaires were equally distributed in 10 administrative Towns. Subsequently equal number of questionnaires (i.e., 30+30=60) were distributed in each Town among two selected Union Councils (Shahdra and Qaisar Town (in RT), Baghbanpura and Shadbagh (in ST), Daroghawala and Salamatpura (in WT), Dharampura and Fatehgarh (in ABT), Bilal Gunj and Anarkali (DGBT), Model Town and Garden Town (in GT), New muslin Town and Samanabad

Table 1. Housing quality indicators, associated factors and related health issues.

Indicator	Associated Factor	Related Health Issues
1. Physical Indicators		
Temperature	Use of cooling products, use of heating products	Heart attack, fever, blood pressure
Noise	Market area, heavy traffic load, loud speaker, generator	Headache, depression, anxiety, lack of sleep, hearing issues, blood pressure
Building features	Open space availability, open space around boundaries, direct exposure to sun, house area, attached/separated kitchen, exhaust fan/chimney in kitchen, attached/separated wash room, open/concealed wiring of electricity supply, water pipes maintenance,, house paint type and conditions, taste of water	Muscular pain, eye sight issues, obesity, asthma, abdominal pain, dysentery, diarrhea, typhoid, lungs, and respiratory diseases, cough, cholera
Sunlight penetration	Open space around boundaries, window presence, house type (detached, separate, semidetached)	Muscular pain, eye sight issues
House accidents	House maintenance roof fall water fall, part of house fall, electrical sparks, gas pipe damage	Fall injury, death due to injury, burning injury, death due to burning injury
Indoor air quality	Windows presence. attached/separate kitchen, exhaust fan, type of stove, inside smoking	Lungs issues, cough, respiratory diseases, asthma, tuberculosis.
Dampness	Roof leakage, water pipes leakages, damp walls, inside laundry	Cough, skin infection, respiratory infections, lungs disease, asthma, tuberculosis.
Ventilation	Window presence, regular opening of windows	Lungs and respiratory disease, asthma, tuberculosis
2. Biological Indicators		
Molds/fungus	Mold presence in any part of house, e.g., wash room, bed room	Skin allergy, cough, respiratory and lung disease, asthma
Pests	Cockroaches, mites, rates, flies	Abdominal pain, skin allergy, lungs diseases cough
Pets	Dogs, cats, birds, cattle	Pet allergy
3. Chemical Indicators		
Insecticides	Frequency usage	Skin infections, heart diseases, lungs cancer
Herbicides	Frequency of usage	Skin infections, heart diseases, lungs cancer
4. Social Indicators		
Neighborhood	Residential area, market area, industrial area, heavy traffic road, contaminated water canal, disposal sites	Depression, anxiety, aggressiveness, malaria, dengue
Overcrowding	Number of persons per room	Aggressiveness, depression, anxiety, headache, blood pressure, tuberculosis

Town (in ST), Awan Town and Sabzazar (in IT), Gajjumatta and Kahna (in NT) and Cantonment area (LC) (Fig. 1).

2.3 Measures of Housing Quality and Health Issues

Based on extensive literature review, the questionnaire was designed by including selected housing quality indicators and factors to investigate their effect on the health of the residents (Table 1). Researcher visited respondents personally to collect data. Respondent's behavior was very good towards answering questionnaire. Some respondents refused to fill up questionnaire; however, many appreciated the exercise by taking interest and filling the questionnaire.

2.4 Data Analysis

Prior to analysis, the data were rechecked and data classification was done for accuracy purposes. The data entry and data tabulation was done using MS Excel and SPSS. Consequently, a database structure was documented which integrates various measures. Afterwards, descriptive, inferential and spatial analyses techniques were applied using SPSS and ArcMap 10.3.

2.4.1 Descriptive Analysis

Descriptive analysis presents a simple summary of whole data in the form of central tendency, mean deviation, frequency distribution and percentages. In present study, descriptive analysis techniques are applied to define elementary features of the data. Descriptive statistics provided unpretentious summaries of sample and measures about study. Three types of techniques were used in descriptive analysis; (a) *Frequencies*, (b) *Proportional Percentages* (c) *Graphical representation* using bar graphs and pie charts.

2.4.2 Inferential Analysis

Inferential analysis is used to determine correlation between housing quality and health issues by using complex designed calculation. Two types of inferential analysis were used in the study.

a) Correlation Analysis

Correlation analysis is a statistical technique which indicate that how strongly two different variables are related to each other. Main results of correlation are defined by value 'correlation coefficient' represented by letter 'r'. The statistical formula for computing correlation coefficient is given below;

$$r = \frac{1}{n-1} \sum \left(\frac{x-\bar{x}}{S_x}\right)\left(\frac{y-\bar{y}}{S_y}\right)$$

Values of correlation coefficient (r) always range between +1 and -1.Value of +1 shows that there exists a positive relationship among variables while value of -1 represents the negative relationship between variables. However, value of 0 denotes that no relationship exists among variables. If there are only two variables to be tested for correlation analysis, then bivariate analysis techniques are used in correlation analysis. Therefore, in present study a bivariate analysis technique of correlation analysis was used to find out the level of relationship between two major variables of the study (housing quality and health issues) by applying means and standard deviation statistics, Pearson's coefficient and two-tailed test of significance.

(b) Pearson's Chi-square Test

Pearson's chi-square test is a probability test to know that how likely there are chances of a match among two observed frequencies. It measures that by what means the distribution of a type of data gets fit into the distribution of another data. A chi-square test is specifically appropriate for categorical/ ordinal/nominal data. Statistical formula for chi-square is given below;

$$X^2 = \sum \frac{(O-E)^2}{E}$$

where,

O = Observed frequency, Σ = Expected frequency and Σ = Sum of all cells

Chi-square test was used to determine relationship among two variables which are organized in a bivariate type of table. The *p*-value of chi-square test is known as a probability estimate. The *p*-value below 0.05 indicates that there is a relationship between two variables whereas *p*-value greater than 0.05 indicate no correlation. In present study, Pearson's chi-square test was applied to find out those observed indicators of housing quality which may have a significant relation with observed health issues by fitting frequencies with each other in cross-tabulation.

2.4.3 Spatial Analysis

Spatial analysis is a type of data analysis which is specifically used to process geographic data. When data are to be related with locations and results are required to show spatial pattern, then, spatial analysis makes it possible by using information of geographic and locational attributes. Geographic Information System (GIS) is best commonly used and user friendly software for spatial analysis. In present study, GIS mapping techniques for interpolation, quantities and charts were used to

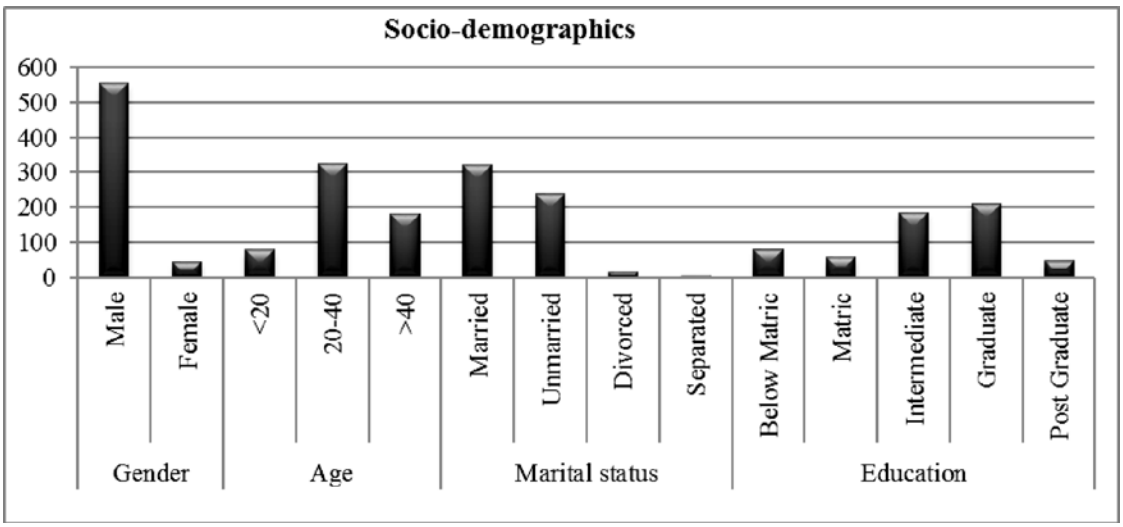

Fig. 2 Socio-demographics of the respondents.

model spatial pattern of housing and health issues in Lahore using ArcMap 10.3 software. For spatial mapping, first of all Union Council wise responses of both poor housing quality (PHQ) indicators and reported health issues (HI) were entered in ArcMap database and associated shape files were generated. Later, these data were used to prepare required maps for spatial analysis from Fig. 4 to Fig. 12.

3. RESULTS AND DISCUSSION

3.1 Socio-demographics

Among 600 residents who responded to questionnaires, 92.5% were male and 7.5% were female. 54.2% of the respondents were among the age group 20-40 years, 13.2% were below 20 years and 30.3% were above 40 years. Married respondents were 55.6%, unmarried 41.1% and a very minute 2.8% were divorced and 0.5% were separated. Generally the respondents were qualified (Fig. 2).

3.2 Residential Information

Residential information was assembled on the basis of information about house type, area type, and family type and planning to move from current residence. Frequency distribution presents that 40.3% residents live in semi-detached houses, almost equal percentage lives in detached and

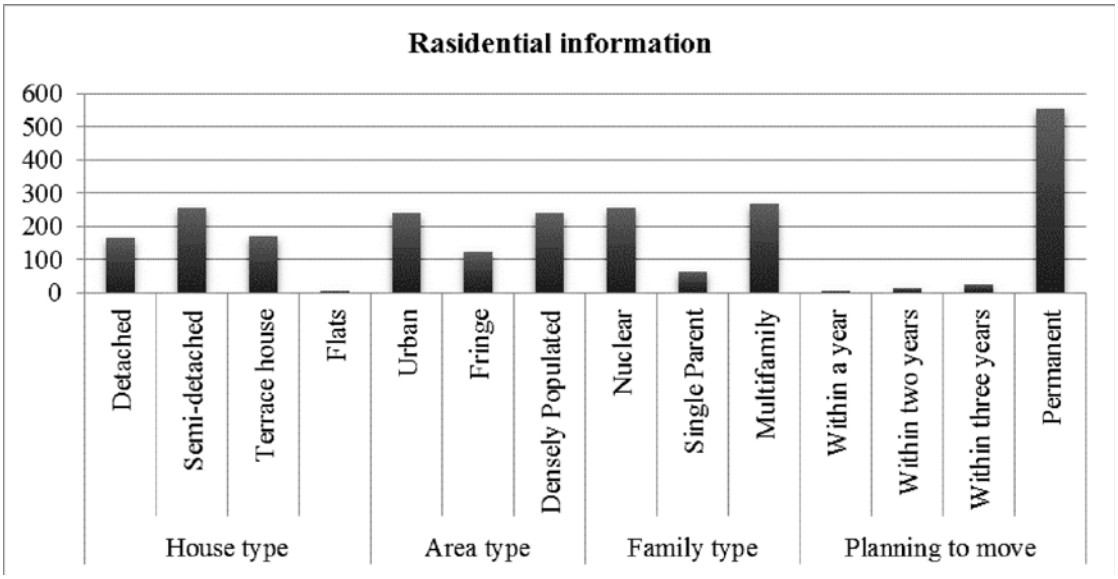

Fig. 3 Residential information.

terrace houses while a small percentage lives in flats. Respondents belonging to fringe area were relatively less in numbers as compared to the respondents of urban area or densely populated area. Majority of the respondents belong to multifamily or nuclear family while belonging to single parent family is very low. The collected data revealed that most of the respondents are permanent residents and only 4.5% residents intended to move from their current residences (Fig. 3).

3.3 Physical Indicators of Poor Housing Quality (PHQ)

Data analysis demonstrated that 27.8% of total respondents do not have open space availability in their houses, 31.8% do not have direct exposure to sun, 63.3% of total respondents do not have open space around the boundaries of house and 18.5% of total respondents have tall building present in both sides of their house boundary. 79.8% of the total respondents have attached kitchen type and 83.5% respondents use attached bathroom. Three types of paints were assessed; oil paints, white wash and distemper. 68.2% respondents have distemper in their houses, although positive response for white wash with 32.0% was also notable, 44.8% complained of not having good paint condition of their houses while 77.8% change paint after two years or more than two years (Table 2).

Table 2. Building aspects of physical indicators.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Open space availability in house	162 (27.8)	438 (72.2)
Direct exposure to sun	191 (31.8)	409 (68.2)
Open space around boundaries	380 (63.3)	220 (36.7)
Tall building around house	489 (81.5)	111 (18.5)
Kitchen type		
Attached	121 (20.1)	479 (79.8)
Separated	478 (79.7)	122 (20.3)
Wash room type		
Attached	99 (16.5)	501 (83.5)
Separate	500 (83.3)	100 (16.7)
Paint type		
Oil paints	566 (94.3)	34 (5.7)
White wash	408 (68)	192 (32)
Distemper	191 (31.8)	409 (68.2)
Good paint condition	269 (44.8)	331 (55.2)
Paint change period		
One year	467 (77.8)	133 (22.2)
Two years or more	133 (22.2)	467 (77.8)
Experienced building collapse	585 (97.5)	21 (2.5)
Apart of house collapsed	589 (98.2)	11 (1.8)
Roof collapsed	597 (99.5)	3 (0.5)
Wall collapsed	593 (98.8)	7 (1.2)
Wall collapsed	593 (98.8)	7 (1.2)
Repairing house damage on immediate basis	163 (27.2)	473 (72.8)

3.3.1 Services Supply

The gas supply through surface-pipe wiring was available in 13.3% houses, of which 2.8% respondents experienced gas pipe damage and in 5 houses gas pipe damage had led to fire incidents. In 11.2% houses electricity is supplied through open wiring, 2% experienced electrical spark/damage and fire due to electrical spark/damage. 74.3% of total respondents are facilitated by district water supply, 78% have own water pump whereas 32.5% experience unpleasant taste in water. 9.8% reported water pipe leakage (Table 3).

Table 3. Services infrastructural aspects of physical indicators.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Gas supply wiring condition		
Under-ground pipes	69 (11.5)	53 (88.5)
Surface-pipes	520 (86.7)	80 (13.3)
Gas pipe damage	583 (97.2)	17 (2.8)
Caused fire	595 (99.2)	5 (0.8)
Electricity wiring condition		
Concealed wiring	65 (10.8)	535 (89.2)
Open wiring	533 (88.8)	67 (11.2)
Electrical spark/damage	588 (98)	12 (2)
Caused fire	598 (99.7)	12 (2.0)
Water supply Condition		
District water supply	154 (25.7)	446 (74.3)
Own water pump	128 (21.3)	472 (78.7)
Unpleasant taste in water	405 (67.5)	195 (32.5)
Water pipes leakage	541 (90.2)	59 (9.8)

3.3.2 Moisture Factors

Frequency distribution of collected data designates that 51.8% of total houses have moisture presence. Analysis signifies that common cause of moisture presence in houses is damp walls with the

Table 4. Moisture factors of physical indicators.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Moisture in house	289 (48.2)	311 (51.8)
Moisture due to roof leakage	540 (90)	60 (10.0)
Moisture due to water pipes leakage	557 (92.8)	43 (7.2)
Moisture due to inside use of laundry	553 (92.2)	47 (7.8)
Moisture due to damp walls	391(65.2)	209 (34.8)

percentage of 34.8%; roof leakage is also notable having 10% of total responses. Contribution of water pipe leakage and inside use of laundry in moisture occurrence is above 7.0% (Table 4).

3.3.3 Indoor Air Quality

Data collected based on indoor air quality indicators show that 77.5% of houses have chimney and Exhaust fan. 98.7% houses use gas stove for cooking. 76.8% houses have windows in every bed room and they open it on regular basis which improve the quality of indoor air. In only 17.7 % of houses people smoke inside house and 21.7% houses has bad smell (Table 5).

Table 5. Indoor air quality factors of physical indicators of housing quality

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Chimney/Exhaust fan presence	135 (22.5)	465 (77.5)
Stove type		
Gas stove	8 (1.3)	592 (98.7)
Wood stove	568 (94.7)	32 (5.3)
Window presence in every bed room	139 (23.2)	461 (76.8)
Window opened regularly	174 (29.0)	426 (71.0)
Inside smoking	494 (82.3)	106 (17.7)
Feel bad smell in house	470 (78.3)	130 (21.7)

3.3.4 Indoor Temperature

About 20% of the respondents reported that their houses are unbearably hot during summer months. Two types of cooling products was mentioned in questionnaire air conditioner and water cooler, 51.5% use air conditioner while 42.7% use water cooler. In winters 17.7% houses are unbearable cold and majority 91.2% use gas heaters as heating product, 4.3% use coal burning (Table 6).

Table 6. Indoor temperature factors of physical indicators of housing quality.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Feel house unbearable hot in summers	479 (79.8)	121 (20.2)
Types of cooling products used		
Air conditioner	291 (48.5)	309 (51.5)
Water cooler	344 (57.3)	256 (42.7)
Feel house unbearable cold in summers	494 (82.3)	106 (17.7)
Types of heating products used		
Gas heater	53 (8.8)	547 (91.2)
Electric heater	566 (94.3)	34 (5.7)
Coal burning	574 (95.7)	26 (4.3)

3.3.5 Indoor Noise

Analysis of physical indicators related to noise proves that more than half (59.3%) feel noise in house, 20.0% respondents complained road traffic passing nearby house as a source of noise, 5.7% declared overcrowding in house is causing noise, 5.0% reported market area near of house, 16.7% reported noise severity on regular basis (Table 7).

Table 7. Indoor noise factors of physical indicators of housing quality.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Feel noise in house	356 (59.3)	244 (40.7)
Noise source		
Road traffic	480 (80)	120 (20)
Overcrowding	566 (94.3)	34 (5.7)
Market area	570 (95.0)	30 (5.0)
Generator	547 (91.2)	53 (8.8)
Loud speaker	543 (90.5)	57 (9.5)
Noise severity		
Regularly	500 (83.3)	100 (16.7)
Occasionally	497 (82.8)	103 (17.2)

3.3.6 Spatial Analysis of Physical Indicators of PHQ

Factors among physical indicators are highly varying in the region. Physical indicators of PHQ were maximum in Ravi Town, Nishtar Town and Aziz Bhatti Town. Gulberg Town, Samanabad Town and Iqbal Town are showing minimum value of poor physical indicators. Moisture presence,

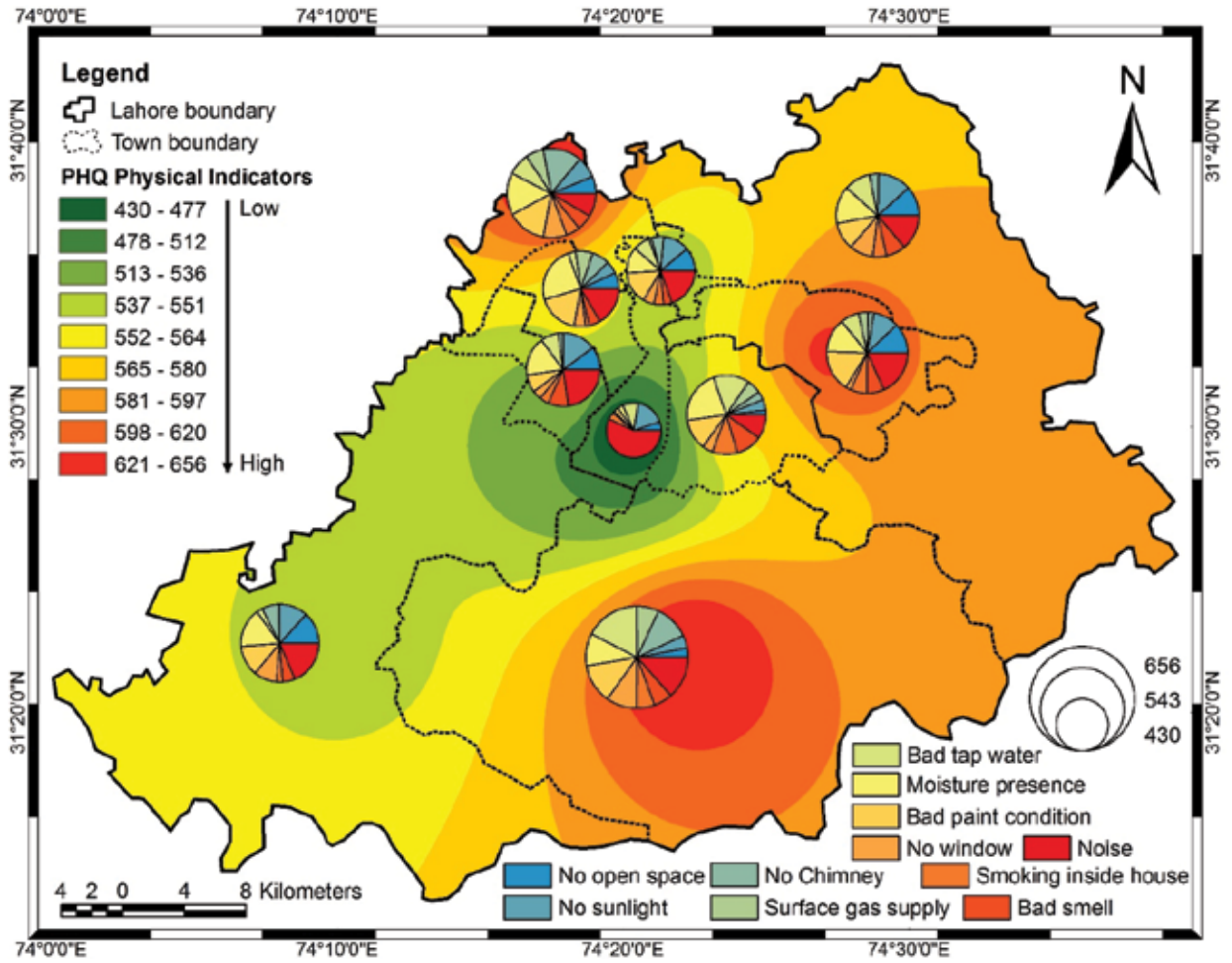

Fig. 4 Spatial distribution of physical indicators in Lahore.

noise and no open space response is higher than the other factors. Furthermore, reported values of moisture presence are highest in Data Gunj Bakhsh, Ravi and Nishtar Town. Highest values of noise are reported in Gulberg Town (Fig. 4).

3.4 Biological Indicators of PHQ

71% of families have fungus/mold presence in their houses, out of which 18.5% claimed to have fungus presence in just their washrooms, 13.5% have rats, 28.8% have cockroaches, 6.2% have mites and 36.7% have flies. 51.5% have pets in their houses of which 23.5% have dogs, 9.5% have cats, 2.8% have cattle and 14.3% have birds (Table 8).

Spatial analysis of biological indicators shows highest number in Ravi Town, Lahore Cantt and Aziz Bhatti Town. The pie chart information indicated that insect pests were in highest proportion and fungus was present in much lower proportion (Fig. 5).

Table 8. Biological indicators of poor housing quality.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Fungus presence in house	174 (29.0)	426 (71.0)
Fungus presence in wash room	489 (81.5)	111 (18.5)
Fungus presence in bed room	531 (88.5)	69 (11.5)
Fungus presence in entire house	584 (97.3)	16 (2.7)
Pests presence in house	432 (72)	168 (28.0)
Rats	519 (86.5)	81 (13.5)
Cockroaches	427 (71.2)	173 (28.8)
Mites	563 (93.8)	37 (6.2)
Flies	380 (63.3)	220 (36.7)
Pets presence	291 (48.5)	309 (51.5)
Dog	459 (76.5)	141 (23.5)
Cat	543 (90.5)	57 (9.5)
Cattle	583 (97.2)	17 (2.8)
Birds	513 (85.5)	87 (14.3)

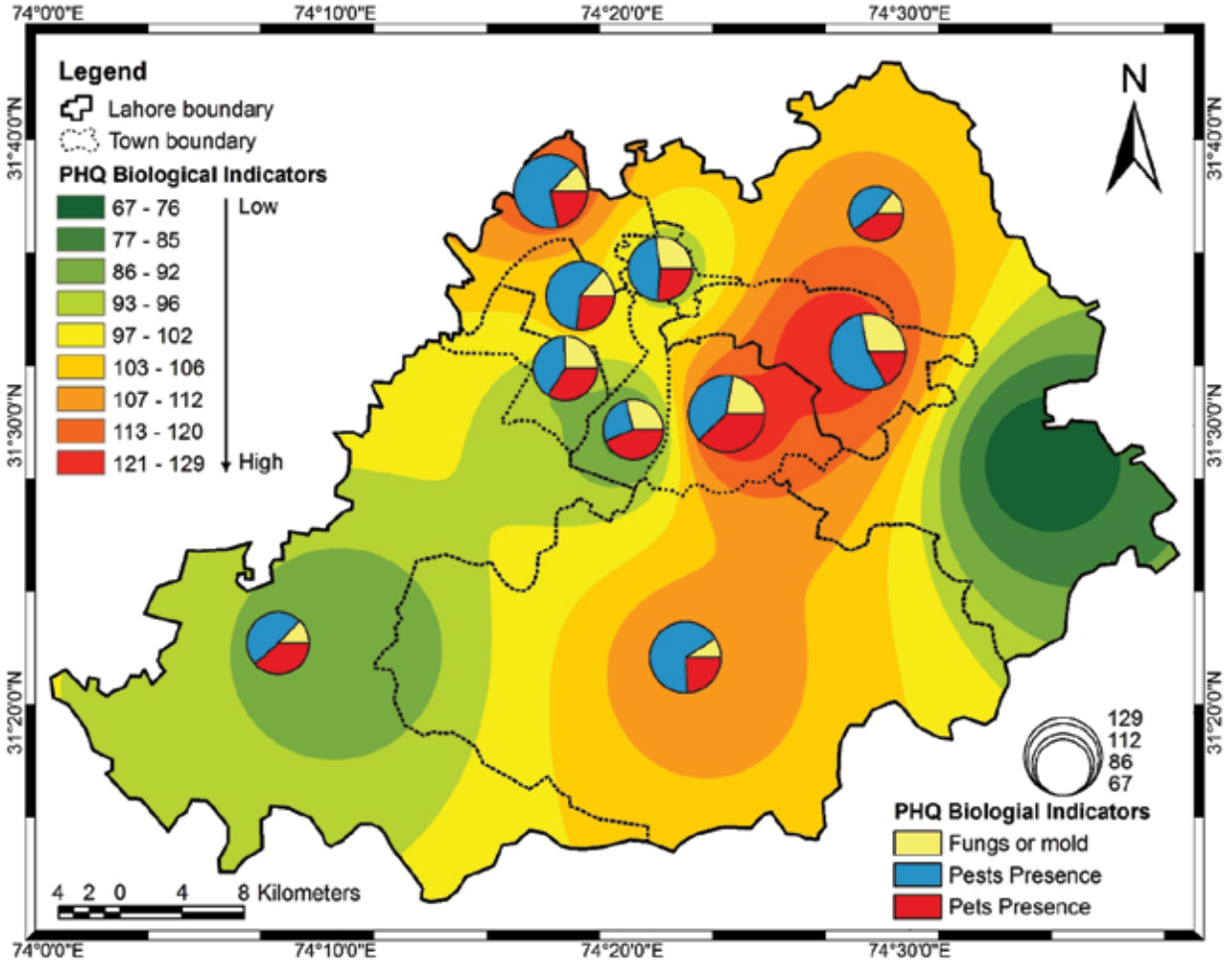

Fig. 5. Spatial distribution of biological indicators in Lahore.

3.5 Social Indicators of PHQ

Data elaborates that 13.7% expressed annoyance to have contaminated water canal in neighborhood of their house and 19.7% complained to have disposal site in near of their house. 16.1% reported overcrowding in houses (Table 9).

Social indicators of poor housing quality also vary in Lahore relative to its Towns. Spatial map illustrates that level of social indicators is highest in Ravi Town as compared to other Towns of Lahore. Gulberg Town and Samanabad Town has lowest level. Factors of market area presence, overcrowding and nearby disposal sites presence are higher (Fig. 6).

3.6 Chemical Indicators of PHQ

Analysis of chemical indicators reveals that 84.7% of total respondents do not use insecticides at their homes. 72.7% of total respondents do not use herbicides but 27.30% use herbicides every year.

Analysis describes that incidences of chemical indicators of housing quality are few in number.

Table 9. Social indicators of housing quality.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Satisfaction of neighborhood safety	42 (7.0)	558 (93.0)
Neighborhood area type		
Residential area	60 (10)	540 (90)
Market area	438 (73)	162 (27)
Industrial area	552 (92)	48 (8)
Heavy traffic road	495 (82.5)	105 (17.5)
Contaminated water canal	518 (86.3)	82 (13.7)
Disposal site	482 (80)	118 (19.7)
House area sufficient for whole family	97 (16.1)	503 (83.8)
One person per room	462 (77)	138 (23)
Two persons per room	235 (39.2)	365 (60.8)
More than two persons per room	515 (85.8)	85 (14.2)

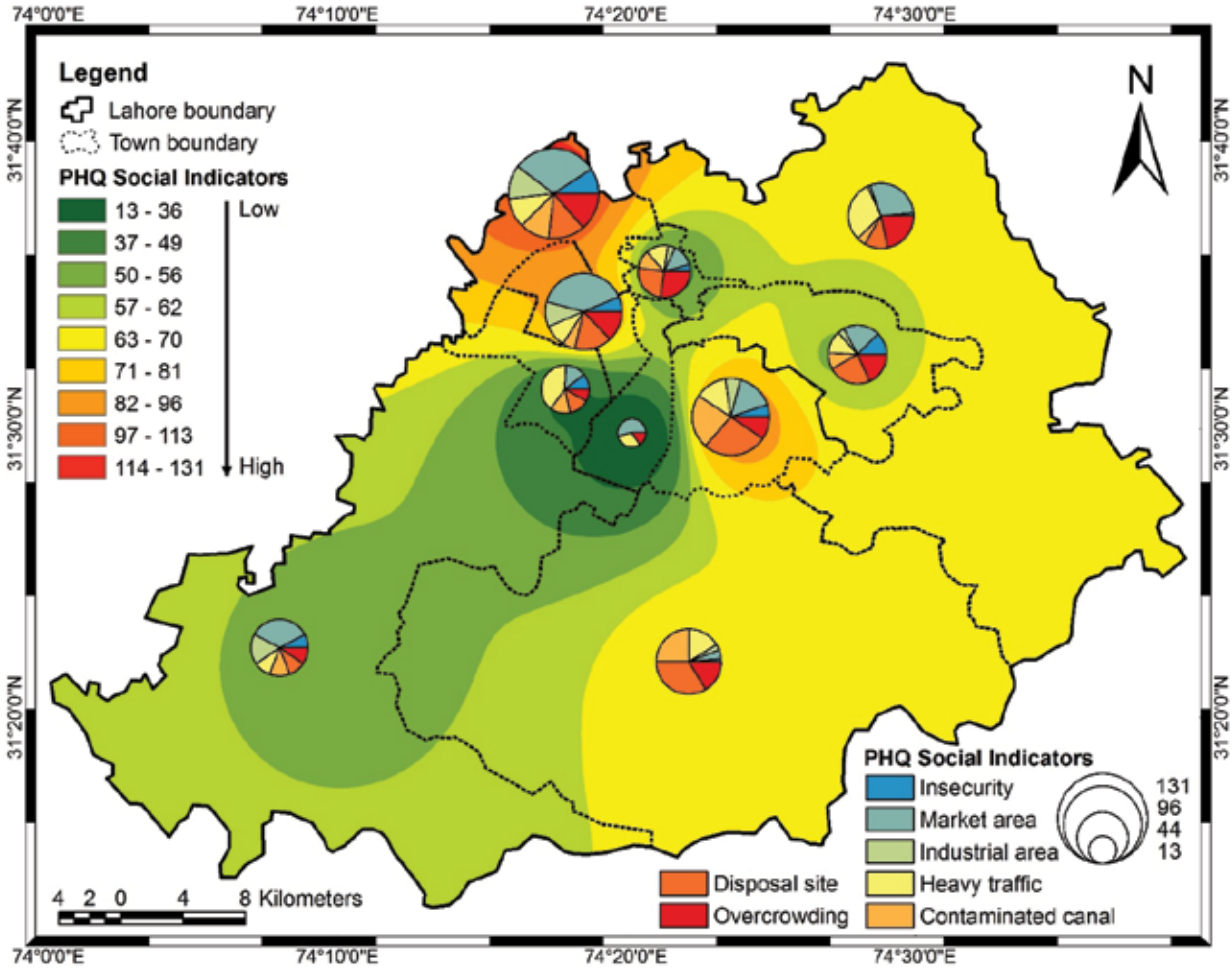

Fig. 6. Spatial distribution of social indicators in Lahore.

However, Gulberg Town, Samanbad Town have highest level of herbicides usage as compared to other Towns. Nishtar Town has negligible response to herbicides (Fig. 7).

3.7 Specific Health Issues (SHI)

Specific health issues (SHI) referred to chronic illness which requires serious medical attention. Gastrointestinal illness was reported by 13.3%. Vector borne diseases included 19.3% responses of malaria. High frequency rate of responses denotes that most of the population suffers from typhoid, malaria and skin allergy (Table 11).

Spatial analysis demonstrates that gastrointestinal illness occurs in majority of persons. Ravi Town has a highest number of gastrointestinal illness and vector borne diseases. SHI are distinctively higher in Ravi Town and Shalamar Town (Fig. 8).

Table 10. Chemical indicators of housing quality.

Variables	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Use of insecticides	508 (84.7)	92 (15.3)
Every week	587 (97.8)	13 (2.2)
Every month	467 (77.8)	133 (22.2)
Every year	235 (39.2)	365 (60.8)
Use of herbicides (per year)	436 (72.7)	164 (27.30)

3.8 General Health Issues (GHI)

GHI refers to those diseases or health issues which are very common to occur and are not fatal. Majority of respondents (45.6%) reported persistent cough and 42.6% claimed eye sight issues. Fever, headache, and blood pressure were reported around 30% which is quite considerable. Self-reported cases of abdominal pain were 26.0%, while 25.5% suffering from muscular pain, and 21% claimed to

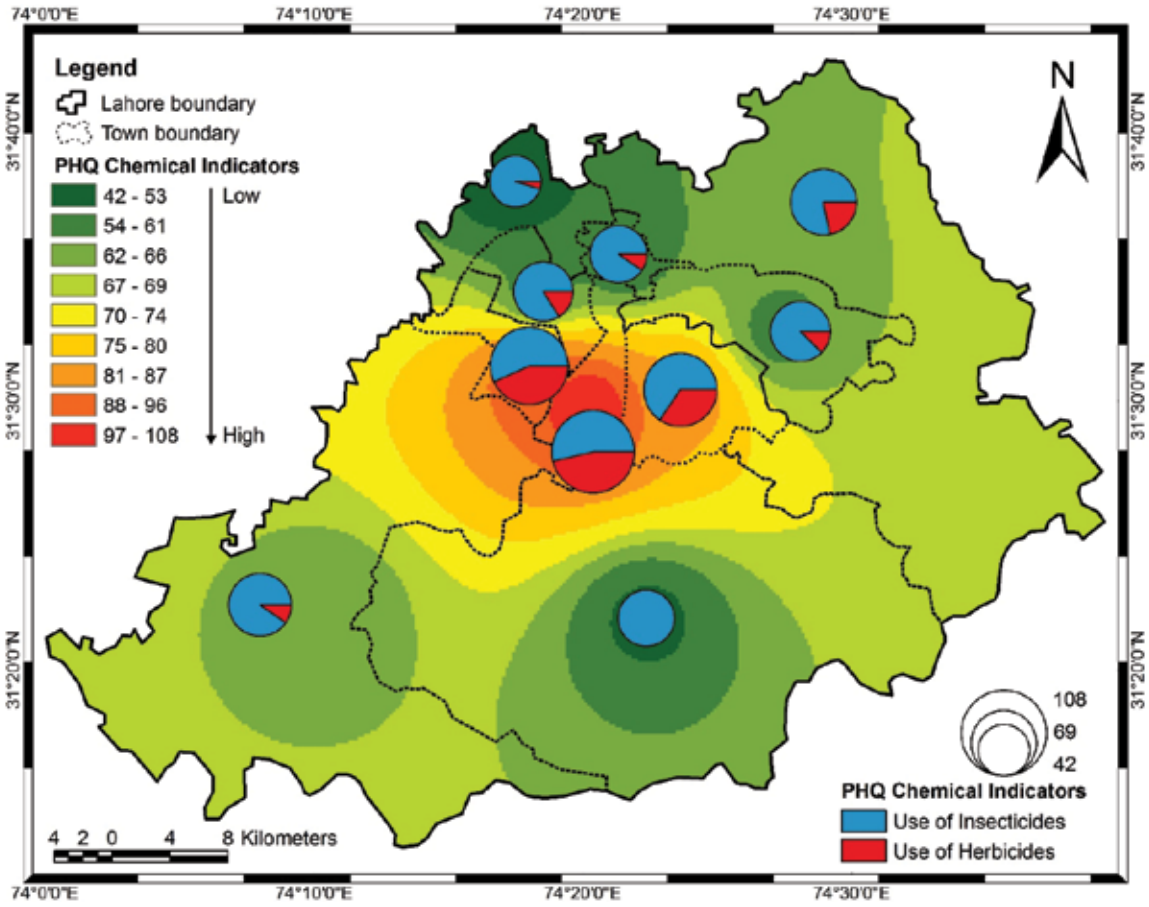

Fig. 7. Spatial distribution of chemical indicators in Lahore.

Table 11. Specific health issues.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Respiratory infections		
Lungs cancer	599 (99.8)	1 (0.2)
Asthma	556 (92.7)	44 (7.3)
Tuberculosis T.B.	566 (92.7)	44 (7.3)
Gastrointestinal illness		
Diarrhea	520 (86.7)	80 (13.3)
Cholera	513 (85.4)	87 (14.5)
Dysentery	578 (96.2)	22 (3.7)
Typhoid	466 (77.5)	134 (22.3)
Vector borne diseases		
Malaria	484 (80.5)	116 (19.3)
Dengue	494 (82.2)	106 (17.6)
Cardio vascular diseases		
Heart attack	529 (88.0)	71 (11.8)
Angina	569 (94.7)	31 (5.2)
Allergies		
Pet allergy	595 (99.0)	5 (0.8)
Skin allergy	480 (79.9)	120 (20.0)
Dust allergy	531 (88.4)	69 (11.5)
Pollen allergy	567 (94.3)	33 (5.5)

have obesity, these frequencies are also noteworthy. Minimum response was given to hearing issues (4.5%) and cold (12.2%). Accidental injuries due to fall and burn were reported by a few respondents (Table 12).

General health issues were highest in Ravi Town. The respondents in Nishtar Town and Wahga Town also reported high incidents of GHI. Gulberg Town has shown lowest value of GHI (i.e., 84). Samanabad Town and Iqbal Town also have low level of GHI. Frequent cough and abdominal pain, week eye sight and muscular pain is reported by majority of persons among general physical health issues (Fig. 9).

3.9 Psychological Health Issues (PHI)

Psychological health issues (PHI) represents those health issues which are related to behavioral problems like aggressiveness, lack of sleep and laziness. Aggressive behavior is highly responded by 28.7% persons as compare to other selected psychological health issues. 21.1% cases reported

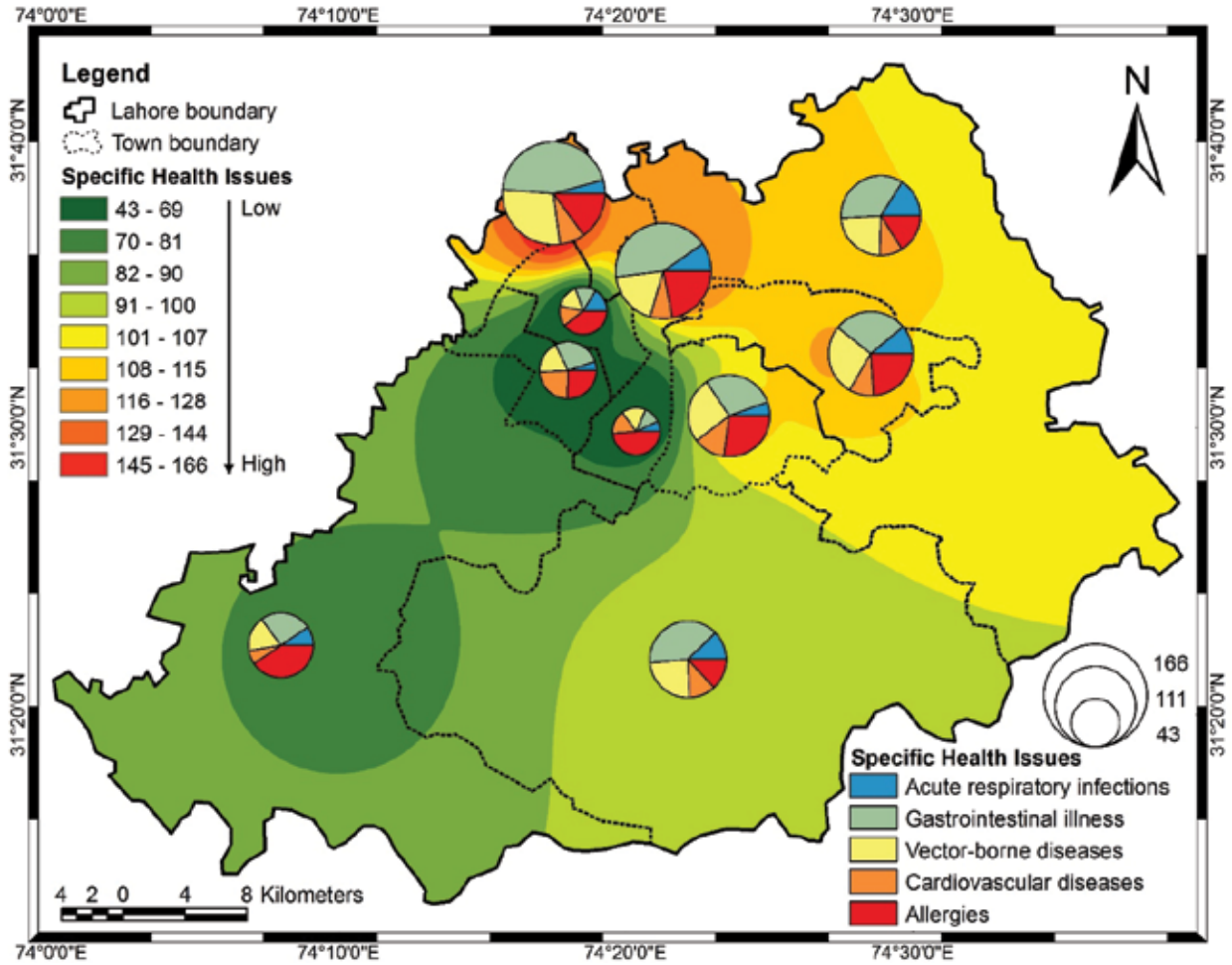

Fig. 8. Spatial distribution of specific health issues in Lahore.

depression which is also considerable. Lack of sleep (13.5%) and laziness (12.3%) were responded similarly (Table 13).

Occurrence of laziness and lack of sleep is less as compared to aggressiveness and depression. Spatial analysis indicate that highest number of psychological issues exists in Ravi Town, Aziz Bhatti Town, Data Gunj Bakhsh Town and Lahore Cantt.

3.10 Inferential Analysis

Inferential analysis was performed to analyze association between indicators of poor housing quality and health issues. Two types of tests were used in inferential analysis.

3.10.1 Pearson's Correlation Analysis

Pearson's correlation analysis is a type of inferential analysis based on technique of statistics, which can be performed to describe that how strongly

Table 12. General health issues observed in the survey.

Variable	No	Yes
	No, (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Eye sight problems	344 (57.2)	256 (42.6)
Abdominal pain	444 (73.9)	156 (26.0)
Muscular pain	447 (74.4)	153 (25.5)
Fever	420 (69.9)	180 (30.0)
Cough	326 (54.3)	274 (45.6)
Burn injury	596 (99.3)	4 (0.7)
Death due to burn injury	599 (99.7)	1 (0.1)
Hearing issues	573 (95.5)	27 (4.5)
Obesity	474 (78.9)	126 (21.0)
Headache	409 (68.1)	191 (31.8)
Blood pressure	417 (69.5)	183 (30.5)
Cold	527 (87.7)	73 (12.2)
Fall injury	590 (98.3)	10 (1.7)
Death due to fall injury	598 (99.7)	2 (0.3)

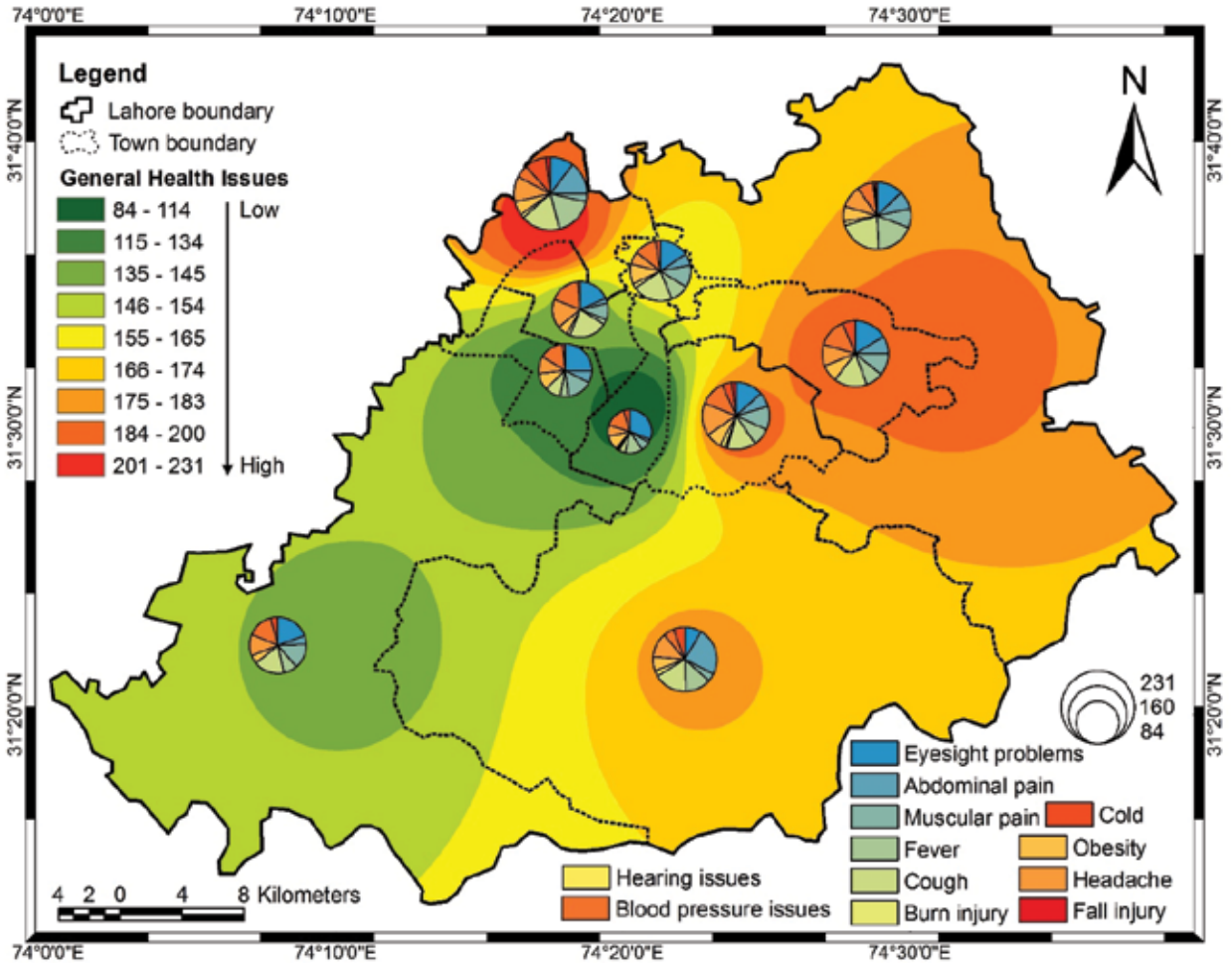

Fig. 9. Spatial distribution of general physical health issues in Lahore.

Table 13. Psychological health issues.

Variable	No	Yes
	No. (%)	No. (%)
Total	**600 (100)**	**600 (100)**
Depression	473 (78.7)	127 (21.1)
Aggressive behavior	428 (71.2)	172 (28.7)
Lack of sleep	519 (86.4)	81 (13.5)
Laziness	526 (87.7)	74 (12.3)

a relationship exists between two variables. The outcomes of Pearson's correlation analysis are precise by significance of "correlation coefficient" (r). Correlation coefficient (r) values range from +1 to -1. The value of +1 shows a positive relationship amongst variables but if the r is -1 then it denotes that there is a negative relationship between variables. Coefficient value (r) of 0 proves null relationship among variables. The analysis indicate a significant positive relationship between housing quality and health issues by showing (r =0.118) at level of $p<0.05$.

3.10.2 Pearson's Chi-square Test

Chi square analysis was applied between indicators of housing quality and selected diseases individually**.** Purpose of Pearson's chi square test was to find out those indicators of housing quality which are probably causing health issues, by fitting different frequencies with each other in cross tabulation. The *P*-value below 0.05 reflects that poor housing quality is causing health problems and if *P*-value is greater than 0.05 then, it shows no correlation between poor housing quality and health issues. The asterisks with *P*-values show three of the most commonly used levels of significance. If a *P*-value is less than 0.05 it is flagged with one asterisk (*). If a *P*-value is less than 0.01 it is flagged with two asterisks (**). If a *P*-value is less than 0.001 it is flagged with three asterisks (***). This test was applied on all observed health issues and all observed housing quality indicators. Finally only those indicators were selected who have shown

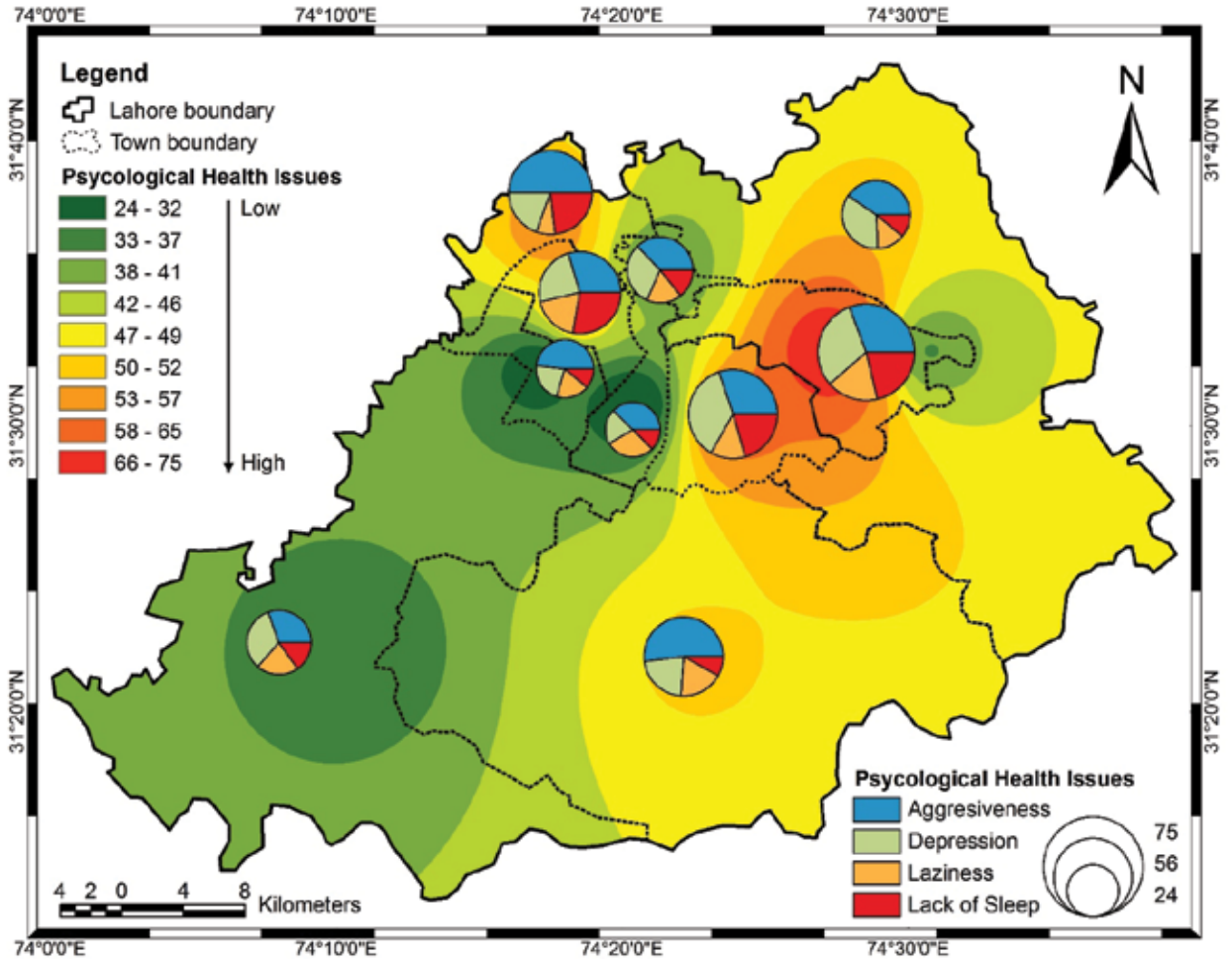

Fig. 10. Spatial distribution of psychological health issues in Lahore.

Table 14. Pearson correlation coefficients of housing quality and health issues.

Variables		r^1	Mean	SD
1-	Housing	-	35.19	5.57
2-	Health	0.118	5.09	2.76

[1]*r* is correlation coefficient, and SD is standard deviation

significant *p*-value, i.e., $P<0.05$ (Table 15).

3.11 Association between Poor Housing Quality (PHQ) and Health Issues (HI)

Poor housing quality (PHQ) indicators in Lahore shows varying trend between the Towns, i.e., increasing number of PHQ indicators represents poor housing condition and thus are related to increased number of health issues in those areas [11-20] (Fig. 12). The proportion of physical indicators of PHQ is higher than other indicators. Spatial analysis of all the factors of PHQ reveals that Ravi Town has highest level of poor housing quality with the frequency of 941 poor indicators, followed by Aziz Bhatti Town, Lahore Cantt and Nishtar Town. However, Gulberg Town represented least value of 631 (Fig. 11). Highest number of health issues was observed in Ravi Town. Households in Shalamar Town, Aziz Bhatti Town, Wahga Town, Lahore Cantt and Nishtar Town are also suffering from multiple health issues. Gulberg Town, Samanabad Town, Iqbal Towns and Data Gunj Bakhsh are observed as comparatively healthy areas (Fig. 12). Finally, our analysis proved that poor condition of houses is associated with poor health. Different poor housing conditions are affecting occurrence of different types of health issues in the study area

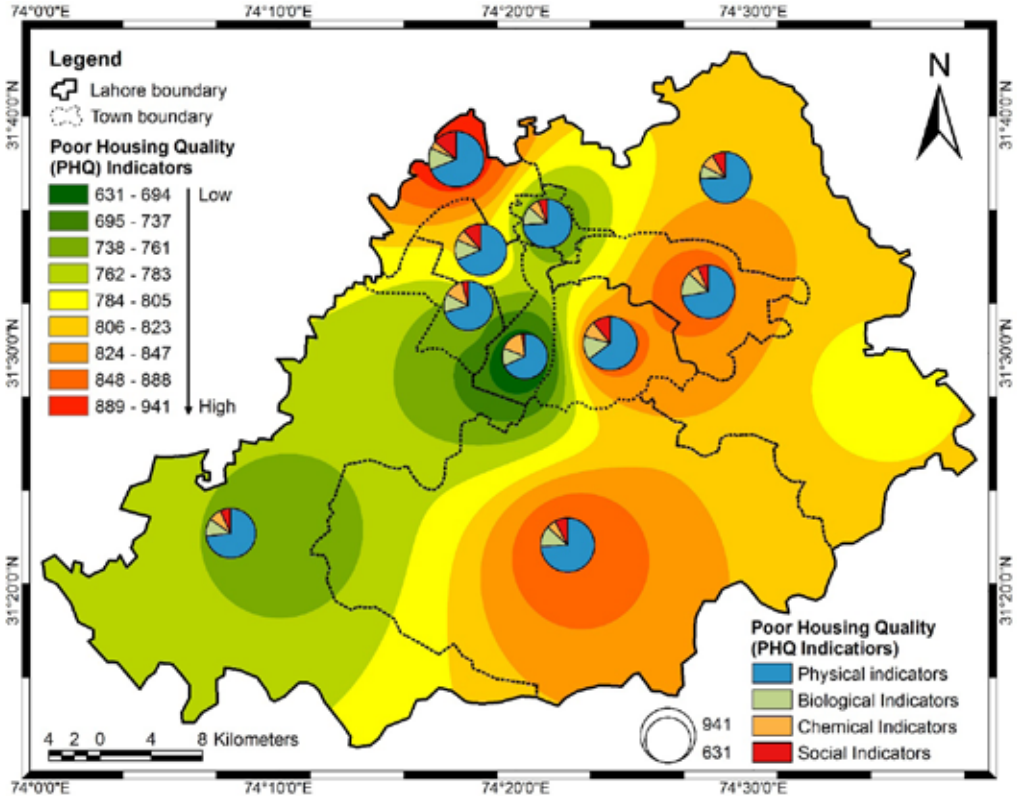

Fig. 11. Spatial distribution of poor housing indicators in Lahore. Red and orange colour exhibit very poor housing conditions related to worse health conditions in these areas (Fig. 12).

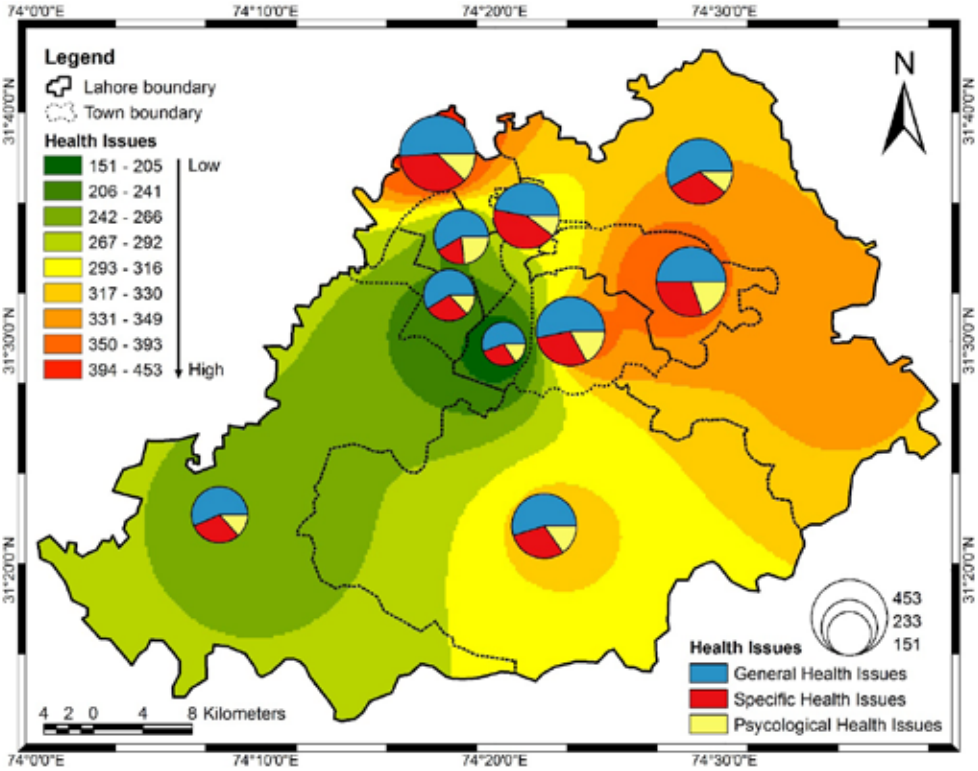

Fig. 12. Spatial distribution of health issues in Lahore. Red and orange colour exhibit high disease incidence areas because of poor housing quality in these areas.

Table 15. Final model of chi-square test giving significant *P*-value between poor housing quality indicators and health issues.

Sr. no.	Estimated relationship of two different frequencies	*P*-value
Factors of housing quality indicators affecting occurrence of abdominal pain		
1	Pests in houses are causing abdominal pain	0.015*
2	Fungus in homes is causing abdominal pain	0.003*
3	Disposal sites near house is a factor causing abdominal pain	0.002*
4	District water supply having unpleasant taste is a cause of abdominal pain	0.000**
Factors of housing quality indicators affecting occurrence of Cholera		
5	District water supply is reason behind cholera occurrence.	0.000***
Factors of housing quality indicators affecting occurrence of diarrhea		
6	District water supply is causing diarrhea	0.002**
7	Attached bathroom usage is causing diarrhea	0.001***
Factors of housing quality indicators affecting occurrence of Typhoid		
8	District water supply is a cause of typhoid	0.005**
Factors of housing quality indicators affecting occurrence of Cough		
9	Moisture presence in houses is causing cough	0.000***
10	White wash in houses is causing cough	0.002**
11	Air conditioner usage is causing cough	0.013*
12	No open space availability in houses is causing cough	0.016*
13	Industries near house is causing cough	0.032*
14	Overcrowding in homes is causing cough	0.005**
Factors of housing quality indicators affecting occurrence of tuberculosis		
15	Moisture presence in houses is causing tuberculosis	0.024*
16	Lack of open space availability in houses is causing tuberculosis	0.001***
17	Pests present in houses area causing tuberculosis	0.011*
Factors of housing quality indicators affecting occurrence of cold		
18	Moisture presence in houses is causing cold	0.043*
19	Usage of Air conditioner is causing cold	0.05*
Factors of housing quality indicators affecting occurrence of weak eyesight		
20	No open space around boundaries is affecting eye sight	0.011*
Factors of housing quality indicators affecting occurrence of skin allergy		
21	White wash in houses is causing skin allergy	0.02*
22	Fungus in houses is causing skin allergy	0.001***
23	Water cooler usage is causing skin allergy	0.005**
24	Overcrowding in houses is causing skin allergy	0.017*
Factors of housing quality indicators affecting occurrence of headache		
25	Moisture in houses is causing headache	0.003**
26	White wash in houses is causing headache	0.003**
27	Overcrowding in houses is causing headache	0.049*
28	Noise in houses in causing headache	0.000***
29	Bad smell in houses is causing headache	0.013*
Factors of housing quality indicators affecting occurrence of malaria		
30	Contaminated canal near house is causes malaria	0.031

Table 15 contd.....

Table 15 (contd.....)

Sr. no.	Estimated relationship of two different frequencies	*P*-value
31	Disposal site near house is causing malaria	0.000***
32	Industries near house is causing malaria	0.002**
Factors of housing quality indicators affecting occurrence of dengue		
33	Market area near house is causing dengue	0.05*
Factors of housing quality indicators affecting occurrence of hearing issues		
34	Noise in house is causing hearing issues	0.000***
Factors of housing quality indicators affecting occurrence of obesity		
35	Lack of open space availability in houses is causing obesity	0.000***
36	Overcrowding in house is causing obesity	0.000***
Factors of housing quality indicators affecting occurrence of blood pressure		
37	Overcrowding in house is causing blood pressure issues	0.000***
38	Fungus in house is causing blood pressure issues	0.001***
Factors of housing quality indicators affecting occurrence of heart attack		
39	Pests presence in house is affecting heart attack occurrence	0.021*
Factors of housing quality indicators affecting occurrence of muscular pain		
40	Fungus presence in house is causing muscular pain	0.029*
41	Lack of open space in house is causing muscular pain	0.000***
42	Insufficient exposure of sun in house is causing muscular pain	0.000***
Factors of housing quality indicators affecting occurrence of burn injury		
43	Gas supply through surface pipes is causing burn injuries	0.03*
Factors of housing quality indicators affecting occurrence of depression		
44	Moisture presence in house is affecting depression occurrence	0.000***
45	Fungus presence in houses is affecting depression occurrence	0.046*
46	Bad smell in house is affecting depression occurrence	0.005**
47	Noise in house is affecting depression occurrence	0.003**
Factors of housing quality indicators affecting occurrence of aggressiveness		
48	Moisture presence in house is affecting occurrence of aggressiveness	0.034*
49	Fungus presence in houses is affecting occurrence of aggressiveness	0.046*
50	Noise in houses is affecting occurrence of aggressiveness	0.000***
Factors of housing quality indicators affecting occurrence of lack of sleep		
51	Noise in house is affecting occurrence of lack of sleep	0.007**

(Table 15). However chemical indicators have not shown any relation with health issues.

3.12 Limitations

Researcher was unable to approach old Anarkali part of Lahore (area of old and critical condition of housing) due to non-cooperative behavior of residents of the colony. Residents didn't answer the questionnaire as they were quite security conscious and also they didn't allow researcher to enter their house to examine and assess housing conditions by self.

4. CONCLUSIONS

This investigation is a first approach to highlight the association between housing quality and health issues in Lahore using public level data. The poor housing conditions in Lahore's households are linked with ill health among the residents; the general public health can be improved with improved housing conditions. The results of this study may attract attention of the local and provincial government authorities to improving housing and health issues in Lahore.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (3): 259–261 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Short Communication

Combining Ability in *Jatropha curcas* L. Genotypes

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1. INTRODUCTION

Jatropha curcas L. is a vegetable oil plant growing under diverse climates throughout the year. This plant can adapt to very low rainfall areas and marginal lands possessing low soil fertility, and could produce high biomass as a potential cover crop to reduce evaporation [1]. The *J. curcas* biodiesel has numerous advantages, such as: more environmental friendly due to its better emission, higher combustion efficiency, biodegradable, and renewable. Oil content in *J. curcas* is rather high which can be used to replace diesel fuel to some extent [2]. thus, the Jatropha biodiesel has the potential to increase an independent fuel supply [3].

In an attempt to provide biodiesel fuel in Indonesia, by the year 2025 the cultivation area under *J. curcas* is intended to be increased to about 2.4×10^6 ha; the available land area for the purpose in the country is a mostly dry and unproductive otherwise [4]. The Directorate General of Plantation has planned for *J. curcas* cultivation especially in West Nusa Tenggara, East Nusa Tenggara, Southeast Sulawesi, Gorontalo, Maluku, and Papua areas [5]. However, a desirable variety of *J. curcas* with high production potential and adaptability in such areas is not available as yet. The success of the breeding program to produce such a leading variety relies predominantly on the available germplasm. The potential of a good genotype source can be exploited through introduction, exploration and hybridization. In an effort for exploration of *J. curcas* during 2005, the Centre for Research and Plantation Development had collected 421 germplasm accessions from East Java, West Nusa Tenggara, East Nusa Tenggara, and South Sulawesi areas [6]. The variety improvement can be attempted by utilizing a proper germplasm source [8]. This research was conducted in the plantation of *J. curcas's* germplasm located in Asembagus – Situbondo, East Java, Indonesia using seven local accessions of *J. curcas*, i.e., HS49, SP16, SP38, SP8, SM33, SP34, and SM35 [7].

2. METHODOLOGY

During flower-initiation stage, the prospective cross crops were covered with gauze. Emasculation of pollen in parent plants was done in early morning while the flowers were subsequently wrapped in transparent plastic bags to avoid contamination by other pollen. Pollen was taken from selected parent plants by cutting the flower containing mature pollen. The hybridization process was conducted in early morning from 5.00 a.m. to 6.00 a.m. The pollinated flowers were labeled numerically as pollination codes and further wrapped with gauze bags to guarantee its purity. After the pollination

Received, January 2017; Accepted, September 2017
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process, the soil moisture was maintained at adequate level. The gauze bags were opened 7 d after the pollination and any non-pollinated flowers were disposed off. Jatropha seeds were gradually harvested by firstly picking yellow fruits, peeling it out immediately after being harvested, and drying the seeds until their moisture was reduce to approximately 7 %.

3. RESULTS AND DISCUSSION

J. curcas oil is a non-edible and renewable oil source. In breeding program, any genetic information of varied germplasms that will be crossed must necessarily be recognized whether through phenotypic or molecular characterization activities. The variation of local *J. curcas* germplasm can be utilized for the variety improvement when there is available information on patterns of genetic variation and phylogenetic relationship among genotypes. Various analytical techniques have been used to study on the genetic relationships as well as individual performance within and among plant species, including Jatropha [9, 10].

From 42 cross combinations tested in this research, there were 10 combinations that did not result any seeds at all. The crosses among accessions that had been done were not entirely successful in obtaining fruits and seeds. The cross between SP8 × HS29 produced highest level of compatibility (88.33 %), followed by the cross between HS49 × SP38 (72.67 %). The highest production of dry seeds was obtained from the cross between SP8 × HS29 (569.14 g), followed by the cross between SM35 × HS49 (449.89 g). The dry weight of 100 seeds from this cross was 65.83 g to 72.84 g.

Pollination is the process by which pollen is transferred to the female reproductive organs of a plant that has been emasculated. The pollination of *J. curcas* was conducted in early morning, after which the flower crown was covered to avoid contamination by other pollen. The pollinated flowers were labeled numerically as pollination codes and further wrapped with gauze bags to guarantee its purity. Any non-pollinated flowers were disposed [11]. In this research, seeds were gradually harvested by firstly picking yellow mature fruits. The use of accessions SP16, SP8, SP38, SP33, SP34, and SM35 as parent plants was apparently less suitable since evidently there were several failures of seed production in some cross combinations.

4. CONCLUSIONS

The crosses among seven *J. curcas* accessions (i.e., HS49, SP16, SP38, SP8, SM33, SP34, and SM35) were not successful in obtaining fruits and seeds; in total, 14 cross combinations did not produce fruits nor seeds at all. The cross between SP38×HS49 produced the highest number of fruits and seeds, followed by the cross between SM35×HS49.

5. ACKNOWLEDGEMENTS

The authors thank Directorate of Research and Community Services of University of Muhammadiyah Malang, Ministry of Research, Technology and Higher Education of Republic of Indonesia, Ministry of Agriculture of Republic of Indonesia, and Indonesian Sweetener and Fiber Crops Research Institute for provision of the requisite research facilities.

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ACKNOWLEDGEMENTS (font size 10)**:** In a brief statement, acknowledge financial support and other assistance.

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2. Bialek, W. & S. Setayeshgar. Cooperative sensitivity and noise in biochemical signaling. *Physical Review Letters* 100**:** 258–263 (2008).
3. Kay, R.R. & C.R.L. Thompson. Forming patterns in development without morphogen gradients: differentiation and sorting. *Cold Spring Harbor Perspectives in Biology* 1: doi: 10.1101/cshperspect.a001503 (2009).

b. **Books**

4. Luellen, W.R. *Fine-Tuning Your Writing*. Wise Owl Publishing Company, Madison, WI, USA (2001).
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c. **Book Chapters**

6. Sarnthein, M.S. & J.D. Stanford. Basal sauropodomorpha: historical and recent phylogenetic developments. In: *The Northern North Atlantic: A Changing Environment.* Schafer, P.R. & W. Schluter (Ed.), Springer, Berlin, Germany, p. 365–410 (2000).
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